BELGIAN SOCIETY FOR MICROBIOLOGY
National Committee for Microbiology of
The Royal Academies of Science and the Arts of Belgium

Contact Forum

Current Highlights in Microbiology

Academy Palace, Brussels

October 30th 2017
# Programme

## Current Highlights in Microbiology

Brussels, October 20, 2017

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ABSTRACTS INVITED LECTURES

PLENARY LECTURES

Assembly of an antibacterial speargun: the Type VI secretion system

Eric Cascales

Aix-Marseille Université Marseille, FR

The Type VI secretion system (T6SS) delivers protein effectors to diverse cell types including prokaryotic and eukaryotic cells, therefore participating in inter-bacterial competition and pathogenesis. The T6SS comprises an envelope-spanning complex anchoring a cytoplasmic tubular edifice. This tubular structure is evolutionarily, functionally and structurally related to the tail of contractile phages. It is composed of an inner tube tipped by a spike complex, and engulfed within a sheath-like structure. This structure assembles onto a platform called "baseplate" that is connected to the membrane sub-complex. The T6SS functions as a speargun: upon contraction of the sheath, the inner tube is propelled towards the target cell, allowing effector delivery. During this presentation, I will present recent results regarding the architecture, biogenesis and function of this fascinating secretion machine.

Ecological principles governing anaerobic degradation of oil

Rainer Meckenstock

Universität Duisburg-Essen DE

Hydrocarbons are both an essential resource for humanity and major pollutants of the environment. Although microorganisms are perfectly adapted to degrade hydrocarbons with or without molecular oxygen, the degradation is obviously limited as documented by long lasting contaminations of groundwater or the presence of oil reservoirs. In the present talk, I will challenge the current perspectives of hydrocarbon degradation in the environment and introduce new concepts of how such processes proceed. This will include the anaerobic degradation of hydrocarbons in groundwater and novel processes of long distance electron transfer. Furthermore, I will transfer these concepts to oil reservoirs and present recent findings of how microbial life occurs in oil and how microbial communities work in the subsurface.
Novel insights in molecular mechanisms of pathogen-host interactions during influenza virus and Staphylococcus aureus co-infection

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Influenza viruses (IV) are the causative agents for severe respiratory diseases resulting in significant morbidity and mortality worldwide. Major complications upon influenza are due to secondary bacterial infection, often leading to severe pneumonia. Typical bacterial species isolated from patients with secondary infections are common colonizers of the nasopharynx, such as Staphylococcus aureus (S. aureus). Given the enormous socio-economic burden caused by IV and S. aureus super-infection, it is mandatory to unravel the underlying disease mechanisms. Descriptive data from clinical studies and animal models have improved our understanding of how co-pathogenesis between IV and bacteria might occur. One hallmark of severe infection is the dysregulation in immune responses accompanied by increased cytokine and chemokine expression resulting in detrimental inflammation, enhanced pathogen load and tissue damage. Additionally, it is well established that pathogen replication and host cytokine responses are controlled by pathogen-regulated signaling events. Nonetheless, the complex interplay of pathogen-pathogen and pathogen-host interactions that affect cellular regulatory mechanisms is only in part understood.

Thus, we established IV and S. aureus co-infection model systems that allow the investigation of cellular signal transduction processes in human lung epithelial cell-lines. By use of these model systems, we could verify enhanced cytokine and chemokine expression, higher pathogen load as well as increased cell damage upon IV and S. aureus co-infection in vitro.

Detailed molecular analysis indicated that S. aureus inhibits IV-induced type I interferon response on the level of STAT-1 phosphorylation resulting in less STAT-1 and STAT-2 dimerization and reduced activation of interferon stimulated genes. In consequence the first line of defense against IV infection is blocked and viral replication is boosted. Further investigation of cell-death mechanisms revealed detrimental cell damage in super-infection scenarios. However, our data surprisingly show that, despite enhanced cell damage in presence of both pathogens, S. aureus did not increase but rather inhibit IV-induced apoptosis. We provide evidence for a S. aureus-mediated switch from apoptosis to necrotic cell death of IV-infected cells promoting bacterial survival and spread.

In conclusion, we already identified different mechanisms of S. aureus-mediated interference with IV-induced signal transduction processes that might contribute to co-virulence.
Our studies blend multiple scientific disciplines elucidating bacterial and host mechanisms that determine the onset, course and outcome of interactions between a host mucosal surface and bacterial pathogens. Using genetics, genomics, biochemistry, structural biology, high-resolution imaging, animal models, clinical studies and combinatorial chemistry, we have illuminated how bacterial intracellular lifestyles and community behaviors play critical roles in urinary tract infection (UTI). We uncovered principles of adhesive pili biogenesis in Gram-negative bacteria of the chaperone/usher pathway; delineating molecular details of donor strand complementation and exchange mechanisms by which subunit folding is coupled with translocation and assembly of pili across the outer membrane. We delineated how uropathogenic E. coli use type 1 pili to invade and establish biofilm-like intracellular bacterial communities within bladder cells subverting extracellular host defenses and how quiescent intracellular reservoirs can seed recurrent infection. We have shown that risk of UTI depends on the specific pairing between diverse uropathogens and hosts and that the outcome of these interaction depends on both gene carriage and transcriptional responses. We identified complex networks governing mucosal epithelial responses that determine disease outcome. Finally, our work has also revealed fundamental insights into catheter-associated urinary tract infections caused by Enterococcus and E. coli. Together, our work is changing the way UTIs are evaluated, re-shaping models of bacterial infections in general and spawning new technologies to design novel vaccines and anti-microbial therapeutics to diagnose, treat and/or prevent UTIs and their sequelae.
The rocky road to success of bedilaquine, a novel powerful addition to the treatment of drug-resistant tuberculosis

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There is a dire need for new drugs to treat TB, especially multi-drug resistant TB (MDR-TB). People with MDR-TB are forced to take up to 20 pills a day with side effects that range from nausea to deafness and psychosis – to have a 50% chance of surviving the infection. In 2012 and 2014, respectively, the FDA and EMA approved bedaquiline, the first dedicated new TB drug to be approved since 1963. The story of its discovery and development is an illustration of the challenges one needs to overcome to achieve better treatments for patients around the world.

Dr Koen Andries (1951) studied Veterinary Sciences and obtained his PhD at the University of Ghent, Belgium. He joined the team of Dr. Paul Janssen in 1982. Using cell-based assays in search of antiviral compounds, his team discovered nanomolar inhibitors of uncoating of rhinoviruses, picomolar inhibitors of fusion of respiratory syncytial virus and several non-nucleoside reverse transcriptase inhibitors of HIV (TIBO’s, alpha-APA’s, and DAPY’s) with high activity against wild-type and resistant HIV-1 strains. Two of these became approved drugs: etravirine - intelence® and rilpivir - edurant®.

Dr Andries also led the team that discovered R207910/TMC207 (bedaquiline – sirturo®), a first in-class new anti-tuberculosis drug, and its unique mechanism of action. Bedaquiline is the first molecule with specific activity against the ATP synthase, one of the most fundamental enzymes in biology, and the first antibiotic known to interfere with the generation of energy. He grandfathered bedaquiline from discovery to early development and eventually full development as the microbiology leader, currently studying mechanisms of resistance to the new drug. He authors 172 papers, 180 abstracts and 32 patents, and is Professor emeritus at the University of Antwerp.
Section A: General Microbiology
Exploring the possibilities of art-science collaborations by using color as research medium

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In the past few decades, an increased number of art-science collaborations have been developed, as well as an increased interest by the society on them. On the one hand, emerging biotechnology not only provides resources, knowledge and methodologies to artistic creations but also generate questions and discussions traditionally limited to the scientific community. On the other hand, scientists may find artistic tools and outcomes a powerful way to communicate their work to a broader audience. Moreover, when artists and scientists work together a space of freedom for unexpected solutions or research lines is generated.

In this context, a cooperative collaboration between the KASK-HoGent and the Center for Microbial Ecology and Technology (CMET, UGent) has been pushing the boundaries between art and science by using a common language in both disciplines: color. To this end, the potential use of color generated by pigment producing bacteria or the use of cell cultures as canvas and tool for creating artistic images have been explored.

Inspiring photographs, colorful bacterial drawings and original designed lab-tools were obtained as outcome, reflecting on the limit between both disciplines, the aesthetic of science and the use of living organism for artistic proposes.

This project aims to establish a dialogue between art-science, questioning traditional parameters of scientific and artistic production and opening new approaches to academic research to improve, optimize and extend the outreach activities in both areas of knowledge.
Molecular characterization of SpoT partners interactions in Caulobacter crescentus

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The (p)ppGpp alarmone is used by bacteria to quickly respond to environmental stress. This alarmone induces various biological modulations allowing cells to survive during the wait for a more favorable context. The α-proteobacteria Caulobacter crescentus exhibits an asymmetric division leading to two phenotypically different daughter cells: a sessile stalked cell and a chemotactically active motile swarmer cell that differentiates later in the sessile form. Upon nitrogen starvation, C. crescentus triggers (p)ppGpp accumulation, which in turn controls the cell cycle by extending the G1 swarmer phase.

Recently, a novel molecular mechanism involving PTSNtr (nitrogen-related phosphotransferase system) has shown to stimulate (p)ppGpp production. PTSNtr system acts as metabolic sensor translating glutamine deprivation signal into (p)ppGpp accumulation. Once phosphorylated, the PTSNtr component EIIA\textsubscript{Ntr}\textendash P directly reduces the (p)ppGpp hydrolase activity of SpoT, the only RelA/SpoT homologue in C. crescentus responsible of the synthesis and degradation of the alarmone. The cellular (p)ppGpp level seems therefore to be increased by direct inhibitory interaction of the phosphorylated form of EIIA\textsubscript{Ntr} with SpoT.

This project aims at understanding the molecular basis of bacterial stress survival mechanisms by unravelling and characterizing the interactions between the nitrogen-related phosphotransferase protein EIIA\textsubscript{Ntr} and the hydrolase/synthetase SpoT.

The production of high purity samples of wild-type and mutated SpoT and EIIA\textsubscript{Ntr} proteins will provide quantitative informations about their interactions, by Isothermal Titration Calorimetry, among others. Diffracting crystals will provide the tridimensional structure of EIIA\textsubscript{Ntr}\textemdash SpoT complex using the power of X-Ray crystallography that will lead to new knowledges regarding EIIA\textsubscript{Ntr} mode of action on SpoT.

References
Protein-DNA contacts for activation and repression by ArgP, a LysR-type (LTTR) transcriptional regulator in Escherichia coli.

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*Escherichia coli* ArgP, is a member of the large family of LysR-type transcriptional regulators (LTTR). In contrast to many LTTRs, ArgP operates with two effector molecules, lysine and arginine, to differentially regulate gene expression. Both effector molecules bind competitively to the same binding site of dimeric ArgP. Effector-free ArgP stimulates transcription of all investigated regulon members, except *argO*. Lysine abolishes this activating effect. ArgP-mediated activation of *argO*, encoding an exporter for arginine and its toxic analogue canavanine, is strictly arginine dependent. Lysine counteracts this co-activator effect of arginine. Lysine-bound ArgP stimulates RNA polymerase recruitment at the *argO* promoter but the complex is non-productive (Laishram and Gowrishankar, 2007). It is presently unclear what distinguishes *argO* from other ArgP targets and how binding of arginine and lysine translates in antagonistic effects on promoter activity.

The ArgP binding site was previously identified as a 50 to 55 bp stretch by DNase I footprinting in the *argO* and *lysP* operators of *E. coli* and the *gdhA* control region of *K. pneumonia* (Laishram and Gowrishankar, 2007; Ruiz et al., 2011; Goss, 2008). However, even for these best-studied targets there is little to no information available on base-specific contacts and potential differences in DNA contacts of effector-free and effector-bound ArgP. For most ArgP targets the effect of arginine and/or lysine was exclusively determined on overall apparent binding affinity, essentially by electrophoretic mobility shift assays, and effects of ligand binding on local ArgP-DNA contacts are poorly documented.

Here we generate high resolution contact maps of effector-free and effector-bound ArgP-DNA interactions and show that *argO* is the only target at which binding of effector-free ArgP overlaps the -35 promoter element. Binding of arginine results in a repositioning of promoter proximal bound ArgP-arg in the *argO* control region. Similar effect was not observed at other ArgP-targets. Furthermore we determine ArgP sequence specificity, stoichiometry of ArgP-DNA complexes and ArgP-induced bending angles with and without effectors. These results provide insight into the molecular mechanisms of ArgP-mediated regulation and a molecular explanation for the unique arginine-dependence of *argO* activation that distinguishes this particular ArgP target from all others.


Functional and structural analysis of AtaT – an acetyltransferase toxin from toxin-antitoxin system

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Toxin-antitoxin (TA) systems are operons of toxic protein and its antidote. TAs can be omnipresent in bacterial mobile genetic elements as well as settled in genomes. It has been demonstrated that TAs prevent loss of genetic elements by phenomenon known as post-segregational killing (PSK). PSK relies on the fact that antitoxin is less stable than toxin and once the genes encoding the operon are lost upon degradation of antitoxin the liberated toxin kills the TA-free progeny. On the other hand, genome-encoded TA systems have been proposed to be part of stress-response pathway leading to persistence as most of the toxins inhibit translation in bacteriostatic fashion and therefore could facilitate entrance to dormancy. We have found novel TA system encoded in enteropathogenic E. coli O157:H7 that is comprised on GNAT-fold acetyltransferase toxin AtaT and RHH-domain antitoxin AtaR. We have demonstrated that AtaT toxin has a unique activity for GNAT-fold enzymes. AtaT acetylates methionine charged on the initiator Met-tRNA^fMet using acetyl-CoenzymeA. Upon acetylation acetyl-Met-tRNA^fMet becomes a dead-end product that is incompatible with translation as it fails interaction with initiation factor IF2 responsible for presenting initiator fMet-tRNA^fMet to the 30S initiation complex. We have solved the structures of the AtaT toxin catalytic mutant Y144F, its antitoxin AtaR, and we have obtained first structural data of AtaT-AtaR interaction which results in the neutralization of AtaT toxicity. Using mutational analysis where we have mapped the interaction sites of AtaT toxin with the target Met-tRNA^fMet. Taken together we provide the first insights of AtaT interactions with the acetyl-coenzymeA, with its target tRNA and with its antitoxin.
A PUTATIVE ADP-RIBOSYLTRANSFERASE TOXIN ASSOCIATED WITH AN RHS SYSTEM INHIBITS CELL DIVISION IN E. COLI

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Contact-dependent growth inhibition (CDI) systems and rearrangement hotspots (RHS) are coding for long conserved proteins containing extremely variable C-terminal domains (CdiA-CT/Rhs-CT). In addition, they also encode small cognate immunity proteins that antagonize the toxic domain activity (CdiI/RhsI). These systems were recently shown to play an important role in inter-bacterial competition as well as biofilm formation. However, they are likely to possess still unknown functions, toxins activities and targets.

