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BELGIAN SOCIETY FOR MICROBIOLOGY

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National Committee for Microbiology
of

The Royal Academies of Science
and the Arts of Belgium

Microbes without Frontiers

Palace of Academies

Brussels

October 18, 2019

Microbes without Frontiers

Brussels, October 18, 2019

08.30	Registration – Poster mounting
09.00	Welcome address: GR Cornelis, President of BSM
09.10	***Session A : General Microbiology*** *- Chair: E Peeters* Plenary speaker: **Sonja-Verena Albers** *Assembly of cell surface appendages of Archaea and their role in motility, adhesion and biofilm formation*
09.55	***Session B: Applied and Environmental Microbiology*** *- Chair: N Boon* Plenary speaker: **Jan Roelof van der Meer** *Integrative and conjugative elements, and their roles in bacterial adaptation to environmental stresses*
10.40	General assembly of BSM effective members
11.00	Coffee break and poster viewing
11.30	***Parallel sessions****: section A (Rubenszaal)* *Chairs Jan Michiels and Laurence Van Melderen* **Pierre Godessart**: Several integral outer membrane proteins are covalently linked to peptidoglycan in *Brucella abortus* **Sander K. Govers:** Nucleoid size scaling and intracellular organization of translation across bacteria **Tatjana Schlechtweg:** Cryptic prophages are important for persistence to high concentrations of ofloxacin in *E. coli* **Ian Vandenbussche**: The role of DNA methylation as epigenetic regulator of gene expression in *Burkholderia cenocepacia* ***Parallel sessions****: section B (Troonzaal)* *Chairs Isabelle George and Nico Boon* **Sofija Andrić***:* Impact of interspecies interactions on the production of biocontrol-related metabolites by *Bacillus velezensis* **Benjamin Horemans***:* Pesticide degrading bacteria for the removal of micropollutants from drinking water. From limitations to opportunities **Marie Legein:** The phyllosphere microbiome of greenhouse crops, a first step towards plant probiotics **Rogiers Tom***:* Microbial community dynamics of a meadow contaminated with metals, natural and artificial radionuclides ***Parallel sessions****: section C (Ockeghemzaal)* *Chairs Xavier Saelens and Paul Cos* **Gang Wang:** The polyamino-isoprenic efflux inhibitor NV716 revives old disused antibiotics against intracellular forms of infection by *Pseudomonas aeruginosa* **Evelien Vanderlinden:** Broad influenza virus inhibitor targeting inosine monophosphate dehydrogenase in a distinct manner from ribavirin **Olga Mineeva-Sangwo**: Unravelling the role of BK polyomavirus variants and of cellular CMP kinase on viral pathogenicity and clearance in kidney transplant recipients **Francesco Amisano**: ß-Lactams Translocation Through *Pseudomonas aeruginosa* Outer Membrane

	Parallel sessions**: section D (Marie-Thérèsezaal)* *Chairs Xavier De Bolle and Laurent Gillet* **Igor Fijalkowski**: Hidden in plain sight - proteogenomic view of bacterial proteoform expression in bacterial infection **Emma Hernandez-Sanabria**: Celecoxib builds up and cues metabolically active bacteria inhabiting the mucosal environment of a simulated human intestinal ecosystem, modulating inflammatory response **Georges Potemberg:* Identification of bacterial genes indispensable to pulmonary Brucella infection in mouse experimental model **Sara Van den Bossche:** The effect of airway epithelial cells on antibiotic efficacy towards cystic fibrosis clinical isolates of *Pseudomonas aeruginosa*
12.45	Lunch and poster viewing
14.30	***Session C: Medical and Veterinary Microbiology** - Chair: X. Saelens* Plenary speaker: **Caroline Goujon** *Interferon and antiviral restriction*
15.15	***Session D: Host and microbial interactions** - Chair: G. Cornelis* Plenary speaker: **Dirk Bumann** Salmonella *single-cell biology in host tissues*
16.00	*Coffee break and poster viewing*
16.30	***BSM honorary Lecture** - Chair: G. Cornelis (introduction) - N Boon* Plenary speaker: **Willy Verstraete** Microbial Ecology and Technology: The Riddles of the Past & the Challenges of the Future
17.15	Announcement of poster and agar-art awards
17.45	End of the meeting

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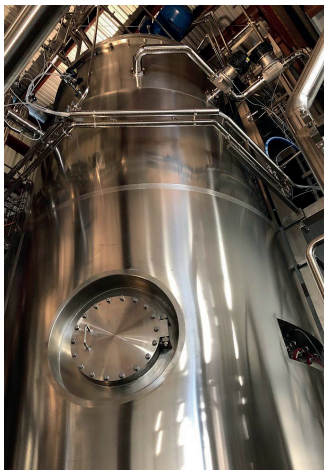

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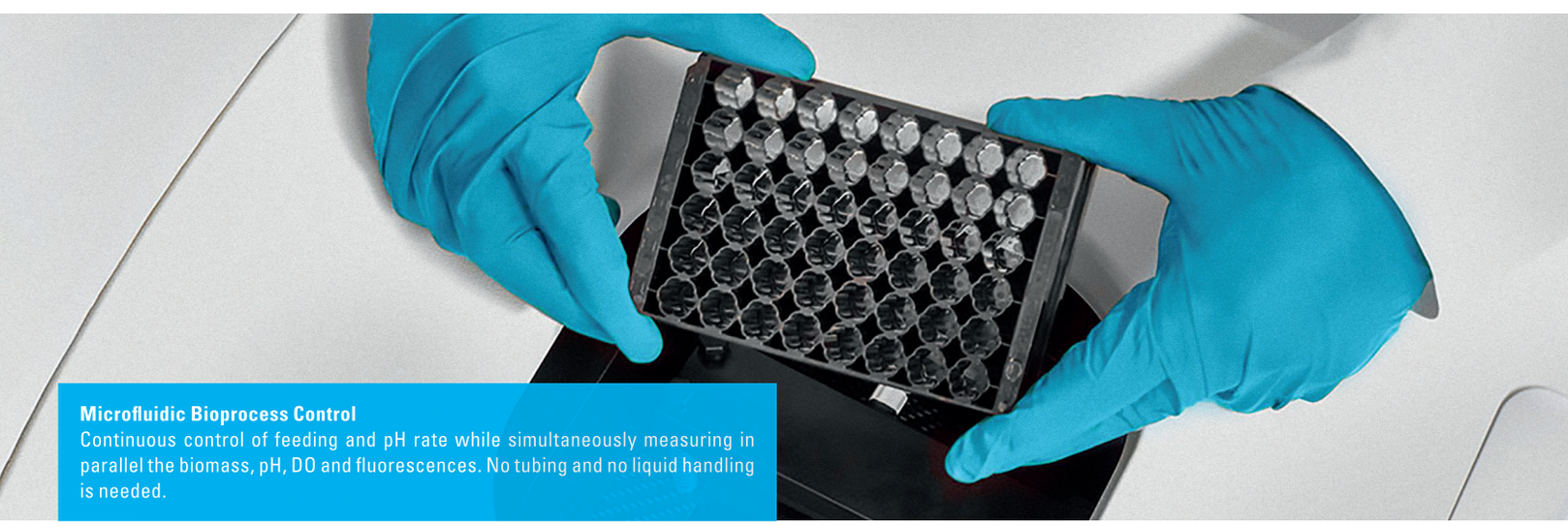

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2018/04

About us

Syngulon is a **synthetic biology startup** developing original genetic technologies using **bacteriocins** to improve microbial fermentation.

Our team of scientists works in different academic laboratories and our R&D programs involve partners from Belgium, Brazil, France, Germany, The Netherlands, and the UK. We think of ourselves as a "**Startup in the Labs**", meaning that we work directly in the labs of our academic partners.

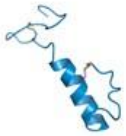
The members of our **Scientific Advisory Board** are active in Belgium, France and the USA.

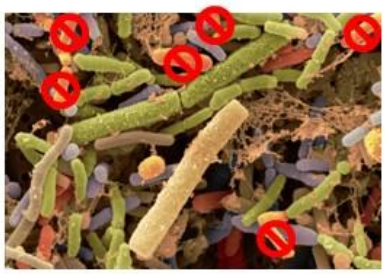
What are bacteriocins? Why use them?

André Gratia

- Discovered in 1925 by Belgian scientist: "**André Gratia** (1893–1950): Forgotten Pioneer of Research into Antimicrobial Agents"
- Heterogenous group of **antimicrobial peptides** produced **ribosomally** by **bacteria**
- Used to **kill related species** to **reduce competition** for **resources** and **space**
- **Not toxic**
- Small peptides that in many cases do not undergo post-translational modification to be active = **Ideal for gene-based peptide engineering**[1]
- Active against **antibiotic-resistant bacteria** (VRE, MRSA…)[2]
- Antimicrobial activity at the **nanomolar range scale**
- (more active than most antibiotics) [2]
- Naturally produced by **probiotic** strains
- **Nisin** is a bacteriocin widely used in the **food industry**
- Availability of broad and **narrow spectrum** of activity

Pediocin PA-1 [3]





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[1]Cotter et al., Nat. Rev. Microbiol., 2013
[2]Mathur et al., *Front. Microbiol.*, 2017
[3]Oppegård et al., *Biochemistry*, 2015

3

Our latest publication: "PARAGEN 1.0: A Standardized Synthetic Gene Library for Fast Cell-Free Bacteriocin Synthesis", Gabant et al., Front. Bioeng. Biotechnol., 06 September 2019

Startup in the labs

At **Syngulon**, we work closely with Universities to develop and improve our technologies. We also focus on generating publishable data related to bacteriocin production and their interactions within microbial networks. We are a "**Startup in the Labs**", meaning that the members of our team perform research directly in our partner labs and have a strong link to academic research. In this way, we can directly work with our academic partners in their own labs, which allows us to have a strong R&D relationship while maintaining our confidentiality.

Main existing academic collaborations:

Pr. Laurence van Melderen, Université libre de Bruxelles – Cellular and Molecular Microbiology

Pr. Bruno André, Université Libre de Bruxelles – Molecular Cell Physiology

Pr. Frank Delvigne, Gembloux Agro-Bio Tech- Liège Université – Microbial Processes and Interactions lab (MiPI)

Pr. Pascal Hols, Université Catholique de Louvain – LIBST (Louvain Institute of Biomolecular Science and Technology) –

Pr. Vitor Pinheiro(*), University College of London – Institute of Structural and Molecular Biology

Pr. Chris Barnes, University College of London – Centre for Computational Statistics and Machine Learning (CSML)

(*) also KULeuven since September 2018

We are open to new collaborations! (please contact: pgabant@syngulon.com)

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Dr. Philippe Gabant, Co-Founder, CSO

Dr. Mohamed El Bakkoury, CTO Yeast
Bertrand Delahaye, R&D Scientist
Thomas Gosset, Ir, R&D Engineer
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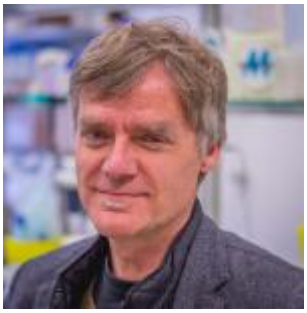
Presentation of plenary speakers

Section A

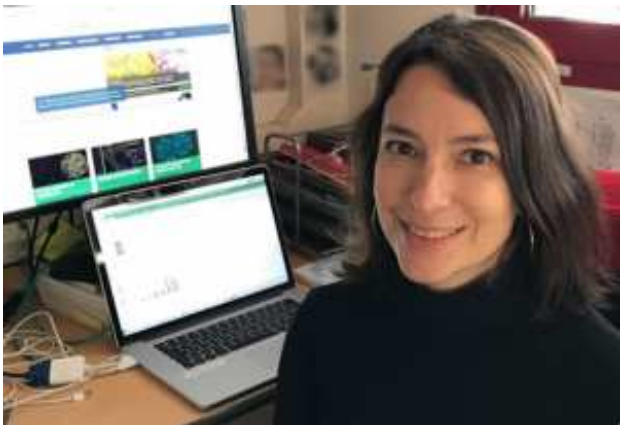
Sonja-Verena Albers will be welcomed as the plenary speaker for section A (General Microbiology). Sonja-Verena Albers is one of the key scientists investigating archaeal microorganisms, prokaryotes that are phylogenetically distinct from bacteria. Already during her "diploma" and PhD studies (at the University of Würzburg (DE) and University of Groningen (NL), respectively), she was fascinated by archaea, with a focus on the model organism *Sulfolobus spp*. Sonja-Verena Albers delivered important contributions to the basic understanding of physiological processes in these organisms such as the assembly and composition of the cell envelope and surface appendages.
A major accomplishment is the unravelling of the structure and function of the archaeal motility structure, named "archaellum". Besides basis science, Sonja-Verena Albers contributed to the field by developing an advanced genetic toolbox for the genetic manipulation of these organisms, which is widely adopted by other scientists. After establishing an independent research group at the Max Planck Institute for Terrestrial Microbiology in Marburg (DE) in 2008, she moved to the Albert-Ludwigs-University of Freiburg (DE) in 2014, where she obtained a full professorship. Recently, she is extending her research focus towards other archaeal model organisms (haloarchaea).

Section B



Jan Roelof van der Meer (University of Lausanne, Switzerland) is the plenary speaker of section B (Applied and Environmental Microbiology). **Jan Roelof van der Meer** studied Environmental Sciences at the Agricultural University in Wageningen, The Netherlands. He performed his PhD research with Alexander Zehnder and Willem de Vos as advisors, still at the Wageningen Agricultural University. After a postdoc at the Dutch Dairy Institute, he became Junior Group Leader in Environmental Microbiology at the Swiss Federal Institute for Aquatic Sciences (Eawag).
In 2003, he moved to the University of Lausanne, in Switzerland. Since 2011,
Prof. van der Meer is Head of the Department of Fundamental Microbiology at the University of Lausanne. His research interests are genetic adaptation mechanisms in bacterial communities under pollution stress, as well as applications of bacteria for environmental benefits, for example, as biosensors or for community engineering.

Section C



Caroline Goujon (Montpellier, Fr.) is the plenary speaker of section C. Her main research interest has always been understanding the cell's natural defense mechanisms against viral infections. She studied at the Ecole Normale Supérieure (ENS) of Lyon (France), and performed her PhD in Prof. Jean-Luc Darlix's laboratory (INSERM / ENS-Lyon), in Dr Andrea Cimarelli's team.

She worked on understanding the restriction of HIV-1 infection in myeloid cells and discovered that an auxiliary protein from HIV-2 (called Vpx) was able to relieve this restriction. This work paved the way to the identification of SAMHD1 as a potent restriction factor of HIV-1. After gaining her PhD in 2007, she joined Prof. Michael Malim's group at King's College London (United Kingdom) in 2008 and was initially awarded a Marie Curie Intra-European Fellowship to study whole-genome expression changes in primary T cells following HIV-1 infection. In parallel to this work, she started to develop an interest in the relationships between the interferon system and HIV-1. Indeed, it had been known for decades that type 1 interferon treatment induced a potent block to HIV-1 infection but the effectors of this block were unknown. She characterized this interferon-induced block in depth and undertook a comparative transcriptome analysis in order to generate a list of interferon-stimulated gene candidates potentially responsible for the inhibition of HIV-1 infection. This approach led, in 2013, to the identification of the GTPase MX2 (also called MxB) as one of the effectors in the HIV-1 interferon block (Goujon et al, Nature 2013). Interestingly, MX2 is homologous to MX1 (or MxA), a very well-known restriction factor able to inhibit a broad range of viruses, including influenza A virus. In 2014, she was awarded the Andy Kaplan prize, which honors the accomplishments of a distinguished postdoctoral scientist in the retrovirology field. In 2015, she moved back to France and started her own lab at the CNRS/Montpellier university co-funded IRIM institute (Institut de Recherche en Infectiologie de Montpellier), thanks to the support of an INSERM researcher permanent position and a CNRS/INSERM ATIP-Avenir grant. Using two major pathogenic viruses as models, HIV-1 and influenza A virus, Caroline was awarded an ERC Starting Grant in 2017 to study the innate cellular defense mechanisms against viral infections. In collaboration with her former lab, she recently identified the short isoform of NCOA7 as a new interferon-induced barrier to endocytosis-mediated viral entry into host cells (Doyle et al, Nature Microbiology 2018).

Section D

Dirk Bumann (Biozentrum, Universität Basel, CH), the plenary speaker for section D is, by training a chemist and a biologist. He did his PhD thesis at the Max Planck Institute of Biochemistry (Martinsried, Germany). He was a post-doctoral fellow at the same institute and later at the Marine Biological Laboratory of Woods Hole (USA). Back to Europe, he became team leader at the Max-Planck Institute for Infection Biology (Berlin) and later independent junior group leader at the Hannover Medical School.

In 2007, he was appointed as Associate Professor for Infection Biology at the Biozentrum (University of Basel) and was promoted to Full Professor in 2015.
His career is marked by deep thinking, great curiosity and innovative approaches such as the use of fluorescence-activated cell sorting to identify bacterial genes expressed in the host, which unraveled the heterogeneity of host-pathogen interactions in vivo.
Unlike many infection biologists, **Dirk Bumann** investigated several different pathogens including *Helicobacter pylori*, *Salmonella enterica*, *Shigella flexneri* and *Pseudomonas aeruginosa* and was among the pioneers in drawing attention to the bacterial metabolism during infection. He received several rewards including the prestigious EMBO Young Investigator award (2006) and the Pettenkofer Prize of the City of Munich (2015). He is an EMBO Member since 2015.

Honorary lecturer

Willy Verstraete (April 25, 1946) is the Belgian honorary lecturer nominated for 2019. He graduated in 1968 from the Gent University as bio-engineer and in 1971, he obtained a Ph D degree in the field of microbiology at the Cornell University, Ithaca (USA). Since 1979, he worked at the Gent University as professor and head of the Laboratory of Microbial Ecology and Technology. In 2011, he became emeritus professor.

His R&D has as central theme: **Microbial Resource Management**; i.e. the design, operation and control of processes mediated by mixed microbial cultures. Willy Verstraete has field experience with respect to drinking water production plants, aerobic wastewater treatment, anaerobic digestion of wastewaters and sludges, solid state fermentation of organic residues and bioremediation processes of soils and sediments. He has also gained experience in various aspects of pre- and probiotics used in human and animal nutrition and in systems which simulate the latter.

In 2005, he received the prestigious Excellence in Science Prize , awarded by the Science Foundation FWO , Belgium .

Since 2014 , he ranks in the list of Highly Cited Researchers , due to the fact that several of his papers belong to the top 1% most cited papers of his field .

In april 2016 , Willy Verstraete was elected President of the Science Foundation FWO , Belgium .

In september 2016 , he received from the International Water Association (IWA) and the International Society for Microbial Ecology (ISME) , the Ardern and Lockett Award for his contributions in the fields of water engineering and microbial ecology .

In october 2016 , Nature Microbiology published a new archaeal phylum Verstraetearchaeota ,recognizing the contributions of his team at the Ghent University to the development of engineered microbial ecosystems .

In 2018 , the Dutch Water Institute KWR proclaimed him as Honorary Fellow for his invigorating resource recovery science and application work. In 2018 he also received the Great Award of the Flemish Government for his contributions to science and science governance.

Abstract Honorary Lecture

Microbial Ecology and Technology : The Riddles of the Past & the Challenges of the Future

W. Verstraete , CMET , Ghent University

Looking back , I very early on struggled with the concepts of the soil as A 'jungle' where apparently billions of micro-organisms survived in a constant struggle for life . Particularly the fact that nitrogen conversions occurred so effectively and had such major consequences for the environment and yet could never be explained on the basis of the numbers of the nitrifiers detected , was most intriguing . In further phase , the story of methanogenesis with conversion reactions against the thermodynamic gradient became a fascination . The latter got us trapped in the challenge to understand how and why microorganisms grow in flocs and granules . Gradually , all of these riddels got 'resolved ' in the magics of the word 'microbiome' .

Looking forward , there is a large load of challenges where the microbial ecologist and technologist has a very prominent role to play. The 2015 Paris climate conference generated hope that society will invest in efforts to deal with a number of issues to avoid further deterioration of the globe . In terms of microbiology , there is a lot to offer .

First , the planet struggles with a major N problem : we apply massive amounts of Haber Bosch mineral fertilizer to produce protein . The remedy is to produce microbial protein , particularly by using hydrogen as the electron donor . The hydrogen is generated by electrolysis driven by green electricity . Concomitantly , by using autotrophic CO2 fixing aerobic hydrogenotrophs , one can capture substantial amounts of CO2 into microbial biomass and use the latter for food , feed, organic fertilizer and a variety of biodegradable materials .

A second issue is the need to prominently engage microbial biotechnology in dealing with the greenhouse effect . Clearly , the diffuse CH4 emissions from soils and intestinal sources must be resolved .Moreover , microbiology can also contribute to massive storage of CO2 via deep undergound conversion of the latter to methane as currently demonstrated in Austria .

A third issue is the ongoing deterioration of the fresh water reserves and the fact that for many residues agricultural soil remains the "final solution and destination". The proposal is to drastically go for the technical combination of reductive gasification for all non-readily biodegradable wastes and couple the latter to clever microbial biotech as the subsequent upgrader of the energy and nutrient containing gases .

Finally , there is an absolute need to convince the public and particularly the regulators to improve the cooperation with 'our microbial friends' in order to decrease the massive use of antibiotics and disinfectants .

ABSTRACTS SHORT TALKS
SECTION A: GENERAL MICROBIOLOGY

A - ST01 - Several integral outer membrane proteins are covalently linked to peptidoglycan in *Brucella abortus*

P. Godessart[1], M. Dieu[2], A. Lannoy[1], S. Van der Verren[3], P. Renard[2,4], H. Remaut[3] and X. De Bolle[1,]*

[1]Research Unit in Microorganisms Biology (URBM)-Namur Research Institute for Life Sciences (NARILIS), University of Namur, rue de Bruxelles 61, 5000 Namur, Belgium. [2]MaSUN, Mass Spectrometry Facility, University of Namur, rue de Bruxelles 61, 5000 Namur, Belgium. [3]VIB-VUB Center for Structural Biology, Building E, Pleinlaan 2, 1050 Brussel, Belgium. [4]Laboratory of Biochemistry and Cell Biology (URBC)-Namur Research Institute for Life Sciences (NARILIS), University of Namur, 61, Rue de Bruxelles, 5000 Namur, Belgium.

[*]Corresponding author: xavier.debolle@unamur.be

Escherichia coli, a Gram negative model bacteria, has an abundant lipoprotein called Lpp, or Braun's lipoprotein, in the outer membrane (OM). Lpp is covalently bound to the peptidoglycan (PG) through the amino group of its C-terminal lysine (Volkmar & Bosch, 1972). This linkage is established by enzymes called L,D-transpeptidases (Ldts). *Brucella abortus* is an intracellular facultative extracellular pathogen and is the etiological agent of bovine brucellosis, a worldwide neglected anthropozoonosis. In *B. abortus*, there is no homolog of Lpp nor any known structure establishing a covalent link between the OM and the PG.

To investigate the existence of OM proteins (Omps) linked to the PG, we purified *B. abortus* PG while preserving proteins that could be covalently linked to PG. Mass spectrometry study showed that several proteins were abundant in the sample. All were predicted to be Omps and while they had no C-terminal lysine, a conserved aspartate was found at the position 2 of the mature protein sequences. Hence, we hypothesized that the link could be between the N-terminus of these proteins and PG. Hybrid fragment of PG and N-terminal protein fragments were successfully identified and sequenced in MS/MS confirming the covalent linkage of several Omps with the PG of *B. abortus*. To identify which Ldts are involved in the anchorage of the Omps to the PG, we established a heterologous *E. coli* system where we co-expressed one Omp (Omp25) and one Ldt of *B. abortus*. This heterologous system allowed us to identify three Ldts able to anchor the Omp of *Brucella* to the PG of *E. coli*. The role of one of these Ldt was also confirmed in *Brucella*. The deletion of this Ldt was also shown to impair the envelope integrity towards heat stress, highlighting the role of OM-PG anchorage in the envelope stability under stress conditions. Finally, the aspartate 2 was shown to have a crucial role in the anchoring, suggesting that it could be involved in the Omp recognition by Ldts, as its punctual mutation to an alanine abolished the Omp25 linking to PG. Preliminary MS data suggest that this mechanism is conserved in *Agrobacterium tumefaciens*. Bioinformatic analysis suggests that it could also be broadly found in Rhizobiales. Overall, we characterised a new OM-PG anchorage system that could be of major significance in the understanding of the envelope resistance towards stress in Rhizobiales.

A – ST02 - Nucleoid size scaling and intracellular organization of translation across bacteria

Sander K. Govers[1,2,*], *William T. Gray*[1,3,*], *Yingjie Xiang*[1,2], *Bradley R. Parry*[1,2], *Manuel Campos*[1,2,5], *Sangjin Kim*[1,4], *and Christine Jacobs-Wagner*[1,2,4,6].

[1]*Microbial Sciences Institute, Yale University, West Haven, CT, USA*

[2]*Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA*

[3]*Department of Pharmacology, Yale University, New Haven, CT, USA*

[4]*Howard Hughes Medical Institute, Yale University, New Haven, CT, USA*

[5]*Laboratoire de Microbiologie et Génétique Moléculaires, Centre de Biologie Intégrative, Centre National de la Recherche Scientifique, Université de Toulouse, UPS, Toulouse, France*

[6]*Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT, USA*

**Equal contribution*

Abstract

The scaling of organelles with cell size is thought to be exclusive to eukaryotes. Here, we demonstrate that similar scaling relationships hold for the bacterial nucleoid. Despite the absence of a nuclear membrane, nucleoid size strongly correlates with cell size, independent of changes in DNA amount and across various nutrient conditions. This correlation is observed in diverse bacteria, revealing a near-constant ratio between nucleoid and cell size for a given species. As in eukaryotes, the nucleocytoplasmic ratio in bacteria varies greatly among species. This spectrum of nucleocytoplasmic ratios is independent of genome size, and instead it appears linked to the average population cell size. Bacteria with different nucleocytoplasmic ratios have a cytoplasm with different biophysical properties, impacting ribosome mobility and localization. Together, our findings identify new organizational principles and biophysical features of bacterial cells, implicating the nucleocytoplasmic ratio and cell size as determinants of the intracellular organization of translation.

A - ST03 - Cryptic prophages are important for persistence to high concentrations of ofloxacin in *E. coli*

Tatjana Schlechtweg[1], Frédéric Goormaghtigh[2], Laurence Van Melderen[1]*

[1]Cellular and molecular microbiology CMM, ULB, Belgium

[2]Biozentrum der Universität Basel, Basel

[*]lvmelder@ulb.ac.be

Together with the current multidrug resistance crisis, bacterial persistence plays an increasingly important role in the failure of antibiotic treatments. Bacterial persisters are rare phenotypic variants that acquired transient tolerance to lethal doses of antibiotics. Due to their transiency and low frequency (± 1/10,000) the properties of persister cells are particularly difficult to characterize and so far, not much is known about the molecular mechanisms of bacterial persistence or the impact of antibiotic concentrations on persisters formation. Our group is especially interested in how a small subpopulation of persister cells is able to elude bactericidal action of ofloxacin, a fluoroquinolone which inhibits DNA replication by binding to DNA Gyrase. The effects of fluoroquinolones result in the induction of the SOS response and cause double-stranded DNA breaks, ultimately leading to cell death. Previous works showed that fluoroquinolone persisters rely on a functional SOS response, as also seen in the largely decreased rate of persisters in SOS-deficient cells (LexA3 mutant).

In this work we investigated the Eagle effect, which describes a paradoxical increase in survival when treating with increasing concentrations of antibiotics. Despite the fact that this phenomenon has been observed under several different conditions and strains, up to this day, the molecular basis behind the paradox remains unknown. We found that while exposure of wild type exponentially growing *E. coli* cultures to extreme concentrations (up to 240-fold the minimum inhibitory concentration; MIC) gave rise to more survivors than intermediate bactericidal concentrations (15-fold MIC), an *E. coli* mutant lacking all nine cryptic prophages (Δ9) no longer shows the paradoxical survival. We then measured cellular induction of SOS by the use of a fluorescent transcriptional reporter (p*sulA::gfp*) and observed that the non-monotonous survival rates inversely correlated with the level of induction of SOS in the wild type strain ($R=-0.8$). Interestingly, we found the lack of paradoxical survival in the Δ9 strain also coincided with the measured SOS induction levels ($R=-0.9$). To investigate the priorly proposed involvement of oxidative stress in killing by antibacterial agents we monitored the production of hydrogen peroxide in single cells using an H_2O_2-specific biosensor (HyPer-3). Our observations suggest a correlation between ofloxacin concentration, induction levels of SOS and oxidative stress in bacterial survival. The molecular mechanisms are currently under investigation. Our results underline previous findings of an important role of the SOS response for persistence and suggest an implication of cryptic prophages in the phenomenon of paradoxical survival at extreme antibiotic concentrations.

A - ST04 - The role of DNA methylation as epigenetic regulator of gene expression in *Burkholderia cenocepacia*

Ian Vandenbussche[a]#, Andrea Sass[a], Marlies Buelens[a], Marta Pinto[b], Olga Mannweiler[b], Leo Eberl[b], Tom Coenye[a]

[a] *Laboratory of Pharmaceutical Microbiology, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium*

[b] *Department of Plant and Microbial Microbiology, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland*

Respiratory tract infections by the opportunistic pathogen *Burkholderia cenocepacia* often lead to severe lung deterioration in cystic fibrosis (CF) patients. New insights in how to tackle these infections might emerge from the field of epigenetics, as DNA methylation has shown to be an important regulator of gene expression. Interestingly, while research on eukaryotic epigenetics seems to skyrocket, comparatively little research has been conducted on epigenetics and DNA methylation in prokaryotes. In the present study we focused on the function and role of specific DNA methyltransferase (MTase) enzymes in *B. cenocepacia* J2315 and K56-2 and their potential role in regulating gene expression. *In silico* predicted DNA MTase genes BCAL3494 and BCAM0992 were deleted in both strains, and phenotypic characteristics of the resulting deletion mutants were determined. ΔBCAL3494 mutants displayed a higher degree of pellicle formation and clustering, and a different biofilm morphology, whereas ΔBCAM0992 mutants showed a decrease in swimming motility. Furthermore, cultures treated with sinefungin, a known DNA MTase inhibitor, exhibited the same changes in phenotype as DNA MTase deletion mutants. Single-Molecule Real-Time Sequencing was used to characterize the methylome of *B. cenocepacia*, and several methylation motifs (CACAG, GTWWAC and GCGGCCGC) were identified. Deletion mutants ΔBCAL3494 and ΔBCAM0992 lacked adenine methylation in the CACAG and GTWWAC motif, respectively, and allowed to link the different DNA MTases to methylation of a specific motif. All genes with methylated motifs in their putative promoter region, and thus potentially under transcriptional regulation by DNA methylation, were determined. qPCR experiments showed an upregulation of several genes in MTase deletion mutants where methylated motifs were absent in the promoter region, and links could be drawn between the function of these genes and the altered phenotypes observed in the deletion mutants. In ΔBCAL3494, expression data showed an upregulation of the BCAM0820 gene, important for biofilm formation in *P. aeruginosa*. In addition, gene BCAL0079 was upregulated in ΔBCAM0992, a gene that already showed to affect motility in *E. coli*. These findings confirm that DNA methylation plays an important role in regulating the expression of *B. cenocepacia* genes involved in biofilm formation and motility.

ABSTRACTS POSTERS
SECTION A: GENERAL MICROBIOLOGY

A01 - Screening for antimicrobial activities of Actinobacteria isolated from cave moonmilk deposits

Delphine Adam[1], Marta Maciejewska[1], Pierre-Hugues Stefanuto[2], Aymeric Naômé[1], Loïc Martinet[1], Delphine Zanella[2], Jean-François Focant[2], and Sébastien Rigali[1]

[1]InBioS, Center for Protein Engineering, Liège University, B-4000, Liège
[2] Organic and Biological Analytical Chemistry Group (CART), Liège University, B-4000, Liège

Caves are subterranean ecosystems that are considered to be extreme habitats for life due to their high oligotrophy. Nevertheless, they are surprisingly full of life. Cave moonmilk deposits host a rich microbiome among which Actinobacteria represent one of the most abundant phyla. In this work, cultivable Actinobacteria were isolated from moonmilk of the "Grotte des Collemboles" in Belgium, and their taxonomic profile and their potential in biosynthesis of antimicrobials were evaluated. Phylogenetic analyses of the isolates revealed that a significant majority of them was affiliated to the genus *Streptomyces* (with some new representatives of other genera) and bioactivity screenings revealed that some isolates exhibited antibacterial and antifungal activities. Interestingly, when grown on the MHA medium, our collection of *Streptomyces* strains was found to display strong growth inhibitory properties against the clinically relevant filamentous fungus *Rasamsonia argillacea*. This mold is a causative agent of chronic infections in patients with cystic fibrosis or chronic granulomatous diseases and displays tolerance to various antifungals. That almost all our isolates (94 %) display high antifungal activity against *R. argillacea* suggests the involvement of one or more molecules commonly produced by streptomycetes. As the inhibition is total in most cases, we suspected the involvement of volatile compounds which was confirmed by repeating the tests using bipartite Petri dishes with a boundary to ensure a physical separation of the *Streptomyces* species and *R. argillacea*. By using gas chromatography-mass spectroscopy technique, we detected various organic volatile compounds and the identification of active compound(s) is currently under investigation.

A02 - Biodiversity of Cyanobacteria and associated microbiome in the BCCM/ULC Culture Collection

Anne-Catherine Ahn [1], Luc Cornet [2], Kim Beets [1], Yannick Lara [3,4], Benoit Durieu [3], Emmanuelle Javaux[4], Denis Baurain [2], Annick Wilmotte[1]*

[1]BCCM/ULC Cyanobacteria collection, InBioS-Centre for Protein Engineering, University of Liège, Liège, Belgium,

[2]InBioS – PhytoSYSTEMS, Eukaryotic Phylogenomics, University of Liège, Liège, Belgium3[2]InBioS - Centre for Protein Engineering, University of Liège, Liège, Belgium,

[3]InBios-Centre for Protein Engineering, University of Liège, Liège, Belgium

[4]UR Astrobiology, University of Liège, Liège, Belgium.

[*]awilmotte@uliege.be

Cyanobacteria are a phylum of photosynthetic bacteria that played an important role in the evolution of the planet by oxygenating its early atmosphere and provoking the Great Oxydation Event around 2.3 billion years ago. Early cyanobacteria were the ancestors of plastids and thus, at the origin of the highly successful algae and plants. Nowadays, they still are the basis of the food chain in many biotopes, as long as there is liquid water, light, air and some minerals. Some cyanobacterial taxa are very resistant to harsh environmental conditions, and thus, grow in polar, hypersaline, alkaline and/or arid biotopes, but also in spatial conditions. Furthermore, they are also a prolific source of secondary compounds with bioactivies.

The BCCM/ULC public collection funded by the Belgian Science Policy Office since 2011 presently includes 224 cyanobacterial strains, with 140 being of Antarctic origin (catalogue: http://bccm.belspo.be/catalogues/ulc-catalogue-search). The strains are unicyanobacterial but not axenic, due to the well known difficulties of purifying them. Morphological identification showed that the strains belong to the orders of Synechococcales, Oscillatoriales, Pleurocapsales, Chroococcidiopsidales and Nostocales. Furthermore, 16S rRNA and ITS sequences of the strains are being characterized. Recent sequencing efforts increased the amount of available 16S rRNA sequences of BCCM/ULC strains to 190. Those sequences belong to 75 OTUs (groups of sequences with > 99 % 16S rRNA similarity), which represents a quite large diversity.

To better characterize the microbiome of the cultures, a metagenomic analysis was performed for 12 polar or subpolar strains and three temperate ones, including three early-branching organisms that will be useful for phylogenomics. The design of a specific metagenomic pipeline enabled the easy recovery of the cyanobacterial genomes from the non-axenic cultures. In parallel, 31 genomes of co-cultivated bacteria (12 nearly complete) from the same cultures were determined. They mostly belonged to Bacteroidetes and Proteobacteria, some of them being very closely related, in spite of sometimes geographically distant sampling sites (Cornet et al. 2018).

In summary, the BCCM/ULC public collection serves as a Biological Resource Centre to conserve *ex situ* and document the biodiversity of cyanobacteria and their microbiomes, as well as a repository for discovery of novel bioactive compounds.

Cornet, L., Bertrand, A., Hanikenne, M., Javaux, E., Wilmotte, A., & Baurain, D. (2018). Metagenomic assembly of new (sub)polar Cyanobacteria and their associated microbiome from non-axenic cultures. Microbial Genomics.4. DOI 10.1099/mgen.0.000212.

A03 - Study of *Streptomyces sp.* carbohydrate catabolic pathways as a means to antibiotic discovery : the ManR example

Sinaeda Anderssen, Aymeric Naômé, Sébastien Rigali

Genome mining of antibiotic-producing bacteria has uncovered a significant number of biosynthetic gene clusters (BGCs) that remain to be linked to a natural product, reviving hope for the discovery of novel bioactive compounds. However, these specialized metabolites are not usually observed in laboratory conditions. Indeed, in their natural habitat, they are produced as a response to specific combinations of environmental cues, most of which are not known and thus hard to mimic in the lab. This highlights the importance of understanding the environmental signals triggering the production of specialized metabolites. A simple change in the nutritional status of the environment necessits rapid adaptation mechanisms, sometimes involving the production of bioactive compounds, linking primary and secondary metabolism. A well-known example is the perception of cellobiose by *Streptomyces scabies*, probably signalling the proximity of a host for this plant-pathogen, inducing the production of the appropriate virulence molecule, thaxtomin. This link is determined by CebR, the common regulatory protein controlling the expression of cellulose utilization genes and the thaxtomin biosynthesis cluster. This duality in function is not an isolated case, which is why our approach focuses on the discovery of novel *cis-trans* relationships for transcription factors involved in primary metabolism. Finding their *cis*-regulatory element is a direct gateway to predicting their primary function (which carbohydrate catabolic pathway they are part of), as well as unveiling their secondary function if there is one (which BGC they control). Here, we present the methodology and show how this elucidated the catabolic pathway of mannan utilization in streptomycetes.

A04 - Heat shock response in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*: gene regulation of heat shock proteins and transcription factors

Rani Baes[1], Liesbeth Lemmens[1], Eveline Peeters[1]*

[1]Research Group of Microbiology, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium

[*]Eveline.Peeters@vub.be

Temperature is a crucial physical parameter for all living organisms. The ability to respond adequately to a sudden temperature rise or drop underlies the survival and fitness of a species. Although it is well understood how bacteria and eukaryotes sense and respond to temperature changes, this is largely enigmatic for archaeal microorganisms. On a molecular level, knowledge on how so-called 'heat shock proteins (HSPs)' are (up-)regulated upon heat shock is lacking for most archaea. This despite the observation that many archaeal species thrive in high-temperature habitats that are typified by large temperature gradients, imposing constant heat- and cold-shock stress on the cells.

With the aim of characterizing the regulatory mechanism behind this heat- and cold-shock response in the thermoacidophilic archaeon *Sulfolobus acidocaldarius,* living in volcanic hot springs and growing optimally at 75°C, a wide exploration of different temperature stress conditions seemed crucial. We questioned which conditions can be considered heat shock for thermophiles and examined the effect of high temperature stress on the gene expression of HSPs and transcription factors (TFs).

