The Royal Academies for Science and the Arts of Belgium RASAB

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

Contact Forum

Microbes and the Global Change

Academy Palace, Brussels

December 11th 2015

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History

The Belgian Society for Microbiology (BSM) is a nonprofit association dedicated to the advancement of microbiological sciences in its broadest sense.

It intends to create a forum for the exchange of information and ideas by people interested in microbiology, whether they are active in fundamental, biomedical, environmental or applied research, addressing bacteria, viruses or fungi.

Founded on 18th November 1996 under the auspices of the National Committee for Microbiology of the Royal Academies for Science and the Arts of Belgium (RASAB), BSM promotes the exchange of scientific information mainly through its meetings, but also by publications in its Newsletter and Blog and through serving as liaison among the specialized fields of microbiology.

BSM membership is open to anyone with interest in microbiology. Currently, BSM counts circa 250 members

Board

Council members are outstanding microbiologists with different microbiology backgrounds and specialized in several domains including molecular, plant and pharmaceutical microbiology, taxonomy, microbial ecology, animal and human virology, and thereby covering many aspects of microbiology. Members are from different universities and institutions located in the different regions of Belgium (Flanders, Brussels and Wallonia).

Council consists of the following members: Chair: Jozef Anné (KU Leuven), Secretary: Paul De Vos (UGent); Treasurer: Tom Coenye (UGent); Tom is also liaison officer for the Dutch Society for Microbiology.

The other Council members are: Spiros Agathos (UCL), Alfons Billiau (KU Leuven); Guy Cornelis (UNamur), Pierre Cornelis (VUB), Paul Cos (UA), FEMS delegate, Herman Favoreel (UGent), Isabelle George, (ULB), David Gillan (UMons), Laurent Gillet (ULg), Natalie Leys (SCK-CEN), Max Mergeay (SCK-CEN), Dominique Schols (KU Leuven), Jos Vanderleyden (KU Leuven).

Activities

The society's annual symposium takes place in Brussels in the prestigious and historical building "Academy Palace" of the RASAB. For these activities internationally renowned microbiologists from Europe or non-European countries are invited to present topics of high current interest. During these meetings junior microbiologists can be selected for short oral communications, while others can show their work in posters. The large attendance of these yearly meetings (between 160 and 200 participants and 60 to over 100 poster presentations) proves that this formula is very successful. Usually, meetings take one day, but occasionally 2-days meetings are organized. *M*ore details on http://www.belsocmicrobio.be.

Advantages

BSM members have free access to BSM activities, subscription to the quarterly E-News letter, and are automatically member of FEMS (Federation of the European Microbiological Societies), which also support members via several types of grants (see http://www.fems-microbiology.org/website/nl/default.asp)

Program

08.30	*Registration – Poster mounting*
09.00	*Welcome address*
09.10	**Alain Préat,** *Res. Grp. - Biogeochemistry & Modeling of the Earth System*, ULB, Brussels *Climate Change : the Rule in the Geological Record*
09.45	**Jan Semenza***, European Centre for Disease Prevention and Control (ECDC), Sweden* *Drivers of infectious disease threat events in Europe*
10.30	Short communications selected abstract **Jean-Francois Sternon,** (URBM*, UNamur)* *Identification of Brucella abortus genes required for growth on plates and macrophage infection using Tn-seq*
10.45	*Coffee break and poster viewing*
11.15	**Mike Jetten,** Ecological Microbiology, Radboud University Nijmegen, The Netherlands *Impossible anaerobic micro-organisms important in the global Methane and Nitrogen Cycles*
12.00	Short communication of selected poster abstract **Bénédicte Machiels,** (*Lab Immunology-Vaccinology, FARAH, ULg*) *A gammaherpesvirus infection protects from allergic asthma development*
12.15	*Lunch and poster viewing*
14.30	**Albert D.M.E. Osterhaus,** University of Veterinary Medicine Hannover, Germany *Emerging and re-emerging viruses: origins and drivers*
15.15	Short communications of selected abstracts (3) **Ruben Props,** (LabMET UGent, SCK•CEN, Mol**)** Measuring the biodiversity of microbial communities by single-cell analysis
15.30	**Louise Hock** (*Lab Food and Environmental Microbiology, UCL, Louvain-la-Neuve*) *How to control emetic Bacillus cereus foodborne intoxications? A phage approach*
15.45	**Igor Stelmach Pessi,** (*Centre for Protein Engineering, University of Liège, Liège*) *Dynamic Responses of Cyanobacterial Communities Following Glacier Retreat in the High Arctic (Svalbard)*
16.00	**Marc Coosemans**, Dep. Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium *Innovative vector Control*
16:40	*General conclusions and presentation of best poster awards*

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Abstracts of invited lectures

Climate Change: the Rule in the Geological Record

Prof. Alain Préat

Res. Grp. - Biogeochemistry & Modeling of the Earth System
Sedimentology & Basin Analysis, Free University of Brussels, Belgium

The first aim of paleoclimate science is to identify from observations of the geological record, the nature of past climate changes. Paleoclimate is probably the oldest discipline in Earth science, it began in the 19th-century, and earlier with the discovery of elephant-like beast in the superficial deposits of Europe and Siberia debate about the intepretation in the 18th-century. The debate was about these surface enviroments of temperate areas shaped by the biblic flood or by glaciers [Préat, 2015 http://www.notre-planete.info/actualites/actu_4356.php]. By the middle of the 20th-century, many climate features associated with the recent ice ages have been identified. Geological processes are critical to the evolution of the climate. The most important issue pertaining the earliest evolution of the Earth's climate is that energy emitted by the sun has progressively increased over 4.6 Ga. Recontructing climate history from the inherently incomplete geological record requires integrated analyses including geochronology, paleomagnetostratigraphy, paleobiology, paleotectonics etc. Climate change in the geological past is the rule, it has been reconstructed using a number of key archives (including sedimentary, geochemical proxies) since billion of years. These records reveal that since its birth the Earth's climate as a rule has been warming up or cooling down with periods of (super)greenhouse and (super)icehouse modes, on scales of thousands to hundreds of million of years. The controlling factors are both cyclic (external or astronomical) and secular (internal to the Earth) and related to plate tectonics. For more than 90 percent of its 4.6 billion-year history, Earth has been too warm, even at the poles, for ice sheets to form. We live in unusual times at least from the cooling at the Eocene-Oligocene boundary (± 34 Ma) with the glaciating Antarctica**.** The Earth was also severely glaciated several times in its history (e.g. about 750 and 535 Ma). As an example of the conditions prevailing in the very warm times, oxygen isotopes suggest that the Archean seawater (4.0-2.5 Ga) coud have experimented hyperthermal environments, with temperatures as high as 55-85°C [Knauth, 2005 Palaeogeography, Palaeoclimatology, Palaeoecology, 219 : 53-69]. Considering the Precambrian as a whole (4.6-0.541 Ga), prior to about 2.2 billion years ago, the amount of oxygen in the atmosphere and surface ocean was small, concentrations of CO_2 were as high as 100-1000 times modern levels, as those of CH_4 which were higher. Complex microbial eocosystems developed during this period (sulfate-reducing bacteria, autotrophic methanogens, fermenting bacteria, anoxygenic phototrophic bacteria) and could have been important contributors to the biological productivity of early Earth. Past about 2.2 Ga the productivity began to be driven by oxygen-producing (micro)organisms.

Drivers of infectious disease threat events in Europe

Jan C. Semenza
European Center for Disease Prevention and Control
Sweden

Background

Globalization and environmental change; social and demographic determinants; and health system capacity are significant drivers of infectious diseases which can also be epidemic precursors of disease. Thus, monitoring changes in these drivers can help anticipate, or even forecast, an upsurge of disease.

Methods:

Four early warning systems for infectious disease threats in Europe have been built with epidemiologic and environmental/climatic data. Disease risk maps were generated using non-linear discriminant analysis (NLDA) to determine the best predictors of disease occurrence.

Findings

1) Malaria re-emerged in Greece in 2009-2012. The environmental suitability of transmission was mapped with NLDA, which guided targeted interventions, and interrupted transmission in 2013. 2) Tick-borne encephalitis (TBE) is a serious public health concern. The TBE risk in southern Sweden for late fall was quantified through the use of vegetation indices and early spring temperature and mapped spatially. This information can be used for vaccination and prevention campaigns. 3) Since 2010, recurrent West Nile fever outbreaks have occurred in South/eastern Europe. Temperature deviations from a thirty year average proved to be associated with these outbreaks. Drivers of subsequent outbreaks were computed through multivariate logistic regression models and included in prediction models. 4) *Vibrio spp*. is a waterborne pathogen that can cause gastrointestinal diseases or wound infections. The environmental suitability for *Vibrio spp*. in coastal waters was computed for the near future with remotely sensed sea surface temperature and salinity data.

Interpretation

Such early warning systems, based on environmental conditions, can help improve and accelerate alert and public health response capabilities and provide the evidence-base for strategic public health action.

Impossible anaerobic micro-organisms important in the global Methane and Nitrogen Cycles

Mike Jetten

Soehngen Institute of Anaerobic Microbiology, Radboud University, Nijmegen, Netherlands

Anaerobic oxidation of ammonium by anammox bacteria or anaerobic oxidation of methane by M*ethylomirabils oxyfera* bacteria and *Methanoperedens* Archaea are recent discoveries in the nitrogen and methane cycle catalyzed by novel so-called impossible microbes [Strous et al 1999 Nature 400: 446-449; Raghoebarsing et al 2006 Nature 440: 918-921; Haroon et al 2013 Nature 501:578-581]. These anaerobic processes share many interesting aspects. Both anaerobic ammonium and methane oxidation processes were once deemed to be biochemically impossible and non-existent in nature, but have now been identified as important players in global nitrogen and methane cycling. Molecular studies showed that anammox bacteria can make the rocket fuel hydrazine by novel multiheme complexes [Kartal et al 2011 Nature 479: 127-130; Dietl et al Nature doi: 10.1038/nature15517] that are located in a unique bacterial organelle surrounded by ladderane lipids [van Niftrik & Jetten 2012 MMBR 76(3):585-596 doi: 10.1128/MMBR.05025-11]. The *M. oxyfera* bacteria turned out to have a new intra-aerobic metabolism. They are able to produce their own oxygen by conversion of 2 NO into O_2 and N_2 by a putative NO dismutase [Ettwig et al 2010 Nature 464: 543-548]. *Methanoperedens* Archaea can couple the reverse methanogenesis pathway to reduction of nitrate. Molecular surveys have indicated that these organisms are wide spread in anaerobic ecosystems around the globe where they most probably interact in an intricate anaerobic food chain. Furthermore all three microorganisms can be applied in sustainable, cost effective wastewater treatment for the removal of methane and nitrogen compounds and are investigated within the center of excellence in anaerobic microbiology (www.anaerobic-microbiology.eu) funded by the Netherlands Gravitation program 024.002.002 SIAM and ERC AG EcoMOM 339880.

Emerging and re-emerging viruses: origins and drivers

Ab Osterhaus

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'RIZ', Hannover, Germany. albert.osterhaus@tiho-hannover.de

Complex relationships between the human and animal species have never ceased to evolve since the emergence of the human species and have resulted in a human-animal interface that has promoted the cross-species transmission, emergence and eventual evolution of a plethora of infectious pathogens. Remarkably, most of the characteristics of the human-animal interface -as we know it today- have been established long before the end of our species pre-historical development took place, to be relentlessly shaped throughout the history of our species. More recently, changes affecting the modern human population worldwide as well as their dramatic impact on the global environment have taken domestication, agriculture, urbanization, industrialization, and colonization to unprecedented levels. This has created a unique global multi-faceted human-animal interface, associated with a major epidemiological transition that is accompanied by an unexpected rise of new and emerging infectious diseases. Until the beginning of the last century, infectious diseases were the major cause of mortality of humankind. Around 1900 infectious diseases caused an estimated fifty percent of all deaths in the western world. In the following decades, this percentage decreased to a few percent. This was largely due to the implementation of public health measures such as the installment of sewage and clean drinking water systems, but also to the development of vaccines and antimicrobial compounds. A major success in this regard was the eradication of smallpox through a worldwide vaccination campaign orchestrated by the World Health Organization (WHO). Stimulated by these successes certain policymakers and scientists predicted that all infectious diseases of humankind would be brought under control. Paradoxically the following decades confronted the world with an ever-increasing number of emerging or re-emerging infectious diseases, some causing true pandemics. Striking examples were the emergence of AIDS, Avian flu, SARS, MERS, and Ebola. Viruses spilling over from animal reservoirs caused these disease outbreaks. A complex mix of predisposing factors in our globalizing world, linked to major changes in our social environment, technology and global ecology, collectively created opportunities for viruses to infect new hosts. Subsequent adaptation to the newly invaded species then paved the way for an unprecedented spread with dramatic consequences for public health, animal health, animal welfare, food supply, economies, and biodiversity. Importantly, these developments are largely paralleled by medical, technological, and scientific progress, continuously spurred by our never-ending combat against pathogens. Investment in a better understanding of the human-animal interface will therefore offer humankind a future head start in the never-ending battle against infectious diseases. Recent events like the emergence in humans of avian influenza, MERS and Ebola**, have highlight the urgent need for this investment.

*Reperant LA, Cornaglia G, Osterhaus AD. *Curr Top Microbiol Immunol. 2013*
**Reperant LA, van de Burgwal LH, Claassen E, Osterhaus AD. *Science 2014*

Innovative vector control: from evaluation to recommendation.

*Marc Coosemans**

Institute of Tropical Medicine of Antwerp
Department of Biomedical Sciences, University of Antwerp

Vector borne diseases such as malaria, leishmaniasis, dengue, chikungunya account for 17% of the estimated global burden of all infectious diseases. Prevention and control of these diseases rely mainly on vector control, particularly mosquito vectors. Substantial progresses have been made during the last decade in controlling malaria and large use of insecticide impregnated bed nets account for 68% of prevented malaria cases. However new vector control tools are required to face the problems of insecticide resistance and the residual transmission, i.e. transmission due to vector avoidance behaviour of insecticide treated surfaces. The public health value of a new vector methods requires substantial evidence. Besides several requirements such as entomological efficacy, safety, users compliance, feasibility of implementation, manufacturability, epidemiological evidence is the most important. The aim of vector control is to achieve a community protection by which all individuals in the community (including non-users) are expected to be protected due to the mass effect on the vector population and on transmission and this can only be demonstrated in Community Cluster randomized trials. The World Health Organization (WHO) has established a Vector Control Advisory Group (VCAG) to review and assess the public health value of new tools, paradigms such as attract-and-kill baits, microbial control of human pathogens in vectors, reducing vector populations through genetic manipulation, vector traps for disease management, lethal house lures, topical and spatial repellents interrupting human-vector contact, but also insecticide treated bed nets and walls to control insecticide (pyrethroid) resistant vector populations. Once VCAG has sufficient evidence of the public health value of the new paradigm, findings are presented at the Malaria Policy Advisory Committee (MPAC) and Strategic and Technical Advisory Group for Neglected Tropical Diseases (STAG). These Committees will further formulate recommendations on when, where and how the intervention should be deployed. New category testing guidelines, mainly based on entomological outcomes, will be further developed for products having a similar Target Product Profile (TPP) as the prototype used for assessing the new paradigm. This will accelerate the approval of other tools within the same class.