We investigated an Rhs-CT toxic domain from X. bovienii predicted to be an ADP-ribosyltransferase and analyzed its effect and mechanism of action in E. coli. We experimentally validated the functionality of the Rhs-CT/RhsI toxin-immunity pair in E. coli. Upon overexpression, the Rhs-CT toxin induces a strong filamentation phenotype while DNA replication and nucleoid segregation appear to be unaffected.

Furthermore, filamentation appears to be independent of the SOS system. Using an FtsZ-GFP fusion, we observed that the Z-ring is destabilized upon toxin overexpression. Altogether, these data indicate that the Rhs-CT toxin directly inhibits cell division. The direct target is under investigation.
Stripping away Bacillus anthracis’ protective coats

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The Bacillus anthracis vegetative surface is covered by a two-dimensional paracrystalline protein array known as S-layer (surface layer). Two mutually exclusive S-layers sequentially appear at the cell surface in a growth phase-dependent manner: the Sap exponential layer and EA1 stationary layer.

Since their discovery, the self-assembling characteristic of these proteins has hampered their ease of handling under non-denaturing conditions and has hitherto proven prohibitive for detailed structural and biophysical characterization. To overcome the protein self-polymerization issue we successfully applied Nanobodies (Nbs) as a bio-tool to control Sap and EA1 polymerization. Using these Nbs as crystallization aid we could accomplish the crystallization and structure determination of Sap, unveiling a new class of S-layer proteins with a novel mechanism of assembly. Excitingly, we were able to identify Nbs capable to inhibit in vitro Sap and EA1 polymerization. When applied in vivo, these Nbs perturb B. anthracis S-layer integrity and cell morphology and growth, making these proteins a promising tool for the development of new strategies to fight anthrax disease.
The role and evolution of the Pco copper homeostasis system in \emph{C. crescentus}

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Despite its essential role as a trace element for any living organism, where it can act as a cofactor for many proteins, copper (Cu) turns toxic at high concentration. It can displace other native metallic ions in metalloproteins, generates unwanted disulfide bonds and triggers a Fenton-like reaction leading to oxidative stresses. Since the toxicity threshold can be reached quickly, there is a strong need for an organism to tightly regulate its intracellular Cu concentration.

The oligotrophic alphaproteobacterium \emph{Caulobacter crescentus} is characterized by an asymmetrical cell cycle, resulting in two morphologically different cells: a swarmer cell, motile, and a stalked cell, sessile. \emph{C. crescentus} produces, in its sessile form, two proteins (PcoA and PcoB) that confer resistance to a Cu stress. We demonstrated \emph{in vitro} that PcoA exhibits a Cu oxidase activity. We also performed cell fractionation to pinpoint the subcellular localizations of both proteins and provided evidence for a periplasmic and outer membrane localization of PcoA and PcoB, respectively. Finally, by using various genetic backgrounds, we confirmed that PcoB likely acts as an Cu efflux pump. We proposed a model summarizing how the PcoAB operon is handling Cu, from its detoxification to its ejection.

In the motile form, though, the Pco system does not seems to be expressed in a concentration important enough to act as a Cu detoxification system. Our results indicate that the swarmer cells are instead relying on negative chemotaxy to flee the Cu stress.

However, several questions remain. Is there an interaction between PcoA and PcoB? Do these two proteins require other actors to function properly? Another puzzling unknown is the evolutionary path of this system. In \emph{E. coli}, where the Pco system has been discovered, it is composed of seven different proteins. The conservation of these proteins seems to vary a lot among the alphaproteobacteria genus. Some species possess homologs for as many as six of these seven proteins, while others have none. Why? Is this conservation related to lifestyle? Do bacteria that don’t harbor any Pco homolog possess other systems to cope with Cu stress, or are they simply less likely to encounter Cu in their environment? Does a correlation exist between different lifestyle categories (such as mammal pathogens or free living bacteria) and the conservation of the Pco system?

All in all, we try to tackle these questions thanks to broad-scale bioinformatics and phylogeny analysis. We might be able to get a better grasp in the evolution of the Pco system in alphaproteobacteria and its potential relation with the lifestyle. We discuss the different hypothesis and their potential implication in the evolution of these bacteria.
Together with the current antibiotic resistance crisis, bacterial persistence appears to play an increasingly important role in the frequent failure of antibiotic treatments. Persister cells are rare bacteria that transiently become drug tolerant, allowing them to survive lethal concentrations of antibiotics. The specific physiology of persisters has been shown to rely on several global molecular mechanisms, such as the SOS response, the stringent response and dormancy.

We aim at a better understanding of the specific molecular mechanisms leading to persistence to ofloxacin in *E. coli* and allowing cells to elude the lethal effect of ofloxacin with the aim of developing new anti-persister treatments.

We showed that on the contrary to most antibiotics, ofloxacin treatment does not yield a biphasic killing curve, but a complex killing curve composed of four different phases. Correlations between metabolic parameters (growth rate, stringent response, SOS response and activation of efflux pumps) and survival during all four phases was measured in order to characterize this complex killing curve. As the SOS response was found to be crucial for 3 out of the 4 phases, we investigated the importance of the SOS response for persistence to ofloxacin at the single-cell level using a microfluidic device. To our knowledge, this is the first time persister bacteria could be tracked before, during and after antibiotic treatment. By using bioinformatics tools, we performed a systematic and quantitative analysis of the shape, fluorescence and division of persister cells and compared it to normal cells. On the contrary to the bulk of the population, we observed the SOS response to be triggered in persister cells only after ofloxacin removal, as a consequence of SOS-independent cell filamentation. Filamentation is followed by the excision of the tip of the cell, which we showed to be DNA-free. Multiple divisions in the core of the filament then rapidly take place, giving rise to a new bacterial population.
ROLE OF CsrA GLOBAL REGULATOR IN UROPATHOGENIC E. COLI BIOFILMS STRUCTURE

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Background
The CsrA protein is a global post-transcriptional regulator controlling carbon fluxes and social behaviors in bacteria. Independent studies have shown that CsrA regulates different processes involved in biofilm formation such as flagella synthesis, exopolysaccharides production or c-di-GMP homeostasis.

Objective
Internal organization of K-12 *Escherichia coli* biofilms had recently been described at cellular resolution using macrocolony model and scanning electron microscopy (SEM) by the group of R. Hengge. Our objective is to decipher the role of CsrA into biofilm organization by characterizing biofilms formed by a mutant deleted for *csrA* in *E. coli* CFT073.

Methods
Using genetics and biochemical approaches and scanning electron microscopy, we analyzed at macro- and microscopic level a collection of mutants deleted for *csrA* and other genes encoding proteins involved in production of extracellular structures such as curli, N-acetylglucosamine (PGA), flagella or participating to c-di-GMP homeostasis.

Conclusions
SEM observations of wild-type macrocolonies revealed very organized structures such as vertical pillars and differentiated horizontal layers. In a mutant deleted for *csrA*, macrocolony organization is lost. We also showed that deletion of *csrA* abolishes negative regulation of the YcdT c-di-GMP cyclase, leading to an increase of intracellular c-di-GMP concentration. In turn, both cellulose and PGA are highly overproduced. We propose that this abundant extracellular matrix is produced anarchically thereby hindering macrocolony internal organization.
Identification of novel interaction partners of the persister regulator Obg

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Background

Chronic infections that are recalcitrant to treatment with antibiotics are posing a major threat to human health. This recalcitrance is partly caused by so-called persister cells. Persisters comprise a small (typically < 0.1%) fraction of transiently antibiotic-tolerant cells that are able to survive prolonged exposure to antibiotic treatment. Although the clinical importance has been demonstrated, the precise molecular mechanisms underlying persistence are not fully unravelled.

Objectives

Previous research in our group has demonstrated a central role for the conserved GTPase Obg in mediating persistence. In the current project, direct interaction partners of Obg are identified to further unravel the Obg-persistence pathway and select specific targets that influence persister formation.

Methods

An innovative photo-crosslinking technique is used to identify novel direct interaction partners of Escherichia coli Obg. The unnatural photo-reactive amino acid p-benzoyl-L-phenylalanine (pBpa) is incorporated at specific locations on the surface of the Obg protein. Following UV radiation, the carbonyl oxygen of pBpa crosslinks to carbon-hydrogen bonds of molecules within a radius of 3 angstrom. Interaction partners of Obg are identified using high resolution liquid chromatography-mass spectrometry. Additionally, a second screening for interaction partners of Obg was carried out using a bacterial two-hybrid system based on the reconstitution of the catalytic domain of Bordetella pertussis adenylate cyclase (CyaA). Subsequently, specific proteins and corresponding genes are tested for their involvement in persistence.

Conclusions

Identification of Obg-interaction partners contributes to the full understanding of the pathway. It is expected that targeting Obg-mediated persistence will significantly reduce the number of persister cells in bacterial populations, thereby facilitating clearing of infections by conventional antibiotics.
Characterization of the molecular mechanisms controlling negative chemotaxis to Cu in *Caulobacter crescentus*

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During their life, 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. A previous study in the lab has shown that in the aquatic alphaproteobacteria *Caulobacter crescentus*, the flagellated cell and the stalked cell, resulting from an asymmetric cell division, adopt different strategies to cope with a high Cu concentration. The sessile stalked cell oxidizes and ejects Cu within a few minutes while the flagellated cell rapidly escapes towards a Cu-free environment (Lawarée *et al.*, 2016).

The signaling network controlling bacterial chemotaxis to heavy metals has remained totally unknown so far. The first part of our project aims at understanding how Cu is sensed by the flagellated cell by (i) determining the intracellular Cu localization using on the first hand a combination of cell fractionation and inductively coupled plasma optical emission spectrometry and on the second hand nanoSIM technology and (ii) identifying the chemoreceptor involved in Cu binding by knocking-out the predicted 19 Methyl-accepting Chemotaxis Proteins (MCPs) from *C. crescentus*. Conserved and non-conserved Che proteins will also be studied in the context of signal transmission to the flagellar motor. In the last part of the project, we will focus on the possible development of a Cu memory by comparing the chemotactic response of naïve flagellated cells and flagellated cells born in a Cu-rich medium.
EXPRESSION OF THE GLOBAL REGULATOR CSRA IS REGULATED BY THE CPXRA TWO-COMPONENT SYSTEM IN E. COLI

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Backgrounds
Global regulators play major roles in response to a variety of intracellular and extracellular signals, allowing bacteria to adapt to environmental changes. Lack of global regulators generally leads to strong defects. We are interested in CsrA, a post-transcriptional global regulator acting by binding target mRNAs and modulating their translation. This regulator is important for cell growth and regulates central carbon metabolism and social behavior pathways.

Transcriptional regulation of \textit{csrA} expression remains largely unknown although it was shown that Sigma70 and RpoS directly regulate \textit{csrA} transcription.

Objectives
The objective is to identify two-component system (TCS) involved in regulation of \textit{csrA} expression.

Methods
Transcriptional fusion was used in different mutant strains to identify \textit{csrA} regulators. Confirmation and characterization of the \textit{csrA} regulation by cpxRA were obtained by western blot, mobility shift assays and footprint experiments.

Conclusions
We focus our study on the cpxRA system, a TCS sensing periplasm and inner membrane stresses through various signals. cpxRA regulate pathways notably involved in envelop repair and homeostasis. Our results show that \textit{csrA} promoter activity is dependent on CpxRA. Phosphorylation state of CpxR is important for \textit{csrA} regulation \textit{in vivo}. We showed that acetyl-phosphate plays an important role in the CpxRA-dependent regulation of \textit{csrA} expression by phosphorylating CpxR independently of CpxA, and that CpxA phosphatase activity counteracted it thanks to its phosphatase activity. Finally, we defined the location and sequence of the two CpxR binding sites on the \textit{csrA} promoter region.
DISSECTION OF A NOVEL METAL RESISTANCE MECHANISM IN CUPRIAVIDUS METALLIDURANS

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Cupriavidus metallidurans strains contain many genes involved in the resistance and processing of heavy metals, which are very well studied and characterized. In addition, studies at SCK•CEN underscored the rapid evolution of C. metallidurans strains towards significantly increased metal resistance, particularly for silver. Our objective is to understand the molecular mechanisms of this increased resistance to silver.

C. metallidurans strains including type strain CH34, its plasmidless derivatives AE104 and NA4, isolated from a silver-sanitized drinking water system, were grown in toxic levels of silver to obtain silver-resistant mutants. The latter were characterized in detail via re-sequencing, oligonucleotide microarrays, RNA-seq and deletion and complementation analyses.

Our data indicate that C. metallidurans is able to adapt rapidly to toxic silver concentrations. Silver-resistant mutants were obtained from different strains, even from strains cured from the main mega-plasmid encoded metal resistance determinants, indicating that the canonical efflux mechanisms are not involved. Whole-genome expression profiling in non-selective growth conditions showed that only a few genes are commonly up-regulated in all obtained silver-resistant mutants and some of them are downregulated in the absence of a two-component system. In addition, deletion mutants and plasmid-based complementation confirmed that a two-component regulatory system and two genes coding for small periplasmic proteins play a central role. These results represent a previously uncharacterized molecular mechanism for metal/silver resistance.
Labyrinthopeptin A1 exerts broad-spectrum antiviral activity against Zika virus.

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Infections with flaviviruses such as dengue (DENV) and Zika (ZIKV) form an increasing risk to a large part of the global human population. This is mainly due to the expansion of the mosquito vector as a result of climate change, urbanization and worldwide traveling. The recent epidemic caused by the emerging ZIKV highlights the importance of the presence of broad-spectrum antivirals, since no specific treatment has made it to the market to date.