We investigated the phenotypical response of *S. acidocaldarius* when exposed to a wide range of high temperature stress conditions (80 - 93.5°C). This was achieved by, at the one hand, cultivating cells at different temperatures from the moment of inoculation onwards and, at the other hand, rapidly heat shocking exponentially growing cultures and performing cell viability and plating assays. We defined a range of temperature stress conditions for which either impaired growth kinetics without loss of viability was observed, and conditions where a rather gradual decrease in cell survival was observed over time. During these experiments, the importance of a well-controllable and robust 'heat shock-medium' was thoroughly investigated and emphasized.

In parallel, we examined the differential gene expression of the most important HSPs (mainly chaperones, protecting the proteome from the effects of high-temperature stress) and some of the TFs by gene expression analysis (qRT-PCR) at the above-defined non-lethal temperature stress conditions. We observed a similar gradual increase in expression levels of most HSP- and TF-genes over time, with the largest differential expression being detected 30 min after heat shock followed by a diminished upregulation after one hour. Whereas many TFs show an increased transcriptional expression level upon heat shock, none of them is shown to specifically regulate HSP gene expression. We found that upon heat shock, some of the most important HSPs are not transcriptionally regulated to a large extent, suggesting the existence of additional regulatory mechanisms on a different level.

A05 - Ca^{2+} regulated substrate-switching in type III protein secretion of EPEC

*Biao Yuan[1], Athina G Portaliou1, **Luit Barkalita1**, Mohamed Belal Hamed[1], and Spyridoula Karamanou[1] and Anastassios Economou[1]**

1KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Bacteriology, B-3000 Leuven, Belgium

**For correspondence:*
tassos.economou@kuleuven.be

Type III secretion system (T3SS) is a protein secretion system used by many Gram-negative bacterial pathogens to deliver toxins into the host eukaryotic cells. The assembly of the injectisome and protein secretion are highly organized and coordinated. Secretion is regulated by environmental cues, such as pH, temperature, and calcium concentration. Moreover, the gatekeeper SctW is an affinity switch that regulates secretion of "middle substrate" translocators or "late substrate" effectors and is itself a secreted pseudo-effector. Here, we probed how calcium depletion switches from translocator to effector secretion via SctW. We explored three hypotheses: SctW being secreted, released from the translocase or undergoing conformational changes. We found deletion mutants of *sctW,* or of its chaperone genes *sepD* and *cesL,* were "blind" to calcium regulation, suggesting that switching-competent SctW must be both stable and membrane-associated. Mutational analysis of SctW revealed its C-domain to be critical for switching. The major translocase component SctV is necessary for SctW/SepD/CesL membrane association, but additional translocase components improve its affinity. Secretion of SctW, was regulated by high calcium similarly to that of translocators and its membrane localization is calcium-independent suggesting that conformational changes in SctW/SepD/CesL are responsible for switching.

A06 - Novel cryptic prophage operon modulates stress resistance in *Escherichia coli* O157:H7

Alexander Cambré[1], Hanne Hendrix[2], Tom Dongmin Kim[1], Bram Van den Bergh[3,4], Dietrich Vanlint[1], Oscar E. Torres Montaguth[1], Elisa Gayán[1], Kristof Vanoirbeek[1], Jan Michiels[3,4], Rob Lavigne[2], Abram Aertsen[1]

[1] *Laboratory of Food Microbiology, KU Leuven, Belgium*

[2] *Laboratory of Gene Technology, KU Leuven, Belgium*

[3] *Centre of Microbial and Plant Genetics, KU Leuven, Belgium*

[4] *Centre for Microbiology, VIB, Belgium*

Prokaryotic genome plasticity has paved the way for an explosive diversification within bacterial species. Part of this plasticity originates from horizontal gene transfer that takes place within the bacterial kingdom, most commonly using bacteriophage elements as exchanging fragments. These phage genes can constitute up to one fifth of the complete prokaryotic genetic blueprint but its influence on the host's physiology remains ill defined.

We serendipitously discovered a cryptic prophage operon (*hpsP-hpsQ*) uniquely found in the notorious foodborne pathogen *Escherichia coli* O157:H7 capable of modulating the host's stress response. However, the mechanism of the physiological modulation by this prophage genetic element remains elusive. To unblur the dynamics of this hijacker's interaction with the resistosome of the host bacterium, we zoomed in on the particularities of these cryptic loci. Since disruption of HpsP functionality led to a desensitization of the *E. coli* O157:H7 host to abiotic stresses, this specific target was subjected to a detailed functional analysis. Both *in vitro* and *in vivo* data support the hypothesis of a hidden DNA binding and nuclease activity in the ATP domain-containing HpsP protein, inflicting double stranded breaks in the targeted genetic material. Surprisingly, these activities can only be fully grasped in non-native backgrounds such as *E. coli* K-12 and *Salmonella* species, possibly indicating the interplay with other *E .coli O157:H7* antifactor proteins in its mode of action. One of these putative interacting partners (HpsQ) was identified and displayed a high degree of cell toxicity in the native *E. coli* O157:H7 background upon overexpression, but lost this phenotype when the *hpsP* operon partner gene was disrupted. This interaction was further confirmed by co-expression of both operon constituents where HpsQ-mediated toxicity was alleviated via a mechanism concealed in the HpsP C-terminus.

Overall, we found a prophage descending operon in *E. coli* O157:H7 which has the ability to modulate the bacterial resistosome via a mechanism mainly leaning on the presence of the active form a single phage protein.

A07 - Replication fork targeting by the Tn*3*-family transposon Tn*4430*: a new model for replicative transposition

Ophélie d'Udekem d'Acoz and Bernard Hallet[1]

[1] *Institut des Sciences de la Vie, Université Catholique de Louvain, Croix du Sud 4/5 L7.07.06, B-1348 Louvain-la-Neuve, Belgium*

A broad analysis of all the sequenced genes found in nature revealed that the most abundant and ubiquitous genes (widely outnumbering essential or housekeeping genes) are genes that encode transposases. While perceived for a long time as parasites, they now appears to confer a selective advantage to their host by disseminating transposable elements thus generating mutations and rearrangements that leads to an increase in biological diversification. Even though transposition is a driving force of evolution, the mechanisms by which the transposase communicates with the host's DNA enzymes are poorly understood.

This research focuses on the Tn*4430* transposon as a paradigm of the large and widespread Tn*3* group of bacterial replicative transposable elements. Recent in vivo and in vitro studies on this element converge on a new "replisome hijacking" model according to which the transposon integrates into replication forks as a direct mechanism to recruit the host replication machinery for its own duplication.

The aim of this project is to validate this model by studying the functional and physical interactions between the transposition and replication machineries *in vivo*. A new transposition assay to construct large genomic libraries of Tn*4430* insertions in different genetic backgrounds of *E. coli* has been developed and the conditions for the Illumina sequencing have been optimized. Supporting the "replisome hijacking" model, preliminary results show that the insertion of the transposon preferentially occurs in regions where the progression of the replication is altered, such as the terminus of replication of the chromosome.

For the first time, the transposase was studied with high-resolution microscopy techniques. These experiences shed new light on how the transposase searches for its target site in living cells. Half of the transposases, surprisingly, bind non-specifically to DNA while the other half diffuses. To further comprehend the whole transposition process, experiments are under way to study the interactions between all the transposition partners (e.g. the transposon, the transposase and the replication machinery).

A08 - Structural Study of Yeast Mitochondrial DNA Transcription Initiation Complex

Brent De Wijngaert[1], Shemaila Sultana[2], Chhaya Dharia[2], Hans Vanbuel[1], Jiayu Shen[2], Smita Patel[2], Kalyan Das[1,*]

[1]Rega Institute for Medical Research, The Department of Microbiology, Immunology and Transplantation, KU Leuven, 3000 Leuven, Belgium [2]Department and University, Belgium

2Department of Biochemistry and Molecular Biology, Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ 08854, USA

[*]e-mail: kalyan.das@kuleuven.be

Transcription of the mitochondrial DNA is carried out by the mitochondrial RNA polymerase (mtRNAP), which is distinct from the RNA polymerases responsible for the nuclear DNA transcription. The mtRNAP is a single polypeptide chain protein conserved across eukaryotes and evolutionary related to the T-odd lineage of bacteriophage RNAPs. For the transcription initiation - (i) a phage RNAP does not require transcription factor, (ii) the yeast mtRNAP (y-mtRNAP; Rpo41) requires one transcription factor Mtf1, and (iii) the human mtRNAP (h-mtRNAP; POLRMT) requires two transcription factors, transcription factors A (TFAM) and transcription factor B2 (TFB2M) for promoter-specific initiation. The structures of transcription initiation complexes (ICs) of T7 RANP and h-mtRNAP have been determined. The structure of y-mtRNAP IC, that has been missing, will help understand the requirement of no factor for T7, one factor for y-mtRNAP, to two factors for h-mtRNAP along the evolution pathway. Also, the potential difference in the transcription initiation process by yeast vs. human mtRNAP may validate y-mtRNAP as a target for designing anti-fungal drugs.

We determined the x-ray crystal structure of y-mtRNAP IC at 3.3 Å resolution. The structure locates the non-template strand of the bubble DNA, which has been disordered in h-mtRNAP IC structure. The structure and biochemical analysis define the key interactions of Mtf1 with mtRNAP and the non-template DNA strand. The crystal structure, however, appears to be not in a catalytic confirmation and the twinned crystal form limits extensive investigation of the IC conformational states. Therefore, we are using single-particle cryo-electron microscopy (cryo-EM) for determining the structure of y-mtRNAP IC in the catalytic conformational state. The crystal structure and the progress on the cryo-EM study will be presented

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A09 - Cpn60.1 (GroEL1) contributes to mycobacterial Crabtree effect: implications for biofilm formation

*Sheng Zeng[1], Patricia Constant[2], Dong Yang[1], Alain Baulard[3], Philippe Lefèvre[1], Mamadou Daffé[2], Ruddy Wattiez[4a], Véronique Fontaine[1*a]*

[1]Microbiology, Bioorganic & Macromolecular Chemistry Research Unit, Faculté de Pharmacie, Université libre de Bruxelles (ULB), Belgium.

[2]Department of Tuberculosis and Infection Biology, Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, 205 Route de Narbonne, 31077 Toulouse, France.

[3]Institut Pasteur de Lille, Université de Lille, CNRS, INSERM, CHU Lille, U1019-UMR 8204, Center for Infection and Immunity of Lille, Lille, France.

[4]Department of Proteomics and Microbiology, University of Mons, Belgium.

[a]Equally contributed.

Abstract

Introduction : Biofilm formation is a survival strategy for microorganisms facing hostile environment. Under biofilm, bacteria are better protected against antibacterial drugs and the immune response, increasing treatment difficulty, as persistent populations recalcitrant to chemotherapy are promoted. Deciphering mechanisms leading to biofilms could thus be beneficial to obtain new antibacterial drug candidates. **Material and Methods:** This research was performed on wild-type or Δ*cpn*60.1 *M. bovis* BCG in biofilm or 7H9 culture. The impact of Cpn60.1 (GroEL1) deficiency was investigate by quantifying various metabolites and the methylglyoxal protein adduct, but also by lipid and proteomic analysis. **Results:** We show that mycobacterial biofilm formation is linked to excess glycerol adaptation and the concomitant establishment of the Crabtree effect. This effect is characterized by respiratory reprogramming, ATP downregulation and secretion of various metabolites including pyruvate, acetate, succinate and glutamate. Interestingly, the biofilm formation and the Crabtree effect were abnormal in the mycobacterial strain deficient for Cpn60.1. Indeed, this mutant strain had a compromised ability to downregulate ATP and secreted more pyruvate, acetate, succinate and glutamate in the culture medium. Importantly, the mutant strain had higher intracellular pyruvate and produced more toxic methylglyoxal, suggesting a glycolytic stress leading to growth stasis and consequently biofilm failure. **Discussion-Conclusion** : This study demonstrates for the first time the link between mycobacterial biofilm formation and the Crabtree effect. The secreted metabolites may be considered as valuable biofilm markers. Cpn60.1 participates in this adaptation by facilitating the ATP downregulation and controlling these metabolic adaptations, thereby restricting overabundance of pyruvate and the upstream methylglyoxal.

A10 - Toxin antitoxin systems in bacterial physiology : the *mqsRA* case

Nathan Fraikin[1], Clothilde J. Rousseau[2], Nathalie Goeders[3]& Laurence Van Melderen[1*]*

[1]Cellular & Molecular Microbiology, Department of Molecular Biology, Université Libre de Bruxelles, Belgium, in Times New Roman 11, italic.

[*]nfraikin@ulb.ac.be

In the last decade, chromosomal toxin-antitoxin (TA) systems have been attributed various biological functions, ranging from programmed cell death or stress response to antimicrobial persistence. These modules, which encode a toxic gene and its cognate antidote, are widely distributed through prokaryotic genomes. While we and others disproved roles for TA systems in various biological processes, including persistence, many of these roles remain ambiguous. Here, we studied the *mqsRA* system, a type II TA system that was previously associated with stress response, biofilm formation and persistence. MqsR, a RNAse, was shown to positively regulate biofilm formation and bile salts tolerance while MqsA was shown to bind the promoters of several genes and repress their transcription. These genes include *csgD*, a global regulator of biofilm formation and curli biosynthesis, *cspD*, a protein associated with persistence and *rpoS*, the general stress response sigma factor. MqsA was also shown to be degraded under oxidative stress, leading to the depression of *rpoS*, increased catalase production and resistance to oxidative stress. Our results show that MqsA does not regulate transcription of *csgD*, *cspD* and *rpoS*. We also show that mqsRA has no effect on biofilm formation, curli production as well as on tolerance and resistance to oxidative stress and bile salts. Moreover, we show that MqsA is degraded neither by oxidative stress nor by other stresses this system was suggested to be implicated in (amino acid starvation, bile salts). Finally, we show that *mqsA* transcription is constitutive and uncoupled from *mqsR* thanks to two novel promoters, thus allowing a constant level of *mqsA* expression and a stable toxin:antitoxin ratio. We also discuss the technical limitations regarding previous literature on *mqsRA*, including the probable comparison of non-isogenic strain and the use of very high-copy overexpression vectors that could have caused misinterpretation of previously published data. Altogether, our data suggests that mqsRA and other type II TA systems do not play a role in stress response or biofilm formation.

A11 - Phosphotransferase-dependent regulation in *Caulobacter crescentus*

Aravind Gopi and Régis Hallez

Bacterial Cell cycle & Development (BCcD), Biology of Microorganisms Research Unit (URBM), Namur Research Institute for Life Science (NARILIS), University of Namur,
61 Rue de Bruxelles, 5000 Namur, Belgium

Nitrogen-related phosphotransferase system (PTS^{Ntr}) has been shown in *Caulobacter crescentus* to trigger the accumulation of the alarmone (p)ppGpp, which interferes with cell cycle progression and cellular differentiation. The PTS^{Ntr}-dependent regulation of (p)ppGpp levels first involves autophosphorylation of EI^{Ntr} upon nitrogen starvation, which triggers the phosphorylation of the downstream PTS^{Ntr} components HPr and $EIIA^{Ntr}$. Once phosphorylated, both proteins modulate activities of the bifunctional (p)ppGpp synthetase/hydrolase SpoT to stimulate (p)ppGpp accumulation (1, 2). Here the project aims at identifying and characterizing other PTS^{Ntr}-dependent mechanisms. To unravel the regulations mediated by HPr and $EIIA^{Ntr}$, a combination of genetic and biochemical approaches were performed. First, we used phosphomimetic and phospho-dead variants of HPr and $EIIA^{Ntr}$ in pulldown experiments to identify their interacting partners by mass spectrometry. An uncharacterized protein has been identified in the pulldown experiment performed with $EIIA^{Ntr}$ and the interaction was further verified using a bacterial two-hybrid system. The role of this conserved uncharacterized protein is currently characterized. Also, a genetic screen in a strain expressing a phosphomimetic variant of $EIIA^{Ntr}$ identified a mutation in a gene involved in N-acetylglucosamine metabolism. Interestingly, there is a *bona fide* PTS system encoded in the *Caulobacter* genome with EIIBC (permease) components predicted to be involved in the uptake of N-acetylglucosamine. Our preliminary results suggest there might be crosstalks between PTS and PTS^{Ntr} systems, suggesting that cell cycle progression and N-acetylglucosamine metabolism would be coordinated.

(1) Ronneau et al., 2016. *Nat Comm* 7:11423
(2) Ronneau et al., 2019 *Nucleic Acids Res, 47(2):843-54*

A12 - GenDisCal: A tool for inter-genomic distance estimation with applications in metagenome analysis of environmental samples

Gleb Goussarov [1,2], Ilse Cleenwerck [2], Mohamed Mysara [1], Natalie Leys [1], Pieter Monsieurs [1,3], Guillaume Tahon[2], Aurélien Carlier[2], Peter Vandamme[2] and Rob Van Houdt [1]

[1] Microbiology Unit, Belgian Nuclear Research Centre (SCK•CEN), Mol, 2400, Belgium
[2] Laboratory of Microbiology and BCCM/LMG Bacteria Collection, Faculty of Sciences, Ghent University, Ghent, 9000, Belgium.
[3] Unit Health, Flemish Institute for Technological Research (VITO), Mol, 2400, Belgium.

Environmental samples obtained from soil and water are likely to contain a large amount of poorly characterized bacterial species, as culturing them can often be difficult. In the near future, third generation sequencing followed by metagenomics assembly will enable reconstruction of the genomes of such bacteria. We have developed a software tool, in addition to a user-friendly graphical user interface, which provides multiple methods for estimating the inter-genomic distance between complete genomes. Among these is a novel method, which we called PaSiT. Here, we discuss the performance of this method based on two large subsets of the publicly available bacterial genomes from RefSeq and one dataset of newly sequenced bacteria. Our tests have shown that it can rapidly compare thousands of genomes and accurately predict whether two genomes belong to the same bacterial species or type. We also briefly explain our plans to apply this method as part of a complete metagenomic pipeline which can parse both second and third generation sequencing data.

A13 - An extended host range displayed by pXO16 from *Bacillus thuringiensis* sv. *israelensis*

Hinnekens Pauline[1], Klèma Marcel Koné[1], Fayad Nancy[1, 2], Leprince Audrey[1] and Mahillon Jacques[1]*

[1]Laboratory of Food and Environmental Microbiology, Earth and Life Institute, Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

[2]Laboratory of Biodiversity and Functional Genomics, Faculty of Science, Université Saint-Joseph de Beyrouth, Beirut, Lebanon.

**jacques.mahillon@uclouvain.be*

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 it enhances pXO16 transfer efficiency, recent work indicated that the aggregation mechanism is not mandatory for the plasmid transfer. When using a pXO16 aggregation-minus mutant, a deficit of pXO16 transfer efficiency was observed [1]. However, it was recently highlighted that colony or filter mating largely compensate such deficit. pXO16 transfer capability was therefore explored under various mating conditions with different members of the *Bacillus cereus* group, *i.e. B. weihenstephanensis* and *B. cytotoxicus* [2]. Using filter mating allowed to enhance, and to extend, pXO16 transfer efficiency and host range, respectively. For instance, pXO16 was shown to transfer itself from *B. thuringiensis* sv. *israelensis* to *B. cytotoxicus*, the thermotolerant and most distantly related member of the *B. cereus* group, at frequencies of 3.3×10^{-3} transconjugants per donor (T/D). It was also shown that pXO16 is able to mobilize the small pUB110 plasmid to *B. cytotoxicus*, to transfer itself back from *B. cytotoxicus* to *B. thuringiensis* sv. *israelensis* and even to circulate among strains of *B. cytotoxicus*. Given the thermotolerant nature of *B. cytotoxicus*, one could raise the question of pXO16 stability at high temperature. In absence of specific selection, it was shown that pXO16 tended to be lost over time at high temperature in *B. cytotoxicus*. All together, these results indicate that pXO16 can potentially "circulate" among all members of the *B. cereus* group. Yet, this is contrasting with pXO16 known natural distribution, which is apparently limited to the *israelensis* serovar of *B. thuringiensis*.

References

1. Makart, L., Gillis, A., Hinnekens, P., Mahillon, J., 2018. A novel T4SS-mediated DNA transfer used by pXO16, a conjugative plasmid from Bacillus thuringiensis serovar israelensis. *Environ. Microbiol.* 20, 1550-1561.

Hinnekens, P., Klèma, M.K., Fayad, N., Leprince, A., Mahillon, J., 2019. pXO16, the large conjugative plasmid from Bacillus thuringiensis serovar israelensis displays an extended host spectrum. *Plasmid* 102, 46-

A14 - Uncovering the choreography of the chromosome during the non-binary cell cycle of the predatory bacterium *Bdellovibrio bacteriovorus*

Jovana Kaljevic[1], *Géraldine Laloux*[1]

[1]*De Duve Institute, UCLouvain, Brussels, Belgium*

jovana.kaljevic@uclouvain.be

Bdellovibrio species are predatory δ-proteobacteria that invade the periplasm of other Gram-negative bacteria. The prey bacterium forms a structure termed "bdelloplast" whereas the predator grows in its periplasm as a filament before dividing at once into a variable number of monoploid cells by a non-binary division process. This remarkable lifestyle is at odds with textbook models of binary proliferation and raises a series of intriguing biological questions. Yet, molecular understanding of how this micro-predator proliferates inside the envelope of other bacteria is still missing. One of the biggest challenges that cells face is to keep chromosome structured and to ensure proper coordination of its replication and segregation before cell division. In the case of *Bdellovibrio*, additional complexity arises as the growing predator must copy its single chromosome multiple times before cell division. Most bacteria possess a single circular chromosome where subcellular location of key landmarks, origin of replication (*ori*) and terminus region (*ter*), varies among species and is critical for cell cycle progression. However, the spatiotemporal arrangement of the *Bdellovibrio* chromosome is mainly unknown. Moreover, the mechanism and spatiotemporal coordination of chromosome segregation with DNA replication and cell division, have remained unexplored in *Bdellovibrio*. Our first goal is to determine how the essential chromosomal regions are positioned throughout the cell cycle of *Bdellovibrio*. The localization of *ori* and *ter* regions is tracked by inserting synthetic sequences with high affinities towards fluorescent proteins which can be observed via microscopy. Our results suggest that the circular chromosome exhibits a specific orientation in the non-proliferating cell that is maintained at least in early stages of predation. Our observations also reveal intriguing dynamics of the level of nucleoid compaction in *Bdellovibrio*. Furthermore, in order to gain insight into the segregation machinery of *Bdellovibrio*, we aimed to investigate the homologues of the tripartite segregation system ParABS using subcellular localization in living cells, bacterial genetics and protein-protein interaction assays. Our recent findings shed light on essential and original features of these predatory bacteria supporting the idea that bacterial nucleoids are complex and dynamic entities tightly coupled to cell cycle progression and growth phase.

A15 - High-speed Atomic Force Microscopy visualization of an archaeal transcription factor binding to DNA using a DNA origami frame

Yeseren Kayacan[1], Andreas Krauss[3], Timothy Januarius[1,2], Heiko Haschke[3], Eveline Peeters[2], Ronnie Willaert[1]*

[1]*Structural Biology Brussels (SBB), Vrije Universiteit Brussel, Belgium*

[2]*Microbiology (MICR), Vrije Universiteit Brussel, Belgium.*

[3]*JPK BioAFM Business, Bruker Nano GmbH, Berlin, Germany.*

[*]*Yeseren.Kayacan@vub.be*

High-speed AFM (HS-AFM) is a unique tool that provides the high spatio-temporal resolution necessary to unravel the geometry and the mode of protein-DNA interactions, without the need for labeling. Structure and dynamics of these interactions can be directly recorded at nanometer spatial and sub-100 ms temporal resolutions in liquid. The insights obtained with HS-AFM into molecular dynamics of biologically relevant, functional processes surpass that of other approaches.

The unique protein-DNA interactions in this study were visualized using DNA origami. The basic principles of DNA origami involve a long single strand of DNA which serves as a scaffold, folded into desired shapes with the help of short oligonucleotides, referred to as staples. The experimental simplicity and the fidelity of this folding process has enabled the fast development of this area in the recent years. In this study, surface-immobilized DNA origami nanostructures are utilized to visualize individual protein molecules as they interact with dsDNA containing their binding site and attached into a DNA origami frame.

The protein of interest was a TetR-family transcriptional regulator of a large cluster of genes encoding fatty acid metabolism functions in the archaeal model organism *Sulfolobus acidocaldarius* (Wang *et al*., 2019). This regulator, $FadR_{Sa,}$ is capable of repressing the expression of a large 23-gene cluster by interacting with only four binding sites. These binding sites are unusual in their positions, with sites at more than 130 bp upstream of the corresponding promoter. This indicates that $FadR_{Sa}$ employs a unique repression mechanism, involving long-range interactions between its different semi-palindromic binding sites. Moreover, dimers of the protein bound to opposite sides of the DNA helix can interact via weak electrostatic interactions and form dimer-of-dimer complexes.

DNA origami frames were designed *in silico* and constructed, with two DNA duplexes with $FadR_{Sa}$ binding sites incorporated into the frames. $FadR_{Sa}$ was able to mediate a connection between two relaxed 74-mer duplexes inside the frame. Qualitative analysis of loose DNA duplexes with AFM height images showed X-shaped structures where two strands interact but not cross each other, only in the presence of $FadR_{Sa}$. These findings were further confirmed by HS-AFM visualization in liquid to mimic physiological conditions, indicating that $FadR_{Sa}$ is able to interact with two binding sites and switch between binding as a dimer and as a dimer-of-dimers. We have demonstrated that the use of HS-AFM with the DNA origami methodology is a robust approach to study dynamic protein-DNA interactions at a single-molecule level.

References

Wang, Kun, et al. "A TetR-family transcription factor regulates fatty acid metabolism in the archaeal model organism Sulfolobus acidocaldarius." *Nature communications* 10.1 (2019): 1542.

A16 - Competence bistability in *Streptococcus salivarius* : complex circuitry for phenotypic heterogeneity

Adrien Knoops[*1], *Laura Ledesma-Garcia, Camille Baquet*[1], *Marie Verhaeghe*[1], *Johann Mignolet*[1], *Florence Vande Capelle*[1], *Philippe Goffin*[2,3], *Laetitia Fontaine*[2,3] *and Pascal Hols*[1].

[1] *UCLouvain - Biochemistry and Genetics of Microorganisms, Croix du Sud, 4-5, bte L7.07.06 - 1348 Louvain-la-Neuve*

[2]*MeKaGene.*

[3]*ULB - Cellular and Molecular Microbiology, Rue des Professeurs Jeneer et Brachet, 12 - 6041 - Gosselies*

[*]*Adrien.knoops@uclouvain.be*

In bacteria, Horizontal Gene Transfer (HGT) allow genetic exchanges between organisms. While we only start to understand how crucial those mechanisms are for bacterial evolution and adaptation, medical issues linked to HGT, such as antimicrobial resistance spread or pathogenicity acquisition, emphasize the need for new investigations.

From a bacterial point of view, human mouth is probably one of the most challenging ecological niche. Continuous physicochemical changes and competition between about 700 microbial species allow only quick-adapting bacteria to survive. In this context, HGT is essential for long-term survival. Following this strategy, Streptococcus salivarius can activate the so-called competence state, triggering bacteriocins production and efficient natural transformation. Because competence will have dramatic effects on the cell physiology, a transcriptional regulatory pathway called ComRS will fine-tune its initiation. This intricate regulatory pathway shows particularly interesting behaviour such as pheromone signal-mediated communication and heterogeneous activation (bistability).

In this work, we investigated bistability using single-cell microscopy. Thanks to overexpressing strains of the main actors of the ComRS pathway, we identified key players of the system. Subsequent disruption of ComRS biochemical pathways such as positive feedback loop or distal transcriptional regulation through histidine kinases allowed us to understand which circuitries were involved in phenotypic heterogeneity. Finally, mathematical modeling of the system based on experimental data provided insights in the importance of gene stochasticity for bacterial phenotypic heterogeneous enhancement and survival at the population level.

A17 - Deciphering the pheromone specificity determinants for competence activation in streptococci

Laura Ledesma-García[1], Jordhan Thuillier[2], Joaquín Guzmán[1], Patrice Soumillion[1], Sylvie Nessler[2] and Pascal Hols[1].

[1]Louvain Institute of Biomolecular Science and technology (LIBST), Biochemistry and Genetics of Microorganisms, Catholic University of Louvain (UCLouvain), 4-5 Croix du Sud., Louvain-La-Neuve, Belgium

[2]Institute of Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette cedex, France

ComRS is the cell-to-cell communication system that tightly regulates competence in a broad number of streptococci groups. Since **competence activation** allows a bacterial subpopulation to transiently acquire exogenous DNA, it has major importance for genome plasticity. In addition, competence development is generally associated with the activation of predatory mechanisms (e.g. bacteriocins production) and biofilm formation that improve the fitness of pathogenic streptococci in the host and will affect to the population dynamics.

The ComRS signalling regulatory system is formed by the transcriptional regulator ComR of the RRNPP superfamily, which displays an N-terminal DNA-binding domain and a C-terminal TPR-domain which interacts with the pheromone ComS or XIP. Moreover, the evolutionary divergence observed among the ComR peptide-binding receptors and the pheromone sequences suggests a peptide-sensor co-evolution. In many cases this polymorphism prevents the cross-talk even between close-related streptococci species.

Previous studies of the ComRS system from *Streptococcus thermophilus* deciphered a new activation model never described for this family of regulators. In this model we demonstrated that the gratuitous activation of competence in absence of pheromone is prevented by ComR DNA-binding domain sequestration through interactions with the TPR-domain. Moreover, those studies disclosed key information about peptide binding and discrimination.

Questions regarding the molecular mechanisms of pheromone recognition have been tackled by structural and mutational analysis of ComRS systems of two close related species: *Streptococcus thermophilus* and *Streptococcus vestibularis*, which belong to the salivarius group but do not show ComRS systems cross-activation. Results have disclosed key information about peptide-ComR binding and discrimination allowing us to highlight the main ComR regions involved in XIP specificity.

In addition, the consequences, by modifying the peptide-specificity of the system, of ComRS cross-talk for interspecies communication and competition are being evaluated.

A18 - $YtrA_{Sa}$, a GntR-family transcription factor, represses two genetic loci encoding membrane proteins in *Sulfolobus acidocaldarius*

Liesbeth Lemmens[1], Laurentijn Tilleman[2], Ezra De Koning[1], Karin Valegård[3], Ann-Christin Lindås[4], Filip Van Nieuwerburgh[2], Dominique Maes[1] and Eveline Peeters[1]

[1] Vrije Universiteit Brussel, Brussels, Belgium. [2] Ghent University, Ghent, Belgium. [3] Uppsala University, Uppsala, Sweden. [4] Stockholm University, Stockholm, Sweden.

E-mail: liesbeth.lemmens@vub.be

Transcription factors (TFs) of the GntR family regulate many physiological and morphological processes in Bacteria, often in response to the nutritional state (1, 2). Based on their variable C-terminal domain, these TFs are subdivided in different subfamilies, including the YtrA subfamily. YtrA-like TFs are characterized by their short C-terminal domain and YtrA encoding genes are typically located in an operon with genes encoding transport proteins (3). Despite their wide distribution in bacterial species, GntR/YtrA-like TFs are rather scarce in Archaea and to our knowledge have not yet been described in these organisms (4). In this work we describe the first archaeal YtrA-like transcription factor to be described, from the thermoacidophilic archaeon *Sulfolobus acidocaldarius*, named $YtrA_{Sa}$.

An *in vivo* genome-wide interaction map of $YtrA_{Sa}$ showed only two highly enriched regions with average fold changes of 24 and 114 for peak 1 and 2, respectively, suggesting that $YtrA_{Sa}$ has a very restricted direct regulon. Peak 1 is located in the promotor region of an operon encoding the transcription factor itself and a putative membrane protein, while peak 2 is located just upstream of the gene predicted to encode another membrane protein. The protein-DNA interaction of $YtrA_{Sa}$ is confirmed *in vitro* by performing electrophoretic mobility shift assays (EMSAs) showing a specific high-affinity binding to DNA probes harboring the control region of both targets. A DNase I footprinting experiment revealed two very similar, 24 bp semi-palindromic recognition binding sites covering the transcriptional start of both target genes, suggesting that $YtrA_{Sa}$ represses transcription by inhibiting RNA polymerase recruitment. In an attempt to check whether this direct binding of the regulator also leads to transcriptional regulation, we employed a comparative transcriptomic analysis for a $ytrA_{Sa}$ overexpression strain and its isogenic wild type by using a RNA-seq approach. This experiment revealed a repression of the two direct targets of $YtrA_{Sa}$, the two membrane-protein encoding genes, and additionally revealed 9 other differentially expressed genes, involved in several metabolic processes. Additionally, the crystal structure of $YtrA_{Sa}$ was determined to a resolution of 3.0 Å. The protein formed a dimer within the crystals. In conclusion, $YtrA_{Sa}$ is transcriptional regulator with a restricted regulon, repressing the expression of two putative membrane-protein encoding genes.

A19 - OmpA and IgaA compete for the lipoprotein stress sensor RcsF across the periplasmic space, regulating Rcs activity

Juliette Létoquart[1,2], Kilian Dekoninck[1,2], Pascal Demange[3], Robin Bevernaegie[4], Benjamin Elias[4], Seung Hyun Cho[1,2,], and Jean-François Collet[1,2,*]*

[1]WELBIO, Avenue Hippocrate 75, 1200 Brussels, Belgium

[2]de Duve Institute, Université catholique de Louvain (UCLouvain), Avenue Hippocrate 75, 1200 Brussels, Belgium

[3]Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, Université Paul Sabatier, BP-64182, F-31077 Toulouse, France

[4]Institut de la Matière Condensée et des Nanosciences (IMCN), Molecular Chemistry, Materials and Catalysis (MOST), Université catholique de Louvain (UCLouvain), Place Louis Pasteur 1 box L4.01.02, 1348 Louvain-la-Neuve, Belgium

**Correspondence and Lead contact: seung.cho@uclouvain.be and jfcollet@uclouvain.be*

Bacteria carefully monitor and maintain the integrity of their cell envelope using signalling system such as the Rcs phosphorelay. *Escherichia coli* RcsF is an outer membrane lipoprotein that detects damage in the outer membrane and the peptidoglycan.

Under stress, RcsF triggers the Rcs signaling cascade by interacting with the inner membrane protein IgaA. In the absence of stress, RcsF is occluded from IgaA by interacting with BamA, the key component of the ß-barrel assembly machinery, and the ß-barrels OmpA, OmpC and OmpF.

How these ß-barrels interact with RcsF is unclear. We investigated the interaction between RcsF and OmpA, a protein which, unlike OmpC and OmpF, adopts a two-domain structure. We found that RcsF interacts with the C-terminal, periplasmic domain of OmpA and that the N-terminal, ß-barrel domain is dispensable for the interaction. We also provide evidence that OmpA competes with IgaA for RcsF binding across the periplasm, supporting a model in which OmpA functions as a buffer for RcsF, fine-tuning Rcs activity. RcsF was previously shown to be surface-exposed; our results indicate that OmpA does not function as a vehicle for RcsF surface exposure.

A20 - Characterization of mechanisms activating (p)ppGpp production upon carbon starvation in *Caulobacter crescentus*

Elie Marchand and Régis Hallez

Bacterial Cell cycle & Development (BCcD), Biology of Microorganisms Research Unit (URBM), Namur Research Institute for Life Science (NARILIS), University of Namur, 61 Rue de Bruxelles, 5000 Namur, Belgium

Coordinating cellular processes in response to fluctuations in nutrient availability is crucial for all living cells, especially for bacteria competing for resources. Recently, our lab identified the nitrogen-related phosphotransferase system (PTS^{Ntr}) as a first mechanism that regulates cell cycle progression and growth upon nitrogen starvation in the α-proteobacterium *Caulobacter crescentus*. This mechanism allows *Caulobacter* cells to sense nitrogen starvation by way of detecting glutamine deprivation and, in response to it, to trigger production of the alarmone (p)ppGpp by the RelA/SpoT Homolog (RSH) enzyme. Although (p)ppGpp accumulates in *Caulobacter* cells starved for nitrogen or carbon, PTS^{Ntr} is blind to carbon limitation. Thus our project aims at identifying and characterizing the carbon-sensitive mechanism regulating (p)ppGpp levels. We first tested the GTP binding protein called ObgE or CgtA as a potential sensor for carbon starvation since it was proposed to be a regulator of RSH in *Escherichia coli*. We also combined high-throughput genetic (Tn-Seq) and biochemical (Pull-down) screens to identify any components of the carbon stress response mechanism. These approaches might allow the identification of genes and proteins responding to a carbon starvation, and therefore contribute to a better knowledge of the bacterial response to nutrient deprivation.

A21 - Impact of protein aggregates on Bacillus subtilis sporulation and germination

Julien Mortier[1], Alexander Cambré[1], Sander Govers[2], Abram Aertsen[1]*

[1] Department of Microbial and Molecular Systems (M²S), KU Leuven, 3001 Leuven, Belgium.

[2] Department of Molecular, Cellular, and Developmental Biology, Yale University, West Haven, CT, USA

[*] abram.aertsen@kuleuven.be

Misfolded or denatured proteins tend to aggregate within the bacterial cell, and can form protein aggregates (PAs) that often become polarly localized. Although generally regarded as debilitating, the actual impact of such PAs on cellular physiology remains poorly studied. In this study, we elaborated a synthetic circuit allowing conditional PA-production in Bacillus subtilis, and investigate whether and to what extent the presence of intracellular PAs interferes with the intricate processes of sporulation and germination.

A22 - Whole Genome DNA Methylation (Methylome) Analysis and Role of Dam DNA Methyltransferase in the Entomopathogenic Bacterium *Photorhabdus luminescens*.

Amaury PAYELLEVILLE[1#], Anne LANOIS[1], Ludovic LEGRAND[2], Sylvie PAGES[1], Dana BLACKBURN[3], David CLARKE[3], Alain GIVAUDAN[1], Julien BRILLARD[1]

(1) DGIMI, INRA, Univ. Montpellier, Montpellier, France.

 # Current : Cellular and Molecular Microbiology, ULB, Belgium

(2) LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France.

(3) Department of Microbiology, University college Cork, Cork, Ireland.

**amaury.payelleville@laposte.net*

Background: DNA methylation is an epigenetic mechanism involved in the pathogenicity of several bacteria as in *Salmonella* or *E. coli*. It decreases the affinity of some transcriptional regulators to their binding site. Thus, two sub-populations can arise, one expressing the gene and the other not, depending on the DNA methylation state. Dam DNA Methyltransferase (MTase), widespread in *Gammaproteobacteria*, methylates the adenine of GATC sites. The role of Dam was investigated in *Photorhabdus luminescens*, an entomopathogenic bacterium in symbiosis with a soil nematode used in biocontrol against crop pests.
Methods: SMRT sequencing (PacBio), which allows identification of the DNA methylation of the whole genome, was performed in *P. luminescens.* Methylome, RNAseq and phenotypic analysis was also performed in a strain overexpressing Dam MTase.
Results: More than 99% of GATC sites of the *P. luminescens* genome are methylated. DNA methylation levels of the whole genome do not change during growth phase. However, the Dam-overexpressing strain displays more methylated GATC sites than the control and most of these sites are located in putative promoter regions. Dam overexpression leads to a decrease in motility and pathogenicity whereas it increases *Photorhabdus* biofilm formation. Symbiosis assays show a decrease in pathogenicity of the complex (overexpressing Dam) whereas no major differences were observed in the symbiosis ability of Dam overexpressing strain. RNAseq analysis revealed that the observed phenotypes are related to differences at the transcriptional level.
Conclusions: The differentially methylated GATC sites found in promoter regions may be involved in the observed differences in phenotypes and gene expression. Combined analysis of phenotypes, transcriptome and methylome provides clues to understand the involvement of Dam DNA methylation in *P. luminescens* life cycle.