*Chairperson of the VCAG

http://www.who.int/neglected_diseases/vector_ecology/VCAG/en/

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List of posters and Location

Abbreviations of topics:

V = Virology

B = Bacteriology

- **BHe = health**
- **BBio = biochem & molecular**
- **BGen/ec = general, environmental, ecological**
- **BDiv = biodiversity/taxonomy**

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20	*Arends Jan B.A., Antonin Prévoteau, Annelies Geirnaert, Levi Buysschaert, Tom Van de Wiele and Korneel Rabaey.* **Two new methods to study anaerobic microbial metabolism & kinetics**	BBio
21	*Bidart de Macedo Maíra, Eveline Torfs, Sven Mangelinckx, Kourosch Abbaspour Tehrani, Kris Huygen, Paul Cos, Davie Cappoen.* **The *Mycobacterium tuberculosis* macrophage infection assay: a versatile tool for the generation of artificial granuloma**	BHe
22	*Castiaux Virginie, Laurence Delbrassinne[2], Xiaojin Liu, Jacques Mahillon.* **Is Cytotoxin K2 from *Bacillus cereus* a *bona fide* enterotoxin?**	BHe
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Poster Abstracts

CLINICAL EVALUATION OF THE EFFICACY OF OZONATED OLIVE OIL FOR TREATMENT OF TOENAIL ONYCHOMYCOSIS

Ozonated olive oil

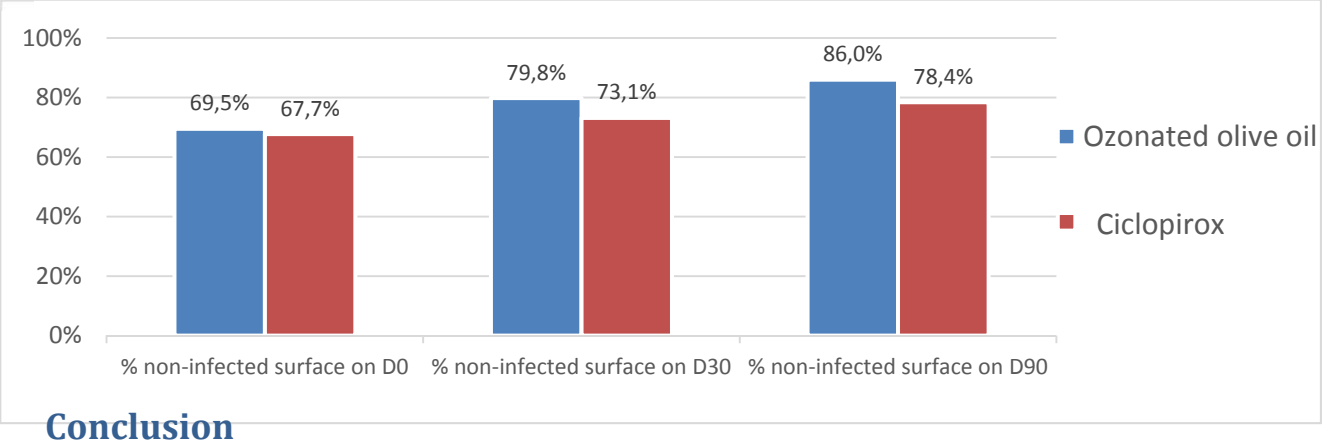
The present study was undertaken to assess the therapeutic effects of topical ozonated olive oil (OOO) for the treatment of toenail onychomycosis. Many commercial products against onychomycosis have a mode of action which relies on acidification of the nail environment and the inhibition of pH-sensitive dermatophytes. Ozonation is an electrochemical oxidation process that turns unsaturated oils into a saturated, acidic, oxidative gel. As OOO is able to create an acidic and oxidative micro-environment in tissue, we wanted to study its ability to acidify the nail and thereby treat onychomycosis.

Study design

This open-label trial was designed to evaluate the efficacy of OOO on onychomycosis with ciclopirox 8% serving as comparator. Briefly, 66 subjects were recruited into a randomized, single center clinical trial. ciclopirox was selected as reference product because it is a standard treatment in case of onychomycosis. Subjects were randomized to either the OOO oil group or the ciclopirox. At day 0, the toenail was sampled for fungal culture in order to ascertain the cause of the infection. The participants were instructed on how to apply the product correctly on their infected toenail at home (for OOO twice daily, for ciclopirox every other day for the first month, not less than twice weekly for the second month and once weekly for the third month). At day 30 and day 90 of the study, toenails ware examined clinically by an investigator and the participants had to answer a subjective evaluation questionnaire.

Results

Both treatments were efficient in improving onychomycosis condition. OOO was globally more efficient than ciclopirox nail lacquer, as reflected by an increase of healthy surface and a decrease of onycholysis, dystrophy, discoloration and nail thickening. The number of subjects with an improvement or a success of their treatment was also higher for the OOO when compared to ciclopirox nail lacquer. Both products were very well appreciated and improved the quality of life of the participants. Both treatments were very well tolerated



Conclusion

Ozonated olive oil performs equal or even slightly better in comparison to ciclopirox 8% nail lacquer in treating toenail onychomycosis. Ozonated olive oil achieves this action through a non-specific, physicochemical mode of action by creating acidic and oxidative conditions in the nail environment.

Main causes of false analytical results in food microbiology laboratories - Conclusions of 7 years of Proficiency Testing

Marleen Abdel Massih[1], Viviane Planchon[2], Marie Polet[3], Katelijne Dierick[3], Jacques Mahillon[1]

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Proficiency Testing (PT) schemes are inter-laboratory comparisons, based on the analysis of identical samples by different laboratories. The purpose of these tests is to assess the analytical performances of the participants and to help determining the possible causes of errors. The results of two Belgian PT providers were analysed in order to identify the main causes of false analytical results in the food microbiology laboratories and to suggest areas of improvement.
Based on the 2009-2015 results of REQUASUD and IPH Proficiency Testing (19 food microbiology PT schemes), the rates of outlying results and the main causes of mistakes were determined. On average, the laboratories failed to accurately detect or enumerate food bacteria in 3 % of the tests, which represents a total of 265 unsatisfactory results out of 8678 PT results. A peculiarity of the REQUASUD and IPH programs is that, after each PT scheme, a follow-up (meeting or individual feedback) was conducted with the participants to examine the causes of these outliers, for educational purposes. Thanks to this active feedback of the laboratories, the causes of outliers could be identified in 74% of the cases and sorted by importance.

The main causes of outliers identified in this study can be classified into three categories: pre-analytical errors (inappropriate handling of samples, timing of analysis), analytical errors (inadequate method, interference of background flora, errors in colony-count or confirmation) and post-analytical errors (calculation and encoding of the final result).

PT schemes represent a privileged observation post to highlight analytical issues, which might otherwise remain unnoticed by the laboratories. This contributes to the educational role of inter-laboratory comparisons. This study supports the hypothesis that regular participation to PT, followed by feedback and appropriate corrective actions, can provide increased confidence in the laboratory analytical results.

Two new methods to study anaerobic microbial metabolism & kinetics

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Anaerobic microorganisms play important roles in global and local biogeochemical processes. Understanding and harnessing these processes will enable us to steer and direct processes towards our benefit, for example in human health (i.e. gut microbial processes), in the framework of the bioeconomy (i.e. anaerobic digestion or fermentation) or as an environmental technology (i.e. CO_2 fixation by means of microbial electrosynthesis). However, a thorough understanding of microbial kinetics and metabolism is needed to select the most beneficial biocatalyst (or consortium of microorganisms) and operational conditions to develop a sensible process.

Therefore, the objective of this work was to develop techniques for screening microorganisms and understand how desired products will stimulate or inhibit growth of the microorganism and/or the conversion of the substrate into the desired product. The developed techniques are not limited to the selected examples but can be expanded to any anaerobic microbial conversion. In this study, the model organisms for CO_2 fixation are *Acetobacterium woodii* and *Clostridium ljungdahlii* whereas *Faecalibacterium prausnitzii* was used as the model organism for colon processes. To assess product inhibition under growing conditions, continuous spectrophotometric anaerobic incubations were performed using a modified technique for growth in 96 well plates (Technique 1). Substrate turnover under non-growing conditions was assessed by using riboflavin (RF) reduction as an electrochemical probe. RF reduction was measured with a rotating disk electrode (RDE; Technique 2). The same experiments, under non-growing conditions, were also performed spectophotometrically. The obtained RF reduction rates could be recalculated into a substrate conversion rate per microorganism for both the RDE and spectrophotometric data.

The developed methods were useful in determining growth rates and product inhibition under growing and non-growing conditions. For example: (i) anaerobic growth of the microorganisms was highly similar in traditional culture tubes compared to well plate incubations, (ii) RF turnover by *F. prausnitzii* as determined electrochemically as well as spectrophotometrically yielded similar metabolic rates for both methods and (iii) endproduct inhibition on the metabolic rate of *F. prausnitzii* could be quantified. (Prévoteau *et al* (2015) Sci. Rep. 5: 11484]. Overall, the spectrophotometric technique was less sensitive and required more time compared to the electrochemical technique but the spectrophotometric technique allows to assess 20 conditions in triplicate at the same time.

The *Mycobacterium tuberculosis* macrophage infection assay: a versatile tool for the generation of artificial granuloma

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Tuberculosis (TB), caused by its etiological agent *Mycobacterium tuberculosis* (*Mtb*) remains a challenge for medicinal chemists and microbiologists around the world. Spread through aerosol, *Mtb* is readily taken up by patrolling alveolar macrophages (aMϕ). To prevent further spread of *Mtb* throughout the host, granuloma structures are formed within the lung around the primary point of infection. In the granuloma, a perfect balance between the host immunity and the bacillus can last for decades to a lifetime in which the bacillus is contained, but persists as a latent infection. When the host immunity is weakened or compromised, the latent infection, turns into active TB. In order to study the effect of small molecules on the persistence of TB within a granuloma structure, we aim to produce new models to study the intracellular growth and to create artificial granulomata. In the first step towards the artificial granuloma formation, it is of paramount importance to study the intracellular replication of *Mtb* within the macrophage. To this extent, an *in vitro* model was developed in which a macrophage cell line is seeded in 24 well plates and subsequently infected with a luminescent H37Ra *Mtb* strain. After infection, the cell layer is washed to remove extracellular *Mtb* and the infected monolayer can be treated with antibiotics. At the desired time point, the monolayer is washed again, lysed and the *Mtb* is collected to be enumerated through luminometry. This provides a tool that can be used to measure the effect of small molecules on the intracellular replication of *Mtb* within a monolayer. Currently the artificial granuloma model is under investigation. In this assay, Mtb is immobilized in solid agar particles. Although the bacillus is shielded from the environment, mycobacterial antigens can freely diffuse through the pores within the agar beads. Co-cultured macrophages will migrate and attach to the agar beads. Provided sufficient time of incubation, the macrophages will ultimately form a spherical monolayer around the bead representing a rudimentary artificial granuloma. When activated primary macrophages are chosen, a model can be envisaged in which effector cells from the adaptive immunity and with the same genetic background are isolated and co-incubated with the artificial granuloma.

Is Cytotoxin K2 from *Bacillus cereus* a *bona fide* enterotoxin ?

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Bacillus cereus is an opportunistic pathogen that can be responsible for severe local or systemic infections. This bacterium may also cause two types of foodborne diseases: the emetic and the diarrhoeal syndromes. The emetic syndrome is due to the production of the emetic toxin cereulide in food before consumption and causes nausea and vomiting. The diarrhoeal syndrome is caused by one or several enterotoxin(s), produced after ingestion of viable cells and/or spores, which elicit diarrhoea by disrupting the integrity of the plasma membrane of epithelial cells in the small intestine. The main putative enterotoxins potentially involved in this type of illness are the tripartite protein haemolysin BL (HBL), the tripartite non-haemolytic enterotoxin (Nhe) and the single protein Cytotoxin K (CytK) [Stenfors Arnesen et al. (2008) FEMS Microbiol. Rev. 32: 579-606]. Two distinct variants of CytK have been reported: CytK1 from the thermotolerant bacterium *Bacillus cytotoxicus* and CytK2 from *B. cereus*. The potential implication of CytK2 in the diarrhoeal syndrome of *B. cereus* pathotypes is assessed in this study.

The occurrence of *cytK2* gene was studied among 190 *B. cereus* isolates from different origins (29 clinical, 30 food toxi-infection, 94 food and 37 environmental samples) in order to evaluate the potential role of CytK2 in diarrhoeal disease associated with *B. cereus*. 70 *cytK2*-positive strains were then assessed for their genetic diversity using Multi-Locus Sequence Typing (MLST) analysis.

Concerning the occurrence of *cytK2* among *B. cereus*, about 47 % of the screened strains were positive. Interestingly, the occurrence of *cytK2* was lower in isolates from foodborne toxi-infections and clinical strains than in samples coming from the environment or from food. The construction of a phylogenetic dendrogram (NJ algorithm) based on the alignment of the *cytK2* genes from the 70 positive strains (including 28 isolates involved in foodborne outbreaks) showed an important diversity, with the foodborne outbreak isolates widely spread throughout the dendrogram. These observations were confirmed by MLST, based on five different loci of housekeeping genes (*PurF*, *GdpD*, *SucC*, *RecF* and *CcpA*) that resulted in the identification of 61 distinct sequence types.

Although it would have been expected that the percentage of *cytK2* positive strains to be higher in foodborne outbreaks and clinical samples than in environmental samples, the opposite observation was made. Also, based on genetic analyses, an important diversity of *cytK2* sequences was shown among the strains involved in foodborne outbreaks. Therefore, the choice of *cytK2* as virulence marker for the diarrhoeal pathotype of *B. cereus* does not seem to be relevant. However, based on the MLST results, a limited number of these outbreak strains grouped into at least two distinct clusters. Therefore, the responsibility of CytK2 in the diarrhoeal syndrome cannot be fully excluded and potential synergistic effects with other putative enterotoxins (e.g. HBL, Nhe or HlyIII) should be further investigated.

Enterotoxicity of *Bacillus cereus*

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Bacillus cereus is an opportunistic pathogen that can be responsible for two types of foodborne diseases: the emetic and the diarrhoeal syndromes. The pathogenesis of the emetic disease is well understood and is due to the production of the emetic toxin cereulide in food before consumption. The pathogenesis of the diarrhoeal syndrome is still unclear. Several enterotoxins have been described as potential candidates. Among them, the three most regularly cited are the tripartite protein haemolysin BL (Hbl), the tripartite non-haemolytic enterotoxin (Nhe) and the Cytotoxin K (CytK). However, none of these molecules is able to explain the diarrhoeal syndrome alone. In addition to these major candidates, a large panel of toxins and virulence factors have also been described as potentially involved, alone or in combination, in the *B. cereus* diarrhoeic pathotype. So far, no correlation could be established between one of these components and the diarrhoeal symptoms. The aim of this study was therefore to pinpoint the *bona fide* molecule(s) responsible for the enterotoxicity of *B. cereus* strains.

Differentiated Caco-2 cells, cultivated in a medium supplemented with foetal bovine serum were used as model of the human intestinal barrier to assess the supernatant cytotoxicity of 70 *B. cereus* strains (food poisoning [40], food [14], environment [13] or of unknown origin [3]). The Caco-2 cells were confronted with the supernatant of *B. cereus* O/N cultures during 3 h. The toxicity effects were then assessed using two tests: the staining of living Caco-2 cells with neutral red and the LDH activity released from damaged Caco-2 cells. For each strain, the genetic determinants of all known putative enterotoxins and virulent factors (i.e. Hbl, Nhe, CytK, EntFM, EntS, BceT, CerO, HlyII, SMase and PiplC) were screened by PCR. Among the 70 supernatants, 42 displayed a significant cytotoxicity on Caco-2 cells. Surprisingly, the proportion of cytotoxic strains was similar regardless the origin of the strain (food poisoning, food or environmental isolates). This study also revealed 10 highly toxic strains, even more active than the positive control strain NVH1230-88. The PCR screening of the genetic determinants of enterotoxins and virulent factors did not give a simple correlation between the presence (and the number) of gene(s) and the cytotoxicity of a strain.

