BELGIAN SOCIETY FOR MICROBIOLOGY

BELGIAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

National Committee for Microbiology

National Committee for Biochemistry and Molecular Biology

of

The Royal Academies of Science

and the Arts of Belgium

Contact Forum

"Microbiome and Host Metabolism"

Academy Palace, Brussels

October 28th 2016

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

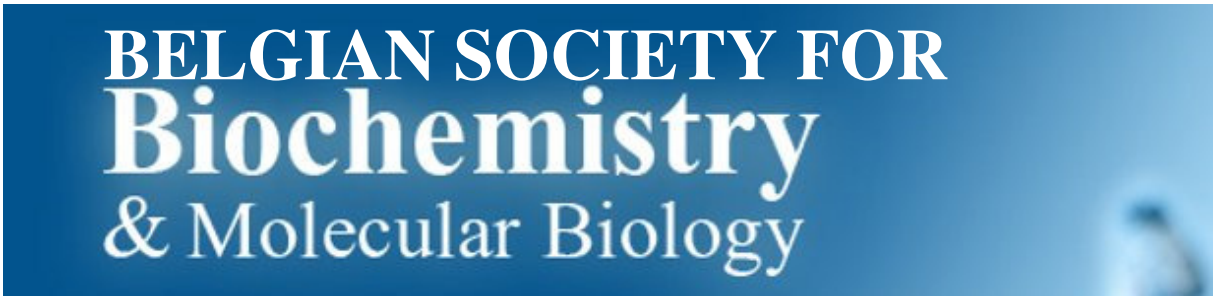
Council members are outstanding microbiologists with different microbiology backgrounds and specialized in several domains including molecular, plant and pharmaceutical microbiology, taxonomy, microbial ecology, animal and human virology, and thereby covering many aspects of microbiology. Members are 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 (SCKCEN), 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 Enews 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.femsmicrobiology.org/website/nl/default.asp)



History

The Belgian Biochemical Society was created at the initiative of Marcel Florkin to enable a Belgian society to join the newly proposed International Union of Biochemistry. On the 15th of September 1951, Edouard J. Bigwood, Jean Brachet, Christian de Duve, Marcel Florkin, Lucien Massart, Paul Putzeys, Laurent Vandendriessche and Claude Lièbecq drafted the statutes of the society on principles similar to those of the Belgian Physiological Society. They were approved by the first general assembly held at the University Foundation in Brussels on the 12th of January 1952. The assembly elected Marcel Florkin as president and Claude Lièbecq as secretary and treasurer.

It also decided to pre-circulate the abstracts of communications in what became known as the Archives Internationales de Physiologie, de Biochimie et de Biophysique and to participate in the creation of the International Union of Biochemistry.

The first scientific meeting of our society took place on the 8th of March 1952 and was chaired by Christian de Duve. It was a joint meeting with the French Société de Chimie Biologique.

In 1955 our Society organized the 3rd International congress of Biochemistry, which was held in Brussels. The organizing committee consisted almost entirely of the members of the board of our Society.

The 10th anniversary of our society was celebrated in 1962 at a joint meeting with the (British) Biochemical Society in the presence of delegates of the Dutch and French biochemical societies.

Two members of the Society, Albert Claude and Christian de Duve, have been awarded the Nobel Prize in 1974.

When the society celebrated its 25th anniversary in 1977, it had organized exactly 100 scientific meetings, with more than 3000 communications, read or presented as posters, and about 100 lectures of general interest. At that time the society had about 700 members.

In 1999 a Society website was created and since 2000 all abstracts are being published in electronic form on the web, keeping the original format of the “Archives”.

At the 50th anniversary, the Society has close to 1000 members. Until then 182 meetings had taken place.

In 2015 a new general board takes office with a new secretary. The board is grateful for all the efforts of Prof. Fred Opperdoes, secretary of the society during the past 17 years. The members list is up-dated. In 2016 the website is moved: www.biochemistry.be

Board

Board members are outstanding scientists with different backgrounds in biochemistry and molecular biology. Members are from different universities and institutions located in the different regions of Belgium (Flanders, Brussels and Wallonia).

Council consists of the following members:

President: Carine Michiels (U. Namur), Secretary: Jan Gettemans (UGent), Treasurer: Yves Engelborghs (KULeuven), 1st vice president: Jason Perret (ULB), 2nd vice president: Sylvia De Wilde (UAntwerpen), publications editor: Anne-Marie Lambeir (UAntwerpen).
The other Council members are: Wanda Guedens (Uhasselt), André Matagne (U. Liège), Joris Messens (VUB), Patrice Soumillion (UCL) and Lionel Tafforeau (U. Mons).
The members of the board of the BSBMB are also represented in the National Committee for Biochemistry and Molecular Biology.

Activities

The society organizes 2 or 3 scientific meetings every year, sometimes in collaboration with sister societies and doctoral schools.

Advantages

BSBMB members have free access to BSBMB activities and are automatically member of FEBS (Federation of the European Biochemical Societies), which also support members via several types of grants (see http://www.febs.org/) and educational and societal activities.

Program

08.30	*Registration – Poster mounting*
09.00	*Welcome address*
09.10	**Patrice D. Cani**, Louvain Drug Research Institute, Metabolism & Nutrition, UCL Obesity, diabetes and cardio-metabolic health: a matter of dialogue with gut microbes?
09.50	**Tom Van de Wiele**, Center for Microbial Ecology and Technology, Ugent Dietary management of the mucosal gut microbiome to maintain gut barrier function and energy homeostasis
10.30	**Elke Lievens**, KU Leuven, Centre for Microbial and Plant Genetics, Heverlee Unraveling the contribution of vaginal Lactobacillus species to the vaginal barrier function and their potential against HSV-2 infection
10.45	**Ilke De Boeck**, UAntwerpen, Dep. Bioscience Engineering, Research group Environmental Ecology and Applied Microbiology, Antwerpen The microbiota of the upper respiratory tract: potential of niche-specific isolates as probiotics
11.00	*Coffee break and poster viewing*
11.30	**Willem de Vos**, Wageningen & Helsinki University, NL & FI Microbes Inside - From Structure to Solutions
12.10	**Francesco Renzi**, URBM, Département de Biologie, Université de Namur, Namur Identification of a new lipoprotein export signal in Gram-negative bacteria
12.25	*Lunch and poster viewing* *General assembly BSM*
14.00	**Gabriele Berg**, Graz University of Technology, AT The plant microbiome and its diverse relationship with the host
14.40	**Jelle Matthijnssens**, Laboratory of Viral Metagenomics, KU Leuven The virome of the honeybee (Apis mellifera) and its role in winter loss.
15.20	**Charles Dumolin**, Laboratory of Microbiology Universiteit Gent Seeking for novel "microbials" with industrial potential via the high throughput isolation platform MiCRoP
15.35	**Doris Vandeputte**, KU Leuven, Dep. Microbiol. and Immunol and VIB, Center for the Biology of Disease, Leuven Population-level study identifies main gut microbiome covariates
15.50	**Leo Lahti**, *Dep Mathematics and Statistics, University of Turku, Finland* Challenges and opportunities in large-scale microbiome profiling studies
16.05	**Eugène Rosenberg & Ilana Zilber-Rosenberg**, Tel Aviv University, Israel The Hologenome Concept
16:55	*General conclusions and presentation of best poster awards*

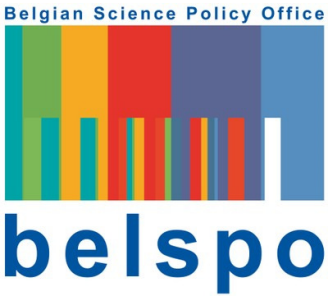
Sponsors


BVBMB
SBBBM


AWK


fwo


fnrs
LA LIBERTÉ DE CHERCHER


Belgian Science Policy Office
belspo


VWR
We Enable Science


BASECLEAR
FOR 100% DNA RESULTS


analis

Abstracts of invited lectures

Obesity, diabetes and cardio-metabolic health: a matter of dialogue with gut microbes?

PATRICE D. CANI

Université catholique de Louvain, Louvain Drug Research Institute, WELBIO- Walloon Excellence in Life Sciences and BIOtechnology, Metabolism and Nutrition Research Group, Brussels, Belgium. European Associated Laboratory "NeuroMicrobiota" (Inserm-Toulouse/UCL-Brussels),

Obesity is characterized by a cluster of metabolic alterations (insulin resistance, diabetes, dyslipidemia, low-grade inflammation) increasing cardiovascular risks.

We and others have shown that gut microbes dialog with host cells and regulate energy, glucose and lipid homeostasis via different mechanisms.

Over the last 15 years, we have investigated the mechanisms by which gut microbes control nutrient absorption and host biology in the context of obesity and associated cardio-metabolic disorders.

We found that gut microbes contribute to the onset of metabolic inflammation, but also control fat mass development and energy homeostasis. For example, we described the concept of metabolic endotoxemia (increased plasma LPS during metabolic syndrome), we found that this was also linked with changes in gut barrier function (e.g., antimicrobial peptides production, mucus layer thickness, innate immune system) or altered endocannabinoid system tone.

Recently, we discovered that prebiotic feeding increases the abundance of a relatively newly identified bacteria, namely *Akkermansia muciniphila.* By using different approaches, we demonstrated that this *A. muciniphila* plays a major role and protects against obesity, diabetes and inflammation in mice. We also strongly associated the abundance of *A. muciniphila* with a better metabolic response after a caloric restriction diet in obese humans. Novel data will be discussed in this context.

Finally, we found that specific alteration of the innate immune systems such as the deletion of MyD88 (specifically in the intestinal epithelial cells or in hepatocyte) may profoundly affect host metabolism via microbiota-dependent associated mechanisms (i.e., changes in bile acids, bioactive lipids, metabolome). The role of bacteria and immunity on obesity will be discussed in both mice and human approaches.

Acknowledgement
This work is supported by ERC Starting Grant ENIGMO 336452, the Grant for medical research from Baillet-Latour Funds 2015, the FRS-FNRS and WELBIO.

Dietary management of the mucosal gut microbiome to maintain gut barrier function and energy homeostasis

TOM VAN DE WIELE

Host-microbe Interaction Technology group, Center for Microbial Ecology and Technology, Faculty of Bioscience Engineering, Ghent University

Recent progress in host-microbiome research has revealed an important interaction of the gut microbiome with host processes that control energy homeostasis and satiety and that may modulate risks for developing obesity, metabolic syndrome and associated cardiometabolic health. A crucial element in this research is the role of mucosal microorganisms that, due to their close proximity to the gut wall, can more profoundly interact with the host compared to the luminal microbiota. Gut barrier and enteroendocrine functions play an important role in this interaction process. Besides the many factors (delivery mode, gender, lifestyle, genetic predisposition) that can affect host-microbe interactions, diet composition certainly is an important determinant. We will illustrate how specific dietary components, like chemical emulsifiers, can negatively impact the microbiome and increase the risk for metabolic disease. Conversely, we will also exemplify how dietary strategies or functional foods can modulate the microbiome towards a composition and functionality that alleviates the risks for gut barrier dysfunction and development of metabolic disease.

Microbes Inside: From Structure to Solutions

WILLEM M. DE VOS

Wageningen and Helsinki University

Since early life, microbes dominate our body in numbers. In the intestinal tract they constitute the largest microbial ecosystem that is close to our heart: our microbes inside. Previous molecular studies have shown that the intestinal microbiota is highly personalized and now have confirmed and extended this in comparative studies with over 10,000 samples that were carefully analyzed on an identical high throughput platform. The collective genome of these microbes contributes considerably to the coding capacity of our system and includes millions of genes in what also has been called our second genome. However, unlike our own genome, the intestinal microbiome is not strictly vertically inherited. Moreover, this personalized organ can be modified by a variety of food and pharma treatments that target its composition, stability and activity. These are instrumental in providing cause-effect relations to complement the abundance of associations that are presently being reported.

This contribution will provide an overview of the present state of the art of the human microbiome and its relation with health and disease. Notably, the microbial colonization events at early life will be described as these are thought to affect later life health. This is illustrated by the pervasive effects on the intestinal microbiota of repeated use of specific antibiotics that is associated with later life asthma and obesity. Finally, specific attention will be given to the successes and future of new avenues to alter the intestinal microbiome, including fecal transplantations and a series of new therapeutic microbes.

The plant microbiome and its diverse relationship with the host

GABRIELE BERG

Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria

The plant microbiome is a key determinant of plant health and productivity. The microbiomes associated with plants form tight networks, which revealed strong species and niche specialization. However, all of them have a relationship with its host: together the host and the indigenous microbiome forms a holo-biont, which is characterized by an intense metabolic interplay. Exemplarily, this relationship and interplay will be presented for mosses, medicinal plants and crops. Analyzing microbiome networks from healthy and diseased plants led to the identification of health indicators. This approach thus serves to open new opportunities for future targeted biocontrol studies and could fuel progress in sustainable agriculture, such as the development of microbial inoculants as biofertilizers, biocontrol, or stress protection products. The plant microbiome has not only an impact on plant health, it also influence the human microbiome, e.g. by raw-eaten fruits, vegetables and herbs. Moreover, it can have a positive impact on the microbiome of our built environment. Taken together, plant microbial networks are powerful networks with an impact on ecohealth.

The virome of the honeybee (Apis mellifera) and its role in winter loss

JELLE MATTHIJNSSENS

Laboratorium Klinische en Epidemiologische Virologie, Rega Instituut, KULeuven

Honeybees (*Apis Mellifera*) are social insects that play a crucial role in pollination of many economically important crops. Honeybee colonies are in decline in Belgium as well as in the rest of Europe and a large portion of this decline is due to winter loss (WL). Environmental factors and various viruses alone or in combination with a *Varroa destructor* parasite infection have been suggested as risk factors for winter loss. Identification of associated pathogens can help to elucidate the mechanisms behind WL and may allow the development of preventive strategies.

In collaboration with the University of Ghent (prof. Dirk de Graaf), we have access to a unique honeybee collection of Belgian honeybees, collected during a large EU-funded project named Epilobee. From this study we have samples from hives that succumbed to WL as well as from hives surviving winter for comparison.

These bees were subjected to viral metagenomics approaches developed in our laboratory, specifically designed to sequence the genomes present in viral particles. This method (named NetoVir) uses filtration, centrifugation and a nuclease treatment to purify viral particles from entire honeybees. After extraction, reverse transcription and random amplification, these genomes were prepared for sequencing using Illumina technology, followed by bio-informatics analyses to analyze the obtained sequence data.

In addition to known pathogens such as Lake Sinai virus, Filamentous virus and Varroa destructor virus-1, we identified a large number of highly divergent novel viruses. These viruses are only very distantly related to Kashmir bee virus (35% amino acid similarity), Khasan virus (25% aa similarity), Bloomfield virus (31% aa similarity) and Kelp fly virus (26% aa similarity). The complete genome of a distant relative of the Kashmir bee virus and the partial genome distantly related to Kelp fly virus are of particular interest since they are members of the *picornavirales*. This order contains most of the pathogenic honeybee viruses.

We are currently completing these viral genomes as well as trying to visualize virions using electron microscopy. qPCR assays will be used to identify their prevalence and viral loads in Flanders, and statistical analyses will be done to identify viruses associated with WL. These viruses will be investigated and characterized further *in vitro* and *in vivo* to investigate their involvement in WL.

The Hologenome Concept of Evolution

EUGENE ROSENBERG AND ILANA ZILBER-ROSENBERG

Tel Aviv University

The hologenome concept of evolution posits that the holobiont (host + symbionts) with its hologenome (host genome + microbiome) is a level of selection in evolution. Humans, animals and plants can no longer be considered individuals by the classical definitions of the term. All are holobionts consisting of the host and diverse symbiotic microorganisms. Microbial symbionts can be transmitted from parent to offspring by a variety of methods, including cytoplasmic inheritance, coprophagy, direct contact during and after birth, and via the environment. Numerous studies have demonstrated that these symbionts contribute to the anatomy, physiology, development, innate and adaptive immunity, behavior and finally also to genetic variation and to the origin and evolution of species. Acquisition of microbes and microbial genes is a powerful mechanism for driving the evolution of complexity. Evolution proceeds both via cooperation and competition, working in parallel.

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

page	Names (first author in alphabetical order) and title	location
20	A. ABDELMALEK, M. HEYNDRICKX AND A. BENSOLTANE Determination of survival and resistance to acidity as probiotic potential of infant and calf faecal Bifidobacteria	BHe
21	M. M. ALI, K. MIJNENDONCKX, N. LEYS, P. MONSIEURS, D. CHARLIER AND R. VAN HOUDT Dissection of novel resistance mechanisms in *Cupriavidus metallidurans*	BGen/ec
22	C. N. ALLONSIUS, D. VANDENHEUVEL, I. CLAES, P. DELPUTTE AND S. LEBEER Interaction between exopolysaccharides from *Lactobacillus rhamnosus* GG and *Candida* species	BHe
23	I. BERVOETS, M. VAN BREMPT, M. DE MEY AND D. CHARLIER Orthogonal gene expression: creating 3 promoter libraries linked to different sigma factors which are orthogonal between each other and towards the host	BBio
24	B. BYLOOS, I. CONINX, O. VAN HOEY, N. NICHOLSON, V. ILYIN, R. VAN HOUDT, C. COCKELL, N. BOON AND N. LEYS Physiology of *Cupriavidus metallidurans* CH34 in the presence of basalt, a lunar-like rock, on Earth and in Space	BGen/ec
25	P. BECKER, P. SZTERNFELD, M. ANDJELKOVIC, R. ANTHONISSEN, L. VERSCHAEVE, M. HENDRICKX, M. RENARD, Y. LARA, B. DURIEU, V. SIMONS, H. D. LAUGHINGHOUSE IV, L. CORNET, D. BAURAIN AND A. WILMOTTE The BCCM/ULC collection to conserve the biodiversity and explore the applied potential of Polar cyanobacteria	BDiv
26	M. BERAUD, G. GIAMBARRESI, D. GILLAN AND R. WATTIEZ Fishing up rare bacterial proteomes using SWATH-MS in synthetic microbial communities	BBio
27	M. CALATAYUD, E. HERNANDEZ-SANABRIA, A. RIZKY FERDINA, N. BOON AND T. VAN DE WIELE *In vitro* adhesion of oral bacteria to mucosal niche of the intestine is impacted by specific food components	BHe
28	A. CARLIER Leaf Nodule Symbiosis: Endophytes as obligate symbionts	BGen/ec
29	D. CHARLIER, M. NADAL AND P. NGUYEN LE MINH Trigger Enzyme PepA of *E. coli*, a transcriptional repressor that generates positive supercoiling	BBio
30	I. CHRISTOPOULOU, K. ROOSE AND X. SAELENS	V

	Protection against influenza A virus and bacteria co-infection by M2e universal flu vaccine	
31	F. COOLS, E. TORFS, B. VANHOUTTE, D. CAPPOEN, L. BONOFIGLIO, M. MOLLERACH AND P. COS Functional characterization of *S. pneumoniae galU* knockout strains: Could the enzyme UDPG:PP be a novel antimicrobial target?	BHe
32	V. CYRIAQUE, S. JACQUIOD, L. RIBER, W. A. AL-SOUD, S. MILANI, D. C. GILLAN, S. J. SØRENSEN AND R. WATTIEZ Heavy metal accumulation shaped presence and potential activity of sediment bacteria	BGen/ec
33	I. DE BOECK, I. J. J. CLAES, D. VANDENHEUVEL, S. WUYTS, E. OERLEMANS, S. WITTOUCK, O. VANDERVEKEN AND S. LEBEER The microbiota of the upper respiratory tract: potential of niche-specific isolates as probiotic	BHe
34	B. DESGUIN, P. SOUMILLION, P. HOLS AND R. P. HAUSINGER The lactate racemase nickel pincer complex	BBio
35	C. DUMOLIN, P. VANDAMME Seeking for novel "microbials"with industrial potential via the high throughput isolation platform MiCRoP	BGen/ec
36	G. FALONY, M. JOOSSENS, S. VIEIRA-SILVA, J. WANG, Y. DARZI, K. FAUST, A. KURILSHIKOV, M. J. BONDER, M. VALLES-COLOMER, D. VANDEPUTTE, R. Y. TITO, S. CHAFFRON, L. RYMENANS, C. VERSPECHT, L. DE SUTTER, G. LIMA-MENDEZ, K. D'HOE, K. JONCKHEERE, D. HOMOLA, R. GARCIA, E. F. TIGCHELAAR, L. EECKHAUDT, J. FU, L. HENCKAERTS, A. ZHERNAKOVA, C. WIJMENGA AND J. RAES Population-level study identifies main gut microbiome covariates	BHe
37	L. GOETGHEBUER, P. SERVAIS AND I. GEORGE Characterization of the metabolic profile of river bacterial strains in pure and mixed cultures	BGen/ec
38	O. HENRIET, C. MEUNIER, P. HENRY AND J. MAHILLON Phosphorus removal in aerobic granular sludge process: Effect of biomass management on efficiency and bacterial diversity	BGen/ec
39	E. HESS, F. RENZI AND G. R. CORNELIS Implications on host-pathogen interactions of host protein deglycosylation by *Capnocytophaga canimorsus*	BHe
40	J. JÖRISSEN, M. VAN DEN BROECK, I. DE BOECK, S. WUYTS, P. VAN DE HEYNING, V. VAN ROMPAEY, A. BOUDEWYNS, O. VANDERVEKEN AND S. LEBEER Screening for probiotic bacteria protective against chronic otitis media with effusion	BHe
41	M. KADARI, D. LAKHLOUFI, V. DELFORGE, H. KOROGLU, P. SMEESTERS AND A. BOTTEAUX Characterization of the role of Spa33, a component of the type 3 secretion system in *Shigella flexneri*	BBio
42	M. S. KHAN, P. SOUMILLION Does homologous oligomerisation promote protein evolution?	BBio
43	S. KIEKENS, A. SASS AND T. COENYE Discovering the physiological role of small RNAs expressed in *burkholderia cenocepacia* biofilms	BBio
44	A. KOLPE, B. SCHEPENS, L. VANDE GINSTE, W. FIERS AND X. SAELENS	V

	FcγR-mediated phagocytosis by broadly protective influenza A virus IgGs	
45	L. LAHTI, W. M. DE VOS AND J. RAES Challenges and opportunities in large-scale microbiome profiling studies	BHe
46	F. LAUBER, G. R. CORNELIS AND F. RENZI Identification of a new lipoprotein export signal in Gram-negative bacteria.	BBio
47	L. LEMMENS, E. DE KONING AND E. PEETERS Molecular characterization of a GntR-like transcription factor involved in heat stress response in *Sulfolobus acidocaldarius*	BBio
48	E. LIEVENS, T. VERHOEVEN, L. PERSOONS, S. CLAES, G. SCHOOFS, J. VANDERLEYDEN, S. LEBEER, D. SCHOLS AND M.I. PETROVA Unraveling the contribution of vaginal *Lactobacillus* species to the vaginal barrier function and their potential against HSV-2 infection	BHe
49	E. LOBET, N. NINANE, C. DEMAZY, K. WILLEMART, P. RENARD, X. DE BOLLE, M. RAES, J-J. LETESSON AND T. ARNOULD Characterization of the relationship between *B. abortus* and mitochondrial population of infected macrophages and non-myeloid cells	BHe
50	L. MARTINET, M. MACIEJEWSKA, D. ADAM, A. NAÔME, AND S. RIGALI Actinobacterial population hosted by moonmilk deposits: demystifying the genesis of these speleothems and their use in traditional medicine	BGen/ec
51	S. MORETTI AND S. LEBEER Bacterial endotoxins in an urban environment: Are they modulating our immune responses?	BHe
52	M. MYSARA, M. NJIMA, N. LEYS, J. RAES, S. MALHOTRA-KUMAR AND P. MONSIEURS From Reads to Operational Taxonomic Units: an Ensemble Processing Pipeline for MiSeq Amplicon Sequencing Data	BDiv
53	C. NANNAN, T. VANZIELEGHEM, P. MODRIE, J. MAHILLON AND T. CARLETTI Staphylococcus epidermidis adhesion: *in vitro* and *in silico* approaches	BHe
54	A. NAÔMÉ, S. ANDERSSEN, S. JOURDAN, P. TOCQUIN, AND S. RIGALI PREDetector 2.0, online and updated version of the Prokaryotic Regulatory Elements Detector tool	BDiv
55	V. NOLLA-ARDEVOL, L. CLUYTS, K. SIMOENS, B. LAMAZE, S. RAFFESTIN, C. LASSEUR, K. BERNAERTS, D. SPRINGAEL AND I. SMETS Start-up of an acidic-thermophilic anaerobic membrane reactor integrated in the MELiSSA loop	BGen/ec
56	E. OERLEMANS, S. WITTOUCK, S. WUYTS, I. CLAES, G. DONDERS AND S. LEBEER The vaginal microbiome in bacterial vaginosis and aerobic vaginitis	BHe
57	A. PARMENTIER, A. BILLIET, G. SMAGGHE, P. VANDAMME, D. DEFORCE, F. VAN NIEUWERBURGH AND I. MEEUS A correlation between a neogregarine parasite and the microbial community of bumblebees	BGen/ec
58	I. S. PESSI, H. D. LAUGHINGHOUSE IV, D. VELÁZQUEZ, Y. LARA AND A. WILMOTTE The 'cyanobiome' of Svalbard, High Arctic	BGen/ec
59	M. I. PETROVA, N. C. E. IMHOLZ, T. L. A. VERHOEVEN, D. SCHOLS, J. VANDERLEYDEN AND S. LEBEER	BHe