The inhibition of viral entry is an attractive antiviral strategy since this restrains an early step in the viral life cycle, inhibiting further dissemination of the pathogen in the body. Flavivirus entry is facilitated by a variety of cellular receptors expressed by susceptible target cells. Examples are Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), and the families of phosphatidylserine receptors T-cell immunoglobulin and mucin domain 1 (TIM) and Tyro 3, Axl and Mer (TAM). Viral entry through TIM and TAM receptors occurs through apoptotic mimicry. In this process, viruses are recognized by phagocytes as apoptotic cells and engulfed for further degradation by exposing the marker for apoptosis phosphatidylserine. Interfering with this mechanism is an interesting antiviral approach.

Labyrinthopeptin A1 (LabyA1) is a bacterial lantibiotic peptide that contains the amino acid labionin. We have previously shown that it possesses broad and potent antiviral activity against human immunodeficiency virus (HIV), Herpes simplex virus (HSV) and DENV in the low micromolar range by interfering with the viral entry process. However, its mechanism of action has largely remained unknown. Here, we demonstrated the antiviral potency of LabyA1 against ZIKV infection in several in vitro experiments. Viability assays and multi-parameter flow cytometry show that this antiviral activity is independent of the ZIKV strain tested or the target cell line used. In our preliminary results, the mechanism of action of LabyA1 seems to act through an interaction with the viral envelope proteins as well as by interacting with the TIM-1 receptor.

Our results suggest that Labyrinthopeptin A1 (LabyA1) possesses broad-spectrum antiviral activity against flaviviruses. This demonstrates the unique and broad antiviral properties of LabyA1 as well as the necessity of further deciphering its mechanism of activity.
Investigating phage-related threats to the MELiSSA loop

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The MELiSSA (Micro-Ecological Life Support Alternative) loop is an artificial ecosystem intended as a regenerative life support system for long-term space missions. It is important to know whether the bacteria that are used in such a system are susceptible to external threats such as infection by bacteriophages or internal ones like induction of prophages present in their genome. DNA stress, such as caused by cosmic radiation, is known to induce prophages in various bacterial species, and factors like the high biodiversity in waste streams and the mixed culture fermentation in the first compartment increase the odds of a bacteriophage being present with a host range that includes *Rhodospirillum rubrum* S1H (the bacterium used in the consecutive compartment).

To investigate those phage related threats, a three pronged approach was taken: 1) the genome of *R. rubrum* was analysed to find regions similar to known prophages, 2) *R. rubrum* was exposed to DNA damage caused by mitomycin C and the cell lysate was analysed for the presence or absence of induced prophages, 3) samples of dissimilar sources of waste (A sample from a pilot of MELiSSA compartment I, two sewage samples, two grease trap samples and a compost sample) were used to investigate the presence of bacteriophages with lytic activity against *R. rubrum*.

The initial investigation *in silico* indicated that the presence of prophages was unlikely, which was confirmed by the absence of induced prophages in the cell lysates.

No bacteriophages with lytic activity against *R. rubrum* were found.
EXPLORING THE ROLE OF MINI-PROTEINS IN BURKHOLDERIA CENOCEPACIA BIOFILM FORMATION AND PERSISTENCE

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Burkholderia cenocepacia J2315 is a member of the Burkholderia cepacia complex (Bcc), a group of opportunistic pathogens that can cause severe lung infections in cystic fibrosis patients. Infections are often difficult to treat due to resistance, biofilm formation and persistence. Bcc bacteria have large multi-replicon genomes (6-9 Mb) and the function of a large fraction of genes annotated as “hypothetical” or “conserved hypothetical” is still unknown. While previous research has predominantly focussed on larger proteins, evidence is accumulating that genes encoding polypeptides with a length smaller than 100-200 amino acids are ubiquitous in the genomes of all living organisms and are involved in various biological processes. The goal of the present study is to elucidate the role of mini-proteins in B. cenocepacia biofilm formation and persistence.

We focused on genes smaller than 300 nucleotides of which the function is unknown. Almost 10% (646) of the genes in the B. cenocepacia genome are smaller than 300 nucleotides and more than half of these are annotated as hypothetical proteins. For 234 of them no similarity could be found with non-hypothetical genes in other bacteria using BLAST (E < 10^-5 and identity > 40%). However, based on available transcriptomic data the majority of these genes were found to be up- or downregulated in stress conditions (treatment with tobramycin, H2O2 or chlorhexidine, low oxygen, low pH, low iron or heat). Using available RNA sequencing data, a list of 27 mini-proteins highly expressed in biofilms (RPKM > 200) was compiled. For 8 of them expression in biofilms was also confirmed using proteomic analysis or translational fusion mutants. Overexpression of two of these mini-proteins had a negative influence on growth, whereas for three other proteins overexpression led to an increase in biofilm biomass. Additionally, overexpression did not have an influence on resistance but for several mini-proteins had an influence on persistence. In conclusion our results confirm that mini-proteins are present in the genome of B. cenocepacia J2315 and indicate that they are involved in various biological processes.
Respiratory complex I variants, selected during evolution under frequent antibiotic treatment, induce bacterial persister cell formation through cytoplasmic acidification

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The rise of antibiotic resistance and lack of new antibiotics targeting pristine targets will soon push mankind into an era where common infections will once again kill millions. In addition to resistance, bacteria can also turn to other strategies to evade antibiotics. We previously showed that bacteria evolve towards extreme levels of antibiotic tolerance under frequent antibiotic exposure by acquiring mutations that increase the numbers of antibiotic-tolerant persister cells. One target is complex I, a membrane-embedded super enzyme of 535kDa and main entry point for electrons from NADH into the respiratory chain. Here, we verified the importance and diversity of complex I variants in conferring extreme persistence levels and decipher their molecular mechanisms. Whole genome sequencing on Escherichia coli evolved in the lab (both lab and pathogenic strains), identified a multitude of mutations in nuo. Surprisingly, all hit nuoLMN and none of the other 10 nuo genes. Genome engineering and antibiotic tolerance assays demonstrated their causality and ruled out overall knock-out effects. Biochemical analyses on purified complex I variants further confirmed stability and validity as entry point of electrons from NADH into the respiratory chain but instead indicated a diminished proton-translocating activity. Flow cytometry and pH-sensitive fluorophores pinpointed the effect specifically towards the pH component as membrane potentials remained unchanged, also explaining the unaffected antibiotic uptake of the mutants. Artificially changing cytoplasmic pH showed the causality of cytoplasmic acidification in the increased antibiotic tolerance. We therefore conclude that complex I variants, selected during frequent antibiotic exposure, influence bacterial persister cell formation through cytoplasmic acidification.
Epigenetic regulation of *Burkholderia cenocepacia* J2315 phenotypes by DNA methyltransferases

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Respiratory tract infections with the opportunistic pathogen *Burkholderia cenocepacia* in cystic fibrosis (CF) patients are associated with an increased morbidity and mortality. Due to high antibiotic resistance, capacity of biofilm formation, and production of a broad range of virulence factors, treatment of these infections are challenging. In the last decade, microbial epigenetics has gained increased attention because of its role in bacterial cell regulation. Epigenetic phenomena are commonly referred to as heritable changes in gene expression without an alteration of the DNA sequence itself. The most extensively studied form of epigenetic regulation of gene expression is DNA methylation, due to the activity of DNA methyltransferase enzymes (MTases). However, a study on the role of specific MTases in *Burkholderia cenocepacia* (J2315) is still lacking. Two putative DNA MTase genes (BCAM0992 and BCAM1036) were selected for further analysis.

In order to evaluate the role of DNA methylation in *B. cenocepacia* J2315, mutants of putative DNA MTases were created and confirmed with Sanger Sequencing. Two conditional mutants (ΔcBCAM0992 and ΔcBCAM1036), which contain a rhamnose-inducible promotor that only initiates transcription in presence of rhamnose, and a deletion mutant (ΔBCAM0992), were constructed. Next, their phenotype was compared to that of the wild-type strain.

Growth curve analysis revealed the mutant strains were not reduced in growth, which allowed identical cultivation parameters for all strains. Crystal violet (CV) staining revealed that in both BCAM0992 mutants biofilm formation was reduced, whereas the ΔcBCAM1036 mutant did not show any difference in this regard. In addition, motility of the ΔBCAM0992 deletion mutant strain appeared to be reduced.

Although these findings are preliminary, they suggest that MTases play a role in regulating gene expression and certain phenotypes in *B. cenocepacia* J2315.
Identification of the target of the Type II ParE2 Toxin in *E. coli*

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Toxin-Antitoxin (TA) systems are small genetic modules consisting in general of two components encoding a stable toxin, and a labile antitoxin. The toxin performs its function by interfering with essential cellular processes such as DNA replication, transcription, translation and peptidoglycan biosynthesis, while antitoxin inhibits synthesis or activity of the toxin. Based on the antitoxin nature and mode of action, TA systems are grouped into 5 types.

Type II TA systems are the most abundant in bacterial genomes and have been extensively studied. In a former study, our group has identified a novel type II antitoxin family named PaaA, which is associated with ParE toxins. Together with a regulator named paaR located upstream of the antitoxin gene, these ORFs constitute a three-component operon. Overexpression of ParE2 toxin leads to cell death in a SOS-dependent manner. Using a ParE2-GFP fusion, it was shown that ParE2-GFP co-localized with the nucleoid, suggesting that ParE2-target complex is associated to DNA. Structure prediction of ParE2 indicates that it is similar to that of *C. crescentus* ParE. Antitoxin PaaA2, which lacks DNA-binding domain, is an atypical member of RelB-antitoxin family. A combination of SAXS and NMR data revealed the highly flexible nature of PaaA2. It harbors two α-helices which serve as molecular recognition elements to wrap itself to ParE2 forming a hetero-dimers. Eight heterodimers form a unique stable hetero-hexadecamer both in solution and in crystal. These findings provide novel insights on TA modules.

The aim of my work is to identify the target of ParE2 toxin. Since the ParE2-target complex interacts with DNA, and the canonical ParERK2 targets on Gyrase, we hypothesize that ParE2 toxin may target on Gyrase or another Type II topoisomerase, topoisomerase IV (topo IV). Type II topoisomerases are essential enzymes to manage the topological structure of DNA.

Purification of ParE2 toxin was done by denaturation and renaturation method to get rid of its cognate antitoxin PaaA2. ParE2 toxin is Co-expressed with its cognate antitoxin PaaA2, then the complex of PaaA2ParE2 was denatured with 8 M urea. Unfolded ParE2 was purified with Ni sepharose, then renatured with low salt buffer. This method enables to get pure ParE2 toxin.

Isothermal titration calorimetry experiment is a technique with the capability of measuring not only the binding affinity but also enthalpic and entropic components that define the affinity. Titration of subunit ParE, which is the subunit of topo IV showed that there is spontaneous and exothermic interaction between ParE2 toxin and ParE. Whether topo IV is the target of ParE2 toxin need further test.
Section B: Applied and Environmental Microbiology
Sigma factors, anti-sigma factors and promoter libraries for orthogonal gene expression and modular control of complex pathways in *Escherichia coli*

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Advances in synthetic biology and metabolic engineering have boosted applications in industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate gene expression and regulation, which results in high metabolic burden for the host organism and low production efficiencies/yields. Nowadays the size and complexity of genetic circuits is growing, but stay limited by a lack of regulatory parts that can be used without interference. Therefore there is need for orthogonal expression and regulation systems to minimise this undesired crosstalk.

In Bacteria the first step in gene expression is transcription initiation where a multi subunit core RNA polymerase binds to a sigma factor to form the holoenzyme. This complex is able to recognise a specific DNA sequence (called a promoter) and initiate transcription. While the bacterial core RNA polymerase is highly conserved, there are numerous sigma factors varying greatly in sequence, size, and promoter specificity. Most bacteria have several alternative sigma factors that all bind competitively to the core enzyme and target the holoenzyme to distinct classes of promoters in order to change the genetic expression program in response to stress conditions.

Here we develop a set of orthogonal expression systems in *Escherichia coli* based on a selection of heterologous sigma factors originating from the Gram-positive sporulating soil bacterium *Bacillus subtilis*. They all belong to the sig-70 family but have different domain make-ups and recognise specific promoter sequences. These expression systems proved to function orthogonal between each other and towards the host. In addition we expand the toolbox by creating promoter libraries for the different sigma factors without loss of their specificity and orthogonal status. As this set is highly orthogonal and covers a wide range of promoter strengths, it enables fine-tuning of multiple independent channels (modular systems) linking the outputs of a circuit to the control of different input signals. Furthermore, this parts toolbox has potential for the assembly of even more complex genetic circuits (/pathways) in the future.

Finally, we tested several anti-sigma factors from *B. subtilis* as orthogonal on/off switches of their cognate sigma factors in *E. coli*. These could add an additional level of control in modular genetic circuits.
Metal effect on conjugation frequency: Assessing the exchange dynamics

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Metal contamination of soil and sediment is a serious environmental issue. Despite elevated metal concentrations, complex microbial communities may progressively adapt to such environments using horizontal gene transfers as recently suggested for a river sediment (Gillan et al., 2015; Jacquiod et al., in press). Plasmids are known to be efficient vehicles for bacterial evolution and adaptation. But it is still unclear how environmental stressors such as metals influence the movement of plasmids by conjugation (Jutkina et al., 2016; Lopatkin et al., 2016; Klümper et al.; 2017). In the present study, we assessed the transfer of a pKJK5 conjugative plasmid carrying or not the pbrTRABCD lead resistance operon in batch co-culture with different lead concentrations (0, 0.5 and 1.5 mM). We quantified each community member (donor, recipient and transconjugant) using flow cytometry. The donor (P.putida KT2440) carries the mCherry gene on its chromosome and the GFP gene on a pKJK5 broad host-range plasmid. Burkholderia xenovorans and Delftia acidovorans were used as recipient cells. The co-cultures were incubated at 16°C during 15 days. We show that the conjugation frequency and plasmid maintenance depend on both metal concentration and the recipient strain.