A23 - A novel master regulator controls production of gas vesicles and antibiotics in *Serratia*.

Alex Quintero-Yanes[1], *Rita Monson*[2] *and George Salmond*[2] *

[1]*Department of Biology, University of Namur, Belgium.*

[2]*Department of Biochemistry, University of Cambridge, UK.*

* *gpcs2@cam.ac.uk*

Serratia sp. ATCC 39006 (S39006) produces virulence factors and bioactive secondary metabolites, such as carbapenem and prodiginine antibiotics. It can swim using flagella and, aided by secretion of biosurfactant compounds, can swarm over semisolid surfaces. Furthermore, S39006 is the only enterobacterium known naturally to produce intracellular, proteinaceous nanostructures, called gas vesicles (GVs), in order to float. Buoyancy is tightly controlled by quorum sensing (QS), oxygen and potassium availability and post-transcriptional regulatory elements, such as the *rsmAB system*. Here a new pleiotropic regulator of buoyancy, motility, secondary metabolism and virulence is described. A putative DeoR-type regulator (FloR) showed opposite phenotypic impacts on GV-driven flotation and flagella-based motility. In addition, production of the *N*-butanoyl-L-homoserine lactone QS signalling molecule and the prodigiosin and carbapenem antibiotics were affected in a mutant. TMT and LC-MS/MS based proteomics revealed that FloR is a high-hierarchy regulator controlling multiple known pleiotropic regulators in S39006.

A24 - Investigations on the role of a conserved polar hub protein in the predatory bacterium *Bdellovibrio bacteriovorus*

Ophélie Remy[1*], *Géraldine Laloux*[1]

[1]*De Duve Institute, UCLouvain, Brussels, Belgium*

[*]*ophelie.remy@uclouvain.be*

The word "predator" may make you think of wolves or lions but did you know that predators also exist among bacteria? In our lab we study a predatory δ-proteobacterium named *Bdellovibrio bacteriovorus,* which uses the periplasm of other Gram-negative bacteria as a niche for proliferation and kills the prey shortly after invasion. Another striking feature of *B.bacteriovorus* is its singular mode of growth, as it elongates as a filament inside its prey and divides at once in a variable number of daughter cells, therefore at odds with the textbook model of binary division. One of the key implications of such non-binary proliferation is that some cells of the progeny do not inherit one of their cell poles from the mother cell but instead harbour two newly-generated poles originating from division septa. This raises the question of how and when the polar asymmetry that is inevitably set in daughter cells upon a binary division event is established in the *B. bacterivorous* progeny. In model bacteria, polarity is determined thanks to "hub" proteins that have the remarkable ability to spontaneously localize at the cell poles, where they recruit several client proteins involved in a variety of cellular events, therefore organising the cell content in space. It is likely that *B. bacteriovorus* also relies on such "hub" proteins: an obvious sign of polarity is the single flagellum that assembles at only one pole of the cell. Thus, to address how the non-binary growing predator sets its polarity, we set out to identify potential polar hub proteins, using a candidate-approach. Here we focus on $DivIVA_{Bb}$, a homolog of a polar hub protein that is mainly found and characterized in Gram-positive bacteria (whereas *B. bacteriovorus* is a Gram-negative species). Since the first step to study the role of a polar protein is to determine if the protein is indeed positioned at cell poles, we explored the localization of $DivIVA_{Bb}$ in *Bdellovibrio bacteriovorus*. We show that, unlike the symmetric bipolar pattern of DivIVA seen in Gram-positive bacteria, $DivIVA_{Bb}$, fused to a C-terminal GFP and produced either constitutively from a plasmid or at native levels from the *divIVA* locus, is predominantly unipolar or asymmetrically bipolar. Our data also suggest that $DivIVA_{Bb}$ localization is highly dynamic, at least in the early stages of the cell cycle. We are now analysing DivIVA localization pattern further, throughout the cycle and using other cellular components as markers to discriminate the two poles. We also aim at providing more insight into the role(s) of DivIVA by finding its partners (*i.e.* its potential polar clients) via pull-down assays and by phenotypically characterizing *divIVA* mutants.

A25 - Study of lipoprotein export in Bacteroidetes.

Francesco Renzi[1] *

[1]*Research Unit in Biology of Microorganisms (URBM), University of Namur, Namur, Belgium.*

**francesco.renzi@unamur.be*

A hallmark of bacteria of the phylum Bacteroidetes is the presence of surface-exposed multi-protein membrane complexes (Sus-like systems). These systems have a crucial role in the biology of Bacteroidetes since they allow the uptake and catabolism of a large variety of nutrients, mainly polysaccharides. Sus-like systems are predominantly composed of surface exposed lipoproteins. This lipoprotein localization is uncommon in most studied Gram-negative bacteria while it is widespread in Bacteroidetes.

Through bioinformatics analyses, we identified a lipoprotein export signal (LES) at the N-terminus of surface-exposed lipoproteins of the dog oral commensal and human pathogen *Capnocytophaga canimorsus*. We showed that, when introduced in sialidase (SiaC), an intracellular lipoprotein, this signal is sufficient to target the protein to the bacterial cell surface. We identified a LES in other Bacteroidetes, namely *Bacteroides fragilis* and *Flavobacterium johnsoniae*, suggesting the existence of a shared novel bacterial lipoprotein export pathway in Bacteroidetes (1).

Our current work focuses at identifying the machinery that allows surface exposure of lipoproteins using *C. canimorsus* and *F. johnsoniae* as model organisms.

To this aim we started several different experimental approaches:

1. Considering the abundance of surface lipoproteins and their crucial role in Bacteroidetes biology, we can hypothesize that the protein or protein complex responsible for lipoprotein export is essential. Therefore, we performed a transposon sequencing (Tn-seq) approach on *C. canimorsus* and *F. johnsoniae* in order to determine the set of essential genes in these species. Among the essential genes of both species we identified a gene encoding for the Omp85 family protein TamL, an homolog of TamA. While TamA is involved in autotransporter biogenesis and is found almost exclusively in Proteobacteria, TamL is a lipoprotein of unknown function exclusively present in Bacteroidetes. In order to find TamL function and in particular to see if it is involved in lipoprotein export, we have recently generated TamL conditional mutants in both *C. canimorsus* and *F. johnsoniae*.

2. Given that surface lipoproteins are crucial for the uptake and metabolism of complex polysaccharides we can hypothesize that their depletion would be tolerated in rich medium in the presence of readily metabolizable (Sus-independent) carbon sources. Deletion of the lipoprotein export machinery would thus not be lethal in this condition. In order to identify the export machinery, we will perform a random transposon mutagenesis in *F. johnsoniae* and monitor lipoprotein surface exposure in the mutants. To this aim, we generated a reporter strain expressing sialidase at its surface (LES-SiaC) and set up an ELISA screen allowing the detection of mutants with reduced or no sialidase at their surface.

3. Transport to the bacterial surface implies that the target lipoprotein interacts transiently and probably rapidly with the export machinery. Trying to identify the export machinery by pull-down assays with a surface lipoprotein as bait could thus have limited possibilities of success. Blocking a lipoprotein during transport would increase the chances to pull it down with the export machinery. To this aim, we fused the fast folding protein glutathione S-transferase (GST) to the N- or C-terminus of SiaC. Preliminary data suggest that SiaC-GST fusion proteins are not trasported to the bacterial surface. The next step will consist in determining whether the fusion proteins are stuck at the inner leaflet of the OM and, if this will be the case, perform pull-down experiments using GST affinity columns to identify the members of the lipoprotein export machinery.

(1) Lauber F., Cornelis G. R., Renzi F. *Identification of a new lipoprotein export signal in Gram-negative bacteria.* 2016 Oct 25, *mBio* vol. 7 no. 5 e01232-16. doi: 10.1128/mBio.01232-16.

A26 - Small RNA BdhR1 co-regulates utilization of carbon sources in *Burkholderia cenocepacia* J2315.

Andrea M. Sass[1], Stijn De Waele[2], Simon Daled[3], Bart Devreese[2], Dieter Deforce[3], Filip Van Nieuwerburgh[3] and Tom Coenye[1]*

[1]Laboratory of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium
[2]Laboratory for Microbiology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium
[3]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium
**andrea.sass@ugent.be*

Small non-coding sRNAs have versatile roles in regulating bacterial metabolism. Four short homologous *Burkholderia cenocepacia* sRNAs strongly expressed under conditions of growth arrest were recently identified. One of these, designated BdhR1, contains a short putative target recognition sequence which is conserved throughout the order *Burkholderiales* and is the reverse complement of the Shine–Dalgarno sequence of a large number of genes involved in transport and metabolism of amino acids and carbohydrates. Overexpression of BdhR1 had a distinct impact on growth, attenuating growth on a variety of substrates such as phenylalanine, tyrosine, glycerol and galactose, while having no effect on growth on other substrates. Transcriptomics and proteomics of BdhR1 overexpression and silencing mutants revealed numerous predicted targets changing expression, notably of genes involved in degradation of aromatic amino acids phenylalanine and tyrosine, and in transport of carbohydrates. The conserved target recognition sequence was essential for growth phenotypes and gene expression changes. Cumulatively, our data point to a role of BdhR1 in regulating the shutdown of metabolism upon nutrient deprivation in *B. cenocepacia*.

A27 - Carrier state dynamics unveil new phage propagation strategy

Ines Staes[1], Kenneth Simoens[2], Gašper Marolt[1,2], William Cenens[1], Sanne Wolput[1], Rob Lavigne[3], Kristel Bernaerts[2], Abram Aertsen[1]*

[1] Department of Microbial and Molecular Systems, Faculty of Bioscience Engineering, KU Leuven, Belgium.

[2] Department of Chemical Engineering, Faculty of Engineering, KU Leuven, Belgium

[3] Department of Biosystems, Faculty of Bioscience Engineering, KU Leuven, Belgium.

**Corresponding author: abram.aertsen@kuleuven.be*

"Upon infection of the host, a temperate phage can make the decision between integrating into the host chromosome or producing new virions". This long-lasting paradigm has been the subject of many studies, attempting to uncover the mechanisms governing this decision. Our research group previously reported the peculiar behavior of phage P22 upon infection of its *Salmonella* Typhimurium host, as commitment to the lysogenic cycle is first preceded by the formation of a phage carrier state. In this state, delay of the integration event of a temperate phage in the host chromosome results in the emergence of a phage free subpopulation that is transiently resistant to superinfection due to cytoplasmic inheritance of immunity factors. We now investigate the implications of these novel phage infection dynamics at population level in chemostats as well as the underlying molecular mechanisms using time-lapse fluorescence microscopy. This revealed that the phage carrier state is able to bring forth increased spread of virions by molecular mechanisms defying the paradigm that high phage-to-cell ratios favor non-lytic development of temperate phages new field campaigns in order to contribute to the understanding of the Antarctic terrestrial microbial ecology.

A28 - Particular interest in the unusual biosynthetic gene cluster in the genome of *Streptomyces lunaelactis*

Déborah Tellatin[1], Loïc Martinet[1], Marta Maciejewska[1], Aymeric Naômé[1], Delphine Adam[1], Monique Carnol[2], Hazel A. Barton[3], Nicolas Smargiasso[4], Edwin De Pauw[4], Marc Hanikenne[5], Denis Baurain[5], and Sébastien Rigali[1]
[1] InBioS – Centre for Protein Engineering, Institut de Chimie B6a, University of Liège, Liège, Belgium, [2] InBioS –Plant and Microbial Ecology, Botany B22, University of Liège, Liège, Belgium, [3] Department of Biology, University of Akron, Akron, OH, USA, [4] MolSys Research Unit, Mass Spectrometry Laboratory, University of Liège, Liège, Belgium, [5] InBioS – Functional Genomics and Plant Molecular Imaging, University of Liège, Liège, Belgium, [6] PhytoSYSTEMS, University of Liège, Liège, Belgium.

Streptomyces lunaelactis is the first *Streptomyces* species isolated from moonmilk deposits of a limestone cave called Grotte des Collemboles (Belgium). Genome mining identified 37 Biosynthetic Gene Clusters (BGCs) for natural products (NPs) many of which being cryptic. One of these NPs is an iron chelator called ferroverdin which confer to this strain its typical deep green coloration. We attempted by genome mining and genetic based approaches to identify the BGC associated with the production of ferroverdins. Typically, one BGC is responsible for the production of one or several highly similar compounds with bioactivities that usually only vary in terms of strength and/or specificity. Surprisingly, we identified that the ferroverdin BGC is also implicated in the production of another kind of specialized (secondary) metabolite known as bagremycin. Our results reveal a case of a BGC that is required for the production of metabolites, namely ferroverdins and bagremycins, which differ in terms of i) chemical composition (nitroso- vs amino-aromatic compounds), ii) structural organization (trimers vs monomers), iii) bioactivity (iron chelators vs antibiotics), iv) localization (intracellular vs intra- and extracellular), and v) conditions eliciting their synthesis (unknown vs iron overload). By revealing a new level of complexity of pathways associated with natural compound production our finding challenges the conventional concept of one BGC being responsible for one type of metabolite.

A29 - Quantifying the dynamics of conjugation at single-cell level

Laetitia Van Wonterghem[1,2], Natalie Verstraeten[1,2], Jan Michiels[1,2]*

[1]KU Leuven Centre of Microbial and Plant Genetics, Leuven, Belgium.

[2]VIB-KU Leuven Center for Microbiology, Leuven, Belgium.

**laetitia.vanwonterghem@kuleuven.vib.be*

Conjugation plays a major role in the dissemination of antibiotic resistance genes. To illustrate, resistance genes to most of our frontline antibiotics are located on conjugative elements. Furthermore, most plasmids found in clinical isolates are transferrable by conjugation. Moreover, conjugation has already been reported to cause treatment failure. Transient exposure of a donor to a gut commensal in a mouse model has been demonstrated to result in plasmid transfer. Combined with the high diversity of resistance genes in the human microbiome, this is most worrisome.

This project aims to elucidate the dynamics of conjugation at the single-cell level using fluorescence microscopy. Based on the image data, we propose a model describing conjugation on a surface. This allows us to quantify the rate and the frequency of conjugation. In contrast to bulk measurements, this method enables to evaluate the influence of the recipient's micro-environment on conjugation. For example, our preliminary results indicate that the number of donor cells surrounding a recipient cell does not affect conjugation.

In conclusion, we strive to gain more insight into the rate and frequency of conjugation at the single-cell level. These results will further clarify how antibiotic resistance genes are disseminated to recipients upon transient exposure to a donor cell.

A30 - The Influence of Circadian Rhythm on Plant Cell Wall Degradation by Lytic Polysaccharide Monooxygenases

Monclaro, A.V. and Cannella, D.[1*]

[1]*Biomass Transformation Laboratory, École Interfacultaire de Bioingénieurs, Université Libre de Bruxelles.*

**david.cannella@ulb.ac.be*

Light plays an important role on fungus development, as it can cause oxidative and heat stress, regulate its growth and also influence the circadian rhythm. Fungi has the capability to respond to light in different ways. One important response is the regulation of the production of extracellular enzymes involved in the degradation of lignocellulose. Stappler *et al.* (2017a) showed that the secretion of proteins of *Trichoderma ressei* was influenced by light. For *cel61b*, there was no significative difference on the number of transcripts under constant light or darkness, but SDS-PAGE gel bands of secreted Cel61B were visible only in darkness. For *cbh1*, under high light intensity, transcripts were upregulated, but the activity of CBH1 was severely decreased, suggesting a level of posttranslational regulation of cellulase biosynthesis under the influence of light. Another study conducted by Stappler *et al.* (2017b) showed that *cel61a* and *cel61b* were located in different genomic clusters and were modulated by light in different ways, specifically with *cel61b* being downregulated by light. Schmoll (2018) has recently proposed that many CAZYmes from *T. reesei* present light-dependent regulation and that the photoreceptors play a role connecting nutrient signaling pathways (e.g. G-protein and cAMP) and enzyme expression. It is worth to mention that the number of light-regulated genes varies in the presence of different carbon sources. Posttranslational modifications, especially phosphorylation, are essential for regulatory processes in the cell. Phosphorylation is also the key posttranslational modification in the control of eukaryotic circadian rhythm. Besides, light can also induce phosphorylation of a range of proteins such as photoreceptors and others (Liu 2005). Considering that the expression and secretion of CAZymes is dependent on the composition of the plant biomass, we propose that: i) Circadian rhythm of fungi play an essential role in the expression and secretion of CAZymes; ii) The interplay between the secretion of these enzymes, specifically LPMOs, and the composition of plant biomass might be regulated by posttranslational modifications, particularly phosphorylation; iii) Finally, circadian rhythm and oxidative stress induced by light might have an interplay. For that, we propose to work with *Neurospora crassa*, as it is a fungus model for studies on plant biomass degradation and for biological circadian studies, and as it also presents 14 AA9 genes.

Stappler E., Walton J. D., Beier S. and Schmoll M. Abundance of Secreted Proteins of *Trichoderma reesei* is Regulated by Light of Different Intensities. Frontiers in Microbiology 2017a;8:1-14.

Stappler E., Dattenböck C., Tisch D. and Schmoll M. Analysis of Light- and Carbon-Specific Transcriptomes Implicates a Class of G-Protein-Coupled Receptors in Cellulose Sensing. mSphere 2017;2:1-19.

Schmoll M. Regulation of plant cell wall degradation by light in Trichoderma. Fungal Biology and Biotechnology 2018:5;1-20.

Liu Y. Analysis of Posttranslational Regulations in the Neurospora Circadian Clock. Methods in Enzymology 2005;393:379-393.

A31 - The polyamino-isoprenic efflux inhibitor NV716 revives old disused antibiotics against intracellular forms of infection by *Pseudomonas aeruginosa*

Gang Wang[1], Jean-Michel Brunel[2], Paul M. Tulkens[1], Jean-Michel Bolla[2], Françoise Van Bambeke[1]

[1] *Université catholique de Louvain, Brussels, Belgium*

[2] *Aix-Marseille Université, Marseille, France*

Objective: WHO considers *P. aeruginosa* (PA) as a priority pathogen for the search of innovative therapies. PA is indeed intrinsically resistant to many antibiotics due to poor outer membrane permeability and/or active efflux. Moreover, it can also adopt specific lifestyles, like intracellular survival, that make it poorly responsive to treatments. Our aim was to evaluate the capacity of efflux pump inhibitors to restore the activity of old, disused antibiotics, against intracellular PA. We compared PABN to original polyamino-isoprenic compounds, namely NV731 and NV716 in combination with doxycycline, chloramphenicol (substrates for efflux), and rifampicin (not substrate).

Methods: See (AAC 2013; 57:2310-2318) for details. Phagocytosis of PAO1 (opsonized with human serum) was allowed for 2 hours using a bacterium: cell ratio of 10:1, after which non-phagocytosed bacteria were eliminated by incubation with gentamicin (50 X MIC) for 1 hour. After washing, infected cells were incubated with antibiotics (0.003-100 x MIC) for 24 hours. Maximal relative efficacy (E_{max}) and apparent static concentrations (C_s) were calculated using the Hill equation of concentration-response curves. Toxicity was assessed by LDH release

Results: See table. NV731 no significant deferent and PABN decreased the MICs of doxycycline and chloramphenicol but not that of rifampicin, while NV716 (2.5 and 10 µM) markedly reduced the MIC of all antibiotics. Intracellularly, C_s of antibiotics alone were close to their MIC in broth; their E_{max} was approx. 2 log decrease in cfu. NV731 and PABN did not modify these parameters, while NV716 (10µM) was able to increase both relative potency (lower C_s value) and maximal efficacy (more negative E_{max} value) for all drugs without causing toxicity for THP-1 cells.

Conclusion: In contrast to PABN and NV731 that act as efflux inhibitors against planktonic bacteria only, NV716 is capable to re-sensitize PA to antibiotics whether substrates (doxycycline, chloramphenicol) or not (rifampicin) for efflux, not only in the broth but also intracellularly. This could be due to its capacity to alter PA membrane integrity (PLoSOne 2016; 11(5):e0154490). NV716 may, therefore, appear as a useful adjuvant to revive the activity of old antibiotics with low antipseudomonal activity against PA infections, including its intracellular persistent forms.

	MIC (mg/L)[a]			**C_s[b]** (mg/L)			**E_{max}[c]** (Δlog cfu from post-phagocytosis inoculum)		
Experimental conditio	DOX	CHL	RIF	DOX	CHL	RIF	DOX	CHL	RIF
Antibiotic (AB) alone	8	32	16	16.0±8.2	37.5±9.3	20.2±4.0	-2.1±0.2	-2.0±0.3	-2.1±0.3
AB + NV716 (2.5µM)	**1**	**2**	**0.25**	17.9±3.7	42.1±7.8	19.7±3.5	-2.4±0.2	-1.9±0.3	-2.2±0.3
AB + NV716 (10µM)	**0.5**	**1**	**0.12**	4.9±1.2*	8.7±5.3*	11.1±2.3*	-2.9±0.5	-2.5±0.3	-2.7±0.4
AB + NV731 (2.5µM)	4	32	16	16.3±1.7	41.1±8.2	26.2±1.0	-2.6±0.2	-2.1±0.2	-2.4±0.4
AB + NV731 (10µM)	4	16	8	14.2±4.0	15.0±9.1*	18.2±3.8	-2.6±0.4	-2.1±0.2	-2.3±0.4
AB + PABN (20mg/L)	**2**	**8**	8	22.9±1.0	34.5±13.8	17.9±1.2	-2.3±0.2	-2.1±0.3	-2.3±0.3

[a] values in bold denote a decrease of at least 2 doubling dilutions vs. AB alone

[b] Cs: apparent static concentration i.e., the extracellular concentration (mg/L) resulting in no apparent bacterial growth (number of cfu identical to the initial [extracellular] or post-phagocytosis [intracellular] inoculum)

[c] Emax: relative maximal efficacy: maximal decrease in inoculum (in $\log_{10}$ units) compared to the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration.

* significantly different ($p < 0.05$) from AB alone

A32 - A plea to preserve microbial diversity in public microbial resource centres

Pierre Becker [1], Peter Chaerle [2], Marijke Hendrickx [1], Wim Vyverman [2], Anne-Catherine Ahn [3], Marleen Bosschaerts[4], Heide.-Marie Daniel[5], Ann Hellemans[6], Anneleen Olbrechts[7], Leen Rigouts[8], Annick Wilmotte[3]*

[1]BCCM/IHEM, Sciensano, Rue J. Wytsmanstraat 14, B-1050 Brussels, Belgium
[2]BCCM/DCG, Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Krijgslaan 281 S8, 9000 Gent, Belgium
[3]BCCM/ULC Cyanobacteria collection, InBioS-Centre for Protein Engineering, University of Liège, Liège, Belgium,
[4]BCCM Coordination Cell, Belgian Science Policy, Brussels, Belgium
[5]BCCM/MUCL, Mycothèque de l'Université catholique de Louvain, Earth and Life Institute, Mycology Laboratory, Université catholique de Louvain, Louvain-la-Neuve, Belgium
[6]BCCM/LMG Bacteria Collection, Laboratory of Microbiology, Faculty of Science, Ghent University, Ghent, Belgium
[7]BCCM/GeneCorner Plasmid Collection, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
[8]BCCM/ITM Mycobacteria Collection, Institute of Tropical Medicine, Antwerp, Belgium
[*]awilmotte@uliege.be

Open science aims at sharing scientific output in order to maximize the impact of research. This allows follow-on studies, facilitates new discoveries, improves reproducibility of experiments and favours transparency of results. Although open data is becoming a well-known concept, less attention is given to the availability of research materials. In life sciences, public microbial collections represent an historical example of open science, thanks to their longstanding experience in the preservation of living microbial strains and their distribution for further scientific investigations or development. These microbial resource centres provide well-characterized, quality-controlled and authenticated strains and associated data (1). In microbiology, the diversity of bacteria, fungi and algae is an invaluable source of applications for the bio-industry. It needs to be secured following (inter)national legislations for future utilizations and research questions. The responsibility to make microorganisms available is shared by researchers, funding agencies and publishers (1). Microbiologists need to be more aware towards strain conservation. Governmental funding policies should request the deposit of strains isolated during financed projects. Regarding publishers, most journals encourage authors to deposit their datasets (codes, sequences, etc) in public repositories but very few specifically require deposit of biological material and cultivated strains in scientific collections. However, this is a key prerequisite to "make it possible to repeat the experiments and perform future research"(2). Editors should therefore implement mechanisms for active agreement by authors to deposit strains when submitting an article. Such mechanisms could follow Transparency and Openness Promotion guidelines (3) for journals that include standards for research materials.

1. P. Becker, M. Bosschaerts, P. Chaerle, H.-M. Daniel, A. Hellemans, A. Olbrechts, L. Rigouts, A. Wilmotte, M. Hendrickx. 2019. Public microbial resources centres: key hubs for FAIR microorganisms and genetic materials. Applied and Environmental Microbiology, AEM.01444-19; DOI: 10.1128/AEM.01444-19
2. https://onlinelibrary.wiley.com/page/journal/15298817/homepage/forauthors.html
3. Nosek BA et al. (2015). Promoting an open research culture. Science 348: 1422-1425.

A33 - A proteomic approach to unveil phage-host interactions during the phage carrier state

Sanne Wolput[1], Oscar Enrique Torres Montaguth[1], William Cenens[1], Angela Makumi[1], Ines Staes[1], Erik Royackers[2], Jean-Paul Noben[2], Rob Lavigne[3] and Abram Aertsen[1]*

[1]Laboratory of Food Microbiology, Department of Microbial and Molecular Systems (M^2S), Faculty of Bioscience Engineering, KU Leuven, Leuven, Belgium
[2]Biomedical Research Institute and Transnational University Limburg, School of Life Sciences, Hasselt University, Diepenbeek, Belgium.
[3]Laboratory of Gene Technology, Department of Biosystems, Faculty of Bioscience Engineering, KU Leuven, Leuven, Belgium

**E-mail: abram.aertsen@kuleuven.be*

Bacteriophages have co-evolved with their bacterial hosts for millions of years, rendering them the most ubiquitous and diversified organisms on the planet. Traditionally, two distinct reproductive strategies are described in literature, the lytic and lysogenic cycle. Our research group previously reported an alternative strategy preceding lysogeny. After infection, the phage genome is maintained in the host cell without immediate integration as a prophage. This peculiar behaviour, called the phage carrier state, allows the emergence of a phage-free subpopulation which is transiently resistant to superinfection. Further evidence for the existence of this carrier state was provided by a novel phage-host interaction that is strictly observed in carrier cells in the temperate phage P22-*Salmonella* Typhimurium model system. The gene product of the P22 ORFan gene *pid* derepresses the *dgoRKAT* operon of the host, which is involved in D-galactonate metabolism and important in virulence and intracellular survival. In search for the molecular mechanism by which Pid derepresses the *dgoRKAT* operon and its impact on the behavior and physiology of *S.* Typhimurium, protein-protein interaction studies have been performed revealing potential interaction partners involved in the regulation, expression and function of *pid*.

A34 - Selection of cyclic peptides inhibitors of carbapenemases through SICLOPP and Selection By Lysis

Sebastian Worms, Patrice Soumillion, Louvain Institute of Biomolecular Science and Technology, UCLouvain

In a world where antibiotic resistance is spreading and discovery of new antibiotics is not proceeding fast enough, finding ways to inhibit resistance to existing antibiotics would offer another way to fight antibiotic-resistant bacteria.

Using the expertise of our laboratory in generation of cyclic peptides, library creation and creation of novel selection approaches to directed evolution we aim to discover peptides that could help fight infections by resistant Gram- bacteria either by inhibiting the enzymes that cause their resistance, or by sensitizing them to Gram+-specific antibiotics such as vancomycin.

Split-intein chemistry was used to create large ($>10^8$) libraries of periplasmic cyclic peptides in *Escherichia coli.* To avoid time-consuming screening of library we developed a novel selection method where strains expressing the carbapenemases from genomic loci were transformed with the library. Subsequent addition of antibiotics lysed cells containing a sensitizing peptide. Following centrifugation to remove live cells, plasmids encoding peptide of interest could be recovered from the supernatant.

ABSTRACTS SHORT TALKS
SECTION B: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

B - ST01- Impact of interspecies interactions on the production of biocontrol-related metabolites by *Bacillus velezensis*

S. Andrić[1], T. Meyer[1], A. Rigolet[1], A. Argüelles Arias[1], S. Steels[1], M. Ongena[1]

[1]Microbial Processes and Interactions laboratory, Gembloux Agro-Bio Tech, University of Liège, Belgium

Biological control of plant pathogens, which is frequently involving microorganisms, is appearing as increasingly important sustainable alternative to the chemical pesticides. Root-associated *Bacillus* sp. are commonly used for this purpose. Besides competition with plant pathogens and ability to stimulate host defence mechanism, members of *Bacillus velezensis* species produce an impressive range of biologically active molecules. As biocontrol activity of *Bacillus* is promising for the plant protection, there is always an effort for its improvement. An important point, that is not enough investigated and could possibly contribute to this aim, is the interaction with other rhizosphere microorganisms such as PGPR bacteria *Pseudomonas* sp.. In this work we showed the impact of *Pseudomonas* sp. secreted metabolites on *B. velezensis* synthesis of antibacterial compounds. Based on observed interaction between these two PGPR partners, different enhanced antibacterial activities of *B. velezensis* were observed against the phytopathogenic bacteria *Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas campestris* pv. *campestris*. By combining analytical, transcriptional and mutational methodologies, we could correlate these enhanced *Bacillus* antibacterial activities upon *Pseudomonas* sensing with the stimulation of specific compounds belonging to different chemical classes such as polyketides and bacteriocins. We demonstrate that the anti-*Clavibacter* activity relies on the amylocyclicin bacteriocin while anti-*Xanthomonas* inhibition is mainly due to two forms of polyketides, (oxy)difficidin and (dihydro)bacillaene. Our results highlight the potential of *B. velezensis* to boost the synthesis of multiple antimicrobial weapons in response to the perception of metabolites from bacterial competitors and reinforces the interest of this species as biocontrol agent.

B - ST02- Pesticide degrading bacteria for the removal of micropollutants from drinking water. From limitations to opportunities.

Benjamin Horemans[a], B. Raes[a], D. Springael[a]

[a] *Department of Earth and Environmental Sciences, KULeuven, Belgium*

Currently, bioremediation strategies are explored for the removal of micro-pollutants such as pesticides in drinking water. The European norm for pesticides and their transformation products (TP) in intake water for drinking water production is exceeded regularly. Drinking water companies are therefore forced to use physico-chemical treatment and close or relocate extraction wells. Much attention goes to the persistent groundwater micro-pollutant 2,6-dichlorobenzamide (BAM), a TP of the banned herbicide dichlobenil. BAM is regularly found in groundwater used for drinking water production at trace level concentrations all over Europe. A sustainable alternative treatment was suggested in which the BAM-degrading bacterium *Aminobacter* sp. MSH1 is inoculated in sand filters exploited in drinking water treatment plants (DWTP) to remove BAM by biodegradation. So far, two pilot scale studies treating intake water in DWTPs were reported. While results were promising, limitations of the approach surfaced as well. We performed detailed studies to understand the impact of the abiotic and biotic environment of DWTPs on strain MSH1. BAM removal by *Aminobacter* sp. MSH1 was found to be controlled by assimilable organic carbon (AOC) impacting the physiology, genotype and ecology of MSH1. AOC, although very low in intake water with estimates of 100 µg/L, is the main source of carbon and energy in the system. MSH1 biofilm studies in continuous flow cells showed that BAM degradation at micro-pollutant concentrations is controlled by AOC-linked growth. MSH1 carries the catabolic genes required for BAM mineralization on two plasmids. Plasmid pBAM1 encodes the amidase BbdA which converts BAM to 2,6-dichlorobenzoic acid (DCBA), while plasmid pBAM2 encodes for enzymes that convert DCBA to TCA cycle intermediates. The availability of AOC in intake water with BAM at trace concentrations leads to MSH1 cells that lack pBAM2. pBAM2 free cells have a higher fitness compared to cells carrying both plasmids resulting into a dominance of a MSH1 subpopulation lacking pBAM2. As plasmid pBAM1 is not lost, AOC in intake water dictates whether BAM is simply converted to DCBA or fully mineralized. In addition, proteomics revealed that the low AOC levels lead to starvation with an impact on the physiology of MSH1 and likely results in decreased specific BAM-degradation at trace concentrations. In addition, AOC plays also a key role in the ability of MSH1 to invade and settle in the indigenous community residing in the sand filters. The assimilable traces of carbon in intake water is as such key in the success of bioaugmentation by MSH1 for micro-pollutant removal. The knowledge gathered so far can help to overcome current limitations and devise strategies to improve bioaugmentation for the removal of BAM from drinking water, and for the removal of micro-pollutants in general.

B – ST03 - The phyllosphere microbiome of greenhouse crops, a first step towards plant probiotics

Marie Legein[1], Wenke Smets[1], Babette Muyshondt[1], Roeland Samson[1], Sarah Lebeer[1]*

[1]ENdEMIC, bio-engineering sciences, University of Antwerp, Belgium

[*]Corresponding author: marie.legein@uantwerpen.be

The phyllosphere is the surface of plants in contact with the atmosphere. It is occupied by bacteria, fungi and other micro-organisms. This microbial community forms intimate interactions with the host plant and has an influence on plant growth and plant health. Phyllosphere commensals can protect the plant in various ways, by exclusion of pathogens trough competition for nutrients and space, production of antibiotic metabolites, hyperparasitism or by triggering the plants immune. Plant growth can be promoted trough production of plant hormones or by increasing the availability of nutrients.

There is a huge potential in harnessing this microbial community to improve crop production and crop protection while decreasing the negative impacts of pesticides and fertilizers on human health and on the environment. This research aims to explore the potential applications of plant probiotics, i.e. bacteria with a beneficial impact on the host plant by altering the phyllosphere microbiome.

16s rRNA gene amplicon sequencing is used to determine the community structure and dynamics over time of the phyllosphere of seven tomato cultivars and three strawberry cultivars, at two different locations. Variations in community structure were monitored over time and linked to cultivar, distance between plants and location, use of pesticides and the occurrence of pathogens. Interestingly, the insects used in the greenhouses for pollination or plant protection have a big impact on the phyllosphere community. Insights in the factors shaping phyllosphere communities are a crucial step towards modulating these communities and the formulation of plant probiotics.

B -ST04 - Microbial community dynamics of a meadow contaminated with metals, natural and artificial radionuclides

*Rogiers Tom[1,2] *; Claesen Jürgen[1]; Van Gompel Axel[1]; Mysara Mohamed[1]; Williamson Adam[2]; Leys Natalie[1]; Van Houdt Rob[1]; Boon Nico[2]; Mijnendonckx Kristel[1]*

[1]*Interdisciplinary biosciences, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium*

[2]*Center for Microbial Ecology and Technology, UGent, Ghent, Belgium*

**tom.rogiers@sckcen.be*

Historical discharges of phosphate and nuclear industry have contaminated certain environmental sites in Belgium with metals and radionuclides, which can be harmful for all residing organisms. This study assesses the impact of metal and radionuclide contamination on the microbial community of a contaminated site located in the Grote Nete river basin.

Eight soil samples expected to have varying contamination levels were taken consecutively on one line perpendicular to the river. In addition, one control sample was taken further in the field. General soil parameters such as pH, moisture content and total organic carbon (TOC) were determined. Moreover, EDXRF was used to measure metal elements, while radionuclides were analysed with gamma spectroscopy. A comparable pH of 5.08 to 5.49 was measured in all samples while clear differences were observed for TOC, varying from 3.3% to 17.1%. A metal and radionuclide contamination gradient was noticed among the samples, which was positively correlated with the moisture content (from 7.19% to 53.8%). The highest concentrations of metals were As and Zn, ranging from respectively 9.60 mg/kg to 2300 mg/kg and 49.40 mg/kg to 3430 mg/kg. The most important radionuclide contamination originated from Ra^{226} with concentrations ranging from 19.2 Bq/kg to 3630 Bq/kg. Correlation analysis of all studied chemical parameters indicated that they could be divided in seven distinct groups.

The microbial community was analysed via 16S rRNA microbial profiling and by scoring their viability on growth medium supplemented with metals. The 16S microbial profiling resulted in over 7000 OTUs in total, showing that the residing microbial community is highly diverse. Unconstrained RCM analysis revealed different groups with close intra-sample clustering. This indicated that the contamination affected the microbial community structure. Moreover, constrained RCM analysis was performed to combine the 16S rRNA amplicon sequencing results with one representative parameter of each of the seven groups of chemical parameters. This confirmed that the different groups in the microbial community could be explained based on the level of contamination. Finally, viable count on 1 mM $NiCl_2$ or 1 mM $ZnSO_4$ showed that the communities originating from contaminated soil sites were better at surviving on these metal stressors in comparison with non-contaminated control communities.

This study shows that metal and radionuclide contamination in the environment affects the residing microbial community. Detailed experiments will be carried out to further investigate the metal resistant communities by screening for the most resistant bacterial species.

ABSTRACTS POSTERS
SECTION B: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

B01 - Selection of Actinobacteria strains for Biocontrol and plant growth promoting activities: Applications on wheat crops.

Wiem ALLOUN[1,2], Noredine KACEM-CHAOUCH[1], Amin SHAVANDI[2], David CANNELLA[2]*

[1]Mycology, Biotechnology and Microbial Activity Lab, University of Constantine 1, Algeria.
[2] Laboratory of Biomass Transformation Lab, 3BIO Unit, Université Libre de Bruxelles (ULB), Belgium.
**e-mail address of the corresponding author: david.cannella@ulb.ac.be*

Fusarium genus remains the principal pathogenic fungi responsible of wheat root-rot causing annual yield losses in Constantine region in Algeria. To face-up the environment toxicity related to the excessive use of chemical fungicides and fertilizers, the investigation of plant growth promoting bacteria as plant inoculants constitutes an environmentally safe and sustainable approach to increase wheat crop production and suppress *Fusarium* growth. This study aims to explore the biocontrol potential and plant growth promotion traits of a collection of Actinobacteria strains for the formulation of a biobased fertilizer and biofungicide and to evaluate its efficiency in pot trials. For that, a total of 102 Actinobacteria strains were isolated from different Algerian niches as well as marine sediments, wheat rhizospheric soil (soil surrounding wheat plant roots) and non-rhizospheric soil. These strains were screened for their *In vitro* antifungal activity against two phytopathogenic fungi belonging to *Fusarium* genus, *F. oxysporum* and *F. culmorum* isolated from rotten wheat plants. 29 strains showed antifungal activitiy and reduced significantly the fungal growth. All the selected 29 strains were able to produce the typical heteroauxin phytohormone IAA (Indole 3-acetic acid) implicated in stimulation of root initiation and cell elongation. Indeed, the IAA production was ranged from 12 to 473,5 µg/ml. Strains Pt-2F, Pt-16, RZB24 and Pt-1 exhibited the highest IAA production with, respectively, 473.5, 420.5, 319.87 and 309 µg/ml. In similar study, Anwar and *al*. (2016) obtained a maximum IAA amount (79.5 µg/ml) from *Streptomyces nobilis WA-3* and Goudjal *and al.* (2013) recorded 127 µg/mL of IAA from *S. rochei PTL2*. All strains gave positive result for ammonia production and amylolytic activity against starch and 9 strains showed a cellulolytic activity. Only strain RZBC, isolated from rhizospheric soil, was able to solubilize inorganic phosphate on Pikovskaya medium amended with Ca_3PO_4 as sole source of inorganic phosphate, to hydrolyse starch and cellulose, it constitutes then a promising plant growth promoting agent. Strain S28, isolated from marine sediments, showed a promising biocontrol ability and the highest Vigor index of inoculated seedlings was observed when wheat seeds were treated with cell suspension of strain S28 corresponding to 7.8×10^6 cfu/ml and a significant increase of root and shoot length was observed in the inoculated plants. Powder formulation of a mixture of RZBC and S28 will be developed using wheat straw and corn starch as solid carriers for further applications in pot and field trials.