	Lectin-like molecules of *Lactobacillus rhamnosus* GG inhibit pathogenic biofilm formation	
60	PHUONG THI ANH ONG, J.-F. STERNON, J.-J. LETESSON AND X. DE BOLLE Cell cycle control of *Brucella abortus* inside human trophoblast cells and its comparison to murine macrophages RAW 264. 7	BHe
61	K. PONCIN, A. ROBA, J.-J. LETESSON AND X. DE BOLLE Alkylating stress on *Brucella abortus* in culture and in infection	BHe
62	J. PRAET, I. MEEUS, G. SMAGGHE AND P. VANDAMME The gut microbiota of bumblebees: a treasure chest of biodiversity and functionality	BGen/ec
63	R. PROPS, P. MONSIEURS, M. MYSARA, L. CLEMENT, V. J. DENEF AND N. BOON Measuring the biodiversity of microbial communities by flow cytometry	BDiv
64	C. RENS, F. LAVAL, M. DAFFE, O. DENIS, R. FRITA, A. BAULARD, R. WATTIEZ, P. LEFEVRE AND V. FONTAINE Drugs synergy and study of the underlining mechanism through proteomic and lipidomic analysis	BBio
65	C. RIGAUTS, E. VANDEPLASSCHE, T. COENYE AND A. CRABBÉ The effect of members of the cystic fibrosis lung microbiome on the *Pseudomonas aeruginosa*-induced epithelial immune response.	BHe
66	Is complex gut metabolism simulable? S. S. RUBIN	BBio
67	A. M. SASS, S. KIEKENS, H. VAN ACKER AND T. COENYE Small regulatory RNAs in *Burkholderia cenocepacia* biofilms	BHe
68	T. SASSEN, J. MAHONY, R. VAN HOUDT, F. MASTROLEO AND D. VAN SINDEREN Long term stability of MELiSSA strains	BGen/ec
69	W. SMETS, K. WUYTS, E. OERLEMANS, S. WUYTS, S. WITTOUCK, S. DENYS, R. SAMSON AND S. LEBEER Air pollution and plant-microbe interactions in the phyllosphere	BGen/ec
70	S. TAVERNIER, M. TUYSUZ, P. RIGOLE, L. STUER, I. VANDECANDELAERE, G. BRACKMAN, A. CRABBÉ AND T. COENYE Antimicrobial susceptibility of *Streptococcus anginosus* in a multispecies biofilm	BHe
71	A. TOUSSAINT AND F. VAN GIJSEGEM BcepMu/B3-like prophages in proteobacteria and pseudomonads	V
72	H. VAN ACKER, S. KIEKENS, A. SASS, A. WATTY AND T. COENYE Exploring the role of mini-proteins in *Burkholderia cenocepacia* biofilm formation and persistence	BHe
73	W. VAN BEECK, M. VERSLEGERS, S. BAATOUT, M. MYSARA, P. MONSIEURS, N. LEYS, S. LEBEER AND F. MASTROLEO Investigations on the potential radio-protective properties of edible cyanobacterium during pelvic irradiation in a mouse model.	BHe
74	E. VAN DAELE, F.M. KERCKHOF, C. BELZER T. VAN DE WIELE The impact of breastmilk and delivery mode on the infant's gut microbiota	BHe
75	F. VAN DEN DRIESSCHE, B. VANHOUTTE, G. BRACKMAN, A. CRABBE, P. RIGOLE, J. VERCRUYSSE, G. VERSTRAETE, D. CAPPOEN, C. VERVAET, P. COS AND T. COENYE	BHe

	Evaluation of combination therapy for *Burkholderia cenocepacia* lung infection in different *in vitro* and *in vivo* models	
76	D. VANDENHEUVEL, C. ALLONSIUS, G. BROECKX, S. KIEKENS, F. KIEKENS, AND S. LEBEER The role of EPS in the processing of probiotics into pharmabiotic formulations	BHe
77	E. VANDEPLASSCHE, T. COENYE AND A. CRABBÉ Antibiotic susceptibility of biofilms is influenced by interspecies interactions in an artificial CF lung microbiome	BHe
78	M. VAN DER HENST, N. FRANCIS, J.-J. LETESSON AND X. DE BOLLE Study of the chromosomes replication of *Brucella abortus*	BHe
79	J. VANDERMAESEN, A. J. DALY, J. M. BAETENS, B. DE BAETS, N. BOON AND D. SPRINGAEL Unravelling the relationship between indigenous community diversity and success of bioaugmentation using synthetic microbial ecosystems	BGen/ec
80	V. VAN EESBEECK, M. MYSARA, R. PROPS, R. VAN HOUDT, P. PETIT, N. LEYS, J. ARMENGAUD, C. RIVASSEAU, J. MAHILLON AND P. MONSIEURS Microbiological analysis of spent nuclear fuel pools: towards the identification of radiation-resistant bacteria	BGen/ec
81	A. VAN HOUT, T. D'HUYS, M. OEYEN, T. VAN LOY AND D. SCHOLS Evaluation of the anti-CXCR4 activity profile of published GPCR inhibitors	V
82	C. VANMARSENILLE, J. ELSEVIERS, G. G. HASSANZADEH, A. MARTEL, F. HAESEBROUCK, F. PASMANS, J.-P. HERNALSTEENS AND H. DE GREVE Protecting chickens against *Campylobacter jejuni* by Camelidae-derived nanobodies	BHe
83	V. VASSEN, M. DEGHELT, J.-J. LETESSON AND X. DE BOLLE Polar insertion of the bacterial envelope components in *Brucella abortus*	BHe
84	J. WENDLAND Synthetic Biology and Biocontrol with Predator Yeasts: an emerging system	BBio
85	J. WILLE, E. TEIRLINCK, K. BRAECKMANS AND T. COENYE Differences between cell responses to various biofilm dispersion methods in *B. cenocepacia*	BHe
86	S. WUYTS, E. OERLEMANS, I. CLAES, I. DE BOECK, S. MORETTI, S. WECKX, B. LIEVENS, L. DE VUYST AND S. LEBEER Exploration of artisan carrot juice fermentation as alternative probiotic carrier	BHe
87	A. YADAV, P. JANSSEN, N. LEYS AND A. CUYPERS Mechanism of radiation resistance in *Arthrospira* sp. PCC 8005	BGen/ec
88	D. YANG, P. SOUMILLION, S. ZENG, A. WOHLKÖNIG, M. S. KHAN, G. VANDENBUSSCHE AND V. FONTAINE Functional characterization of the TesA of *Mycobacterium bovis BCG*	BBio
89	S. ZENG, C. RENS, D. YANG AND V. FONTAINE The *Mycobacterium bovis* BCG chaperonin 60.1 is not involved in hypoxic dormancy but affects biofilm growth	BBio

Abstracts of participants

Determination of survival and resistance to acidity as probiotic potential of infant and calf faecal Bifidobacteria

A. ABDELMALEK[1], M. HEYNDRICKX[2] AND A. BENSOLTANE[1]

[1]laboratory of food and industrial microbiology laboratory bioremediation and phytoremediation experimental biotoxicology Oran university Algeria; [2]Institute for agriculture and fisheries research (ILVO), Technology and food science Unit Melle , Belgium

Bifidobacteria are an important part of the normal faecal flora and may provide health-promoting benefits to the host. High bifidobacterial counts are especially important in newborns early in infancy (human and animal). The aim of this study is to determine the level of bifidobacteria in infant faeces and calf faeces, and compare the resistance of common *Bifidobacterium* spp.to acidity. Forty samples from twenty breast-fed infants in the age between 5 to 180 days and twenty five samples from ten calves between 5 to 60 days were investigated. Bifidobacteria and other bacterial groups were determined by cultivation on MRS medium and TPY medium. Faecal samples were examined for the activity of fructoso-6-phosphate phosphoketolase (F6PPK) and for other enzymatic reactions using the API-ZYM kit. Nine infants had high numbers of bifidobacteria (usually higher than 9 log CFU/g) in their faeces. Five infants did not contain detectable amounts of bifidobacteria in their faecal samples. The remaining six individuals had low counts of bifidobacteria (3–6 log CFU/g). Most negative infants possessed major amounts of clostridia in their faecal flora. All calves contain high numbers of Bifidobacteria in their faecal samples. Detection of F6PPK, agalactosidase and α-glucosidase activities could routinely be used for the rapid and simple detection of bifidobacteria in all faecal samples. Acid tolerance was determined by introducing Bifidobacterium to pH-adjusted skimmed milk and enumerating during storage at 4°C. The viability of the organism decreased during storage. *Bifidobacterium* spp. isolated from calf faeces showed superior survival abilities compared with their isolates from infant faeces.

Dissection of novel resistance mechanisms in *Cupriavidus metallidurans*

M. M. ALI[1,2], K. MIJNENDONCKX[1] , N. LEYS[1], P. MONSIEURS[1], D. CHARLIER[2] AND R. VAN HOUDT[1]

[1]Unit of Microbiology, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium; [2]Research Group of Microbiology, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussel, Belgium

Cupriavidus metallidurans strains, which belong to the *Burkholderiaceae* family, are characterized by multiple metal resistances and have been mostly isolated from industrial sites linked to mining, metallurgic and chemical industries. Studies at SCK•CEN underscored the rapid evolution of *C. metallidurans* strains towards significantly increased metal resistance. Surprisingly, the canonical mechanisms did not participate in this adaptive evolution. In contrast, a novel and unique resistance mechanism, involving an uncharacterized small periplasmic protein, was discovered.

In this work, we investigated silver resistance. Different *C. metallidurans* strains including type strain CH34, its plasmidless derivative AE104 and strain NA4, which was isolated form the silver-sanitized drinking water of International Space Station (ISS), were grown in toxic concentration of silver to obtain silver-resistant mutants. Since silver-resistant mutants were even found in the plasmidless AE104 strain, the dominant silver-resistant mechanism does not include the canonical efflux mechanisms encoded by the megaplasmids. Whole-genome expression profiling was used to compare the up- and downregulated genes of the silver-resistant mutants with their respective parents. Only eight genes were commonly upregulated in all silver-resistant mutants and no commonly downregulated genes were observed. Deletion mutants were constructed for these genes and observations confirmed that deletion of the two-component system *agrRS* and a *copQ*-like gene coding for a small periplasmic protein render susceptibility to silver. Furthermore, plasmid-based complementation restored resistance to silver. Further investigation is ongoing with the other differentially expressed genes and to validate the DNA-binding properties of AgrR.

Altogether, our data indicate that *C. metallidurans* is able to adapt rapidly to toxic silver concentrations without mediation of its known silver efflux pumps. Although the mechanism that confers increased silver resistance is still not fully understood, these results indicate differential regulation via a two-component regulatory system and the involvement of a family of small periplasmic proteins.

Interaction between exopolysaccharides from *Lactobacillus rhamnosus* GG and *Candida* species

C. N. ALLONSIUS[1], D. VANDENHEUVEL[1], I. CLAES[1], P. DELPUTTE[2] AND S. LEBEER[1]

[1]University of Antwerp, Department of Bioscience Engineering, Research Group Environmental Ecology and Applied Microbiology, Groenenborgerlaan 171, B-2020 Antwerp; [2]University of Antwerp, Department of Biomedical Sciences, Laboratory of Microbiology, Parasitology and Hygiene, Universiteitsplein 1, B-2610 Wilrijk

The health-promoting properties of probiotics can be attributed to different mechanisms, including antipathogenic activities. The exact molecules conferring these activities remain largely unknown. We aim to unravel the role of the long, galactose-rich extracellular polysaccharides (EPS) of the model probiotic strain *Lactobacillus rhamnosus* GG (LGG) in its antipathogenic capacity against *Candida* species.

The effect of LGG wild-type on the growth and adhesion to epithelial cells of *C. albicans* was compared to the effects of its isogenic EPS mutant and isolated EPS. Well-diffusion assays, spot assays, time-course analysis experiments and hyphal induction assays were performed to assess the direct antipathogenic activity. Indirect antipathogenic effects were studied by competition, displacement and exclusion assays with the vaginal epithelial cell line VK2/E6E7.

We found that LGG could inhibit *C. albicans* growth and hyphal formation, but no differences with the EPS-deficient mutant were observed. Isolated EPS molecules from LGG could not inhibit fungal growth, but were able to prolong the lag phase of *C. albicans's* growth. The EPS was also able to reduce hyphal formation of *C. albicans*. Next, we investigated the effect of LGG and the isogenic EPS mutant on adhesion of *C. albicans* to VK2/E6E7 monolayers. While LGG wild-type was able the reduce the adhesion in competition assays with approximately 50%, no effect for the EPS mutant was observed. In exclusion and displacement assays, the effects of LGG and EPS mutant were also similar, with reductions between 25% and 35%. However, the isolated EPS molecules from LGG could also reduce adhesion with 30% in competition assays.

Taken together, these data suggest that LGG and its isolated EPS have potential as antifungal strategy against *C. albicans*.

Orthogonal gene expression: creating 3 promoter libraries linked to different sigma factors which are orthogonal between each other and towards the host

I. BERVOETS[1], M. VAN BREMPT[2], M. DE MEY[2] AND D. CHARLIER[1]

[1]Research group of Microbiology, Vrije Universiteit Brussel, Belgium and [2]Centre of Industrial Biotechnology and Biocatalysis, Universiteit Gent, Belgium

Technological advances in synthetic biology, systems biology, and metabolic engineering have boosted applications of industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate regulatory mechanisms. Hence, fine-tuning expression of synthesis pathways in specific conditions is mandatory together with the decoupling of host systems.

Here we develop an orthogonal expression system in *Escherichia coli* with three heterologous sigma factors that function orthogonal between each other and towards the host organism. Furthermore, for each of these sigma factors, a promoter library will be created that retains this orthogonal feature.

First, several sigma factors originating from *Bacillus subtilis* were tested for their orthogonality in *E. coli* on the level of promoter recognition, by using a red-fluorescent reporter system. Secondly, the potential of sigma factors, and mutants thereof, from *B. subtilis* to work together with the *E. coli* core RNA polymerase was tested. This was accomplished by expressing these proteins together with their promoters; three distinct promoters for each factor. Subsequently, cross talk between the heterologous sigma factors and the promoters was evaluated by examining all combinations. Based on the results three heterologous sigma factors with one of their respective promoters were chosen for further work. For each of these factors a promoter library will be generated that remains orthogonal towards the host and towards the alternative sigma factors. The promoter libraries will be attained by randomising part of the promoter, one strategy will be to randomise regions of 5 nucleotides throughout the promoter while the second strategy will be randomisation of the complete linker sequence. Subsequently the promoter strengths will be measured through sequential rounds of fluorescent-activated cell sorting (FACS) to result in a set of orthogonal promoters for each sigma factor, covering a range of different strengths.

The use of these different orthogonal sigma factors combined with a range of promoter strengths for each factor offers the opportunity to fine-tune expression of different genes in heterologous pathways.

Physiology of *Cupriavidus metallidurans* CH34 in the presence of basalt, a lunar-like rock, on Earth and in Space

B. BYLOOS[1,2], I. CONINX[1], O. VAN HOEY[3], N. NICHOLSON[4], V. ILYIN[5], R. VAN HOUDT[1], C. COCKELL[4], N. BOON[2] AND N. LEYS[1]

[1]Microbiology Unit, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgiu, [2]Center of Microbial Ecology and Technology, University of Ghent, Ghent, Belgium, [3]Research in Dosimetric Applications, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium, [4]UK Centre for Astrobiology, School of Physics and Astronomy, University of Edinburgh, Edinburgh, United Kingdom and [5]Institute of Medical and Biological Problems of Russian Academy of Sciences (IMBP RAS), Moscow, Russia

Microbe-mineral interactions, which have been already applied on Earth, have also become of interest for space exploration as microorganisms could biomine elements from extra-terrestrial materials which could be used in life-support.Therefore, this research is aimed to identify the interaction of bacteria with basalt, a volcanic lunar-like analogue and look at the impact of micro-gravity and space radiation on these interactions.

The physiology and interaction of the soil bacterium *Cupriavidus metallidurans* CH34 in the presence of basalt was analyzed trough growth and survival experiments. Survival of CH34 was monitored, with and without basalt, in mineral water. After 3 months, cell physiology was analyzed (by flow cytometry), as well as cultivability, element release and biofilm formation (by scanning electron microscopy). To study the influence of micro-gravity on these interactions the survival setup was also send as a flight experiment onboard the Russian FOTON M4 capsule, launched in July 2014. For growth, cells were grown in medium with and without essential elements in the presence and absence of basalt and followed up with flow cytometry, plate counting and OD measurements.

CH34 cells survived relatively well in mineral water at ambient conditions for 3 months on ground. Storage in mineral water did have a significant impact on cell physiology and energy status: 61% of cells of the stored cell suspensions had lost their cell membrane potential and only 7% were still active. These stored cells also contained less ATP and more PHB, compared to the cells at the start. These changes impacted cultivability as viable cell counts dropped one log indicating a transition into a more 'dormant' state. Both basalt and flight conditions counteract some of these effects and cells in the presence of basalt exposed to flight conditions showed less changes in physiology, with only 2% of the cells which lost its cell membrane potential and 34% of the cells which were still active leading to a higher cultivability. Microbe-mineral interaction and biofilm formation were slightly influenced by spaceflight as less biofilm was present on the basalt from the flight conditions. For the growth experiments, it was seen that CH34 was able to grow in the presence of basalt and basalt could be a source for essential elements supporting its growth. In contrast, results obtained in another setup (different type of basalt,…) showed significant growth limitation of CH34 in these conditions and additional growth experiments will be performed to look at the impact of different kinds of basalt and setups on growth.

Acknowledgements
This work was supported by the European Space Agency (ESA-PRODEX) and the Belgian Science Policy (Belspo) through the E-GEM/BIOROCK project. We thank Prof. C. Cockell and.Prof. K. Finster for the project coordination.

In vitro adhesion of oral bacteria to mucosal niche of the intestine is impacted by specific food components

M. CALATAYUD, E. HERNANDEZ-SANABRIA, A. RIZKY FERDINA, N. BOON AND T. VAN DE WIELE

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

The oral cavity is the main gateway to the human body, potentially impacting the gut communities due to microbial transfer. The alimentary bolus, composed by chewed food mixed with saliva, arrives in the stomach and intestinal tract, undergoing harsh conditions. As a result, diet-associated microbes or oral commensals may influence the diversity of microbial lineages in the gut. Previous findings suggest that the ingestion of saliva containing large loads of bacteria can influence the microbial ecosystems in the small intestinal [1].

Food is a key modulator of the human gut microbiome. However, little is known about the interplay between food, oral bacteria and host. This research evaluated the effect of food components (oil, meat and sugar) in the survival of oral bacteria after mouth, stomach and small intestine digestion. Further, the effect of food in bacterial adhesion to the mucosal surface of the small intestine was evaluated.

We developed an *in vitro* setup resembling the bacterial transit through the upper gastrointestinal tract and small intestine. Fourteen species representing the oral bacterial community underwent a standardized static *in vitro* digestion in absence or presence of meat, oil or sugar. Controls without oral bacteria were run simultaneously. Samples from oral, stomach and small intestine digestions were obtained for each treatment, diluted in filter sterilized water and stained for live/death cell count by flow cytometry [2].

A co-culture of Caco-2 and HT29-MTX cell lines in a proportion of 90/10 was used to simulate the small intestine, as previously described [3]. Fluid from the small intestinal digestion was diluted in MEM to 10^4 live bacteria/ml and added to the cell monolayer. After 48 hours of incubation, the medium was removed and cell monolayers were washed twice with PBS. The mucus and mucus-adhered bacteria were obtained by N-acetylcysteine treatment [4]. The cellular layer was trypsinzed and vortexed 1 min. Viable/death bacteria were quantified in mucus and cellular samples as described before.

In fasted state, the number of live bacteria after gastric and small intestinal digestion was reduced from 7.6 to 6.7 and 5 log units, respectively. The presence of meat after gastric and small intestinal digestion increased the number of viable bacterial cells to ~ 7 log units ($p < 0.001$), while oil and sugar did not affect bacterial survival as compared when food was not provided (control).

The adhesion of bacteria to the small intestine mucosal surface was significantly increased by the presence of meat (1.53 ± 0.06 log units) compared with the control condition (1.3 ± 0.04 log units, $p < 0.001$).

These results show the relevance of considering the gastrointestinal tract as a continuum without compartmentalizing different segments as independent elements. In addition, the food interaction with oral microbiota could affect the survival and adhesion to the small intestine, thus interplaying with resident microbiome in distal sites. Further research is being performed to evaluate the host-microbiome interaction at small intestinal level and the possible effects on health or disease conditions.

1 ARIMATSU, K. ET AL. SCI REP-UK 4, DOI:ARTN 4828. 10.1038/SREP04828 (2014).
2 VAN NEVEL, ET AL. JOURNAL OF MICROBIOLOGICAL METHODS 94, 73-76 (2013).
3 CALATAYUD, M., ET AL. CHEMICAL RESEARCH IN TOXICOLOGY 25, 2654-2662, (2012).
4 MAHLER, G. J., ET AL.. THE JOURNAL OF NUTRITIONAL BIOCHEMISTRY 20, 494-502 (2009).

The BCCM/ULC collection to conserve the biodiversity and explore the applied potential of Polar cyanobacteria

P. BECKER[1], P. SZTERNFELD[2], M. ANDJELKOVIC[2], R. ANTHONISSEN[3], L. VERSCHAEVE[3], M. HENDRICKX[1], M. RENARD[4], Y. LARA[4], B. DURIEU[4], V. SIMONS[5], H. D. LAUGHINGHOUSE IV[4,6], L. CORNET[7], D. BAURAIN[7] AND A. WILMOTTE[4]

[1]BCCM/IHEM biomedical fungi and yeasts collection, Mycology and Aerobiology Laboratory, Scientific Institute of Public Health, Brussels, Belgium; [2]Chemical Residues and Contaminants Laboratory, Scientific Institute of Public Health, Brussels, Belgium;[3]Toxicology Laboratory, Scientific Institute of Public Health, Brussels, Belgium; [4]BCCM/ULC cyanobacteria collection, Centre for Protein Engineering, University of Liège, Liège, Belgium; [5]BCCM/MUCL environmental and applied mycology, Université catholique de Louvain, Croix du Sud 2, Louvain-la-Neuve, Belgium; [6]Institute for Bioscience & Biotechnology Research – IBBR, Rockville, USA ; [7]Phylogenomics of eukaryotes, Department of Life Sciences, University of Liège, 4000 Liège, Belgium

In the Polar Regions, Cyanobacteria represent key primary producers and are the main drivers of the food webs in a wide range of aquatic to terrestrial habitats. For example, they build benthic microbial mats in lakes and soil crusts in terrestrial biotopes. They may present interesting features to survive freeze/thaw cycles, seasonally contrasted light intensities, high UV radiations, dessication and other stresses.