The ability of bacterial communities to form biofilms and to biodegrade plastics

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Because of its indispensable role in human life, plastic production increases every year. Most of them are then released in landfills or in the sea and scientists estimate that 5 trillions of plastic pieces (macro and micro particles) are today in the marine environment [1]. These synthetic polymers cause large ecological and health impacts [2]. Nevertheless, some microorganisms are able to grow on plastics and some bacteria are even able to degrade them. In this work, bacterial communities from plastics released in the marine environment (Mediterranean Sea, Corsica) and in water from a wastewater treatment plant (Wasmüel, Belgium) were analysed by 16S rRNA sequencing and by denaturing gradient gel electrophoresis. We also enriched bacteria able to degrade plastics using mculture media with low carbon contents. Plastic films used for this enrichment were the four most produced plastics in Europe namely, the Low Density PolyEthylene (LDPE), the PolyEthylene Terephthalate (PET), the PolyStyrene (PS) and the PolyVinyl Chloride (PVC).

Results from 16S rRNA sequencing performed on marine samples (floating plastics, sediment plastics and sediments) show that the two major phyla on plastic samples and on sediments are Bacteroidetes and Proteobacteria. Moreover, bacterial communities on sediments were different from those found on plastics, as shown by comparison of diversity indices and by the taxonomic profile. These results confirm that plastics are a distinct environmental niche. Interestingly, bacterial communities on floating plastics were different from those in sediments.

After 2 months of enrichment (marine samples), two main bacteria were selected on the LDPE: Alcanivorax borkumensis and Microbulbifer sp. The first microorganism is already known for its ability to degrade hydrocarbon chains and the second one for its complex polysaccharide degradation. So, these bacteria are potential candidates for the degradation of LDPE. The biofilm structure and degradation signs of plastic surface were observed by scanning electron microscopy (SEM). Some holes and cracks were observed on several samples. With regards to the wastewater treatment samples, biofilms contained a very high bacterial richness and SEM revealed signs of degradation on the 4 types of plastics.

Phylogenetic and functional analysis of linuron mineralizing microbiota in on-farm biopurification systems through a combination of DNA Stable Isotope Probing and Next Generation Sequencing

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Most studies on bacterial biodegradation of recalcitrant organic compounds rely on retrospect analysis of isolated bacteria, meaning that the unculturable majority is in most cases not considered. This calls for a community level analysis that includes the unculturable bacteria in the environment. Recent advances in culture independent research, such as next generation sequencing techniques, allow us to study those neglected 99% and paint a more complete picture of environmental processes, such as pesticide degradation. Such a study, however, proves difficult. Environmental samples are rich in bacterial diversity and screening a community DNA sample for active degraders is a tremendous task. This problem can be reduced by selecting the active degraders prior to the analysis through Stable Isotope Probing (SIP).

In SIP a heavy isotope labeled compound is used as a substrate for the investigated environmental sample. When the compound is mineralized, this heavy isotope will be incorporated into the cell components of the mineralizing community, including their DNA. This labeled DNA can be separated from the lighter, unlabeled, DNA and will represent a sample containing only the genetic material of bacteria involved in the degradation of the investigated compound.

By combining SIP with Illumina MiSeq sequencing of the 16s rRNA gene, the bacterial population involved in the degradation of the commonly used phenylurea herbicide linuron could be directly identified in this study. The results were further confirmed through cloning of the 16s rRNA gene and subsequent Sanger sequencing. The results show, that the linuron degrading genus identified in the studied material, is not closely related to previously identified degraders of this compound.

This illustrates the potential of this method to identify unknown degraders of contaminants in an environmental sample, providing valuable information for potential bioaugmentation efforts. Furthermore, this study provides an example of how information obtained through cultured bacteria, might produce a skewed picture of the environmental reality.
INSPIRATION - INTRODUCTION OF SPIRULINA IN EQUATORIAL AFRICA TO IMPROVE LOCAL NUTRITION

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In the frame of long-haul manned space exploration mission, the European Space Agency (ESA) together with academic and industrial partners are developing a high tech recycling system that will enable the production of fresh food, water and oxygen from organic and inorganic wastes produced during the mission. The system is based on the joint work of higher plants and microorganisms inhabiting bioreactors. Among the latter, the cyanobacterium Arthrospira sp. a.k.a. ‘Spirulina’, naturally growing in African lakes, will be specifically used for oxygen and super food production (Lasseur et al., 2010).

Other important aspects of the MELiSSA project are the benefits for Earth applications and the education of young people. In that purpose, SCK•CEN, one of the founding members of the MELiSSA consortium, set up with a local NGO in D.R. Congo the INSPIRATION project. With INSPIRATION, it is aimed to combat malnutrition in Congo by local production of Spirulina in villages. This type of cyanobacterium is rich in proteins, vitamins, essential fatty acids and minerals and therefore ideal as a food supplement. Moreover, Spirulina is fairly easy to grow in open ponds. During the presentation of this poster, we will share our experience in transforming the high tech lab experience into low tech field applications that can be useful in the bush of Equatorial Africa.

The COSMOS experiments: studying the EFFECTS of geological consolidation on a microbial community.

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Geological disposal of nuclear waste into clay host rocks is considered as a reliable and feasible option. In Belgium, such a disposal system could be located in poorly indurated clay hosts, such as the Boom Clay (BC) or the Ypresian clays. Geological consolidated clays contain very little space (small pores and small sizes of the pore throats) to accommodate or to allow transport of microorganisms. However, during construction of a disposal facility, microorganisms will be provided with increased space inside the excavated disturbed zone and might proliferate if sufficient nutrients and minerals are available. Because of the high plasticity of BC (and of the Ypresian clays), it is expected that voids and fractures will fill and close rapidly and that (re)consolidation will, in a very short time period, restore the soil density. Hence, porosity will be a parameter that returns relatively fast to its initial state. By doing so, it is very likely that space restriction is amongst the first parameters to limit a possibly present and active microbial community.

COSMOS, an acronym for “Consolidation by Oedometers Shifting Microbiological Optimum towards Stress”, aims to study the impact of (re)consolidation of a BC-slurry inoculated with a microbial community collected from the water of a filter of a piezometer installed in the surrounding BC of the HADES underground laboratory (Mol, Belgium).

Reconsolidation of an inoculated BC-slurry was achieved in so called hermetically closed oedometers. Besides consolidation, these oedometers were also adapted to allow percolation, i.e. forced water flow, through the inoculated BC-system with Boom Clay Pore Water (BCPW) amended with acetate, as main electron donor, and nitrate, as principal electron acceptor, to feed the microbial community in the clay. During the consolidation and percolation phases of the COSMOS experiments, a number of physico-chemical and microbial parameters were online logged to monitor the immediate impact of consolidation on a thriving microbial community. After 18 months of stepwise increasing the consolidation to the final level, a level equal to the governing in situ effective stress ($\approx 22$ bar) at the depth level of the underground HADES laboratory, the consolidated BC-cores were unloaded out of the oedometers and immediately cut into thin ($\approx 1$ mm) discrete BC-slices. Subsamples of these BC-slices were used to analyse the evolution inside the BC-cores of important physico-chemical and microbiologically parameters. Geochemical analyses reveal that in almost all collected percolates nitrate and intermediate formed nitrite were absent and, thus most probably converted into reduced gaseous nitrogen species. The extra added acetate was also absent and thus more than likely used by microbes as electron donor in this process. Intracellular. Metagenomic 16S rDNA analyses of subsamples of the initial Boom Clay slurry, some of the squeezed water samples and of the first percolates, reveals that the microbial communities were dominated by *Pseudomonas* sp., and *Undibacterium* sp.. Many species of both genera are known to be strongly involved in the reduction of nitrate to nitrite. *Pseudomonas* species often go further, and are able to perform either a part or the complete sequence of the denitrification process, i.e. the reduction of nitrite into NO, N₂O or even N₂ gas. Analyses after the recovery and slicing of the consolidated BC core (22 bar, bulk density of 2 g/cm³) show detectable amounts of intracellular ATP in resuspended subsamples. Besides, out of all resuspensions of the BC slices, microbes could be revived during both, aerobic– and anaerobic, cultivations attempts.

The COSMOS-experiments have shown geochemical evidences of the consumption and reduction of respectively acetate and nitrate during the consolidation and the percolation phase. The metagenomics study indicate that the inoculated BC-slurries were dominated by nitrate reducing genera like *Pseudomonas* and *Undibacterium*. The COSMOS experiments have also undoubtedly shown that microorganisms are revivable out of BC-slurries that have been (re)consolidated to the same magnitude as the governing in situ effective stress ($\approx 22$ bar, , wet clay density of 2 g/cm³) at the HADES underground laboratory level.

Acknowledgement:
This work is performed in close cooperation with, and with the financial support of ONDRAF/NIRAS, the Belgian Agency for Radioactive waste and Fissile materials, as part of the programme on geological disposal of high-level/long-lived radioactive waste that is carried out by ONDRAF/NIRAS.
Methanogenic archaea residing in Boom Clay borehole water

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Deep geological disposal of radioactive waste has been considered in many countries as a potential safe long-term solution. In Belgium, Boom Clay has suitable physico-chemical characteristics to serve as natural barrier for the disposal of radioactive waste and is studied extensively in the High Activity Disposal Experimental Site (HADES) underground research facility. One of the in situ experiments – the PRACLAY Heater test – simulates all phases (construction, backfilling, saturation, sealing, heating) of the gallery lifetime on a large scale. For at least ten years, the test gallery will be heated up to 80°C to demonstrate that the thermal load generated by the heat-emitting radioactive waste will not jeopardize the safety functions of the host rock. To assess the effect of the heating on the physico-chemical characteristics of the clay, a sampling system was used specifically designed to allow simultaneous in-situ sampling of dissolved gases and pore water. In addition, this design is suitable to implement the analysis of the microbial community residing in Boom Clay piezometer water. Although it is clear that microorganisms will be ubiquitously present in a radioactive waste repository, it remains unclear whether they can be active as the conditions are far from optimal (e.g. high temperature, high pH, space restriction). In this study, the effect of temperature on the microbial population residing in piezometers installed in the PRACLAY gallery is investigated.

Before heating of the gallery, intracellular ATP measurements demonstrated that the microbial activity remained constant during 2.5 years, with an average of 4.5 x 10^5 cells/ml. Heating of the gallery to 80°C for 1 year resulted in a water temperature of 57°C in the piezometer and a drop in microbial activity to 8.6 x 10^4 cells/ml. In addition, DGGE profiles showed that this temperature induced a clear shift in the bacterial and archaeal community. Interestingly, before heating, CO₂ levels decreased in time and a clear correlation with CH₄ was observed suggesting the presence of methanogenic archaea. Indeed, sequencing of the DGGE bands showed the presence of a species from the genus Methanomassiliicoccus, known to produce methane. The genus was still present after heating of the gallery, demonstrating possible methanogenic activity at higher temperature.

This study indicates that Boom Clay piezometer water is hosting methanogenic archaea and that methane production was induced after installation of a piezometer. After 1 year at 57°C, microbial activity decreased and is accompanied with a change in the microbial community.

Acknowledgement: This work is partly performed in close cooperation with, and with the financial support of ONDRAF/NIRAS, the Belgian Agency for Radioactive Waste and Fissile Materials, as part of the programme on geological disposal/surface disposal that is carried out by ONDRAF/NIRAS.
The development of high-throughput sequencing technologies has revolutionized the field of microbial ecology via 16S RNA amplicon sequencing approaches. Clustering those amplicon sequencing data into Operational Taxonomic Units (OTUs) is one of the most commonly used approaches to approximate a bacterial species. Since a 97% 16S rRNA sequence similarity has been widely used in bacterial taxonomy as one of the criteria to delineate species, this cut-off is often applied when clustering amplicon reads into OTUs. However, where this cut-off is derived based on full-length 16S rRNA genes, the amplicons obtained with current high-throughput sequencing approaches in general only rely on one or two variable regions within this 16S rRNA gene. Therefore, within this work we assess the paradigm that applying a clustering step using a sequence similarity cut-off of 97% would lead to OTUs accurately corresponding to species. We show that the robustness of this species cut-off is questionable when applied to short amplicons that are only representing a small part of the full 16S rRNA gene. Indeed, the selected amplicon might be evolutionary more conserved for a specific taxonomic lineage, leading to the merging of different species at the OTU level. Based on our observations we claim that integrating the differential evolutional rates of taxonomic lineages by defining a taxonomic dependent OTU cut-off score, provides a more accurate correspondence between OTUs and species.
Identification of the active core bacterial populations in two thermophilic acidogenic reactors for space application.

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The Micro-Ecological Life Support System Alternative (MELiSSA) is a project from European Space Agency (ESA), with the objective to gain knowledge for the future development of a regenerative life support system for long term space missions. MELiSSA provides a model system for the study and development of a closed life support systems targeting complete recycling based on the combined activity of different living organisms and inspired on a lake ecosystem [1]. Based on this “aquatic” ecosystem, MELiSSA is a loop-system, comprising four compartments (C1 to C4) and the crew. The C1 compartment, in which the solid waste is liquefied by thermophilic anaerobic bacteria and which produces ammonium, volatile fatty acids (VFA’s), CO2 and minerals, has proven its potential efficiency [2].

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. To obtain such a knowledge of the C1 compartment, metagenomics, metatranscriptomics and metaproteomics will be used to describe its microbial community regarding composition and functionality.

For the work presented here, we obtained DNA and RNA samples at regular time points from two independent thermophilic acidogenic anaerobic reactors (pH 5.5; 55°C) which were used to study the evolution of the C1 bacterial community over time. DNA and cDNA amplicon libraries of the 16S rRNA gene were sequenced with the MiSeq platform in order to identify the changes in the microbial composition overtime. By studying the evolution of the bacterial populations of the two reactors, we intend to identify the core group of bacteria involved in the conversion of the specific MELiSSA substrate (plant material and human fecal matter) into VFAs and CO2.