Keywords. Biological control, *Fusarium*, Actinobacteria, growth promotion, powder formulation.

B02 - Characterization of a non toxic pyomelanin pigment produced by *Y. lipolytica* and its potential application in cosmetics

Imen Ben Tahar[1], Małgorzata Kus-Liśkiewicz,[2], Yannick Lara[3],Emanuelle Javaux[3], Patrick Fickers[1]

[1]Microbial Processes and Interactions, TERRA Teaching and Research Centre, University of Liège - Gembloux Agro Bio Tech, Avenue de la Faculté, 2. B-5030 Gembloux, Belgium
[2]Faculty of Biotechnology, University of Rzeszow, Pigonia 1, 35-310 Rzeszow, Poland

[3]Early Life Traces & Evolution – Astrobiology, UR Astrobiology, Geology Department, University of Liège, Belgium

[*]imen_bentahar@yahoo.fr

Melanin is an heterogeneous polymer produced by several organisms *via* different biochemical pathways. Based on their biosynthesis pathway, they were classified into three groups: eumelanins (black or brown pigments), pheomelanins (yellow-red pigments) and allomelanins which includes different pigments such as DHN-melanins (grey-green pigments) and pyomelanins (brown pigments). Although melanins are not essential for growth, they confer to cells a high protection against different environmental stresses, principally UV radiations, oxidative stress and toxic effect of heavy metals. In humans, eumelanin accumulates in keratinocytes and confers to skin a photoprotection toward UV-induced skin damages.

In this study, we report on the ability of the strain *Y. lipolytica* W29 to produce melanin pigment at the yield of 0.5 mg/mL. On the basis of different physico-chemical assays, the pigment has been identified as pyomelanin and its synthesis was found to occur from tyrosine by the so-called HGA-melanin pathway. Pyomelanin production starts with an enzymatic conversion of L-tyrosine to HGA *via* 4-hydroxyphenylpyruvate. Subsequently, a series of non-enzymatic reactions leads to the formation of pyomelanin.

The purified pyomelanin exhibits radical scavenging activity with IC_{50} of 230 µg/mL, which highlighted its antioxidant capacity. Moreover, the purified pigment was also characterized as non-cytotoxic toward two mammalian cell lines, namely the mouse fibroblast NIH3T3 and human keratinocytes HaCaT. The outcome of this set of experiments suggests that pyomelanin shows a series of interesting properties which make it a great choice for different biotechnological applications. In this context, the incorporation of pyomelanin in different commercial sunscreens yielded to an increase of their SPF, highlighting its potential utilization as UV-filter in cosmetic preparations.

Keywords: pyomelanin, *Y. lipolytica;* HGA-melanin pathway; antioxidant; non-cytotoxic

B03 - First insight of microbial interactions between heterotrophic bacteria from river water

Mathias Bonal[1], Marie Vanderkelen[1], Pierre Servais[1], Didier Gonze[2], Karoline Faust[3] & Isabelle George[1]*

[1]*Laboratory of Ecology of Aquatic Systems, ULB, Belgium*

[2]*Unit of Theoretical Chronobiology, ULB, Belgium*

[3]*Laboratory of Microbial Systems Biology, KU Leuven, Belgium*

[*]*igeorge@ulb.ac.be*

Rivers represent aquatic ecosystems among the most suffering from anthropogenic activities, by their regular and important exposure to massive inputs of organic matter and chemical pollutants. Heterotrophic bacteria play a key role in rivers' ''self-purification'' by their action of mineralization of organic matter and export of carbon to the atmosphere, and besides they form an important trophic link as they are involved in the microbial food web. However, studies conducted till then only focus on ecological characterization of river waters at time *t* (field studies) or are limited to a small number of species (lab studies), and in both cases they don't lead to a fine understanding of the intrinsic functioning of the microbiome (the role of each species and how they interact). Therefore, we aimed in this research project to study the microbial interactions amongst a simplified model community of 15 bacterial strains (frequent genera in river waters worldwide) to get an insight of how the community is structured and how each strain interacts with each other.

We analysed the growth of each strain alone and in co-culture (105 in total) in R2B medium in batch experiments, in order to obtain the growth rate (μ) and plateau (K) of each monoculture and coculture. Two series of experiments were carried on, in which the cultures of mono- and co-cultures were followed by $OD_{600\ nm}$ over 65 h and incubated in 96-well microplates; in the first set of experiments the microplates were let inside the spectrophotometer and the temperature was not controlled (ranging from 24 to 29 °C), whereas in the second set of experiments they were let at 20 °C in an incubator and taken out only to take OD measurements. The aim was to compare, for each coculture, the growth rate of the co-culture $\mu_{A\&B}$ with the sum of the growth rates of the two monocultures $\mu_A + \mu_B$ to infer the interaction between the two strains A and B (Freilich *et al.*, 2011); the same calculation and comparison was made with K (de Vos *et al.*, 2017). We calculated the values of μ and K from the growth curves using three different methods: (i) manual calculation of the slope of the beginning of the exponential phase on the semi-ln growth graphs (μ) and manual reading of the value of the plateau on the $OD_{600\ nm}$ growth graph (K); then (ii) *in silico* using a fitting function based on a logistic equation provided by the package *growthcurver* in R (μ & K); eventually (iii) *in silico* using a fitting function based on a model formulated by Baranyi in 1995 provided by the package *growthrates* in R (μ only).

We concluded that high temperatures seem to lead of high values of μ and low values of K for the monocultures. Depending on the calculation method, he matrices were all different except for the fact that they all displayed a majority of negative interactions (competitions). We found potential positive interactions in all of them, which concur with what a previous PhD student in our lab suggested, *i.e.* the strains grow better when they grow within the community as a whole than when they grow separately (Goetghebuer *et al.*, 2017 and 2019). However, a master student studying a sub-community (3 strains taken from our community) in Colony Forming Units (R2-agar) over the past months found that there were positive interactions between some pairs, whereas in our matrices we always observed negative interactions in these pairs of strains. She also observed asymmetric interactions between these pairs, *i.e.* the type of interaction, or its intensity, was overall different in one direction (A → B) than in the other one (B → A). Using a comparison of μ and K of mono- *versus* co-cultures as done in the literature, we therefore observed the limits of this method which doesn't allow to assess the asymmetry of the interactions.

B04 - The microbial community of *H. illucens* larvae: what have we learned?

Jeroen De Smet[1], Enya Wynants[1,2], Leen Van Campenhout[1]*

[1] KU Leuven, Faculty of Engineering Technology, Department of Microbial and Molecular Systems (M2S), Lab4Food, Campus Geel, Geel, Belgium.

[2] Current affiliation: R&D Department, Biobest Belgium N.V, Westerlo, Belgium.

[*] Corresponding author: jeroen.desmet@kuleuven.be

The larvae of *Hermetia illucens* have become the mostly used insect species in industry for the bioconversion of organic waste. This economic relevance is translated in a rapid increase in scientific research being published on this species. The last two years more papers were published on the topic than in the past three decades, dealing with many aspects of larval productivity. Of particular interest are the recent advances on the larval gut physiology[1] and its microbial community[2,3]. They indicate that the larval gut microbiota should be considered as an extra organ capable of degrading wastes and generate beneficial products for the larvae[4].

Here, we present the results of our ongoing efforts to explore the gut microbiota of the larvae of *H. illucens* and how substrate spiking with specific food pathogens affects the microbiological safety of said larvae. To this end, we investigated the substrate, the larvae and their residue, both on laboratory scale and in production scale facilities at several locations[3]. As the substrate composition differs greatly between rearing locations, large differences in the microbial load were observed. Little similarity between the microbiota of the larvae and the substrate at one facility or between the larvae at different facilities were observed. Nevertheless, 48 operational taxonomic units (OTUs) were omnipresent in all facilities, of which most belonged to the phyla *Firmicutes* and *Proteobacteria*. These OTUs are also compared to available datasets of other recent studies to get a broad view on the presence of these 48 OTUs in these diverse datasets to explore the hypothesized claim of a core microbiota residing in the larval gut containing important symbiotic bacteria[5]. In a second experiment, we spiked the substrate with a high amount of a food pathogen, more specific *Salmonella* spp., to mimic a severe microbiological contamination in the rearing cycle. Using selective plate counts we then analyzed if this contamination also affects the larvae. Our results hints at potential interactions occurring between the larvae and specific food pathogens. These might be exploited in the future to manipulate the microbiological safety of the larvae.

We can conclude that the rapid increase in data on the microbial communities in the larvae warrants a thorough meta-analysis to advance from the current narratively describing towards a more in-depth understanding of the microbial community dynamics towards control and applications for industry. At the same time the results of the spiking experiment also call for safety measurements during rearing to ensure the microbiological safety of the larvae.

1. Bonelli, M. *et al. Front. Physiol.* **10,** 204 (2019).
2. Bruno, D. *et al. Appl. Environ. Microbiol.* **85,** e01864-18 (2019).
3. Wynants, E. *et al. Microb. Ecol.* 1–18 (2018). doi:10.1007/s00248-018-1286-x
4. Jiang, C.-L. *et al. Microb. Biotechnol.* **12,** 528–543 (2019).
5. De Smet, J., Wynants, E., Cos, P. & Van Campenhout, L. *Appl. Environ. Microbiol.* **84,** e02722-17 (2018).

B05 - Skin microbiome analysis in chronic inflammatory skin conditions to study the potential of topical probiotics

Lize Delanghe[1], Ingmar Claes[2], Tim Henkens[2], Julien Lambert[3], Sarah Lebeer[1]*

[1]*Department of Bioscience Engineering, University of Antwerp, Belgium*

[2]*YUN Probiotherapy, Belgium*

[3]*Department of Dermatology and Venereology, University Hospital Antwerp/University of Antwerp, Belgium*

[*]*lize.delanghe@uantwerpen.be*

Chronic inflammatory skin conditions such as acne and atopic dermatitis (AD) affect an increasing number of children and adults worldwide. Their etiology is complex, with the manifestation of the disease being influenced by various genetic and immune mechanisms acting in concert with environmental factors. In addition, patients also demonstrate an altered skin microbiome. A reduction in the skin microbial diversity is often observed and flares are characterized by an abundance of skin pathobionts such as *Staphylococcus aureus* and *Cutibacterium* (or *Propionibacterium) acnes.* Topical probiotics could have a positive effect on the skin microbiome and function by inhibiting pathogens, restoring the skin microbiome and/or barrier function and executing anti-inflammatory effects on the skin cells.

In an ongoing collaboration between the University of Antwerp and the biotech company YUN, the skin microbiome of Belgian children and young adults with AD and acne is being studied to explore the potential of topical probiotics. YUN developed a revolutionizing technique allowing to formulate skin creams containing live bacteria. In *vitro* experiments confirmed that the freeze-dried bacteria still show adhesion capacity to human epidermal keratinocytes. A proof-of-concept study with topically applied live lactobacilli in patients with mild-to-moderate acne symptoms has already shown promising results according to symptom improvement and skin microbiome modulation (BioRxiv).

This research is now extended towards other skin conditions. Previous research has already shown that the inhibition of *S. aureus* resulted in symptom improvement and *in vitro* application of probiotic strains such as *Lactobacillus rhamnosus* GG has been shown to inhibit *S. aureus* growth and toxicity. This implicates that life biotherapeutic products are promising to answer the need for new and effective therapies for atopic dermatitis. Herefore, more in depth exploration of the working mechanism, biomarkers and skin microbiome is crucial. A skin microbiome analysis in 120 Belgian children using next generation sequencing is now scheduled to obtain in depth data of the composition of the skin microbiome and identify possible biomarkers for AD in children. In parallel, a proof-of-concept placebo-controlled study with topically applied live lactobacilli in children with AD will give more insights in the working mechanisms of lactobacilli on AD skin whereafter an *in vitro* selection for the most optimal probiotic strains will be the next step towards an effective probiotherapy for AD.

B06 - Understanding Strain to Strain Interactions that Affect the Bioaugmentation Success of a Pesticide Degrading Bacterium in a Microbial Community

*Siyao Du[1], Benjamin Horemans[1], Dirk Springael[1]**

[1] Division of Soil and Water management, Department of Earth and Environmental Sciences, Faculty of Bioscience Engineering, KU Leuven, Leuven, Belgium

** dirk.springael@kuleuven.be*

Interactions between microbes are of crucial importance in the well-functioning of microbial communities. Microbial organisms that are deliberately introduced into an environment to perform beneficial functions have to survive and establish, i.e., successfully invade, within the resident community of the target environment. Beneficial bacteria are for instance pollutant degrading specialist bacteria used in bioaugmentation for improving bioremediation of polluted ecosystems. Research at KU Leuven that aimed at understanding how bacterial invasion depends on the interactions between invader and resident community members within a bioaugmentation context, showed that various resident bacterial strains affected the establishment/functionality of a pesticide degrader in different ways. Some of the residents increased pollutant degradation while others had an negative effect. Moreover, several effects assigned at a particular resident strain were still at play at higher resident strain richness, i.e., when multiple residents were present. These observation are of importance for fundamentally understanding microbial invasion in an ecological perspective as well as from a biotechnological perspective e.g. for improving bioaugmentation. Especially, beneficial strains might be used as co-inoculants to improve invasion of the pollutant degrader. However, the underlying mechanisms of the interactions are not known and it remains unclear whether or not these interactions are universal for other invading species and in particular for other bacteria of interest for bioaugmentation. This research aims at answering these questions.

This research will be studied using *Aminobacter* sp. strain MSH1 that degrades the common groundwater micro-pollutant 2,6-dichlorobenzamide (BAM) and a resident community of ten DWTP sand filter isolates, as a model system. Ten resident strain were in previous work shown to affect, either positively or negatively, MSH1 biodegradation activity. Focus will be on resident strains that positively affect MSH1 activity, designated as benefactor strains. Different communities of MSH1 and resident strains with and without benefactor strains will be assembled and the effect of the assemblage and the presence of specific strains on MSH1 pesticide degrading activity will be determined as well as the effect on developing resident community composition in order to indicate resident member interactions. Techniques that will be used are flow cytometry and quantitative PCR for determining (specific) cell counts, UPLC-MS/MS analysis for determining BAM degradation activity, Illumina 16S rRNA gene amplicon sequencing and bio-informatical community composition/co-occurrence analysis to determine community composition and indicate interactions.

B07 - Occurrence of pesticides in groundwater in and around graveyards and pesticide biodegradation capacity of the corresponding top soils

Lana Geukens[1], Bram Verreydt[1], Benjamin Horemans[1], Jan Walstra[2], Bas van Wesemael[3], Vanessa Heyvaert[2], Marijke Huysmans[4], Cas Neyens[4], Bertrand Leterme[5], Diederik Jacques[5], Erik Smolders[1], Dirk Springael[1]*

[1]*Division of Soil and Water Management, KU Leuven, Leuven, Belgium*

[2]*Royal Belgian Institute of Natural Sciences, OD Earth & History of Life, Geological Survey of Belgium, Brussels, Belgium*

[3]*Earth and Life Institute, UCL, Louvain-la-Neuve, Belgium*

[4]*Department of Hydrology and Hydraulic Engineering, VUB, Brussels, Belgium*

[5]*Institute for Environment, Health and Safety, SCK-CEN, Mol, Belgium.*

[*]*dirk.springael@kuleuven.be*

In addition to their application on agricultural land, pesticides are or were used at various public places such as graveyards, but the impact of such activities on groundwater contamination has been less studied compared to agricultural land. In this context, a groundwater screening was performed at multiple graveyard sites in Flanders and Wallonia, for the occurrence of pesticide residues in the phreatic groundwater. The most detected compound (eight out of eight sites screened) was 2,6-dichlorobenzamide (BAM), a known transformation product of dichlobenil (DB), which is a herbicide commonly used for private purposes. DB was banned since 2009, but strongly sorbs onto soil particles and hence provides a long-term 'source' of the highly mobile metabolite BAM, which leaches to and contaminates the groundwater. Given that microbiota are known for their high adaptability as it comes to degradation of xenobiotic organic compounds (XOCs), such as pesticides, we hypothesise that the long-term application of DB in these public places might have resulted into the development of DB/BAM-degrading microbiota.

To study this, topsoil samples were taken in and around the examined graveyards at locations where DB had been applied. Currently, these soil samples are being examined for the presence of DB, their capacity to transform DB into BAM and their capacity to degrade/mineralise BAM.

B08 - Interactions between bacterial populations lead to an adjustment of their individual phenotypic diversities.

Jasmine Heyse[1], Ruben Props[1], Peter Rubbens[2], Benjamin Buysschaert[1], Willem Waegeman[2], Nico Boon[1].

[1] *Center for Microbial Technology and Ecology (CMET), Ghent University, Belgium*

[2] *KERMIT, Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Belgium*

Ecosystems are often characterized by their diversity. The classical approach to assess diversity is to inspect the genetic composition of a community. However, there is a broad level of diversity at an even finer scale: the phenotypic diversity. The phenotype of an organism is a combination of all its observable traits and is assumed to be related to the functionality of the organism. In literature, there is a growing interest in understanding how phenotypic diversity is manifesting itself and what its potential importance might be in both natural and engineered microbial ecosystems. Currently, our knowledge regarding factors that influence this phenotypic diversity is limited.

The goal of our study was to evaluate whether microbial interactions between sympatric bacterial populations can lead to changes in phenotypic diversity of the interacting populations. In order to characterize phenotypic diversity in a controlled environment, synthetic microbial communities were created in transwell systems using two drinking water isolates as model organisms. We applied two single-cell technologies, flow cytometry and Raman spectroscopy, to quantify the phenotypic diversity of the microbial populations. Our results show that interactions between sympatric bacterial populations lead to an adjustment of the phenotypic diversities of the individual populations. This finding opens a future for research on phenotypic diversity and microbial interactions. Moreover, the experimental set-up that was used in this study can be used to study both biotic and abiotic factors that might influence phenotypic diversity of microbes. This can be within the context of both engineered or natural ecosystems. We hypothesized that the individual phenotypic diversity of a genotype is a function of the other genotypes in the community and that this might be related to niche separation between the genotypes. Within the framework of our current study, this hypothesis can be further evaluated at higher genotypic richness.

B09 - *Bacillus weihenstephanensis* can readily improve its endospore heat resistance without compromising its psychrotolerant vegetative growth characteristics

Tom Dongmin Kim[1]°, Katrien Begyn[2]°, Fatima Taghlaoui[2], Marc Heyndrickx[3], Andrea Rajkovic[2], Frank Devlieghere[2], Chris Michiels[1] and Abram Aertsen[1]*

1 Laboratory of Food Microbiology, Department of Microbial and Molecular systems, Faculty of Bioscience Engineering, KU Leuven, Leuven, Belgium

2 Research unit Food Microbiology and Food Preservation (FMFP-UGent), Department of Food Technology, Food Safety and Health, Faculty Bioscience Engineering, Ghent University, Ghent, Belgium

3 Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) - Technology and Food Science Unit, Melle, Belgium & Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke,

[*]abram.aertsen@kuleuven.be

Proper elimination of bacterial endospores in foods and the food processing environment is of concern due to their extreme recalcitrance to various stresses. Often, sporicidal treatments are insufficient to eradicate all the existing endospores and might therefore serve as a selection pressure for improved endospore resistance. To address this possibility, we employed a directed evolution approach on *Bacillus weihenstephanensis* endospores with wet heat as a stressor, as it is one of the most widely used stresses for spore inactivation. The model organism *B. weihenstephanensis* is a psychrotrophic member of the *Bacillus cereus sensu lato* group, which is an important foodborne pathogen causing emetic or diarrheal type of intoxication. Three independent lineages of *B. weihenstephanensis* LMG 18989 endospores were iteratively subjected to sporicidal heat stress with intermittent germination and sporulation steps. After a number of such cycles, mutant clones isolated from each lineage were confirmed for their increased endospore heat resistance. Vegetative cells of these confirmed mutants were subsequently checked for their growth at 7°C, heat resistance at 50°C and toxin production. Increased spore heat resistance of mutants yielded 96- to 500-fold higher survival than the wild type at 97°C for 5 minutes. Nevertheless, despite this improved heat resistance at the endospore level, the isolated mutants were still perfectly able to grow at 7°C with comparable growth kinetics as the parental strain. Similarly, vegetative cell heat resistance of two selected isolates did not deviate from those of controls. While there was no difference between the wild type and the mutants in the level of non-hemolytic enterotoxin (Nhe), HBL enterotoxin was absent in 2/3 resistant mutants and increased in one of the remaining mutants. However, also 2/2 of the control lineages (i.e. those serially passaged in the absence of any stress) lacked HBL production, indicating an unexpected impact of serial passaging on toxin production that merits further detailed investigation. In conclusion, the fact that *B. weihenstephanensis* can readily improve its endospore heat resistance (and thus its survival in the food production chain) without compromising its psychrotolerant vegetative growth (and thus its proliferation under refrigerated temperatures) is an important concern for food safety.

° both authors contributed equally.

B10 - Identification of the endolysin encoded by Deep-Blue phage infecting *Bacillus weihenstephanensis*

Leprince Audrey[1], Hock Louise[1], Gillis Annika[1,2] and Mahillon Jacques[1]*

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

[*]Audrey.leprince@uclouvain.be

Since several years, there is a renewed interest in phages and their derived proteins for various applications. Among the 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 host cell 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 thus influencing the host spectrum, whereas the N-terminal part contains the Enzymatically Active Domain (EAD) that cleaves conserved bonds in the peptidoglycan. This study focuses on the endolysin encoded by Deep-Blue [1] phage infecting B. weihenstephanensis and belonging the Myoviridae family. In silico analyses revealed that Deep-Blue gp221 is a good endolysin candidate. This protein has a Peptidase_M15_4 conserved domain from the VanY superfamily as EAD, which is also present in the endolysins of phages B4 [2] (LysB4) and Phrodo [3] (PlyP56) infecting members of the Bacillus cereus group. As for the CBD, two SH3 domains were detected at the C terminus end of gp221. The putative endolysin was cloned and expressed to assess its lytic activity. Similarly the CDB was fused to an N-terminal GFP and expressed in order to evaluate its binding capacities. Gp221 has a broader spectrum than its related phage and showed lytic activities not only against B. weihenstephanensis but also against other members of the B. cereus group that cannot be infected by Deep-Blue phage. Additionally, gp221 CBD is able to bind the surface of several strains of the B. cereus group. Characterization studies of both endolysins, including host spectrum, pH, temperature and salt concentration for optimal activity will be presented and discussed.

[1] Hock L., Leprince A., Tournay M., Gillis A., Mahillon J. 2019. Food Control, in press.

[2] Son B., Yun J., Lim J. A., Shin H., Heu S., Ryu S. 2012. BMC Microbiol 12, 33.

[3] Etobayeva I., Linden S., Alem F., Harb L., Rizkalla L., et al. 2018. Viruses 10, 276.

B11 - Characterization of the molecular mechanisms controlling negative chemotaxis to Cu in *Caulobacter crescentus*

Gwennaëlle Louis, Pauline Cherry, Thomas Lamot and Jean-Yves Matroule

URBM – Narilis/ILEE, UNamur, rue de Bruxelles, 61, Namur, Belgium

Most of pathogenic, symbiotic and free-living bacteria are likely to be exposed to toxic concentrations of heavy metals, such as copper (Cu), requiring a rapid response to maintain their fitness. We have previously shown that the dimorphic alpha-proteobacterium *Caulobacter crescentus* uses a bi-modal strategy to cope with toxic Cu concentrations. The sessile stalked cell triggers Cu oxidation and efflux within a few minutes while the motile swarmer cell rapidly flees from the Cu source (Lawarée *et al.*, 2016).

The molecular mechanisms underlying bacterial chemotaxis upon heavy metals exposure have remained unknown so far. A high cellular Cu concentration is essential for *C. crescentus* negative chemotaxis, suggesting that a Cu sensory system monitors intracellular variations of Cu concentration. The preferential accumulation of Cu in the periplasm measured by inductively coupled plasma optical emission spectrometry (ICP-OES) in cell fractionates suggests that Cu sensing could occur in the periplasm. One could also expect a sensing of slight variations of Cu concentration in the cytoplasm that could provide a higher sensitivity in Cu detection.

A bioinformatics analysis reveals the presence of 19 chemoreceptors (MCPs – Methyl-accepting Chemotaxis Proteins) encoding genes in *C. crescentus* genome. Twelve of these MCPs are predicted to localize in the inner membrane and the remaining 7 MCPs are likely cytoplasmic. The invalidation of the 19 genes coupled to the analysis of Cu chemotaxis of the resulting single mutants by Live Chemotaxis Imaging (LCI) led us to isolate the Cu chemotaxis-defective *mcpR* mutant. A thorough characterization of this candidate is undergoing in order to determine whether McpR binds Cu or a Cu-loaded chaperone/transporter via its sensor domain.

Several lines of evidence support the idea that Cu chemotaxis may rely on the sensing of Cu-induced ROS. By using the rxYFP biosensor, we showed that Cu impacts the GSH/GS-SG balance, indicative of ROS production. In addition, a genetic screen of a transpositional mutants library realized in our lab identified a Cu sensitive mutant with an insertion in the superoxide dismutase encoding *sodA* gene. In order to verify the involvement of ROS in Cu chemotaxis, we are currently investigating the chemotactic behaviour of SOD and catalase knock-out and overexpressing strains. The potential implication of McpR in ROS sensing via its PAS domain will be assessed by exposing the *mcpR* mutant and a *mcpR PAS* mutant to the superoxide anion inducer paraquat.

In parallel to LCI, we also setup a Microfluidic CellASIC system to monitor bacterial chemotactic response at the single cell level. We can monitor many parameters like the swimming speed of the bacteria but also the traveled distance and the number of direction changes per bacteria. In presence of Cu, we could imagine that the bacteria increase their number of direction changes if Cu is sensed as a chemorepellent. Some preliminary data show that when bacteria are exposed to Cu, their number of direction changes doubles after 4 minutes. With this method, we will be able to compare the chemotactic behavior of naïve and Cu born swarmer cells in order to identify a potential long-term Cu memory.

B12 - Metallic copper rapidly inactivates the metal-resistant *Cupriavidus metallidurans*

Laurens Maertens[1,2*], *Ilse Coninx*[1], *Natalie Leys*[1], *Jean-Yves Matroule*[2] and *Rob Van Houdt*[1]

[1]*Microbiology Unit, SCK-CEN, Belgium*

[2]*URBM, UNamur, Belgium*

[*]*laurens.maertens@sckcen.be*

The disinfecting properties of copper have been recognized for centuries, which led to its recognition as the first solid antimicrobial material by the US Environmental Protection Agency in 2008. The ESA backed BIOFILMS project aims to use metallic copper surfaces with different surface topologies to combat biofilm formation aboard the International Space Station and future manned spaceflight.

We studied the effects of metallic copper on *Cupriavidus metallidurans* CH34, an extremely metal-resistant strain, and a species which has been isolated from different sources on-board the ISS. We opted for a "wet contact" approach to mimic the drinking water reservoirs aboard the ISS, where bacterial contaminations are persistently present. *C. metallidurans* CH34 cultures were washed and resuspended in sterile mineral water (OD_{600} of 0.1), and added to conical centrifuge tubes containing either a copper plate, a stainless steel plate, or no plate. This setup was placed on an orbital shaker at 30 °C. Viable counts and flow cytometry samples gave insight into the biological state of the planktonic cells. The biofilm formed on the metal plate was visualized with fluorescence microscopy after 9 days, which marked the end of the experiment.

In the no-plate control condition, no decrease in viable counts was observed. In addition, flow cytometry did not indicate an increase in the percentage of dead cells in the planktonic phase. In the stainless steel condition, a 2-log decrease in viable count was seen within 3 hours. However, there was again no increase in the percentage of dead cells, which could indicate a partitioning of cells from the liquid phase to the stainless steel plate. After 5 hours, viable counts increased again to reach a plateau around $2x10^7$ CFU/ml. In the copper plate condition, a near 5-log decrease of viable counts was seen within 5 hours. After 5 hours, viable counts increased to 10^6 CFU/ml at 48 hours, but then dropped steadily to reach 10^2 CFU/ml after 9 days. The initial decrease in viable counts was attributed to a killing effect of the copper plate, with the subsequent increase in viable counts possibly explained by bacterial growth on the nutrients of lysed cells and an activation of copper resistance mechanisms. The steady drop of viable counts during the remainder of the experiment could be ascribed to a lack of nutrients and toxic copper concentrations to maintain copper homeostasis, although this has yet to be confirmed experimentally. Flow cytometry confirmed the killing of cells by the copper plate, as the percentage of dead cells in the planktonic phase increased gradually throughout the experiment. Biofilms formed on the stainless steel plate contained much more biomass than biofilms formed on the copper plate, and in addition, this biomass consisted of a higher percentage of live cells, confirming the killing effect of the copper plate.

In conclusion, metallic copper seems to be efficient in killing even extremely metal-resistant bacteria, highlighting its value as antimicrobial surface. Follow-up research will focus on the copper-specific resistance mechanisms of *C. metallidurans* CH34 and their relevance in contact with metallic copper.

B13 - Single strain selection for bacterial biochemical production

Paul Matthay[1,2], Natalie Verstraeten[1,2], Jan Michiels[1,2]*

[1] *KU Leuven Center of Microbial and Plant Genetics, Leuven, Belgium.*

[2] *VIB-KU Leuven Center for Microbiology, Leuven, Belgium.*

[*] *paul.matthay@kuleuven.vib.be*

The global demand for petrochemicals is continuously rising. These chemical products derived from petroleum are used in the production of plastics, the manufacturing of fuels and the synthesis and purification of drugs. However, the use of petrochemicals comes with high environmental and health concerns. Burning petrochemicals as fuels, for example, leads to acid rain, air pollution and enhancement of the greenhouse effect. Combined with oil fields running dry, this illustrates the urgent need for sustainable alternatives. Oleochemicals (for example fatty acids) represent a green and imperishable alternative for petrochemicals with the possibility to be produced in a more continuous and sustainable way. Unfortunately, these days they are mainly produced though distillation of plant and animal oils, which is not sustainable in large-scale industrial settings. However, recent advances in genetic and metabolic engineering pave the way for bacterial biocatalysis to be used for sustainable, large-scale, industrial oleochemical production. In this case, relevant characteristics of the microorganism (such as high production rates) must be optimized. Since increased production of biochemicals is usually not advantageous to the microorganism, commonly used strain optimization strategies (based on selection of the fittest) cannot be used. Therefore, we propose to implement a revolutionary microbe optimization platform based on single amino acid changes in combination with automated selection of individual strains using picodroplet microfluidics.

To develop the rapid microbe optimization platform we will use the model organism *Escherichia coli* for which a plethora of genetic information and biotechnological tools are available. As a plus, *E. coli* can grow on a variety of feedstock and it easily reaches high cell densities. In our workflow, target enzymes with a known effect on the production of fatty acids will be selected based on rational arguments. Every codon in the encoding candidate genes will be replaced by all possible alternatives using state-of-the-art genome editing methods, which will result in large mutant libraries. Once constructed, the mutant libraries will be screened using an available picodroplet microfluidics setup to select mutants with increased fatty acid yield. This state-of-the-art screening approach is fast and high-throughput. As an added advantage, putative mutants with increased fatty acid yield are compartmentalized in individual droplets and have equal opportunities to grow, allowing to better maintain the diversity of mutations. By monitoring single cells in separate droplets and combining with a sensitive, fluorescence-based detection method for fatty acids, we will select on fatty acid production and not on cell growth. In a final step, mutations that increase fatty acid yield will be identified by sequencing and combined to create an optimized biocatalyst strain.

In addition to creating an optimal fatty acid biocatalyst, this project will result in the development of a rapid microbe optimization platform with a broad range of possible industrial applications. It therefore holds great promise to find sustainable alternatives for petrochemical approaches.

B14 - Investigation of the diversity and community composition of phyllosphere bacterial communities in urban parks across Europe.

Babette Muyshondt[1], Karen Wuyts[2], Wenke Smets [3], Anskje Van Mensel [4],Roeland Samson [5] , Sarah Lebeer [6]*

[1] *University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium Department and University, Belgium.*

[2] *University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium.*

[3] *University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium Department and University, Belgium.*

[4] *University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium*

[5] *University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium*

[6] *University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium*

[*] *e-mail of the corresponding author: babette.muyshondt@uantwerpen.be*

Urban green areas have been shown to support native biodiversity, enhance ecosystem functions and provide important ecosystem services. Moreover, urban green infrastructure contributes to human well-being. Nevertheless, urban areas are subject to several urban disturbance factors, as they are characterized by highly polluted air and often highly fragmented and isolated. These factors impact both abiotic and biotic conditions, and taxonomic and functional biodiversity tends to become more homogenous. In the framework of the Biodiversa project BIOVEINS, we investigated the diversity and community composition of phyllosphere bacteria on trees in urban green areas. Phyllosphere bacteria are known to influence plant health, performance and growth, but also human health through aerosolization to the atmosphere and degradation of air pollutants. Tree leaves were sampled in urban green patches in six cities throughout Europe, from Lisbon to Tartu, in summer 2018. Care was taken that samples from patches in a range of size and structural connectivity were included. Leaves were washed and further processed for DNA extraction and DNA sequencing based on the 16S gene. In addition, biomagnetic analysis were performed to assess the particulate matter exposure and leaf characteristics such as wettability and nutrient leaching were determined. Currently, samples are being processed and data analysed in order to evaluate the effect of urban stressors on leaf bacterial communities.

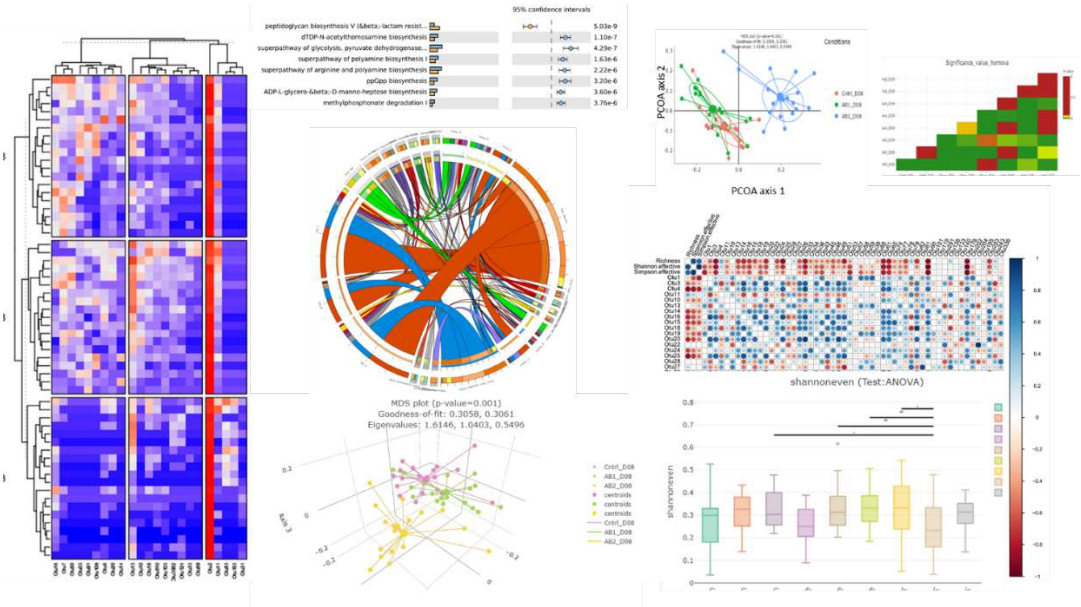
B15 - OCToPUS pipeline: from reads to diversity analysis.

Mohamed Mysara[1], *Natalie Leys*[1], *and Pieter Monsieurs*[1]

[1] *Unit of Microbiology, Belgian Nuclear Research Centre (SCK-CEN), Mol, Belgium.*

Bacteria plays a vital role in industry, from the production of traditional foods such as yoghurt, cheese, and vinegar; to the production substances such as drugs and vitamins. They also affect the human health, being involved in the immune responses and food digestion and contributing to the pathogenicity of various diseases. Several important aspects of bacteria in various environments are poorly understood, making the identification and monitoring microbial communities of utter importance to the industry and patient care. Recent developments in new high-throughput sequencing technologies have revolutionized molecular biology, including the study of microorganisms without the need for culturing them in the lab, an approach often referred to as **metagenomics**. Such **metagenomics** applications allow the simultaneous high-throughput analysis of genetic material of most of the microbes present in a given sample, without the need for culturing the bacteria first. Although this approach has nowadays been adopted in many project, it is far from straightforward necessitating various **bioinformatics** optimization.

In this work, we aimed at optimizing and standardizing the analysis of 16S metagenomics with a final aim to develop a pipeline to starts from the raw sequencing reads and deliver their microbial composition. Therefore, different tools were developed to deal with chimera (Mysara, Saeys, *et al.*, 2015) and sequencing errors (Mysara, Leys, *et al.*, 2015; Mysara *et al.*, 2016), each of them found to outperform the other existing state-of-the-art tools. Additionally, a new method was introduced to bring closer correspondence between the number of microorganisms detected and the actual diversity within the samples (Mysara, Vandamme, *et al.*, 2017). A one stop-shop software, named OCToPUS, assembles these various algorithms, thereby leading to a highly accurate assessment of microbial diversity starting from the raw sequencing reads (Mysara, Njima, *et al.*, 2017). These tailored algorithms have already been successfully applied to assess complex microbial communities in a wide range of environments such as deep subsurface geological clay formations (**HADES, Boom clay, Belgium; and Mont Terri**, Opalinus clay Swiss, Moors *et al.* 2013), the human gut microbiome after radiotherapy treatment, and **cooling water circuits of nuclear reactors** (Props *et al.*, 2016), as such revealing microbial diversity at **unseen depth**.



B16 - Microbial Communities in the Metalliferous Soil from Auby (France)

Haixia Pan, Ruddy Wattiez and D. C. Gillan

Laboratory of Proteomics and Microbiology, Mons, Belgium

The aim of the present study was to investigate a rhizosphere microbial community in a metal-contaminated soil using shotgun metagenomics in order to highlight the most important metal resistance systems. The selected soil, located in Auby (North of France), is dominated by plant species such as *Arabidopsis halleri* and *Armeria maritima* and has been contaminated by Zn, Cd, Cu and Pb since the arrival of the "Compagnie Royale Asturienne des Mines" (today the Nyrstar-Umicore plant) in 1868. For more than 150 years, with the development of the plant, heavy-metal-rich dusts from ore processing were deposited in the soils of the sector. Today the Auby soil features up to 40 000 mg kg^{-1} of Zn, one of the highest value known for Zn in natural soils. A total of 33.10^6 reads of 252 ± 32 pb were obtained by the Illumina sequencing technology. After analysis by the MG-RAST platform (M5NR database; alignment length of 15bp; e-value: e-5) results indicate that 7 bacterial genera represent 25% of the reads. These genera are Candidatus *Solibacter*, a member of the Acidobacteria (9.4% of the reads), followed by *Gemmatimonas* (4.6% of the reads), *Streptomyces* (2.9%), *Chitinophaga* (2.7%), *Nitrospira* (2.0%), *Burkholderia* (1.9%) and *Conexibacter* (1.9%). The most abundant metal resistant systems were three-components efflux pumps like CzcCBA (67 061 reads) and cation diffusion facilitators like CzcD. Most of these zinc resistance systems were produced by Candidatus *Solibacter* (12329 reads), *Pseudomonas* (5703 reads) and *Rhodopseudomonas* (5122 reads).