This study did not allow identifying specific genes playing a key role in the pathogenicity of *B. cereus* strains. This result could be explained by the fact that the molecule responsible for the symptoms has not been discovered yet. Therefore, the *B. cereus* secretome should be studied in details. Furthermore, a co-culture of Caco-2 cells with the HT-29-5M21 cell line, which is able to produce a mucus layer, should be tested. This co-culture should allow assessing the protective effect of the mucus layer on the intestinal barrier against the enterotoxin attack.

Bacillus spp. potato crop protection

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The management of potato crop diseases, and in particular the late blight, has always been a major issue in Europe. Farmers often resort to a massive use of pesticides, which induces tremendous direct and indirect (environmental) costs. They must find sustainable alternatives to face both economic losses due to disease pressure, while complying with the reinforcement of the European environmental policies. One of these alternatives is the *Integrated Pest Management* (IPM), which encompasses a large set of solutions, including purposeful fertilization, sufficiently long rotation, or the utilization of resistant cultivars. Among them, the development of alternative crop protection strategies has led to study microbial agents with proven antagonistic abilities. Bacteria from the *Bacillus* genus are frequently used in IPM strategy and not only because of their resilience and survival ability associated with their endospores, but also for the number of bioactive molecules they produce. The present study aims at characterizing and isolating new potential antifungal metabolites produced by *Bacillus* spp. bacteria that were selected for their antagonistic activity against six major potato pathogens: *Phytophthora infestans, Fusarium solani, Alternaria solani, Rhizoctonia solani, Pectobacterium carotovorum* and *Streptomyces scabies.* These bacilli were identified by sequencing their 16S rRNA gene, and preliminary genetic characterizations indicated that they do not represent any pathogenic potential to vertebrates or plants. The study of the activities displayed by the selected strains indicated that a broad range of secondary metabolites is produced: enzymes, lipo-peptides and siderophores, among others. Currently, these strains are being intensively studied in order to identify, by HPLC- and GC-MS, the different diffusible metabolites and volatile compounds produced. Eventually, the genetic determinants of these novel antifungal metabolites active against *P. infestans* will be identified and then confirmed through the generation of knockout mutants.

Molecular diversity of microorganisms in microbial mats of Antarctic lakes

CCAMBIO partners:Igor Stelmach Pessi[1], Yannick Lara[1] and Annick Wilmotte[1], Maxime Sweetlove[2], Elie Verleyen[2], Dagmar Obbels[2], Pieter Vanormelingen[2] and Wim Vyverman[2], Bjorn Tytgat[3] and Anne Willems[3], Bart Van De Vijver[4], Anton Van De Putte[5]

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The BelSPO project CCAMBIO aims to study the biogeographical distribution of microorganisms in lacustrine microbial mats using a combination of techniques including microscopical observations (light and electronic), strain isolation, and molecular diversity assessment using Next Generation Sequencing (NGS). The samples were collected in different Antarctic and sub-Antarctic biogeographical regions. A multivariate analysis of diatoms based on their morphology showed that these regions hosted different diatom flora and that the broad-scale biogeographical zoning is strikingly congruent with that found in plants and animals). Endemic diatom taxa were also observed, and a multigene molecular phylogeny of *Pinnularia borealis* showed a high genetic diversity. A new Scenedesmacean species was described from Antarctica, *Chodatodesmus australis*. A comparison of the bacterial diversity retrieved by cultivation or NGS showed a complementarity of both approaches and differences when different variable regions of the 16S rRNA gene were used. Novel and unclassified sequences, also observed by other authors, were obtained. Pilot studies were conducted for the microeukaryotes and cyanobacteria to select the most adequate NGS protocols and bioinformatic pipelines. The use of 'artificial communities' made with known strains to validate the NGS experiments is advised. For the eukaryotes, the local OTU richness decreased with increasing latitude , corresponding to a six-fold decrease in average richness from 150 OTUs/sample (46° S) to 25 OTUs/sample (84°S). This is in agreement with macroecological patterns observed in macroscopic organisms. The cyanobacterial community structures differed considerably between the 6 studied samples (sub-Antarctic, Eastern Antarctica, Transantarctic Mountains), with only one common OTU that corresponds to the widely distributed *Leptolyngbya antarctica* (strain BCCM/ULC041). One outcome of the project is to deposit the microbial diversity data in the "Microbial Antarctic Resource System (MARS)" presently developed into the webportal 'biodiversity.aq'. MARS will provide a linkage to existing molecular depositories used by the community and facilitate the discovery, access and analysis of molecular microbial diversity (meta)data generated by Antarctic researchers (http://mars.biodiversity.aq/).

Halophilic and alkaliphilic bacteria associated to *Caroxylon* sp. in the arid environment of the Sahara

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Relatively few studies have been devoted to the microbial diversity of the Sahara desert. We have investigated two sites near Bechar, in South-West Algeria. In this region, colliery spoil heaps, witnesses of a past coal mining activity, are colonized by a Saharan-Mediterranean vegetation dominated by a halophyte genus, *Caroxylon*. This shrub is also frequent in natural areas of the desert. In the natural site studied, as well as on the colliery heaps, the bulk soils have the physico-chemical characteristics of desert soils: sandy, dry, in majority saline, alkaline (pH 8.24 to 9.25), and poor in organic matter (0.018 to 0.12%). Because of the alkalinity and high salinity in soils, we were interested in identifying the halophilic and alkaliphilic bacteria associated to the rhizosphere of the C*aroxylon* shrub. Rhizospheric and bulk soil samples were taken in two locations: a natural area and a spoil heap.

A total of 110 bacterial strains (48 isolated from the colliery spoil heap and 62 isolated from the natural desert) were isolated on Luria Bertani Agar medium supplemented with 5, 10, 15 or 20% NaCl, at a pH adjusted to 8.5. Eighty were identified on the basis of their 16S rDNA sequence. They belong to 13 genera (Firmicutes (44%), Proteobacteria (39%) and Actinobacteria (17%)). Ten genera were identified in the collection of non-rhizospheric isolates and nine genera in the rhizospheric soil; six genera are shared. The *dominant species* were moderate halophiles belonging to the family *Bacillaceae*, *Halobacillus* (36%), *Bacillus* (19%) *and Oceanobacillus* (19%). In addition some isolates were alkaliphilic bacteria belonging to the genera *Citricoccus, Georgenia* and *Zhihengliuella*.

Key words: halophilic bacteria, alkaliphilic bacteria, *Caroxylon* sp., Sahara desert, spoil heap.

GPCR cell-based fluorescence assays using the Fluorescence Imaging Plate Reader (FLIPR) Tetra

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The chemokine receptor CXC receptor 4 (CXCR4), belonging to the G protein-coupled receptor (GPCR) family, is an important therapeutic target because of its involvement in numerous human pathological processes including several types of cancer and HIV viral entry. Anti-CXCR4 drug discovery can be achieved by measuring the stimulating (agonist) or inhibiting (antagonist) effect of compounds on the transient receptor-mediated calcium ion signaling in cells overexpressing CXCR4. The Fluorescence Imaging Plate Reader (FLIPR) Tetra (Molecular Devices) provides a high-throughput fluorescent cell based platform for the evaluation of such intracellular calcium fluxes. A unique feature of the FLIPR technology lies in its ability to simultaneously measure the fluorescence kinetics in all wells of a microplate. Here we performed both ratiometric (using Fura-2 calcium indicator, excitation: 340 and 380 nm, emission: 505 nm) and non-ratiometric (using Fluo-2 dye, excitation: 488 nm, emission: 515 nm) calcium measurements to evaluate the inhibitory capacity of three specific small molecule CXCR4 inhibitors, namely the bicyclam AMD3100 [Schols *et al*. (1997) J. Exp. Med. 186(8): 1383-8], the dihydrochloride IT1t [Wu *et al.* (2010) Science. 330(6007): 1006-71] and the compound WZ811 [Zhan *et al*. (2007) J.Med.Chem. 50(23): 5655-64]. By measuring the fluorescent emission of Fura-2 at two different excitation wavelengths, a discrimination between the intracellularly bound calcium and the free calcium form can be made. This property reduces the potential side-effects of uneven dye loading, dye leakage, photobleaching, as well as problems associated with measuring the calcium concentration in cells of unequal thickness. On the other side, single wavelength calcium indicators (*e.g.* Fluo-2) tend to have a broader dynamic range. Both assays similarly showed that AMD3100 and IT1t dose-dependently inhibited the CXC chemokine ligand 12 (CXCL12) induced calcium flux measured in CXCR4 positive cells with IC50 values in the low nanomolar range. In contrast to previous reports, the compound WZ811 showed no antagonistic activity in these assays. No agonistic activity was observed with all three compounds.

These CXCR4 inhibitors, and others that are currently being identified on the FLIPR Tetra platform, may prove to become valuable lead compounds that might reduce HIV infection rate and transmission and can also be potentially applied in many other diseases such as inflammation and cancer.

Sphingomonads and heavy metals

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Sphingomonads strains are often isolated for their ability to degrade organic molecules and recalcitrant pollutants. Yet, we have regularly isolated Sphingomonads from environments polluted with copper and zinc: mining sites, contaminated sediments, metallophytes, etc. Those isolates have the particularity to be highly resistant to copper and zinc ions, even when they were isolated on a selective streptomycin medium (Vanbroekhoven et al. (2004) Environmental Microbiology 6:1123-1136), instead of in the presence of zinc or copper. The metal-resistant Sphingomonads are phylogeneticaly very diverse. The three genera: *Sphingomonas*, *Sphingobium* and *Novosphingobium* are represented between the 28 independent isolates in the collection. Many metal-resistant isolates represent new species based on their 16S rDNA sequence.

The genome of one of the copper-and zinc-resistant isolates was completely sequenced. It is an endophytic *Sphingomonas* of the *S. paucimobilis* group. The strain harbours several plasmids, two of which appear specialized for the response to heavy metals, with multiple repeats of gene clusters devoted to the resistance to zinc and copper.

Comparison and evolution of the plasmidome in metal-contaminated microbial communities

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Environmental plasmids in two different freshwater sediments were purified. The stations were located in Férin (northen France, low metal levels) and near a Pb/Zn foundry (MetalEurop, northen France). Plasmids were extracted using a mini AX kit. Then, chromosomal DNA was digested with a plasmid safe DNAse. Finally, plasmid DNA was amplified with the Phi29 polymerase and sequenced by the Illumina technology (2 x 150 pb, shotgun sequencing). A quantity of ± 31 x 10Exp6 reads were obtained in each station. After MG-RAST analysis it appeared that the proportion of reads assigned to *Pseudomonas, Burkholderia, Stenotrophomonas, Cupriavidus* and *Ralstonia* were very different in the two stations, contrary to the results obtained previously with total DNA (Gillan et al. 2015 Environ Microbiol 17:1991). On the functional side, reads assigned to the SEED category Virulence, Disease and Defense (among which metal resistance genes) were over-represented in MetalEurop. Sediments from Férin were also placed in microcosms, contaminated with metals (Zn or a mix of Zn, Pb, Cu, Cd) and incubated during 6 months. The plasmidome was then extracted and sequenced again. Microcosms were also followed by viable counts, quantitative PCR (*czcA* gene levels, a Zn/Cd efflux pump) and DGGE. The structure of the community changed significantly and a decrease in equitability was observed in some microcosms. Levels of *czcA* increased in Zn-contaminated microcosms and these changed were confirmed by metagenomics of the plasmidome.

VC is a Research Fellow of the FNRS. This work was supported by an FNRS grant to D.C.G. and R.W. (FRFC Nr 2.4577.12). This work was also partially financed by a Pole d'Attraction Interuniversitaire (PAI) n° P7/25.

Metal complexes of pyridine-fused macrocyclic polyamines as selective antagonists of the chemokine receptor CXCR4

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The chemokine receptor CXCR4 acts as a key cell surface receptor in multiple forms of cancer, HIV infections and a variety of other pathologies, such as rheumatoid arthritis and asthma, which led to a growing interest in developing CXCR4 inhibitors. To date, plerixafor (AMD3100, Mozobil) is the only CXCR4 antagonist approved for clinical use as a hematopoietic stem cell (HSC) mobilizing agent that disrupts the anchoring signal of stem cells in the bone marrow. It consists of two cyclam macrocyclic units linked by a phenyl ring and is believed to act as a pro-drug that coordinates metal ions after administration in humans. Macrocyclic polyamines and their metal complexes are known to exert anti-cancer, but also anti-HIV activity, mainly by acting as HIV entry inhibitors through specific blocking of the CXCR4 receptor.

In this study, we tested the anti-CXCR4 activity of pyridine-fused polyamines, another class of macrocyclic compounds. Initially, we studied these macrocyclic polyamines with or without metal complexation as lead compounds for the inhibition of CXCR4 in two experimental set-ups: the anti-HIV activity against a CXCR4-using HIV strain, and the CXCL12-CXCR4 binding inhibition.

The $MnCl_2$ complex of a new pentaazacyclopentadecane with one (SH06) or two (SH08) fused carbocyclic ring(s) and the $ZnCl_2$ complex with two fused carbocyclic rings (SH39) were found to have the greatest potency as antagonists of the chemokine receptor CXCR4, as reflected in their nanomolar to low micromolar IC_{50} values in pure CXCL12-CXCR4 binding inhibition and anti-HIV activity against a CXCR4-using HIV strain, as well as in (*i*) CXCL12-stimulated calcium mobilization, (*ii*) CXCL12-induced endocytosis of CXCR4 and (*iii*) CXCL12-dependent chemotaxis of CXCR4-positive T cells.

The anti-CXCR4 activity profile of these novel metal-complexed pentaazacyclopentadecane structures makes them promising lead molecules for the control of tumor growth, metastasis and invasion, as well as for the induction of stem cell mobilization.

Single Nucleotide Polymorphism Genotyping and Distribution of *Coxiella burnetii* from Field Samples in Belgium

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Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium with a worldwide distribution. *C. burnetii* can be found in vertebrate and invertebrate hosts, such as ticks, but ruminants are considered the primary source of infection for humans. The genotypic characterization of *C. burnetii* is of primary importance in the understanding of Q fever epidemiology. Indeed, it provides useful information about the strains circulating at a farm, region or country level. Molecular epidemiology can be used to identify the source of infection for animals and humans. In Belgium the disease is enzootic in the domestic ruminant population, while sporadic cases are detected in humans. The aim of the present study was to investigate the strains of *C. burnetii* circulating in caprine and bovine Belgian farms, using a single-nucleotide-polymorphism (SNP) technique. Direct genotyping was applied to different samples (bulk tank milk, individual milk, vaginal swab, fetal product, air sample).

Besides the identification of well-known SNP genotypes, unreported ones were found in bovine and caprine samples, increasing the variability of the strains found in both species in Belgium. Moreover, multiple genotypes were detected contemporarily in caprine farms at different years of sampling and by using different samples. The presence of multiple *C. burnetii* strains within a farm has been reported by several authors using different genotyping methods (SNP, MLVA). However, the epidemiological and pathological implications of the circulation of different strains are still unknown. . It would be interesting to highlight the origin of the different strains within a farm, including different hypotheses of investigation such as the introduction by animal live trade (different geographic origins), or the influence of the sample used for genotyping (BTM, vaginal swab, air sample). Interestingly, certain SNP genotypes were detected in both bovine and caprine samples, raising the question of an inter-species transmission of the pathogen.