References:

Antibiotic resistance along the Zenne River

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Indiscriminate overuse of antibiotics has led to the increase of antibiotic-resistant (AR) bacteria. Urban rivers are impacted ecosystems which may play an important role as reservoirs for AR bacteria. The main objective of this study was therefore to describe the prevalence of antibiotic resistance along a sewage-polluted urban river, the Zenne River (Belgium). Seven sites were selected to study the prevalence of AR Escherichia coli and autochthonous bacteria over a 1-year period. Culture-dependent and -independent methods were used to estimate E. coli and heterotrophic bacteria resistant to four antibiotics (amoxicillin, sulfamethoxazole, nalidixic acid and tetracycline). The antibiotic resistance genes (ARGs), sul1, sul2, tetW, tetO, blaTEM and qnrS were also quantified in both particle-attached (PAB) and free-living (FLB) bacteria. Our results evidenced an effect of the treated wastewaters on the spread of antibiotic resistance along the river. Although an increase in the abundance of both AR E. coli and resistant heterotrophic bacteria was observed from upstream to downstream sites, the differences were only significant for AR E. coli. We also found a significant positive regression between AR E. coli and resistant heterotrophic bacteria, suggesting that fecal bacteria released by wastewater treatment plants (WWTPs) could play a role in the spread of AR determinants among autochthonous communities. In general, the concentration of ARGs increased from upstream to downstream sites for both particle-attached (PAB) and free-living (FLB) bacteria. Particularly, a significant increase in the abundance of four among six ARGs analyzed was observed after crossing Brussels. Significant positive correlations between the concentrations of tetracycline and their corresponding ARGs were observed. The comparative analysis of ARGs in different fractions revealed a significantly higher abundance in PAB compared to FLB for tetO and sul2 genes. This study evidenced that urban activities may increase the spread of antibiotic resistance even in an already impacted river.
Flow cytometric monitoring of bacterioplankton phenotypic diversity predicts high population-specific feeding rates by invasive dreissenid mussels

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Recent advances in microbial flow cytometry analysis have allowed the calculation of phenotypic diversity estimates from minute amounts of sample. Here, we validated this approach for high-diversity freshwater environments. We then demonstrated its efficacy in detecting subtle transitions in the phenotypic properties of a natural bacterioplankton community when subjected to feeding pressure by invasive dreissenid mussels (IDMs). Based on a data set of parallel flow cytometry and 16S rRNA gene amplicon data from various environments, the phenotypic diversity was shown to be highly correlated to the taxonomic diversity. This allowed the application of the phenotypic diversity as the sole metric to infer changes in both the phenotypic and taxonomic diversity. Using the flow cytometry approach, a significant decrease in the diversity (11.6 ± 4.1%) was detected within one hour of feeding. We further demonstrate that this diversity loss was caused by selective feeding of IDMs upon high nucleic acid (HNA) populations at a clearance rate that is comparable to that of laboratory strains. Based on known characteristics of HNA populations this selective behavior is predicted to directly impact ecosystem function, as it drives the bacterioplankton community towards a less productive and less diverse state.
**Fermented carrots: A novel food matrix for probiotic interventions**

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Fermentation is humankind's first venture in biotechnology, mainly developed for food preservation purposes. With the introduction of refrigerators, the household use of fermentation to preserve vegetables and milk dropped drastically. However, artisanal fermentations are regaining popularity among top-chefs and general populations for their postulated digestive benefits and rich flavours. The probiotic fermentation market is mainly dominated by dairy products (e.g. yoghurt, fermented milk drinks), with commercially available probiotic starter cultures. An increasing number of meta-analyses have shown that these probiotics could be a valuable health-care strategy for the prevention and treatment of gastro-intestinal, acute respiratory tract infections with also positive indications for oral health and even depression. However, not all clinical trials with probiotics have been positive. While this can—at least partially—be explained by the fact that not all individuals are responders to specific probiotic treatment, it is also clear that probiotic strain selection and formulation needs to be further improved based on more thorough molecular knowledge.

The increase of veganism and people suffering from milk protein allergies also promotes the search for alternative probiotic food matrices. Fermented carrots and juice can fill this position due to the high presence of lactic acid bacteria (LAB) after three days of fermentation, with additional benefits such as the high amount of nutrients e.g. vitamins, antioxidant, fibres, and the low amount of proteins and sugars. The carrot fermentation however is to this date mainly a spontaneous process, characterized by an unwanted initial growth of *Enterobacteriaceae*. Although our RNA-based 16S amplicon sequencing approach showed that these *Enterobacteriaceae* are not really metabolically active at the later time points of fermentation, the fact that biogenic amines were detected points towards the need of better controllable fermentations, e.g. with starter cultures. So far, more than 400 LABs were isolated from previous carrot juice fermentations and we currently explore their fermentation capacity, as well as additional functionalities, such as capacity to persist in the human body. In addition, the capacity of LABs from other niches to ferment carrot juice is explored. Current results indicate that the LAB isolated from previous carrot juice fermentation show a superior capacity to ferment carrots compared to the human isolates. A *Leuconostoc* strain isolated out of previous carrot juice fermentations showed the fastest decline of pH, reaching pH <5 after the first day. After 30 days of fermentation, the juices with LAB isolated from previous carrot juice fermentations as starter culture, also had a higher relative abundance of *Lactobacillus* species compared to juices with LAB from other niches as starter culture.

Further screening and characterisation of LAB from different niches will lead to the identification of suitable probiotic starter cultures which can control the fermentation process, substantiating fermented carrots as a suitable food matrix for probiotic intervention.
Assessing the microbiological diversity in the cooling waters of a nuclear research reactor

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The BR2 nuclear research reactor comprises different watery environments: the primary cooling circuit consists of a closed loop containing water that cools down the fuel rods in the reactor core. An open basin surrounds the reactor vessel, and whenever fuel is loaded onto or unloaded from the core matrix, an exchange occurs between the basin and the primary circuit water. Spent nuclear fuel is stored in the contiguous spent nuclear fuel pool (SNFP) in order to cool down before being safely disposed. Remarkably, despite the low-nutrient environment combined with the highly radioactive character of the water and the presence of dissolved radioactive metals, microbial growth 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 described environments of the BR2 nuclear research reactor. In parallel, this research also focuses on following up these communities over time during and outside reactor operation to monitor the long-term effect of ionizing radiation. Finally, this project also aims at a phenotypical characterization of the prevailing species.

For the characterization and the follow-up of the bacterial communities, a 16S rRNA amplicon sequencing approach was adopted. Results from a long-term follow-up experiment highlighted a clear shift in the bacterial community profile during and outside reactor operation, both for the basin and the primary water. More specifically for the basin water, the profiles observed across multiple shutdown periods appeared to be quite similar. In contrast to this, the community interestingly showed drastic variation when compared across different reactor operation cycles. This could be due to the change in physico-chemical parameters that these waters undergo when transitioning from one state to the other.

With regard to the phenotypical characterization, strains were isolated from the basin and the SNFP and subsequently subjected to ionizing radiation in a gamma irradiation facility to test their radiation resistance. This experiment showed that all 12 tested strains tolerated a dose of 300 Gy, but only 8% of the strains was able to cope with a dose of 2100 Gy, indicating large variability in radiation resistance between different strains, and as such not necessarily a high radiation tolerance to survive in the basin and the spent nuclear fuel pool.
MECHANISM OF RADIATION RESISTANCE IN THE CYANOBACTERIUM ARTHROSPIRA SP. PCC 8005

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Background

The cyanobacterium *Arthrospira* is globally used as feed- and foodstock owing to its high nutritional value. The MIC group at SCK•CEN studies *Arthrospira* sp. strain PCC 8005 as a principal organism and edible endproduct of the MELiSSA bioreactor, a life support system developed by the European Space Agency. Particular caveats to study this highly versatile organism are its assay-interfering autofluorescence and the lack of a genetic system.

Objectives

Two important aspects of our research are (i) to understand the genetic and biochemical pathways involved in the resistance of strain PCC 8005 to extreme high doses of ionising radiation (IR) and (ii) to define optimal growth conditions for *Arthrospira* to conserve its beneficial properties in radiation-intensive space environments.

Methods

To study the effect of radiation on growth and morphology and to check whether extreme IR-resistance is a general trait in *Arthrospira*, different strains of *Arthrospira* were exposed to increasing doses of gamma radiation and analyzed for culture-based growth recovery, morphological changes, and cellular and molecular effects. For the latter we used LC-MS/ESI-TOF metabolic profiling and TEM microscopy. The strains were also studied for changes in the cellular content of proteins, pigments, carbohydrates, and fatty acids in response to radiation.

Conclusions

We found that resistance to IR in *Arthrospira* strains is not just confined to our strain PCC 8005 but that this property was also present in all other Arthrospira strains investigated (i.e. all strains witstood 300 Gy) albeit that some strains were more resistant than others, in the range of 300 – 5000 Gy). Even the two morphotypes of *Arthrospira* sp. strain PCC 8005 (straight versus helical trichomes) show, in terms of growth recovery, distinct sensitivities towards IR although no apparent physiological or cellular change could be observed immediately after radiation. Through comparison of the two morphotypes of the *Arthrospira sp. PCC 8005*, showing different sensitivities towards radiation, we observed a higher amount of light harvesting antenna and cellular content of hydrophilic and lipophilic proteins in the more resistant morphotype paired with a lower amount of the stress protectant trehalose. To further understand this complex response, we are now designing experiments for an indepth analysis using RNAsseq and mass spectrometry.

Keywords

Radiation resistance, *Arthrospira* sp. PCC 8005, Genetic and Biochemical pathways
Section C : Medical and Veterinary Microbiology
Challenges in varicella vaccine

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Over the last decades, refusal behavior to vaccination had arisen. Without any doubt, this anti-vaccination movement could cause an erosion of the public trust in the efficiency and safety of vaccination programmes. As known, vaccination is voluntary or compulsory. A plethora of infectious diseases were almost eradicated after successful vaccination programs in many developed and other countries. Varicella zoster virus (VZV) is belonging to the herpesviruses which collects 8 herpesviruses species-specific known to affect humans. The virus causes chickenpox (varicella) which is a disease mainly affecting school and pre-school children adults and herpes zoster (shingles) in older adults; shingles is rare in children; shingles is rare in children. VZV proliferate in the lungs, and causing different symptoms. However, after the primary active infection (chickenpox), the virus turn into dormant state in the nerves, including the cranial nerve ganglia, dorsal root ganglia, and autonomic ganglia for many years. VZV can reactivate to cause neurologic conditions, after many years of recovery. A live-attenuated vaccine was produced (1995) and has been recommended a first routine use for immunization of children 12 to 18 months of age. A second dose of the vaccine is received at 4 through 6 years old. General population is protected from varicella by vaccination; changes in the current epidemiology of the disease are anticipated. The vaccine can be mono-associated to the attenuated varicella virus or polyvalent combined with other virus or bacteria Measles, Mumps, Rubella, and Varicella Vaccine (MMRV). Although varicella is considered a mild disease, it may cause severe complications, leading to hospitalization in about 2–6% of infected subjects. However, it should be noted that most hospitalized individuals due to VZV infection have no history of underlying disease. Varicella infection in immunocompromised patients and healthy adults is associated with increased morbidity and mortality. Congenital infection can result in fetal varicella syndrome in up to 2% of cases, if the mother develops varicella during weeks 8–20 of gestation. Climate factors seem also to influence varicella incidence. The average duration of hospitalization ranges between 3 and 8 days. To evaluate further the relation between the efficacy of the vaccination, pre and after vaccination serology testing was offered systematically in populations groups which entered our study. Although a slight increase in the peak incidence age of varicella has been observed post-universal varicella implementation, the incidence rates among adolescents and adults have decreased when compared with the pre-vaccination era.
Galleria mellonella as an alternative in vivo model for streptococcal virulence studies and antimicrobial drug screening.

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Recently, Galleria mellonella larvae have been introduced as an alternative model to study microbial infections. Wax moth larvae are easy to maintain, relatively cheap and do not require specialized lab equipment. Larvae are capable of surviving at 37°C, which makes them suitable host organisms to study human pathogens. Also, the immune system of G. mellonella larvae resembles the vertebrate innate immune system, which allows the study of early host-pathogen interactions.

Here, we present the use of a streptococcal G. mellonella infection model for two purposes. Firstly, it can be used to investigate differences in virulence between streptococcal serotypes and/or strains and secondly it can be used for the early in vivo evaluation of novel antistreptococcal molecules. Our findings show a strong correlation between instilled bacterial inoculum and host survival as well as a distinct difference in virulence of several streptococcal serotypes. Importantly, the host survival increases after treatment with conventional therapeutics in a dose-dependent manner.

Currently, a novel luminescent streptococcal strain is being developed. This strain will allow for a reliable, fast and easy quantification of the bacterial burden, which to date remains an issue. Implementation of this model in the drug discovery process will reduce the overall cost and allow for a more stringent selection of in vitro bio active compounds to be tested in a subsequent murine infection model, hereby reducing the number of vertebrate used in the early drug discovery phase.
Drug resistant pattern of bacterial isolates in infected wounds at Bahir Dar Regional health research laboratory center, Northwest Ethiopia

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Background An increased antibiotic resistance of bacterial isolates from wound infections is a major therapeutic challenge for clinicians. The aim of this study was to describe bacterial isolates that caused wound infection and determine their current antimicrobial susceptibility pattern.

Methods: We analyzed the records of 380 wound swab samples that have been cultured at Bahir Dar regional health research laboratory from January 2013 to December, 2015. Swabs from different wound types were collected aseptically. Antimicrobial susceptibility test was performed using disc diffusion technique as per the standard protocol. Bacteriological and socio-demographic data were collected using a standard data collection format. The data was cleared, entered and analyzed for descriptive statistics using SPSS version 20.

Result: The overall bacterial isolation rate in this study was at 61.6% (234/380). About 123 (52.6%) of the isolates were gram positive cocci and 111 (47.4%) were gram negative rods. The predominant isolate was S. aureus at 100 (42.7%) followed by E. coli 33 (14.1%), P. aeruginosa 26 (11.1%) and S. pyogenes 23 (9.8%). The overall rate of multidrug resistant (MDR) bacterial pathogens that caused wound infection was 54.7%. Out of these, 35 (15.1%) of the isolates were resistant to more than five antibiotics. Ampicillin had the highest resistance rate at 85.9% among gram negative isolates. Whereas the highest resistance rate among gram positive isolates was in erythromycin at 31.1%.

Conclusion: In the studied region, higher frequency of mono and multi drug resistance of bacterial pathogens that caused wound infection was documented. Thus, a new method to the causative agent and antimicrobial susceptibility testing surveillance in areas where there is no culture facility is needed to assist the health professionals in the selection of appropriate antibiotics.