B17 - Genetic and phenotypic characterization of multidrug-resistant clinical isolates of *Acinetobacter baumannii*.

Chantal Philippe [1], *Mona Bové* [2], *Pierre Bogaerts* [3], *Te-din Huang* [3], *Tom Coenye* [2], *Gérald Glupczynski* [3], *Xavier De Bolle* [1] & *Charles Van der Henst* [1,4*]

[1] *Research Unit in Biology of Microorganisms (URBM), Narilis University of Namur (UNamur), Namur, Belgium*
[2] *Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium*
[3] *Belgian National Reference Laboratory for Monitoring of Antimicrobial Resistance in Gram-Negative Bacteria, CHU UCL Namur-Site Mont Godinne, Yvoir, Belgium*
[4] *VIB-VUB Center for Structural Biology – Brussels, Belgium*
[*] *charles.vanderhenst@unamur.be*

The opportunistic pathogen *Acinetobacter baumannii* represents a severe public health threat due to its impressive resistance arsenal to the last resort antibiotics and to harsh conditions such as desiccation, disinfectants and the human immune system. Despite an established clinical relevance, the pathogenicity of this biosafety level 2 bacterium remains poorly understood.

In order to study its virulence and stress resistance potential, few reference strains are commonly used worldwide, yet this bacterium is known to be highly heterogeneous amongst different isolates. The aim of this project is to compare the reference strains with modern clinical isolates. Phenotypic characterization strongly suggests that capsule production levels are heterogeneous amongst the modern clinical isolates, which is not the case for the reference strains. Infections using the *Galleria mellonella* multicellular model show that the virulence potential of the modern clinical isolates is very heterogeneous, ranging from an avirulent background to highly virulent isolates. However, using the amoeba *Acanthamoeba castellanii* as a phagocytic cellular model, we show that the majority of the *A. baumannii* strains resists predation by producing a mucoid phenotype.

This project shows that to study specific aspects of *A. baumannii* virulence and resistance arsenal, the reference strains are not representative of the diversity amongst the current clinical isolates. In addition, the amoeba *A. castellanii* represents a promising cellular model to better understand *A. baumannii*. Comparative genomics approaches based on phenotypic clustering are currently undertaken to decipher the molecular mechanisms governing the resistances and the virulence arsenal of *A. baumannii*.

B18 - Phenoflow : an R package for the flow cytometric characterization of natural and synthetic microbial communities

Props Ruben[1,3], Kerckhof F-M[1], Rubbens P[4], Monsieurs P[2], Waegeman W[4], Denef V[3], Boon N[1]*

[1] Center for Microbial Ecology and Technology (CMET), Ghent University, Ghent, 9000, Belgium

[2] Unit Health, Flemish Institute for Technological Research (VITO), Mol, Belgium

[3] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, 48109, USA;

[4] KERMIT, Department of Data Analysis and Mathematical Modelling, Ghent University, Gent, 9000, Belgium

[*]Nico.Boon@UGent.be

Background. Characterizing microbial community structure (e.g., diversity) in natural, engineered and synthetic environments is important for ecosystem monitoring, (bio-)process performance, and hypothesis testing. Although conventional assays by means of molecular techniques have resulted in major advances, these procedures remain slow, labor-intensive and susceptible to multiple sources of laboratory and data processing bias. Growing interest in highly resolved temporal surveys of microbial community structure necessitate rapid, inexpensive and robust analytical platforms that require limited computational effort.

Here we introduce ***Phenoflow***, an R-based toolbox for the advanced analysis of microbial flow cytometry data. ***Phenoflow*** contains:

- unsupervised computational methods for estimating microbial diversity based on sensitive single-cell measurements of phenotypic attributes;
- supervised computational methods for retrieving the community composition of low-complexity synthetic microbial communities.

Both were developed to allow fast first-line assessments of microbial community dynamics (i.e. within minutes) without demanding extensive sample preparation and downstream data processing.

Methods & Results. For estimating the diversity of environmental microbial communities we developed a data processing pipeline that fits bivariate kernel density functions to phenotypic parameter combinations of nucleic-acid stained microbial community data and concatenates them into a one-dimensional phenotypic fingerprint . By calculating established diversity metrics (i.e. Hill numbers) from such phenotypic fingerprints, we construct an alternative interpretation of the microbial diversity that incorporates distinct phenotypic traits underlying cell-to-cell heterogeneity (i.e. morphology and nucleic acid content). Validation through surveys of various engineered and natural aquatic ecosystems demonstrated that our approach delivered diversity profiles that are strongly correlated with the reference diversity, as estimated by 16S rRNA gene amplicon sequencing. For synthetic microbial communities such as laboratory cocultures, a Random Forest wrapper was implemented for classifying individual cells to strain labels. Using this approach, the composition of low-complexity synthetic microbial communities (< 6 strains) could be retrieved with accuracies >80%. In addition, this approach aids in the experimental design of synthetic ecosystem studies as the workflow allows determining communities for which the constituent strains can be optimally detected.

Conclusions. Phenoflow provides (i) the first computational approach for estimating diversity proxies, and (ii) the first supervised approach for resolving community composition of synthetic microbial communities, based on flow cytometry data. This allows a fast, robust and low-cost analysis workflow for monitoring the microbial community structure of natural, engineered and synthetic ecosystems. Furthermore, our approach offers perspectives for the development of online and in situ monitoring systems for aquatic ecosystems.

B19 - Plant immunization by the *Bacillus* lipopeptide surfactin: insights into the mechanistics of perception at the plasma membrane

Jelena Pršić[1], Marc Ongena[1], Magali Deleu[2], Anthony Arguelles[1], Thibault Meyer[1]*

[1]University of Liège, Faculty Gembloux Agro-Bio Tech, Research Unit Microbial Processes and Interactions (MiPI), Bâtiment TERRA, Avenue de la Faculté 2B, B-5030 Gembloux, Belgium

[2]University of Liège, Faculty Gembloux Agro-Bio Tech, Laboratory of Molecular Biophysics at Interfaces (LBMI), Passage des Déportés, 2-5030 Gembloux, Belgium

[*]jelena.prsic@student.uliege.be

Some *Bacillus* species are well known and widely studied as plant growth promoting and biocontrol agents. Main way for these bacteria to act is through their secondary metabolites among which cyclic lipopeptides (CLPs) are important. Not only that CLPs act as antibacterial and antifungal substances but also, they can activate induced systemic resistance (ISR) in plants. In the case of ISR, the CLP surfactin has a leading role since it is the only compound formed by the *Bacillus* strains tested retaining elicitor potential on various plants species. However, the exact way this lipopeptide acts as plant immunity elicitor is still not clear. Previous studies have shown that surfactin perception by plant cells is not mediated via pattern-recognition receptors but rather that it interacts with the lipid phase of the plasma membrane (PM) composed of phosphoglycerolipids, sphingolipids and sterols. Our biophysical data indicate that the structure- and dose-dependent activity of surfactin relies on a quite specific interaction with sphingolipid-enriched domains in the outer leaflet of the PM with an impact on lipid organization and PM structural and rheological properties. This is supported by functional assays showing that ROS-inducing activity of the lipopeptide is significantly lower on mutants of *Arabidopsis* affected in their content in glucosylceramide-type sphingolipids. According to the priming concept, SF does not directly trigger a strong defensive response in the treated tissues nor causes fitness cost but gently prepare the plant for mounting a robust defense once the pathogen is perceived.

B20 - Multinuclearity as a fitness advantage in *Acanthamoeba castellanii*

Théo Quinet[1], Corry Charlier[2], Karine Van Doninck[1] & Charles Van der Henst[1, 3, 4]*

[1]*Laboratory of Evolutionary Genetics and Ecology, UNamur, Belgium.*

[2]*Electronic microscopy service, UNamur, Belgium.*

[3]*Research Unit in Biology of Microorganisms, UNamur, Belgium.*

[4]*Center for Structural Biology, VIB-VUB, Belgium.*

[*] *charles.vanderhenst@unamur.be*

Multinuclearity is a spread phenomenon across the living world. It is frequently associated with a variety of advantages. In this study, we decided to investigate multinuclearity in amoebae with the infection model *Acanthamoeba castellanii* that could be an important vector of diseases but still remains poorly characterized. We observed that amoebae under agitation are giant multinucleated cells. Those cells can solve their multinuclearity by a non-mitotic like process when put at rest with no agitation. The progeny of those multinucleated cells is more numerous than uninucleated amoeba. We propose multinuclearity as a fitness advantage to colonize new environments, which is biologically relevant regarding the lifestyle conditions of *Acanthamoeba castellanii*.

B21 - *Aminobacter* sp. MSH1, an excellent candidate for BAM removal in DWTPs: unraveling its degradation pathway

Bart Raes[1], Benjamin Horemans[1], Daniel Rentsch[2], Jeroen T'Syen[1], Maarten G. K. Ghequire[3], René De Mot[3], Ruddy Wattiez[4], Hans-Peter E. Kohler[5], Dirk Springael[1]*

[1] Division of Soil and Water management, Department of Earth and Environmental Sciences, Faculty of Bioscience Engineering, KU Leuven, Leuven, Belgium

[2] Laboratory for Functional Polymers, Empa, Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland

[3] Centre of Microbial and Plant Genetics, KU Leuven, Leuven, Belgium

[4] Department of Proteomics and Microbiology, University of Mons, Mons, Belgium

[5] Department of Environmental microbiology, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

Groundwater in various European countries is often contaminated with the extremely recalcitrant pesticide metabolite 2,6-dichlorobenzamide (BAM). BAM is frequently detected above the EU-limit of 0.1 µg/L and as a consequence, groundwater extraction wells are closed or expensive treatment steps are included in drinking water treatment plants (DWTPs) for pollutant removal (e.g. activated carbon filtration). Bioremediation is considered as a sustainable alternative method for BAM removal. The BAM-degrading *Aminobacter sp.* MSH1 is a promising candidate for bioaugmentation in DWTPs, i.e., by their inoculation in dedicated modules of the DWTPs.

In order to use strain MSH1 for BAM removal in DWTPs it is essential to elucidate the BAM degradation pathway. Currently, only the first step in the degradation pathway is known. BAM is converted into 2,6-dichlorobenzoic acid (DCBA) by an amidase enzyme. However, it is unclear how further metabolization occurs.

In this work, we tried to unravel the downstream pathway after the formation of DCBA. Mutants of strain MSH1 that lack part of the degradation pathway were isolated and used in degradation experiments of DCBA, in order to identify DCBA degradation products by means of UPLC-MS/MS and NMR. As such, five new metabolites were identified. In addition, various constructs of strain MSH1 were made that lack all but one enzyme putatively involved in the downstream pathway. As such, attempts were made to investigate each of the enzymes function in the degradation pathway by using them in conversion experiments of DCBA and the newly identified metabolites.

1. Ellegaard-Jensen, L., et al., *Groundwater contamination with 2,6-dichlorobenzamide (BAM) and perspectives for its microbial removal.* Applied Microbiology and Biotechnology, 2017. **101**(13): p. 5235-5245.
2. T'Syen, J., et al., *Identification of the amidase BbdA that initiates biodegradation of the groundwater micropollutant 2,6-dichlorobenzamide (BAM) in Aminobacter sp. MSH1.* Environmental Science & Technology, 2015.

B22 - Spontaneous mutation in the sensor kinase *czcS2* of the bacterium *Cupriavidus metallidurans* results in increased resistance to uranium

*Rogiers Tom[1,2] *; Giménez Del Rey Francisco Javier[3], Williamson Adam[2]; Leys Natalie[1]; Van Houdt Rob[1]; Boon Nico[2]; Mijnendonckx Kristel[1]*

[1]*Microbiology Unit, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium*

[2]*Centre for Microbial Ecology and Technology, UGent, Ghent, Belgium*

[3]*University of Granada, Granada, Spain*

**tom.rogiers@sckcen.be*

The bacterium *Cupriavidus metallidurans* is mostly isolated from industrial sites linked to mining, metallurgic and chemical industries, and is a well-known model organism for metal resistance. The interaction of *C. metallidurans* with uranium (U^{238}) has been demonstrated, however, neither the genes or proteins involved nor the precise mechanism is known. The goal of this study is to unravel the molecular uranium resistance mechanisms in the strain *Cupriavidus metallidurans* NA4.

Via a laboratory evolution experiment, a spontaneous *C. metallidurans* NA4 uranium resistant mutant NA4U was obtained. This mutant strain resists up to 1000 µM of $UO_2(NO_3)_2$, while the wild type strain can only resist up to 125 µM. When *C. metallidurans* NA4U was grown in the presence of 250 µM $UO_2(NO_3)_2$, it retained 98% to its biomass after 24 hours. On the other hand, NA4U was unable to retain uranium from the medium when carbonyl cyanide m-chlorophenyl hydrazone (CCCP) was used to block its electron transport chain. This confirms that *C. metallidurans* NA4U is using an active mechanism to remove $UO_2(NO_3)_2$ from the supernatant. Furthermore, TEM HAADF microscopy combined with EDX analysis revealed that uranium was located at the NA4U cells, more specifically in the periplasm and at the cell membrane. Also, a correlation with phosphorous was seen, suggesting that NA4U is immobilising uranium in the form of uranium phosphate deposits.

To determine the genetic circuit underlying this resistance phenotype, whole genome sequencing of NA4U was performed. This revealed a 999 bp deletion in *czcS2*, coding for a sensor histidine kinase of the two-component system *czcR2S2*, inactivating CzcS2. To investigate the role of this mutation in the uranium resistance phenotype, an insertional deletion mutant of *czcS2* was made in the parental strain NA4. The minimal inhibitory concentration (MIC) for uranium of NA4Δ*czcS2* increased from 250µM to 1mM, demonstrating that the mutation in *czcS2* is sufficient to increase the uranium resistance potential of *C. metallidurans* NA4. Moreover, TEM HAADF microscopy combined with EDX analysis revealed uranium phosphate deposits after growing NA4Δ*czcS2* in the presence of 250 µM $UO_2(NO_3)_2$, confirming that this mutation results in a similar resistance mechanism.

Furthermore, whole transcriptome sequencing of NA4U compared to the parental strain in basic growth conditions indicated the constitutive upregulation of the response regulator *czcR2* in NA4U, suggesting an important role for CzcR2 in the resistance mechanism. This was confirmed by an insertional deletion mutant of *czcR2S2* in NA4U, which showed a decrease in the MIC for uranium to 500 µM. However, as the uranium resistance of NA4UΔ*czcR2S2* did not decrease to that of the parental strain, it suggests that other regulatory mechanisms might be at play. Indeed, possible cross-regulation of CzcR2 with other response regulators *agrR* and *copR2*, which are also constitutively upregulated in NA4U, was confirmed. The role of these response regulators is still under investigation.

Our study demonstrates that in the bacterium *C. metallidurans* a single mutation results in an increased resistance to uranium, showing the adaptation potential of this bacterium to toxic metals. Moreover, this research helps in assessing the microbial impact on the long-term behaviour of radionuclides in contaminated environments. In addition, the acquired knowledge could be used to determine and enhance bioremediation potential.

B23 - *manta* - a clustering algorithm for weighted ecological networks

Lisa Röttjers[1], *Karoline Faust*[1]*

[1]*Laboratory of Molecular Bacteriology, KU Leuven, Belgium*

[*]*Karoline.faust@kuleuven.be*

Microbial network inference and analysis has become a successful approach to generate biological hypotheses from microbial sequencing data. Network clustering is a crucial step in this analysis. Here, we present a novel heuristic flow-based network clustering algorithm, which equals or outperforms existing algorithms on noise-free synthetic data and performs well when a large percentage of the data are shuffled. *manta* comes with unique strengths such as the ability to identify nodes that do not strictly belong to a cluster, to exploit negative edges and to assess the robustness of cluster membership. We demonstrate in two case studies how these properties help to gain a better understanding of the microbial community under study. *manta* does not require parameter tuning, is straightforward to install and run, and can easily be combined with existing microbial network inference tools. We therefore expect it to be useful in a wide range of microbial network applications.

B24 - A planetary microbial brain that operates on the Earth climate system

Sergio Rubin[1]

[1]*Georges Lemaître Centre for Earth and Climate Research, Earth and Life Institute, University catholique de Louvain, Belgium.*

[*]*sergio.rubin@uclouvain.be*

This work present the hypothesis of the existence of a single planetary microbial network operating on the Earth climate system. The present hypothesis is termed the 'planetary microbial brain' because of certain similarities with the large-scale neural connectivity and integration for cognitive operations. The formulation is almost entirely speculative and should therefore be regarded, at least initially, primarily as a pedagogical way for systematizing a number of facts about Earth climate dynamics and the microbial world acting on the the planetary-scale. The hypothesis put forward might also prove useful for suggesting new experimental approaches to understanding sophisticated planetary microbial coordinated action. Microbial metabolism for the past four billions of years ago plays a central role in primary production, biogeochemical cycles and geological processes that support and modulates the Earth's climate dynamics and the geophysilogical bounded habitable system. Microbes are everywhere, the so-called Gaia microbiome, they cannot live out of communities, in isolation, unless extremely artifitial medium cultures are set up. They reach anticipative and coordinated actions not only by quorum sensing and horizontal gene transfer, but the can communicate by non-chemical and non-contact cell-to-cell means; by electric, electromagnetic, waves and by photonic means. All this open the possibility that non exergonic-endergonic sophisticated microbial brain-like coordinated actions may have an impact on macro-diversity and on climate stability and tipping points on fast timescales. At least, microbial activity in general, and specifically in this way of brain-like action is underappreciated in the current climate models. This hypothesis may aslo contribute to the recent biological developments in Gaia theory on which the Earth climate system is regarded as making anticipative active inferences about flucntuation of sun and cosmic radiation to resist dissipation and persist alive.

B25 - Untargeted metabolomics reveals the metabolic profile of *Bifidobacterium bifidum* in biofilm and planktonic states

Faizan Ahmed Sadiq[*]*, Wei Chen, Hao Zhang, Jianxin Zhao*

School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China

** faizan_nri@yahoo.co.uk* **Section:** Applied & Environmental Microbiology section

Abstract

The human gut harbours trillions of microbes which outnumber the host body cells and play important roles in immunity, physiology, and metabolism of the host. Mounting evidence suggests that many gut microbial strains reside in the form of biofilms on the surface of mucosa or epithelial cells. *Bifidobacterium bifidum* strains are believed to the first colonisers of the human gut particularly during the first two years of breast-fed infants and are considered of great importance because of their purported health-promoting effects. *B. bifidum* strains have been reported to form biofilms on mucosa, epithelial cells, and food residues in the gut lumen. This is the first study which provided metabolic insights into *B. bifidum* biofilm and planktonic states using untargeted metabolomics. The biofilm was formed on a mucin-coated microtiter plate and scrapped after 24 h incubation. The two states were clearly distinguishable by principal component analysis (PCA). A total of 173 metabolites of *B. bifidum* were differentially expressed in the biofilm state compared with planktonic cells. However, out of these 173 metabolites, 49 metabolites showed $\log_2$ fold changes of ≥ 1 in the planktonic state compared with biofilms, and similarly only 17 metabolites were significantly ($P < 0.05$) more expressed with $\log_2$ fold changes of ≥ 1 in biofilms as compared with planktonic cells. Using pathway analysis, it was found that aminoacyl-tRNA biosynthesis, alanine, aspartate and glutamate metabolism, nitrogen metabolism, citrate cycle (TCA), and arginine and proline metabolism were among the five most affected metabolic pathways that were down regulated in biofilms compared with planktonic cells. Among amino acids, L-histidine, L-alanine, and D-methionine were significantly more expressed, with log2 fold changes of ≥ 1, in biofilms compared to planktonic cells. Nucleotides (adenine, guanine, thymine, and uracil) were prevalent in biofilms as compared to planktonic cells. Similarly, poly-N-acetylglucosamine, an exopolysaccharide which serves as a major component of biofilm matrix, was more expressed in biofilms (log2 fold change of 4). These comprehensive insights emphasise the fact that metabolic claims of the probiotic *B. bifidum* strains should be state-dependent.

Key words: Metabolomics, Gut bacteria, Biofilms, Bifidobacteria, Probiotics

B26 - MICROBIAN: Microbiome diversity and function in the Sør Rondane Mountains, East Antarctica

Valentina Savaglia [1,3], *Sam Lambrechts[2]*, *Bjorn Tygtat[3]*, *Elie Verleyen[3]*, *Anne Willems [2]*, *Wim Vyverman[3]*, *Annick Wilmotte[1]*

[1]*InBioS-Centre for Protein Engineering, University of Liège, 4000 Liège, Belgium,*

[2]*Laboratory of Protistology & Aquatic Ecology, University of Ghent, 9000 Ghent, Belgium*

[3]*Laboratory of Microbiology, University of Ghent, 9000 Ghent, Belgium*

[*]*valentina.savaglia@uliege.be*

The scarce ice-free areas in Antarctica are among the most extreme terrestrial environments on. Here, life is dominated by microbes in strongly truncated foodwebs. Inland nunataks in East Antarctica, like the Sør Rondane Mountains (SRM), are far less studied than those in more coastal locations and in the McMurdo Dry Valleys. This is surprising given their long-term exposure and their potential role as ice-free refugia during Neogene and Pleistocene glacial maxima. The SRM encompass a large range of terrestrial habitats differing in geology and soil characteristics, exposure time and microclimatic conditions. Within the BelSPO funded project MICROBIAN, two field campaigns have been carried out during the Austral summers of 2018 and 2019 and another one is planned in January-February 2020. A total of ~ 300 samples have been collected for microbiome diversity analysis in different nunataks 200 km around the Princess Elisabeth Station. During the first MICROBIAN campaign, a total of 126 samples were collected along gradients in bedrock type, moisture availability, exposure and microclimatic conditions. The samples ranged from barren bedrock to substrates covered by biofilms and well-developed biological soil crusts consisting of lichens, mosses and microalgal mats. In addition, Open Top Chambers and Snow Fences were installed to mimick the impacts of future climate warming, and the baseline samples were collected. These samples are the object of a preliminary study in which the bacterial diversity was characterized by amplicon sequencing using general primers targeting the 16S rRNA gene and the Illumina MiSeq platform (2x300 bp). Datasets were subsequentially processed using a well-established in-house bioinformatics pipeline. Principal coordinate analysis (PCoA) plot based on Bray-Curtis similarities of Hellinger (square root) transformed OTU abundance data evenly subsampled to 5000 reads was used to statistically analyse the resulting OTU dataset. Based on this preliminary study conducted over more than 100 samples, the type of bedrock seems to be one of the most important abiotic factors that shape the diversity of bacteria. Additional analyses will be carried out on environmental parameters of the sampled soils, and on data from the new field campaigns in order to contribute to the understanding of the Antarctic terrestrial microbial ecology.

B27 - Bacterial colonization on weathered monuments: a case study on Lede Stone

Laurenz Schröer[1,2], Tim De Kock[1], Nico Boon[2] and Veerle Cnudde[1]*

[1] Ghent University, PProGRess-UGCT, Department of Geology, Ghent, Belgium
[2] Ghent University, Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Ghent, Belgium
[*]Laurenz.Schroer@UGent.be

Belgian city centers consist of important heritage made of natural building stones. These stones are oligotrophic and exposed to intense conditions such as extreme temperature variations, desiccation, UV radiation and pollution. This makes it an extreme habitat for organisms to thrive but bacteria and other microorganisms still colonize these building stones, causing biodeterioration. On top of that, our monuments undergo further forms of chemical and physical deterioration. Air pollution is one of the main actors, leading for example to the typical gypsum crusts in limestones. Little is known about the microbial habitat and the impact of bacteria on specific forms of weathering such as the gypsum crusts. Therefore, the outer layer of weathered Lede stone, a sandy limestone has been sampled at two historic monuments: one in the polluted urban environment (City hall, Ghent, Belgium) and one on the countryside (Castle of Berlare, Belgium). The stones of Ghent contain thick botryoidal gypsum crusts while the crusts in Berlare are thin. DNA extraction, 16S rRNA gene amplification followed by Illumina Mi-Seq Next Generation Sequencing (NGS) revealed several extremophiles. High abundances of Rubrobacter, Deinococcus-Thermus and Thermomicrobiales in several samples indicate a high tolerance for radiation and high temperatures. The isolation campaign in Ghent and Berlare captured many of the dominating genera such as Arthrobacter, Blastococcus and Noviherbaspirillum. Some of them are adapted to extreme cold temperatures. Culture dependent and independent techniques revealed in both localities a variable, diverse, but similar microbial community. It did not identify a significant impact of pollution and the gypsum crusts. The isolates verify the NGS data and next steps will include lab-based experiments to test their influence on stone degradation and potential gypsum crust formation.

B28 - *In vitro* activity of *Lactobacillus* isolates from Belgian women against Group B Streptococcus: mechanisms of action and strain-specific differences

Spacova Irina[1], Gasper Melanie[2], Oerlemans Eline[1], Armistead Blair[2], Brokaw Alyssa[2], Rajagopal Lakshmi[2,3], Jaspan Heather[2,3], Lebeer Sarah[1]*

[1]*Department of Bioscience Engineering, University of Antwerp, Belgium*

[2]*Center for Global Infectious Disease Research, Seattle Children's Research Institute, USA*

[3]*Department of Global Health, University of Washington, USA*

[*]*sarah.lebeer@uantwerpen.be*

Background: Rectovaginal colonization with Group B Streptococcus (GBS, *Streptococcus agalactiae*) poses a major threat for successful pregnancies and for the health of neonates. Recent microbiome and *in vitro* data strongly suggest that lactobacilli inhabiting the female genital tract can influence GBS colonization in a species- and strain-specific manner.

Objective: In this study, we explore the GBS inhibition properties of several *Lactobacillus* strains isolated from Belgian women with different health statuses. Data were compared to those obtained using commercial probiotic and certain representative vaginal *Lactobacillus* strains.

Methods: Eight *Lactobacillus* strains were tested for inhibition using the GBS strain COH1. Four vaginal *Lactobacillus* isolates were obtained from Belgian women with different health status. The other four isolates were *Lactobacillus rhamnosus* GG and *L. rhamnosus* GR-1 (representative strains commercially available as probiotics), and *L. crispatus* JV-V01 and *L. iners* UPII 143-D (representative inhabitants of the female genital tract). Anti-GBS properties of lactobacilli were evaluated by the following methods: 1. Time-course GBS growth inhibition assays using live lactobacilli or *Lactobacillus* supernatants (either native, pH-adjusted, proteinase K-treated, fractionated +/-3 kDa); 2. Adhesion and adhesion competition assays with GBS and human vaginal epithelial cells (hVEC); 3. Fluorescence and/or light microscopy with fluorescent GBS and *Lactobacillus* strains on hVEC.

Results: The tested *Lactobacillus* isolates and their supernatants showed varying degrees of GBS growth and adhesion inhibition. Less pronounced GBS growth and/or adhesion inhibition was demonstrated by two *Lactobacillus* isolates from women with aerobic vaginitis and bacterial vaginosis, as well as *L. crispatus* JV-V01 and *L. iners* UPII 143-D. The degree of GBS growth reduction correlated with *Lactobacillus* supernatant acidification and was affected by pH adjustment, while supernatant treatment with proteinase K or fractionation did not significantly influence its anti-GBS activity. *Lactobacillus* AMB-V16 isolated from a healthy Belgian woman, and commercial probiotics *L. rhamnosus* GG and *L. rhamnosus* GR-1 showed the most efficient GBS growth inhibition and media acidification. These strains also adhered well to hVEC and significantly diminished adhesion of live GBS to hVEC. Adhesion properties of lactobacilli and the influence of the best performing *Lactobacillus* strains on GBS adhesion were confirmed by light and/or fluorescence microscopy.

Conclusion: Vaginal *Lactobacillus* strains differentially affect GBS growth and adhesion *in vitro*, which might have important consequences for GBS colonization of the vaginal niche and its prevention. Acidification of the surrounding environment by lactobacilli was confirmed to be the major factor contributing to GBS growth inhibition, and novel strain-specific insights into *Lactobacillus* mechanisms of action against GBS were obtained. Currently, immunological effects of simultaneous GBS and *Lactobacillus* application to hVEC are being explored to confirm the potential beneficial effects of selected vaginal *Lactobacillus* strains on host cells.

B29 - Genetic Tool Development For Sulfolobus acidocaldarius, a Thermoacidophilic Archaeon

David Sybers[1], *Janne Swinnen*[1], *Eveline Peeters*[1]

1. Research group of Microbiology, Faculty of Sciences, Vrije Universiteit Brussel, Brussels, Belgium

Extremophilic microorganisms have always been considered to have a large industrial potential. They allow the implementation of microbial fermentations in extreme conditions that are compatible with harsh industrial processes. However, limited biological knowledge and a lack of engineering tools hamper their further development into industrial platform organisms.

Here, we expand the genetic toolbox for the thermoacidophilic archaeon *Sulfolobus acidocaldarius*, which grows optimally at 75°C and pH 2-3. The current toolbox contains a basic *E. coli – Sulfolobus* shuttle vector based on a native cryptic plasmid, a uracil-based auxotrophic selection system, a thermostable beta-galactosidase as a reporter and several inducible promoters including a maltose-inducible promoter. Despite the availability of these valuable tools, genetic engineering of *S. acidocaldarius* still encounters several roadblocks. These include low efficiencies in transformation and genome modification, background growth when selecting for uracil auxotrophy and unstable plasmid retention over longer periods of time. In order to lift some of these roadblocks, we focus on developing a modular *E. coli- Sulfolobus* shuttle vector repertoire with well-characterized and optimized modules, including optimized replication modules and novel selection modules for use in *Sulfolobus* spp. Additionally, the vector size is minimized and the transformation protocol optimized in order to increase transformation efficiencies.

The expansion of the genetic toolbox available for *Sulfolobus* spp. will facilitate the use of state-of-the-art metabolic engineering and synthetic biology methods, enabling their development as extremophilic cell factories for industrial applications.

B30 - Effect Of Climate Change On Soil Microbiome And its Activity

Lyudmyla Symochko[1,2], Olga Gafiak[1] Volodymyr Patyka[3]*

[1]Faculty of Biology, Uzhhorod National University, Voloshyna Str. 32, 88000, Uzhhorod, Ukraine

[2]Research and Development Centre of Molecular Microbiology and Mucosal Immunology, Uzhhorod National University, Narodna Square.1, 88000Uzhhorod, Ukraine;

[32]D.K. Zabolotny Institute of Microbiology and Virology, Akad. Zabolotnoho Str, 154, 03680, Kyiv, Ukraine

**Corresponding author: Lyudmyla Symochko, E-mail:lyudmilassem@gmail.com*

However, understanding the responses of microbial communities to climate change is complicated by the vast and largely unexplored diversity of microbiota found in the terrestrial environment. Climate change will have both direct and indirect effects on terrestrial microbial communities and their functions. The effects of increased CO_2 levels on microbial communities are often indirect, as they are mediated by cascading effects on plant metabolism, growth and diversity, and the associated changes in soil physicochemical properties such as soil moisture and resource quality. Microbial processes in soil have a key role in the global fluxes of biogenic greenhouse gases (carbon dioxide, methane and nitrous oxide) and are likely to respond rapidly to climate change. Important parameters of the soil microbiome are the number and functional diversity of microorganisms, soil respiration (CO_2 emission) and enzymatic activity. Well known that more than 1 billion tones of carbon are added to the atmosphere each year trough change of land use. The purpose of our studies was to investigate the dynamics of CO_2 emission from soils of agrogenic, postagrogenic and natural ecosystems and they soil microbiome. In the transformation of arable soils to postagrogenic category changing the flow of major biogenic elements in the ecosystem, including carbon. Self-restoration of abandoned cropland can be considered as a combination of natural processes aimed at achieving homeostasis by the ecosystem. Monitoring studies of the emission of carbon dioxide from soddy-podzolic soils and analisis of soil microbiome were conducted from 2010 to 2017 in dynamics. Were isolated 468 dominanting bacteria, among them 79 antibiotic resistant bacteria. All isolates were multi-drug resistant, of which greater than 74,5% were resistant to 9 antibiotics. Multi-resistance were such pathogenic and conditionally pathogenic bacteria as: *Enterococcus faecium*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Escherichia coli,* Bacillus licheniformis, Serratia fonticola, *Hafnia* alvei, Bacillus cereus, Bacillus megaterium and Clostridium difficile. The maximum level of intensity of carbon dioxide emissions from soils of the studied ecosystems was fixed from the beginning of May to the end of June, due to a favorable combination of abiotic factors for the activity of the soil microbiota. The amount of carbon dioxide produced by virgin soddy-podzolic soils averaged - 79.55 (mg CO_2 / kg soil / day); postagrogenic - 64.25 (mg CO_2 / kg soil / day); agrogenic - 52.18 (mg CO_2 / kg soil / day). In post-agrogenic soils, the value of the total CO_2 emission for vegetation was greater than in agrogenic soils. This is explained by the absence of alienation of primary production, as well as by phytogenic and microbiogenic successions, which leads to a gradual restoration of the natural state of soils and the accumulation of carbon in the post-agrogenic ecosystems.

B31 - Definition and characterization of the metabolic interactions within the microbial community of the MELiSSA waste degradation compartment C1

Tinh Nguyen[1], Claude-Gilles Dussap [2], Laurent Poughon[2], Dirk Springael[1]*

[1] Department of Earth and Environmental Sciences, KU Leuven, Belgium.

[2] Institut Pascal, Université Clermont Auvergne, France.

[*] e-mail of the corresponding author: dirk.springael@kuleuven.be

The Micro-Ecological Life Support System Alternative (MELiSSA) is a concept developed by the European Space Agency (ESA), that evolved out of the need for a regenerative life support system for long term space missions. The concept is inspired on a lake ecosystem and is conceived as a closed loop system consisting of 4 biological compartments (C1 and C4) that through combined activity of different organisms recycles organic waste to new food for the space crew. The robustness of the MELiSSA loop relies on the building of robust, structured and predictive mathematical models which can only be implemented through a deep knowledge of the composition, behavior, metabolisms, kinetics, limitations, inhibitions, etc. of each subsystem. The C1 compartment is the first compartment in the cycle. A thermophilic anaerobic microbial consortium liquefies the solid waste and produces ammonium, volatile fatty acids (VFA's), CO2 and minerals. The overall aim of this research is to obtain a validated biological metabolic model that describes the metabolic interactions within the complex microbial community of the MELiSSA waste degradation compartment C1. The research will start from available background information from the host lab that describes a preliminary microbial network reporting on the potential key-stone species and functionality (mainly based on high abundancy) in the C1 reactor at steady state performance deduced from metaomic data acquired from multiple reactor operations under standard conditions. More specifically, the research will validate and detail this preliminary microbial network, the corresponding metabolic pathways and the identified key species/functions/biomarkers of C1.

B32 - Shrimp pond bottom treatment strategies determined by use of microelectrodes

Funda Torun[1], Bárbara Hostins[2], Jessica Teske[2],Peter De Schryver[2], Jo De Vrieze[1], Nico Boon[1]*

[1] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, Ghent 9000, Belgium

[2] INVE Technologies NV, Hoogveld 93, Dendermonde, Belgium

[*]Nico.Boon@UGent.be

The production of shrimp is often performed in earthen outdoor ponds in which the high input of feed and faeces on the bottom results in deterioration of the water which results in a negative impact on the animals and the environment. Treatment strategies generally focus on oxidizing the organic matter using chemical oxygen booster such as percarbonate or providing an alternative electron acceptor, *i.e.*, nitrate when oxygen deficient conditions in the pond are present. Molybdate can be also used as a sulphate analogue to inhibit sulphate reduction process that leads to production of hydrogen sulphide (H_2S) which is crucially toxic during the growth of shrimp.

In this study three different treatment compounds, *i.e.*, sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), sodium nitrate ($NaNO_3$) and sodium percarbonate ($Na_2CO_3 \cdot 1.5H_2O_2$) at 50 mg/L were tested in comparison to a control that did not include any treatment. The test was conducted for 7 days with a light dynamics of 12 light on/off without any aeration input. The set-up was consisted of 250 mL beakers (7 cm diameter and 9.5 cm height) that was filled with about 3.5 cm deep dry sediment to represent the earthen shrimp pond (collected from Yzermonde nature reserve from a tidal pond that was used for oyster growing in the past). Over the sediment, beakers were filled with 5 cm deep instant ocean at 20 g/L (about 200 mL in total). For the simulation of the waste accumulation, 501 mg shrimp feed (Crevetec Grower 2) and 13.23 mL concentrated shrimp faeces (dry weight 4.55 %) representing the bottom waste produced during 90 days of shrimp culture added in the beginning of the test. Sediment depth profiles of oxygen, H_2S and pH profiles were measured using microelectrodes every day for 7 days of incubation time.

The most significant impact was reported with 50 mg/L sodium molybdate treatment where the both water phase and sediment depth had less H_2S concentrations. For water-sediment interface this was 73% less H_2S than the control and for the sediment depth it was 47% less H_2S. This impact could be measured for first 3 days of the incubation and also observed in the colour of the water column as reddish brown turbidity (colour did not transfer to filtered samples and more visible on the top layer of the sediment). Molybdate treated samples also had sulphate concentrations 16±4% more than the control indicating an inhibition in sulphate reduction. Nitrate and percarbonate treatments had less turbidity in the water column, yet no significant difference in H_2S concentrations in the sediment could be measured for these treatments except slight less H_2S concentrations in the water column compared to the control.

Molybdate addition to the shrimp pond showed a potential to control H_2S accumulation both in the sediment and water-sediment interface where shrimp dwell. This study shows a potential use of microelectrodes to investigate the impact of treatments during waste accumulation in shrimp ponds.

B33 - Novel phage-encoded miniDNases as an inspiration for enzyme-based biotechnological and antimicrobial tools

Marie Van der Gucht[1], Hanne Hendrix[1], Vincent De Maesschalck[1], Kaatje Schroven[1], Jeroen Wagemans[1], Rob Lavigne[1]*

Laboratory of Gene Technology, Biosystems, KU Leuven, Belgium

**rob.lavigne@kuleuven.be*

Bacterial viruses, better known as bacteriophages, are the natural enemies of bacteria. By continuously coevolving with their bacterial hosts, they developed highly efficient strategies to manipulate the host metabolism and promote their own propagation. These strategies can serve as a great inspiration for researchers to develop new antimicrobial strategies and biotechnological tools. However, due to the lack of homology to known genes, functional annotation of predicted phage genes is highly challenging and many host-manipulating strategies remain elusive.

In the present study, an attempt was made to elucidate the function of the 9 kDa protein 'gp5', which is expressed by the *Pseudomonas aeruginosa* infecting phage LUZ19 and all its related *Phikmvvirus* members. While gp5 was shown to inhibit the growth of *P. aeruginosa* upon intracellular expression, *in vitro* assessment of the purified protein revealed that it acts as a nuclease. This nuclease activity was subsequently characterized by qualitative agarose gel-based assays and quantitative hyperchromicity-based assays, indicating that gp5 is a non-specific DNase with an acidic pH optimum. Moreover, this 'miniDNase' shows innovative properties, like high thermostability and resistance to denaturation, making it an interesting tool for many applications that require the removal of DNA[1].

One such application could be the use of miniDNases to target microbial biofilms by degrading the extracellular DNA (eDNA) in the biofilm matrix. eDNA plays a pivotal role in structure and increased tolerance of many microbial biofilms, making it an attractive and broad-spectrum biofilm target[2]. With the use of the high-throughput Calgary device and crystal violet staining, we showed that gp5 is capable of significantly preventing and disrupting 24 h old *P. aeruginosa* biofilms. These findings indicate the potential of miniDNases to be used as antibiofilm strategies, which will be further investigated in the future.