Global Microbial Networks and Gaia Autopoiesis Theory

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Climate change represents the major scientific and technological challenge for 21st century. The key aspect is that the environment is not *instructive*, externally or internally. The satisfy condition of the environment viewed not purely as inert non cognitive, but from Gaia theory suggest that is created not as a mechanism, but primary as a large scale network of microbial metabolism. More formally as an autopoietic-(M,R) systemic unity minimizer of free energy which is at the edge of zero thermodynamical, informational and geometrical entropy generation. While industrial engineered functional work mechanisms of the technological anthropocene behaves in the contrary way, i.e increasing entropy and maximizing surprise, therefore suggesting unequivocally that the probability of the internal Gaia entropy decreasing becomes exponentially smaller with time. Here I purpose how to understand the Gaia as autopoietic-(M,R) systemic unity minimizer of free energy, based on the connectome of large scale global microbial metabolism networks, but also the climate impact of shut-down/turn-off machines, soil microbial-non-technological waste management, de-mechanization (biologization) of agriculture, medicine and science, that certainty will replace mechanisms for metabolism in order to improve long-term average of minimization entropy, therefore climate prediction and safe human habitability.

New drugs against superbugs: mining the parvome of Gram-negative non-fermenting bacteria for novel anti-infective compounds

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The problem of widespread resistance against commonly used antibiotics has been a growing concern. In addition, many pathogens have an increased tolerance to antibiotics due to the formation of biofilms. High-throughput drug discovery screens of libraries of synthetic compounds have proven largely unsuccessful and recent discoveries of potent new antimicrobial compounds from bacteria indicate that we can consider new ways of exploring natural resources in search for new bioactive compounds. Gram-negative non-fermenting (GNNF) bacteria have largely been overlooked in this field; yet, several studies suggest that this group is an underexploited source of new anti-infective compounds.

Therefore, the present research project aims to analyze a diverse collection of GNNF bacteria for antimicrobial, anti-quorum sensing and anti-biofilm activity and isolate and characterize the bioactive metabolites responsible for the observed activities. The existing GNNF culture collection at the LM-UGent (Laboratory of Microbiology, Faculty of Sciences, UGent) is unique due to its diversity, both genetically and in terms of isolation source, but also because of the extensive knowledge available for each isolate. This collection will be tested for antimicrobial production (AMP) activity against a test panel of medically relevant organisms by means of an agar-overlay assay. The collection will also be examined for quorum sensing (QS)-interfering activity by performing a screen that relies on a *Pseudomonas aeruginosa* QSIS2 biosensor. Positive isolates will then be further analyzed to assess the mode of action: quorum quenching (QQ; enzymatic degradation of the acyl homoserine lactone signal) or QS inhibition (QSI; production of small compounds that interfere with QS in another way), or both.

Murid herpesvirus 4 infection protects mice from the development of pneumovirus induced immunopathologies

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Gammaherpesviruses are highly prevalent pathogens that establish lifelong latency. However, little is known about how these viruses imprint the immune system of their host. Here, we used Murid herpesvirus 4 (MuHV-4) to investigate the impact of gammaherpesvirus infections on the development of an anti-pneumovirus vaccine-induced Th2-skewed immunopathology. Briefly, the vaccine-enhanced disease was induced in mice by a subcutaneous vaccination with formalin-inactivated antigens of pneumonia virus of mice (FI PVM) followed by an intranasal infection with wild-type PVM. This homologous host-pathogen model was used to mimic the human respiratory syncytial virus (RSV) vaccine-enhanced disease, as observed in children during past vaccinal trials. Interestingly, using this model, we observed that MuHV-4 infection, either before or after the FI PVM vaccination, prevented the development of the PVM-induced immunopathology while the protection against PVM infection was unaffected. Notably, we observed significant lower BALF levels of total leukocytes, eosinophils, and Th2 cytokines IL-4, IL-5 and IL-13 in MuHV-4 infected mice than in mock-infected ones. This protective impact against the immunopathology was maintained over time and required pulmonary MuHV-4 replication. Moreover, MuHV-4 infection also conferred protection in non-vaccinated mice against the lethal wild-type PVM infection. This protection was associated with an improved PVM-specific CD8 cytotoxic response that we observed in lungs of MuHV-4 imprinted mice. Altogether, these results open perspectives for vaccination against pneumoviruses and highlight that some so-called pathogens could be revealed in the end as beneficial for their host.

No, no, there's no limit! Exhaustive isolation of microorganisms using MICROP

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Microorganisms are the most diverse group of life present on earth and are ubiquitous. Terrestrial ecosystems are the habitats where the most diverse communities can be found. Traditionally this diversity has been studied via standard microbial plating techniques. The development of culture independent technologies led to a revolution in microbial diversity insights. It became clear that in many biological samples most of the diversity present could not be cultivated under standard laboratory conditions, a phenomenon known as the "great plate count anomaly" (Staley and Konopka, 1985; Davis *et al.*, 2005). This makes the isolation and cultivation of the "biological dark matter" a topic of great interest, and may lead to a so-called cultural revolution (Kurd, 2013). In order to obtain a larger cultivated fraction recent research focused on the development of new isolation techniques based on mimicking natural conditions or in situ incubation (Stewart, 2012). This development of new techniques is a promising evolution to find new diversity, yet the standard plating techniques are not yet fully deployed to reach the maximum diversity possible. For example, simply altering culturing conditions still reveals new diversity (Connon and Giovannoni, 2002; Janssen *et al.*, 2002). A further development of the standard plating techniques into a high-throughput mode of action would allow to screen a large number of growth conditions, and to culture a large fraction of the yet uncultured organism. The present project aims to develop and test in a proof-of-concept manner a high-throughput semi-automated, miniaturized culturomics pipeline (MiCRoP). The development of this pipeline has been in progress during several years in the LM-UGent research group and is centered around a colony picker K2. Further dereplication into operational isolation units (OIUs) of the obtained isolates is performed via MALDI-TOF mass spectrometry. The obtained OIUs will be further identified, functionally characterized and preserved by using this pipeline, which should allow to test >100 different growth conditions leading to >10.000 isolates per sample analyzed. By taking standard cultivation methods to the next level, insight in the factors influencing the cultivation of the yet uncultured organisms might be obtained as well.

Function of two copper resistance proteins: The role of PcoA and PcoB in *Caulobacter crescentus*

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Despite its essential role as a trace element for any living organism, copper (Cu) turns toxic at high concentration, where (i) it displaces other native metallic ions in metalloproteins (Osman & Cavet, 2008), (ii) it generates unwanted disulfide bonds (Hiniker, Collet, & Bardwell, 2005), and (iii) it triggers a Fenton-like reaction leading to an oxidative stress (Osman & Cavet, 2008). Since the toxicity threshold can be reached quickly, there is a strong need for an organism bacterium to tightly regulate its intracellular Cu concentration.

A previous study in the lab has shown that the free living aquatic alphaproteobacteria *Caulobacter crescentus* encodes a two-protein system (PcoA and PcoB) involved in the regulation of Cu homeostasis. Here we characterized more precisely the function of both PcoA and PcoB. We demonstrated *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 confirm that PcoB likely acts as an Cu efflux pump. We propose a model summarizing how the PcoAB operon is handling Cu, from its detoxification to its ejection.

We also discuss the genetic conservation of *pcoA* and *pcoB* genes in the alphaproteobacteria class, which harbors bacteria with different life styles.

Uncovering novel tectiviruses preying on the *Bacillus cereus* group and their impact on ecological traits of their hosts

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Tectiviridae is a rare phage family comprising non-enveloped tail-less phages, with a linear dsDNA located within a lipid-containing membrane, covered by a rigid icosahedral protein capsid. Several tectiviruses have been found infecting the *Bacillus cereus* group and they display the unique characteristic of behaving as linear plasmids during their lysogenic cycle. Despite the significant contributions of phages in different biological processes, little is known about the dealings taking place between tectiviruses and their *B. cereus sensu lato* (*s.l.*) hosts. Therefore, this work focuses on characterizing the interactions between tectiviruses and the *B. cereus* group, mainly by assessing their occurrence, genetic diversity and addressing the question of whether or not temperate tectiviruses influence their hosts' life traits. Screening of a worldwide collection of *B. cereus s.l.* strains and propagation tests indicated that tectiviruses occurred in less than 3 % of the bacterial isolates. Notwithstanding this limited distribution, partial DNA sequencing uncovered novel tectiviruses, some of them with ORFans in regulatory regions involved in lysogeny maintenance. The host range analyses along with the molecular data revealed that tectiviruses in the *B. cereus* group can be clustered into two major groups: the ones infecting *Bacillus anthracis* and those isolated from other *B. cereus* group members. Moreover, interesting tectiviruses – and plasmid-related molecules – with recombinant characteristics have been discovered by analyses of whole genome sequences. Additionally, it was found that tectiviral lysogeny had a significant influence on the bacterial growth, sporulation rate, biofilm formation, and swarming motility of their *Bacillus thuringiensis* host, all of which are traits involved in the survival and colonization of this bacterium in different environmental habitats. Overall, these findings provide evidence that not only tectiviruses are more diverse than previously thought, but they also have ecological roles in the already complex life cycle of *B. thuringiensis* and kin.

Characterisation of oral murine adenovirus type 1 infection in mouse and evaluation of the protection induced against a respiratory homologous infection

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Oral vaccination offers many immunological and practical advantages. Nevertheless, oral immunization may be hampered by tolerance mechanisms. A solution to this problem could lie in the use of vectors such as viral vectors. Since the 1970s, alive, orally administered adenovirus serotype 4 and 7 vaccines are effectively used to protect United States military personnel from severe respiratory diseases caused by the same viruses. Replication-competent adenoviruses appear therefore as promising vectors for the development of oral vaccines. Until now, as human adenoviruses replicate efficiently only in a highly restricted host range, researches on this topic have suffered from the lack of reliable animal models. In this study, we used Murine adenovirus type 1 (MAV-1) to characterize adenovirus oral infection in mice. Briefly, while we did not observe any clinical signs associated with the oral administration of the virus, viral DNA was detected by qPCR in various organs, showing that the virus efficiently infects mice by the oral route. This infection was associated with an increase in MAV-1 specific and neutralizing antibodies over time. We finally evaluated the protection induced by MAV-1 oral infection against a respiratory challenge with the same virus. Clinical observations and histological analyses showed that orally immunized mice were protected against the severe symptoms observed after intranasal infection of naive mice. Altogether, these results show that MAV-1 offers a reliable model for oral vaccination based on replicative adenoviruses. This model provides a precious tool for studying the potential of orally administered adenoviruses as vaccine platforms.

Predicting the dynamics and biodegradation potential of river microbial communities in anthropized watersheds: a wet lab approach.

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River waters represent less than 0.0002 % of global water resources but they are essential for human life. Yet they are particularly exposed to anthropogenic stresses and risks of degradation. In a context of diminishing access to clean resources, lotic microbial communities play a key role in water self-purification. However they remain largely uncharacterized, and the link between lotic bacterial diversity and bacterial community stability and performance has not been studied. The goal of our project is to determine which factors drive the response of lotic microbial communities to organic pollution and how they tolerate, resist, exploit this pollution and/or go back to original status (resilience) downstream sewage outfalls. More specifically, the role of (i) native community structure (both phylogenetic and functional), (ii) wastewater bacteria released with organic matter and (iii) type of disturbance (regular/occasional, low/high loads) will be studied using bacterial strains isolated from the Zenne river, Belgium. Random artificial assemblages of these strains will be used to test the effect of microbial community structure (richness, evenness, architecture, metabolic diversity…) on community performance and recovery from stress. We hypothesize that such simplified, "wet lab" approaches can help to develop predictive tools of the dynamics and activity of bacterial communities in urban watersheds and *in fine* contribute to a better protection and restoration of river ecosystem services.

Labyrinthopeptins, a novel class of lantibiotics, exhibit broad and potent anti-dengue virus activity

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Introduction. Lantibiotics are ribosomally synthesized peptides, produced by bacteria, that contain the noncanonical amino acid lanthionine and many of them exhibit potent antibacterial activity. The best known lantibiotic is nisin, a preservative used in the food industry for over 40 years which has yet to encounter significant bacterial resistance. The labyrinthopeptins are a novel class of a prototype peptide of carbacyclic type III lantibiotics isolated from *Actinomadura namibiensis* containing labionin, a posttranslationally modified triamino acid. The labyrinthopeptin A2 (LabyA2) was reported to have pronounced activity in a neuropathic pain mouse model and moderate anti-herpetic activity (Meindl *et al*, 2010). Recent studies from our laboratory showed that labyrinthopeptin A1 (LabyA1) demonstrated broad anti-HIV and anti-HSV activity and, therefore, has a potential as novel lead candidate for microbicidal applications (Férir *et al*, 2013). Here we focus on the antiviral activity of the labyrinthopeptins LabyA1 and LabyA2 against dengue virus (DENV) infection.

Methods. Various cellular assays and different methods (qRT-PCR, flow cytometry, Bio-Plex human 27 cytokine/chemokine assays) were used to investigate the antiviral properties of LabyA1 and LabyA2. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is described to be an important cellular receptor for DENV to enter and infect human cells. Therefore, we used in addition to the DC-SIGN transfected Raji cell line, monocyte-derived DCs (MDDCs), which are naturally DC-SIGN expressing cells and physiologically relevant target cells. Furthermore, since the liver is the main target organ for DENV infection, we performed antiviral replication assays with L-SIGN, a receptor mainly expressed on liver cells. Here we used Raji cells (Raji/L-SIGN$^+$) and a human hepatoma cell line Huh-7. Surface plasmon resonance (SPR) studies were performed to study the interaction of the labyrinthopeptins with the envelope of DENV-2.

Results. We observed with LabyA1 a dose-dependent inhibition of the four serotypes of DENV in Raji/DC-SIGN$^+$ cells, Raji/L-SIGN$^+$ cells and in MDDCs (EC_{50}: 0.6 – 3.2 µg/ml). However, LabyA2 had only weak, if any, antiviral acitivity (EC_{50}: 7.7 – >100 µg/ml). In addition, LabyA1 demonstrated a dose dependent inhibition of DENV-2 infection in the Huh-7 cell line (IC_{50}: 3.8 µg/ml). Time of drug addition assays revealed that LabyA1 interacts with DENV and not with cellular membrane proteins, such as DC-SIGN. SPR studies showed that LabyA1 interacts with the DENV envelope. The mechanism of action of LabyA1 is clearly different from carbohydrate-binding agents (CBA) as an *in vitro* generated CBA resistant virus missing both N-glycosylation sites, proved not to be cross-resistant to LabyA1 (EC_{50}: 0.48 µg/ml). In a fusion assay with C6/36 mosquito cells, we demonstrated that LabyA1 inhibited DENV-induced fusion, suggesting an interaction with the fusion region of the E-protein. Fluorescent microsphere human bio-plex cytokine/chemokine assays revealed that LabyA1 inhibited the production of high amounts of inflammatory cytokines compared to untreated DENV-infected MDDCs, such as CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, TNF-α and IFN-γ.

Conclusion. Our results suggest that LabyA1 is a novel and potent DENV entry inhibitor and is thereby also an interesting peptide for deciphering the complex DENV entry process.

Improving phosphorus removal in aerobic granular sludge processes through selective microbial management

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Aerobic Granular Sludge (AGS) represents an innovative strategy to treat wastewater. It relies upon the formation of microbial consortia held together by a self-produced exopolymeric matrix. The major advantages of this process are an excellent settling capacity and high biomass retention derived from the growth of dense bacterial populations. Aerobic granules have been reported to settle down ten times faster than flocks and the process was accommodating a biomass concentration five times higher than that of activated sludge. Biological phosphorus removal in AGS reactors is largely performed by bacteria known as Polyphosphate Accumulating Organisms (PAOs). In aerobic or anoxic conditions, these bacteria accumulate phosphorus as intracellular poly-phosphate inclusions. The Beta-proteobacteria species *Candidatus* Accumulibacter phosphatis has been identified as the main actor in phosphorus removal. In AGS reactors performing enhanced biological phosphorus removal, it often represents the most abundant species with relative frequencies regularly exceeding a quarter of the bacterial population.