Key words: wound infection, bacterial isolates, culture, drug susceptibility.
Identification of virulent *Capnocytophaga canimorsus* isolates by capsular typing

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*Capnocytophaga canimorsus* is a dog oral commensal causing human septicemia that often evolve to a septic shock despite an adequate antibiotic therapy. *C. canimorsus* sepsis are associated with a mortality rate as high as 30% and significant morbidity [1]. Estimations of *C. canimorsus* infections prevalence are low and vary from 0.5 to 4.1 cases per million inhabitants per year [2]. However while splenectomy and alcohol abuse are common predisposing factors, up to 40% of patients presented no obvious risk factor, thereby implying that *C. canimorsus* cannot solely be considered as an opportunistic pathogen [1]. *C. canimorsus* was recently shown to be endowed with a capsular polysaccharide implicated in the resistance to the innate immune system of the host [3]. We developed the first *C. canimorsus* capsular serotyping scheme [4]. We described 11 different capsular serovars (A to K), which allowed the typing of 101/101 *C. canimorsus* strains isolated worldwide from human infections. Only 21/55 strains isolated from Swiss, Belgian and Swedish dog mouths were typed, indicating a vast capsular repertoire in the species. Strikingly, three serovars only (A, B and C) covered 91% (92/101) of the human isolates tested while they covered only 7.3% of the dog isolates (4/55). This enrichment in clinical isolates was statistically significant strongly suggesting that the serovars A, B and C are more virulent for human than other serovars. A retrospective analysis of clinical records from 73 Finnish *C. canimorsus* infected patients showed that serovars A, B and C were equally dangerous. Capsular typing could allow to identify virulent strains in dogs, which could contribute to the prevention of these infections. To this end, we developed a PCR typing method based on the amplification of specific capsular genes.

Molecular epidemiology of *Legionella pneumophila* in Belgium from 2011 to 2016


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**Background:** *Legionella pneumophila* (*Lpn*) is the etiological agent of legionnaires' disease. This microorganism can be found in natural aquatic environment as well as in artificial water systems. Infection occurs mainly through inhalation of contaminated aerosols. To discriminate between *Lpn* strains, Sequence Based Typing (SBT) has been widely used as a typing method. In this study, we have investigated by SBT clinical and environmental isolates of *Lpn* collected from 2011 to 2016 in the Belgian National Reference Centre.

**Materials/methods:** *Lpn* isolates of respiratory samples (*n*=115) and related environmental samples (*n*=10) were genotyped using the SBT protocol of the European Working Group for Legionella Infections (EWGLI). The eBURST algorithm v3 was applied to assign the same group to STs that share at least five of seven SBT loci.

**Results:** Clinical isolates of *Lpn* serogroup 1 (*Sg1*) (*n*=109, 95.5%) were classified into 39 STs (Simpson's index of diversity: 0.9). The most frequent STs were ST1 (24.8%) and ST47 (19.3%). The other serogroups (*n*=6, 4.5%) were represented by 5 distinct STs. Among all serogroups, six STs were newly characterised. The eBURST analysis showed that *Lpn* *Sg1* isolates were distributed into six clonal complexes (CCs) and five singletons. The main lineages were CC1 (*n*=46, 42.2 %) and CC932 (*n*=26, 23.9 %). For 10 patients, environmental isolates were available for comparison. In 5 cases, environmental source could be confirmed based on identical ST results. In one case, no similarity was found. In the remaining cases, a matching with the very frequent ST1 should be further analysed with other techniques.

**Conclusion:** This study shows that ST1 and ST47, belonging respectively to CC1 and CC932, are the most frequent STs in Belgium, confirming previous epidemiological observations (Vekens *et al*., 2012) and in agreement with the epidemiology in northwest Europe. The SBT of *Lpn* isolates has updated the Belgian database and could confirm the link between clinical and environmental samples.
An artificial sebum model to explore *Propionibacterium acnes* biofilm

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Colonization of the skin’s pilosebaceous units by *Propionibacterium acnes* is one of the major contributing factors in the pathogenesis of acne and it has been shown that *P. acnes* can form biofilms in these skin appendages. This biofilm formation could explain why acne is a chronic disease and why long-term antimicrobial therapy is needed. Additionally, *P. acnes* can also produce various virulence factors including host tissue degrading enzymes, such as proteases and lipases, and the co-hemolytic CAMP factors.

In order to elucidate the role of *P. acnes* in acne, growth and biofilm formation were investigated in a new *in vitro* model with artificial sebum, resembling the nutrient-poor, hydrophobic micro-environment that this bacterium colonizes. Using both the microtiter plate model and the artificial sebum model, biofilm formation, virulence, and antibiotic susceptibility of a collection of *P. acnes* strains were investigated.

Growth and biofilm formation by *P. acnes* in the artificial sebum model were evaluated using conventional plating techniques, and subsequently confirmed using fluorescence microscopy after LIVE/DEAD staining. Production of lipases and proteases was assessed using fluorescence based assays, while the production of CAMP factors was verified using an adjusted form of the CAMP test. Finally, the susceptibility of *P. acnes* biofilms grown in the different model systems towards compounds used as anti-acne therapies (including clindamycin and erythromycin) was determined.

Our results demonstrate that the artificial sebum model supports growth and biofilm formation of *P. acnes*. Moreover, the production of aforementioned virulence factors was verified in both biofilm grown in a MTP and on artificial sebum. Lastly, our results indicate that common anti-acne antimicrobials are not able to eradicate biofilm grown in a MTP and on artificial sebum.
Implementation of the *Galleria mellonella* larvae model to evaluate phage therapy against *Klebsiella pneumoniae*


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*Klebsiella pneumoniae* (*K. pneumoniae*) is an encapsulated Gram negative bacillus belonging to the family *Enterobacteriaceae*. *K. pneumoniae* strains display a large number of capsular serotypes. It is an opportunistic pathogen causing severe therapeutic challenges due to the continuous emergence of multidrug resistant strains. The aim of this work is to develop an infection model of *Galleria mellonella* larvae with *K. pneumoniae* strains of interest to evaluate phage therapy strategies.

Three selected bacteriophages have been isolated from wastewater plants of Paris against two *K. pneumoniae* strains of epidemiological interest, two against the ST258 (phages K?-ULIP47 and K?-ULIP54) and one against ST23 (phage K1-ULIP33). The host range of these three bacteriophages shows a high specificity for the *K.* capsular type. In a first *in vivo* experiment, 110 larvae have been used to assess the optimal inoculation dose of *K. pneumoniae* to use for phage therapy experiments. A dose of 10⁴ CFU/10µl results in a 70-90% killing of larvae in 4 days. In the second and the third experiments, 480 larvae were inoculated to assess both prophylactic and curative treatment of these bacteriophages. More than 80% of survival is observed in the larvae treated with the bacteriophages at a multiplicity of infection of 10 compared to the non-treated *K. pneumoniae* infected control in which over 90% of larvae died. The group of larvae inoculated with bacteriophages only showed comparable survival rate as the PBS control group. Both prophylactic and curative groups showed similar survival rates.

These results show that *G. mellonella* could be used as a preliminary model to test phage therapy against *Klebsiella* infection.
Staphylococcal chromosome cassette mec typing of methicillin resistant staphylococci from different samples collected in European, African and North-American countries.

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Staphylococci are one of the most prevalent bacterial agents worldwide, which are responsible for several human and animal pathologies. Antimicrobial treatment against staphylococci has led to the selection of Methicillin Resistant Staphylococci (MRS) which represent a potential hazard in public health via the inter- transferability of the mobile “Staphylococcal Chromosome Cassette” (SCC) carrying the mec genes encoding the resistance. The aim of this study was the identification of the MRS and the typing of their SCC mec in a collection of isolates coming from European, African and North-American countries.

A total of 1269 staphylococci were isolated between 2005 and 2014 from different samples in Belgium (n=731), Italy (n=45), Switzerland (n=25), Senegal (n=90), Niger (n=256) Canada (n=90) and Japan (n=32). Isolates were tested for their growth on “Chrom MRSA ID®” agar, for the presence of mecA and mecC genes (by hybridization and PCR) and for the SCC mec typing (by PCR).

A total of 71 S. aureus isolates grew on “Chrom MRSA ID®” agar, including 38 mecA-positive and 33 mec-negative isolates. Twelve non S. aureus staphylococci (NAS) grew on “Chrom MRSA ID®” agar, but only one carried mecA. Additionally, 29 S. aureus isolates and 16 NAS isolates carried mecA but did not grow on “Chrom MRSA ID®” agar. No isolate was tested positive for mecC gene. The mecA-positive isolates (n=84) were considered MRS and subjected to SCC mec typing. The MR S. aureus (MRSA) carried SCC mec types II (n=8), III (n=2), IV (=27), V (n=7), VII (n=3) or non-typeable (n=20), while the MRNAS carried types III (n=1), IV (=5), V (n=4), or non-typeable (n=7). Most MRS isolates were recovered from Belgium (MRSA: n=58; MRNAS: n=16), Japan (MRSA: n=5) and Niger (MRSA: n=4; MRNAS: n=1).

A diversity of SCC mec types were found in MRS isolates from different countries. Interestingly, some MRS carried non-typeable SCC mec types that could represent new variants. Further analysis by whole genome sequencing will be done in order to investigate these potential novel cassettes.
Quinones and a novel bio sensor to report oxidative stress in *Mycobacterium tuberculosis*

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A series of novel azaanthraquinones immediately attracted our interest due to its favorable in vitro bioactivity profile against *Mycobacterium tuberculosis* H37Ra. Furthermore, it is hypothesized they could act as redox cyclers, putatively targeting mycothione reductase (Mtr), and interfere with the bacillary redox homeostasis and hence its ability to cope with oxidative stress. In addition, the reaction of these compounds with Mtr could result in an array of free radicals and thus an increase in intracellular reactive oxygen species (ROS) level, to which the bacillus ultimately succumbs. To study the direct effect of the quinone analogs on the bacillary redox homeostasis, we developed a panel of three new *Mycobacterium marinum* reporter strains. Despite the difference in ecological niche, the bacillary redox system of *Mycobacterium marinum* is nearly identical to *Mycobacterium tuberculosis*. In the recombinant strains, transcription of the FurA/KatG mediated detoxification system is coupled to the expression of eGFP whilst the expression of mCherry is transcribed from the strong constitutive mycobacterial promoter P1. The ratio of the Green/Red light emitted by the reporter strain can be used as an indicator of the overall bacillary redox state. Validation of the model has been conducted by the use of first- and second-line anti-TB drugs as well as the use of different concentrations of oxidative stress produced by hydrogen peroxide. Evaluation of the azaanthraquinones is currently ongoing. In future perspectives, we envisage to use this model to study the effect on the bacillary redox homeostasis inside bone-marrow derived macrophages.
Evaluating the response of multispecies biofilms to antibiotics using novel selective media

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The lungs of cystic fibrosis (CF) patients are chronically colonized by a polymicrobial biofilm community, leading to infections that are difficult to treat with available antibiotics. Lower microbial diversity in the CF airways is typically associated with negative health outcomes. Hence, choosing antibiotics that minimally affect microbial diversity may result in health benefits. To understand the dynamics of polymicrobial community composition in response to antibiotic therapy, *in vitro* studies have provided valuable insights. To this end, culture-dependent quantification of individual bacteria from defined multispecies biofilms is frequently carried out by plating on selective media. However, the influence of the selective agents in these media on quantitative recovery before or after antibiotic treatment is often unknown.

In the present study we developed selective media for six bacterial species that are frequently co-isolated from the CF lung, i.e. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus anginosus*, *Achromobacter xylosoxidans*, *Rothia mucilaginosa*, and *Gemella haemolysans*. We show that certain supplemenations to selective media strongly influence quantitative recovery of (un)treated biofilms. Hence, the developed media were optimized for selectivity and quantitative recovery before or after treatment with antibiotics of four major classes, i.e. ceftazidime, ciprofloxacin, colistin, or tobramycin.

The novel selective media were applied to determine the community composition of multispecies biofilms before and after treatment with ceftazidime, ciprofloxacin, colistin, or tobramycin. Results showed that in the untreated multispecies biofilm, each strain was highly abundant (6 to 8 log CFU/mL per strain). Following treatment of the multispecies biofilm with antibiotic concentrations that target *P. aeruginosa*, the community composition was strongly influenced. Especially after ceftazidime and tobramycin treatment, the relative abundance of all community members was decreased. Ciprofloxacin treatment on the other hand affected the individual species to a lesser extent resulting in a higher microbial richness and evenness in the community. In contrast, colistin affected solely Gram-negative species which led to a high richness but low evenness in the community.

In conclusion, we developed a novel tool to determine the influence of antibiotic treatment on individual bacteria within multispecies biofilm communities which allowed us to demonstrate that antibiotics that target the major CF pathogen *P. aeruginosa* may have differential effects on co-colonizing species.
Antibiotic Tolerance of dispersed *P. aeruginosa* Cells

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During biofilm dispersion, sessile cells leave the biofilm and return to a planktonic lifestyle via the so-called dispersed state. In this state they show properties that are different from those of planktonic cultures and biofilms. It is known that dispersed cells are more virulent than planktonic cells. Therefore, it is necessary to know the vulnerability of these cells to antibiotics. In the present study we investigated the susceptibility of *Pseudomonas aeruginosa* cells dispersed from biofilms by exposure to glutamate. The antibiotics used are tobramycin and meropenem.

*P. aeruginosa* PAO1 biofilms were cultivated in flow cells with minimal medium containing 1.8 mM glutamate as the sole carbon source. To induce dispersion, a ten-fold higher glutamate concentration was used in the same medium. Spontaneous and glutamate dispersed cells were collected and treated with 10 µg/ml tobramycin or 10 µg/ml meropenem for 1 to 5 hours; every hour the number of surviving cells was determined using plate counts. Planktonic cultures that were exposed to the same treatment served as controls. The remaining biofilms were treated with antibiotics for 24h. Fluorescence microscopy was used to visualize biofilms and flow cytometry was used to assess cell viability.