References:
[1] Hendrix, Van der Gucht, Aertsen, Lavigne (2019). WO 2019/020731 A2
[2] Okshevsky M. *et al.* (2015), *Curr. Op. in Biotech.* 33: 73-80

B34- Effect of radiation and temperature on the microbial community in the cooling water of a nuclear reactor

Valérie Van Eesbeeck[1,2], Mohamed Mysara[1], Ruben Props[1], Rob Van Houdt[1], Pauline Petit[3], Natalie Leys[1], Jean Armengaud[3], Corinne Rivasseau[3], Jacques Mahillon[2], Pieter Monsieurs[1]

[1] *Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium*

[2] *Université Catholique de Louvain, UCL, Louvain-la-Neuve, Belgium*

[3] *Commissariat à l'énergie atomique, CEA, Grenoble, France*

The BR2 nuclear research reactor consists of different watery environments, one of which is an open basin surrounding the reactor vessel. The water in this basin has a shielding effect on the radiation originating in the reactor core, where nuclear fission takes place during reactor operation. Remarkably, despite the ultra-high purity of the water due to constant filtering and deionization, combined with the high radioactivity exposure, microbial growth in these environments is not fully prevented. Indeed, several microbes appear to be able to survive and thrive in such conditions. Microorganisms identified in those environments thus provide a unique opportunity to acquire new insights into survival strategies and radiation-resistance mechanisms.

The objective of this work is to explore the bacterial communities present in the basin water of the BR2 nuclear research reactor. In order to accomplish this, the bacterial population was followed up over time during and outside reactor operation to monitor its dynamics in this unique, never-before-studied environment.

For the characterization and the follow-up of the bacterial communities, a 16S rRNA amplicon sequencing approach was adopted. Results from two long-term follow-up experiments highlighted a clear shift in the bacterial community profile during and outside reactor operation. Interestingly, the profiles for both experiments appeared to be quite similar, notwithstanding the fact that the two sampling campaigns were separated by a one-year interval. This indicates that the system is very robust.

During reactor operation, the bacterial community is mostly dominated by two OTUs that were taxonomically assigned to an unclassified Gammaproteobacterium and *Pelomonas*, respectively. During this phase, either one or the other becomes prevalent, or both are equally abundant. When the reactor goes into shutdown, the community clearly shifts to become dominated by an OTU assigned to *Methylobacterium*. This interesting finding can be explained by the change in physico-chemical parameters like flow rate, temperature and most importantly radiation that occurs when the reactor transitions from one phase to the other. In addition, exposure to radiation also causes the bacterial population to steeply decrease in number, before it can slowly recover during reactor shutdown.

To conclude, we have been able to shed some light on this unique system for the first time and thoroughly explored its dynamics. In a next step, we will further investigate the radiation resistance potential of some interesting isolated strains.

B35 - Acid robustness engineering in *Escherichia coli*

Stefanie Van Riet[1], *Wubishet Tadesse*[1], *Abram Aertsen*[1*]

[1]*Laboratory of Food Microbiology, Department of Microbial and Molecular systems (M^2S), Faculty of Bioscience Engineering, KU Leuven, Leuven, Belgium*

[*]*Corresponding author: abram.aertsen@kuleuven.be*

Bio-production is becoming an industrially relevant approach to produce all kinds of medically or industrially relevant products. Because of the growing concerns about fossil resources and climate change, there is a shift from the fossil-based industry towards a bio-based industry. Micro-organisms can be metabolically engineered to efficiently convert renewable carbon sources into all sorts of chemicals. To be competitive with the traditional petroleum refinery processes, much effort is invested to increase the yield of these processes. One method is increasing robustness of the used cell factory, as the cells should be able to withstand the product and production conditions. One of the important stresses during bio-production in bio-reactors is acid stress, caused by acidic end-products or as a side effect of suboptimal mixing, which causes lack of oxygen and thus fermentation. To increase production yields, synthetic biology approaches can be used for robustness engineering towards an acid-tolerant bacterial chassis. To gather an inventory of parts to engineer acid robustness, we performed directed evolution to identify novel acid resistance mechanisms in *Escherichia coli*. As such, five independent lineages of *E. coli* MG1655 were iteratively subjected to severe acid shocks with intermittent resuscitation periods. We observed that acid resistance could readily and reproducibly be improved, since each of the five lineages independently yielded mutants with > 500-fold resistance compared to the parental strain. Whole genome sequencing and further genetic analysis of these different mutants revealed new mutational roads towards activation of the acid fitness island, as well as novel resistance mechanisms that are independent from activation of this island. Further investigation of these findings will yield strategic engineering routes towards acid tolerant industrial processing strains.

B36 - Toxic Cyanobacterial Blooms in Brussels: a case study

W. H. R. Van Hassel[1,3], B. Huybrechts[1], M. Andjelkovic[2], A. Wilmotte[3]

[1]*Organic Contaminants and Additives, Sciensano, Leuvensesteenweg 19, Tervuren, Belgium*

[2]*Risk and Health Impact Assessment, Sciensano, Rue Juliette Wytsmanstraat 14, Brussels, Belgium*

[3]*Department of Life Sciences, University of Liège, Allée du six Août 11, Liège, Belgium Corresponding author: wannes.vanhassel@sciensano.be*

In Brussels, cyanobacterial blooms are quite frequently observed in urban lakes and ponds, as already shown during the B-BLOOMS project in 2007-10. If such a bloom consists of toxic cyanobacteria, this may have an adverse impact on the water quality and poses a threat to public and animal health, mostly during recreation activities. The cyanotoxins may also be ingested by animals (dogs, ducks, fish...) or be transported by aerosols. Thus, the detection of toxigenic cyanobacteria and the produced toxins is a crucial step to assess the risk for public health and to decide on the management measures to limit exposure.
Therefore, we developed a method to detect and quantify intra- and extracellular toxins in the samples by HPCL-MS/MS. We selected 8 microcystin congeners and nodularin for this analysis. These are hydrophobic cyanobacterial toxins, of which microcystin-LR is known to be the most prevalent and most studied cyanobacterial toxin in western Europe.
The method was tested on 4 samples of different ponds in Brussels where visual inspection indicated the presence of a bloom in September. The cyanobacterial genera *Microcystis* and *Planktothrix* were identified based on morphology and several microcystin variants were indeed detected in the samples. After separating the cells and lake water, the DNA was also extracted and the presence of *mcy* genes was tested, as they are responsible for the synthesis of microcystins.
In the future, we intend to implement our analysis in cooperation with the regional authorities to better assess the impacts of toxic blooms.

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B37 - Cold seep sediment enrichment yields piezotolerant, obligate hydrocarbon degraders.

Josefien Van Landuyt[1], Lorenzo Cimmino[3], Charles Dumolin[2], Ioanna Chatzigiannidou[1], Felix Taveirne, Valérie Mattelin[1], Yu Zhang[5], Peter Vandamme[2], Alberto Scoma[4], Adam Williamson, Nico Boon[1]

[1] *Centre for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium*

[2] *Laboratory of Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium*

[3] *Laboratory for Environmental Biotechnology (LBE), Ecole polytechnique fédérale de Lausanne (EPFL), CH B2 407 (Bâtiment CH), Station 6, CH-1015 Lausanne, Switzerland*

[4] *Biological and Chemical Engineering (BCE), Department of Engineering, Aarhus University, Hangøvej 2, 8200 Aarhus, Denmark*

[5] *Laboratory of Microbial Oceanography, Shanghai Jiao Tong University*

地址：上海市 闵行区 东川路800号 上海交通大学 木兰船建大楼 B308

[*] *Correspondence to: Nico Boon, e-mail: Nico.Boon@UGent.be*

Deep-sea environments can get contaminated with recalcitrant petroleum hydrocarbons through different anthropogenic activities. The effects of the hydrostatic pressure in the deep sea on microbial oil degradation, the option of choice for full remediation of these environments, is poorly understood. Moreover, so far, efficient piezotolerant oil degraders, i.e. interesting bioaugmentation candidates, have been rarely isolated. Here we performed long-term enrichments (100 days) of sediment taken from a natural cold seep while providing optimal conditions to sustain high hydrocarbon degradation rates (i.e. oxygen, mesophilic temperatures [20°C] and medium chain alkanes [dodecane] in abundance). Through enrichments performed at high hydrostatic pressure (HHP) [10 MPa] and ambient pressure (AP) [0.1 MPa] and by using control enrichments with marine broth, we demonstrated that both pressure and carbon source can have a big impact on community structure. In contrast to previous studies, hydrocarbonoclastic OTUs remained dominant at both AP and HHP, suggesting piezotolerance of these OTUs over the supplied pressure range. Twenty-three isolates were obtained after isolation and dereplication using matrix-assisted laser deionisation/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). After re-cultivation at HHP, a hydrocarbonoclastic bacteria, identified as *Alcanivorax venustensis* strain #1, showed promising piezotolerance in axenic culture as well. Furthermore, preliminary co-cultivation tests showed synergistic growth between some isolates, a promising result for future bioaugmentation tools. Overall, more insight into the effect of HHP on oil degrading communities was obtained as well as several interesting isolates, among others a piezotolerant *Alcanivorax*, with which future deep-sea synthetic community remediation could be further investigated.

B38 - Bacterial shift of frozen broiler meat

*Zhongjia Yu, Pieter-Jan Kerkhof, Kurt Houf**

Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

* *kurt.houf@ugent.be*

Broiler meat frozen by both commercial and domestic freezers is widely consumed in the world. However, for the bacterial shifts on both commercial and domestic frozen broiler meats, including contamination level and diversity, the monitor regulations and studies are still lacking. To assess these shifts, the present study examined the level of mesophiles, psychrotrophs and Enterobacteriaceae on frozen broiler carcasses stored in long term (up to 6 months). Also, the present study, using MALDI-TOF MS identification and 16S rRNA amplicon sequencing, examined the bacterial shifts on frozen broiler meat stored in short term (up to 7 days), including the contamination level (TAB, TANAB, LAB, presumptive *Pseudomonas* and *E. coli*) and general bacterial diversity. As results, after long-term frozen storage, the level of mesophiles and Enterobacteriaceae decreased intensively while the level of psychrotrophs did not change a lot. By contrast, after a short-term storage, only the levels of LAB and *E. coli* decreased significantly while these shifts were dependent on the conditions of freezers. Interestingly, one *Salmonella*-negative meat sample turned to be positive after co-frozen with a positive sample in the short-term test. *Pseudomonas* spp. was always major flora isolated from these samples regardless of the frozen time and isolation temperatures when MALDI-TOF MS identification was applied. Due to the effect of freezing, the relative abundance of *Escherichia* spp., *Actinetobacter* spp. and *Carnobacterium* spp. decreased but the relative abundance of fungi and *Psychrobacter* spp. increased. Through 16S rRNA amplicon sequencing, regardless of freezing, *Pseudomonas* spp. was the most dominant flora in plate washing samples whereas in stomachered homogenates samples, *Bacteroides* spp., *Faecalibacterium* spp., Lactobacillus spp., Prevotella spp., Ruminococcus spp., Streptococcus spp., unknown bacteria, unknown Lachnosipiraceae, unknown Rikenellaceae and unknown Ruminococcaceae were present in dominance ($p<0.05$). Moreover, the storage time and freezers affect on species richness but not much on OTU richness. In conclusion, both commercial quick-frozen and domestic-frozen could reduce the contamination level on broiler carcasses, whereas bacterial cross-contamination and migration might exist in domestic-frozen storage. From the microbiological aspect, the domestic frozen carcasses cannot be simply regard as commercial frozen broiler carcasses due to the difference of initial contamination and freezers condition. The condition of freezers impacts on the microbiological quality of frozen meat but the correlation needs to be investigated further.

B39 - Optimization and identification of cultivation conditions and the possibility of producing probiotic microorganisms producing GABA (gamma-aminobutyric acid) from indigenous dairy of west of Iran and using in whey protein drink

Fatemeh Zarei[1], Leila Nateghi[2], Mohamad Reza Eshaghi[3], Maryam Ebrahimi Taj Abadi[4]*

[1] Halal Research Center Islamic Republic of Tehran, Iran, Phone: +98-9129569137, Email: zarei.fatemeh@gmail.com
[2] Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, Phone: +98-9125878775, Email: l.nathegi@iauvaramin.ac.ir,
[2]Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, Phone: +98-9121768519, Email: m.eshaghi@iauvaramin.ac.ir
[3]Department of Biology, Islamic Azad University, Tehran, Iran, Phone: +98-9121088517, m.tajabadi@iauvaramin.ac.ir

Abstract : Isolation and identification of probiotic bacteria from native strain have a very important role in the food industry as well as increasing the health of the community. Lactic acid bacteria play a pivotal role in fermentation processes. One of the metabolites produced by this starin of bacteria is gamma aminobutyric acid (GABA). It has a variety of physiological functions such as the induction of hypertensive, diuretic, anti-depression, and tranquilizing effects. The general objective of this research was to optimize and identify the conditions of cultivation and the possibility of producing probiotic microorganisms producing GABA from indigenous Iran to be utilized in whey protein drink. In the first part of this study, 50 bacterial samples from indigenous dairy products (yogurt, dough, cheese and butter) were isolated in western Iran. Initial diagnostic tests including stain gram, oxidase and catalase tests were performed to detect lactic acid bacteria. Results showed that only 10 strains were gram positive, negative catalase and negative oxidase, and were known as Lactic Acid Bacteria (LAB). Then, probiotic properties including acid resistance, bile, gastric juice, and hemolysis inactivity and L-arginine hydrolysis were evaluated. Three LAB samples with strong probiotic properties were selected from them, followed by identification of the primers with 16SrDNA by sequencing and drawing phylogeny tree, the results showed that *Lactobacillus paracaosi, Lactobacillus plantarum and Pediococos acidilactici* strains isolated from yogurt and dough and cheese, respectively, had the highest probiotic properties. In the second part, the concentration of GABA production by probiotic bacteria was evaluated by HPLC in two media of MRS Broth and whey protein. Based on HPLC results, *Lactobacillus plantarum*, grown in MRS broth had shown the highest concentration of GABA production with 115.4 ppm. In order to increase the amount of GABA produced, the conditions of the culture medium (temperature range (30 to 50 °C), pH (4 to 6), time (12 to 72 h) and glutamic acid concentration (25 to 250 mM) were optimized. The optimized conditions resulted in an increase in the production of GABA to reach170.942 ppm. In the third part of this study, the bacterium was added under optimal conditions of culture medium at 37.77 °C, pH 5.19, 250 mM glutamic acid and 72 hours in whey protein drink containing concentrate of banana and strawberry, and the viability properties, production GABA and its sensory evaluation within 30 days. The results of this study showed that the highest viability rate on the on day 30 of whey drink with strawberry at 25°C was ($8.1Log_{10}$cfu / ml) and the highest amount of GABA on day 30^{th} of production of whey drink containing banana stored at 25°C (195.5ppm). A sample of whey drink containing banana stored at 25°C was recognized as a superior treatment due to higher levels of GABA. In conclusion, Using indigenous and resistant species of probiotic bacteria and optimizing bacterial growth conditions, more GABA can be produced in food products and a positive step towards the development of pragmatic products and the promotion of consumer health.

Keywords: GABA, *Lactobacillus plantarum*, Probiotic, Response method, Whey protein

B40 - Reverse Photosynthesis for Brussel – RE4BRU

Magri S[1]., Kadowaki M.A.S.[1] & Cannella D.[1]

[1]Biomass Transformation Lab (BTL), Ecole interfacultaire de bioingénieurs, Université Libre de Bruxelles (ULB), Belgium

[*]silvia.magri@ulb.ac.be

Photosynthetic organisms have evolved complex electron-transfer systems to convert light energy into primary biomass. A recent discovery [1] showed that the light can also trigger the opposite reaction breaking-down carbohydrates by means of dioxygen and chlorophyll. This process, named Reverse Photosynthesis, is driven by Lytic Polysaccharide MonoOxygenases (LPMOs), copper-dependent redox enzymes produced by a wide range of fungi and bacteria. Upon activation through reduction, LPMOs are able to oxidize the most recalcitrant polysaccharides as cellulose and chitin, have a strong synergistic effect on biomass degradation when combined with hydrolases and their activity is dramatically increased by light supply. In this frame the Reverse Photosynthesis for Brussels - RE4BRU project is born from the collaboration of two main universities of Belgium such as Université Libre de Bruxelles (ULB) and Université Catholique de Louvain (UCLouvain) and supported by the Brussels Institute for Research and Innovation. RE4BRU is focused on the recovery of plant and food waste from urban area of Bruxelles and their valorisation into chemical platform using green conversion technologies. At the Biomass Transformation Laboratory (BTL-ULB) we will focus on the heterologous expression of monocomponent enzymes for biomass degradation into oligo- and monosaccharides using the innovative Reverse Photosynthesis setup and on the two ultimate bioconversion steps through lactic acid fermentation or production of tissue healing hydrogel for biomedical applications. Here we report the cloning, expression and purification of five enzymes using *Escherichia coli* and *Pichia pastoris* as cell-factories. This expression platform will be used to prepare specific formulations of enzymic blends. These enzyme cocktails will be investigated in order to optimize the global light-to-product quantum of the process using statistical experimental design.

[1] D. Cannella et al. 2016. Light-driven oxidation of polysaccharides by photosynthetic pigments and a metalloenzyme. Nature Communications 7, article number: 11134; doi: 10.1038/ncomms11134

ABSTRACTS SHORT TALKS
SECTION C: MEDICAL AND VETERINARY MICROBIOLOGY

C - ST01 - ß-Lactams Translocation Through *Pseudomonas aeruginosa* Outer Membrane

F. Amisano[1], P. Mercuri[1], J.M. Frère[1] and M. Galleni[1]*

[1]Macromolécules Biologiques, Centre for Protein Engineering, InBioS, University of Liège, Belgium

[*] mgalleni@uliege.be

Permeation of small molecules through *P. aeruginosa* outer membrane is an important issue, still poorly understood. Its importance stems from the fact that the low permeability of *P. aeruginosa* is one of the causes of its intrinsic resistance to different antibiotics such as β-lactams. For example, the deletion of the specific porin OprD can lead to strains highly resistant towards imipenem. Many studies have characterized different single channels and their roles in determining antibiotic permeation, but we still lack a global view of the effects of single/multiple porin(s) deletion(s) for the translocation of antibiotics in *P. aeruginosa*.

With the purpose of gaining relevant evidence on the matter, BlaR-CTD, the C-terminal domain of a highly sensitive penicillin binding protein from *Bacillus licheniformis*, was expressed in the periplasmic space of *P. aeruginosa* PAO1. We were then able to perform a direct measure of the β-lactam accumulation in the periplasmic space of the bacteria. The permeability coefficients of the external membrane of *P. aeruginosa* to different antibiotics belonging to the penicillin, cephalosporin and carbapenem sub-families were measured for *P. aeruginosa* PAO1 and different mutant strains, lacking in one or multiple porins.

Quantitative real-time reverse transcription-PCR (qRT-PCR) was also performed to determine the expression of different porins at four moments of cellular growth and to comprehend how these changes can modulate the antibiotic uptake.

We noticed a 150-fold reduction of imipenem permeability in strains where OprD was deleted. The permeability coefficient *p* for *P. aeruginosa* PAO1, *P. aeruginosa* TNP004 (mutant where OprD is downregulated) and *P. aeruginosa* PAO1 ΔoprD were $2.0 \cdot 10^{-6}$ cm/s, $1.4 \cdot 10^{-8}$ cm/s and $1.4 \cdot 10^{-8}$ cm/s respectively. These results are in good agreement with MICs values that increase from 1 to 8 μg/mL when OprD is poorly or not expressed. We also pointed out that the absence of OprD did not affect the permeability coefficient of *P. aeruginosa* PAO1 for meropenem and biapenem, differently from the MICs that rise from 0,5 to 4 μg/mL for both antibiotics. However, for these antibiotics, we measured permeability coefficients in *P. aeruginosa* PAO1 ΔoprD ΔopdP; Interestingly, the *p* values decreased to $6.5 \cdot 10^{-10}$ and $1.6 \cdot 10^{-8}$ cm/s respectively. Interactions of OpdP and meropenem were already described but we identify the involvement of OpdP in biapenem uptake.
qRT-PCR allowed us to observe that, in absence of OprD, the strain increases the expression of the related porin OpdP; we also showed a different ratio between OprD and OpdP porins as a function of growth phase in *P. aeruginosa* PAO1. Thus, in accordance with the experimental results obtained by permeability coefficient determination for meropenem and biapenem.

This work allowed us to quantify the permeability of the outer membrane in *P. aeruginosa* and contributes to the modelisation of the intrinsic resistance of *P. aeruginosa* to β-lactams.

C - ST02 - The polyamino-isoprenic efflux inhibitor NV716 revives old disused antibiotics against intracellular forms of infection by *Pseudomonas aeruginosa*

Gang Wang[1], Jean-Michel Brunel[2], Paul M. Tulkens[1], Jean-Michel Bolla[2], Françoise Van Bambeke[1], [1]Université catholique de Louvain, Brussels, Belgium[2] Aix-Marseille Université, Marseille, France

Objective: WHO considers *P. aeruginosa* (PA) as a priority pathogen for the search of innovative therapies. PA is indeed intrinsically resistant to many antibiotics due to poor outer membrane permeability and/or active efflux. Moreover, it can also adopt specific lifestyles, like intracellular survival, that make it poorly responsive to treatments. Our aim was to evaluate the capacity of efflux pump inhibitors to restore the activity of old, disused antibiotics, against intracellular PA. We compared PABN to original polyamino-isoprenic compounds, namely NV731 and NV716 in combination with doxycycline, chloramphenicol (substrates for efflux), and rifampicin (not substrate).
Methods: See (AAC 2013; 57:2310-2318) for details. Phagocytosis of PAO1 (opsonized with human serum) was allowed for 2 hours using a bacterium: cell ratio of 10:1, after which non-phagocytosed bacteria were eliminated by incubation with gentamicin (50 X MIC) for 1 hour. After washing, infected cells were incubated with antibiotics (0.003-100 x MIC) for 24 hours. Maximal relative efficacy (E_{max}) and apparent static concentrations (C_s) were calculated using the Hill equation of concentration-response curves. Toxicity was assessed by LDH release
Results: See table. NV731 no significant deferent and PABN decreased the MICs of doxycycline and chloramphenicol but not that of rifampicin, while NV716 (2.5 and 10 µM) markedly reduced the MIC of all antibiotics. Intracellularly, C_s of antibiotics alone were close to their MIC in broth; their E_{max} was approx. 2 log decrease in cfu. NV731 and PABN did not modify these parameters, while NV716 (10µM) was able to increase both relative potency (lower C_s value) and maximal efficacy (more negative E_{max} value) for all drugs without causing toxicity for THP-1 cells.
Conclusion: In contrast to PABN and NV731 that act as efflux inhibitors against planktonic bacteria only, NV716 is capable to re-sensitize PA to antibiotics whether substrates (doxycycline, chloramphenicol) or not (rifampicin) for efflux, not only in the broth but also intracellularly. This could be due to its capacity to alter PA membrane integrity (PLoSOne 2016; 11(5):e0154490). NV716 may, therefore, appear as a useful adjuvant to revive the activity of old antibiotics with low antipseudomonal activity against PA infections, including its intracellular persistent forms.

	MIC (mg/L)[a]			**C_s[b]** (mg/L)			**E_{max}[c]** (Δlog cfu from post-phagocytosis inoculum)		
Experimental condition	DOX	CHL	RIF	DOX	CHL	RIF	DOX	CHL	RIF
Antibiotic (AB) alone	8	32	16	16.0±8.2	37.5±9.3	20.2±4.0	-2.1±0.2	-2.0±0.3	-2.1±0.3
AB + NV716 (2.5µM)	**1**	**2**	**0.25**	17.9±3.7	42.1±7.8	19.7±3.5	-2.4±0.2	-1.9±0.3	-2.2±0.3
AB + NV716 (10µM)	**0.5**	**1**	**0.125**	4.9±1.2*	8.7±5.3*	11.1±2.3*	-2.9±0.5*	-2.5±0.3*	-2.7±0.4*
AB + NV731 (2.5µM)	4	32	16	16.3±1.7	41.1±8.2	26.2±1.0	-2.6±0.2	-2.1±0.2	-2.4±0.4
AB + NV731 (10µM)	4	16	8	14.2±4.0	15.0±9.1*	18.2±3.8	-2.6±0.4	-2.1±0.2	-2.3±0.4
AB + PABN (20mg/L)	**2**	**8**	8	22.9±1.0	34.5±13.8	17.9±1.2	-2.3±0.2	-2.1±0.3	-2.3±0.3

[a] values in bold denote a decrease of at least 2 doubling dilutions vs. AB alone
[b] Cs: apparent static concentration i.e., the extracellular concentration (mg/L) resulting in no apparent bacterial growth (number of cfu identical to the initial [extracellular] or post-phagocytosis [intracellular] inoculum)
[c] Emax: relative maximal efficacy: maximal decrease in inoculum (in log_{10} units) compared to the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration.
* significantly different ($p < 0.05$) from AB alone

C - ST03 - Unravelling the role of BK polyomavirus variants and of cellular CMP kinase on viral pathogenicity and clearance in kidney transplant recipients

Olga Mineeva-Sangwo[1], Dimitrios Topalis[1], Maarten Naesens[2,3], Katrien Lagrou[4], Graciela Andrei[1], and Robert Snoeck[1]*

[1]*Rega Institute for Medical Research, KU Leuven, Leuven, Belgium.*

[2]*Laboratory of Nephrology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium.*

[3]*Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium.*

[4]*Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium*

[*]*olga.mineevasangwo@kuleuven.be*

Introduction: BK polyomavirus (BKPyV) is the causative agent of polyomavirus associated nephropathy (PVAN) in kidney transplant recipient (KTR) and hemorrhagic cystitis in bone marrow transplant patients. BKPyV is highly prevalent in the general population (>85%), exhibiting latency in the kidneys. The immunosuppressed state of KTRs often leads to reactivation of the virus and PVAN, which is associated with a significant risk of graft loss. Several protocols for the management of PVAN have been suggested, although the evidence base is low. The combination of lowering immunosuppression and cidofovir (CDV; an anti-DNA virus agent) permits in some cases the control of viral replication preserving the integrity of the graft. However, in some patients, this strategy does not result in control of the viral replication.

Aims: On the one hand, this study is focused on the characterization of heterogeneous BKPyV pool obtained from clinical samples of KTRs. On the other hand, the *Cmpk1* gene coding for an enzyme responsible for the activation of CDV is genotyped using RNA extract from kidney biopsy specimens. Several publications highlighted the association between polymorphism in the *Cmpk1* gene and the altered activation of certain nucleotide analogues. In addition, the question, whether the co-infection with other DNA viruses can contribute to PVAN and/or to a negative outcome of a CDV-based treatment, will also be addressed in this work. All together, the information collected will help to understand differential BKPyV replication and inhibition patterns after CDV treatment observed in KTRs.

Methods: Two set of primers were designed and further used to amplify the whole genome of BKPyV from kidney biopsy, urine and plasma samples collected at 3 months after transplantation. The amplified samples were purified with AMPure XP SPRI beads and sequenced using Illumina Miseq system (pair-end sequencing; 300 cycles) in order to identify genetic variation within a heterogeneous BKPyV pool of clinical samples. At the same time, Sanger sequencing was used for CMPK1 genotyping following the cDNA synthesis and *Cmpk1*-specific PCR amplification protocol.

Results: The results of quantitative PCR were analysed for the presence of BKPyV in urine and plasma samples as well as those of large tumor antigen staining on kidney biopsy specimen for the confirmation of nephropathy. Accordingly, from 1,024 kidney transplantation events performed during the period 2008-2017 at the University Hospitals Leuven, 397 have been identified as BKPyV-positive in plasma (viremia) and 214 in urine (viruria). The remaining 413 KTRs were associated with the absence of BKPyV in both urine and plasma. From the BKPyV-positive group, 195 available DNA samples (kidney biopsy) were analysed for the presence of BKPyV by PCR. In addition, 145 out of 220 available RNA samples were proceeded from this group.

Conclusions: Two specific amino acid changes in CMPK1 (Gln48His and Asn83Ser) were identified in 14 RNA samples so far. The impact of identified mutations on CMPK1 enzymatic activity and their role in negative outcome of CDV-based treatment still has to be determined. The analysis of the NGS data for BKPyV required a setup of an in-house bioinformatics pipeline. Two approaches will be applied, a de novo whole BKPyV genome assembly and a whole BKPyV genome alignment versus Dunlop strain (a reference strain for BKPyV; GenBank V01108). This pipeline is still in development.

C - ST04 - Broad influenza virus inhibitor targeting inosine monophosphate dehydrogenase in a distinct manner from ribavirin

Evelien Vanderlinden[1], Leentje Persoons[1], Ria Van Berwaer[1], Arnaud Marchand[2], Patrick Chaltin[2,3], Lieve Naesens[1]*

[1]KU Leuven, Rega Institute for Medical Research, Leuven, Belgium

[2]CISTIM Leuven vzw, Bioincubator 2, Leuven, Belgium

[3]KU Leuven, Centre for Drug Design and Discovery (CD3), Bioincubator 2, Leuven, Belgium

[*]evelien.vanderlinden@kuleuven.be

Seasonal influenza is caused by influenza A and B viruses and associated with significant morbidity and mortality. Antiviral therapy to suppress severe influenza infections was long dominated by inhibitors of the viral neuraminidase, yet is currently undergoing a dramatic change with the advent of viral polymerase inhibitors. However, since drug-resistant influenza viruses can arise for both drug classes, fundamentally different antiviral strategies are still needed.

To identify novel inhibitors of influenza A and B viruses, we performed high-throughput screening using the minigenome assay, which quantifies influenza virus polymerase activity in transfected cells. Further evaluation of the hit compounds for their potential to inhibit influenza virus replication in Madin-Darby canine kidney (MDCK) cells, led to selection of CIM116859. This molecule exhibited potent activity against a broad panel of influenza A/H1N1, A/H3N2 and B viruses, with EC_{50} values of 0.089 - 2.2 µM. It was devoid of cytotoxicity at 25 µM, yielding a selectivity index of 11 - 280. In addition, CIM116859 displayed moderate inhibition of respiratory syncytial virus (RSV) in HeLa cells (EC_{50} of 5 µM), whereas no activity was noted against several other RNA viruses.

Time-of-addition experiments with influenza virus revealed that CIM116859 acts between 1 and 5 h p.i., which coincides with viral RNA synthesis. However, the compound did not directly inhibit the viral polymerase in enzymatic assays measuring RNA elongation or cap-snatching. To assess whether CIM116859 may have an indirect effect on viral RNA synthesis, we conducted HPLC analysis to measure its potential effect on cellular NTP pools. After 24 h incubation with CIM116859, the cellular GTP level was drastically decreased in MDCK cells (by 89% at 1 µM), and to a lesser extent in HeLa and Vero cells (at 5 µM: decrease of 78% and 49%, respectively). This strong GTP depletion indicated that CIM116859 might interfere with *de novo* synthesis of GMP, more precisely with the inosine monophosphate dehydrogenase (IMPDH) enzyme that converts inosine monophosphate (IMP) to xanthosine monophosphate (XMP) in an NAD^+-dependent reaction. This assumption was validated in influenza virus-infected MDCK cells, since combining CIM116859 with guanosine led to significant reduction of its antiviral effect, while guanine, hypoxanthine and inosine had no effect. Nevertheless, in enzymatic assays with IMPDH type I and type II, CIM116859 was found to have no direct inhibitory effect, suggesting that CIM116859 might require metabolic activation in order to inhibit the IMPDH reaction. This is reminiscent of ribavirin, a well-known IMPDH inhibitor that requires conversion to its monophosphate to interact at the IMP binding site of IMPDH. Interestingly, combining CIM116859 with ribavirin resulted in a synergistic antiviral effect in influenza virus-infected cells. This suggests that the IMPDH interaction mode of CIM116859 (or its active metabolite) may differ from that of ribavirin monophosphate, for instance by blocking the NAD^+-binding site or by mediating allosteric inhibition of the IMPDH enzyme.

To conclude, our investigation identified CIM116859 as a strong inhibitor of influenza A and B viruses. We provided strong support for an indirect inhibitory effect of CIM116859 on the IMPDH reaction, in a manner distinct from that of ribavirin.

ABSTRACTS POSTERS
SECTION C: MEDICAL AND VETERINARY MICROBIOLOGY

C01 Effect of different carbon sources on antibiotic susceptibility of *Pseudomonas aeruginosa* PAO1 in CF patient sputum

Xuerui Bao 1, Tom Coenye 1

[1] Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium
Contact: Xuerui Bao, xuerui.bao@ugent.be

Cystic fibrosis (CF) is a genetically inherited disease characterized by the absence of the CF transmembrane conductance regulator. It leads to the production of sticky mucus, especially in the respiratory tract, facilitating chronic lung infections. *Pseudomonas aeruginosa* acts as important multidrug resistant primary pathogen that is particularly dangerous for CF patients. Once the bacteria colonize the lung of CF patients, they will form biofilms and it is difficult to eradicate them.

According to several studies, some carbon sources can increase antibiotic efficacy by changing the bacterial metabolism. However, in these studies, the medium used is most often a minimal medium, which is from a physicochemical point of view very different from the CF sputum. To increase our understanding of how extra carbon sources influence the bacterial antibiotic susceptibility, *Pseudomonas aeruginosa* PAO1 was cultivated in synthetic CF sputum medium (SCFM2) mimicking CF patient sputum, which was combined with antibiotics from different classes.

We determined MIC of tobramycin, ciprofloxacin and ceftazidime in SCFM2 for PAO1. The results indicate that the MICs of tobramycin, ciprofloxacin and ceftazidime for PAO1 are 5 µg/ml, 0.6 µg/ml and 3.125 µg/ml respectively. Concentrations that are equal to 0.5 × MICs of each antibiotic were used to determine the effect of the different carbon sources on the antibiotic susceptibility. The 78 different carbon sources, including carbohydrates, aromatic compounds, organic acids, amino acids and alcohols, were supplemented to SCFM2 in a final carbon concentration of 60 mM. PAO1 treated with 0.5 × MICs of antibiotics and incubated in normal SCFM2 without extra carbon source served as control. Following the culture of 96-well plate, macrodilution method was used to determine the CFU concentration. Our data shows that antibiotic susceptibility of PAO1 is influenced by the different carbon sources. Carbon sources resulting in large difference compared with control were identified as those, in which CFU concentrations of PAO1 were 10 times lower than that of the control. They are betaine HCl, citric acid, L-malic acid, acetohydroxamic acid and trimethylamine-n-oxide trihydrate for PAO1 treated with 2.5 µg/ml tobramycin; D,L-malic acidfor PAO1 cultured together with 0.3 µg/ml of ciprofloxacin; and citric acid for PAO1 with the addition of 1.56 µg/ml of ceftazidime. The results suggest the possibility of reducing antibiotic susceptibility of PAO1 in CF patient sputum by adding different carbon sources.

C02. Sensitivity of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* to a combination therapy of antibiotics and potentiators in synthetic cystic fibrosis sputum medium

Mona Bové[1] *, Tom Coenye*[1]

[1] *Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium*

Contact: Mona Bové, Mona.Bove@ugent.be

Cystic fibrosis (CF) is an inheritable disease occurring in 1 of every 3000 births with an average life expectancy of 37 years with sufficient health care. Colonization of the lungs of CF patients with pathogens, who form biofilm aggregates, results in recurring inflammation, destruction of lung tissue and eventually death by respiratory failure. The efficacy of antimicrobial treatments is hampered by tolerance associated with growth of the organisms in biofilm aggregates. In addition, besides failure due to biofilm related tolerance, pathogens show an increasing resistance to currently available therapies. One of the strategies to increase the sensitivity of bacteria to antibiotics is the use of quorum sensing inhibitors (QSI) or potentiators. These compounds can inhibit the tools pathogens need to be virulent, like biofilm formation and toxin production, but do not have a killing effect. In order to mimic the in vivo conditions, the antimicrobial susceptibility of *P. aeruginosa* (PAO1) and *B. cenocepacia* (J2315) was assessed in a previously described synthetic CF sputum medium (SCFM2). This medium was designed to resemble CF sputum and allows formation of biofilm aggregates that resemble those found in CF sputum. The MIC of tobramycin for PAO1 was reduced 4 fold from 10 to 2,5 µg/ml after the addition of 30 µg/ml of the QSI furanone C30. J2315, a strain that is highly resistant to most antibiotics, became 64 times more sensitive to aztreonam when combined with 400 µg/ml of the non-mevalonate pathway inhibitor FR-900098, as the MIC was reduced from >2048 to 32 µg/ml. The next step was to evaluate the ability of these compounds to eradicate a 24h old biofilm. For PAO1 combining 100 and 200 µg/ml of C30 with 20 and 10 µg/ml of tobramycin respectively, gave a 5 log reduction in CFU. For J2315 potentiating effect of FR-900098 could not be found when added in a concentration of up to 1000 µg/ml, to aztreonam, ceftazidime, meropenem or amikacin. The present study demonstrates that a combination therapy of C30 and tobramycin can potentially inhibit the growth of *P. aeruginosa* in the CF sputum, and significantly reduces the amount of bacteria present in biofilm aggregates formed by the pathogen. These results indicate that the combination FR-900098/aztreonam, has the potential to inhibit the growth of *B. cenocepacia* in the CF sputum but is less likely suited to combat existing *B. cenocepacia* infections.

C03. Treatment of in vitro oral biofilms with chlorhexidine results in an altered community composition and metabolic activity.

Ioanna Chatzigiannidou[1], Xantha Coene[1], Wim Teughels[2], Tom Van de Wiele[1], Nico Boon[1]*

[1] Center for Microbial Ecology and Technology (CMET), Ghent University

[2]Department of Oral Health Sciences, KU Leuven

[*]nico.boon@ugent.be

The oral microbiome consists of many commensal and beneficial microorganisms that reside in the mouth by forming biofilms. Environmental perturbations can destabilize this commensal biofilm and stimulate the outgrowth of pathogenic strains, leading to disease (e.g. dental caries, periodontitis). Oral diseases are commonly treated with antiseptics (e.g. chlorhexidine). The use of broad-spectrum antimicrobials disturbs the total microbial community and cannot guarantee a shift to a healthy state. This study aims to identify the effect of antiseptics on the composition and metabolism of oral biofilms.

We used two different in vitro models: a synthetic 14-species community (more controllable) and poly-microbial communities from tongue swab samples (more representative). The biofilms were daily treated with 0.12% chlorhexidine for 5 minutes. Microbial survival was assessed with live/dead flow cytometry. Community composition was defined with PMA-qPCR and 16S rRNA gene amplicon sequencing for the synthetic and the multi-species communities respectively. Metabolic activity was monitored by organic acid production.

The chlorhexidine treatments resulted in a temporal 3 log decrease of the bacterial concentration. However, within 2 days, the biofilms regrew reaching similar levels as the initial concentration, yet with a different composition and a different metabolic profile. The 14-strains community shifted to a high *Streptococcus* dominance and increased lactate production (+22mM). On the other hand, the response from the tongue swab microbiota displayed pronounced donor variability with one of the samples moving towards a *Fusobacterium* monodominance and a higher butyrate production (+1mM).

The biofilms treated with chlorhexidine exhibited a pattern of kill and regrowth maintaining similar bacterial concentrations. At the same time, in both models the composition and metabolic activity shifted in an inoculum dependent manner. In some cases, disease associated traits were even increased. All the above confirms our hypothesis and highlights the need for alternative treatments that selectively target the disease-associated bacteria in the biofilm without targeting the beneficial bacteria.