In this study, we aimed to improve the phosphorus removal in AGS sequential batch reactors (SBR) by a differential selection of the granules containing the highest proportion of PAOs. The abundance of PAOs in granules with different size and density were analysed by PCR-DGGE, pyro-sequencing and qPCR. Granules with intermediate densities contained the highest proportion of *Ca*. A. phosphatis with a 16S rRNA gene frequency up to 48.3 %, corresponding to 7.9 log PAO-specific 16S rRNA gene copies per ng DNA. On the contrary, the size of the granules had little influence on the abundance of PAOs.

Starting with an acetate fed AGS reactor with unstable P removal efficiency, a modification of the purge procedure was applied to remove both the slowest and fastest settling granules and to maintain a homogeneous population of granules with intermediate densities. It resulted in the improvement and stabilization of P removal over 90 %. This microbial management procedure resulted in a shift in bacterial populations and a decrease of the bacterial diversity. This study offers a new approach of biomass management to further improve phosphorus removal in SBR.

Exploring the diversity of extremely halophilic archaea in food-grade salts

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Salting is one of the oldest means of food preservation: adding salt decreases water activity and inhibits microbial development. Worldwide production of salt is led by China (24%), Europe (21%) and USA (17%). Applications for food and feed represent 3% of the European consumption. Salted and fermented food products often contain halophilic archaea. The presence of *Halorubrum* and *Halosarcina* species has been reported in brines during fermentation of table olives [Abriouel et al. (2011) Int. J Food Microbiol. 144: 487-496]. Histamine-degrading archaea belonging to genera *Halobacterium* and *Natrinema* were also reported in salted-fermented fishery products and *Haloarcula marismortui* was isolated from salted anchovies [Moschetti et al. (2006) Ann. Microbiol. 56: 119-127; Tapingkae et al. (2010) Enzyme Microb. Tech. 46: 92-99.]. Kimchi, a Korean fermented food, was shown to maintain archaeal population of the *Natronococcus*, *Natrialba*, *Halosimplex*, *Halobiforma* and *Halococcus* genera [Chang et al. (2008) Int. J. Food Microbiol. 126: 159-166.]. The salt itself has proven to contain viable microbial cells [Minegishi et al. (2010) Int. J. Syst. Evol. Microbiol. 60: 2513-2516]. However, to our knowledge, no studies have investigated the occurrence and diversity of archaea in food-grade salts. The archaeal diversity of 26 commercial food-grade salts from worldwide origin was assessed by culture on four different solid media. Colony forming units (CFU) were counted and phenotypically distinct isolates were identified by 16S rRNA gene sequencing. Additionally, high-throughput sequencing was performed on nine of these salts.

Viable archaea were observed in 14 salts and colony counts reached more than 10^5 CFU/g in three salts. All archaeal isolates identified by 16S rRNA gene sequencing belonged to the *Halobacteriaceae* family and were related to 16 distinct genera among which *Haloarcula*, *Halobacterium*, *Halarchaeum* and *Halorubrum* were the most represented. High-throughput sequencing generated extremely different profiles for each salt. Four of them contained a single major genus (*Halorubrum*, *Halonotius* or *Haloarcula*) while the others had three or more genera of similar occurrence. The number of distinct genera per salt ranged from 21 to 27. *Halorubrum* spp. had a significant contribution to the archaeal diversity in 7 salts; this correlates with its frequent occurrence in crystallization ponds. On the contrary, *Haloquadratum walsbyi*, the halophilic archaea most commonly found in solar salterns, was a minor actor of the food-grade salt diversity. This indicates that the crystallization process can modify the archaeal diversity, possibly due to distinct survival capabilities in extremely low water activity environments. Although not primarily sought, halophilic bacteria were also recovered from four food-grade salts.

The surprisingly high content of viable archaea in popular food-grade salts frequently used in food preparation raises the question of their fate after ingestion. Therefore, their survival and potential activity in human intestinal tract should now be considered and further investigated.

A comparative study of respiratory syncytial virus (RSV) infection of murine macrophage cell lines reveals remarkable differences in susceptibility.

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RSV is responsible for 64 million infections/year and severe bronchiolitis in children and elderly. Besides this acute pathology, RSV is also linked to chronic pulmonary problems, like asthma and recurrent wheezing. There is a clear link between the pathology and the immune response, so various research groups have studied the possible role of macrophages, which are prominent cells of the lung immune system and appear to be permissive for RSV. Published results however are often contradictory. A viable explanation is that diverse types of macrophages cell lines were used which may exhibit different sensitivities towards RSV infection. The aim of this study is to evaluate the susceptibility of different macrophage cell lines for RSV.

The murine macrophage cell lines MH-S, RAW 264.7 and J774 were infected with the RSV strain A2 and fixed at 2 and 24h p.i. Cells were permeabilized and RSV antigens were stained with a polyclonal anti-RSV serum followed by an AF488-labelled conjugate. Both MH-S (2%) and RAW 264.7 (0,4%) cells showed clear staining of RSV-antigens in the cytoplasm 24h p.i. The number of infected cells was low, yet significant since the staining was more intense compared to the staining at 2h p.i., indicating that new RSV-antigens were synthesized and that cells were indeed infected. J774 cells showed no positive signal of RSV-antigens.

A double staining showed that RAW 264.7 cells, in contrast to MH-S cells, express no RSV-antigens on the surface. This suggests an abortive RSV-infection in RAW 264.7 cells. This was confirmed by inoculating HEp-2 cells with supernatants of infected cells, collected 24 and 72h p.i. The percentage of infected HEp-2 cells increased from 1,5 to 5% when inoculated with supernatants of MH-S from 24 or 72h p.i. This in contrast to HEp-2 cells inoculated with supernatants of RAW 264.7 cells, where the percentage of infected cells varied between 1,4 and 1,2%. In conclusion, the RSV-infection with the A2 strain varies among macrophage cell lines and only MH-S appear to be fully susceptible, yet in a low percentage of the cells.

How to control emetic *Bacillus cereus* foodborne intoxications ? A phage approach

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Bacillus cereus is a bacterial foodborne pathogen implicated in more than 1,000 intoxications each year in Europe (EFSA and ECDC, 2015). The ubiquity and the sporulation capacity of this bacterium, as well as the extreme resistance (pH, heat and protease) of its emetic toxin, the cereulide, prevent an efficient sanitation by conventional methods. Therefore, novel means to control the emetic pathotypes of *B. cereus* are necessary. Thanks to their specificity for bacteria and their safety for humans, bacterial viruses, known as bacteriophages (or phages) seem to be a good alternative for the biocontrol of emetic *B. cereus* population in food matrices. In order to establish a collection of bacteriophages specific to the emetic pathotype of *B. cereus*, ten phages were isolated among about 300 samples of various origins (*e.g.* soil, food, animal faeces, wastewater treatment plant) according to their presumptive lytic properties and their host spectra. Although none of isolated bacteriophages was able to lyse the entire collection of 155 emetic strains tested, combinations (or cocktails) of some of these phages could lyse the majority of the emetic *B. cereus* and their application to prevent foodborne intoxications looks promising. However, before being use in food matrices, they have to satisfy certain requirements to certify their safety and efficiency in foodstuffs. The genome sequences of the selected phages are currently being studied to confirm that they are truly virulent (*versus* mutated temperate phages) and that they are devoid of bacterial virulent (pathogenic) genes. Their morphology and growth parameters (e.g. multiplicity of infection, adsorption constant, eclipse period, latent period, burst size) are also under investigation, in optimal laboratory conditions. The main features of our best candidate phages will be reported and discussed. Ultimately, their efficacy in controlling the emetic strains of *B. cereus* will be evaluated in food matrices, in real life conditions.

Love everlasting?
Successful partnerships between methanotrophs and other bacteria that withstand the rigors of time.

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Methane (CH_4) is an important greenhouse gas, and the majority (ca. 60%) of emissions comes from anthropogenic sources [Kirschke, Bousquet et al. (2013) Nat Geosci 6: 813-823]. One strategy for methane mitigation and recovery is the use of methanotrophic bacteria (MOB). MOB form a subgroup of the methylotrophs and possess the unique ability to use CH_4 as a sole carbon and energy source. Recently more and more evidence is accumulating that a methanotrophic microbiome rather than an individual methanotrophic ecosystem partner is responsible for biological aerobic methane oxidation [Oshkin, Beck et al. (2015) ISME J 9: 1119-1129]. These interactions can be very specific [Stock, Hoefman et al. (2013) RES MICROBIOL 164: 1045-1054; Ho, de Roy et al. (2014) ISME J 8: 1945-1948] and it is not entirely elucidated yet what determines the success of these partnerships. As exploitation and engineering of these microbiomes could lead to sustainable mitigation and recovery of methane, we set up synthetic partnerships of known methanotrophic- (α- and γ-proteobacterial) and non-methanotrophic bacteria and investigated their behavior upon repeated cycles of co-cultivation. To follow individual partners in time 16S rRNA gene based DGGE and qPCR as well as pmoA based qPCR were employed. Additionally we investigated methane removal rates from the headspace of batch reactors. We found out that after repeated sub-cultivation specific partners were selected by the MOB. Methane removal rates and the lag time until methane was removed varied considerably among repeated cycles of co-cultivation. In another setup a repeatedly co-cultivated couple of MOB and partner were challenged with several new partners with varying degrees of competitiveness. Preliminary results indicate that indeed differences in methane oxiditation rates and carbon dioxide production can be detected. qPCR results of individual composition have still to be generated as well as metaproteomics analysis to investigate partner adaptation over time. A better understanding of these interactions in successful partnerships will enable a broader biotechnological application of this interesting functional microbial clade while both mitigating and recovering CH_4-derived carbon.

Murid herpesvirus 4 ORF63 is involved in the translocation of incoming capsids to the nucleus

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Gammaherpesviruses architecture are important human and animal pathogens. Despite they display the classical of herpesviruses, the function of the most of their structural proteins is still poorly defined. This is especially true for the tegument proteins. Interestingly, a potential role in immune evasion has recently been proposed for the tegument protein encoded by ORF63. However, this study did not involve the construction of an ORF63 knockout strain and the significance of these results remains unknown. In this project, we wanted therefore to better define the importance of ORF63 in the lifecycle of Murid Herpesvirus 4 (MuHV-4). We showed that a lack of ORF63 was associated with a severe viral growth deficit both in vitro and in vivo. The latter deficit was mainly associated with a defect of replication in the lung but did not appear to be due to a reduced ability to establish the latency. On a functional point of view, inhibition of caspase-1 or inflammasome did not restore the growth of the ORF63 deficient mutant suggesting that the observed deficit was not associated with the immune evasion mechanism identified previously. Moreover, this growth deficit was also not associated with a defect in virion egress from the infected cells. In contrast, it appeared that MuHV-4 ORF63 deficient mutants failed to address most of their capsids to the nucleus during entry, suggesting that ORF63 plays a role in capsid movement along the microtubule network. In the future, ORF63 could therefore be considered as a target to block gammaherpesvirus infection at a very early stage.

Antibody-induced internalization of RSV F protein expressed on the surface of infected cells and cells expressing a recombinant protein

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Antibody-induced internalization is an important process that may modulate the surface expression of viral glycoproteins. It has been described for several viruses, including herpesviruses and measles virus. Also for respiratory syncytial virus (RSV), it was shown that upon binding of polyclonal RSV-specific antibodies to RSV antigens expressed on the surface of infected HEp-2 cells, internalization of these RSV antigen-antibody complexes may occur. Further research was performed to determine whether only one or both RSV major surface proteins F and G undergo internalization. RSV-infected cells and cells transfected with plasmids encoding RSV F or G were incubated with polyclonal or monoclonal RSV-specific antibodies. Both strategies resulted in a clear uptake of RSV antigen-antibody complexes in a time-dependent manner. The process was rapid and reached a maximum after 90 minutes. In addition, flow cytometric analysis after induction of internalization showed a clear reduction in surface expressed RSV antigens. By using infected cells with biotin-labelled surface proteins, a clear difference was observed between internalization of the RSV F protein induced by antibodies and spontaneous endocytosis, confirming that antibody binding triggers internalization.

Of the RSV surface proteins, RSV F is the most conserved and the main target of neutralizing antibodies, including palivizumab which is the only approved immunoprophylaxis. Current research into the development of new immunoprophylaxis and vaccines is mainly focused on the RSV F protein. Inhibitors of endocytic mechanisms and dominant-negative proteins were used to identify the mechanism through which RSV F antigen-antibody complexes are internalized. The findings of these experiments suggest a clathrin-dependent mechanism. Internalization was induced by different neutralizing pre- and/or postfusion RSV F-specific antibodies, including palivizumab, indicating that this process is epitope-independent. For herpesviruses, antibody-induced internalization was shown to interfere with antibody-dependent complement-mediated lysis. It remains to be determined whether this process for RSV can act as an immune evasion mechanism and play a role in the partial failure of RSV antibody responses and immunoprophylaxis. In this context, experiments are ongoing to identify the responsible endocytic motifs in the cytosolic domains. By using a BAC-based RSV rescue system, the development of recombinant viruses deficient in RSV F internalization could be achieved.

Bovine herpesvirus 4 modulates its beta-1,6-N-acetylglucosaminyltransferase activity through alternative splicing

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Carbohydrates play major roles in host-virus interactions. It is therefore not surprising that, during co-evolution with their hosts, viruses have developed sophisticated mechanisms to hijack to their profit different pathways of glycan synthesis. Thus, the Bo17 gene of Bovine herpesvirus 4 (BoHV-4) encodes a homologue of the cellular core 2 β-1,6-N-acetylglucosaminyltransferase-mucin type (C2GnT-M) which is a key player for the synthesis of complex O-glycans. Surprisingly, we show in this study that, as opposed to what is observed for the cellular enzyme, two different messenger RNAs are encoded by the Bo17 gene of all available BoHV-4 strains. While the first one corresponds to the entire coding sequence of the Bo17 gene, the second results from the splicing of a 138 bp intron encoding critical residues of the enzyme. Antibodies generated against the Bo17 C-terminus showed that the two forms of Bo17 are expressed in BoHV-4 infected cells, but enzymatic assays revealed that the spliced form is not active. In order to reveal the function of these two forms, we then generated recombinant strains expressing only the long or the short form of Bo17 and showed by glycomic analyses that BoHV-4 uses alternative splicing to markedly regulate the cellular core 2 branching activity of infected cells. We therefore postulate that the relative abundance of active/inactive forms of pBo17 in Golgi oligomeric complexes may define the global level of C2GnT-M activity in the infected cell. These results suggest the existence of new mechanisms to regulate the activity of glycosyltransferases from the Golgi apparatus.

Bacterial community composition in three freshwater reservoirs of different alkalinity and trophic status.

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Abstract

In spite of the knowledge recently gained on bacterial community composition (BCC) and their ecological drivers in natural freshwater environments (i.e., lakes), there is a lack of knowledge on man-constructed reservoirs. In order to investigate the factors controlling the BCC in different Belgian reservoirs, we sampled three freshwater reservoirs with contrasted physical and chemical characteristics and trophic status nearly covering a whole year period. The BCC was analysed by means of 454 pyrosequencing analyses of the 16S rRNA gene. In parallel, a complete dataset of environmental parameters, cell quantification and phytoplankton community composition (by means of pigment content analysis) was also collected.