The dispersed cells and planktonic cultures showed a 5 log reduction after 5 hours treatment with tobramycin except the glutamate dispersed cells which showed only a 3.5 log reduction. The difference between glutamate dispersed cells and planktonic cultures was already significant after 1 hour treatment. Tobramycin had the same effect on spontaneously dispersed cells and on planktonic cells. In contrast, the dispersed cells and planktonic cultures showed a 2 log decrease during meropenem treatment without significant difference between each other.

To conclude, induced dispersed cells show an enhanced antibiotic tolerance against tobramycin (but not against meropenem) while the spontaneously dispersed cells did not.
Bacterial persistence promotes the evolution of antibiotic resistance by increasing mutation rates

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Persisters are transiently antibiotic-tolerant variants that complicate the treatment of bacterial infections. Both theory and experiments have suggested that persistence facilitates genetic resistance by providing an evolutionary reservoir of viable cells. We show a strong positive correlation between levels of persistence and the likelihood to become genetically resistant in natural and lab strains of *E. coli*. Furthermore, our data show that this effect comes about not only by an increased availability of viable cells, but also because persistence appears to be pleiotropically linked with mutation rates. We argue that this heightened mutation rate is not coincidental, but likely driven by the fact that high persistence and high mutation rates are both favored in environments that are frequently struck by antibiotics. A theoretical model further demonstrates that increased survival and mutation rates strongly affect the likelihood of evolving clinical resistance. Overall, these results suggest that the battle against antibiotic resistance will strongly benefit from incorporating anti-persister therapies.
Section D: Host and microbial interactions
Immunomodulatory and antipathogenic activity of lactobacilli and their exopolysaccharides

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Our knowledge on the human microbiota has been vastly expanding in recent years. Molecular insights into their mutual interactions and with our immune system are, however, still lagging, especially regarding beneficial bacteria. We aim to unravel the role of the extracellular polysaccharides (EPS) of lactobacilli in their antipathogenic and immunomodulatory activity. These glycoconjugates show great strain-specificity in their presence and composition, which makes them excellent candidates for conferring strain-specific health effects to lactobacilli.

Before performing immunomodulation assays, we checked the presence of endotoxins in our isolated EPS samples with the recombinant Factor C assay, since these could interfere in these assays. Samples with minimal endotoxin contamination were used to investigate the effect of these EPS on the production of cytokines by antigen-presenting cells, namely differentiated THP-1 cells.

The antipathogenic effects of EPS against the major fungal pathogen, Candida albicans, were investigated using well-diffusion assays and optical density measurements for growth inhibition on solid media and in suspension, respectively. We also explored the ability of EPS to inhibit hyphal morphogenesis, a crucial virulence step of C. albicans. Finally, we determined the level of inhibition of pathogen adhesion to human epithelial cells when co-incubated with the EPS.

Our data indicate that Lactobacillus EPS shows potential as antifungal strategy against C. albicans, especially reducing the hyphal morphogenesis and adhesion of this pathogen. Furthermore, preliminary data show that EPS of several lactobacilli can differentially influence the cytokine expression in THP-1 macrophages and this is currently being further explored.
Measurement of 1, 3, 5, 6 and 7 substituted 4-hydroxyquinolin-2(1H)-ones activity in vitro and in macrophages using reporter mycobacteria strain

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Quinoline-based compounds have attracted the attention of medicinal chemists due to their versatile bioactivity. As part of our drug development program to identify novel antitubercular drug candidates, we have discovered the potency of 4-hydroxyquinolin-2(1H)-ones against intracellular Mycobacterium tuberculosis. To provide an efficient experimental tool able to detect the in vitro intracellular effect of the small molecules on the mycobacterial replication, an adapted macrophage infection assay has been constructed. This model is based on the infection of primary macrophages with a double reporter M. marinum strain. M. marinum was chosen as it is closely related to M. tuberculosis with a close resemblance in the genetic organization of the genes involved in the resistance to oxidative species, ensuring its survival in host cells. The double reporter strain is both luminescent and fluorescent which enables easy quantification by plate reader while the DsRed-Express fluorophore allows for qualitative evaluation by both the cell analyzer and fluorescent microscopy. In our investigation of the quinoline based compounds, we have found that derivatives carrying a 3-phenyl substituent were favored exhibiting low MIC and no signs of cytotoxicity or genotoxicity. The compound 6-fluoro-4-hydroxy-3-phenylquinolin-2(1H)-one was selected as the most promising member of the library with a MIC of 3.2 µM. Although the activity was not in the same order as the first line antituberculosis drugs, the library proved that the 4-hydroxy-2(1H)-quinolone can be altered by structural modification and change in substituents of the heterocyclic scaffold, representing an interesting group of antitubercular compounds.
Improving the in vitro tools for oral bioaccessibility and bioavailability assessment: impact of the salivary and colonic microbiome.

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Keywords: oral microbiome, gut microbiome, gastrointestinal digestion, bio-accessibility; in vitro models; environmental pollutants

Background

The oral cavity contains hundreds of different bacterial species, which interact with microbiomes from other parts of the human body, especially the intestinal tract. Previous findings suggest that the ingestion of saliva containing large loads of bacteria can influence the microbial ecosystems in the small intestine, or even the colon. Saliva and salivary microorganisms contain an enzymatic array to initiate the digestion of food compounds, as gluten or starch. In addition, saliva can affect the stomach pH by its buffering activity. The release of food compounds, including food toxicants, during the gastrointestinal digestion, can be affected by the presence of saliva in the stomach, a factor not always weighed in in vitro gastrointestinal methods.

We developed a new ecosystemic in vitro model including the salivary and colon microbiomes in the gastrointestinal digestion and applied to test the in vitro bioaccessibility and bioavailability of a model environmental pollutant (arsenic).

Methods

A four-stage gastrointestinal model containing the oral, gastric, small intestinal and colonic environments was applied to three food matrices (rice, mussels, and nori seaweed), naturally containing arsenic. The oral and colonic reactors were inoculated with salivary and fecal samples from healthy human donors. Control reactors without microorganisms were run in parallel. Supernatants from the small intestine and colon digestion were applied to a co-culture model of Caco-2 and HT29-MTX cells in Transwells® and arsenic transport and cellular uptake were assessed.

Conclusion

Salivary microorganisms affected significantly the in vitro oral bioaccessibility and bioavailability of arsenic in a food-dependent way. The current standardized in vitro methods for evaluating bioaccessibility are lacking a key element in the digestion process (oral and gut microorganisms), which could affect the in vitro/in vivo translation of results. The combination of digestion models, including microbial metabolic potency, with models of the gut epithelium, can offer a more accurate prediction of, specifically, As bioavailability, and in a broader context, environmental pollutants, drugs or food compounds.
Breaking the bond between *Helicobacter pylori* and its host

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Background:

*Helicobacter pylori* is a human stomach pathogen infecting over half the world’s population. The infection is generally associated with asymptotic gastritis, but can progress to peptic ulcers and stomach cancers. To sustain infection, the bacteria maintain an intimate interaction with the stomach epithelial cells and overlaying mucus by using a set of structurally conserved autotransporter-like adhesins called Hops.

Objectives:

A highly conserved C-terminal domain is believed to represent the Hop transmembrane domain, but lacks typical features seen in classical autotransporters. We here set out to investigate the Hop architecture and transport route to the outer membrane.

Methods:

We use X-ray crystallography, directed mutagenesis and localization studies to delineate the Hop passenger and transmembrane domain. Recombinant expression in *E. coli* is used to determine the minimal Hop fragment that is targeted to the outer membrane as a stable β-barrel.

Conclusions:

We found that Hops represent a novel family of autotransporter-like adhesins with a conserved discontinuous β-barrel, interrupted by the α-helical passenger domain in extracellular loop 1. The Hop architecture is incompatible with prevailing models for autotransporter insertion into and passenger transport across the outer membrane, leading us to further investigate the route and mechanism of Hop biogenesis as a means of targeting this family of primary *Helicobacter pylori* virulence factors.
A new dimension in host-microbe interactions – How does the host influence antibiotic efficacy?

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Pseudomonas aeruginosa is a major health threat for immunocompromised people, including for patients with cystic fibrosis (CF). In CF patients, conventional antibiotics against P. aeruginosa are ineffective most of the time, and novel treatment options are urgently needed.

We previously demonstrated that when biofilms of P. aeruginosa PAO1 were formed on lung epithelial cells in an in vivo-like three-dimensional (3D) model, the efficacy of various antimicrobial agents was altered. In the present study, we were particularly interested in the increased aminoglycoside efficacy in the presence of 3D lung epithelial cells.

We demonstrate that conditioned medium of 3D lung epithelial cells (3D-CM) improves the efficacy of aminoglycosides against P. aeruginosa PAO1, suggesting the involvement of extracellular host compound(s). In contrast, conditioned medium of 2D (monolayer) cells did not potentiate aminoglycosides. Tobramycin activity was also enhanced by 3D-CM for 6/8 (75%) tested P. aeruginosa strains (including the cystic fibrosis lung isolates), and for 3/3 (100%) strains belonging to other Pseudomonas spp. (including P. putida KT2440). In contrast, only 2/7 (30%) non-Pseudomonas strains were more susceptible to tobramycin or gentamicin in the presence of 3D-CM. In order to shed light into the mode of action, intracellular tobramycin levels were evaluated using BODIPY-labeled tobramycin and flow cytometry analysis, revealing a higher proportion of tobramycin-positive P. aeruginosa in the presence of 3D-CM. Physical and chemical treatment of 3D-CM indicated that the compound(s) are small peptides. Differential high resolution MS/MS analysis was performed to determine the nature of the peptide(s) that enhanced aminoglycoside efficacy.

Our findings indicate that when lung epithelial cells are cultured under in vivo-like conditions, compounds are produced that influence antibiotic efficacy. Not only does this study provide insights into the role of the host in antibiotic efficacy, it might lead to novel approaches to treat P. aeruginosa infections.
Studying the potential beneficial role of lactic acid bacteria in the upper respiratory tract

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Interest in the beneficial functions of the microbiota in different human body niches, such as the upper respiratory tract (URT), has boomed within the last years. A better understanding of the microbiota in this niche might offer new insights into the role of this microbiota in health and disease.

This project aims to characterize the healthy versus diseased microbiome of the upper respiratory tract (URT) using MiSeq (Illumina) 16S rRNA gene amplicon sequencing and cultivation-dependent methods, with chronic rhinosinusitis (CRS)-patients as case study. Furthermore, we aim to cultivate beneficial lactic acid bacteria (LAB) from the nasopharynx of healthy individuals in order to explore their potential as URT probiotics.

Our analysis of 100 healthy adults showed that the ‘healthy’ URT microbiome is a low bacterial diversity niche often dominated by common opportunistic nasopharyngeal pathogens. We could observe five bacterial community types dependent on the dominant genera, some of them belonging to the LAB: Moraxella-, pathogenic LAB Streptococcus-, Fusobacterium- or Haemophilus-dominated or an intermixed type of Staphylococcus, Corynebacterium and the specific LAB Dolosigranulum. We isolated from our healthy participants several LABs via selective cultivation methods. Further characterization of these species is currently going on, e.g. adhesion assays, antipathogenic screening, … to explore their potential as URT probiotics.
Molecular diversity of *Photorhabdus* sp. and *Xenorhabdus* sp. bacteria symbiotically associated with entomopathogenic nematodes retrieved from soil in Benin.

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Entomopathogenic nematodes (EPNs) are commonly used to control many insect pests across the world. *Xenorhabdus* and *Photorhabdus* species are bacterial symbionts of EPNs. Indigenous EPN have been proven in many researches to be more effective than the exotic ones. To establish any biological control program in Benin against insect pests which represent a huge constraint to agriculture (especially citrus and mango orchards), the first step was to investigate the diversity of local EPN as well as their associated symbiotic bacteria as the latter play a key role in the insect infection process.

In years 2012 and 2015 soil sampling was performed across the country to detect local presence of EPN. Several strains of *Heterorhabditis* and *Steinernema* nematodes were retrieved from soil. In this study, all symbiotic bacteria associated with collected EPNs were isolated, purified and characterized molecularly and morphologically. DNA was extracted from each bacterial strain and 5 protein coding genes were targeted in addition to the 16S rRNA gene. In total we amplified and partially sequenced the RecA, GyrB, DnaN, InfB and Gltx genes and phylogenetic relatedness were reconstructed to see the molecular position of the bacteria strains among the described ones. In total 43 symbiotic bacterial strains were isolated. Results showed that bacteria were distributed into two main clusters, *Photorhabdus* and *Xenorhabdus*. One bacterial strain clustered within *Serratia* genus. *Xenorhabdus* strains appeared to cluster with *X. indica* and they share more than 98% nucleotides identity value based on the 16S rRNA sequences information while bacteria in the *Photorhabdus* group, based on the same gene, were distributed in two different sub-clusters within *Photorhabdus luminescens* clade with closest neighbor *P. luminescens* subsp. *luminescens*. Molecular analysis of the concatenated sequences of *Photorhabdus* strains based on the 5 housekeeping genes demonstrated that those strains share with the known subspecies of *P. luminescens*, less than 97 % nucleotides identity which is the threshold proposed by Tailliez et al. 2010 to split subspecies within a *Photorhabdus* species group. This infers that the *Photorhabdus* strains represent two new sub-species within the *P. luminescens* group. Phenotypic characters based on the Api 20E, 20 NE and 50CH (biomerieux) and biolab plates were also assessed to confirm the novelty of the *Photorhabdus* strains. *In vivo* pathogenicity test was also carried out to confirm the ability of the isolated bacterial strains to kill insect pest.

Keywords: Symbiotic bacteria, biological control, housekeeping genes. *Serratia sp.*
CELECOXIB SUPPLEMENTATION IMPACTS ACTIVITIES OF IN VITRO GUT MICROBIAL ECOSYSTEM

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Interest in developing colon-targeted drug delivery systems has increased in recent years. These formulations are intended for gastrointestinal related diseases, such as colorectal cancer and inflammatory bowel disease. Cyclooxygenase-2 (COX-2) inflammatory pathways have been demonstrated to be altered on colon carcinogenesis (CRC). COX-2 inhibitors (coxibs), such as celecoxib, have successfully reduced COX-2 and prevented CRC recurrence. Alas, their continuous administration may increase the risk of a cardiovascular event. Currently, little information is available on how inter-individual variations in colon microbiota impact overall celecoxib disposition. Thus, gaining insight into their metabolic impact on the gut is of great importance. Characterisation of the players involved in drug disposition in the gut will contribute to ensure optimal efficacy and safety profiles.