C04. Daptomycin enhances nafcillin and ceftaroline activity against *S. epidermidis* biofilms

Yvan Diaz Iglesias[1], Françoise Van Bambeke[1]*

[1]Université catholique de Louvain, Brussels, Belgium

**francoise.vanbambeke@uclouvain.be*

Objectives: to study the activity of the combination of daptomycine with nafcillin and ceftaroline against *Staphylococcus epidermidis (S.e.)* biofilms, which can play a role in joint infections.

Methods: *S.e.* YBara was used. Biofilms were grown in 96-well plates in TGN (TSB + 1% Glucose + 2% NaCl). Mature biofilms were exposed during 24h to daptomycin [DAP], nafcillin [NAF] or ceftaroline [CPT] alone using a broad range of conc. (0.001-1000 mg/L to obtain full conc.-response curves). To assess the combination, mature biofilms were exposed during 24h to a fix concentration of daptomycine (1 or 10mg/L, corresponding approximately to free C_{min} and C_{max} in human serum) and a broad range of beta-lactams. Biomass was evaluated by crystal violet staining and bacterial viability, using the resazurin assay. Pharmacodynamic parameters (E_{max}; maximal efficacy [reduction in viability/biomass for an infinitely large antibiotic conc.]; C_{50}; conc. causing 50% reduction in viability/biomass) were calculated based on the Hill equation of the regression fitted to the data.

Results: First, antibiotics were tested alone against 24h biofilms, all drugs showed concentration-dependent activity, with only daptomycin causing almost complete reduction in viability (maximal efficacy 97%) while beta-lactams had a maximal effect close to 50 % reduction.

Then, beta-lactams were combined with daptomycin. For nafcillin, we observed a synergistic effect with the highest concentration of daptomycine and a gain of 25% of reduction, the C_{50} became infinitively low (figure right panel). For ceftaroline, an additive effect was observed with 1 mg/L daptomycine (figure, left panel), while indifference was observed with 10 mg/L daptomycin (efficacy similar to that of the most active drug in the combination, i.e. daptomycin).

Conclusions: In this model, nafcillin was most active against viability of *S.e.* biofilm when combined with daptomycin while ceftaroline only shows additive effect when combined to daptomycin.

C05. The polyamino-isoprenic efflux inhibitor NV716 re-sensitizes old disused antibiotics against *Pseudomonas aeruginosa*

Gang Wang[1], Jean-Michel Brunel[2], Paul M. Tulkens[1], Jean-Michel Bolla[2], Françoise Van Bambeke[1]

[1]Université catholique de Louvain, Brussels, Belgium

[2] Aix-Marseille Université, Marseille, France

Objective: WHO considers *Pseudomonas aeruginosa* (PA) as a priority pathogen for the search of innovative therapies. PA is indeed intrinsically resistant to many antibiotics due to poor outer membrane (OM) permeability and/or active efflux (mediated essentially by 4 pumps belonging to the RND family, namely MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM). Our aim was to evaluate the capacity of efflux pump inhibitors (EPIs) to restore the activity of old, disused antibiotics, against PA. We compared PAβN to original polyamino-isoprenic compounds, namely NV731 and NV716, in combination with ciprofloxacin (CIP), doxycycline (DOX), chloramphenicol (CHL), all substrates for efflux, and rifampicin (RIF; not substrate).

Methods: Susceptibility testing: MICs were measured by serial 2-fold microdilution in CA-MHB with or without EPIs against reference strains, PAO1 (wild type) and its derivative PA403 (deletion mutant of PAO1; no expression of the four aforementioned pumps), and clinical isolates. NPN uptake: The uptake of the lipophilic probe N-phenyl-1–naphtylamine (10µM NPN, substrate for MexAB-OprM) after 10 min incubation was used to evaluate the MexAB-OprM pumping activity in reference strains. 10 mM Mg^{2+} was added in selected experiments to stabilize the LPS layer by cross-bridging.

Results: See table. In reference strains, the MIC of CIP, DOX and CHL were 3-5 doubling dilutions lower in PA403 compared to PAO1. NV731 only decreased the MIC of DOX against PA403, and PAβN, the MIC of CIP, DOX and CHL against PAO1 and that of RIF against PA403. NV716 markedly reduced the MIC of all drugs except that of CIP against PA403, while NV716 effects on CHL MICs were reduced in PAO1 and abolished in PA403 in the presence of 10 mM Mg^{2+}. In clinical isolates, MIC_{50} and MIC_{90} of all drugs were high, and significantly reduced by NV716 for all drugs and by PAβN or NV731 for DOX only. In PAO1 and PA403, NV731 had no effect on NPN accumulation while PAβN and NV716 markedly increased NPN accumulation; yet, the effect of NV716 was inhibited by 10 mM Mg^{2+} in PA403 but not in PAO1.

Conclusion: In contrast to NV731 that has almost no effect and PAβN that acts essentially as an EPI, NV716 is able to re-sensitize both PAO1 and PA403 to antibiotics whether substrates (DOX, CHL and CIP) or not (RIF) for efflux (lower MIC_{50} and MIC_{90} for all drugs). The fact that the effects of NV716 on MICs and NPN accumulation are counteracted by Mg^{2+} especially in PA403 (no efflux pump expressed) suggests an additional mode of action consisting in a destabilization of the OM. According to our data, NV716 may appear as a useful adjuvant to revive the activity of old drugs with low antipseudomonal activity against PA infections.

Experimental conditions	**MIC (mg/L)[a] of Antibiotics**							
PAO1	**CIP**		**DOX**		**CHL**		**RIF**	
Antibiotic (AB) alone	0.25		32		32/(128)[d]		16	
AB + NV716 (2.5µM)	**0.0625**		**2**		**2 /(32)**		**0.25**	
AB + NV731 (2.5µM)	0.25		16		32		16	
AB + PAβN (40 µM)	**0.0625**		**8**		**8**		8	
PA403								
AB alone	0.008		1		2/(16)		16	
AB + NV716 (2.5µM)	0.008		**0.063**		**0.5**/(8)		**0.25**	
AB + NV731 (2.5µM)	0.008		**0.25**		1		16	
AB + PAβN (40 µM)	0.008		1		2		**4**	
Clinical isolates (n=67)	**MIC_{50}[b]**	**MIC_{90}[c]**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
AB alone	0.5	8	16	64	64	256	16	32
AB+PAβN(40 µM)	0.25	4	**4**	**8**	32	128	8	16
AB+716(2.5µM)	**0.125**	**2**	**0.5**	**2**	**2**	**16**	**1**	**4**
AB+731(2.5µM)	0.5	8	**4**	16	32	128	16	16

[a] values in bold denote a decrease of at least 2 doubling dilutions vs. ABs alone

[b] MIC_{50} and [c] MIC_{90}: antibiotic concentration that inhibits the growth of 50% and 90% of clinical isolates respectively.

[d] values between brackets are MICs measured with 10 mM Mg^{2+}

C06. SECOND TRIMESTER ABORTION CAUSED BY SNEATHIA AMNII: A CASE REPORT

L. Gruwier[1], A. Sprenkels[1], S. Hulsbosch[1], A. Vankeerberghen[2], R. Cartuyvels[1]*

[1]Jessa hospital, Hasselt, Belgium
[2]OLV hospital Aalst, Belgium

[*]lien.gruwier@gmail.com

Sneathia amnii (formerly designated as *Leptotrichia amnionii*) was first described in 2002 in the USA. Members of the genus *Sneathia* can be part of the normal flora of the genitourinary tract, but have been implicated in invasive (mostly gynaecological) infections. To our knowledge, we hereby present the first case of *S. amnii* infection in Belgium, in a woman with spontaneous second trimester septic abortion. *S. amnii* was identified as the causative micro-organism by 16S-RNA gene sequencing after extracting bacterial DNA directly from positive blood cultures.

C07. Exploring the virulence pattern and antibiotic resistance of *Escherichia coli* strains isolated from diarrheal stool in Benin

Houngbégnon Olivia[1] (oliviahoungbegnon@yahoo.fr), Dougnon Victorien[1], Mariani-Kurkdjian Patricia[2], Bankolé Honoré[1]

[1]*Reasearch Unit in Applied Microbiology and Pharmacology of natural substances, Reasearch Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, 01 PO BOX 2009 Cotonou, Benin*

[2]*Service de Microbiologie, CNR associé Escherichia coli, Hôpital Robert Debré, 48 Boulevard Sérurier, 75019 Paris, France*

Abstract:

This research study aims to probing the virulence genes of *Escherichia coli* isolated from children's diarrheal stool samples upon admission in two hospitals in Cotonou, Benin. A collection of 100 *E. coli* strains were isolated and characterized for five intestinal virulent genes through a multiplex PCR. The characterization was supplemented by a survey of the antibiotic-resistance of these strains. Among the studied virulence genes, only the intimine coding gene, *eae* gene was found in a proportion of 9%. Moreover, *E. coli* strains show higher resistance to Ampicillin (82%), Tetracycline (79%), Trimethoprim Sulfamethoxazole (77%), Amoxicillin + Clavulanic Acid (75%) and strong sensitivity to Imipenem. By way of conclusion, the positive *eae*-isolation of *E. coli* implies that this pathogen is an important etiology of gastroenteritis in Benin.

Keywords: Virulent genes, *Escherichia coli*, Antibiotics- resistance, Benin

C08. Fragment Screening of Catalytically Active HIV-1 Reverse Transcriptase with Bound DNA Template-Primer

Sergio E. Martinez[1,2], Abhimanyu Singh[1,2], Brent De Wijngaert[1,2], Hans Vanbuel[1,2], Kalyan Das[1,2*]

[1]*Division of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, 3000 Leuven, Belgium*
[2]*Department of Microbiology, Immunology and Transplantation, KU Leuven, Belgium*

[*]*kalyan.das@kuleuven.be*

HIV-1 reverse transcriptase (RT) copies the RNA viral genome into a DNA copy that is integrated into the host cell chromosome. Given the importance of this step in the infection process, RT is a target of many of the drugs used to treat HIV. These RT inhibitors in clinical use come in two groups: the nucleos(t)ide RT inhibitors (NRTIs) have a base and a cyclic or acyclic ribose ring, but lack a 3' hydroxyl group, and non-nucleoside RT inhibitors (NNRTIs) that bind to a common hydrophobic pocket near the polymerase active site.

Emergence of resistance mutations and side effects to the current drugs necessitate the need for new HIV drugs. Structural biology plays a pivotal role in drug design. RT slides over a dsDNA or an RNA/DNA substrate in the process of DNA polymerization. Sliding of RT over DNA in our RT/DNA crosslinking complex results in a new crystal form that traps two distinct conformations of the enzyme, and each of the two states can be a drug target. The states are - (i) following a nucleotide incorporation and prior to translocation (N-state) and (ii) when the 3'-end of the primer is positioned one nucleotide short of the priming site (i.e. P-1 state). This crystal form helps understand how the enzyme slides over RT to accomplish DNA polymerization.

Recently, we have conducted a systematic fragment screening by X-ray crystallography using the one of a kind, state-of-the-art XChem facility at Diamond Light Source (UK); the programme was funded by iNEXT. This project targets two distinct conformational states of RT/DNA complex for the binding of drug-like fragments. In this study, about 300 individual fragments are soaked into individual crystals. The structures and the progress in the fragment-screening project will be presented.

C09. Mobilization of microflora commensals and their weapons for therapy

J. Mignolet[1,2], G. Cerckel[2], J. Damoczi[2], P. Gabant[1], P. Hols[2]

[1]Syngulon, Rue du Bois de Saint-Jean 15/1, B-4102 Seraing, Belgium.

[2]University of Louvain (LIBST/BGM) 5 Croix du Sud B-1348 Louvain-La-Neuve Belgium

With the specter of resurgence of pathogens due to the propagation of antibiotic resistance genes, innovative antimicrobial strategies are needed. In a synthetic biology scope, we assessed the potential of bacteriocins, a set of miscellaneous peptide-based bacterium killers, and considered their use in cocktails to curb emergence of new resistance. Complementarily, we investigated how we might exploit prevailing bacterial commensals such as *Streptococcus salivarius* and deliberately mobilize their bacteriocin arsenal 'on site' to cure multi-resistant infections or finely reshape the endogenous microbiota for prophylaxis purposes. In this species, the cytoplasmic regulator ComR couples competence for natural transformation to bacteriocin-mediated predation. We designed a robust genetic screen to unveil potent/optimized signaling pheromones disconnecting predation from competence in order to use the antimicrobial properties of our model organism without enhancing its capacity to mutate or acquire new genes. We described a paralogous sensor duo, ScuR and SarF, which circumvents ComR in order to desynchronize competence and predation. Finally, we dissected the switchable selectivity toward their pheromone and operating sequences at the subtle molecular level. This highlights how bacteria discriminate between relevant pheromones in a cacophony of signaling molecules to execute appropriate behavior.

C10. A unique class of lignin derivatives displays broad anti-HIV activity by interacting with the viral envelope

Merel Oeyen[1], Sam Noppen[1], Emiel Vanhulle[1], Sandra Claes[1], Bernt O. Myrvold[2], Kurt Vermeire[1], Dominique Schols[1]*

[1]Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Department of Microbiology, Immunology and Transplantation, KU Leuven, Herestraat 49, 3000 Leuven, Belgium

[2]Borregaard LignoTech, P.O. Box 162, N-1709 Sarpsborg, Norway

[*]dominique.schols@kuleuven.be

We have previously shown that lignosulfonic acid, a commercially available lignin derivative, possesses broad antiviral activity against human immunodeficiency virus (HIV) and Herpes simplex virus (HSV) by preventing viral entry into susceptible target cells. Because of the interesting safety profile as potential microbicide, we now determined the antiviral activity of a series of lignosulfonates in order to understand better which molecular features can contribute to their antiviral activity. Here, 24 structurally different lignosulfonates were evaluated for their capacity to inhibit HIV and HSV transmission and replication in various cellular assays. These derivatives differ in origin (hardwood or softwood), counter-ion used during sulphite processing (Na^+, Ca^{2+}, or NH_4^+), sulphur content, carboxylic acid percentage, and molecular weight fraction, which allowed to determine structure-activity relationships. We demonstrate that the broad antiviral activity of lignosulfonates is mainly dependent on their molecular weight and that their mechanism of action is based on interactions with the viral envelope glycoproteins. This makes the lignosulfonates a potential low-cost microbicide that protects women from sexual HIV and HSV transmission and thus prevents life-long infection.

C11. Understanding PBP5 regulation and β-lactam resistance mechanism in *Enterococcus hirae and faecium*

*Alice Raymackers, Olivier Verlaine, Ana Amoroso, Bernard Joris**

Laboratory of Bacterial physiology and genetic - CIP - InBioS - ULiège, Institut de Chimie, Allée du 6 Août 13, Liège, Belgium

Corresponding author: alice.raymackers@doct.uliege.be

Enterococci are among the leading causes of nosocomial infections. They present intrinsic resistance to β-lactams. These antibiotics inhibit the final step of peptidoglycan synthesis by binding covalently to the membrane PBPs (Penicillin-Binding Proteins). In *E. faecium* and *E. hira*e, low affinity PBP5 is responsible for resistance to β-lactams. The intrinsic resistance is further enhanced by overexpression of PBP5, combined with other essential factors that contribute to high level resistance that remain still unknown. In *E. hirae* and *E. faecium*, *pbp5* is included in an operon composed by 3 genes: *ftsW* and *lcpA* both situated upstream *pbp5*. *ftsW* (Filamenting Temperature Sensitive W) encodes for a membrane protein featuring 10 membrane-spanning segments belonging to the SEDS (Shape, Elongation, Division and Sporulation) family. LcpA's function is still unknown but it appears that it interacts with peptidoglycan through non-ionic interactions and catalyses the transfer of a rhamnose containing polysaccharide (like the Enterococcal polysaccharide antigen) from a lipidic transporter, associated with a SEDS protein, such as FtsW, on *E. hirae* peptidoglycan.

With the aim of identifying the unknown element/s involved in β-lactams resistance, *E. hirae* mutants were selected on penicillin gradients of increasing concentrations. Shifts of the previously described operon between strains, allowed to identify mutations responsible for *pbp5* overexpression. Furthermore, a Mg^{2+}-riboswitch, called M-box, was identified *in silico* upstream the operon. The contribution of Mg^{2+} to Mbox conformation was characterized *in vitro* and *in vivo*. Finally, wide genome sequencing of hyper-resistant mutants allowed to highlight other genes potentially involved in resistance: their contribution was confirmed by gene knock-out and further mutant complementation. Overall, results indicated that high level resistance in enterococci is a multifactorial event.

C12. Viral Compartmentalization and Rapid Evolution of Drug-resistant Herpes Simplex Virus (HSV-1) Infection in a Hematopoietic Stem Cell Transplant (HSCT) Patient

Hanna Schalkwijk[1], Marijke Reynders[2], Dominik Selleslag[3], Sarah Gillemot[1], Robert Snoeck[1], and Graciela Andrei[1]*

[1]Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Belgium

[2]Department of Laboratory Medicine, AZ Sint-Jan Brugge, Belgium

[3]Department of Internal Medicine, AZ Sint-Jan Brugge, Belgium

[*] Hanna.Schalkwijk@kuleuven.be

Background. HSV-1 is a prevalent human pathogen that can cause severe disease in immunocompromised patients. These patients often require prolonged antiviral therapy increasing the risk of drug-resistance. Investigating the evolution of HSV infections is crucial for understanding pathogenesis and emergence of drug-resistance.

Objectives. HSV-1 compartmentalization, viral evolution, emergence of drug-resistance and heterogeneity of viral populations were investigated in an HSCT recipient in order to improve the management of herpesvirus infections.

Materials and Methods. Five HSV-1 isolates were recovered from an HSCT patient who had recurrent orofacial infections treated consecutively with acyclovir, foscavir and cidofovir. Drug-resistance was determined by genotyping [conventional Sanger sequencing of the viral thymidine kinase (TK) and DNA polymerase (DP) genes and phenotyping (drug-susceptibility profile)]. Next generation sequencing (NGS) was performed on the clinical isolates in order to quantify the viral variants present. Plaque purification was performed on the clinical isolates in order to isolate homogeneous populations of the viral variants. One representative viral clone per variant was selected and drug-susceptibility profiles were performed retrospectively.

Results: Prospective analysis of the HSV-1 isolates showed evolution of the drug-resistance mutations over time resulting in acyclovir resistance in all five isolates and foscarnet resistance in three of the isolates. In one of the isolates, heterogeneous populations of TK and DP mutants were detected by Sanger sequencing. NGS performed retrospectively showed heterogeneity of HSV-1 viral populations in most viral isolates. A minor emerging population of foscarnet resistant virus could be identified by NGS, which was not detected by Sanger Sequencing. In order to study the effects of the mutations detected in the isolates, plaque purification was performed on the clinical isolates to obtain clones of each viral variant present. In total, six different types of viral clones harboring different drug-resistant mutations were obtained. Two isolates from distinct body sites recovered on day 126 post-transplantation showed different viral populations (compartmentalization). While only one type of viral clone, i.e. TK(T183P+R222H)+DP(L778M), could be obtained from one of the body sites, four distinct types of viral clones were obtained from the other body site, i.e. TK(T183P)+DP(L778M), TK(A189V)+DP(L778M), TK(T183P)+DP(L802F) and TK(A189V)+DP(L802F). For each viral variant, one representative clone was selected to perform a drug-susceptibility profile. This showed that the previously undescribed T183P amino acid change in the viral TK had no effect on drug-susceptibility, pointing to a natural polymorphism phenotype.

Conclusion: A rapid viral evolution was observed with six different viral variants (TK mutant, DP mutant, or double mutant) appearing within 4 months. Compartmentalization and hetrogeneity of viral populations was observed. Characterization of drug-resistance at multiple time points and at multiple body sites is useful to adjust antiviral therapy and avoid emergence of multi-drug resistance.

C13. CRISPR/Cas9 editing of the polyomavirus tumor antigens inhibits Merkel cell carcinoma growth in vitro

Arturo Temblador[1,], Dimitrios Topalis[1], Graciela Andrei[1], Robert Snoeck[1]*

[1]Laboratory of Virology and Chemotherapy, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, KU Leuven, Belgium

**arturojesus.tembladorperez@kuleuven.be*

Background

Merkel cell carcinoma (MCC) is a rare but aggressive type of skin cancer with a mortality rate between 33-46%. Around 80% of MCC cases are associated with integration of the Merkel cell polyomavirus (MCPyV) DNA in the genome of the tumor cells and clonal expansion of transformed cells. MCPyV-positive ($MCPyV^+$) tumor cells constitutively express two main viral oncoproteins: the small (sT) and large (LT) tumor antigens (TAs). The integrated virus harbours truncating mutations in the sequence of the LT that hamper viral replication but leave intact the retinoblastoma-binding domain, which is required to promote cell growth and tumor progression. Among the diverse functions of MCPyV sT, the LT-stabilization domain (LSD) avoids degradation of the LT and cellular oncoproteins. The aim of this study was to investigate the CRISPR/Cas9-mediated genome editing of the viral tumor oncoproteins as a new approach to impair proliferation of tumor cells. Additionally, further effects of this disruption were examined in $MCPyV^+$ MCC cell lines.

Methods

Two $MCPyV^+$ cell lines (MS-1 and WAGA) were transfected with three different vectors expressing the Cas9 endonuclease and one specific sgRNA: two sgRNAs were designed to target the genomic sequence of the viral TAs (the sT/LT-sgRNA and LT-sgRNA) and a non-targeting sgRNA (ctr-sgRNA) was included as negative control. In addition, we evaluated the off-target activity of the targeting sgRNAs by transfecting HEK293T cells, a $MCPyV^-$ cell line that expresses the LT of SV40.

Results

The TAs-targeting sgRNAs (sT/LT-sgRNA and LT-sgRNA) induced frameshift mutations in the sequence of the MCPyV TAs that led to decrease levels of LT protein. TAs-targeting also impaired cell proliferation, caused cell cycle arrest and increased apoptosis, whereas the control HEK293T cells remained unaffected, as well as those cells expressing the non-targeting sgRNA (ctr-sgRNA). Moreover, WAGA cells had an altered expression of cellular proteins involved in cell cycle progression after CRISPR/Cas9 editing of the viral oncoproteins.

Conclusions

The use of CRISPR/Cas9 system to target MCPyV TAs selectively impaired the proliferation of $MCPyV^+$ cells, supporting its further validation as a potential therapeutic strategy for $MCPyV^+$ MCC lesions refractory to classic treatment options. Our data confirmed previous findings regarding the importance of the viral TAs to support the growth of MCC cell lines. In addition, we obtained more insights into the involvement of MCPyV LT in the mechanisms of cell cycle regulation.

C14. *In vitro* and *in vivo* assessment of lytic bacteriophages against *Staphylococcus aureus* causing bovine mastitis

Ngassam Tchamba C.[1], Duprez J.N.[1], Fergestad M.[2], De Visscher A.[3], L'Abee-Lund T. [2], De Vliegher S. [3], Wasteson Y. [2], Blanchard Y.[4], Lavigne R.[5], Chanishvili N.[6], Cassart D.[7], Mainil J.[1], Thiry D.[1]

[1]*Department of Infectious and Parasitic Diseases, Bacteriology, FARAH, ULiège, Belgium*

[2]*Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Norway*

[3]*M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health,, Faculty of Veterinary Medicine, Ghent University, Belgium*

[4] *Viral Genetics and Bio-security Unit, ANSES, Ploufragan-Plouzané laboratory, France*

[5] *Laboratory of Gene Technology, Department of Biosystems, KU Leuven, Heverlee, Belgium*

[6]*R & D Department, Eliava Institute of Bacteriophages, T'bilisi,Georgia*

[7] *Department of Morphology and Pathology, FARAH, ULiège, Belgium*

Corresponding author: *damien.thiry@uliege.be*

The use of antibiotic against *Staphylococcus aureus* causing bovine mastitis can lead to the emergence of Livestock-Associated Methicillin Resistant *Staphylococcus aureus* (LA-MRA) which represent a potential hazard for public health. A biological antimicrobial agent known as bacteriophage has been identified as a potential solution to tackle this problem. The aim of this study was to assess the efficacy of four lytic bacteriophages on a collection of *Staphylococcus aureus* isolated from cow with mastitis in Belgium and Norway, by *in vitro* and *in vivo* assays (Galleria mellonella larvae and mice model). Between May and December 2016, 10 *Staphylococcus aureus* isolates including 5 MRSA and 5 methicillin sensitive *S. aureus* (MSSA) were isolated from milk samples of cows with mastitis in Belgium and Norway. After phenotypic and genetic characterization, the isolates were assessed *in vitro* for their susceptibility to four lytic bacteriophages (Romulus, Remus, ISP and DSM105264) and then *in vivo* on *Galleria melonella* larvae and on a murine mastitis model. The *in vitro* assay has shown the lytic activity of these 4 bacteriophages on 9 *S. aureus strains,* while the *in vivo* assay has shown that the larvae survival rate was below 50% after 4 days post inoculation and that the recovery of the mice mastitis was incomplete after 48h post inoculation. However, a statistically significant difference was observed in the results between infected-PBS treated and infected-phage treated groups in the *G. mellonella* and the murine models suggesting an effect of the phage therapy on mastitis caused by *S. aureus*.

C15. Triplex PCR to detect CTX-M-1, CTX-M-2 and CTX-M-9 extended-spectrum-β-lactamase-encoding genes in bovine *Escherichia coli* isolates

Guérin V.[1], Thiry D.[1], Duprez J-N.[1], Mainil J.[1]*

1. Bacteriology, Department of Infectious and Parasitic Diseases, FARAH and Faculty of Veterinary Medicine, ULiège, Belgium

[*]*damien.thiry@uliege.be*

Extended-spectrum-BLA (ESBL) confer a resistance to some antibiotics classified as critical in human medicine, like 3rd/4th generation cephalosporins. Though the use of critical antibiotics in livestock is regulated since 2016, it remains important to follow the evolution of ESBL resistance, especially in enterobacteria. The aim of the study was therefore to identify the ESBL-encoding genes in *Escherichia (E.) coli* from young calves in Wallonia with an ESBL phenotype at the disk diffusion assay. During 2 calving seasons (A: 2017-2018 and B: 2018-2019), 152 (A) and 161 (B) *E. coli* with ESBL resistance profiles were collected at ARSIA from calves with enteritis or septicaemia. Of them, 50 were tested with microarrays and only $bla_{\text{CTX-M}}$ genes, coding for cefotaximases, were detected. Based on these results, all 313 *E. coli* of the collection were tested with PCR for the different genes coding for the CTX-M-1, CTX-M-2 and CTX-M-9 ESBL groups: 103 (A) and 84 (B) were positive for the CTX-M-1 group; 26 (A) and 24 (B) isolates, for the CTX-M-2 group; 25 (A) and 37 (B) isolates, for the CTX-M-9 group; and 4 (A) and 5 (B) isolates were negative. In 4 (A) and 1 (B) isolates, genes coding for CTX-M-1 and CTX-M-2 groups were simultaneously detected. In conclusion, the $bla_{CTX\text{-}M}$ genes are the most prevalent ESBL-encoding genes in our collection and of them, the genes coding for the CTX-M-1 group are the most prevalent, as described in the literature. The PCR-negative *E. coli* will be further tested with PCR for the other $bla_{CTX\text{-}M}$ genes, if any. Moreover, a third collect of isolates is already planned during the next calving season to follow the prevalence of ESBL *E. coli*.

C16. Identification of Shigatoxigenic (STEC) and enteropathogenic (EPEC) *Escherichia coli* O80 in young calves with diarrhea.

Habets A.[1], Duprez J-N.[1], Iguchi A.[2], Saulmont M.[3], Lucas P.[4], Blanchard Y.[4], Thiry D.[1], Mainil J.[1]

[1]*Bacteriology, Parasitic and Infectious Disease, FARAH, ULiège.*
[2]*Department of Animal and Grassland Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan.*

[3]*Regional Agency for Animal for Health and Identification, Ciney, Belgium.*

[4]*Viral Genetics and Bio-security Unit, ANSES, Ploufragan-Plouzané laboratory, Ploufragan, France*

Corresponding author: *damien.thiry@uliege.be*

Escherichia coli producing the Shiga toxins (STEC) and/or the attaching-effacing (AE) lesion (EPEC) cause enteritis and (bloody) diarrhea in young calves and in humans. STEC and EPEC can belong to 7 serogroups frequently identified worldwide: O26, O103, O111, O121, O145, O157 and O165. Beside these classical "gang of 7", unconventional serogroups can be identified as previously demonstrated with the zoonotic O80 EPEC detected for the first time in diarrheic calves in Belgium between 2008 and 2015. The purposes of this project were (i) to identify 7 unconventional serogroups among 279 STEC and EPEC isolated between 2008 and 2017 from diarrheic calves at ARSIA (ii) to identify the virulence genes and the MLST, and to confirm the serotypes of a selection of these strains by whole genome sequencing. Two triplex PCRs have been applied either for the O146_O182_O183 serogroups or for the O123/186_O156_O177 serogroups and one uniplex PCR for the O80 serogroup. So far, the first triplex PCR identified 4 O182-positive and 2 O183 positive. The second triplex PCR identified 12 O123/186-positive, 3 O156-positive and 29 O177-positive STEC and EPEC. The uniplex PCR identified 20 O80-positive EPEC and STEC. The MLST 29, 300, 342 and 765 were detected. The variants *eaeζ*, *stx1a* and *stx1c* were predominantly found. In addition to the *eae*, *stx1* and *stx2* genes, many other virulence genes were found. The further steps of this study will be to search after the O80 serogroup among STEC and EPEC isolated from healthy cattle and to answer the following question: are these calf EPEC true EPEC, STEC derivatives that lost *stx* genes or STEC precursors that could acquire *stx* genes in the future?

C17. The effect of fosmidomycin prodrugs against Acinetobacter baumannii biofilms

Frits van Charante[1], Charlotte Courtens[2], Serge van Calenbergh[2], Tom Coenye[1]

[1] Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

[2] Laboratory of Medicinal Chemistry, Ghent University, Ghent, Belgium

Contact: Frits van Charante, Frits.Vancharante@UGent.be

Isoprenoids can be produced through the mevalonate and/or the non-mevalonate pathway and multiple bacterial pathogens exclusively use the non-mevalonate pathway for isoprenoid synthesis. One of these pathogens is *Acinetobacter baumannii*, which is a responsible for various types of (nosocomial) infections. With increasing antibiotic resistance, also *A. baumannii* infections are becoming more difficult to treat, and novel drugs are needed. Fosmidomycin was originally developed as an antimalarial drug that targets the non-mevalonate pathway for isoprenoid synthesis and as *A. baumannii* uses this pathway fosmidomycin and its (prodrug) derivatives are interesting candidates for novel antimicrobial therapy.

The aim of this study is to verify the activity of the fosmidomycin prodrug CC366, identified in a previous screening, against biofilms of *A. baumannii*.

The prodrug was evaluated for its ability to inhibit biofilm formation as well as for its biofilm-eradicating ability, using a 96 well microtiter plate model and nine *A. baumannii* strains. The activity of the compound was subsequently also investigated in a more complex artificial dermis model that simulates the wound environment in vitro. Finally, a simple *in vivo* model, *C elegans*, was used to verify activity in an *in vivo* system.

The CC366 prodrug is able to fully inhibit biofilm formation 6 of the tested strains in the 96 well microtiter plate model at 16 µg/mL. This includes 4 strains that are resistant to ceftazidime at 16 µg/mL. Biofilm eradication by CC366 was similar to that achieved by aztreonam and ceftazidime at 16 or 32 µg/mL, up to about 60% eradication. In the artificial dermis model inhibition with CC366 resulted in a 3 log reduction in CFU at 32 µg/mL. In the *C. elegans* model CC366 was able to prevent death of the nematodes even when an antibiotic resistant strain was used.

The CC366 prodrug is an effective compound for inhibiting biofilm formation from *A. baumannii*. We are currently investigating the compound in further models and the possibilities of integrating it into wound dressings.

C18. Applying machine learning-based algorithms in metaproteomics leads to an increased protein and species identification rate

Tim Van Den Bossche[1,2], Ana C. S. Silva[1,2], Bernhard Y. Renard[3], Thilo Muth[3] and Lennart Martens[1,2]*

[1] *CompOmics, VIB – UGent Center for Medical Biotechnology, Ghent, Belgium*

[2] *Department of Biomolecular Medicine, Ghent University, Belgium*

[3] *MF1-Bioinformatics, Robert Koch Institute, Berlin, Germany*

[*] *Lennart.Martens@UGent.be*

Metaproteomics, the study of the collective proteome of whole microbial ecosystems, has seen substantial growth over the past few years. This growth is driven by the increased awareness that metagenomics and metatranscriptomics can be powerfully supplemented with protein analysis in order to resolve the main functional components driving these microbial ecosystems (Wilmes et al., 2015). This is, for example, clearly illustrated by the combined efforts of the Integrative Human Microbiome Project (iHMP) Research Consortium. Despite this increased awareness, the field still suffers from low identification rates in comparison to single-species proteomics. The underlying challenge here, is a lack of sequence resolution in the current identification algorithms, which are typically designed for single-species proteomics (Colaert et al. 2011, Muth et al. 2015).

To solve this issue, we applied the recently developed, machine learning-based ReScore algorithm on several multi-species, metaproteomics datasets (Silva et al., 2019). ReScore is a post-processing tool that re-evaluates peptide-to-spectrum-matches (PSMs) based on predicted fragment ion peak intensities. To achieve this, ReScore combines two, well-established machine learning-based algorithms: Percolator, which re-scores PSMs based on the search engine output (Käll et al., 2007), and MS2PIP, which predicts fragment ion peak intensities given a peptide's sequence, charge and modifications (Degroeve et al., 2013). In the ReScore algorithm, the search engine-dependent features of Percolator are replaced with intensity features of MS2PIP. When ReScore is applied on metaproteomics datasets, it performs similar to Percolator. However, when both feature sets from Percolator and MS2PIP are combined, a significant improvement can be achieved.

When the updated ReScore algorithm is applied on metaproteomics datasets, our results show that ReScore leads to an increased identification rate, ranging from the number of PSMs to the taxonomical level, while the false discovery rate (FDR) remains under full control as validated in an entrapment experiment with Pyrococcus furiosus (Vaudel et al., 2012).

ABSTRACTS SHORT TALKS
SECTION D: HOST AND MICROBIAL INTERACTIONS

D - ST01 - Hidden in plain sight - proteogenomic view of bacterial proteoform expression in bacterial infection.

Igor Fijalkowski[1*], *Veronique Jonckheere*[1], *Patrick Willems*[1], *Daria Fijalkowska*[1], *Petra Van Damme*[1*]

[1]*Department of Biochemistry and Microbiology, UGent, Belgium*

[*]*Petra.VanDamme@UGent.be*, *Igor.Fijalkowski@UGent.be*

The rapid increase in the number of sequenced bacterial genomes has rendered the use of automated gene prediction algorithms indispensable in modern genomics. Despite their utility, these programs have been shown, by us and others, to underestimate the complexity of bacterial proteomes. In particular, the incorrect assignment of translation initiation sites and the existence of alternative initiation sites giving rise to previously undetected protein variants (N-terminal proteoforms) along with the multitude of small open reading frames (sORFs) has been largely overlooked. With recent advancements in the high-throughput techniques utilizing next generation sequencing to study protein translation, unravelling the specific repertoire of expressed proteins by the bacterial cell became possible with an unprecedented accuracy. In particular, ribosome profiling allowed for the deep sequencing of mRNA fragments covered by ribosomes during protein synthesis, providing a snapshot of cellular translational activity at the moment of sample collection. With the new addition to the ribo-seq toolkit-retapamulin-assisted ribosome profiling, additional information on the genomic locations where translation initiation occurs can be elucidated. By combining ribosome profiling with retapamulin, as a specific blocker of bacterial translation initiation, this tool allows to not only improve currently available gene annotations, but also to do this in a condition-specific manner.

In this work, we combined the use of (retapamulin-assisted) ribosome profiling and proteomics (proteogenomics) to investigate the translational activity of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) under a wide range of conditions previously shown to be representative of various stages of host cell infection and to gain valuable insights into the true complexity of its proteome. Based on previously available transcriptomics data [1], we selected a representative and complementary subset of infection-relevant growth conditions next to a series of environmental stresses (low pH, anaerobic shock, low Mg^{2+}, nitric oxide shock, sodium chloride shock) in order to create comprehensive description of translational activity of *Salmonella* to improve the existing genome annotation. As a result, we confidently detected over 170 previously unannotated translation initiation events giving rise to nearly 130 alternative proteoforms (gene extensions, truncations, incorrectly assigned start sites) and 45 novel ORFs (intergenic, out-of-frame). Various newly delineated ORFs have been evaluated at multiple OMICs levels including transcriptomics, proteomics and additional metadata (e.g. conservation, presence of SD-like sequences).

With this work we demonstrate a refined proteogenomic workflow aiding in correcting existing errors in genome annotations and in investigation of complex biological processes, such as bacterial infection. With the combined use of multiple state-of the art OMICs technologies we provide the most comprehensive snapshot of *Salmonella* gene expression under infection-relevant conditions.

Reference:

1. Srikumar, S., et al., *RNA-seq Brings New Insights to the Intra-Macrophage Transcriptome of Salmonella Typhimurium.* PLoS Pathog, 2015. **11**(11): p. e1005262.

D - ST02 - Celecoxib builds up and cues metabolically active bacteria inhabiting the mucosal environment of a simulated human intestinal ecosystem, modulating inflammatory response

Emma Hernandez-Sanabria[1], Fabian Mermans[1], Ruben Props[1], Marta Calatayud Arroyo[1], Laurent Leclercq[2], Jan Snoeys [2], and Tom Van de Wiele[1]*

[1] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium. [2] Janssen Research & Development, A Division of Janssen Pharmaceutica NV, Turnhoutseweg 30, Beerse, Antwerp, 2340, Belgium.

** Tom.VandeWiele@Ugent.be*

Chronic inflammation is one of the hallmarks of colon cancer initiation and evidence supports the critical role of the pro-inflammatory enzyme COX-2 on tumorigenesis. Further, gut microbiota and their metabolites are interrelated with carcinogenic pathways and may interfere with the pharmacodynamics of anti-inflammatory drugs. Given this interplay, the gut microbiome represents a "druggable target" for ensuring delivery and efficacy. This study aimed at elucidating how this birectional flow impacts the functional and taxonomical features of the gut microbiome and the anti-inflammatory potency of celecoxib (a COX-2 inhibitor). We developed an in vitro platform to explore the interface between microbiome, mucus layer and epithelium, coupling a dynamic gut model with a cell model to mimic the intestinal metabolism of a colon-targeted celecoxib formulation. Long-term monitoring of a clinical dose of celecoxib using the Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME) indicated that functional and taxonomical inter-individual variability of the microbiome was preserved. Celecoxib-sensitive communities showed altered butyrate production, bacterial community structure and bacterial protein synthesis activities, indicating that celecoxib impacts in vitro fermentation in a donor-dependent manner. Additionally, celecoxib triggered taxa displacement from the luminal to the mucosal environment. However, bacteria-exposed celecoxib decreased inflammation and preserved barrier function in a cell model of gut inflammation, even after treatment withdrawal. Our results suggest that variability in gut microbiota features may result in heterogenous response to anti-inflammatory therapy. Strategies for guaranteeing efficient therapeutic interventions will require increased know-how of microbiome-mediated pharmacokinetics and may potentially involve screening patient's microbiome.

D - ST03 - Identification of bacterial genes indispensable to pulmonary Brucella infection in mouse experimental model

Potemberg G.[1,2*], *Galia M.*[1], *Demars A.*[1], *De Bolle X.*[1], *Muraille E*[2].