The BCCM/ULC public collection funded by the Belgian Science Policy Office since 2011 aims to gather a representative portion of the polar cyanobacterial diversity with different ecological origins (limnetic mats, soil crusts, cryoconites, endoliths…). It makes it available for researchers to study the taxonomy, evolution, adaptations to harsh environmental conditions, and genomic make-up. It presently includes 226 cyanobacterial strains, with 119 being of Antarctic origin (catalogue: http://bccm.belspo.be/catalogues/ulc-catalogue-search). An ISO 9001 certificate was obtained for the public deposition and distribution of strains, as part of the multi-site certification for the BCCM consortium.

The morphological identification shows that the strains belong to the orders Synechococcales, Oscillatoriales, Pleurocapsales, Chroococcidiopsidales and Nostocales. The 16S rRNA and ITS sequences of the strains are being characterized. Our results show that the Antarctic strains are positioned into 25 OTUs (sequences with > 97,5% 16S rRNA similarity), and thus, represent a quite large diversity.

In addition, cyanobacteria are known to produce a wide range of secondary metabolites (e.g. alkaloids, cyclic and linear peptides, polyketides) with bioactive potential. Among these bioactive metabolites, some display antibiotic, anticancer or antifungal effects. In collaboration with the BCCM/IHEM collection of biomedical fungi, a screening of cyanobacterial strains from BCCM/ULC was performed in order to discover potential new antifungal drugs. The analysis of a first set of methanol extracts from 15 different strains put in evidence the antifungal activity of a *Phormidium priestleyi* isolate. The latter remains active up to 0.5% (v/v) of fungal culture and was able to inhibit the growth of various fungal species among *Candida*, *Cryptococcus*, *Aspergillus*, and *Penicillium*. The raw extract was subjected to HPLC and a fraction containing the active molecule was obtained. This molecule appeared to be a thermostable hydrophobic compound. Moreover, *in vitro* toxicological analyses suggest that the compound has a general cytotoxic effect that could be inhibited by the mammalian metabolism. Further analyses are needed to identify the molecule and to determine if it could be a candidate for a new antifungal drug.

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

Fishing up rare bacterial proteomes using SWATH-MS in synthetic microbial communities

M. BERAUD[1], G. GIAMBARRESI[1], D. GILLAN[1] AND R. WATTIEZ[1]

[1]*Service de protéomique et de microbiologie, Université de Mons, Belgique*

Within ecosystems, rare bacteria could be a reservoir of genes and functions helping community survival. However functional description of rare bacteria within complex communities using omics methods remains a challenge.

During the present research, we designed a synthetic 9-species community based on a marine community observed in muddy sediments. This community was cultivated in a medium simulating marine conditions and stressed using zinc 0.5 mM. DNA and proteins were then extracted after 28 days to assess taxonomical and functional biodiversity of the community using Quantitative-PCR and proteomics (data-dependent LC-MS/MS; DDA-MS) respectively. The DDA-MS approach failed to catch the proteome for most of the rare species since *Pseudomonas putida* was dominant (more than 60 % as DNA equivalent).

We then used another approach termed SWATH acquisition method (Data-Independent Acquisition-MS) to analyse the proteome of the community. Using SWATH–MS, we successfully fished up the proteins from rare bacteria in the synthetic community. The number of proteins observed for low abundance species was two times more abundant when compared with the DDA-MS approach. The global quantification accuracy was also increased. As a result, we were able to conclude on the effect of metals on rare species in the synthetic community.

SWATH-MS represents a major breakthrough in the field of functional microbiology. It is a promising technique to reach the full functional description of a community.

Acknowledgements
This work is financially supported by Pole d'Attraction Interuniversitaire (PAI) P7/25 MRM (Microbial Resource Management in engineered and natural ecosystems).

Leaf Nodule Symbiosis: Endophytes as obligate symbionts

A. CARLIER

Laboratory of Microbiology, Ghent University, Ghent, Belgium

Bacteria of the genus *Burkholderia* establish an intimate symbiosis with plant species of the *Rubiaceae* and *Primulaceae* families. The bacteria are contained within specialized galls or nodules within the leaves. Because the plants cannot develop beyond the seedling stage without their leaf nodule bacteria, the symbiosis is widely considered obligatory. We sequenced the genomes of leaf nodule symbionts of *Rubiaceae* and *Primulaceae* species to gain insight into the nature of the symbiosis. The genomes of the *Burkholderia* leaf nodule bacteria are heavily eroded with an abundance of IS elements and pseudogenes, similar to many obligate mutualistic or parasitic bacteria. Comparative genomic analyses revealed that secondary metabolism is a key to the symbiosis, with symbionts secreting cytotoxic and insecticidal peptides or cyclitol compounds. Interestingly, genes coding for the synthesis of kirkamide, an insecticidal cyclitol produced by symbionts of *Rubiaceae* species, show evidence of recent, intra-clade horizontal gene transfer. This indicates that substantial gene flow can still occur at the early stages following host-restriction in leaf nodule symbioses, in contrast to animal/bacteria obligate symbioses. Phylogenetic analyses suggest that host switching events and plasmid conjugative transfers could have promoted these HGT. Genomic analysis of leaf nodule symbionts gives, for the first time, new insights in the genome evolution of obligate symbionts in their early stages of the association with plants.

Trigger Enzyme PepA of *E. coli*, a transcriptional repressor that generates positive supercoiling

D. CHARLIER[1], M. NADAL[2] AND P. NGUYEN LE MINH[1]

[1] Research group of Microbiology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium and [2]Institut de Génétique et Microbiologie, Université Paris-Sud, Centre Universitaire d'Orsay, F-91405, Orsay Cedex, France.

Hexameric *E. coli* Leucine-aminopeptidase A (PepA) is a trigger enzyme (COMMICHAU AND STÜLKE, 2008) endowed with catalytic activity and DNA-binding properties prominent in the resolution of ColE1 and pSC101 plasmid multimers (Alén *et al*., 1997) and transcriptional regulation of the *carAB* operon, encoding the unique carbamoylphosphate synthase of *E. coli* (CHARLIER *et al*., 1995). Previous studies by DNase I footprinting and atomic force microscopy (AFM) had both indicated a pronounced deformation of the *carAB* control region upon PepA binding, suggestive of DNA wrapping (CHARLIER et al., 1995, NGUYEN LE MINH *et al*., 2009). On the basis of this observation and previous work, PepA was believed to play a major, although merely architectural role in the formation of the synaptic complexes involved in site-specific DNA recombination and in the elaboration of a higher order regulatory protein-*car*P1 DNA complex.

Here we use single-round *in vitro* transcription and DNA topology assays to further unravel the molecular mechanism of PepA-mediated regulation.

The *in vitro* transcription assays performed with supercoiled template and purified components demonstrate that PepA is a repressor in its own right, which specifically inhibits transcription initiation at *car*P1. Furthermore, DNA topology studies performed on artificial DNA mini-circles by means of various topoisomerases with different substrate specificities demonstrate that PepA binding induces positive supercoiling . Such topological changes may serve as a regulatory mechanism that allows swifter response and multi-layered control of promoter activity in concert with other regulatory components known to be involved in the control of *car*P1.

Acknowledgements

This work was financially supported by the Research Foundation Flanders (FWO-Vlaanderen, grant to DC and postdoctoral fellowship to P. NLM) and the Vlaamse Gemeenschapscommissie.

References

COMMICHAU, F. M. and STÜLKE, J. (2008) *Mol. Microbiol*. 67: 692-702.

ALÉN, C., SHERRATT, D.J. and COLLOMS, S.D. (1997) *EMBO J*. 16: 5188-5197.

CHARLIER, D, GHOLAMREZA HASSANZADEH GH., KHOLTI, A., GIGOT, D., PIÉRARD, A. and GLANSDORFF, N. (1995) *J. Mol. Biol*. 250: 392-406.

NGUYEN LE MINH, P., DEVROEDE, N., MASSANT, J., MAES, D. AND CHARLIER, D. (2009) *Nucleic Acids Res*. 37: 1463-1476.

Protection against influenza A virus and bacteria co-infection by M2e universal flu vaccine

I. CHRISTOPOULOU, K. ROOSE AND X. SAELENS

Department of Biomedical Molecular Biology, Ghent University; Medical Biotechnology Center, VIB, Ghent

Co-infection with *Streptococcus pneumoniae* or *Staphylococcus aureus*, is a frequent disease-exacerbating event during seasonal and pandemic influenza. Influenza can facilitate infection with these bacteria by different mechanisms. Therefore, we hypothesized that influenza vaccine use can reduce the incidence of so called secondary bacterial pneumonia. However, influenza vaccine efficacy is not normally evaluated for such an outcome. Our lab developed a universal influenza A vaccine based on the extracellular domain of matrix protein 2 (M2e). Here we assessed the potential cross-protection, using a mouse model, of M2e immunization against co-infection with *Staphylococcus aureus*.

Female BALB/c mice were immunized twice intraperitoneally with recombinantly produced M2e-virus-like particles (M2e-VLPs) or control VLPs alone. Vaccinated mice were challenged with a sublethal dose of PR8 (H1N1) influenza virus followed by *S. aureus* inoculation seven days later. Mice that were infected only with PR8 virus or *S. aureus* were included as control groups. Mice were monitored for 22 days post viral challenge for mortality and morbidity. Seroconversion was monitored by ELISA, virus and bacterial titers were measured in the lungs by plaque assay and plating out on semi-solid media, respectively. Flow cytometry was used to determine T cell responses in the lungs and spleen.

Vaccination of mice with M2e-VLPs resulted in the induction of M2e-specific serum IgG, and was associated with strongly reduced morbidity and mortality after influenza-*S. aureus* co-infection. M2e-immunized mice also had reduced virus and bacterial loads in the lungs. IFNγ-producing cells were elevated in the M2e-VLPs immunized group compared to control. Mice infected only with bacteria had higher percentages of T helper 17 cells than influenza virus-*S.aureus* co-infected mice, suggesting that influenza virus infection dampens this type of T cell response, which is important for bacterial clearance.

In summary, we showed that vaccination with M2e-VLPs universal vaccine protects against disease and inflammation in an influenza virus-bacteria co-infection model. To our knowledge, this is the first time that protection by an M2e-vaccine is assessed in a dual influenza-bacteria challenge model.

Functional characterization of *S. pneumoniae galU* knockout strains: Could the enzyme UDPG:PP be a novel antimicrobial target?

F. COOLS[1], E. TORFS[1], B. VANHOUTTE[1], D. CAPPOEN[1], L. BONOFIGLIO[2], M. MOLLERACH[2] AND P. COS[1]

[1]Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Belgium and [2]Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina.

Streptococcus pneumoniae has been recognized as one of the major bacterial agents of several infectious mucosal and invasive diseases, such as community acquired pneumonia, otitis media and meningitis. Despite available antibiotic treatments, there is a significant mortality from pneumococcal infections, with rates approaching 40% in susceptible hosts (WHO Publication, 2012). The most important virulence factor of *S. pneumoniae* is its polysaccharide capsule, which prevents opsonization by complement factors, adhesion and macrophage phagocytosis (GENO et al., 2015). The enzyme UTP-glucose-1-phosphate uridylyltransferase (UDPG:PP) is encoded by the g*alU* gene and catalyzes the formation of uridine diphosphate glucose (UDP-Glc) from glucose 1-phosphate (Glc-1P). UDP-Glc is the substrate for the synthesis of UDP-glucuronic acid, and is also required for the interconversion of galactose and glucose by way of the Leloir pathway, making it a key component in the biosynthetic pathway of pneumococcal capsular polysaccharides (FREY, 1996, MOLLERACH et al., 1998). Also in other bacterial species UDPG:PP has a relevant role in virulence, since UDP-Glc is the main glucosyl donor in lipopolysaccharide and capsule biosynthesis. UDPG:PP is widely distributed among animals, plants and other microorganisms but the eukaryotic UDPG:PPs are evolutionary completely unrelated to their prokaryotic counterparts (FLORES-DIAZ et al., 1997). Therefore, UDPG:PP might be a valuable novel target in fighting bacterial diseases.

In this study, several characteristics of 5 different pneumococcal *galU* knockout strains and their non-mutated parent strains were evaluated. Firstly, planktonic and biofilm growth was studied. There was no difference in planktonic growth between parent strains and their respective knockouts, but there were some significant differences in biofilm formation. Because adhesion is the first step towards biofilm formation and pathogenesis, the adhesion to A549 lung epithelial cells was tested. Also, adhesion to RAW246.7 macrophages and macrophage phagocytosis was studied. Our results show that there is an influence of the *galU* gene product, since deletion of the gene makes the bacteria more prone to phagocytosis and increases their adhesion to both epithelial cells and macrophages in most cases. These data give a first indication that UDPG:PP is involved in pathogenesis and thus might be a novel antimicrobial target, but more experiments are needed.

Acknowledgements

All streptococcal strains were a kind gift by Prof. Dr. M. Mollerach, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina.

References

FLORES-DIAZ, M., ALAPE-GIRON, A., PERSSON, B., POLLESELLO, P., MOOS, M., VON EICHEL-STREIBEL, C., THELESAM, M., FLORIN, I. (1997) *J Biol Chem*. 272:23784-23791.

FREY, P.A. (1996) *FASEB J*. 10:461-470.

GENO, K.A., GILBERT, G. L., SING, J. Y., SKOVSTED, I. C., KLUGMAN, K. P., JONES, C., KONRADSEN H.B., NAHM, M. H. (2015) *Clin Microbiol Rev*. 28:871-899.

MOLLERACH, M., LOPEZ, R., GARCIA, E. (1998) *J Exp Med*. 188:2047-2056.

WHO PUBLICATION. (2012) *Vaccine*. 30:4717-4718.

Heavy metal accumulation shaped presence and potential activity of sediment bacteria

V. CYRIAQUE[1], S. JACQUIOD[2], L. RIBER[3], W. A. AL-SOUD[2], S. MILANI[2], D. C. GILLAN[1], S. J. SØRENSEN[2] AND R. WATTIEZ[1]

[1]Proteomics and Microbiology Lab, Research Institute for Biosciences, UMONS, Mons, Belgium; [2]Microbiology section, Department of Biology, University of Copenhagen, Copenhagen, Denmark and [3]Functional genomics section, Department of Biology, University of Copenhagen, Copenhagen, Denmark

Heavy metal contamination of soils and sediments constitutes a serious issue because of biotoxicity and bioaccumulation. Recent methodological improvement of 16S rRNA profiling has improved analytical accuracy and revealed heavy metals as drivers of microbial community structure. For the present study, metal-contaminated river sediments sampled nearby the MetalEurop foundry (Northern France) were compared to unpolluted sediments collected upstream (Férin). The MetalEurop foundry rejected mainly zinc, copper, cadmium and lead directly in the air and the river during a century. DNA and RNA were extracted from sediments and cDNA was synthetized. DNA and cDNA were submitted to Illumina® MiSeq® sequencing (2x250 bp). Results show that 70% of the bacterial reads (DNA and cDNA) were common between the control and the contaminated sediments. This can be explained by the constant influx of bacteria from the upstream river. However, contaminated sediment harboured a significantly different potentially active community (RNA signal). Moreover, while the amount of extracted DNA was lower in contaminated sediments, the richness and Chao-1 indexes were more elevated when compared to Férin. These results suggest that constant input of bacteria is complemented with metal selection uncovering a broader spectrum of the community. Bacterial tolerance ranges were characterized using the macro-ecological concept of Functional Response Group by clustering OTUs based on their cDNA/DNA abundance patterns, revealing 6 groups that highlight the taxonomic richness of the contaminated sediments.

The microbiota of the upper respiratory tract: potential of niche-specific isolates as probiotic

I. DE BOECK[1], I. J. J. CLAES[1], D. VANDENHEUVEL[1], S. WUYTS[1], E. OERLEMANS[1], S. WITTOUCK[1], O. VANDERVEKEN[2,3] AND S. LEBEER[1]

[1]University of Antwerp, Department of Bioscience Engineering, Research group Environmental Ecology and Applied Microbiology, Antwerp, Belgium; [2]University of Antwerp, Faculty of Medicine and Health sciences, translational neurosciences, Antwerp, Belgium;[3] Antwerp University Hospital, ENT, Head and Neck Surgery and Communication disorders, Edegem, Belgium

Upper respiratory tract infections (URTI) have a major impact on public health and account for half of all prescribed antibiotics in many countries. Antibiotics have a large impact on the global issue of antibiotic resistance, and also disturb our normal microbiota that plays an important role for human health. Despite the high use of antibiotics, the pathogenic role for bacteria in several URT diseases remains unclear. The current understanding indicates that URT diseases are often polymicrobial whereby the healthy URT microbiota is disturbed. To better elucidate the contribution of the microbiota in URT diseases and to design more targeted microbial approaches, a better understanding of the composition and diversity of the 'healthy URT microbiota' is necessary.

In this study, MiSeq (Illumina) 16S *rRNA* gene amplicon sequencing is used to characterize the URT microbiome of subjects without URT symptoms (healthy URT). In addition, selective cultivation methods are used to isolate beneficial microbes from the nasopharynx of healthy individuals and explore their potential as URT probiotics.

The nose and nasopharynx of URT-healthy subjects showed to be often dominated by opportunistic pathogens, such as *Staphylococcus* species. The fact that opportunistic pathogens can also be dominant in healthy subjects indicates the importance of host- and microbiome factors in preventing these potential pathogens to cause disease. To identify such microbiome factors, we cultivated beneficial microbes from the healthy URT. Currently, 8 *Lactobacillus* isolates are screened for their potential as URT probiotics. Our results show that all isolates show antipathogenic effects against *Staphylococcus aureus*, an opportunistic pathogen often associated with URTI. Furthermore, adhesion assays with Calu3 (epithelial cells, ATCC® HTB-55™) cells show that these isolates are able to bind to these airway epithelial cells to different extent. Increased adherence can be postulated to increase their potential to interact with the host and exert probiotic effects. Screening of the immunomodulatory effects of the isolates is currently going on. In conclusion, we isolated beneficial microbes from the healthy URT which show potential to be used as URT probiotics.

The lactate racemase nickel pincer complex

B. DESGUIN[1,2], P. SOUMILLION[2], P. HOLS[2] AND R. P. HAUSINGER[1]

[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA; [2]Institute of Life Sciences, Université catholique de Louvain, B-1348 Louvain-La-Neuve, BELGIUM

sLactate racemase (Lar) catalyze the inversion of stereochemistry of lactate, giving the organism the ability to use or produce both isomers (L- and D-lactate) indifferently. Lactic acid racemization is involved in lactate metabolism and cell wall assembly of many microorganisms. We show that the racemase is a nickel-dependent enzyme with a novel α/β fold which requires three accessory proteins (LarB, LarC, and LarE) for its activation.[1] Lar requires nickel, but the nickel-binding site and the role of the accessory proteins remain enigmatic.

We combined mass spectrometry and x-ray crystallography to show that Lar from *Lactobacillus plantarum* possesses an organometallic nickel-containing prosthetic group. A nicotinic acid mononucleotide derivative is tethered to Lys184 and forms a tridentate pincer complex that coordinates nickel through one metal-carbon and two metal-sulfur bonds, with His200 as another ligand. Although similar complexes have been previously synthesized, there was no prior evidence for the existence of pincer cofactors in enzymes.[2]

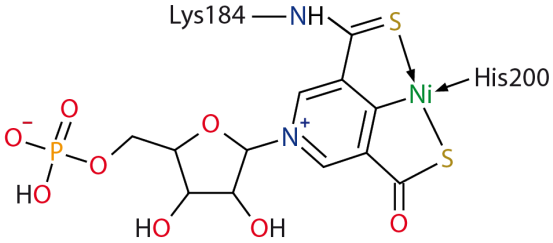


Figure 1. Structure of the lactate racemase cofactor: the pyridinium-3-thioamide-5-thiocarboxylic acid mononucleotide Ni pincer.

Synthesis of the enzyme-bound cofactor requires LarB, LarC, and LarE which are widely distributed in microorganisms. The functions of the accessory proteins are unknown, but the LarB C-terminus resembles PurE carboxylase/mutase, LarC binds Ni and could act in Ni delivery or storage, and LarE is a putative ATP-utilizing enzyme of the PP-loop superfamily. Here we show that LarB carboxylates the pyridinium ring of nicotinic acid adenine dinucleotide (NaAD) and cleaves the phosphoanhydride bond to release AMP. The resulting biscarboxylic acid intermediate is transformed into a bisthiocarboxylic acid species by two single-turnover reactions in which sacrificial desulfurization of LarE converts its conserved Cys176 into dehydroalanine. Our results identify a new metabolic pathway from NaAD using unprecedented carboxylase and sulfur transferase reactions to form the organic component of the (SCS)Ni(II) pincer cofactor of LarA. In species where *larA* is absent, this pathway could be used to generate a pincer complex in other enzymes.[3]

References

1. B. DESGUIN *et al. Nat. Commun.* (2014), 5, 3615.
2. B. DESGUIN *et al. Science.* (2015), 349, 66.
3. B. DESGUIN *et al. Proc. Natl. Acad. Sci. U. S. A.* (2016), Epub 2016 Apr 25.

Seeking for novel "microbials"with industrial potential via the high throughput isolation platform MiCRoP

C. DUMOLIN AND P. VANDAMME

Laboratory of Microbiology UGent, Universiteit Gent, Belgium

Microorganisms are the most diverse group of life present on earth and are ubiquitous. Traditionally this diversity was studied via standard microbial plating techniques. The development of culture independent technologies led to a revolution in microbial diversity insights. It has become clear that in many biological samples the majority of microbial cells (up to 99%) cannot 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). However, standard plating techniques are not yet fully deployed to reach the maximum diversity possible. For example, simply altering culturing conditions still reveals new microbial diversity (CONNON AND GIOVANNONI, 2002; JANSSEN *et al.*, 2002). 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 de-replication into operational isolation units (OIUs) of the obtained isolates is performed via MALDI-TOF MS and up to 750 isolates can be processed in a single week. The obtained OIUs can be further identified, functionally characterized and preserved by using this pipeline. In a proof of concept study, 1500 isolates from a highly enriched HOB reactor were de-replicated using numerical comparison and clustering of MALDI-TOF MS spectra in BioNumerics7 or by means of an in house developed computational program, MOIDePus, and yielded a set of 46 unique OIUs. We now aim to determine the cultivable microbial diversity in a forest soil sample by analyzing some 500 isolates from each of >100 different growth conditions.

Population-level study identifies main gut microbiome covariates

G. FALONY[1,2], M. JOOSSENS[1,2,3], S. VIEIRA-SILVA[1,2], J. WANG[1,2], Y. DARZI[1,2,3], K. FAUST[1,2,3], A. KURILSHIKOV[4,5], M. J. BONDER[6], M. VALLES-COLOMER[1,2], D. VANDEPUTTE[1,2,3], R. Y. TITO[1,2,3], S. CHAFFRON[1,2,3], L. RYMENANS[1,2,3], C. VERSPECHT[1,2], L. DE SUTTER[1,2,3], G. LIMA-MENDEZ[1,2], K. D'HOE[1,2,3], K. JONCKHEERE[2,3], D. HOMOLA[2,3], R. GARCIA[2,3], E. F. TIGCHELAAR[6,7], L. EECKHAUDT[2,3], J. FU[6,8], L. HENCKAERTS[1,9], A. ZHERNAKOVA[6,7], C. WIJMENGA[6] AND J. RAES[1,2,3]

[1]KU Leuven–University of Leuven, Department of Microbiology and Immunology, Leuven, Belgium; [2]VIB, Center for the Biology of Disease, Leuven, Belgium; [3]Vrije Universiteit Brussel, Faculty of Sciences and Bioengineering Sciences, Microbiology Unit, Brussels, Belgium; [4]Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia; [5]Novosibirsk State University, Novosibirsk, Russia; [6]University of Groningen, University Medical Center Groningen, Department of Genetics, 9700 RB Groningen, Netherlands; [7]Top Institute Food and Nutrition, Wageningen, Netherlands; [8]University of Groningen, University Medical Center Groningen, Department of Pediatrics, 9700 RB Groningen, Netherlands; [9]KU Leuven–University Hospitals Leuven, Department of General Internal Medicine, Leuven, Belgium

For the benefit of future clinical studies, it is critical to establish what constitutes a "normal" gut microbiome, if it exists at all. To identify and characterize major microbiome-associated variables, the Flemish Gut Flora Project (FGFP) initiated a large-scale cross-sectional fecal sampling effort in Flanders, Belgium. FGFP collection protocols combined rigorous sampling logistics, including frozen sample collection and cold chain monitoring, with exhaustive phenotyping through online questionnaires, standardized anamnesis and health assessment by general medical practitioners (GPs), and extended clinical blood profiling.