This project evaluated the effect of clinical concentrations of celecoxib on the in vitro colon microbiota. We determined the baseline microbiota activities and metabolic response, to reveal whether microbial drug metabolism impacts the conversion process.

In vitro batch culture experiments were conducted to assess the short-term effect of celecoxib on the activity and composition of the colon microbial community. Faecal slurries from eight volunteers were supplied with 100 mg/ml of celecoxib and anaerobically incubated for 16h, to simulate the transit time of the proximal colon. Short-chain fatty acids (SCFAs) were considered benchmarks of gut microbial functionality and determined by gas chromatography. Liquid chromatography-mass spectrometry (LC-MS) was used to determine celecoxib recovery. Total RNA was applied to perform qRT-PCR of the bacterial 16S rRNA gene and to evaluate the metabolically active population. Community composition and structure were screened using high-throughput amplicon sequencing.

SCFA production showed overall shift in functionality compared to control incubations, suggesting that celecoxib shifts in vitro fermentation, in a donor-dependent manner. Celecoxib significantly decreased total SCFA and butyrate ($P < 0.001$), but not copy number of 16S rRNA gene in all donors. Thus, although total active bacterial population was not significantly different between treatments, composition and structure were influenced. LC-MS outcome implied bacterial transformation of celecoxib, but quantification of the metabolised amount varied due to a donor-dependent extraction efficiency. However, bacterially-metabolised celecoxib could decrease IL-8 production. Higher TEER values confirmed decrease in the inflammatory response. Reduction in butyrate may have negative implications, as a result of its protective properties for colonocytes. Our study provides preliminary information about the microbiota interplay on the efficacy of colon-targeted coxibs.
Specificity of *Helicobacter pylori* towards human host receptors

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*Helicobacter pylori* chronically infects half of the world population; thereby causing chronic gastritis, duodenal ulcers and gastric cancer. On its surface *H. pylori* expresses a family of *Helicobacter* outer membrane proteins (Hops) to mediate attachment to the gastric epithelial cell surface and overlying mucins, thereby avoiding the harsh environment of the stomach lumen. Members of the Hop family include the blood group antigen binding adhesin (BabA) and HopQ that mediate the attachment to respectively stomach glycoreceptors containing ABO/Lewis b blood group antigens and members of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family. The presence of BabA is linked to disease-associated strains, whereas CEACAMs are upregulated during gastritis. Also *H. pylori* has an extreme genetic diversity, with particularly high rates of adaptive evolution in genes for adherence to host receptors.

We obtained structures of the BabA and HopQ adhesin domains that shows a conserved α-helical core domain. ABO/Lewis b bound representative isoforms of BabA identified blood group binding involves a highly polymorphic 4-stranded β-sheet domain that is unique to BabA and critically depends on a disulphide-bound loop in the binding site. Two diversity loops dynamically control blood group and type 1 chain glycan specificity, respectively. The ligand-bound structures provide a molecular rationalization of the observed blood group antigen polymorphisms in *H. pylori* clinical isolates. Recently the structure of the HopQ-CEACAM1 complex was obtained (unpublished results) and allowed to explain the specificity of HopQ towards specific human CEACAM variants.


Identification of bacterial gene indispensables to the early phase of pulmonary infection by *Brucella melitensis* in mice

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Identification of factors that promote susceptibility to a pathogen could contribute to the development of more effective prophylactic and therapeutic strategies to control the spread and establishment of a pathogen in the host in a chronic manner.

Infection with *Brucella* bacteria is responsible for brucellosis, a worldwide zoonosis that can cause serious complications in humans without treatment. The chronicity and recurrence of this infection despite antibiotic treatments result in significant morbidity.

In the experimental murine model, following an intranasal infection, *Brucella* invades the pulmonary alveolar macrophages and persists several days in this organ before disseminating in the host. Our results suggest that early immune control can contain infection and reduces pulmonary bacterial load. To better understand what is essential to the bacteria to survive the immune response and to replicate, we have combined an *in-situ* visualization approach of bacterial infection using a fluorescent reporter system with a sequencing approach of Transposon (Tn-seq) in kinetics. Our first observations in fluorescence microscopy suggest that replicative bacteria are more sensitive to the immune response. A fraction of the non growing bacteria that survive the first 24h, later give rise to an intensive proliferation in some alveolar macrophages. Non replication for *Brucella* may favor its immune escape to persist. Tn-Seq analysis revealed several genes candidates as essential for the survival of the bacteria during the first 24 hours in the host. Notably, the whole lipopolysaccharides synthesis pathway and some genes implicated in different kind of stress response regulation seems required for survival.
The capsular polysaccharide of the human pathogen *Capnocytophaga canimorsus*.

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*Capnocytophaga canimorsus* are gram-negative bacteria living as commensals in the mouth of dogs and cats. *C. canimorsus* cause rare but life-threatening generalized infections in humans that have been in contact with a dog or a cat. Infections mainly start with flu-like symptoms but can rapidly evolve in fatal septicemia with mortality as high as 40%.

Here we present the first evidence of a capsular polysaccharide (CPS) at the surface of *C. canimorsus* (Cc5), a strain isolated from a fulminant septicemia. We provide genetic and chemical data showing that the capsule is related to the lipooligosaccharide (LOS) O-antigen and composed of the same polysaccharide units.

Furthermore, we show that the presence of the CPS increases Cc5 survival in human serum, resistance to CAMPs and reduces the uptake by macrophages. We found this latter effect to be a consequence of increased adhesion of bacteria lacking the CPS to macrophages thus suggesting that the CPS reduces the ability of *C. canimorsus* to adhere to host cells.

Although most of the work was done with strain Cc5, we show that the presence of a capsule is most likely a commonality in *C. canimorsus*.

Unfortunately, to date, no reliable animal model exists for *C. canimorsus* infections and thus the role of the CPS *in vivo* could not be determined. Nevertheless our *in vitro* results clearly suggest that the CPS could play a major role in *C. canimorsus* infections probably at their onset conferring protection against the bactericidal effect of serum and phagocytosis.

Finally, we could show that antibodies targeting the LOS O-antigen and the CPS increase the killing of Cc5 in human serum thus suggesting a potential protective role for the host against these tremendous bacterial infections.

Overall these findings provide a new major element in the understanding of the pathogenesis of *C. canimorsus*.
Functional role of the type 1 pilus rod structure in mediating host-pathogen interactions

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Uropathogenic *E. coli* (UPEC), which cause urinary tract infections (UTI), utilize type 1 pili, a chaperone usher pathway (CUP) pilus, to cause UTI and colonize the gut. The pilus rod, comprised of repeating FimA subunits, provides a structural scaffold for displaying the tip adhesin, FimH. We solved the 4.2 Å resolution structure of the type 1 pilus rod using cryo-electron microscopy. Residues forming the interactive surfaces that determine the mechanical properties of the rod were maintained by selection based on a global alignment of *fimA* sequences. We identified mutations that did not alter pilus production in vitro but reduced the force required to unwind the rod. UPEC expressing these mutant pili were significantly attenuated in bladder infection and intestinal colonization in mice. This study elucidates an unappreciated functional role for the molecular spring-like property of type 1 pilus rods in host-pathogen interactions and carries important implications for other pilus-mediated diseases.
Study of chromosomes replication in the pathogen Brucella abortus

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Brucella spp. are facultative intracellular bacteria responsible for Brucellosis, a worldwide anthropozoonosis. This neglected disease is found in a variety of mammals and humans are considered as accidental hosts. The genome of B. abortus is divided in two chromosomes named chromosome I (ChrI) and chromosome II (ChrII) with a size of 2.1 Mb and 1.2 Mb respectively.

Recently several tools were developed to monitor the state of the chromosomes replication throughout the cell cycle of B. abortus at the single cell level. Indeed, by highlighting proteins involved in the partitioning system of chromosomal replication origins (oriI and oriII), we are able to determine the number of replication origins of both chromosomes indicating if the bacterium is in G1 phase or in S/G2 phase (Deghelt et al., 2014). The study of the two chromosomes replication patterns revealed that the chromosome I initiates its replication first, indicating a coordination between the replication of both chromosomes (Deghelt et al., 2014; De Bolle et al., 2015).

Using these reporter strains to infect RAW 264.7 macrophages and HeLa cells, we showed that B. abortus presents a biphasic infection process. Indeed, the infection is characterized by a first non-proliferative step where bacteria are arrested in G1 phase -being the invasion form of B. abortus in these host cells- followed by a proliferative step where bacteria grow and replicate in the endoplasmic reticulum (Deghelt et al., 2014).

Since the cell cycle of B. abortus seems to be linked to its virulence we are interested to investigate the regulation this cell cycle and more precisely the control of the chromosomes replication in rich medium and during infection.

Previous work highlighted the role of (p)ppGpp in the response to environmental changes and more particularly to nutritional stresses. Overproduction of (p)ppGpp in Caulobacter crescentus leads to a growth defect and a G1 arrest (Lesley and Shapiro 2008). In bacteria, the synthesis and degradation of this alarmone are controlled by RelA/SpoT protein family. In Escherichia coli RelA produces (p)ppGpp from GTP and ATP while SpoT can both synthesize and hydrolize (p)ppGpp. Some other bacteria (including C. crescentus and B. abortus) have only one protein having both synthetase and hydrolase activities, these proteins are named RSH for RelA SpoT Homologues. A Δrsh mutant of Brucella suis is unable to replicate in THP-1 macrophages, suggesting that (p)ppGpp could play a major role in the adaptation of Brucella to its intracellular niche (Dozot et al., 2006). Here we overexpressed the relA gene from E. coli in B. abortus, which resulted in a growth defect in rich culture medium. The growth defect of this strain was also observed during an infection of RAW 264.7 macrophages. Moreover, flow cytometry analysis of the relA-expressing B. abortus strain shows an increase of the proportion of G1 bacteria in culture. The generation of B. abortus rsh mutants for hydrolase and/or synthetase activities combined with reporter fusions for oriI and oriII would be interesting in order to investigate more deeply the role of (p)ppGpp in the control of chromosomal replication in culture and in infection.
Genomic, metabolic and in planta transcriptomic comparison of the potato soft rot enteropathogens *Dickeya solani* and *Dickeya dianthicola*

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Blackleg or soft rot caused by pectinolytic enterobacteria is one of the most devastating bacterial diseases of potato. While *Pectobacterium* was the prominent etiological agent in Europe, since a few decades there was an increased incidence of bacteria belonging to the *Dickeya* genus in the occurrence of this disease, with the successive emergence of *Dickeya dianthicola* and the newly characterized *Dickeya solani* species.

We performed a genomic, metabolic and in planta transcriptomic comparison between one isolate of each of these two emerging species. *D. solani* and *D. dianthicola* have more than a thousand specific genes that are often regrouped in genomic regions. Several of these genomic regions regroup genes involved in transport and metabolism, and this may be related to the differences in metabolic abilities identified for both *Dickeya* strains. 800 *D. dianthicola* and 1100 *D. solani* genes were differentially expressed in potato tubers as compared to in vitro growth in rich medium. These include several genes belonging to specific genomic regions, pointing to a role of these gene clusters in interactions with plants. Interestingly, the expression profiles of several genes common to both species, including virulence genes, differed in the two strains both in vitro and in planta. This may be related to the presence of dozens of specific regulatory proteins encoding genes in both strains, even if the genes encoding the global regulators important for plant infection are conserved in both species.
Insertion of new components in the heterogeneous outer membrane of 
Brucella abortus 

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The outer membrane of Gram-negative bacteria is known as an asymmetric bilayer with lipopolysaccharides (LPS) on the outer leaflet facing the external environment. This structure acts as a permeability barrier and is among other things involved in the interactions of pathogens with their hosts. Brucella abortus is an α-proteobacterium and one of the etiological agents of brucellosis, a worldwide spread zoonosis infecting domestic and wild life cattle. Bacteria belonging to the order Rhizobiales are characterized by unipolar growth. Labeling of the envelope using Texas red succinimidyl ester (TRSE) and subsequently growth in the absence of the dye revealed the new pole and the constriction site as proposed growth region in the B. abortus envelope.

We are interested in the insertion of the different layers of the bacterial envelope in the unipolar growing bacterium B. abortus represented by outer membrane proteins (Omp), peptidoglycan (PG) and LPS as well as the general organization and heterogeneity of the OM.

We monitored growth of the B. abortus 544 envelope by labeling smooth (S)-LPS and the major outer membrane protein Omp25. In order to monitor the incorporation of newly synthesized PG, bacteria were either short or long pulse labeled with the fluorescent D-amino acid HCC-amino-D-alanine (HADA). Additionally, the insertion of new LPS was shown using the rough (R) strain B. abortus 544 Δgmd in which the synthesis of the O-chain, and thus S-LPS, was inducible under the control of the Escherichia coli lac promoter. Labeling experiments of the different envelope layers showed that new S-LPS, Omp25 as well as PG are incorporated at the new pole and the constriction site.

Moreover, the general topography of the outer membrane of B. abortus was studied. The heterogeneity of the outer membrane of bacteria was examined by immunofluorescence (IF) and atomic force microscopy (AFM). IF experiments showed that R-LPS molecules are heterogeneously distributed on the surface of the bacterium. Additionally, the presence of these R-LPS patches could be correlated with an irregular surface structure by combining fluorescence microscopy and AFM. The area of the wild type enriched in R-LPS showed an around 2 times higher average in roughness in comparison to a R-LPS devoid area of the same bacterium.

Finally, a second abundant Omp of B. abortus, Omp2b, was localized by IF with a monoclonal antibody directed against this structure. The observed heterogeneous localization of Omp2b could be correlated with the presence of R-LPS patches supporting the model of a heterogeneous outer membrane.

In conclusion, the different layers of the envelope of B. abortus are incorporated at the new pole and the constriction site. The OM consists out of S- and R-LPS as well out of Omp that can be also heterogeneous distributed along the surface. These will contribute to a heterogeneous structure of the OM.