BCC in the analysed reservoirs resembled that of epilimnetic waters of natural freshwater lakes with presence of *Actinobacteria*, *Alpha*- and *Betaproteobacteria*, *Cytophaga–Flavobacteria–Bacteroidetes* and *Verrucomicrobia* groups. Our results evidenced that the retrieved BCC was strongly influenced by pH, alkalinity and organic carbon content, whereas comparatively little change was observed within a reservoir among layers in stratified conditions. Furthermore, even presenting differences in their physico-chemical characteristics and phytoplankton community composition, the three analysed reservoirs harbour a core microbiome composed of cosmopolitan bacterial groups. Observed dissimilarities arise from differences in the typology of organic carbon content.

Photoferrotrophy and Fe-cycling in a freshwater column

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Emerging insight shows that ferruginous (anoxic and iron-rich) conditions dominated ocean chemistry throughout the first 3.5 billion years of Earth evolution. Before the evolution and proliferation of oxygenic photosynthesis, biological production in the ferruginous oceans was likely driven by photoferrotrophic bacteria that oxidize ferrous iron {Fe(II)} to harness energy from sunlight, and fix inorganic carbon into biomass. Photoferrotrophy may have fuelled Earth's early biosphere providing energy to drive microbial growth and evolution over billions of years. However, modern ferruginous water masses are rare, but detailed examination of these oddities could yield important insights into the early evolution of life on Earth and its impact on global element cycles.

Here, we show that an active community of pelagic photoferrotrophs comprises nearly one third of the total microbial community in illuminated ferruginous waters of Kabuno Bay (KB), East Africa (DR Congo). These photoferrotrophs produce oxidized iron {Fe(III)} and biomass, and support a diverse pelagic microbial community present in the water column of KB. The isolated specie from KB is highly similar to the sole photoferrotroph member of the *Chlorobi* cultured representative to date (*Chlorobium ferrooxidans*). Both laboratory and in situ incubations experiments evidenced that, at modest light levels, rates of photoferrotrophy in KB exceed those predicted for early Earth primary production, and are sufficient to generate Earth's largest sedimentary iron ore deposits (i.e., Banded Iron Formations). In this way, photoferrotrophs could contribute to the overall oxidation of the Earth's surface prior to the oxygenation of the atmosphere.

A gammaherpesvirus infection protects from allergic asthma development

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The innate immune system has a key role in initiating and in regulating polarization of immune responses. Although progress has been made to understand the role of innate immunity in inducing protective responses against pathogens, little is known about how these infections modulate subsequent heterologous immune responses. Interestingly, the hygiene hypothesis postulates that exposure to some microbes early in life prevents the later development of allergic diseases. In particular, while some viral infections exacerbate asthma lesions, others could induce protection against allergic sensitization. Here, we investigated how the highly prevalent gammaherpesvirus infections affect the subsequent development of allergic asthma. Our results demonstrate that infection by Murid herpesvirus 4 (MuHV-4) inhibits the development of House Dust Mites (HDM)-induced airway allergy in mice by modulating the function of innate immune cells involved in sensitization against HDM. Thus, while MuHV-4 infection does not influence HDM uptake by dendritic cells (DCs) nor their migration to the draining lymph nodes, it significantly decreases MHCII expression on migratory DCs and affects their capacity to prime a HDM-specific Th2 response. Initial polarization of the lung immune responses results of complex and still incompletely defined interactions between innate immune cells. Among these cells, alveolar macrophages (AM) could play a prominent role as they represent the most abundant leucocytes found in alveoli and distal airspaces. In our model, we observed by FACS analysis some strong phenotypic modifications induced on AMs following infection such as an increased expression of MHCII and decreased expression of siglec F. Beside these changes, differences in the AM gene expression profile were revealed by RNA sequencing and could be linked to the reduced capacity of DCs to initiate a Th2 response against HDM. Indeed, adoptive transfer of bone marrow-derived DCs (BMDC), co-cultivated with AMs from MuHV-4 infected mice demonstrated the loss of ability of these BMDC to induce HDM allergic sensitisation. Altogether, our results demonstrate the strong imprinting of a gammaherpesvirus infection on lung innate immune cells and the subsequent consequences on asthma development. In the future, this model could allow us to highlight key steps of the Th2 polarization against respiratory allergens and to develop new strategies against allergic asthma.

pXO16 from *Bacillus thuringiensis* serovar *israelensis*: almost 350-kb of *terra incognita*

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The conjugative plasmid pXO16 (350 kb) from *Bacillus thuringiensis* serovar *israelensis* encodes an aggregation-mediated conjugation system and is able of transferring itself and other non-conjugative and non-mobilizable plasmids in a fast and very efficient manner. Even though its conjugative kinetics and capabilities have been extensively studied, the genetic bases for this unique transfer system remain largely unknown. In this study, CDS were predicted from the plasmid sequence and their putative functions were examined with a particular attention to possible conjugative and aggregation functions. Sequence analysis of pXO16 displayed unexpected characteristics for a conjugative plasmid [Makart et al. (2015) Plasmid 80: 8-15]: its 467 putative CDS (88 % of its sequence) are all in the same orientation and more than two third of them showed no homologue in the databases. These features are usually shared by jumbo phages. Concerning conjugative functions, no significant type IV secretion system homologues were found, strongly suggesting that pXO16 encodes an unforeseen conjugative system.

Using double-recombination mutagenesis, knockout of three selected regions was performed: a 25-kb region including 5 cell-surface-associated CDS, one FtsK-like protein CDS and the gene coding for a putative S-layer protein. Transfer frequencies and aggregation phenotype of these mutants were then analysed, together with the modification of their host cell surface. The 25-kb cell-surface-associated knockout mutant was not able to produce macroscopic aggregation but transfer was still detected, yet at lower frequencies (1,000-fold for conjugative transfer and 100-fold for mobilization). This proves that aggregation is important but not essential in pXO16 transfer. The other mutant phenotypes and their surface properties will also be discussed in more details and should give more insights on the conjugative mechanism encoded by pXO16.

H_2-Oxidizing Bacteria for Single Cell Protein production and sustainable nitrogen cycling

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Hydrogen-oxidizing bacteria offer the possibility to autotrophically capture NH_4^+-N and CO_2 by using H_2 as electron donor and O_2 as electron acceptor. Hydrogen produced by green energy sources such as wind and solar energy powered water electrolysis can potentially support a shift from resource dissipation to resource recovery and upcycle in wastewater treatment. Indeed, instead of dissipating the reactive nitrogen contained in wastewater by means of conventional nitrification-denitrification, the latter can be instead recovered and upcycled back to high quality edible microbial protein suitable as protein-rich feed additive: single cell protein (SCP). The process offers also the possibility of capturing the CO_2 contained e.g. in the biogas produced during anaerobic digestion, therefore reducing overall the greenhouse gas emissions (GHG) of wastewater treatment plants (WWTP). The present research focuses on enriching, characterizing and implementing in practice (SCP production from lab to pilot scale) a microbiome where autotrophic H_2-oxidizing bacteria constitute the core (primary consumers), supported in its functionality by other heterotrophic bacteria. As already demonstrated for methane oxidizing bacteria, the presence of heterotrophic satellites (secondary consumers) can help increasing the functionality of the microbiome by e.g. removing inhibitory metabolites or regulating oxygen levels.
The aim of this first experimental phase was to selectively enrich a mixed microbial community with H_2-oxidizing bacteria. A first set of experiments was carried out in a lab-scale setup implementing a closed 0.5 L fermenter operating in batch conditions. The closed-gas recirculation system was set and operated at the optimal physical-chemical conditions reported in literature for H_2-oxidizing bacteria.
The main parameters affecting the microbial growth were studied, aiming at the highest biomass yields. The parameters studied were: sludge retention time (SRT), O_2 concentration, gas recirculation rate, macro and micro nutrients concentration; mixotrophic conditions. The efficiency of the process was monitored mainly in terms of biomass yield, defined as the cell dry weight (CDW) produced per gram of hydrogen COD (H_2-COD). The yield varied from a minimum of 0.10 g to a maximum of 0.25 g CDW/g H_2-COD, approaching the maximum yield of 0.30 g CDW/g H_2-COD reported in literature for axenic cultures of H_2-oxidizing bacteria.

Once the mixed microbial community became highly enriched in H_2-oxidizing bacteria, further studies were conducted in a 5 L Sartorius Biostat-A reactor to estimate the first important biotechnological process parameters for SCP and biopolymer production. In summary, biomass concentrations as high as 15 g CDW/L were achieved, with volumetric productivities of the order of 2-3 g CDW/L·d and a specific biomass yield of about 0.2 g CDW/g H_2-COD. The produced biomass was also characterized by an average crude protein level of about 70% CDW, whereas the average PHA (polyhydroxyalkanoate) content of the biomass ranged between 20 and 25% of the cell dry weight. In order to assess the quality of the protein accumulated by the microorganisms, the biomass was harvested, dried and analysed for essential amino acids compositions.
Concomitantly to the bench scale studies, a more fundamental investigation was conducted on the enriched microbial community by means of an innovative microtiter plate reactor. The ongoing line of investigation aims at characterizing the microbial community in its main biokinetic parameters by means of miniaturized reactor system. The latter allows continuous optical density measurement under a continuous and controlled gas flow on a multiple number of replicates, therefore rendering a high throughput screening of the microbial community.
In conclusion, the first results indicate that the production of edible microbial protein by the HOB microbiome developed so far can represent an innovative mean to supply high value protein while incorporating reactive nitrogen (possibly recovered from wastewater) and capturing carbon dioxide emissions from the treatment facilities.

Reconciliation between Operational Taxonomic Units and Species Boundaries.

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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 previously been used in bacterial taxonomy as one of the criteria to delineate species, this value has become a widely used cut-off 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.

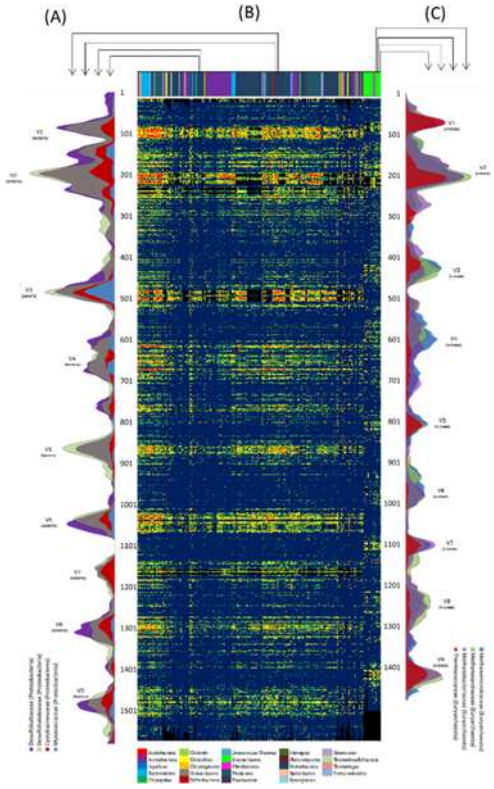

Figure. Illustration of the different levels of conservation within the 16S rRNA gene compared between all prokaryotic families. Within the heatmap each column represents one of the taxonomic families, and each row represents the position within the 16S rRNA gene from the 5' start site (top) until the 3' end of the gene (bottom). The color code of the cells reflects the alignment bit-score (as derived using the SSU-ALIGN algorithm, and reflecting the degree of conservation), where red indicates the most variable and blue reflects the most conserved positions as shown in section (B). To emphasize the variation within closely related families, four families belonging to the same bacterial class (Deltaproteobacteria) and four belonging to the same archaeal class (Euryarchaeota) were selected and the normalized bit-scores (bit-score averaged over a 30-nucleotide window) were plotted against the position within the 16S rRNA gene , as shown section A and C.

Cell cycle control of *Brucella abortus* inside human trophoblast cells

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The characterization of the trafficking of intracellular *Brucella* strains inside host cells is incomplete yet and many interesting mechanisms still need to be studied and discovered. It is possible to follow the growth of single bacteria inside infected cells by labelling bacteria with Texas-Red succinimidyl ester (TRSE) since the unipolar growth generates new non-labelled parts on the surface of *Brucella*. In addition, the replication and segregation of replication origins of both chromosomes of *Brucella*, named *oriI* and *oriII*, could be observed by using fluorescent fusions to ParB and RepB, putatively binding to *parS* and *repS* sequences located close to *oriI* and *oriII*, respectively. Besides, it was revealed that replication and segregation of *oriI* always occurs before *oriII* in tested conditions. Hence a ParB fusion is sufficient to monitor G1 phase or the initiation of S phase in the cell cycle of *Brucella*. By using these methods and tools, in previous studies we have found that nearly 80% of the intracellular *B. abortus* block their growth and their cell cycle at the G1 stage during the first 6 hours post infection (PI) and resume their growth and replication at 8 hours PI in HeLa cells. Anderson *et al*. (1986) found that trophoblasts of a pregnant goat contained numerous massively *Brucella abortus* at 5 days post-inoculation. This means that trophoblasts are a natural site for *B. abortus* replication. Therefore, it would be important to continue to expand the discoveries of intracellular growth and cell cycle of *B. abortus* in the other cell types such as murine RAW 264.7 macrophages and trophoblasts which are more relevant to the natural host cell infections. Our results in human trophoblast cell line JEG-3 showed that more than 60% of intracellular *B. abortus* started their growth already at 2h PI, contrasting with the results of 80% of bacteria arrested their growth within HeLa cells or RAW 264.7 macrophages. This means that trophoblasts provides a different environment than either HeLa cells or RAW 264.7 macrophages for *B. abortus* to grow and reach their replication niche. Besides, one more interesting observation on the growth of *B. abortus* inside JEG-3 was the small propotion of bacteria that generated daughter cells at 8h PI, although most of bacteria started to grow already at 2h PI. Since the doubling time of *B. abortus* in rich medium around 3-3.5 hours, this observation suggest that growth is severely impaired between 2 and 8 h PI in JEG-3 cells. Thus we propose that inside JEG-3 cells, most of *B. abortus*, display 3 stages of growth (instead of 2 stages in HeLa cells), i.e. starting growth, after that growth arrest, and then later resume the growth and generation of the daughter cells. We also found that DNA replication of intracellular *B. abortus* was different between HeLa cells and JEG-3 cells. In HeLa cells, 80% of bacteria were in G1 phase after 2 h, but only 40% of bacteria were in G1 phase after 2 h in JEG-3 cells. These data suggest that the coordination between cell cycle and cellular infection could vary from one host cell type to the other, which further underlines the complexity of the infection process.