Based on microbiome phylogenetic profiling of 1106 individuals through 16S ribosomal RNA (rRNA) gene amplicon sequencing, sixty-nine clinical and questionnaire-based covariates were found associated to microbiota compositional variation. Despite differences in study population and sample analysis, 24 matching covariates were found to be significantly associated with microbiome composition in the Dutch LifeLines-DEEP study (LLDeep; N = 1135), leading to an overall replication success rate of 92%. All covariates identified correlated with alpha-diversity measures and individual taxa abundances. However, the predictive power of these linear covariate-based models was limited, as they only explained 1.50 to 14.74% of genus abundance variation. This suggests the influence of additional, currently unknown covariates as well as intrinsic microbial ecological processes such as founder effects, species interactions, and dynamics. Stool consistency, as measured by self-assessed Bristol stool scale (BSS) score, emerged as the top feature covarying with fecal microbiome composition. Moreover we confirmed previously reported associations of stool consistency with microbiota richness, prevalence of Prevotella-enterotyped samples, and *Akkermansia* and *Methanobrevibacter* abundances.

Overall, we show that some of the medical conditions targeted by fecal microbiota research have much smaller microbiome effect sizes than commonly assumed. However, some of the covariates that we identified (such as BSS and medication) are currently largely ignored and should be taken into account in future clinical studies. The results of this study form a solid basis for the development of microbiome research as a clinical and diagnostic field.

Characterization of the metabolic profile of river bacterial strains in pure and mixed cultures

L. GOETGHEBUER, P. SERVAIS AND I. GEORGE

Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles

Even though river and stream waters represent only 0.006% of global freshwater resources, they are essential for human life and largely exploited. In a context of diminishing access to clean resources, there is an increasing necessity to maintain water quality by a sustainable management of water bodies. Microbial communities play a key role in water purification. They are primary drivers, with archaea, of biogenic element cycles and ecosystem processes. However these communities remain largely uncharacterized, and the link between lotic bacterial diversity and performance (defined here as the conversion of organic carbon into new microbial biomass) has not been studied. In order to understand the diversity-performance relationship, we built a synthetic community composed of 20 'typical' freshwater bacterial species isolated from the Zenne River (Brussels, Belgium). The carbon source utilization profile of each strain was compared in Biolog Phenotype MicroArrays PM1 and PM2A microplates that allowed to test 190 C-sources. Although isolated on the same culture medium (R2A), all strains showed different carbon utilization profiles. The (dis)similarity between profiles was not related to the phylogenetic (dis)similarity between strains. The lack of functional redundancy was further illustrated in experiments where the 20 strains were mixed in even to very uneven communities and grown in R2B medium: in all conditions, mixed communities performed better (showed higher final bacterial biomass) than pure strains. We will further analyze the temporal dynamics of individual strains in these mixed communities to identify possible interspecies relationships.

Phosphorus removal in aerobic granular sludge process: Effect of biomass management on efficiency and bacterial diversity

O. HENRIET[1], C. MEUNIER[2], P. HENRY[2] AND J. MAHILLON[1]

[1]Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, Louvain-la-Neuve, Belgium; [2]CEBEDEAU, Research and expertise center for water, Allée de la découverte, 11 (B53), Quartier Polytech 1, B-4000 Liège, Belgium

Aerobic Granular Sludge (AGS) represents an innovative strategy to treat wastewater. The granules are formed by microbial consortia enclosed in a self-produced exopolymeric matrix. The advantages of this process over activated sludge are excellent settling abilities and high biomass retention. The biological phosphorus removal in AGS reactors is performed by bacteria known as Polyphosphate Accumulating Organisms (PAO).

In this research, a new methodology of PAO enrichment was explored. First, subgroups of PAO-rich granules were identified by performing isopycnic centrifugation on granular sludge (density-based separation) combined with quantitative PCR and 16S amplicon sequencing. Second, the abundance of these granules was increased through non-segregating microbial management.

The first granules were observed after 45 days. The formation of granules coincided with a sharp decrease of the SVI_5. An important variability in phosphorus removal was observed and frequent peaks with P concentration higher than 25 mg/L were recorded in the effluent. The high-throughput sequencing approach revealed that 16S rRNA gene frequency of *Candidatus* Accumulibacter, a major PAO in AGS processes, was dominant in dense granules where it reached 45.1%. A qPCR analysis revealed that these granules contained 6.8 log copies of PAO-specific 16S rRNA gene per ng of DNA. On the opposite, the lighter granules contained only 4.9 log copies per ng of DNA.

Two different strategies of biomass management were applied. The first strategy involved a high selective pressure (short settling time) to select fast settling granules. It resulted in a decrease of the average phosphorus removal to ca. 18%. The P content in these granules was high (102 mg P/g dry suspended solids) which suggested that the biomass was probably close to saturation in polyphosphate. The second strategy was designed to reduce the selective pressure before performing a homogeneous purge of the solids. Hence, this procedure removed not only very slow settling (wash-out with the effluent) but also fast settling granules (removed from the settling bed). Quickly after the start of this procedure, P removal improved and stabilized over 90% until the end of the experiment. The P content of the biomass was 84 ± 5 mg P/g dry suspended solids, which suggested a better turnover of PAO biomass. This is likely to be due to the hold of dense granule seeds that were not yet fully saturated in polyphosphate. Overall, this study offers a new approach of biomass management in AGS process.

Implications on host-pathogen interactions of host protein deglycosylation by *Capnocytophaga canimorsus*

E. HESS, F. RENZI AND G. R. CORNELIS

Unité de Recherche en Biologie des Micro-organismes, Université de Namur, Belgium

Capnocytophaga canimorsus, a member of the Bacteroidetes phylum, is a dog oral commensal causing rare but extremely severe infections in humans. *C. canimorsus* is feeding on the sugar moieties from host N-linked glycoproteins. Protein deglycosylation is achieved by the glycoprotein deglycosylation (Gpd) complex (RENZI *et al*., 2011). This complex is encoded by a polysaccharide utilization locus (PUL), a hallmark of Bacteroidetes. The aim of this work was to understand the implications of deglycosylation on *C. canimorsus* interactions with host cells.

We show that our type strain *Cc5* adheres to epithelial, endothelial and immune cells and adhesion is mainly mediated by the Gpd complex. *Cc5* adheres to host membrane glycoproteins as inhibition of protein glycosylation in HEK293 cells led to a decreased adhesion. Removal of serum, leaving cell glycoproteins as the only feeding source, increased adhesion. If a glycoprotein was added to the serum-depleted medium, adhesion decreased. We conclude that adhesion to host cells is linked to feeding through the Gpd complex.

Cc5 feeds on J774 macrophages membrane glycoproteins (MALLY *et al*., 2008) and is partially resistant to phagocytosis (SHIN *et al*., 2009). We show that deglycosylation of J774 did not impair phagocytosis. Phagocytosis ratio of WT and Gpd mutant was similar but bacteria lacking the Gpd complex adhered less to cells resulting in fewer bacteria taken up. Deglycosylation of IgG by the streptococcal enzyme EndoS inhibits opsonophagocytosis (COLIN *et al*., 2002). Opsonizing IgG bound to WT but not to mutant bacteria were deglycosylated but when incubated with J774 WT *Cc5* were not more protected. Yet in a simplified model, deglycosylation by *Cc5* of anti-D IgG prevented the uptake of opsonized red blood cells by THP-1 cells.

In conclusion, the Gpd complex promoted adhesion to host cells but deglycosylation of phagocytes or IgG was not found to be beneficial for bacteria.

References

RENZI F., MANFREDI P., MALLY M., MOES S., JENO P., and CORNELIS G. R., (2011) *PLoS Pathog*. 7:e1002118.
MALLY M., SHIN W., PAROZ C., LANDMANN R., and CORNELIS G. R., (2008) *PLoS Pathog*. 4:e1000164.
SHIN H., MALLY M., MEYER S., FLETCHER C., PAROZ C., ZAEHRINGER U., and CORNELIS G. R., (2009) *Infect. Immun*. 77:2262-2271.
COLIN M., SVENSSON M. D., SJOHOLM A. G., JENSENIUS J. C., SJOBRING U., and OLSEN A., (2002) *Infect. Immun*. 70:6646-6651.

Screening for probiotic bacteria protective against chronic otitis media with effusion

J. JÖRISSEN[1], M. VAN DEN BROECK[1], I. DE BOECK[1], S. WUYTS[1], P. VAN DE HEYNING[2,3], V. VAN ROMPAEY[2,3], A. BOUDEWYNS[2,3], O. VANDERVEKEN[2,3] AND S. LEBEER[1]

[1]University of Antwerp, Department of Bioscience Engineering, Research Group Environmental Ecology and Applied Microbiology, Antwerp, Belgium; [2]University of Antwerp, Faculty of Medicine and Health Sciences, Translational Neurosciences, Antwerp, Belgium; [3]Antwerp University Hospital, Department of Otorhinolaryngology, Head and Neck Surgery, Edegem, Belgium

Otitis media with effusion (OME) is a common childhood disease characterized by accumulation of fluid in the middle ear space due to Eustachian tube dysfunction. When this condition lasts for more than 3 months and is associated with hearing loss, surgical treatment by placement of ventilation tubes may be considered. In these patients, a disequilibrium of the upper respiratory tract (URT) microbiota with overgrowth of some pathobionts, i.e. bacteria that are present in the healthy microbiota, but that can cause disease under certain circumstances, may contribute to the condition.

We hypothesize that the URT microbiota can be modulated to a health-protective state through inoculation of probiotic bacteria able to grow and establish in this niche.

To test this hypothesis, we aim to compare the microbiota of the middle ear cavity, nasopharynx and adenoids of children suffering from chronic OME and receiving ventilation tubes, and children without OME scheduled for surgery that gives access to the healthy middle ear space (e.g. cochlear implant recipients).

All samples undergo 16S rRNA gene amplicon sequencing (MiSeq), which is currently optimized for these low-biomass and highly mucoid samples. In addition, we will isolate bacteria from the healthy participants and test their probiotic potential against *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* which are commonly associated with otitis media. Also, our in-house collection of lactic acid bacteria originating from different habitats is screened against these pathobionts.

The long-term aim of this study is the identification and isolation of bacteria protective against OME and other common URT diseases and their development into probiotic food or medicinal products.

Acknowledgements

This work is part of and financed through the IWT-SBO project ProCure (coordinator S. Lebeer)

Characterization of the role of Spa33, a component of the type 3 secretion system in *Shigella flexneri*

M. KADARI, D. LAKHLOUFI, V. DELFORGE, H. KOROGLU, P. SMEESTERS AND A. BOTTEAUX

Laboratory of Molecular Bacteriology, Faculty of Medicine, Free University of Brussels, Belgium (ULB)

Shigella flexneri, a gram-negative bacterium, is the causative agent of the shigellosis or bacillary dysentery in humans. Shigellosis is an invasive disease of the colonic epithelium caused by a severe inflammatory reaction and subsequent mucosal destruction (SANSONETTI, 2001). The invasion and dissemination in epithelial cells of *Shigella* is mainly dependent of a type 3 secretion system (T3SS) which mediates translocation of virulence proteins into host cells (CORNELIS, 2000).

Type 3 secretion apparatus (T3SA) are composed of three major parts: an extracellular portion (the needle), a basal body and a cytoplasmic bulb (C-ring) (BLOCKER, 2001). After cell contact, proteins (called translocators) are secreted to form a pore (translocation pore) in the host cell membrane (BLOCKER, 1999; VEENENDAAL, 2007). This pore serves as a gate for secreted virulence proteins (called effectors) to gain access to host cell cytoplasm. The mechanism underlying T3SS activation by host cell contact is still misunderstood but implicates the transmission of a signal from the tip of the needle to the base, resulting in the secretion of cytoplasmic protein (MxiC), which serves as an internal plug before cell contact (BOTTEAUX, 2009, MARTINEZ-ARGUDO, 2010).

Spa33 (33-kDa) has been identified as an essential C-ring component of *Shigella* T3SS since the *spa33* mutant (Δ*spa33*) is unable to form needle and to secrete any proteins (MORITA-ISHIHARA, 2006). In the present study, we have identified an alternative translation initiation site (GTG) inside the*spa33* gene (encoding a valine at position 192) leading to the expression of a short C-terminal fragment (12-kDa), called here $Spa33^{C}$. To determine the role of $Spa33^{C}$ in T3SS, we have mutated the valine 192 ($Spa33^{V192A}$) and found that the resulting strain, lacking $Spa33^{C}$, does not secrete any proteins *in vitro.* Nevertheless, the introduction of $Spa33^{C}$ in this strain restores secretion of translocators but not that of effectors. The blocking of effectors was shown to be a consequence of the secretion defect of MxiC since inactivation of the *mxiC* gene in this strain rescues the effectors secretion. Our results suggest that Spa33 is implicated in the signal transmission leading to the secretion of MxiC. Moreover, we have shown that MxiC interacts with $Spa33^{C}$ strengthening our model in which Spa33 and MxiC act together to control the type 3 secretion after host cell contact.

References:

BLOCKER, A. *et al.* (2001) *MolMicrobiol*. 39(3):652-63.
BLOCKER, A. *et al.* (1999) *J Cell Biol.* 147(3):683-93.
BOTTEAUX, A. *et al.* (2010*) MolMicrobiol*. 71(2):449-60.
CHERRADI, Y. *et al.* (2016) *MolMicrobiol*.87(6):1183-99.
CORNELIS, G.R. AND VAN GIJSEGEM, F. (2000) *Annu Rev Microbiol.* 54:735-74.
MARTINEZ-ARGUDO, I. AND BLOCKER, A.J. (2010) *MolMicrobiol.* 78(6):1365-78.
MORITA-ISHIHARA, T. *et al.* (2006) *J Biol Chem.* 281(1):599-607.
SANSONETTI, P.J. (2001) *Am J PhysiolGastrointest Liver Physiol.* 280 (3):G319-23.
VEENENDAAL, A.K. *et al.* (2007) *MolMicrobiol.63* (6):1719-30.

Does homologous oligomerisation promote protein evolution?

M. S. KHAN[1, 2], P. SOUMILLION[1]

[1]*Institute of Life Sciences (ISV), Group of Biochemistry, Biophysics and Genetics of Microorganisms (BBGM), Université catholique de Louvain (UCL), Louvain la Neuve Belgium;* [2] *Faculty of Biological Sciences, University of Dhaka (DU), Dhaka 1000, Bangladesh*

In this project, the potential role of homologous oligomerization on the evolutionary origins of proteins will be studied by simulating an evolutionary scenario starting by the duplication of a gene encoding the D-malate dehydrogenase (DmlA) of *Escherichia coli.* We will study the potential of the system to evolve towards isocitrate dehydrogenase (IDH) or isopropylmalate dehydrogenase (IPMDH) activity with the directed evolution approach in monogenic and digenic scenarios in an engineered strain of *E. coli*, B3 (HB101Δ*idh::kan(Km^r)*Δ*ipmdh* Δ*dmlA*). Two catalytic mutant versions of DmlA (D224A and D248A) are constructed in compatible vectors and introduced (single vector or both vectors) in the B3 strain which is not able to grow in a media when only D malate is supplied as sole carbon source. In monogenic system, only inactive homodimeric DmlA is produced but in digenic system, heterodimers (D248A-D224'A) with one active site restored due to random assembly of DmlA subunits allow B3 to grow on D-malate. About 20 % of the proteins form heteromers in *vivo* as depicted from the lysate activity compared with the wild type DmlA. Purified heterodimer is 4.5 fold less active (k_{cat} $0.80s^{-1}$) and less stable than wild type DmlA. To demonstrate the advantage of heteromerization and chaperone effect, the DmlA mutant library will be produced by error prone PCR and will be introduced in B3 strain either alone or together with the wild type and colonies will be selected for IDH or IPMDH activity. Higher frequency of clones in digeneic scenario will support that the heteromerization is favoured and there is chaperone effect involved to stabilize the new protein complex. Next, both plasmid and phagemid library of DmlA will be introduced in B3 either alone or both libraries together. We are expecting higher frequency of colonies in digenic scenario referring to the combinatorial advantages of oligomerization.

Discovering the physiological role of small RNAs expressed in *burkholderia cenocepacia* biofilms

S. KIEKENS, A. SASS AND T. COENYE

Laboratory of Pharmaceutical Microbiology, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium

Burkholderia cenocepacia is an opportunistic respiratory pathogen able to infect immunocompromised patients including cystic fibrosis patients. It is highly transmissible among the CF community and infection of CF patients may lead to the development of 'cepacia syndrome', characterized by a rapid decline in lung function and often leading to a fatal necrotizing pneumonia. Infections with *B. cenocepacia* are difficult to treat, which can be due to the high innate resistance of the bacteria against antimicrobial agents and their ability to form biofilms.

In the present study we identified 60 putative small RNAs expressed in *B. cenocepacia* strain J2315 biofilms by differential RNA sequencing. We performed qPCR and northern blotting to confirm the expression and the size of 11 of these sRNAs. To investigate the biological role of one of these, nc35, deletion and overexpression mutants were constructed. We tested biofilm formation and carbon source utilisation in these mutants and exposed them to different stress factors, including oxidative stress and envelope stress and exposed them to different classes of antimicrobial substances.

No difference in biofilm mass or activity was found by using techniques such as crystal violet staining and Cell-Titer blue viability assay, but biofilm morphology was different from that of the wild type. We also exposed planktonic cells to different classes of antimicrobial substances to examine whether the sRNA plays a role in resistance and membrane stability. The nc35 deletion mutant is more susceptible to ceftazidime, meropenem, minocycline and tobramycin. Sensitivity to oxidative stress was measured by exposing biofilm grown cells to hydrogen peroxide or sodium hypochlorite; the biofilm of the Δnc35 mutant was found to be more sensitive to sodium hypochlorite than the wild type.

We identified and confirmed the expression of 11 small RNAs in *B. cenocepacia* strain J2315. A different phenotype of the Δnc35 mutant, compared to the wild type, was observed after exposure to sodium hypochlorite and to antimicrobial substances. Further research will give us a better insight in the specific role of this sRNA and its possible targets.

FcγR-mediated phagocytosis by broadly protective influenza A virus IgGs

A. KOLPE[1,2], B. SCHEPENS[1,2], L. VANDE GINSTE[1,2], W. FIERS[1,2] AND X. SAELENS[1,2]

[1]Medical Biotechnology Center, VIB, Ghent, B-9052, Belgium; [2]Department of Biomedical Molecular Biology, Ghent University, Ghent, B-9052, Belgium

Worldwide influenza epidemics result in substantial morbidity and the deaths of 250,000 – 500,000 people annually, with the young and elderly representing the majority of this mortality. Vaccination is the most effective method to prevent infection, but influenza vaccines must be reformulated annually because of antigenic drift in HA, the immunogenic glycoprotein to which the majority of the humoral anti-influenza immune response is directed. Neutralizing antibodies against influenza viruses have traditionally been thought to provide protection by interfering with virus entry and/or fusion with host cells, events that can rely solely on antibody variable region binding to viral spike proteins. The *in vivo* mechanism of antibody-based inhibition of virus replication is more complex and involves not only Fab recognition of viral epitopes but also Fc interactions with the FcγR system.

We have previously developed a flu vaccine candidate that is based on the conserved ectodomain of matrix protein 2 (M2e) of influenza A virus (IAV). We have demonstrated that influenza M2e-specific IgGs require activating FcgRs for protection. In order to understand FcγR-mediated phagocytosis by broadly protective IAV non neutralizing IgGs, we have developed an *in vitro* assay in which IAV infected cells or M2-expressing epithelial cells can be phagocytosed by immortalized macrophages in the presence of M2e specific IgG2a mAbs or M2e convalescent immune sera from mice. FACS and confocal analysis revealed that phagocytosis of IAV infected or M2-expressing HEK-293T by macrophages is significantly enhanced in the presence of M2e specific IgG2a mAb. Anti M2e antibodies can initiate antibody mediated phagocytosis of target cells that are infected with different IAV subtypes by immortalized macrophages. In conclusion, our results show that M2e-specific antibodies confer heterologous protection and act through antibody-dependent cell-mediated phagocytosis.

Challenges and opportunities in large-scale microbiome profiling studies

L. LAHTI[1,2,3], W. M. DE VOS[2] AND J. RAES[3]

[1]Department of Mathematics and Statistics, University of Turku, Finland; [2]Laboratory of Microbiology, Wageningen University, The Netherlands; [3]VIB11 vzw Center for the Biology of Disease, KU Leuven, Belgium

Our body is a home for diverse microbial communities that have a profound impact on our physiology and well-being. Whereas the composition and function of these microbial communities have been studied extensively, we have only a limited understanding of the individuality and temporal dynamics governing this complex ecosystem. The available longitudinal time series remain limited to relatively small numbers of individuals or time points. Together with the remarkable individual variation this sets challenges for analysis.

The recent accumulation of large-scale population cohorts is opening new opportunities for microbiome profiling studies. Combining cross-sectional observations across large populations can help to uncover population-level variation that extends beyond the temporal dynamics within individuals (FAUST *et al*. Curr. Op. Microbiol. 15:56-66, 2015). Hence, large-scale population cohorts have a key role in understanding human microbiome assembly and function. I will discuss recent findings based on the Flemish Gut Flora Project (VIB/KU Leuven, Belgium) and the HITChip Atlas of the human intestinal microbiota (Wageningen University, The Netherlands). These uniquely large and standardized data resources for studying the intestinal microbiota cover altogether over 10,000 intestinal samples across 5000 individuals and a thousand species-like bacterial phylotypes, together with comprehensive background information on the research subjects.

The analyses shed new light on the remarkable inter-individual variability of the microbial fingerprints (FALONY *et al*. Science 352:560-564, 2016). In particular, the findings indicate specific subpopulations within the intestinal microbiota that exhibit contrasting, stable configurations of low and high abundance, with associations to host physiology and health (LAHTI *et al*. Nat. Comm. 5:4344, 2014). Such bi-stable sub-communities appear robust to dietary interventions (O'KEEFE *et al*. Nat. Comm. 6:6342, 2015) and exhibit notable differences in their stability and contributions to the overall community composition. Host factors such as age can, however, affect the resilience of the alternative states and move the system towards a tipping point of an abrupt switch between the contrasting states, with potential implications for microbiome manipulation and personalized treatments.

Establishing links between community composition, stability and host health presents a fundamental challenge for microbiome studies. I discuss the resources, opportunities and challenges encountered in large-scale microbiome profiling studies based on recent collaboration with partners from Belgium (VIB/KU Leuven), The Netherlands (Wageningen University) and Finland (University of Turku & Helsinki).

Identification of a new lipoprotein export signal in Gram-negative bacteria.

F. LAUBER, G. R. CORNELIS AND F. RENZI

Unité de Recherche en Biologie des Microorganismes (URBM), Département de Biologie, Université de Namur, Namur, Belgium

Bacteria of the phylum Bacteroidetes, including commensals and opportunistic pathogens, harbor abundant surface-exposed multi-protein membrane complexes (Sus-like systems) involved in carbohydrate acquisition. These complexes have been mostly linked to commensalism and in some instances they have also been shown to play a role in pathogenesis. Sus-like systems are mainly composed of lipoproteins anchored to the outer membrane and facing the external milieu. This lipoprotein localization is uncommon in most studied Gram-negative bacteria while it is widespread in Bacteroidetes. Little is known on how these complexes assemble and in particular on how lipoproteins reach the bacterial surface. Here, by bioinformatic analyses, we identify a lipoprotein export signal (LES) at the N-terminus of surface-exposed lipoproteins of the human pathogen *Capnocytophaga canimorsus* corresponding to K-$(D/E)_2$ or Q-A-$(D/E)_2$. We show that, when introduced in sialidase SiaC, an intracellular lipoprotein, this signal is sufficient to target the protein to the cell surface. Mutational analysis of the LES in this reporter system showed that the amino acid composition, the position of the signal sequence and the global charge are critical for lipoprotein surface transport. These findings were further confirmed by the analysis of the LES of mucinase MucG, a naturally surface exposed *C. canimorsus* lipoprotein. Furthermore, we identify a LES in *Bacteroides fragilis* and *Flavobacterium johnsoniae* surface lipoproteins that allow *C. canimorsus* surface protein exposure, thus suggesting that Bacteroidetes share a common new bacterial lipoprotein export pathway that flips lipoproteins across the outer membrane.