[1]*Microorganisms Biology Research Unit, University of Namur, Belgium.*

[2]*Laboratory of Parasitology, Université Libre de Bruxelles, Belgium.*

[*]*georges.potemberg@unamur.be*

Infection by the bacteria of the genus *Brucella* called brucellosis are common worldwide for both mammals and humans. Causing notably sterility and abortion, containment of the disease rely on mass slaughter of the cattle. Moreover chronicity and recurrence of this infection cause significant morbidity in human despite long and costly antibiotic treatments. In the actual context with still no efficient and save vaccine available, brucellosis still represents huge economical losses for endemic countries. The rational development of a safe efficient attenuated vaccine against *Brucella* infections require the identification of the virulence genes that are indispensable to *in vivo* replication of the bacteria.

In a well characterized intranasal infection model in the mouse mimicking the natural airborne infection, we have described the infection dynamic. Using fluorescent tracker, we are able to monitor the bacterial multiplication *in situ* and to determine the different phase of infection. During an intranasal infection, the alveolar macrophages (AM) are the major infected cell type but only a small proportion of the infected AM (5-15%) are permissive to the infection. Bacteria entering replication during the first 24h are massively eliminated but this important selective pressure can be partially lifted by genetic immune deficiencies for IL17RA$^{-/-}$ (TH17 immunity) or γδ T cells or even alteration of the immune response by inducing asthmatic phenotype (TH2 immunity).

An extensive identification of all the essential genes required for growth on rich media or the genes conditionally required for survival during *in vitro* (murine RAW macrophages) or *in vivo* (mouse) infection and this has been performed at different early key timepoint of the infectious cycle by using transposon sequencing (Tn-Seq) technique. On the 3140 *B. melitensis* genes, 643 are required for extracellular growth on rich media. An additional 179 genes are indispensable for survival in the mouse lungs up to 5 days post infection. Only half of these genes can be identified using the standard in vitro model, illustrating the limitation of such *in vitro* approach to identify the requirements for host environment adaptation. The application of clustering analysis illustrate that most of these genes identified can be reframed into comprehensive pathways or involved in linked functions. Lipopolysaccharides synthesis, the synthesis of some amino acids, the β oxidation of fatty acids and the cytochrome C oxidase seems notably specifically important facing host environment. We have now a clearer idea of the minimal requirements for the bacteria to successfully infect its host. Even though, by applying this approach in immunodeficiency or asthmatic condition, we now know that the essentiality of some genes can be lifted. Genetic deletion of the some selected genes (10) candidates validates the results of our Tn-Seq analyses. These comparative analyses have the potential to identify attenuated mutants strains that could trigger protective immunity without the ability to spread or becoming chronic or to be fully virulent even in immunocompromised individuals.

D - ST04 - The effect of airway epithelial cells on antibiotic efficacy towards cystic fibrosis clinical isolates of *Pseudomonas aeruginosa*

Sara Van den Bossche [1], *Charlotte Rigauts* [1], *Tom Coenye* [1], *Aurélie Crabbé* [1]

[1] *Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium*
Contact: Sara Van den Bossche, smavdnbo.vandenbossche@ugent.be

Pseudomonas aeruginosa is considered as one of the most important pathogens in the pathology of cystic fibrosis (CF), as 80% of the adult population is infected with this pathogen which is strongly correlated with a higher morbidity and mortality. In the CF lungs, *P. aeruginosa* forms biofilms, which are highly tolerant and resistant to antibiotics and result in chronic infections. Thus, providing an effective treatment for these infections is an important aspect in improving the quality of life and prolonging the life expectancy of CF patients.

The use of antibiotics is generally recommended for the treatment of *P. aeruginosa* chronic lung infections, but does not always lead to clinical improvements. Indeed, a main gap exists between the efficacy of antibiotics *in vitro* and in CF patients. A reason for this discrepancy might be that environmental factors of the CF lung are not considered when evaluating antibiotic efficacy. This includes host cells, such as lung epithelial cells, which have been previously shown to modulate the efficacy of antibiotics. However, it remains unknown whether this effect varies depending on the patient from whom the lung epithelial cells were derived (carrying a different genetic background).

To address this research question, a method was developed to co-culture *in vivo*-like three-dimensional
(3-D) models of different airway epithelial cell lines (including the CF cell lines CFBE and IB3 – derived from patients and the CF-corrected cell lines CFBE-wt and S9) with clinical CF isolates of *P. aeruginosa* (including AA2, AA44, AMT0060-2, AMT0060-3, E206 and E207) to perform biofilm eradication studies with minimal effect on host cell viability (as determined by light microscopy LDH assay). Biofilm formation in the presence of airway epithelial cells was confirmed by immunofluorescence microscopy. The efficacy of antibiotics (tobramycin, colistin and ceftazidim) to eradicate biofilms was determined in the presence or absence of the different epithelial cells. Host-associated biofilms were treated with the antibiotics in different concentrations (2xMIC and concentrations reported *in vivo*). All experiments were performed in microaerophilic conditions (3% O_2, 5% CO_2) relevant for the CF lung. Quantification of biofilms was done by plating.

The results show that airway epithelial cells can influence the response of *P. aeruginosa* biofilms to antibiotics. For most strains, tobramycin and colistin less effectively eradicated biofilms in the presence of airway epithelial cells compared to a plastic surface. In contrast, ciprofloxacin was more effective in the presence of airway epithelial cells. When comparing CF cells (IB3) with non-CF cells (S9); there was more eradication by tobramycin when biofilms were grown on non-CF cells (S9), while the opposite was observed after treatment with colistin. Finally, we compared the eradication of biofilms (strain AMT0060-2) by tobramycin when grown on airway epithelial cells from patients with different CFTR genotypes (CFBE (homozygous ΔF508) and IB3 (heterozygous ΔF508 and W1282X)). Our data indicate a difference in biofilm eradication efficiency, with a more effective biofilm eradication in the presence of CFBE cells compared to IB3 cells. To our knowledge, this is the first report that indicates that the effect of airway epithelial cells on antibiotic efficacy may vary between patients.

ABSTRACTS POSTERS
SECTION D: HOST AND MICROBIAL INTERACTIONS

D01 - TOWARDS A DEEPER UNDERSTANDING OF BURKHOLDERIA CENOCEPACIA'S VIRULENCE

AUTHORS

Stijn De Waele (1), Miguel Valvano (2) & Bart Devreese (1)

ORGANISATIONS

Lab of microbiology, Protein research Unit, Ghent University (1)
Karel Lodewijk Ledeganckstraat 35, 9000 Ghent

Wellcome-Wolfson Institute of Experimental Medicine, Queen's University Belfast (2)
97 Lisburn Rd, Belfast BT9 7BL, United Kingdom

PRESENTING AUTHOR

Stijn De Waele, Teaching assistant, Ghent University, stijn.Dewaele@ugent.be

Cystic fibrosis (CF) is a genetic disease with an approximated birth prevalence of 1:2300 for non-Hispanic Caucasians. This disease is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, leading to the formation of a sticky mucus layer in the airways and the pancreas. Although the life expectancy of patients has been increased through improved diagnosis and healthcare, this disease remains incurable and leads to the acquisition of life-threatening lung infections. These infections are caused, among others, by opportunistic pathogens, for example *Burkholderia cenocepacia* (*B. cenocepacia*). This pathogen can cause the fatal cepacia syndrome, a lethal complication, and makes the patient unsuitable for the needed lung transplantation.

B. cenocepacia survives inside macrophages, and this survival depends on the secretion of virulence factors, among which two (metallo)proteases. So far, their extracellular function in pathogenesis is known for degrading human immunity-related proteins and tissue-related proteins. Despite their importance in virulence, relatively little is known about their exact structure, function and role in intracellular pathogenesis. In this study we will characterize these two metalloproteases, which are needed for its intracellular survival. In addition, proteases are amongst the top list of "wanted" enzymes and have applications in detergent, food and textile industries. During this project, the application of these proteases will be valorized for industrial use. So far, by modifying the recombinant expression of the proteases we could accomplish a shorter and less labor-intensive protocol of protein expression, leading to enough yield to decipher their specificity. The next steps of this project will be to study their intracellular targets, potential applications and decipher potential protease synergy.

D02 - SUBTLE GENETIC ADAPTATIONS DRIVING PATHOGENICITY BEHAVIOUR IN *STREPTOMYCES SCABIES*

Benoit Deflandre[1], *Samuel Jourdan*[1], *Sinaeda Anderssen*[1], *Elodie Tenconi*[1], *Isolde M. Francis*[2], *Rosemary Loria*[3], *Sébastien Rigali*[1]

1. InBioS – Centre for Protein Engineering, Liège University, Institut de Chimie, Liège, Belgium.
2. Department of Biology, California State University Bakersfield, Bakersfield, California, USA.
3. Department of Plant Pathology, University of Florida, Gainesville, Florida, USA.

Most of the species included in the *Streptomyces* genus are soil-dwelling saprophytic bacteria that play an active role in the organic matter turnover. Only a few species are classified as plant pathogens causing the common scab disease affecting tuber and root crops, with *Streptomyces scabies* being the model species.
The phytotoxin thaxtomin A, a nitrated diketopiperazine, was identified as the main virulence determinant produced by *S. scabies*. Both cellobiose and cellotriose (cellulose oligosaccharides) have been reported as inducers of thaxtomin production, acting as allosteric effectors of the transcriptional repressor CebR, allowing the transcription of thaxtomin biosynthetic genes. However, using oligosaccharides from a polymer that is ubiquitous in soil as pathogenicity inducers appears to be a surprising strategy. Additional regulation layers appear to be necessary to ensure that thaxtomin A production, matching the switch to the pathogenic lifestyle, is triggered at the right time. Indeed, the microorganism should be in favourable environmental conditions that are worth of the energy investment required to produce this secondary metabolite.
Here, we present a series of results and hypotheses indicating how *S. scabies* has adapted to distinguish signals originating from an expanding root network associated with a potential host growing nearby, or from breakdown products of decaying lignocellulose. While the main regulatory pathway and the dependence on cellulose oligosaccharides for thaxtomin production have already been highlighted, some molecular features, sometimes caused by subtle genetic adaptations, appear to play a crucial role in *S. scabies* pathogenicity.

D03 - The virulence of a highly necrotizing clinical strain of *Streptococcus pyogenes*

Geoffrey Deneubourg[1], Dalila Lakhloufi[1], Catherine Neerdaels[1], Virginie Imbault[2], Valérie Delforge[1], Mark Davies[3], David Communi[2], Pierre Smeesters[1] and Anne Botteaux[1*] (* Contributed equally)*

1: Molecular Bacteriology Lab, Free University of Brussels, Belgium

2: Mass Spectrometry and Proteomics facility, IRIBHM, Free University of Brussels, Belgium

3: Doherty Institute, University of Melbourne, Australia

Background: Group A *Streptococcus (*GAS) causes more than 500.000 deaths per year. GAS is responsible for a broad spectrum of diseases ranging from pharyngitis to life-threatening invasive diseases. GAS virulence is due to a complex interplay between host factors and bacterial factors, including DNases and proteases.

Objectives: To investigate the contribution of DNases to virulence and pathogenesis of invasive GAS disease using an isolate recovered from a severe clinical presentation. As the clinical isolate (called L01) has 4 DNases (Sdn, Spd1, Spd3 and Spd4), we want to investigate their individual role in the L01 pathology, as well as their potential cumulative effect. We also want to see if other factors are involved in L01 pathogenesis.

Methods: We have cloned and produced 3 of these DNases (Sdn, Spd1, Spd3) and their mutated version as recombinant proteins. We have tested the L01 fitness and the competition with other bacteria by CFU enumeration. We have also monitored the expression of these DNases by RT-qPCR in different conditions. After, we have tested the strain ability to degrade elastin and collagen in enzymatic assays. Finally, we tried to monitor the survive of our strain in whole blood in comparison of a reference strain.

Results: We have shown that the 4 DNases, even if all expressed in the exponential phase, are more expressed during the stationary phase. Three of them are active DNases and we have mutated their catalytic residue. We have also shown that the presence of exogenous DNA gives a fitness advantage to the L01 in comparison with the L01 without DNA. Also, it seems that the L01 survives better than the reference strain in whole blood.

D04 - Dual and triple epithelial co-culture model systems with donor-derived microbiota and THP-1 macrophages to mimic host-microbe interactions in the human sinonasal cavities

Charlotte De Rudder[1,a], Marta Calatayud Arroyo[a], Sarah Lebeer[b], Tom Van de Wiele[2,a]

[1]Presenting author, [2]Corresponding author

[a]Center for Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

[b]Laboratory for Applied Microbiology and Biotechnology, Antwerp University, Groenenborgerlaan 171, 2020 Antwerp, Belgium

The epithelium of the human sinonasal cavities is colonised by a diverse microbial community, modulating epithelial development and immune priming, and playing a role in respiratory disease. Here we present an *in vitro* approach enabling a three day co-culture of differentiated Calu-3 respiratory epithelial cells with healthy donor-derived bacterial communities ($n_{donor} = 2$). We also assessed how the incorporation of macrophage-like cells could have a steering effect on both epithelial cells and the microbial community. Inoculation of donor-derived microbiota in our experimental set-up did not pose cytotoxic stress on the epithelial cell layers as demonstrated by low (< 10 %) lactate dehydrogenase release, comparable to a sterile control. Epithelial integrity of the differentiated Calu-3 cells was maintained as well, with no differences in transepithelial electrical resistance observed between co-culture with donor-derived microbiota and a sterile control. Interindividual variability in nasal microbiota background resulted in a variable immune response in our model system. The introduction of macrophage-like cells caused increasing IL-8 levels in cell layers inoculated with material from donor 1, whereas for donor 2 a mild decrease in IL-8 levels was noted after 72 h. Inclusion and co-culture of THP-1 derived macrophages did not alter phylogenetic diversity, yet donor-independent shifts towards higher *Moraxella* sp. and *Mycoplasma* sp. abundance were observed, while phenotypic diversity was also increased. Our results demonstrate that co-culture of differentiated airway epithelial cells with a healthy donor-derived nasal community is a viable strategy to mimic host-microbe interactions in the human upper respiratory tract. Importantly, including an immune component allowed us to study host-microbe interactions in the upper respiratory tract more in depth.

D05 - Unraveling the mixed proteome landscapes of *Salmonella* infected host subpopulations

Ursula Fels[1,2,3], Patrick Willems[1], Kris Gevaert[2,3], Petra Van Damme[1**]*

[1]*Department of Biochemistry and Microbiology, Ghent University, Belgium.*

[2]*Department of Biomolecular Medicine, Ghent University, Belgium.*

[3]*VIB Center for Medical Biotechnology, Ghent, Belgium.*

*UrsulaAstrid.Fels@ugent.be, **Petra.VanDamme@ugent.be*

Salmonella enterica subspecies are foodborne pathogenic bacteria that can infect a wide range of hosts causing diverse diseases ranging from gastroenteritis to life-threatening typhoid fever in a host-specific manner. In the past years, the emergence of antimicrobial resistant serotypes represents a global public health concern, highlighting the importance of understanding bacterial infection mechanisms to develop alternative therapies [1]. Being a facultative intracellular pathogen, *Salmonella* can infect and proliferate inside a diverse range of host cells (e.g. epithelial cells and macrophages among others). In the course of its intracellular lifestyle, this pathogen was thought to reside inside specific vacuolar compartments, named *Salmonella* containing vacuoles (SCV) [2], but recent studies have reported the existence of different *Salmonella* subpopulations in phagocytic (i.e. macrophages) and non-phagocytic (i.e. epithelial) host cells [3].

We aim at profiling the proteome of *Salmonella*-infected host subpopulations and the host response to increase our understanding of infection biology. For this purpose, we monitored different *Salmonella* subpopulations using a fluorescence dilution (FD) approach that enables the isolation of host cells enriched for specific bacterial subpopulations by means of fluorescence-activated cell sorting (FACS). pFCcGi [4] transformed *Salmonella* displaying constitutive mCherry and arabinose-inducible eGFP expression was used for routine infections. This way we were able to assign growing and non-growing distinct subpopulations. Moreover, by adding cefotaxime, an antibiotic that specifically targets and kills growing bacteria, we were able to enrich for non-growing subpopulations, and sort the non-growing but metabolically active subpopulation, so-called persisters [5].

References:

1. Zaman, S.B., et al., *A Review on Antibiotic Resistance: Alarm Bells are Ringing.* Cureus, 2017. **9**(6): p. e1403-e1403.
2. Takeuchi, A. and H. Sprinz, *Electron-Microscope Studies of Experimental Salmonella Infection in the Preconditioned Guinea Pig: II. Response of the Intestinal Mucosa to the Invasion by Salmonella typhimurium.* The American journal of pathology, 1967. **51**(1): p. 137-161.
3. Castanheira, S. and F. Garcia-Del Portillo, *Salmonella Populations inside Host Cells.* Front Cell Infect Microbiol, 2017. **7**: p. 432.
4. Helaine, S., et al., *Dynamics of intracellular bacterial replication at the single cell level.* Proc Natl Acad Sci U S A, 2010. **107**(8): p. 3746-51.
5. Helaine, S., et al., *Internalization of Salmonella by macrophages induces formation of nonreplicating persisters.* Science, 2014. **343**(6167): p. 204-8.

D06 - Bacterial endophytes to protect plants against cold temperatures

Sonia Garcia Mendez[1], Prof. Sofie Goormachtig[2] and Prof. Anne Willems[3]
[1] Laboratory of Microbiology University of Ghent, Gent, Belgium; VIB Plants Systems Biology, Gent, Belgium
[2] Ghent University - VIB Plants Systems Biology, Gent, Belgium
[3] Laboratory of Microbiology University of Ghent, Gent, Belgium
* *Sonia.GarciaMendez@UGent.be*

Environmental factors have a great impact on crop production due to their effect on plant growth. Finding an environmental friendly way to overcome adverse environmental conditions has gained great importance during the past years. One solution is the use of Plant Growth Promoting Rhizobacteria (PGPR), free living bacteria that have beneficial effects on plant health. In this study we want to determine the importance and nature of the contributions of bacterial endophytes to the survival and growth of plants under cold temperatures. For that purpose, the microbiome of *Arabidopsis thaliana, Valerianella locusta* and *Poa annua* grown under normal and cold conditions is being studied. Through the comparison of the results from the 16S rRNA amplicon sequencing that are being obtained for *Valerianella locusta* plants, and that will be acquired in a nearby future for the other two pants, we want to identify which bacterial populations are being favored in cold conditions. We already conducted a bacterial isolation campaign to cultivate endophytes from these plants so that representative strains can be tested for their ability to promote plant growth under cold conditions. The use of MALDI TOF mass spectrometry and 16S rRNA sequencing has provided insights in the taxonomic diversity of the cultivable endophytes isolated from the three plants and showed they belong to different phyla which are common in soil, such as Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes.

D07 - Decreased diversity of butyrate-producing bacteria followed by functional adjustment occurs in the microbiome of the simulated proximal colon upon long-term treatment with aspirin

Emma Hernandez-Sanabria[1], Maud Van Belleghem[1], Stephanie Kourula[2], Laurent Leclercq[2], Jan Snoeys [2], and Tom Van de Wiele[1]

[1] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium. [2] Janssen Research & Development, A Division of Janssen Pharmaceutica NV, Turnhoutseweg 30, Beerse, Antwerp, 2340, Belgium.

** Tom.VandeWiele@Ugent.be*

Fate and activity of anti-inflammatory drugs are driven not only by the host but also by our gut microbiome. Microbiota-mediated drug transformations and drug-mediated adaptations in the microbiome have been demonstrated to impact response to inflammation. One of the most promising chemopreventive drugs associated with decreased risk of colorectal cancer (CRC) is aspirin (acetylsalicylic acid). Although modulation of the gut microbiome has been proposed to decrease aspirin bioavailability, mechanistic evidence of this interaction is missing. Thus, we aimed at elucidating how short- and long-term supplementation of aspirin influenced bacterial cell count, community composition and functionality using a dynamic gut model including a mucosal compartment. Faecal incubations were employed to monitor short-term response to clinical concentrations of aspirin and subjects showing opposite fermentation patterns were monitored with the Mucosal Simulator of the Human Intestinal Microbial Ecosystem (M-SHIME) for 6 weeks. Aspirin shifted the community composition and metrics in both lumen and mucin compartments but opposite impact on butyrate production was observed. *Roseburia inulinivorans* was the only butyrate-producing bacteria with high expression of butyrate-associated genes in the control treatment. Salicylates have been demonstrated to be quorum sensing molecules and mucin colonisation of *R. inulinivorans* involves biofilm formation through quorum-sensing mechanisms. Hence, alterations both in functionality and bacterial composition, together with reduced bacterial load after long-term supplementation may be associated with these community interactions. Deeper insights into the mechanisms involved in the drug-microbiota interactions will be fundamental for developing targeted pharmacological interventions to enhance drug efficacy and decrease side effects.

D08 - Mining the bacterial inducers for plant defense and shikonin production in plants: an in-silico guided approach.

Henry Naranjo[1], Anne Willems[1]

[1] Laboratory of Microbiology, Department of Biochemistry and Microbiology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium.

[*] HenryDavid.NaranjoBenavides@UGent.be

Several species from the Boraginaceae plant family are used since ancient times for their medicinal properties due to the presence of secondary metabolites such as shikonin (produced in the roots of the plant). Different induction systems for the production of shikonin in plants have been described and the metabolic pathway partially elucidated. However, the role of shikonin in nature has not been fully understood but it is suggested to be part of the plant defense system against pathogens and some abiotic factors.

Different plant defense elicitors known as Microbe Associated Molecular Patterns (MAMPs) and some plant endogenous molecules referred as Damage Associated Molecular Patterns (DAMPs) are described in relation to a wide diversity of microorganisms associated with plants. MAMPs and DAMPs are not only related to pathogenic bacteria but also to non-pathogenic symbionts like endophytes.

In the present study we compared the genomes of several endophytic bacteria isolated from the roots of *Alkanna tinctoria* growing in wild conditions in order to mine for MAMPs or DAMPs related to bacteria that could be responsible for the plant defense and shikonin induction.

Based on the genomic comparison, well described MAMPs like flagellin (flg22) and EF-Tu factor are evenly present in the bacterial genomes. Besides, type II, IV and VI secretion systems are also represented in many isolates. Enzymes related with the degradation of pectins from plant cell wall (CAZy PL1, PL3, PL4, PL9, GH28, CE12), that potentially generate DAMP-like molecules known as oligogalacturonides are less represented among the genomes but found to be enriched in some bacterial groups like Chitinophagales, Burkholderiales, Sphingobacteriales and Pseudomonadales. Oligogalacturonides were previously recognized to induce shikonin production in the Boraginaceae plant *Lithospermum erythrorhizon*. In the future, the significance of the bacteria predicted to degrade pectins and other complex polysaccharides from plant origin will be tested in-vitro and in-planta for induction of shikonin production.

D09 - Biofilm formation and matrix constituents of *S. aureus* clinical isolates collected from persistent or recurrent infections in Vietnam

Tiep Khac Nguyen[1, 3], Nhung Hong Pham[2], Hoang Anh Nguyen[3], Maria Angeles Argudin[4]
Paul M. Tulkens[1] and Françoise Van Bambeke[1]*

[1]Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium.
[2]Microbiology Laboratory, Bach Mai Hospital, Hanoi, Vietnam.
[3]Hanoi University of Pharmacy, Hanoi, Vietnam
[4]Centre national de référence des staphylocoques, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium.
** francoise.vanbambeke@uclouvain.be>*

Introduction- Objective

Staphylococcus aureus causes difficult-to-treat infections, partly due to its capacity to adopt modes of life recalcitrant to antibiotics, like biofilms. Our aim was to quantify biofilm formation by 23 isolates collected from patients with persistent or recurrent infections in Vietnam in comparison with 2 reference strains, and to determine their matrix constituents.

Materials and methods

Reference strains: ATCC25923 (MSSA), ATCC33591 (MRSA). Clinical isolates: collected at the Bach Mai Hospital (Hanoi, Vietnam) from patients who were still infected after 5 days of treatment with an active antibiotic or presented with a recurrence of a previous infection. Biofilms were grown in 96-well plates at 37°C for 24 h in TGN (TSB supplemented with 2% NaCl and 1% glucose). After washing with sterile phosphate buffer saline (PBS), bacteria were collected in sterile water, sonicated and quantified by CFU counting on TSA. Biomass was quantified by crystal violet staining, with reading of the absorbance at 570 nm (Bauer *et al.,* AAC (2013) 50:2726-37)
Polysaccharide content of the matrix was evaluated using Calcofluor White (CFW), a fluorophore that binds preferentially to β-1,3 and β-1,4 polysaccharides, with fluorescence read at $\lambda_{exc360nm}$/ $\lambda_{em460nm}$ (Stiefel *et al.* Appl Microbiol Biotechnol (2016) 100: 4135-45). Proteins were quantified using the Quick Start Bradford Protein Assay, and measure the absorbance at 595 nm. Biofilm extracellular DNA (eDNA) was purified (Kreth *et al.* J Bacteriol (2009) 191: 6281-91) and measured using a NanoDrop™ spectrophotometer. *icaA* expression was quantified by real-time PCR, with RNAs isolated from 3 h-old biofilms, converted to cDNA, and amplified using SYBR Green Master Mix.

Results

All clinical isolates were high biofilm producers, with crystal violet staining values being globally 2 or 3 times higher than for the reference strains ATCC33591 and ATCC25923 respectively, and CFU counts being ten times (1 $\log_{10}$) higher than for ATCC25923 but similar to those recovered from ATCC33591 biofilms. Polysaccharides, proteins and eDNA were present in variable proportions in the matrix.
A strong correlation was evidenced between CV absorbance and CFW fluorescence or protein content but not with eDNA. CFW fluorescence was correlated with the level of expression of *icaA*.

Conclusion

All clinical isolates were high biofilm producers, with matrix abundance being related to the amount of polysaccharides and proteins but not of eDNA. Poly-*N*-acetylglucosamine, produced by the enzymes encoded by the *icaADBC* operon, is probably the main contributing polysaccharide.

Keywords
Staphylococcus aureus, biofilm,biomass, polysaccharides

D10 - Biosynthetic adaptations and morphological alterations of *Brucella abortus* inside host cells

Agnès Roba and Xavier De Bolle,*

Research Unit in Microorganisms Biology (URBM), Narilis , University of Namur, Namur, Belgium.

**agnes.roba@unamur.be*

Bacteria of the genus *Brucella* are intracellular vacuolar pathogens that cause the wordwide zoonosis brucellosis. Inside host cells, *Brucella* replicates in a compartment derived from the endoplasmic reticulum (ER). Bacterial growth in the ER was recently investigated using Tn-seq, a transpositional saturating mutagenesis allowing the identification of genes necessary for survival and growth in a given condition (Sternon et al., 2018). Tn-seq on *B. abortus* in RAW 264.7 macrophages revealed that only a few pathways (namely those for pyrimidines, histidine and branched-chain amino acids (BCAA) biosynthesis) seem to be required for growth in the ER.

Deletion of selected enzymes from histidine and BCAA biosynthesis pathways leads to auxotrophy for those amino acids in minimal medium. Besides, deletion mutants in the histidine biosynthesis pathways are attenuated at 24h post-infection in HeLa cells and RAW 264.7 macrophages. The investigation of pathways that are crucial or dispensable for *B. abortus* allows a better description of the nutrient sources available for *B. abortus* growth inside several host cell types.

Surprisingly, a deletion mutant for the imidazole-glycerol phosphate dehydratase HisB has a very atypical chain-like morphology when inside HeLa cells. This mutant also displayed the same morphology in rich culture medium, but it was not observed when another enzyme of the pathway was mutated (HisC), even though it was also auxotroph for histidine. This characteristic morphology is intriguing considering that *B. abortus* displays unipolar growth. This chaining phenotype is currently under investigation to understand how it is generated.

D11 - In Vitro Polymicrobial Bacteria-Fungal Biofilm Model in the Context of Prosthetic Joint Infections

Ruiz-Sorribas, A.[1]; Poilvache, H.[2]; Cornu, O.[2]; Van Bambeke, F.[1]

[1]Pharmacologie cellulaire et moléculaire (LDRI), UCLouvain, Brussels.

[2]Neuromuscoeskeletal lab (IREC), UCLouvain, Brussels.

albert.ruiz@uclouvain.be

Introduction. The number of hip and knee arthroplasties in the developed countries is increasing. Prosthetic joint infections (PJIs) are one of the most severe complications, which require additional surgeries, due to the failure of antibiotherapies.

The etiology of PJI is diverse but associated with the formation of biofilms. Among them, an average of 20% are considered polymicrobial, which have an even further reduced sensitivity to antimicrobials due to interactions among species that reinforce the biofilms.

Hypothesis and aims. To set up a three-species biofilm model pertinent of PJIs, including a representative Gram-positive, Gram-negative, and fungal species.

Methodology. A co-culture of *Staphylococcus aureus* ATCC25923 or *Staphylococcus epidermidis* ATCC35984 (most frequently isolated pathogens), *Escherichia coli* ATCC47076 (model for Enterobacteriaceae), and *Candida albicans* ATCC24433 (model for fungi), was performed in 96-wells plates. Biofilm formation was evaluated by assessing the total biomass (staining with crystal violet), and culturable cells (cfu counting on selective media). Fluorescence microscopy was performed on biofilms cultured on titanium coupons.

Results. The conditions for which biomass and culturable cells were the most stable are a 48-hours grown in RPMI + 1% glucose buffered with 50mM KH_2PO_4 / 74.1mM Na_2HPO_4, with inocula of $1.5*10^7$: $6.0*10^6$: $2.5*10^6$ cfu/mL for *S. aureus* or *S .epidermidis* : *E. coli* : *C. albicans* respectively. Based on fluorescence microscopy pictures, maximal thickness was 40 µm. The corresponding dual-species biofilms showed a higher biomass in the case of *S. aureus* or *S. epidermidis* : *C. albicans* and a lower biomass in the case of *E. coli* : *C. albicans*.

Conclusion. We have set up two 3-species models with representative species for the majority of pathogens isolated in PJI. Our models are stable and repeatable, and will be used to study the response to antimicrobials.

D12 - Pelvic radiation induces multimodal responses in the mouse gut microbiome and intestine

Charlotte Segers[1,2], Mieke Verslegers[1], Mohamed Mysara[1], Sarah Baatout[1,3], Natalie Leys[1], Sarah Lebeer[2] and Felice Mastroleo[1]*

[1]Belgian Nuclear Research Centre, SCK•CEN, Interdisciplinary Biosciences group, Mol, Belgium

[2]University of Antwerp, Department of Bioscience Engineering, Antwerp, Belgium

[3]University of Ghent, Department of Bioscience Engineering, Ghent, Belgium

[*]felice.mastroleo@sckcen.be

Background: Dysbiosis of the gut microbiome is well known to be evoked by pelvic radiotherapy. Yet, modulating the resident communities by probiotic consumption has become an appealing means to promote host health by either restoring the host-microbe balance or preventing dysbiosis. Unfortunately, human trials testing adjuvant microbial therapies have been yielding contradictory results.

Objective: The aim of this study was to develop a mouse model of pelvic radiation-induced intestinal toxicity and microbial dysbiosis for future microbial therapy development to prevent the adverse effects of pelvic radiation.

Methods: Eight weeks old, male C75Bl/6 mice were exposed to pelvic radiation with an acute X-ray dose of 12 Gy whilst closely monitored for food intake and body weight. Fecal samples were longitudinally collected before, and one day and seven days after exposure. Microbiota profiles were characterized based on 16S rRNA sequencing using the Illumina MiSeq platform. In parallel, intestinal toxicity was evaluated one, three and seven days post-irradiation on mid jejunum, distal ileum and proximal colon.

Results: Dysbiosis was observed in irradiated mice, which was characterized by both an increase in α- and β-diversity. Additionally, irradiated mice showed maximum 16.8% decreased body weight seven days after 12 Gy of X-ray exposure, which was at least partly due to a compromised food intake. Furthermore, the crypt apoptosis index in jejunum, ileum and colon was increased one day following irradiation, which may tend to shorten jejunum and ileum villi length three days post-irradiation. Also, an increase in ileum crypt depth was observed, indicative of repair after injury. Seven days following pelvic irradiation, ileum villi length and total mucosal thickness had restored and even increased, suggesting that our procedure did not affect the ability of stem cells to regenerate damaged crypts in the long term. This observation was confirmed by a significant increase in proliferative ($Ki67^{+}$) crypt cells.

Conclusion and perspective: This study provides a model for future microbial therapy development to prevent the adverse effects of pelvic radiation. To make this model conclusive, near future research will focus on high throughput proteomics to discover inflammatory biomarkers induced by X-ray exposure. These results should lead to a better understanding of pelvic radiation-induced effects to the healthy intestine.

D13 - Disulfide bond formation in *Brucella abortus* periplasm

C. Servais and X. De Bolle
URBM – UNamur, rue de Bruxelles 61, Namur, Belgium
Contact: caroline.servais@unamur.be and xavier.debolle@unamur.be

The formation of one or more disulfide bond is essential for the stability and the functionality of many proteins, such as proteins from the envelope or proteins involved in virulence (Landeta *et al*., 2018). In the Gram negative model *Escherichia coli*, the machinery catalysing the formation of disulphide bonds is found in the periplasm and is mainly composed of four proteins named DsbA, DsbB, DsbC and DsbD. DsbA is a soluble oxidoreductase that reacts with the proteins cystein residues as they enter the periplasmic space. To remain active, DsbA needs to be reoxidized by DsbB, a transmembrane protein of the inner membrane. DsbB then shuttle electrons to the membrane bound quinones. DsbC and DsbD can participate to the refolding of periplasmic proteins (Kadokura *et al*., 2010). In *B. abortus*, we found one homolog of DsbA, two DsbB paralogs (that we name DsbB1 and DsbB2) but no homolog of DsbC or DsbD.

To investigate the role of this system in *Brucella abortus,* an intracellular pathogen responsible for brucellosis, we performed RAW 264.7 macrophages infection with deletion mutants for *dsbB1* and/or *dsbB2*. The deletion mutant for *dsbA* could not be obtained, suggesting that *dsbA* is essential for growth in the tested conditions. Colony Forming Units (CFU) counting along macrophages infection suggest that DsbB1 is required for intracellular survival and proliferation, while *ΔdsbB2* does not seem to have any defect. A *dsbB1-dsbB2* double mutant behaves like the *dsbB1* mutant in macrophages. On the contrary, a single *dsbB2* mutant does not generate a different CFU counting pattern compared to the wild-type strain. These data indicate that while an intact DsbB1 is not needed for growth in culture medium, it is required for survival inside host cell, indicating that periplasmic functions are crucial during infection. The role of DsbB2 and the conditions in which it is produced are currently under investigation.

References

Kadokura H, Beckwith J. 2010. « Mechanisms of oxidative protein folding in the bacterial cell envelope. » *Antioxidants & Redox Signaling* 13(8):1231–1246.
Landeta C, Boyd D, and Beckwith J. 2018. « Disulfide bond formation in prokaryotes. » *Nature microbiology* 3(3):270-280.

D14 - Elucidating the effector interactomes of bacterial pathogens

Veronique Jonckheere[1], Ursula Fels[1,2,3], Margaux De Meyer[1], Petra Van Damme[1]*

[1]Department of Biochemistry and Microbiology, Ghent University, Belgium.

[2]Department of Biomolecular Medicine, Ghent University, Belgium.

[2]VIB Center for Medical Biotechnology, Ghent, Belgium.

[*]e-mail of the corresponding author

Salmonella enterica serovar *Typhimurium* (*S.* Typhimurium) is a foodborne facultative intracellular pathogen which cause gastroenteritis and systematic diseases in a broad range of mammalian hosts. Central to *S.* Typhimurium infection is its ability to invade and colonize a wide variety of host cell types, including non-phagocytic intestinal epithelial cells and phagocytic cells. This virulence trait of *Salmonella* relies on Type III secretion system (T3SS)-mediated host delivery of effector proteins. However, despite the identification of many *Salmonella* effectors and the acknowledgment of their significant role in virulence, relatively little is known about their specific mechanisms of action and their host-cell targets.

For this, we developed endogenous proximity-based biotin labeling (BioID) in bacteria. Using λ-red based homologues recombineering we engineered *Salmonella* SL1344 strains with biotin ligases translationally fused to endogenous *gene* loci of interest (Datsenko and Wanner 2000).

Our results indicate that endogenous BioID represents an innovative proxeome strategy for studying bacterial protein complexes and which for the first time, enables the capture of host interactors by natively T3SS-delivered effectors.

Overall, the characterization of pathogen virulence factors and their interactors will contribute to the development of innovative therapeutics and diagnostics for multiple models of human infectious diseases.

Reference:

Datsenko, K. A. and B. L. Wanner (2000). "One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products." Proc Natl Acad Sci U S A 97(12): 6640-6645.

D15 - Assessment of Colorectal Cancer Progression and Gut Microbiome Dynamics in a Mouse Model

Shari Wouters [1, 2], Mieke Verslegers [1], Mieke Neefs [1], Sarah Baatout [1,3], Natalie Leys [1], Surbhi Malhotra-Kumar [2], Samir Kumar-Singh [2] , Mohamed Mysara [1]*

[1] Belgian Nuclear Research Centre, SCK•CEN, Interdisciplinary Biosciences group, Mol, Belgium.

[2] University of Antwerp, Faculty of Medicine and Health Sciences, Antwerp, Belgium.

[3] University of Ghent, Faculty of Bioscience Engineering, Ghent, Belgium.

[*] mohamed.mysara.ahmed@sckcen.be

Colorectal cancer (CRC) is the third most common identified malignancy and thus represents an important health and socioeconomical burden. Various studies have systemically showed that CRC patients are confronted with intestinal dysbiosis, a significant shift in the composition of the gut microbiome. In addition, follow-up studies based on experimental models have shown that a dysbiotic gut community provokes enhanced CRC development. To put a halt to this vicious cycle, re-establishment of a healthy gut microbiome could be key. As a first step towards this goal, experimental data on microbial composition needs to be acquired through the use of a reproducible CRC mouse model.

The first objective in our study was the implementation of a CRC mouse model (as described by Zackular *et al.,* 2013) through characterisation of the microbial, histological, molecular and clinical status. In parallel, the microbial variation between non-homogenised aliquots of mice faecal pellets was assessed.

Mice received a single intraperitoneal injection of the carcinogen azoxymethane (AOM) followed by three cycles of water-administered dextran sodium sulphate (DSS). Mice were sacrificed and colon tissue was collected at 6 dedicated time points to allow temporal follow up of tumour number, histopathological hallmarks and RNA/protein alterations, while fresh faecal pellets were collected daily for microbial analysis. Animals were clinically monitored throughout the course of the experiment.

In accordance to literature, AOM-injected mice showed consistent body weight loss following each round of DSS treatment. Additionally, clinical signs of inflammation such as diarrhoea and rectal bleeding were present after each round of DSS treatment. The groups exposed to three rounds of DSS had a median of 6 (IQR = 3.5 – 8) macroscopically identified tumours per mouse. The majority of these tumours were located in the distal colon and rectum. Preliminary analysis of 16S sequencing data showed little variation in microbial community between aliquots of the same faecal pellet, allowing for multiple analyses on a single faecal pellet. Interestingly, a clear shift was observed when comparing baseline samples and samples collected after three cycles of DSS.

Based on the available data, characterisation of the AOM/DSS mouse model for colitis-associated cancer was successful and its reproducibility is guaranteed. However, additional analysis is required for full characterisation of the chronic inflammatory state, temporal dysbiosis and how these are linked to treatment regimes.