Dynamic Responses of Cyanobacterial Communities Following Glacier Retreat in the High Arctic (Svalbard)

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Glacier retreat due to global warming has been observed in all the cryosphere, including the north and south poles as well as high mountain ranges like the Alps and the Andes, systematically exposing new ice-free terrestrial habitats for the colonization by pioneering organisms. Plant succession following glacier retreat has been extensively studied in the Arctic, but there is very little knowledge about the earlier stages of soil development before the establishment of plant communities, in which the soil nutrient cycling is dominated by microbial processes. This study aims on studying shifts in cyanobacterial communities associated with retreating glaciers in Petunia Bay (Svalbard, High Arctic). Soil samples were collected in the forefields of the Ebba, Hørbye and Ragnar glaciers, along transects covering a time period of 100 years of glacier retreat. DNA was extracted from the samples and the V3-V4 region of the 16S rRNA gene was amplified using the cyanobacteria-specific primers CYA359F and CYA781R. Amplicons were sequenced using the 454 GS FLX+ Titanium platform. A total of 152,525 quality-filtered sequences were obtained for 24 samples (6355 ± 3885 per sample), which were grouped into 145 OTUs at 97.5% similarity. Pseudanabaenales was the most OTU-rich order (96 OTUs), followed by Chroococcales (13 OTUs), Oscillatoriales (7 OTUs), Synechococcales (6 OTUs), Nostocales (5 OTUs) and Gloeobacterales (3 OTUs). At the genus level, OTUs were assigned to *Leptolyngbya* (21 OTUs), *Phormidium* (3 OTUs), *Nostoc*, *Pseudanabaena* (2 OTUs each), *Chroococcidiopsis* and *Microcoleus* (1 OTU each). OTU richness increased significantly with soil age in the Ebba and Ragnar forefields, while an inverse correlation was observed in the Hørbye forefield (Pearson r, $p < 0.05$). Newer soils (< 30 years) in the Ebba and Ragnar forefields were composed by only three genera (*Leptolyngbya*, *Phormidium* and *Nostoc*), while older soils (40-100 years) were characterized by a higher cyanobacterial diversity that also included *Pseudanabaena*, *Chroococcidiopsis* and *Microcoleus*. Interestingly, the opposite was observed in the Ragnar forefield. Soil physico-chemical composition differed and also had contrasting effects on cyanobacterial diversity along the three glacier forefields. Soils from the Ragnar forefield were significantly more nutrient-depleted, with lower water, organic carbon, N-NO_3 and Ca contents in comparison to the other two glaciers (one-way ANOVA, $p < 0.05$). OTU richness was positively correlated to total organic carbon and N-NO_3 contents in this forefield but it was negatively correlated with these parameters in the Ebba and Hørbye forefields (Pearson r, $p < 0.05$). These preliminary results highlight a dynamic response of cyanobacterial communities following glacier retreat. Cyanobacterial diversity increases over time in soils with lower nutrient contents, but decreases as nutrients start to accumulate. Further beta-diversity analyses are being currently carried out in order to investigate deeper the effect of soil development on the structure of the cyanobacterial communities, and identify possible key species characteristic of each habitat. Understanding the relationship between cyanobacterial community structure and soil development will provide us with crucial knowledge on how cyanobacteria influence and respond to changes in environmental conditions. The study of cyanobacterial succession in high-latitude ecosystems is of special interest, where the effect of global warming on microbial processes is expected to be the highest.

Alkylating stress on *Brucella abortus* in culture and in infection

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Brucella abortus is a Gram negative alpha-proteobacterium that is responsible for brucellosis, a worldwide zoonosis affecting both cattle and humans [Moreno & Moriyón (2006) in The Prokaryotes, Springer New York, pp 315-456]. The trafficking of this class III pathogen has recently been described as globally biphasic in HeLa cells and RAW 264.7 macrophages. Indeed, bacteria enter host cells as newborn, *i.e.* in G1 phase of their cell cycle (1n), and remain so for 6-8 h before to reach their endoplasmic reticulum-derived replicative niche where they resume their cell cycle and actively proliferate [Deghelt *et al.* (2014) Nat. Commun. 5:4366]. One hypothesis to explain why G1 block has been selected through evolution is that this particular state of the cell cycle, which consists of stable gDNA without any ongoing replication fork, could enable the bacterium to better resist intracellular stresses targeting its genome. In this context, we decided to study the alkylating stress and its impact on *B. abortus* both in culture and in infection. Deletion mutants for eight genes involved in alkylated DNA repair (based on their homology with *Escherichia coli*) where constructed (Δ*ada*1, Δ*ada2*, Δ*ogt*, Δ*tagA*, Δ*alkA*, Δ*alkB*, Δ*xthA*1, Δ*xthA*2 simple mutants and Δ*ada1*Δ*ada2*, Δ*xthA*1Δ*xthA*2, Δ*alkA*Δ*tagA* double mutant, as well as Δ*ada*1Δ*ada*2Δ*ogt* triple mutant) and characterized for their growth in culture in the presence of methyl methanesulfonate, an alkylanting agent. Based on this experiment, we can propose a model in which *alkA* is involved in endogenous alkylating stress resistance, whereas *xthA*1, *ogt*, *tagA* and *alkB* are involved in exogenous alkylating stress resistance. RAW 264.7 macrophages were also infected with these mutant strains to assess whether their gene deletion would affect *B. abortus* survival in host cells. Both Δ*alkA* and Δ*alkB* were attenuated at 24 h post-infection, which suggests that, in addition to endogenous alkylating stress, *B. abortus* also has to face macrophage-derived alkylating stress. To our knowledge, it would be the first time that alkylating stress is directly pointed out as a stress affecting bacterial survival in host cells.

The gut microbiota of pollinators: An unknown and unexplored treasure chest of biodiversity

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Bumble bees are important pollinators of tomatoes, sweet pepper and many other commercial crops and wild plants. There is currently great concern about their worldwide decline and that of other pollinators like butterflies and hoverflies.[1] These declines may have a detrimental economic impact and may create an instable ecosystem. Ongoing declines are presumably caused by a combination of climate change, change in agricultural practices, pesticide and insecticide use and pathogen spill over from commercial bees.[2] A good mitigation strategy to decrease or reverse this phenomenon should therefore combine the augmentation of nest and hibernation sites, the reduction of insecticide use and the direct promotion of bumble bee health. Recent studies showed that the gut microbiota may play a beneficial role in the health of bumble bees.[3] The gut microbiota of bumble bees has predominantly been studied by metagenomics approaches and consists of few and very specific bacterial species and include *Snodgrassella alvi*, *Gilliamella apicola* and *Bifidobacterium bombi*. These symbiotic gut bacteria may contribute to the health of bees by helping with the digestion of pollen, the detoxification of compounds and pathogen inhibition.

Therefore, an inventarization of the cultivable bacteria in the gut of bumble bees has been made. Subsequently, the functionality of these bacteria in the bumble bee gut and their potential to improve bumble bee health is being investigated by pathogen inhibition assays (well diffusion and overlay assays) and a pectin degradation assay. In the future, the beneficial effects of a set of potentially probiotic strains on bumble bee health will be further explored by microcolony experiments.

References

1. Grixti JC, Wong LT, Cameron SA, Favret C. Decline of bumble bees (Bombus) in the North American Midwest. *Biol. Conserv.* 2009;142(1):75-84. doi:10.1016/j.biocon.2008.09.027.

2. Colla SR, Otterstatter MC, Gegear RJ, Thomson JD. Plight of the bumble bee: Pathogen spillover from commercial to wild populations. *Biol. Conserv.* 2006;129(4):461-467. doi:10.1016/j.biocon.2005.11.013.

3. Hamdi C, Balloi a., Essanaa J, et al. Gut microbiome dysbiosis and honeybee health. *J. Appl. Entomol.* 2011;135(7):524-533. doi:10.1111/j.1439-0418.2010.01609.x.

Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river

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Antibiotics are emerging contaminants, which wing to their bioactivity, may lead to short-term and long-term alterations of natural microbial communities in aquatic environment. We investigated the effects of antibiotics on biofilm bacterial communities in the Llobregat River (Northeast Spain). Three sampling sites were selected: two less polluted sites and one hotspot. River water was collected from each site and used both as inoculum and medium for growing biofilms in independent mesocosms. After 25 days of biofilm colonization, we exposed the colonized biofilms to river waters from the downstream sites (progressively contaminated by antibiotics). A control from each site was maintained where the growing biofilm was always exposed to water from the same site. The bacterial community composition, bacterial live/dead ratio and extracellular enzyme activities of the biofilms were measured before and 9 days after exposing the biofilms to increasing contaminated waters. Sixteen antibiotic compounds were detected in the water from the three sampling sites. At each site, the antibiotics present in the highest concentrations were sulfonamides, followed by quinolones and macrolides. Bacterial communities of biofilms grown with the three river waters differed markedly in their structure, but less so in terms of functional descriptors. After switching the medium water to increasing pollution, biofilms exhibited increased levels of actinobacteria (HGC), a trend that was associated to the higher antibiotic concentrations in the water. These biofilms also showed increased bacterial mortality, and decreased extracellular leucine-aminopeptidase and alkaline phosphatase. There was a significant correlation between antibiotic concentrations and biofilm responses. Our results indicate that the continuous entrance of antibiotics in running waters cause significant structural and functional changes in microbial attached communities.

Measuring the biodiversity of microbial communities by single-cell analysis

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Measuring the microbial diversity in natural environments is pivotal for ecosystem characterization and ecological hypothesis testing. Despite the availability of high-throughput sequencing technologies, there exists a need for assessing the diversity on a rapid, inexpensive and database-independent platform with limited computational effort. Here, we demonstrate that sensitive single-cell measurements of phenotypic attributes, obtained via flow cytometry, can provide fast first-line assessments of microbial diversity dynamics, without demanding extensive sample preparation and downstream data processing. By calculating established diversity metrics from whole community cytometric data, we constructed an alternative interpretation of microbial diversity that incorporates distinct phenotypic traits underlying cell-to-cell heterogeneity (i.e., morphology and nucleic acid content). Using 16S rRNA gene amplicon sequencing as a benchmark, we evaluated the extent to which our developed biodiversity indices capture shifts in the taxonomic composition of microbial communities. For this purpose we studied an open model ecosystem that was constrained by reproducible and controlled environmental conditions. The model was a cooling water ecosystem that was part of a discontinuously operated nuclear research reactor and was monitored throughout two 25- to 30-day reactor cycles. Our newly developed approach delivered temporal profiles strongly correlated with the benchmark diversity and was exceptionally powerful for detecting highly dynamic shifts in biodiversity. As such, we showed that our advanced analysis of flow cytometry data represents a novel and powerful resource, capable of strengthening the quality and amount of information gathered from microbiome research.

Surface-water, oil-degrading predominant genus *Alcanivorax* is inactivated by hydrostatic pressure under water (10MPa, 1000m)

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Microbial communities play a prominent role in biogeochemical cycles and have the potential to exert a feedback on climate changes (Gutknecht et al., 2012). The deep-sea environment represents a unique reservoir of biodiversity on Earth, holding largely unknown forms of microbial life. As economic pressure to access deepwater, fossil reservers pushes towards the use of less tested technologies, the risk of oil contamination is not expected to decrease in the forthcoming future (Thibodeaux et al., 2011). Although petroleum enters the environment also through natural sources, the impact of accidental oil spills in a restricted space- and time-frame is critically higher. Naturally, the gaseous fraction will move up to the atmosphere, while the majority of the spill will spread horizontally to form a thin layer over water (Gaines et al., 1999). Much attention is generally paid to this petroleum fraction that may eventually affect costal zones, while very little is known about the fate of oil in deeper seawaters featured by enhanced hydrostatic pressure. Notwithstanding the apparently counterintuitive concept of sinking oil, a large fraction of the hydrocarbons do precipitate to the seafloor. Bacterial and phytoplankton blooming on oil and their production of exopolymeric substances form particles made of oil droplets and microbes that sink to the bottom of the sea (Passow et al., 2012). This phenomenon was recognized as the main cause for oil transport to the seafloor (~1500m) in the Deepwater Horizon spill (Fed. Inter. Sol., 2010), where ~10% of the total spilled oil (~$5*10^6$ barrels) presently resides (Valentine et al., 2014).

Oil-spills worldwide are largely dominated by the ubiquitous genus *Alcanivorax.* This hydrocarbonoclastic microbe may constitute up to 90% of the bacterial population growing on spilled oil (Yakimov et al., 2007) and is considered a model organism in the bioremediation field. The time-dependent reaction towards oil implies that a large fraction of the bacterial bloom on the sea surface will eventually sink together with oil droplets, thus exposing *Alcanivorax* cells to increasing hydrostatic pressure. What is the fate of this oil fraction? Does *Alcanivorax* account for its degradation and hold the same bioremediation potential at increasing pressure?

In the present study, the impact of 0.1, 5 and 10MPa (equivalent to surface water, 500 and 1000m) on the physiology and molecular response of 3 axenic *Alcanivorax* species was tested. *A. jadensis* KS_339, *A. dieselolei* KS_293 and *A. borkumensis* SK2 showed a piezosensitive profile, with decreasing growth rates under increasing pressure, being the impact already statistically significant under 5MPa (P <0.05). Their biodegradation capacity under pressure was dramatically lowered, as indicated by the limited impact on the pH value with respect to sterile controls. However, pressure affected the 3 *Alcanivorax* strains in a different way. In particular, *A. dieselolei* showed a remarkable structural resistance, with high levels of intact cells (above 75% of the total cell number), increased PO_4^{3-} uptake per cell and constant specific degradation rates (CO_2 per cell). Hence, its growth was actually slowed down while its viability and activity were not affected. On the other hand, a pressure of 5MPa was lethal for *A. borkumensis*, with a dramatic drop in intact (10%) and total cell number. Surprisingly, a further increase to 10MPa promoted growth again and improved intact cell number (P <0.05), indicating the triggering of a pressure-resistance mechanism. Transcriptomic analysis revealed that protein synthesis, ATP generation and respiration were highly impacted under 10MPa in both these latter strains. Expression of genes related with ribosomes and translational factors implicated in the binding of amino-acyl t-RNA to ribosomes was upregulated, as also observed for genes related with all subunits constituting the F_1F_0 ATP complex. Most interestingly, pressure appeared to trigger alternative respiration pathways making use of ubiquinone-cytochromes c and Na^+ transporters rather than cytochromes c and b. In *A. borkumensis* renewed growth between 5 and 10MPa was supported by the production of the osmolite ectoine, which increased from 0.45 to 4.71 fmoles $cell^{-1}$ (0.1 *vs* 10MPa). In agreement, neither gene expression nor osmolite accumulation were affected by pressure in the structurally resistant *A. dieselolei*.

These findings indicate a general impediment in *Alcanivorax* metabolism and bioremediation capacity under mild pressure. Interplay between the aforementioned impacted pathways potentially shapes the structure of microbial communities at mild depth and may select for efficient oil degraders in sub-surface waters.

What's out there? Metagenomics diversity assessment of soil

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It has been estimated that the number of prokaryotic species per gram of soil ranges from 100,000 to 1,000,000, but only a very small portion can be cultured in the laboratory. Microbial ecology struggles to keep pace with plant and animal ecology in resolving community composition and functional features. Recent advances in sequencing technology and related bioinformatics tools now makes it possible to attempt metagenomics studies of soil ecosystems.

Prior to undertake any real analysis, we wanted to test the prediction power of Kraken, a taxonomical classifier for metagenomics. For this purpose we simulated a metagenome dataset on the basis of more than 12,245 complete genomes from the NCBI database. To those genomes we also added 500,000 artificial genomes randomly generated from the letters A, C, G and T: this way we recreated the microbial "dark matter", i.e. the unknown diversity present in soil. We then converted the 512,245 genomes into a metagenome dataset and we assessed the performance of Kraken.

The results show about 98% accuracy at the family, genus as well as species level for each classified reads.

Currently, we are running this tool to analyze a real dataset from a soil sample taken from the Gontrode forest in Belgium. Preliminary results already allow us to test out certain hypotheses pertaining to the impact of an acidic environment on fungal and bacterial diversity.

Identification of *Brucella abortus* genes required for growth on plates and macrophage infection using Tn-seq

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Bacteria from the *Brucella* genus are gram negative intracellular pathogens responsible for Brucellosis, one of the most widespread anthropo-zoonosis. *Brucella* spp. are intracellular pathogens and despite their tremendous impact on health and economy no human vaccine is currently available and little is known about molecular mechanisms underlying the infection process.

Here, we have performed a large scale Tn-seq experiment in order to identify all genes that are essential for growth on plates and for infection of RAW 264.7 macrophages by *B. abortus*. Briefly, a hypersaturating transpositional mutagenesis was conducted with a miniTn*5* derivative to generate a large collection of roughly 3.10^6 transpositional mutants. Once generated, this library was used to infect RAW 264.7 macrophages. The transposon insertion sites of all those mutants were determined by high throughput sequencing both before and after infection, allowing us to generate a transposon tolerance map for each of these two conditions. On such maps, a very low frequency of transposon insertion at a given locus highlights the requirement of this locus for either growth on plate (*i.e.* if the locus was found before infection) or for infection (*i.e.* if the locus was found after infection).