Molecular characterization of a GntR-like transcription factor involved in heat stress response in *Sulfolobus acidocaldarius*

L. LEMMENS, E. DE KONING AND E. PEETERS

Department of Bioengineering Sciences, research group of Microbiology. Vrije Universiteit Brussel, Brussels, Belgium

Archaea, commonly referred to as "the third domain of life", are characterized by several extremophilic species, living for example in hot springs and salt lakes. However they are also found in mainstream habitats. *Sulfolobus acidocaldarius* is an aerobic hyperthermoacidophilic crenarchaeon isolated from solfataras, growing optimally at 78°C and pH 2-3. The transcriptional machinery of *Sulfolobus* is, as in all Archaea, a simple version of the eukaryotic RNA polymerase II transcription machinery, while the transcription factors on the contrary are bacteria-like. The characterization of the mode of action of transcriptional regulation in this organism could lead to a better understanding of archaeal physiology and to an extended transcriptional toolbox for gene expression engineering of *Sulfolobus.* Living in a dynamic ecosystem with certain highly disturbed environmental conditions, such as temperature, *Sulfolobus* is subjected to high levels of stress. An adequate gene regulation in response to these stress conditions is crucial for the microbial fitness and survival. This work focuses on the characterization of a GntR-like transcription factor, encoded by *Saci_1851*, involved in regulation of heat shock response.

Saci_1851 belongs to the YtrA subfamily of the GntR transcription factors. *Saci_1851* is encoded in an operon with a membrane protein, as most bacterial members of the YtrA subfamily, and its promotor region contains the typical YtrA recognition motif GTNNNTANNNNNTANNNCA, located 15 bp upstream the transcription start of the gene. The *Saci_1851*-encoding gene shows a 34-fold upregulation after a heat shock, in which the temperature was increased from 75°C tot 90°C, as demonstrated by qRT-PCR analysis. The largest difference in expression is observed 5 minutes after temperature shift. The Saci_1851 protein was recombinantly purified followed by the analysis of *in vitro* protein-DNA binding with a DNA probe representing the 100-bp upstream sequence with respect to the operon encoding its own gene and the membrane protein. Upon the addition of 10 nM to 150 nM Saci_1851 protein one specific protein-DNA complex was observed. When the protein concentration was increased up to 250-1000 nM, multiple non-specific complexes are formed. An in gel footprint shows protection of a 15 bp semi palindromic region covering the transcriptional start.

Based on these results we propose that *saci_1851* is a transcription factor, possibly involved in heat shock response, that performs an autoregulation and that regulates a putative membrane protein, which is possibly involved in the physiological response to heat shock stress.

Unraveling the contribution of vaginal *Lactobacillus* species to the vaginal barrier function and their potential against HSV-2 infection

E. LIEVENS[1,2,3], T. VERHOEVEN[1], L. PERSOONS[2], S. CLAES[2], G. SCHOOFS[2], J. VANDERLEYDEN[1], S. LEBEER[1,3], D. SCHOLS[2] AND M.I. PETROVA[1,3]

[1] KU Leuven, Centre for Microbial and Plant Genetics, Heverlee, Belgium; [2] KU Leuven, Rega institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium; [3] University of Antwerp, Department of Bioscience Engineering, Antwerp, Belgium.

Although the vaginal microbiota of most healthy women is dominated by *Lactobacillus* species, the mechanisms by which they contribute to vaginal health are poorly described. A better molecular understanding of lactobacilli adaptation to the vaginal niche and their role in the protection against various pathogens could be provided by studying host-*Lactobacillus* and *Lactobacillus*-pathogen interactions respectively.

To unravel the role of lactobacilli in the vaginal barrier function, their adhesion capacity to and influence on the immune response of vaginal epithelial VK2/E6E7 cells was investigated. Their adhesion showed to be strain-specific and a high adhesion capacity was correlated with a strong auto-aggregation phenotype. A pro-inflammatory cytokine response of VK2/E6E7 cells was induced by *L. reuteri* RC-14 and *L. plantarum* CMPG5300 as determined by increased expression of IL8/TNF and TNF respectively. Moreover, TNF induction in VK2/E6E7 cells by *L. plantarum* CMPG5300 was confirmed by a dedicated cytokine PCR array and an upregulated IL-6 expression was also observed. The role of TLR2 in this induced cytokine profile is currently under investigation as well as the capacity of *L. plantarum* CMPG5300 to induce a stronger antiviral immune response following viral challenge. The array results also indicate a decreased expression of Fas ligand suggesting a downregulation of Fas-induced apoptosis in VK2/E6E7 cells.

Lactobacilli could also contribute to the vaginal barrier by (in)directly competing with pathogens such as herpes simplex virus type 2 (HSV-2). The potential co-aggregation of *Lactobacillus* and HSV-2, which could prevent binding to VEC, was investigated by using a specifically designed infection assay. A significant lower infection was detected for 3 out of 10 *Lactobacillus* strains, which cannot be attributed to lactic acid, soluble factors or acidic pH. However, as flow cytometric analysis could not validate this co-aggregation, it is hypothesized that HSV-2 could be captured within *Lactobacillus* auto-aggregates as a result of which the virus cannot reach its target cells.

Characterization of the relationship between *B. abortus* and mitochondrial population of infected macrophages and non-myeloid cells

E. LOBET[1], N. NINANE[1], C. DEMAZY[1], K. WILLEMART[2], P. RENARD[1], X. DE BOLLE[2], M. RAES[1], J-J. LETESSON[2] AND T. ARNOULD[1]

[1]Unité de Recherche en Biologie Cellulaire - Narilis, Unamur; [2]Unité de Recherche en Biologie des Microorganismes - UNamur

Brucella species are Gram-negative facultative intracellular bacteria responsible for a worldwide zoonotic disease known as brucellosis. *Brucella*, once in its host cells, traffics inside a vacuole called BCV (*Brucella* Containing Vacuole) that is able to interact with several components of the endosomal pathway. Afterwards, *Brucella* reaches a "permissive niche" sharing some properties and markers with the endoplasmic reticulum and in which bacteria multiply massively. This massive replication is susceptible to expose some organelles to stress or alter their functions during infection.

As the role of mitochondria in innate immune response is now recognized, we hypothesized that mitochondria might be affected and/or play a role during *Brucella* infection, intracellular trafficking and/or virulence. To our knowledge, the effect of *Brucella* on mitochondrial population of infected cells has never been systematically studied, but few indirect arguments do exist to support a putative effect of *Brucella* on the organelle such as a transcriptomic study performed on *Brucella* infected RAW264.7 macrophages showing that different nuclear genes encoding mitochondrial proteins are down-regulated after 4 h post-infection (HE *et al.*, Infect Immun, 2006) or a proteomic study suggesting that there is an physical interaction between BCVs and mitochondria (FUGIER *et al.*, PLOS Pathog, 2009).

Among the different mitochondrial features we analysed, we showed that the mitochondrial morphology is affected in infected cells. Indeed, we observed a strong fragmentation of the mitochondrial network both in HeLa and RAW264.7 cells at 48 hours post-infection.

We are currently studying the effectors of the mitochondrial fission and fusion in those cells to determine how this fragmentation occurs as well as the effect of an alteration of the mitochondrial morphology on *Brucella* replication. We are also interested in analysing what could be the effect of this mitochondrial fragmentation on host cell physiology during infection such as their susceptibility to encountered stresses (e.g. apoptosis inducers).

In conclusion, this work should contribute to a better understanding of the putative crosstalk that exists between *Brucella* infection and mitochondrial function, especially during its trafficking in both myeloid and non myeloid cells. Altogether, the results of this fundamental research should highlight new molecular mechanisms involved in *Brucella* infection.

Actinobacterial population hosted by moonmilk deposits: demystifying the genesis of these speleothems and their use in traditional medicine

L. MARTINET[1], M. MACIEJEWSKA[1], D. ADAM[1], A. NAÔME[1], AND S. RIGALI[1]

InBioS, Centre for Protein Engineering, University of Liège, B-4000 Liège, Belgium

Moonmilk speleothems of limestone caves are mainly composed of fine calcium carbonate crystals with different textures ranging from pasty to hard. Filamentous actinobacteria are believed to actively participate in the formation of these carbonate deposits although their real input in the genesis of moonmilk is difficult to estimate which often leads to controversial hypotheses to explain their origin and the mechanisms (biotic versus abiotic) involved. Interestingly, ancient medical texts and common believes reported that moonmilk had therapeutical properties, thereby suggesting that its filamentous endemic actinobacterial population might be a source of natural products useful in treatment of human disease. The purposes of our work is to investigate the microbial population hosted by moonmilk deposits in order to i) understand how they might play a role in the genesis of these speleothems, ii) identify the active substances present in moonmilk deposits which could explain their use in traditional therapies, and iii) isolate actinobacteria from different moonmilk deposits in order to identify the compounds with antimicrobial properties they are able to produce. Overall, the project aims to unravel the role of actinobacteria in the genesis of cave carbonate deposits, and demystify the therapeutic properties of moonmilk with the hope of finding new compounds active against multi-drug resistant microorganisms.

Bacterial endotoxins in an urban environment: Are they modulating our immune responses?

S. MORETTI[1] AND S. LEBEER[1]

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

Our lungs represent one of the largest interfaces between the human host and the external environment. Although not visible by the naked eye, the air is teeming with bacteria. In an urban environment, bacterial endotoxins (derived from the outer membrane of Gram negative bacteria) may associate with pollutants such as particulate matter (PM). This co-stimulation of our immune system by endotoxins and PM may play a crucial role in inflammatory respiratory disease development. In literature, the study of urban, ambient endotoxins has very likely been underestimated due to shortcomings in collection and quantification strategies.

Here we present an impinger-based sampling strategy which is more targeted for microbial sampling and endotoxin recovery, with a quantification method (recombinant Factor C assay) less likely to be influenced by the numerous other PM components. Subsequently, 87 outdoor air samples were collected within the city of Antwerp from 11 monitoring sites representing urban traffic, urban green, and industrial areas. Our combined approach yielded substantially higher endotoxin concentrations (ranging from 0.45-93 EU/m^3) than studies applying filter-based methods. With this increased recovery, we investigated the pro-inflammatory capacity of the samples *in vitro* using the human macrophage-like cell line U937 by monitoring gene expression using RT qPCR. In contrast to urban green locations, urban traffic and industrial areas showed a heightened and varied immune response for IL-8, TNFα, and IL-1β. Interestingly, these markers showed similar responses for both of the urban green samplings sites: one located opposite the traffic intense ring of Antwerp, and the other geographically removed from the city centre and with low traffic intensity. This may suggest a possible health benefit of green areas, even within busy urban areas. Furthermore, despite urban traffic and green areas showing similar endotoxin concentrations, positive correlations between endotoxin concentration and Il-1β were observed in the traffic and not the green locations. These possible synergistic effects are currently being investigated, with the interaction between endotoxins and traffic-related transition metals being the key-focus.

From Reads to Operational Taxonomic Units: an Ensemble Processing Pipeline for MiSeq Amplicon Sequencing Data

M. MYSARA[1], M. NJIMA[2], N. LEYS[2], J. RAES[3,4,5], S. MALHOTRA-KUMAR[1] AND P. MONSIEURS[2]

[1]Laboratory of Medical Microbiology, Vaccine & infectious Disease Institute, University of Antwerp, Wilrijk, Belgium; [2]Unit of Microbiology, Belgian Nuclear Research Centre (SCK-CEN), Mol, Belgium; [3]Department of Bioscience Engineering, Vrije Universiteit Brussel, Brussels, Belgium; [4]VIB Center for the Biology of Disease, VIB, Leuven, Belgium; [5]Department of Microbiology and Immunology, REGA institute, KU Leuven, Belgium

The development of high-throughput sequencing technologies has provided microbial ecologists with an efficient approach to assess bacterial diversity at an unseen depth, particularly with the recent advances in the Illumina MiSeq sequencing platform. However, analysing such high-throughput data is posing important computational challenges, requiring specialized bioinformatics solutions at different stages during the processing pipeline, such as assembly of paired-end reads, chimera removal, correction of sequencing errors and clustering of those sequences into Operational Taxonomic Units (OTUs). Individual algorithms grappling with each of those challenges have been combined into various bioinformatics pipelines, such as mothur, QIIME, LotuS and USEARCH. Using a set of well-described bacterial mock communities, state-of-the-art pipelines for Illumina MiSeq amplicon sequencing data are benchmarked at the level of the amount of sequences retained, computational cost, error rate and quality of the OTUs. In addition, a new pipeline called OCToPUS is introduced, which is making an optimal combination of different algorithms. Noticeable variability is observed between the different pipelines in respect to the monitored performance parameters, where in general the amount of retained reads is found to be inversely proportional to the quality of the reads. By contrast, OCToPUS achieves the lowest error rate, minimum number of spurious OTUs, and the closest correspondence to the existing community, while retaining the uppermost number of reads when compared to other pipelines. The newly introduced pipeline translates Illumina MiSeq amplicon sequencing data into high-quality and reliable OTUs, with improved performance and accuracy compared to the existing pipelines.

Staphylococcus epidermidis adhesion: *in vitro* and *in silico* approaches

C. NANNAN[1], T. VANZIELEGHEM[1], P. MODRIE[1], J. MAHILLON[1] AND T. CARLETTI[2]

[1]Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, Louvain-la-Neuve, Belgium; [2]Departmement of Mathematics and Namur Center for Complex Systems – naXys, University of Namur, Namur, Belgium

Staphylococcus epidermidis is now recognized as a leading cause of nosocomial infections associated to indwelling medical devices such as implants, central venous catheters or artificial heart valves. Its virulence potential is directly linked to its abilities to adhere to the surface of the biomaterial and to form a biofilm. These microbial communities display a high resistance capacity against conventional antibiotic treatments and host immune defences so that the removal and the replacement of the infected device are usually required to treat the infection.

This research aimed to study the adhesion of *S. epidermidis*, the first step in the formation of these infectious biofilms. Two complementary approaches have been developed: an *in vitro* flow cell chamber and an *in silico* model that represents the reality within the flow cell.

The adhesion of six *S. epidermidis* strains in the flow cell was studied on a surface of polydimethylsiloxane (PDMS) that was either uncoated or coated with tryptic soy broth (TSB), bovine serum adult 10% or fibrinogen 100mg/ml. The results showed that the bacterial adhesion is promoted on uncoated PDMS compared to the surfaces coated with proteins. Besides, an increase of the adhering bacteria number was observed from the inlet to the outlet of the flow cell, regardless of the strain or the surface used.

The second heading of this research consisted in a model development in order to better understand the dynamics of the bacterial adhesion under flow conditions. This modelling process translated the trends of adhesion for six *S. epidermidis* strains on the four distinct surfaces, in terms of two probabilities of adhesion. Indeed, when a bacterium touches the surface of the flow cell, it can adhere instantaneously with a probability of direct adhesion p_d or not adhere with a probability $1\text{-}p_d$. In this case, the liquid carries the bacterium towards the outlet of the flow cell; this, in turn, gives the bacterium another chance to adhere on the surface, with a probability of rolling adhesion, p_{roll}. These parameters have been combined in a reliable and statistically robust model which has been validated for the reality experimentally observed.

PREDetector 2.0, online and updated version of the Prokaryotic Regulatory Elements Detector tool

A. NAÔMÉ[1], S. ANDERSSEN[1], S. JOURDAN[1], P. TOCQUIN[2], AND S. RIGALI[1]

[1]*InBioS, Centre for Protein Engineering, University of Liège, B-4000 Liège, Belgium*; [2] *InBioS, Laboratory of Plant Physiology, University of Liège, Chemin de la Vallée 4, B-4000 Liège, Belgium*

Transcription factors (TF) control gene expression by interacting with DNA sequences - the *cis* elements or TF-binding sites (TFBS) – which interfere in positive or negative ways with the RNA polymerase. The genome-wide distribution map of the *cis* elements of a TF provides an estimation of the spectrum - wide or narrow - of the targeted processes. At a broader scale, unveiling the *cis-trans* regulatory networks is key to understanding the molecular mechanisms that control elicitor-dependent and proper spatio-temporal gene transcription which could help understanding the behavior, the developmental and physiological capabilities of microorganisms. Reduced costs associated with genome sequencing projects together with available collections of known TFBS in specialized databases made the computational prediction of regulons prior to any other genome-wide investigation popular in domains related to system biology.

PREDetector 2.0 is the online version of a software aimed at identifying putative TFBSs on bacterial genomes. The philosophy of version 1.0 of the software is maintained that is, to propose a tool where users can freely fix the DNA-motif screening parameters, and provide a statistical means to estimate the reliability of the prediction. The most remarkable improvement in this new version resides in the novel functionality that allows real time modification of the screening parameters (threshold score, genomic region investigated, …) which automatically updates the list of identified putative TFBSs. This new version also includes the following additional options: (i) access to genome sequences from different databases, (ii) access to weight matrices from public repositories, (iii) visualization of the predicted hits in their genomic context, (iv) grouping of hits identified in the same region, (v) possibility to store the performed jobs, and (vi) automated result reporting in various formats. PREDetector 2.0 is available at http://predetector.fsc.ulg.ac.be/.

Demonstration on how to use our software will be performed on demand.

Start-up of an acidic-thermophilic anaerobic membrane reactor integrated in the MELiSSA loop

V. NOLLA-ARDEVOL[1], L. CLUYTS[2], K. SIMOENS[2], B. LAMAZE[3], S. RAFFESTIN[3], C. LASSEUR[3], K. BERNAERTS[2], D. SPRINGAEL[1] AND I. SMETS[2]

[1]Department of Earth and Environmental Sciences, Division of Soil and Water Management; KU Leuven; Kasteelpark Arenberg 20, 3001 Leuven, Belgium; [2]Chemical Engineering Department, Division of Bio- & chemical systems Technology, Reactor Engineering and Safety, KU Leuven, Celestijnenlaan 200F, 3001 Leuven, Belgium; [3]ESA-ESTEC, Keplerlaan 1, 2201 AZ Noordwijk, The Netherlands

Human presence in space is currently limited to the Low Earth Orbit, and the necessary consumables such as food, water and air are regularly resupplied. However, in long term space missions nutrient, carbon and water recycling is crucial for life support. The Micro-Ecological Life Support System Alternative (MELiSSA) project is a research line by the European Space Agency (ESA), which aims to gain knowledge for the development of regenerative life support systems for long term space missions. MELiSSA provides a model system for the study and development of a closed life support systems targeting complete recycling based on the combined activity of different living organisms (GODIA, 2002). MELiSSA is a loop-system, comprising four main compartments (C1 to C4) and the crew. The C1 compartment, in which the solid waste is liquefied by thermophilic anaerobic bacteria and which produces ammonium, volatile fatty acids (VFA's), CO_2 and minerals, has proven its potential efficiency. The robustness of the MELiSSA loop relies on mathematical models which can only be implemented through a deep knowledge of each sub-system (composition, behavior, metabolisms, kinetics, inhibition, etc…). This project aims at a thorough description of the C1 compartment regarding the identification of the microbial community and its functionality based on metagenomic, metatranscriptomics and metaproteomics in order to pinpoint several markers that can be used in mathematical modelling and for control purposes of the C1 system.

In the present work, we introduce the preliminary results on the start-up of the C1 compartment. A 25L anaerobic membrane reactor was operated at thermophilic (55ºC) and acidic conditions (pH 5.5) after having been inoculated with sludge from the MELiSSA Pilot Plant (Universitat Autònoma de Barcelona) and fed with simulated human waste (1/4th person waste containing non-edible parts of plants (mimicked by lettuce, red beet and straw), feces and toilet paper). After a two week adaption process, microbial activity in the membrane reactor has gradually increased. The latter has been observed by a solubilization of the supplied organic matter and by a gradual increase in the VFA concentration profile in which, acetate and butyric acid are predominant. Due to a high buffering capacity of the reactor's medium, the acidification of the medium, which was expected to indicate bacterial activity, is masked. This observation indicates that during this first phase of the start-up process, the bacterial biomass is still low and more time is required to achieve a significant bacterial biomass volume. As the reactor is operated under acidic conditions, which inhibits methanogenesis, no methane gas was measured in the reactor's headspace. On-line detection of CO_2 gas in the headspace was limited due to interference with water but its concentration has been gradually increasing. In addition to the constant monitoring of the process through the measurement of physicochemical parameters such as VFAs and CO_2, samples for DNA, RNA and proteomics from the start-up period have been stored and are currently being analyzed. The results obtained from this analysis will be used to identify potential molecular markers in order to develop a mathematical model for the predictive control of the MELiSSA loop.

Acknowledgements

This work is supported by the ESA contracts 4000104470/11/NL/JC and 4000113144/14/NL/PA. Francesc Gòdia and his team of the Universitat Autònoma de Barcelona and Olivier Gerbi from Sherpa Engineering are greatly acknowledged for the inoculum and the technical support, respectively. The scientific responsibility is assumed by its authors.

References

GODIA, F., ALBIOL, J., MONTESINOS, J. L., PEREZ, J., CREUS, N., CABELLO, F., MONTRAS, A., MASOT, A., AND LASSEUR, C.H. (2002). J. Biotechnol. 99, 319–330.

The vaginal microbiome in bacterial vaginosis and aerobic vaginitis

E. OERLEMANS[1], S. WITTOUCK[1], S. WUYTS[1], I. CLAES[1], G. DONDERS[2] AND S. LEBEER[1]

[1]University of Antwerp, department of Bioscience Engineering, research group Environmental Ecology and Microbiology, Antwerp, Belgium; [2]University hospital of Antwerp, department of Gynaecology and Obstetrics, Antwerp, Belgium

Like many other niches in and on the human body, the vaginal tract is inhabited by numerous micro-organisms. In most women, the healthy vaginal microbial community is characterized by a low complexity (i.e. limited number of abundant species) and often a dominance of *Lactobacillus* species with an apparent protective role. In some medical conditions, this community is disturbed, and an increase in abundance of other bacterial families and yeasts and a decrease in abundance of lactobacilli can be observed. An important example is bacterial vaginosis (BV), a common condition with important complications for human reproduction and quality of life, although not all patients exhibit symptoms. The microbiome in BV, as studied by both culture and next-generation sequencing (NGS), is characterized by an overgrowth of mainly anaerobic bacteria such as *Gardnerella vaginalis, Prevotella* spp., *Atopobium vaginae* and *Mobiluncus* species. In contrast to BV, aerobic vaginitis (AV) has only been described fairly recently. This condition is not yet well recognized. AV and BV show some similar symptoms, such as a foul-smelling vaginal discharge, but are distinct conditions with different vaginal bacterial communities. The bacterial community of AV was –until now- not yet studied by NGS, but culture and microscopy suggest a depletion of lactobacilli and an increased presence of *Streptococcus agalactiae, Staphylococcus aureus*, *Escherichia coli* and Enterococci.

Here, we characterized the vaginal microbiome of 52 women, including healthy subjects and patients suffering from AV, BV or *Candida* infection. Three cc of sterile saline was used for cervicovaginal lavage, after which the fluid was used for DNA extraction. The extracted bacterial DNA was used for *16S rRNA* amplicon sequencing of the V4 region using Illumina Miseq to determine the bacterial community. The obtained data were analysed using the dada2 bioinformatic pipeline. Our results indicate a more diverse community and an increase in alpha-diversity compared to the normal *Lactobacillus*-dominated microbiota in BV but not in AV. Furthermore, a distinct *Gardnerella* OTU was found in the BV samples, different from the *Gardnerella* OTU in AV or a normal *Lactobacillus*-dominated microbiota. The AV samples tested so far (n = 10) showed a lot of heterogeneity, indicating the need to increase sample numbers. Also, it illustrates the need for complementary approaches including techniques accounting for absolute numbers of OTUs and live microbes. Ultimately, we hope that more knowledge about microbiome-specific profiles of both AV and BV will help clinicians in the search for more targeted treatments.

A correlation between a neogregarine parasite and the microbial community of bumblebees

A. PARMENTIER[1], A. BILLIET[1], G. SMAGGHE[1], P. VANDAMME[2], D. DEFORCE[3], F. VAN NIEUWERBURGH[3] AND I. MEEUS[1]

[1]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium; [2]Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, Belgium; [3]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

Bumblebees are important pollinators in temperate and cold regions of the world. They pollinate a range of wild and agricultural flowering plants, and contribute to the plant diversity and human food supply. Hereby bumblebees provide valuable ecosystem services.

The characterized gut microbial communities of bumblebees and honeybees have a highly specialized but species-poor community. These gut bacterial communities revealed related functions to nutrition, pathogen defense and immune response, showing their important role in the host's health status. To better understand these host-microbe interactions, the dependency of the environmental context needs to be considered, especially the presence of parasitic species.

Both the gut and fat body microbial communities of foraging bumblebees *(Bombus terrestris*) originating from two locations were analyzed, using the 16S ribosomal amplicon sequencing with the Illumina technology. Also the relation with the neogregarine parasite *Apicystis bombi* was explored. *A. bombi* infection disturbs the microbial association network, as positive correlations between the Operational Taxonomic Units (OTUs) residing in the gut and fat body increase from 0.18% to 0.69%. Also the OTU identified as Arsenophonus *sp.*, which has a location dependent interaction with *A. bombi*, is a possible candidate to influence the bee health in collaboration with *A. bombi*.

The 'cyanobiome' of Svalbard, High Arctic

I. S. PESSI[1], H. D. LAUGHINGHOUSE IV[1,2], D. VELÁZQUEZ[1,3], Y. LARA[1] AND A. WILMOTTE[1]

[1]Centre for Protein Engineering, University of Liège, Liège, Belgium, [2]Department of Arctic Biology, The University Centre in Svalbard, Longyearbyen, Norway and [3]Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Spain

Over the last decades, the Arctic has experienced a warming trend that is nearly twice as high as the global average, a phenomenon known as 'Arctic amplification'. Milder temperatures have lead to the decline of snow and ice coverage and have increased the rates of river discharge, permafrost thawing and glacier retreat. The impact of warmer temperatures on Arctic ecosystems is still unclear. Cyanobacteria are the key primary producers in freshwater and terrestrial Arctic ecosystems, being ubiquitously found in coastal and inland lakes, meltwater streams, cryoconite holes and soils. In these generally simple ecosystems, they are the driver for numerous ecological functions, such as forming the base of the food web, fixation of atmospheric nitrogen, carbon sequestration and biomass accumulation. For a better understanding of the impacts of climate change on Arctic ecosystems, baseline knowledge on cyanobacterial diversity and distribution is crucial. Here we investigate, for the first time, the biogeographic patterns of cyanobacterial communities across the Svalbard archipelago (High Arctic, 74–81°N), using 454 pyrosequencing of partial 16S rRNA gene sequences. A total of 72 microbial mats and biocrusts samples were collected from 8 different locations. After bioinformatics analyses, 481 cyanobacterial OTUs (97.5% similarity threshold) were obtained. Pseudanabaenales was the most OTU-rich order, followed by Chroococcales, Oscillatoriales, Nostocales, Synechococcales and Gloeobacterales. Community structure at this taxonomic level was fairly homogeneous across the different locations, and no trend was observed at the phylotype level as well. These results suggest that cyanobacterial communities do not display any biogeographic pattern within Svalbard. Nevertheless, when compared to data obtained for similar Antarctic samples, a clear separation in community structure was observed. Arctic communities had generally higher phylotype richness than the Antarctic ones (42 ± 22 and 21 ± 11 OTUs, respectively), and multivariate analyses clearly discriminated between the two Polar Regions. Within Svalbard, a group of 21–35 OTUs represented the 'core cyanobiome', i.e. were found in more than half of the samples. These were mainly related to filamentous taxa such as *Microcoleus*, *Leptolyngbya* and *Pseudanabaena*, but phylotypes affiliated with the nitrogen-fixing *Nostoc* were also found. Further phylogenetic and biogeographic analyses are currently being carried out, in order to give additional insights into the global distribution and potential ecological functions of this Arctic cyanobiome.

Lectin-like molecules of *Lactobacillus rhamnosus* GG inhibit pathogenic biofilm formation

M. I. PETROVA[1,2], N. C. E. IMHOLZ[1], T. L. A. VERHOEVEN[1], D. SCHOLS[1], J. VANDERLEYDEN[1] AND S. LEBEER[2]

[1]KU Leuven, Belgium; [2]Antwerpen University, Belgium

Increased antibiotic resistance has catalyzed the research on new antibacterial molecules and alternative strategies, such as the application of beneficial bacteria. Since lectin molecules have unique sugar-recognizing capacities, and pathogens are often decorated with sugars that affect their survival and infectivity, we explored whether lectins from the probiotic strain *Lactobacillus rhamnosus* GG have antipathogenic properties.

The genome sequence of *L. rhamnosus* GG was screened for the presence of lectin-like proteins. Two genes, *LGG_RS02780* and *LGG_RS02750,* encoding for polypeptides with an *N*-terminal conserved L-type lectin domain were detected and designated Llp1 (lectin-like protein 1) and Llp2. The capacity of Llp1 and Llp2 to inhibit biofilm formation of various pathogens was investigated. Sugar specificity was determined by Sepharose beads assays and glycan array screening.

The isolated lectin domains of Llp1 and Llp2 possess pronounced inhibitory activity against biofilm formation by various pathogens, including clinical *Salmonella* species and uropathogenic *E. coli,* with Llp2 being more active than Llp1. In addition, sugar binding assays with Llp1 and Llp2 indicate specificity for complex glycans. Both proteins are also involved in the adhesion capacity of *L. rhamnosus* GG to gastrointestinal and vaginal epithelial cells.

In conclusion, lectins isolated from or expressed by beneficial lactobacilli could be considered as promising bio-active ingredients for improved prophylaxis of urogenital and gastrointestinal infections.

Cell cycle control of *Brucella abortus* inside human trophoblast cells and its comparison to murine macrophages RAW 264. 7

PHUONG THI ANH ONG, J.-F. STERNON, J.-J. LETESSON AND X. DE BOLLE

Research Unit in Biology of Microorganisms – University of Namur, Belgium

The intracellular *Brucella* pathogens cause a zoonosis named Brucellosis all over the world. 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 or murine RAW 264.7 macrophages. ANDERSON *et al*. (1986) showed that trophoblasts of a pregnant goat contained numerous *B. abortus* at 5 days post-inoculation, demonstrating that trophoblasts are a natural site for *B. abortus* proliferation. Our results in human trophoblast cell line JEG-3 revealed that more than 60% of intracellular *B. abortus* started their growth early during infection (2 hours PI), contrasting with the results of 80% of bacteria that arrested their growth within HeLa cells or RAW 264.7 macrophages. Furthermore, a minority of *B. abortus* started to generate the daughter cells at 8 hours PI in JEG-3 cells. 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 hours 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), that are starting growth, then growth arrest, and later resuming of growth and generation of daughter cells. In addition, initial growth is correlated with a low proportion of G1 bacteria in JEG-3 cells at 2 h PI, since most of bacteria display a duplication of chromosome I replication origin at early times post-infection. These data suggest that the coordination between cell cycle and cellular infection could vary from macrophages to trophoblasts, which further underlines the complexity of the infection process. This suggest that bacterial adaptation to the intracellular niches in macrophages and trophoblasts is different. The genes required for this adaptation were investigated using Tn-seq, a saturating transpositional mutagenesis of the *B. abortus* genome followed by deep sequencing to identify transposons insertion sites. By comparing Tn-seq analysis of the infections between RAW 264.7 macrophages and trophoblasts JEG-3 at 5 hours PI, we figured out the different genes required in these two cell types. Most of these genes are related to cell cycle, purine biosynthesis or polysaccharide biosynthesis. Some interesting candidates such as *pleC* (histidine kinase/phosphatase predicted to be involved in cell cycle control), *wbkF* (glycosyl transferase involved in O-chain synthesis), *pdeA* (phosphodiesterase of cyclic-di-GMP, also predicted to involved in cell cycle control) were chosen for further investigation, in order to increase our knowledge on the bacterial adaptations to the infections of different host cells.

References

DEGHELT, M. *et al. (2014). Nat. Commun.* 5: 4366.
ANDERSON, T. D., *et al*. (1986). *Vet. Pathol.* 23: 227-239.

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

K. PONCIN, A. ROBA, J.-J. LETESSON AND X. DE BOLLE

URBM, UNamur, Belgium

Brucella abortus is responsible for brucellosis in domestic and wild life bovines. These bacteria are facultative intracellular pathogens. The trafficking of *B. abortus* has recently been described as globally biphasic in HeLa cells and RAW 264.7 macrophages (DEGHELT *et al*., 2014). The first stage of the infection, which happens in compartments derived from the endocytic pathway, is usually thought as being particularly stressful for the bacterium. Indeed, *Brucella* encounters acidic pH, starvation, but probably also reactive oxygen and nitrogen species (ROOP *et al*., 2009).

The characterization of deletion mutants for genes involved in DNA repair suggested that *Brucella* has to face another type of stress inside its host cells, namely the alkylating stress, which mainly results in DNA aberrant methylation and mutations. In particular, the Δ*alkB* mutant displays a clear attenuation at 24 h PI in HeLa cells and in RAW 264.7 macrophages. In order to investigate the occurrence of an alkylating stress on bacterial DNA during infection, a fluorescent reporter system was set up to probe the presence of this alkylating stress directly inside host cells, at the single cell level. This tool is based on the Adaptive System of *E. coli*, well known for its function in sensing *E. coli* alkylated DNA (MIELECKI & GRZESIUK, 2014). A higher activity of the reporter has been observed in the presence of the alkylating agent methyl methanesulfonate in culture, and during the first stage of *Brucella* HeLa cells infection.

To our knowledge, it would be the first time that alkylating stress is directly pointed out as a stress affecting bacterial survival during infection.

The gut microbiota of bumblebees: a treasure chest of biodiversity and functionality

J. PRAET[1], I. MEEUS[2], G. SMAGGHE[2] AND P. VANDAMME[1]

[1]Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium; [2]Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium

There is currently great concern about worldwide bee declines which may have a detrimental economic impact and may create an instable ecosystem. These declines are presumably caused by a combination of climate change, changes in agricultural practices, pesticide and insecticide use and pathogen spill over from commercial bees. A good mitigation strategy should therefore include the augmentation of nest and hibernation sites, the reduction of insecticide use and the direct promotion of bee health.

Bumblebees are important pollinators of many commercial crops and wild plants. Their gut microbiota has predominantly been analyzed through metagenomics studies and consists of few and very specific bacterial species including *Snodgrassella alvi*, *Gilliamella apicola* and *Lactobacillus bombicola*. These symbiotic gut bacteria may contribute to bee health by supporting the digestion of pollen and the detoxification of pesticide residues and through pathogen inhibition.

In our study we aim to isolate, characterize and exploit the biotechnological potential of the bumblebee gut microbiota. An extensive isolation and characterization campaign was performed to inventorize the cultivable bumblebee gut bacteria. Their functionality and potential to improve bumblebee health is being investigated by pathogen inhibition assays, a pectin degradation assay and whole genome sequence mining. The present data show that especially lactic acid bacteria including *Lactobacillus bombicola*, *Weissella bombi*, *Fructobacillus fructosus* and *F. tropeaoli* inhibit notorious bee pathogens like *Paenibacillus* larvae, Melissococcus *plutonius* and *Crithidia bombi*. Ultimately, the effects of a set of potentially probiotic strains on bumblebee health in naive conditions and after pathogen exposure will be studied through microcolony experiments.

Measuring the biodiversity of microbial communities by flow cytometry

R. PROPS[1,2,3], P. MONSIEURS[2], M. MYSARA[2], L. CLEMENT[4], V. J. DENEF[3] AND N. BOON[1]

[1]Center for Microbial Ecology and Technology (CMET), Ghent University, Gent, Belgium; [2]Department of Environment, Health and Safety, Belgian Nuclear Research Centre (SCK•CEN), Mol, Belgium, [3]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor (MI), U.S.A., [4]Department of Applied Mathematics, Informatics and Statistics, Ghent University, Gent, Belgium

Measuring the microbial diversity in natural and engineered environments is important for ecosystem characterization, ecosystem monitoring and hypothesis testing. Although the conventional assessment through single marker gene surveys has resulted in major advances, the complete procedure remains slow (i.e. weeks to months), labour-intensive and susceptible to multiple sources of laboratory and data processing bias. Growing interest, in highly resolved, temporal surveys of microbial diversity, necessitates rapid, inexpensive and robust analytical platforms that require limited computational effort.

Here, we demonstrate that sensitive single-cell measurements of phenotypic attributes, obtained via flow cytometry, can provide fast (i.e. within minutes) first-line assessments of microbial diversity dynamics, without demanding extensive sample preparation and downstream data processing. We developed a data processing pipeline that fits bivariate kernel density functions to phenotypic parameter combinations of an entire microbial community and concatenates them to a single one-dimensional phenotypic fingerprint. By calculating established diversity metrics from such phenotypic fingerprints, we construct an alternative interpretation of the microbial diversity that incorporates distinct phenotypic traits underlying cell-to-cell heterogeneity (i.e. morphology and nucleic acid content).

Based on a detailed longitudinal study of a highly dynamic microbial ecosystem, our approach delivered temporal alpha diversity profiles that strongly correlated with the reference diversity, as estimated by 16S rRNA amplicon sequencing. This strongly suggests that the distribution of a limited amount of phenotypic features within a microbial community already provides sufficient resolving power for the measurement of diversity dynamics at the species level.

We present a fast, robust analysis method for monitoring the microbial biodiversity of natural and engineered ecosystems that correlates well with the conventional marker gene surveys (PROPS 2016a, PROPS 2016b). Our work has both applied and fundamental implications that stretch from ecosystem monitoring and studies on microbial community dynamics, to supervised sampling strategies. Furthermore, our approach offers perspectives for the development of online and in situ monitoring systems for microbial ecosystems.

References

PROPS R, KERCKHOF FM, RUBBENS P, DE VRIEZE J, HERNANDEZ SANABRIA E, WAEGEMAN W, MONSIEURS P, HAMMES F, BOON N. *ISME J*. 2016a. Epub ahead of print.

PROPS R, MONSIEURS P, MYSARA M, CLEMENT L, BOON N. *Methods in Ecology and Evolution.* 2016b. Epub ahead of print.

Drugs synergy and study of the underlining mechanism through proteomic and lipidomic analysis

C. RENS[1], F. LAVAL[2], M. DAFFE[2], O. DENIS[3], R. FRITA[4], A. BAULARD[4], R. WATTIEZ[5], P. LEFEVRE[1] AND V. FONTAINE[1]

[1]Université Libre de Bruxelles (ULB), Unit of Pharmaceutical Microbiology and Hygiene, Belgium, [2]Institute of Pharmacology and Structural Biology, University of Toulouse, CNRS, University Paul Sabatier (UMR 5089), Department of "Tuberculosis and Infection Biology", France, [3]Laboratory of Bacteriology, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles (ULB), Belgium, [4]Institut Pasteur de Lille, Université de Lille, CNRS, Inserm, CHU Lille, France and [5]Department of Proteomics and Microbiology, University of Mons, Belgium

Tuberculosis (TB) is still a cause of great concern, killing globally more than 1 million people annually. The emergence of multi drug resistant strains calls for new treatments. We performed susceptibility assays using already FDA-approved drugs in combination with vancomycin or anti-TB drugs. We identified three successful combinations against *Mycobacterium tuberculosis*. The lipid and protein analyses of *M. tuberculosis* exposed or not to these drugs allowed us to better understand the underlying mechanisms.

Acknowledgements
Céline Rens was supported by the "Amis des Instituts Pasteur à Bruxelles

The effect of members of the cystic fibrosis lung microbiome on the *Pseudomonas aeruginosa*-induced epithelial immune response

C. RIGAUTS, E. VANDEPLASSCHE, T. COENYE AND A. CRABBÉ

Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

Pseudomonas aeruginosa is the most common pathogen colonizing the lungs of cystic fibrosis (CF) patients. Besides *P. aeruginosa*, a variety of other microorganisms are present in the CF lung. The thick mucus layer covering the lung epithelium creates an ideal environment for polymicrobial biofilm-forming communities. This biofilm mode of growth makes them more resistant to antibiotic treatment as well as to the immune system. The inability of the CF lung to clear microbial communities causes a continuous stimulation of the immune system, which is believed to be driven at least in part by *P. aeruginosa*. The accumulation of immune mediators, caused mainly by a sustained neutrophil influx, along with bacterial virulence factors eventually leads to irreversible lung damage.

The first goal of this study was to evaluate the epithelial immune response induced by planktonic or sessile *P. aeruginosa* cells. Secondly, we wanted to investigate how this immune response is influenced by the presence of other CF lung microbiome members.

The production of interleukin-8 (IL-8) by an organotypic three-dimensional (3-D) lung epithelial model was evaluated after infection with *P. aeruginosa* biofilms or planktonic cells by using an IL-8 ELISA test-kit. First, mixed biofilms were generated from *P. aeruginosa* PAO1 (a laboratory strain) or CF127 (a hyper-biofilm forming CF lung isolate) co-cultured with five other bacteria commonly found in the CF lungs: *Staphylococcus aureus*, *Streptococcus anginosus*, *Rothia mucilaginosa*, *Achromobacter xylosoxidans* and *Gemella haemolysans*. Quantification of *P. aeruginosa* in single and polymicrobial biofilms was performed using flow cytometry and by plating.

Preliminary results show that the IL-8 production by 3-D lung epithelial cells infected with sessile *P. aeruginosa* differs from that of epithelial cells infected with planktonic cells. The latter induced a three times higher IL-8 response. There is no significant difference in the number of *P. aeruginosa* PAO1 cells in single and mixed species biofilms. In contrast, *P. aeruginosa* CF127 produced less biofilm when co-cultured with *R. mucilaginosa*, but not when co-cultured with other species.

We are currently measuring the IL-8 production of 3-D lung epithelium in response to *P. aeruginosa* co-cultured with other CF lung microbiome members.

Is complex gut metabolism simulable?

S. S. RUBIN

Université catholique de Louvain, Belgique; Caracol de la Realidad, Université Populare de Caen, France; Centro Nacional de Investigaciones Biotecnologicas - CNIB, Bolivia; Gent Universiteit, Data Analysis Department, België

The gut metabolism is a symbiotic, thus evolutionary a holonomic system. Its dynamics depends on more than the collection of distinguished 'separable' cellular and molecular fractions. In other words, the dynamics of the gut metabolism is of a systemic character and is complex because it is analytically non-fractionable, and hence non-simulable. However different experimental and theoretical models neglect it and argue the possibility of its simulability from effective synthesis by a direct inverse analysis of molecular and cellular fractions. Furthermore, based on these synthetic communities, some studies using artificial models of the gut metabolism propose assessments of bioaccessibility and bioavailability for food technology and pharmacology. Here I will show that even the multi-stability dynamics of the holonomic gut metabolism depicted by a system of non-linear differential equations (dynamical systems) are not sufficient. Moreover I will show that experimental models lack real representation because they are based on the idea that if a gut system exhibits '*enough*' of the behaviors we see in natural gut system, the artificial one must be a natural one. In other words, such models based on synthetic communities are either scientifically recreative, scientifically incorrect, or they have links with pharmacological and 'biotechnological' 'patent seeking' goals. To tackle biological realization of the symbiotic and holonomic gut metabolism, one ought not to be seeking synthetic implementations of what the relations *are*, but, rather, one ought to be seeking interpretations of what the relations *do.*

Small regulatory RNAs in *Burkholderia cenocepacia* biofilms

A. M. SASS, S. KIEKENS, H. VAN ACKER AND T. COENYE

Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

Burkholderia cenocepacia is a member of the *Burkholderia cepacia* complex (Bcc), a group of closely related opportunistic pathogens infecting cystic fibrosis patients and immunocompromised individuals. Bcc infections are very difficult to treat due to the high innate antimicrobial resistance of this group of bacteria. Biofilm formation has been reported for many Bcc strains, further contributing to their recalcitrance.

In recent years, an increasing number of small non-coding RNAs has been discovered in prokaryotes. Non-coding RNAs regulate gene expression post-transcriptionally, either by binding to other mRNAs or by binding to proteins. Non-coding RNAs often target regulatory elements and thus fine-tune the respective regulatory processes. Among the regulatory cascades influenced by non-coding RNAs are global developmental processes such as biofilm formation.

In *Burkholderia* species, non-coding RNAs are to date largely uncharacterised. To identify small non-coding RNAs expressed in *Burkholderia cenocepacia* biofilms, transcription start sites within its genome were mapped by differential RNA sequencing [1]. 158 transcription start sites located in intergenic regions produced a short transcript not associated with a coding sequence. These were further examined for: presence of a pronounced secondary structure, a rho-independent terminator and a high degree of conservation within Bcc bacteria; properties indicating a regulatory small RNA. 15 short transcripts were then chosen for further analysis. The size of these candidate regulatory small RNAs was confirmed by Northern blotting and RACE. qPCR and Northern blotting showed that expression of numerous small RNAs was increased in biofilms, starvation and under certain stress conditions. *In silico* target analysis indicated that the selected small RNAs possibly regulate membrane proteins, proteins involved in carbon metabolism and transcriptional regulators. The role of small RNAs in *Burkholderia* metabolism is further investigated using knock-out mutants; confirmation of sRNA targets is performed using RNA-Seq and eGFP-fusion proteins.

References

[1] SASS, A.M. *et al*. Genome-wide transcription start site profiling in biofilm-grown *Burkholderia cenocepacia* J2315. BMC Genomics 16:775 (2015)

Long term stability of MELiSSA strains

T. SASSEN[1,2], J. MAHONY[2], R. VAN HOUDT[1], F. MASTROLEO[1] AND D. VAN SINDEREN[2,3]

[1]SCK•CEN, Mol, Belgium, research unit for Microbiology; [2]Department of Microbiology and [3]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland

The acronym MELiSSA means 'Micro Ecological Life Support System Alternative'. It refers to the ESA research program aiming to develop a 5-compartments artificial ecosystem to support long-term space exploration missions[1]. Environmental factors including radiation, pH or other stress factors, as well as genetic drift might influence the parameters of the control model or even activate dormant prophages in the genome of the MELiSSA bacteria.

Focusing on the edible bacteria *Arthrospira* sp. PCC8005 and *Rhodospirillum rubrum* S1H genomes, the present study aimed to identify putative mobile genetic elements, i.e. insertion sequences (IS) as well as prophages and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR).

Several high profile targets including IS and CRISPR which will likely influence genetic stability have been identified using bioinformatics. These targets can be used together with inverse PCR, qPCR or used in AFLP analysis to study genetic drift during long term cultivation. Combined with insights in the induction of possible prophages, this will lead to comprehensive insights into culture stability in compartment II and IVa of the MELiSSA loop.

Reference

1. HENDRICKX L, DE WEVER H, HERMANS V, MASTROLEO F, MORIN N, WILMOTTE A, JANSSEN P, MERGEAY M. 2006. Microbial ecology of the closed artificial ecosystem MELiSSA (Micro-Ecological Life Support System Alternative): reinventing and compartmentalizing the Earth's food and oxygen regeneration system for long-haul space exploration missions. *Res Microbiol*. 157(1):77-86.

Acknowledgements

This work is funded by the MELiSSA Foundation.

Air pollution and plant-microbe interactions in the phyllosphere

W. SMETS[1], K. WUYTS[1], E. OERLEMANS[1], S. WUYTS[1,2], S. WITTOUCK[1], S. DENYS[3], R. SAMSON[1] AND S. LEBEER[1]

[1]University of Antwerp, Dept. Bioscience Engineering, Environmental Ecology and Applied Microbiology, Antwerp, Belgium; [2]Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium; [3]University of Antwerp, Dept. Bioscience Engineering, Sustainable Energy, Air and Water Technology, Antwerp, Belgium

The surface of plant leaves, also termed the phyllosphere, is a unique habitat for microbes. The bacterial composition of the phyllosphere seems to depend on the plant host species, leaf characteristics, season, climate, and location of the plant host. In this study, we investigated the effect of an urban environment and air pollution on the composition of phyllosphere communities. Leaves were sampled from 18 ivy plants (*Hedera sp.*) and 56 London plane trees (*Platanus* × *acerifolia*) in the city of Antwerp and its surroundings. The bacterial community composition was determined using 16S rRNA gene sequencing on the Illumina MiSeq platform. Leaf biomagnetic analyses were used to estimate exposure to particulate matter, a major component of air pollution. We found that many of the dominant taxa of the ivy phyllosphere communities were strongly correlated with the leaf biomagnetic signal. Interestingly, some of these taxa indicate differences in the available substrates on ivy leaves of the city compared to a less urbanized environment. However, dominant taxa of London plane tree communities were not correlated with the biomagnetic signal - a proxy for leaf deposited particulate matter –, although they showed a strong correlation with car traffic intensity. This further suggests that gaseous traffic-related pollutants affect the phyllosphere bacteria. Furthermore, leaf characteristics, such as chlorophyll content and leaf wettability were linked with the plane tree phyllosphere composition. Although interesting associations were found, the mechanisms by which land use, car traffic intensity and air pollutants affect the phyllosphere community composition remain to be explored.