With this method, we found 491 candidate genes out of 3419 to be essential for the growth of *B. abortus* on plates, with an asymmetrical repartition between chromosome I and chromosome II, and 165 candidate genes attenuated in infection. Among these attenuated candidates, we found typical components of the virulence arsenal of *B. abortus* such as for example the type IV secretion system *virB*, and the outer membrane homeostasis two component system *bvrS/R*. Several uncharacterized genes and pathways have been identified and are currently being investigated.

Nitrous oxide emission by the non-denitrifying, nitrite ammonifier *Bacillus licheniformis*

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Firmicutes have the capacity to remove excess nitrate from the environment via either denitrification, dissimilatory nitrate reduction to ammonium or both. The recent renewed interest in their nitrogen metabolism has revealed many interesting features, the most striking being their wide variety of dissimilatory nitrate reduction pathways. In the present study, nitrous oxide production from *Bacillus licheniformis*, a ubiquitous Gram-positive, spore-forming species with many industrial applications, is investigated. *B. licheniformis* has long been considered a denitrifier but physiological experiments on three different strains demonstrated that nitrous oxide is not produced from nitrate in stoichiometric amounts, rather ammonium is the most important end-product, produced during fermentation. Genome analyses confirmed the lack of a nitrite reductase to nitric oxide, the key enzyme of denitrification. Based on the gene inventory and building on knowledge from other non-denitrifying nitrous oxide emitters, hypothetical pathways for nitrous oxide production, involving NarG, NirB, qNor and Hmp, are proposed. In addition, all publically available genomes of *B. licheniformis* demonstrated similar gene inventories, with specific duplications of the *nar* operon, *narK* and *hmp* genes as well as NarG phylogeny supporting the evolutionary separation of previously described distinct BALI1 and BALI2 lineages.

Dissimilatory nitrate reductions in N_2O producing *Bacillus azotoformans* LMG 9581^T

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N_2O is a potent greenhouse gas and a contributor to ozone layer destructions. There are three N_2O producing microbial processes that have been studied for now: denitrificaion, dissimilatory nitrate reduction to ammonium (DNRA), and nitrification. The former two respiratory pathways are previously thought to be mutually exclusive, until recently it has been recognized that bacteria can contain the gene inventory for both denitrification and DNRA, such as *Shewanella loihica* PV-4 [Yoon, S., R. A. Sanford and F. E. Löffler (2013) Applied and Environmental Microbiology 79(8): 2818-2822] *B.azotoformans* LMG9581^T [Heylen, K. and J. Keltjens (2012) Front Microbiol 3: 371] and some other organisms [Sanford *et al*, (2012) Proceedings of the National Academy of Sciences 109(48): 19709-19714]. Detailed studies of these microorganisms could shed light on the differentiating environmental drivers of both processes without interference of organism-specific variation. In our study, we found that *Bacillus azotoformans* showed a remarkable redundancy of dissimilatory nitrogen reduction, with multiple copies of each denitrification gene as well as *nrfAH*, but has reduced capacity for nitrogen assimilation, with no *nas* operon nor *amtB* gene. We hypothesized that (i) ammonium could not be used as sole nitrogen source, and (ii) DNRA, if expressed, would compensate for nitrogen assimilation during anaerobic growth. Batch experiments were conducted with various C/N ratios, and different concentrations of nitrate, nitrite, ammonium and yeast extract. Growth was assumed to be supported by ammonium as nitrogen source but required yeast extract added to the medium. Nevertheless, ammonium had a clear effect on growth rate, while yeast extract concentration determined main growth yield. No ammonium was produced via DNRA in nitrogen limiting conditions, but limited ammonium amounts were consumed when ammonium is supplemented. Comparable to *Shewanella loihica*, we generally observed a denitrification phenotype for all growth conditions tested, with all supplied nitrate converted to nitrous oxide (acetylene inhibition method).

In conclusion, the Firmicute *B. azotoformans* requires organic nitrogen for assimilation and does not use DNRA to compensate for the lack of a *nas* operon in nitrogen limiting conditions tested. The environmental drivers triggering DNRA will be studied using continuous culturing experiment and compared to those for the Gammaproteobacterial *S. loihica*.

The azanthraquinone/diaryltriazene couple to target the bacillary redox homeostasis of intracellular *Mycobacterium tuberculosis*

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The predominant intracellular lifecycle of the weakly gram-positive *Mycobacterium tuberculosis* (*Mtb*) complicates the treatment of tuberculosis (TB) with conventional antibiotics. Residing and replicating within the phagosome of the macrophage host cell, the bacillus is partially shielded from harmful xenobiotics. In addition to the ability to prevent phagosome/lysosome fusion, the bacillus has adapted a unique detoxification system to counter the onset of reactive oxygen/nitrogen species produced by the macrophage to clear the invading pathogen.
In this collaborative research we investigate the possibility to target intracellularly replicating *Mtb* with an innovative compound couple. In this strategy, the azanthraquinone lead candidate acts as a redox cycler, targeting mycothione reductase, and abrogating the bacillary redox homeostasis and hence its ability to cope with oxidative stress. The 1,3-diaryltriazene lead candidate on the other hand acts as an activator of the host macrophage, inducing the NO synthase and the NADPH oxidase within the phagosome membrane of the macrophage.

For the azanthraquinone lead candidate we observed nano-molar mycobacteriocidal activity *in vitro* and confirmed this activity *in vivo*. The compound was active against multi-drug resistant *Mtb* and could lower de bacillary load within *Mtb* infected macrophages without affecting host cell viability. Apart from its ability to lower the bacillary load inside *Mtb* infected RAW 264.7 macrophage cell line at concentrations as low as 1 µM, the 1,3-diaryltriazene lead candidate elicited elevated levels of HO– radicals without influencing the viability of the cells.

In the future this compound couple will be studied for its ability to target metabolically dormant bacilli, but also whether implementation of this compound couple can shorten the treatment with or lower the dose of conventional TB antibiotics.

The development of *Pseudomonas* and *Burkholderia* lung infection mouse models as a tool for therapeutic research.

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Pseudomonas aeruginosa, ***Burkholderia cenocepacia*** and ***Burkholderia multivorans*** can cause opportunistic infections in patients suffering from Cystic fibrosis (CF). Although individually treatable, the ability of these pathogens to form pulmonary biofilms increases their bacterial resistance towards antimicrobials. Within the protective biofilm structure, antimicrobial treatment with conventional antibiotics becomes less trivial. As a result, chronic infected CF patients often have a higher mortality rate and often the need for a lung transplant occurs sooner as the co-incidence of the bacterial infection results in a faster decline in lung functionality.

Within a scientific collaboration between the University of Antwerp, Leuven and Gent University, a pioneering therapeutic strategy is under investigation in which existing antimicrobials are combined with a **potentiator**. This potentiator is a compound that makes bacteria more vulnerable to conventional antimicrobials by disrupting **microbial tolerance mechanisms.** To generate the necessary proof of concept, murine infection models were optimized and validated. These models mimic the chronic pulmonary infection and allow enumeration of the bacterial burden of *P. aeruginosa*, *B. cenocepacia* and *B. multivorans* within the lung.

As mice are inherently more resistant to *Burkholderia* infections than humans, an adequate suppression of cell-mediated immunity is crucial to induce an established *Burkholderia* lung infection. Therefore, the immunosuppression protocol was optimized by testing different dose schedules of cyclophosphamide (CPM) in Swiss and BALB/c mice. By measuring the total amount of leukocytes in the blood during immunosuppression, clear leukopenia could be demonstrated when the mice were administered 150mg/kg CPM four days before infection and 50mg/kg CPM, two days before infection and on the day of infection. Due to the genetic heterogeneity, the susceptibility of Swiss mice for infection with *B. cenocepacia* can vary greatly, decreasing the reproducibility and making the Swiss mice a less suitable model for this study. The more genetically homogeneous BALB/c inbred mice were chosen as they generate more reproducible *Burkholderia* infection burdens. Immunosuppressed BALB/c mice inoculated intratracheally with 10^6 CFU *B. cenocepacia* demonstrated a lung burden of $1{,}8.10^8$ CFU/g lung three days post infection (5 independent repeats, 4 mice per repeat). A dose titration of tobramycin (30, 60 and 120mg/kg) was performed to validate whether this model was sensitive enough to evaluate efficiency of the antimicrobials combined with a potentiator. A significant reduction of 1,83 $\log_{10}$ in lung burden could be demonstrated when mice were intranasally treated with 120mg/kg tobramycin (2 independent repeats, 4 mice per repeat). Immunosuppressed BALB/c mice inoculated intratracheally with 10^6 CFU *B. multivorans* demonstrated a lung burden of 4.10^8 CFU/g lung after five days postinfection (3 independent repeats, 4 mice per repeat). Currently experiments are ongoing to validate this *B. multivorans* respiratory model with tobramycin.

In addition, a chronic *P. aeruginosa* mouse infection model is being optimized in which bacteria are encapsulated in spherical alginate beads (30µm) to prevent rapid clearance of the bacteria from the lungs and to mimic the biofilm-like structures of *Pseudomonas* in the CF lung. BALB/c mice inoculated intratracheally with 10^6 CFU *P. aeruginosa* alginate beads demonstrated a lung burden of $1{,}8.10^8$ CFU/g lung after four days postinfection. Currently experiments are ongoing to validate this *P. aeruginosa* respiratory model with tobramycin.

The validated *B. cenocepacia* respiratory model is currently used to evaluate an existing antimicrobial combined with a potentiator.

Characterization of the dynamics of *Brucella abortus* outer membrane components

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Brucella abortus is a gram-negative bacterium that, together with other Brucella species, is causing a worldwide spread zoonosis called brucellosis. In contrast to *Escherichia coli*, bacteria belonging to the order Rhizobiales are characterized by unipolar growth [Brown *et al* (2012) PNAS 109: 1697-701]. This can be visualized by the use of Texas red succinimidyl ester (TRSE), which covalently binds to the outer membrane components, presumably proteins. If TRSE is washed and bacteria are incubated in rich medium, growth is restarted and the newly incorporated unlabeled envelope is detected at the new pole and the constriction side. Since the new material is incorporated only at the new growing pole, one could think that also lipopolysaccharide molecules (LPS) are inserted equally at this part of the bacterial envelope. To test this hypothesis, bacteria were labeled with a monoclonal antibody directed against the O-chain of LPS, and visualized by the secondary antibody anti-mouse Alexa488. The labeling of *Brucella abortus* 544 showed a homogenous distribution over the surface. After 2 hours of growth, a clearly unlabeled zone representing the new incorporated S-LPS molecules was visible at one pole, which was identified as the new pole since it is systematically localized at the opposite pole compared to the old pole marker PdhS-mCherry. By the addition of the secondary antibody only after 2 hours of growth, a homogenous signal is observed. This is consistent with the expected mobility of LPS in the outer membrane. This mobility is restricted if the secondary antibody crosslinks primary antibodies. In contrast to the mobility of LPS, the same experiment using a monoclonal antibody directed against Omp25 showed that this protein is not mobile.

Unlike as smooth bacteria possessing S-LPS, rough bacteria with R-LPS without the O-polysaccharides can enter more efficiently during the infection process, but these are not able to survive inside the host cells [Allen *et al* (1998) Infect Immun 66: 1008-16]. To further study the role of LPS, a Δ*wzm* mutant was created, which is not able to transport the O-polysaccharide from the cytoplasm to the periplasm and therefore possess R-LPS. During infection of RAW264.7 macrophages, the Δ*wzm* strain showed higher colony forming units (CFU) after 2 hours post infection (PI) compared to the wild type strain. This leads to the hypothesis that these bacteria can enter better into this cell type, suggesting that adhesion sites present on the bacteria surface are accessible in rough but not in smooth strains, or that the O-chain has the ability to inhibit phagocytosis of *B. abortus*.

Extreme example of niche differentiation: new methanotrophic species within methylotrophic genus *Methyloceanibacter*

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Methanotrophs (MOB) are widespread on earth contributing significantly to the reduction of emission of methane, a potent greenhouse gas, to the atmosphere. The key enzyme, methane monooxygenase, enables MOB to use methane as their sole source of carbon and energy and exists in both a soluble (sMMO) and particulate (pMMO) forms. It also differentiates MOB from other methylotrophic bacteria only capable of growth on methanol or other more oxidized C1-compounds. Despite the global relevance of the mitigation of methane, little is known about aerobic MOB in marine environment, besides the dominance of pMMO-type MOB and only a handful of well described cultures.

Marine sediments from the North Sea were sampled across an increasing salinity gradient and a decreasing nitrogen gradient and over 200 enrichments were set up to avoid bias inherent to molecular detection of MOB. After significant methane oxidation was observed shotgun sequencing of five enrichments was performed. Two enrichments revealed the presence of a methanotrophs and a methylotrophs, both closely related with the recently described methylotrophic species *Methyloceanibacter caenitipedi* Gela4, obtained from marine sediment collected near a hydrothermal vent in Japan (Takeuchi et al., 2014). An isolation effort to retrieve these organisms resulted in forty-one isolates belonging to the genus *Methyloceanibacter,* representing four distinct genotypes based on rep-PCR fingerprinting, designated Veki 1, 2, 3 & 4. Genome analyses and growth on methane as sole carbon and energy source confirmed the methane oxidizing capacity of strain Veki1. All strains were able to growth on methanol, showing a significant variation in both growth rate and yield. Furthermore, clear strain-dependent ecophysiological differences were observed for temperature, pH, salinity, carbon and nitrogen preferences. Genomic in silico DNA-DNA hybridization and average nucleotide identity (ANI) revealed that the different strains represent four novel, distinct species within the genus *Methyloceanibacter*, despite a high 16S rRNA gene sequence similarity (>97%).

These results provide the first evidence for an extreme example of microdiversity, namely a methanotroph within methylotrophic congenerics in *Methyloceanibacter*. Microdiversity, i.e. metabolic versatility among closely related microorganisms, has been well described for other marine genera such as *Prochlorococcus*, *Vibrio*, and SAR, with cultured strains having distinct pigmentation, maximum growth rates, metal tolerances, nutrient utilization and photophysiological characteristics. Furthermore, Veki 1 is the first marine methanotrophic isolate and the third genus described thus far containing only the soluble form of the methane monooxygenase.

Gp150 promotes sexual transmission of Murid Herpesvirus-4.

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Gammaherpesviruses are important pathogens in human and veterinary medicine. During co-evolution with their hosts, they developed many strategies allowing them to shed infectious particles in presence of immune response. Understanding these strategies is likely to be important to control infection. Interestingly, we recently observed that Murid herpesvirus 4 (MuHV-4), a gammaherpesvirus infecting laboratory mice, could be sexually transmitted between mice. This model offers therefore the opportunity to understand the mechanisms underlying natural transmission. Some of these mechanisms could rely on the glycoprotein 150 (gp150), which could limit virus neutralization and promote the release of infectious particles from cells. In this study, we tested therefore the importance of gp150 in the context of MuHV-4 sexual transmission. Briefly, female mice were infected with WT or gp150- strains expressing luciferase. They were imaged with an *in vivo* imaging system to follow infection. When lytic replication was observed in the genital tract, infected females were mated with naïve males to compare the capacity of transmission of the two strains. Our results show that, while the gp150- strain has no deficit in reaching and replicating in the female genital tract, it displays a major deficit of sexual transmission in comparison with WT virions. Interestingly, this deficit appears to reflect a deficit of virions release from vaginal epithelial cells. Altogether, our results show that, while gp150 is not required for efficient dissemination and maintenance of MuHV-4 within its host, it is essential for efficient transmission, by promoting the releasing of infectious particles from the mucosal cells.