Antimicrobial susceptibility of Streptococcus anginosus in a multispecies biofilm

S. TAVERNIER[1], M. TUYSUZ[1,2], P. RIGOLE[1], L. STUER[1], I. VANDECANDELAERE[1], G. BRACKMAN[1], A. CRABBÉ[1] AND T. COENYE[1]

[1]Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium; [2]Department of Pharmaceutical Microbiology, Istanbul University, Istanbul, Turkey

Chronic lung infections are a major cause of morbidity and mortality in patients with cystic fibrosis (CF). In childhood, *Staphylococcus aureus* is typically the most prevalent organism, while in adults, *Pseudomonas aeruginosa* is the major pathogen. *Streptococcus anginosus* also plays a significant role in exacerbations of respiratory symptoms. Patients are frequently co-colonized with these three bacterial species, yet little is known about whether antibiotic susceptibility of one species is influenced by the presence of others.

In the present study, we compared the susceptibility of *S. anginosus*, *S. aureus* and *P. aeruginosa* to various antibiotics when grown in monospecies biofilms or in multispecies biofilms with all three species together. Subsequently, we determined the susceptibility of *S. anginosus* biofilms grown and treated in cell-free supernatant of mono- and multispecies biofilms, in order to determine whether secreted factors are involved.

Our results show that differences in susceptibility between *S. anginosus* grown in mono- or multispecies biofilms are antibiotic and concentration dependent. Interestingly, S. *anginosus* becomes less susceptible to several antibiotics that interfere with cell wall synthesis (amoxicillin, imipenem, cefepime and meropenem) in a multispecies biofilm, while for tobramycin and ciprofloxacin, no difference in susceptibility is observed. In addition, our findings indicate that a factor secreted by *S. aureus* has a major influence on the decreased susceptibility of *S. anginosus* against these antibiotics. In ongoing experiments, multiple *S. aureus* strains are being tested to determine whether the effect on the susceptibility of *S. anginosus* is *S. aureus* strain-dependent.

BcepMu/B3-like prophages in proteobacteria and pseudomonads

A. TOUSSAINT[1] AND F. VAN GIJSEGEM[2]

[1]Génétique et Physiologie Bactérienne, Université Libre de Bruxelles, IBMM-DBM, 12 Rue des Professeurs Jeneer et Brachet, B 6041 Gosselies, Belgium; [2]IEES UMR1392, UPMC Bât A 7e étage Case 237, 7 Quai Saint-Bernard, F-75252 PARIS Cedex FRANCE

Transposable phages and prophages in the Saltoviridae family have been detected in a large fraction of bacterial phyla. Escherichia coli phage Mu and Burkholderia cepacia BcepMu (or Pseudomonas aeruginosa phage B3) stand as the paradigms for two genetic organisations of the Saltoviridae genomes, with the immunity repressor vs. late positive regulator at the left end, and two vs. three transposition proteins (including one DDE recombinase) respectively. Transposition proteins of the two types are distant enough to belong to distinct protein famlilies (Uniprot, ACLAME etc.) and contain different pfam domains.

The first BcepMu/B3-like prophage in a proteobacterium was described in Pectobacterium atrosepticum (ECA41, EVANS *et al*., 2010). More recently we identified a very similar prophage in the course of annotating the genomic sequence of a related plant pathogen, Dickeya dianthicola RNS04.9.

Starting with this prophage nucleotide sequence, we could identify many complete BcepMu/B3-like prophages in other enterobacteria. The vast majority appear to be functional since they are flanked by a 6 bp direct repeat, as reported by Evans et al. 2010, for ECA41 and contrary to Mu-like (por)phages, which generate a 5 bp direct repeat.

Amazingly, B3-like prophages in E.coli carry an invertible region and its cognate invertase, as do Mu and its close relative D108, which as a result can switch host range (VAN DE PUTTE *et al*., 1980).

The B3-like prophages described here and the newly identified Mu-like phages and prophages in pseudomonads (CAZARES *et al*. 2014) enlarge the Saltoviridae family and should allow for a more refined classification of these ubiquitous “re-arrangers” of host genomes.

References

EVANS, T. J., COULTHURST S.J., KOMITOPOULOU, E. AND SALMOND, G.P. 2010, FEMS Microbiol Lett 304: 195–202.

VAN DE PUTTE, P., CRAMER, S. AND GIPHART-GASSLER, M. 1980, Nature 286: 218-222.

CAZARES, A., MENDOZA-HERNANDÈZ, G. AND GUARNEROS, G. 2014, BMC Genomics 15: 1146.

Exploring the role of mini-proteins in *Burkholderia cenocepacia* biofilm formation and persistence

H. VAN ACKER, S. KIEKENS, A. SASS, A. WATTY AND T. COENYE

Laboratorium voor Farmaceutische Microbiologie, Universiteit Gent, Gent, Belgium

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

We focused on genes smaller than 300 nucleotides of which the function is unknown. Almost 10 % (646) of the genes in the *B. cenocepacia* J2315 genome are smaller than 300 nucleotides and more than half of these are annotated as hypothetical proteins. For 234 of them no similarity could be found with non-hypothetical genes in other bacteria using BLAST ($E < 10\text{-}5$ and identity > 40 %). However, based on available transcriptomic data the majority of these genes were found to be differentially expressed in stress conditions (treatment with tobramycin, H_2O_2 or chlorhexidine, low oxygen, low pH, low iron or heat). Using available RNA sequencing data, a list of 27 mini-proteins highly expressed in biofilms (RPKM > 200) was compiled and those are currently being studied more in detail. Preliminary results indicate that overexpression of some of these mini-proteins leads to an increase in biofilm biomass. Additionally, overexpression of several mini-proteins increased the number of persisters. Translational GFP reporter fusion mutants and flow cytometry was used to measure protein levels. For several mini-proteins the expression was also higher after treatment with Tob, Cip or H_2O_2.

Our results confirm that mini-proteins are present in the genome of *B. cenocepacia* J2315 and indicate that they are involved in various biological processes.

Investigations on the potential radio-protective properties of edible cyanobacterium during pelvic irradiation in a mouse model.

W. VAN BEECK[1, 2, 3], M. VERSLEGERS[2], S. BAATOUT[2], M. MYSARA[1], P. MONSIEURS[1], N. LEYS[1], S. LEBEER[3] AND F. MASTROLEO[1]

[1]SCK•CEN, Mol, Belgium, research unit for Microbiology; [2]SCK•CEN, Mol, Belgium, research unit for Radiobiology; [3]University of Antwerp, Antwerp, Belgium, ENdEMIC research group

Exposure to pelvic irradiation result in several side effects including an increased oxidative stress[1], increased inflammation and, dysbiosis of the gut microbiota resulting in diarrhea. Due to the ability to resist high doses of radiation (up to 6400Gy[2]) and its anti-oxidative capacity, the cyanobacterium *Arthrospira* sp. seems promising to treat these side effects. As such, we aimed to investigate the possible radio-protective properties of *Arthrospira sp.* by assessing its effects on: (i) ileum morphology, (ii) inflammation and (iii) gut microbiome composition. Here for, male mice received *Arthrospira* sp. supplemented chow prior to local 8Gy X-ray pelvic irradiation. These mice showed a significant decrease in villi length, but no change inflammatory response. In this pilot study, different high end points were used to evaluate the potential radio-protective properties of the cyanobacterium but to really understand the mechanism, more in depth techniques have to be used e.g. immunological staining for apoptosis.

References

1. RILEY P. 1994. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *International journal of radiation biology*, 65(1):27-33.

2. BADRI H, MONSIEURS P, CONINX I, WATTIEZ R, LEYS N. 2015. Molecular investigation of the radiation resistance of edible cyanobacterium *Arthrospira* sp. PCC 8005. *MicrobiologyOpen*.

The impact of breastmilk and delivery mode on the infant's gut microbiota

E. VAN DAELE[1,2], F.M. KERCKHOF[2], C. BELZER[1] T. VAN DE WIELE[2]

[1]Laboratory of Microbiology, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands and [2]CMET, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

Neonatal colonisation drives postnatal gut maturation and induces the immune system, thus possibly impacting (long term) health (RODRÍGUEZ *et al.*, 2015). The babies' first encounter with a dense bacterial population happens during birth. Dependent on delivery mode, the newborn is exposed to either the mother's vaginal and fecal flora or the microbiota of the skin and hospital surroundings (DOMINGUEZ-BELLO *et al.*, 2010). The exact impact on later health and mechanisms to overcome this difference remain unclear.

Here, we investigated the microbiome composition of stool and breastmilk samples from 30 mother-infant pairs after 3 months of exclusive breastfeeding. Mothers and infant pairs were stratified according to delivery mode. The samples were analysed using DGGE and Illumina sequencing of the 16S rRNA gene. The evenness of the microbiota in the infants' fecal samples was lower in comparison to the mothers'. Along with a detected lower richness in the infant gut, this indicates a less mature community structure dominated by a few species. Interestingly, a cluster analysis of the gut microbiome composition did not reveal differences according to delivery mode. The presumed postnatal shift was either not picked up by these techniques or three months of exclusive breastfeeding could have erased this .

Taking a closer look at the microbial content of breastmilk, evenness was lower when the mother underwent c-section in comparison with vaginal delivery. More research is needed to substantiate this difference in milk microbiomes and to identify the breastmilk microbiota source(s) as it suggests a possible microbial transfer route between mother and child. Intriguingly two breastmilk samples after c-section delivery gave results similar to the vaginal group. Taking the meta-data into account, these outliers were the women who had an emergency c-section. C-section comprises two distinct biological processes namely the emergency and planned C-section. Preceding an emergency C-section, in contrast to a planned delivery, the mother undergoes labor with or without hormonal changes, rupture of the membranes and contractions. As the biological process prior to the surgical procedure is completely different, this could also affect the microbiota. We suggest that further subdivision of mode of delivery into 3 categories should be taken into account for further research because this can be an important determinant for the development of the infant gut microbiota.

References

DOMINGUEZ-BELLO, M. G., COSTELLO, E. K., CONTRERAS, M., MAGRIS, M., HIDALGO, G., FIERER, N., & KNIGHT, R. (2010) *Proceedings of the National Academy of Sciences 107*:26

RODRÍGUEZ, J. M., MURPHY, K., STANTON, C., ROSS, R. P., KOBER, O. I., JUGE, N., COLLADO, M. C. (2015) *Microbial Ecology in Health & Disease*, *26:*0

Evaluation of combination therapy for *Burkholderia cenocepacia* lung infection in different *in vitro* and *in vivo* models.

F. VAN DEN DRIESSCHE[1], B. VANHOUTTE[2], G. BRACKMAN[1], A. CRABBE[1], P. RIGOLE[1], J. VERCRUYSSE[3], G. VERSTRAETE[3], D. CAPPOEN[2], C. VERVAET[3], P. COS[2] AND T. COENYE[1]

[1]*Laboratory of Pharmaceutical Microbiology, University of Gent, Belgium;* [2]*Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Belgium;* [3]*Laboratory of Pharmaceutical Technology, University of Gent, Belgium*

Species belonging to the *Burkholderia cepacia* complex (BCC) are opportunistic pathogens mainly known for causing chronic lung infections in cystic fibrosis (CF) patients and in patients with chronic granulomatous disease (LIPUMA, 2005). *Burkholderia cenocepacia* belongs to the BCC and accounts for approximately 45% of BCC infections in CF patients in the United States (LIPUMA, 2005). The complex interaction between *B. cenocepacia* and the CF lung is reflected by the various clinical outcomes and disease severity, from transient colonization to necrotizing pneumonia and sepsis resulting in early death. Respiratory infections caused by *B. cenocepacia* are also associated with lower survival following lung transplantation (ALEXANDER *et al*., 2008; MURRAY *et al*., 2012). Antimicrobial therapy against these infections frequently fails due to *B. cenocepacia's* resistance to many antibiotics and the induction of biofilm-specific tolerance mechanisms (COENYE, 2010). Compounds that improve the activity of antibiotics have been called helper compounds, potentiators, adjuvants, or resistance breakers.

The aim of the present study was to identify compounds that reverse tolerance and/or resistance towards tobramycin by increasing the susceptibility of *B. cenocepacia* biofilms. The efficacy of an alternative antimicrobial strategy was therefore evaluated for *B. cenocepacia* lung infections using *in vitro* and *in vivo* models. A screening of the NIH Clinical Collection 1&2 was performed against biofilms of *B. cenocepacia* formed in 96-well microtiter plates in the presence of tobramycin to identify repurposing candidates with potentiator activity. The efficacy of selected hits was evaluated in a three-dimensional (3D) organotypic human lung epithelial cell culture model. The *in vivo* effect was evaluated in the invertebrate *Galleria mellonella* and in a murine model of a *B. cenocepacia* invasive lung infection. The screening resulted in 60 hits that potentiated the activity of tobramycin against *B. cenocepacia* biofilms, including four imidazoles of which econazole and miconazole were selected for further investigation. However, a potentiator effect was not observed in the 3D organotypic human lung epithelial cell culture model. Combination treatment was also not able to increase survival of infected *G. mellonella*. Also in mice, there was no added value for the combination treatment. Although potentiators of tobramycin with activity against biofilms of *B. cenocepacia* were identified in a repurposing screen, the *in vitro* activity could not be confirmed nor in a more sophisticated *in vitro* model, neither *in vivo*.

This stresses the importance of validating hits resulting from *in vitro* studies in physiologically relevant model systems.

References

ALEXANDER, B. D., PETZOLD, E. W., RELLER, L. B., PALMER, S. M., DAVIS, R. D., WOODS, C. W. AND LIPUMA, J. J. (2008). *Am J Transplant* 8, 1025–1030.

COENYE, T. (2010). *Future Microbiol* 5, 1087–1099.

LIPUMA, J. J. (2005). *Curr Opin Pulm Med* 11, 1070–5287.

MURRAY, S., CHARBENEAU, J., MARSHALL, B. C. AND LIPUMA, J. J. (2012). *Am J Respir Crit Care Med* 178, 363–371.

The role of EPS in the processing of probiotics into pharmabiotic formulations

D. VANDENHEUVEL[1], C. ALLONSIUS[1], G. BROECKX[1,2], S. KIEKENS[1,2], F. KIEKENS[2], AND S. LEBEER[1]

[1]University of Antwerp, Department of Bioscience Engineering, Environmental Ecology and Applied Microbiology, Groenenborgerlaan 171, 2020 Antwerp, Belgium; [2]University of Antwerp, Department of Pharmaceutical Sciences, Laboratory of Pharmaceutical Technology and Biopharmacy, Universiteitsplein 1 blg A, 2610 Antwerp, Belgium

External cell-wall appendages (fimbriae, pili, flagella, EPS) are key factors in many of the health-beneficial effects of probiotics, including alteration of host immune responses, improvements of the epithelial barrier, and adhesion-competition with pathogens (SEGERS & LEBEER, 2014). The fate of cell-wall exopolysaccharides when probiotics are processed into functional foods or pharmabiotics is hardly investigated. Indeed, probiotic bacteria can lose their cell-wall appendages when they are subjected to external stress. Even commonly used formulation and processing techniques in the pharmaceutical sector and the food industry (e.g. centrifugation, filtration, freeze drying, spray drying, milling, tableting) can result in the loss of these sensitive structures, thereby altering or impeding the health-beneficial effect (BROECKX *et al.*, 2016). This should therefore be kept in mind when probiotics are marketed and health claims are stipulated.

We want to investigate the importance of EPS in the processing of probiotics. The presence and intactness of this external layer of polysaccharides is important for two main reasons. Firstly, EPS can act as an interaction partner between the probiotic bacterium and its surrounding, providing the probiotic effect. Secondly, EPS can provide protection against detrimental stresses during processing steps, increasing the viability of the probiotic bacteria and/or the intactness of other cell-wall molecules.

Strain-specific differences in survival after processing are studied using wild-type strains of *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* GR-1, each showing a different EPS, and their cell-wall EPS deficient mutants. These strains are processed using lab-scale equipment, mimicking relevant industrial and pharmaceutical formulation and processing techniques. As such, key points in the processing process can be identified, and optimized. The importance of the EPS will be evaluated based on cell viability. The results can provide a proof of concept for the importance of cell-wall structures in the survival and the probiotic effect of processed pharmabiotics.

References

SEGERS, M.E. AND LEBEER, S. (2014). *Micr. Cell Fact.*, 13(S1), S7.

BROECKX, G., VANDENHEUVEL, D., CLAES, I., LEBEER, S. AND KIEKENS, F. (2016) *Int. J. Pharm.*, 505(1-2), 303-318.

Antibiotic susceptibility of biofilms is influenced by interspecies interactions in an artificial CF lung microbiome

E. VANDEPLASSCHE[1], T. COENYE[1] AND A. CRABBÉ[1]

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

Chronic lung infections are the main cause of morbidity and mortality in cystic fibrosis (CF) patients. The source of these continuing infections and subsequent inflammation is a polymicrobial community, known as the CF lung microbiome, which forms biofilms that are highly resistant to antimicrobial therapy. Interactions between the different bacterial species of the CF microbiome may influence the virulence, persistence or antibiotic susceptibility of the community, yet are mostly unexplored.

The aim of the present study is to examine how biofilm formation and drug susceptibility of the CF lung microbiome is affected by interspecies interactions.

We addressed this research question by co-culturing bacterial species that are frequently isolated from the CF lung. Together, these bacteria make up an artificial CF lung microbiome (ACM), consisting of *Pseudomonas aeruginosa* AA2 (an early CF infection strain), *Staphylococcus aureus* (MRSA), *Streptococcus anginosus*, *Achromobacter xylosoxidans*, *Rothia mucilaginosa*, and *Gemella haemolysans*. Mono species biofilms of each strain, dual species biofilms with AA2, as well as multispecies biofilms of all six strains together were formed. The effect on biofilm formation and susceptibility to antibiotic treatment with various antimicrobials was investigated in these different conditions.

In the absence of treatment, *S. aureus* formed significantly less biofilm when co-cultured with either AA2 or the combined ACM compared to a mono species biofilm. *R. mucilaginosa* and *G. haemolysans* formed significantly less biofilm co-cultured with AA2 compared to their respective mono species biofilm. In the ACM however, *G. haemolysans* biofilm formation increased compared to the dual species biofilm.

Furthermore, our results point to significant differences in the antibiotic susceptibility of ACM when grown in a polymicrobial biofilm. When *S. aureus* was treated with ceftazidime, ciprofloxacin or colistin, this pathogen became more susceptible in a multispecies biofilm with the combined ACM, compared to a treated mono species biofilm of *S. aureus* or dual species biofilm of *S. aureus* with AA2. When treated with ciprofloxacin or colistin, *G. haemolysans* became more susceptible in a multispecies and in a dual species biofilm compared to the treated mono species biofilm. When treated with tobramycin, *S. aureus* and *R. mucilaginosa* became more susceptible in a dual species biofilm with AA2 compared to their respective mono species biofilm. In the ACM however, *G. haemolysans* showed increased biofilm formation compared to the mono species and the dual species biofilm.

No significant differences were observed for *S. anginosus*.

Experiments to unravel alterations in *A. xylosoxidans* are ongoing.

We conclude that biofilm formation and antibiotic susceptibility of ACM is influenced by interspecies interactions. Therefore, studying antibiotic susceptibility in the context of the microbiome may open up avenues towards personalised antimicrobial therapies based on microbiome composition in CF patients

Study of the chromosomes replication of Brucella abortus

M. VAN DER HENST, N. FRANCIS, J.-J. LETESSON AND X. DE BOLLE

Unité de Recherche en Biologie des Micro-organismes (URBM), Université de Namur, Belgique

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

Recently some tools were developed to manage the state of the chromosomes replication throughout the cell cycle of B. abortus at the single cell level. By highlighting proteins involved in the partitioning system of chromosomal replication origins, we are able to determine the number of replication origins of both chromosomes indicating if the bacterium is in G1 phase or in S/G2 phase. The study of the two chromosomes replication patterns revealed that the chromosome I initiates its replication first, indicating a coordination between the replication of both chromosomes.

Using these reporter strains to infect RAW 264.7 macrophages and HeLa cells, we showed that B. abortus presents a biphasic infection process. Indeed, the infection is characterized by a first non-proliferative step where bacteria are arrested in G1 phase followed by a proliferative step where bacteria grow and replicate in a compartment derived from the endoplasmic reticulum.

In addition, experiments have shown that the G1 bacteria constitute the preferential form for invasion since the number of G1 bacteria present in host cells at 15 minutes post infection were drastically increased compared to classical bacterial culture in rich medium (DEGHELT *et al.*, 2014).

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

CtrA is an essential response regulator conserved in many alpha-proteobacteria. In Caulobacter crescentus, this factor is able to prevent the replication by binding the replication origin. Recently, a ChIP-seq on CtrA has been done in B. abortus highlighting the genomic targets of this factor. Among these targets, some are involved in the regulation of the cell cycle and more precisely in the replication. For example, the promoter of dnaA (replication initiator of ChrI) and repC (replication initiator of ChrII) were shown to be bound by CtrA. A depletion strain for CtrA in B. abortus has been constructed in order to investigate more deeply the role of this factor. CtrA-depleted bacteria present branching morphologies that are consistent with division defects, arguing that CtrA is involved in cell cycle control in B. abortus. To test the involvment of CtrA in chromosomes replication we decided to construct a CtrA depletion strain highlighting both replication origins. We showed that ChrI replication still occur in bacteria where CtrA is absent indicating that this later is not involved in positive regulation of the replication. CtrA-depletion strain highlighting ChrI and ChrII origins will be characterized to determine if the coordination between replication initiation of both chromosomes is conserved when CtrA is absent.

Unravelling the relationship between indigenous community diversity and success of bioaugmentation using synthetic microbial ecosystems

J. VANDERMAESEN[1], A. J. DALY[2], J. M. BAETENS[2], B. DE BAETS[2], N. BOON[3] AND D. SPRINGAEL[1]

[1]Division of Soil and Water Management, KU Leuven, Kasteelpark Arenberg 20 bus 2459, B-3001 Heverlee, Belgium; [2]Research Unit Knowledge-based Systems (KERMIT), Ghent University, Coupure links 653, B-9000 Gent, Belgium; [3]Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure links 653, B-9000 Gent, Belgium

According to EU guidelines, pesticide residues like 2,6-dichlorobenzamide (BAM) in drinking water should be below 0.1 µg/L. This is currently achieved by activated carbon filtration. A more economical approach is bioaugmentation of sand filter units in drinking water treatment plants with *Aminobacter sp.* MSH1 that uses BAM as a sole source of C, N and energy. Successful bioaugmentation depends on both biotic and abiotic factors. Amongst those, interactions with members of the indigenous microbial community and the diversity of the community are suggested to play a major role. To investigate the fundamental principles behind this hypothesis, MSH1 was combined with synthetic microbial communities, consisting of different bacterial strains isolated from drinking water sand filters, at differing richness levels in sand microcosms. After an initial competition phase during which acetate was provided as the sole carbon source, ^{14}C-labelled BAM was added and $^{14}CO_2$ produced by MSH1 through BAM mineralization was monitored. To quantify the success of bioaugmentation, the survival of MSH1 was determined at the end of the competition phase and kinetic parameters were derived from cumulative mineralization curves as a measure of MSH1's functionality. In dual species communities, the success of bioaugmentation depended on interspecies interactions between MSH1 and its opponent. Most of the observed interactions were of a competitive nature and corresponded to the intrinsic competitiveness of the strain with which MSH1 was combined. However, cooperative interactions also occurred, which positively affected the survival and functionality of MSH1. The effect of those cooperative interactions overruled the negative effects of other strains in triple-species communities, which was not in accordance with the intrinsic competitiveness of those particular strains. When we increased the richness of the synthetic communities with which MSH1 was combined, an overall negative correlation was found between community richness and the survival and functionality of MSH1. However, the presence of specific strains that interacted with MSH1 in a cooperative way dominantly affected the results and counteracted the negative effect of increasing richness. Hence, the survival and functionality of MSH1 highly depended on the composition of the community. In conclusion, the use of synthetic microbial ecosystems unraveled how diversity effects and interspecies interactions influenced the success of bioaugmentation.

Microbiological analysis of spent nuclear fuel pools: towards the identification of radiation-resistant bacteria

V. VAN EESBEECK[1,2], M. MYSARA[1], R. PROPS[1], R. VAN HOUDT[1], P. PETIT[3], N. LEYS[1], J. ARMENGAUD[3], C. RIVASSEAU[3], J. MAHILLON[2] AND P. MONSIEURS[1]

[1]Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium; [2]Université Catholique de Louvain, UCL, Louvain-la-Neuve, Belgium ; [3]Commissariat à l'énergie atomique, CEA, Grenoble, France

After being used as energy source inside power plants, spent nuclear fuel must be stored underwater in so-called "spent nuclear fuel pools" (SNFPs) in order to cool down before being safely disposed. Interestingly, despite the highly oligotrophic and radioactive nature of the water, microbial growth is not fully prevented. Microorganisms identified in such environments provide a unique opportunity to acquire new insights into radiation-resistance mechanisms. A detailed characterization of highly resistant strains might result in identifying good candidates to be used in bioremediation processes for radionuclide-contaminated environments.

The objective of our project is to inventory and monitor the bacterial communities present in SNFPs of French and Belgian reactors over time. Furthermore, this project also aims at a phenotypical characterization of the most abundant species.

In this work, we present the inventory of the bacterial communities we analyzed in one SNFP as well as a secondary pool surrounding the reactor vessel. Since a culture-independent approach focusing on characterizing the entire microbial population is currently lacking, we chose to inventory the communities present in those pools using a 16S rRNA amplicon sequencing approach. First results highlighted the presence of bacteria mainly belonging to the Alpha- and Betaproteobacteria. For the phenotypic characterization, we isolated and cultured the most abundant strains, which were then tested for their resistance towards radiation using a gamma irradiation facility. Preliminary results showed that all strains tolerated a dose of 300 Gy, but only a single strain from the SNFP was able to cope with a dose of 2100 Gy.

Next to bacteria in planktonic form, biofilms present on the walls and the fuel rods stored in the SNFP will also be analyzed in the near future. This will be done using a combination of culture-based and culture-independent (16S rRNA amplicon sequencing) methods.

Evaluation of the anti-CXCR4 activity profile of published GPCR inhibitors

A. VAN HOUT, T. D'HUYS, M. OEYEN, T. VAN LOY AND D. SCHOLS

Laboratory of Virology and Chemotherapy, Department of Microbiology and Immunology, Rega Institute, KU Leuven, Belgium

The CXC chemokine receptor 4 (CXCR4), a seven transmembrane G protein-coupled receptor (GPCR), is activated by the CXC chemokine ligand 12 (CXCL12) and regulates multiple developmental and physiological processes including hematopoiesis and migration of immune cells. CXCR4 is also a major receptor for human immunodeficiency virus (HIV) entry and pathogenesis. The dysregulation of the CXCL12/CXCR4 signaling axis contributes to many human diseases. For instance, CXCR4 is often overexpressed on tumor cells and contributes to cancer progression by mediating tumor cell survival, proliferation, metastasis and tumor-related angiogenesis (*e.g.* in gastrointestinal, breast and lung cancer). Accumulating evidence also indicates a role of CXCR4 in inflammatory diseases, like rheumatoid arthritis and inflammatory bowel disease. Hence, CXCR4 is considered as a very attractive target for therapeutic intervention in many disease areas. During the last decades, numerous agents (*e.g.* anti-CXCR4 peptides and small molecule antagonists, CXCL12 peptide analogs and anti-CXCR4 antibodies) capable of inhibiting CXCR4 functioning have been published in experimental and clinical studies. Here, we evaluated the activity of a selection of agents previously described to inhibit CXCR4 signaling and functioning. In total, 11 products were included in this study: the anti-CXCR4 peptides T22 and its derivatives T140 and TC14012, the small molecule antagonists Me6TREN, Gambogic acid (GA), IT1t, AMD3100 and derivatives (AMD3465, AMD11070, WZ811) and, finally, the CXCL12 peptide analog CTCE-9908. We investigated to what extent these compounds directly act on CXCR4 or, alternatively, affect the CXCL12/CXCR4 signaling in an indirect manner. In addition, the variety of biological assays and experimental conditions applied in previous studies, makes it very often difficult to compare their relative potency in inhibiting CXCR4 signaling. Therefore, their anti-CXCR4 receptor activity was evaluated in several *in vitro* CXCR4-related pharmacological and functional assays to study their CXCR4 binding capacity and their potential inhibitory effect on CXCL12-induced calcium signaling, CXCR4 internalization, chemotaxis and HIV replication.

Our results suggested that the CXCR4 antagonists T22, T140, TC14012, IT1t, AMD3100, AMD3465 and AMD11070 potently inhibit CXCR4 signaling and function via a direct interaction with CXCR4. In contrast, Me6TREN, GA, WZ811 and CTCE-9908 showed no or very weak inhibition of CXCR4 in our assays and are likely to inhibit CXCL12/CXCR4 signaling in an indirect manner. In agreement with this, the first group of inhibitors was shown to potently inhibit HIV replication while the latter one demonstrated almost no antiviral activity.

Protecting chickens against *Campylobacter jejuni* by Camelidae-derived nanobodies

C. VANMARSENILLE[1,2,3], J. ELSEVIERS[4], G. G. HASSANZADEH[4], A. MARTEL[3], F. HAESEBROUCK[3], F. PASMANS[3], J.-P. HERNALSTEENS[2] AND H. DE GREVE[1]

[1]Structural Molecular Microbiology, VIB, Vrije Universiteit Brussel; [2]Genetische Virologie, Vrije Universiteit Brussel; [3]Department of Pathology, Bacteriology and Avian diseases, Ghent University; [4]VIB Nanobody Service Facility, Vrije Universiteit Brussel

Campylobacter jejuni is a Gram-negative microaerophilic bacterium and is the major cause of human gastro-enteritis worldwide. It is a zoonotic pathogen and chickens are generally accepted as the most important source of infections. Since the use of antibiotics in animal feeds is no longer an acceptable option and the prevention is limited to hygiene measures, novel control methods are needed.

The purpose of this research is to investigate whether colonization of chickens by *C. jejuni* can be controlled by passive vaccination in a cost effective manner by using nanobodies, the antigen-binding domains of heavy-chain antibodies. Anti-*Campylobacter* nanobodies were identified after immunization of an alpaca with heat-killed *C. jejuni* cells. The binding of these nanobodies on different *C. jejuni* strains, isolated from chickens and their environment as well as human clinical isolates, was verified. Nanobodies that are able to bind all of the tested strains were selected for further research. We presumed that these nanobodies recognize conserved epitopes expressed on *C. jejuni*. The ability of these nanobodies to agglutinate *Campylobacter* cells was tested. The nanobodies were multimerised by linking them to magnetic beads.

The results confirm that the nanobodies recognize antigens on living *C. jejuni* cells and that multimers of the nanobodies are able to agglutinate the *C. jejuni* cells. The nanobodies with a broad *Campylobacter* specificity can be interesting for diagnostic and therapeutic applications, such as for the reduction or inhibition of the colonization of chickens by *C. jejuni*.

Acknowledgements

The research that yielded these results, was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RF 13/6272 CampyNanoCure

Polar insertion of the bacterial envelope components in Brucella abortus

V. VASSEN[1], M. DEGHELT[1], J.-J. LETESSON[1] AND X. DE BOLLE[1]

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

Brucella abortus is a Gram-negative α-proteobacteria and one of the causing agents of brucellosis, a worldwide spread zoonosis. Like several Rhizobiales tested so far, B. abortus is characterized by unipolar growth, which is in contrast to the laterally-growing Escherichia coli. The regions of new envelope growth can be visualized by using Texas red succinimidyl ester (TRSE), which covalently binds to the outer membrane components, presumably proteins. If B. abortus is labeled with TRSE and growth is restarted, the newly incorporated (unlabeled) envelope is detected at the new pole and the constriction site, the proposed growth sites of B. abortus.

We are interested in the insertion of the different layers of the bacterial envelope represented by lipopolysaccharides (LPS), outer membrane proteins (Omp) and peptidoglycan (PG). To investigate the localization of bacterial envelope components, we made use of the collection of monoclonal antibodies directed against different surface structures available in the host laboratory.

Bacteria labeled with an antibody directed against the O-chain of Brucella smooth LPS (S-LPS) showed after 2 hours of growth a clearly unlabeled zone at one pole, representing the new incorporated S-LPS molecules. This pole could be identified as the new pole, since it is localized at the opposite pole than the old pole marker PdhS-mCherry. S-LPS molecules are also showed to be mobile in the outer membrane. In contrast, the same experiment using an antibody directed against Brucella Omp25 showed that this protein is (i) inserted at the new pole and the constriction site and (ii) not mobile in the outer membrane.

The polar insertion of S-LPS molecules was also demonstrated with an inducible S-LPS strain. In this strain, the production of the O-chain was induced with isopropyl-thiogalactoside (IPTG) in the rough mutant strain B. abortus Δgmd complemented with the gmd coding sequence under the control of the E. coli lac promoter. The newly synthesized S-LPS were only detected at the new pole.

B. abortus has only one penicillin-binding transpeptidase of the PBP2/3 family called FtsI. In B. abortus, YFP-FtsI foci are detectable at the new pole and the division site. This suggests that the biosynthesis of PG is occurring at these growth sides. Sites of maturation of newly incorporated PG were determined by the use of a fluorescent derivative of D-alanine called HADA. This dye is actively incorporated in the immature parts of the PG mesh. Short and long pulse labeling with HADA showed that the active side of PG synthesis is localized at the new pole and the constriction site. Mostly, this site was co-localized with YFP-FtsI. In an exponential growing culture, 82.3% of the bacteria showed an incorporation of new PG at the new pole, representing newborn cells starting to grow. Within this, 85.7% of the bacteria showed a dispersed signal covering the whole bacterial surface. This is probably due to the maturation process allowing the insertion of new PG units in the existing mesh that was proposed to occur in young daughter cells.

Acknowledgements
V. Vassen and M. Deghelt are/were recipient of a FRIA fellowship.

Synthetic Biology and Biocontrol with Predator Yeasts: an emerging system

J. WENDLAND

Vrije Universiteit Brussel, Belgium

Predatory yeasts are unique in their ability to form small penetration pegs, with which they are able to penetrate and, consequently, kill fungal prey cells, such as *Saccharomyces cerevisiae,* and *Candida albicans*. They were first identified only in 1997 by André Lachance. The predators are ascomycete fungi and form the genus *Saccharomycopsis*.

The major goals of our studies are to understand the molecular mechanisms of predation of *Saccharomycopsis,* which include peg formation, prey-penetration and utilization of prey compounds and on the other hand to explore the host range of individual *Saccharomycopsis* species. These species potentially represent a new weapon in the arsenal of biocontrol agents that may well be suited to be used against fungal plant pathogens.

We have generated high quality draft genome sequences of five predator species. For this Illumina and PacBio sequencing was performed. Predacious behaviour is initiated by nutrient deficiency as the *Saccharomycopsis* species are auxotrophic for methionine. This is wired in the genome by loss of genes that e.g. *Saccharomyces cerevisiae* uses for sulphate uptake and assimilation. Genome data also provides evidence that predator yeasts are members of the CTG clade, that is they decode the CTG codon into serine instead of leucine.

FACS analyses indicate that these species are haploid. The life cycle of predator yeasts includes mating and sporulation. Interestingly, mating occurs just prior to sporulation.

Genome data also provides hints of amplified gene families that encode enzymes that may promote predator-prey attachment, cell wall dissolution, and nutrient acquisition. These gene families encode flocculins, chitinases and proteases.

Chromatin capture sequencing indicated the position of centromeric loci that were otherwise not detected due to lack of synteny to other annotated genomes.

We are currently establishing molecular tools to initiate gene-function analyses. For this we have established a synthetic marker gene, *SAK1*, providing resistance to the antibiotic G418.

Current progress will be presented.

Acknowledgements
This research is financially supported by the EU-ITN-FungiBrain and the Vrije Universiteit Brussel.

References
LACHANCE M.A. AND PANG W.M. 1997. Predacious yeasts. Yeast 13:225-232.

Differences between cell responses to various biofilm dispersion methods in *B. cenocepacia*

J. WILLE[1], E. TEIRLINCK[2], K. BRAECKMANS[2] AND T. COENYE[1]

[1]Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium 2 Laboratory for General Biochemistry and Physical Pharmacy, Ghent University, Ghent, Belgium

Biofilm living bacteria are more resistant to antibiotics compared to planktonic cultures resulting in treatment failure. A method to increase antibiotic efficacy is to induce biofilm disruption. These disruptions can either be spontaneous, chemically induced or physical. In spontaneous dispersion, bacteria will leave the biofilm because of environmental changes due to the metabolic activity of the biofilm. Chemical induced dispersion occurs after the administration of a chemical that will generate stress. In physical disruption, a force will disrupt the biofilm by erosion or sloughing. Our aim is to study the kinetics of dispersion in *Burkholderia cenocepacia* biofilms and to determine whether there are profound differences between cell responses in the various disruption methods.

To determine spontaneous dispersion in *B. cenocepacia* J2315, biofilms were grown in microtiter plates using different growth media (LB and Mueller Hinton). Cell numbers of both supernatant and biofilm were quantified every four hours by plating. An increase in cell number in the supernatant, while biofilm cell numbers would stagnate or decline, could indicate dispersion. Such an increase was noticed in supernatant after 4 h incubating until 20 h of incubation.

To demonstrate chemical induced dispersion, different chemical agents (NaCl, MgCl2, CaCl2, sodium nitroprusside (SNP), SDS, Triton X-100, Tween 80 and EDTA) were chosen based on their biofilm dispersion capacity in other organisms. To determine whether these compounds have growth inhibiting and anti-microbial activities on *B. cenocepacia*, MIC values and sub-inhibitory anti-microbial activity were determined on planktonic cultures. Multiple sub-inhibitory and non-anti-microbial concentrations are now being tested on *B. cenocepacia* J2315 biofilms to induce dispersion.

To induce physical disruption, Vapor Nano Bubbles (VNB's) were chosen. VNB's are created when gold nano particles (AuNP) are irradiated by a laser. This leads to thermal energy that will vaporize the water around the AuNP resulting in a vapour nano bubble around the AuNP. Coating a biofilm with AuNP's followed by a laser irradiation which creates VNB's, causes a disruption of the biofilm which was noticed via microscopy and is quantified via plating.

To be able to do high throughput cell quantification, cell viability flow cytometry has been optimized for *B. cenocepacia* J2315.

Exploration of artisan carrot juice fermentation as alternative probiotic carrier

S. WUYTS[1,2], E. OERLEMANS[1], I. CLAES[1], I. DE BOECK[1], S. MORETTI[1], S. WECKX[2], B. LIEVENS[3], L. DE VUYST[2] AND S. LEBEER[1]

[1]University of Antwerp, Research Group Environmental Ecology and Applied Microbiology, Department of Bioscience Engineering, Groenenborgerlaan 171, B-2020 Antwerp, Belgium; [2]Vrije Universiteit Brussel, Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Pleinlaan 2, B-1050 Brussels, Belgium; [3]KU Leuven, Campus De Nayer, Laboratory for Process Microbial Ecology and Bioinspirational Management (PME&BIM), Department of Microbial and Molecular Systems (M^2S), Jan De Nayerlaan 5, B-2860 Sint-Katelijne-Waver, Belgium

Spontaneous fermented vegetables have regained popularity among chefs and home-fermenting enthusiasts. This is probably due to their rich flavours and postulated health benefits. However, the microbiology of these functional foods remains understudied, especially in the light of the latest next-generation sequencing technologies.

Therefore, in this study the microbial characteristics and dynamics of more than 200 spontaneous carrot juice fermentation samples collected from a Michelin star restaurant, two laboratory fermentations and a citizen science fermentation project (Ferme Pekes) were examined, using a combined culture-dependent, culture-independent and metabolite target analysis approach.

The results indicated that the fermentation process was consistent in progress and had a clear tipping point between 3 to 10 days. Culture-dependent methods showed that high numbers of lactic acid bacteria (LAB) were found, even up to 10^9 CFU/mL, while *Enterobacteriaceae* were outcompeted in almost all fermentations. This result indicated the high potential of vegetable juices as an alternative matrix for probiotic administration. The dynamics of the fermentation process were further assessed using high-throughput 16S ribosomal RNA (rRNA) gene sequencing and showed that first *Leuconostoc* and then *Lactobacillus* species mediated the carrot juice fermentation process. Furthermore, the presence of *Weissella* and *Lactococcus* species could be detected in some fermentations. Finally, LAB isolated from these spontaneous fermentation processes showed different cytokine-inducing and *Salmonella*-inhibiting capacities. In light of this, fermenting carrot juice could be a way to promote contact with diverse potential probiotic strains with multiple immune-modulating actions.

Mechanism of radiation resistance in *Arthrospira* sp. PCC 8005

A. YADAV[1,2], P. JANSSEN[1] , N. LEYS[1] AND A. CUYPERS[2]

[1]Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium, [2]University of Hasselt, Belgium

The oxygenic, photosynthetic cyanobacterium *Arthrospira* typically resides in alkaline environments (e.g. soda lakes) and has a wide array of uses in the natural and commercial world. It produces essential fatty acids and a range of minerals, pigments, vitamins, and carbohydrates while being edible owing to a low purine to protein ratio. Being such a useful source of food and oxygen, via solar energy-driven carbon fixation and oxidation of water, it is intensively studied by the MIC group at SCK•CEN in frame of MELiSSA, an ESA-funded project on life support in Space. Our research aims to define optimal growth conditions of *Arthrospira* to conserve its nutritious properties and oxygenic and carbon-reducing capacities in radiation-intensive space environments. Particular challenges to study this organism are its multicellularity and gliding mobility, a complex, thick cell wall, a genome stocked with multiple CRISPR/cas elements and a plethora of restriction-modification systems preventing DNA transformation hence genetic manipulation, and assay-interfering autofluoroscence.

In this work we want to study the genetic and biochemical pathways involved in the resistance of strain PCC 8005 - used in the MELiSSA loop - to extreme gamma radiation (up to 5 kGy). We are focusing on trancriptomics (including ncRNAs), DNA damage, protein modification, ROS avoidance and detoxification, and antioxidants composition. Our primary approaches are to establish a catalogue of all the genes and pathways known for other IR (ionizing radiation) resistant organisms, to test IR-resistance in different *Arthrospira* strains and species, to compare their genomes and relate any genomic differences with a variable IR sensitivity towards IR, and to study molecular and cellular responses of *Arthrospira* exposed to IR during at least one cycle of photosynthetic growth i.e. under light.

To study the effect of radiation on growth and morphology and to check whether IR-resistance is a general trait in *Arthrospira sp*. or not, 15 strains of *Arthrospira* isolated from different geographic locations were exposed to increasing doses of radiation and were analysed for growth recovery and morphological changes after irradiation. From this we conclude that IR-resistance is not a common trait for *Arthrospira* species. We found that strain O.9.13 was most sensitive to IR while strains *SAG* and *Safia* are more resistant than *Arthrospira* sp. PCC 8005. The two morphotypes of *Arthrospira* sp. PCC 8005 (straight versus helical trichomes) were also shown to have different sensitivities towards IR. Furthermore, there is no morpholgical change observed immediately after radiation except for some filament breakage. These strains will be studied by us further for radiation-induced cellular and molecular effects using LC-MS/ESI-TOF metabolic profiling and TEM microscopy.

Functional characterization of the TesA of *Mycobacterium bovis BCG*

D. YANG[1], P. SOUMILLION[2], S. ZENG[1], A. WOHLKÖNIG[3], M. S. KHAN[2], G. VANDENBUSSCHE[4] AND V. FONTAINE[1]

[1]Unit of Pharmaceutical Microbiology and Hygiene, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium; [2]Biochemistry, Biophysics, and Genetics of Microorganisms, Institute of life sciences, Université Catholique de Louvain (U.C.L.), Louvain-la-Neuve, Belgium; [3]VIB Structural Biology Research Center, Vrije Universiteit Brussel (V.U.B), Brussels, Belgium; [4]Laboratory for the structure and function of biological membranes, Faculty of science, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium

Mycobacterium bovis BCG is closely related to the pathogenic *Mycobacterium tuberculosis,* the causative agent of human tuberculosis. Both harbor a thick cell wall with a high lipid content, contributing to the mycobacterial pathogenicity. Among these lipids, the phthiocerol dimycocerosate (PDIM) is considered as one of the major virulence element. The biosynthesis of PDIM is complex and requires involvement of multiple enzymes, encoded in the "virulence gene cluster". Among them, the Thioesterase A (TesA), is a type II thioesterase involved in the synthesis of the phthiocerol chain. The functional and structural basis of TesA remains unclear. In our study, we constructed a new plasmid using a modified pET-15b vector allowing to express His-tag protein and to cut ultimately the His-tag using the HRV 3C protease. We verified the recombinant TesA protein by mass spectrometry and determined its enzymatic activity. We tested four substrates: Palmitoyl-CoA, decanoyl-CoA, malonyl-CoA and HMG-CoA. We observed that BSA addition improved the thioesterase reaction. The activity of TesA with palmitoyl-CoA followed a typical Michaelis-Menten kinetic, on the opposite to our observation with decanoyl-CoA.

The *Mycobacterium bovis* BCG chaperonin 60.1 is not involved in hypoxic dormancy but affects biofilm growth

S. ZENG, C. RENS, D. YANG AND V. FONTAINE

Unit of Pharmaceutical Microbiology and Hygiene, Université Libre de Bruxelles (ULB), Brussels, Belgium

Background: *Mycobacterium bovis* BCG is closely related with the pathogenic *Mycobacterium tuberculosis*. Both contain 2 chaperonin 60s genes (*cpn*60), namely *cpn*60.1 and *cpn*60.2. Although not essential for bacterial survival, Cpn60.1 plays a role in maintaining cell wall integrity potentially due to its involvement in outer membrane PDIM lipid biosynthesis. It is still unknown if Cpn60.1 plays a role in hypoxic dormancy.

Objectives: First, to investigate whether Cpn60.1 is vital for the bacterial adaptation into dormancy under hypoxic stress. Secondly, to understand the roles of Cpn60.1, PGL and PDIM in mycobacterial biofilm growth.

Methods: Using the Wayne dormancy model, we examined the growth and viability of *Wild type (Wt)*, *Δcpn60.1* and *complemented Δcpn60.1 M. bovis* BCG. Mutants *M. bovis* BCG deficient in PDIM and PGL lipids were also tested in this model. We compared the transcription levels of dormancy related genes from the various strains by real-time PCR. Furthermore, antibiotic susceptibility assays were performed to assess the impact of Cpn60.1 and PDIM on drug resistance. In addition, we cultured all the mutants strains in a Petri dish biofilm growth model to understand the role of Cpn60.1, PDIM and PGL in biofilm formation. Additionally, the impact of glycerol concentration was also investigated. Finally, sliding motility test were performed in order to assess the bacterial surface hydrophobicity.

Results: The growth and survival of *Δcpn60.1 M. bovis* BCG are similar to that of *Wt* BCG in the Wayne dormancy model. However, *Δcpn60.1* BCG is more susceptible to some antimycobacterial drugs. Cpn60.1, PDIM and PGL lipids are involved in mature biofilms formation. Glycerol concentration differently affected *Wt* BCG and *Δcpn60.1* BCG biofilm growth.

Conclusions: Cpn60.1, PDIMs and PGLs are nonessential for the mycobacterial adaptation into hypoxic dormancy. However, Cpn60.1 seems important for some drug resistance. Both PDIM and PGL contribute to biofilm maturation. Due to different surface hydrophobicity, various BCG strains require different conditions for optimal biofilm growth.

Abbreviations: PDIMs, phthiocerol dimycocerosates; PGLs, phenolic glycolipids