BELGIAN SOCIETY FOR MICROBIOLOGY

NATIONAL COMMITTEE FOR MICROBIOLOGY
of
THE ROYAL ACADEMIES OF SCIENCE AND
THE ARTS OF BELGIUM

Vlaams
Kennis- en
Cultuurforum

ANALYZING COMPLEX MICROBIAL COMMUNITIES AND THEIR HOST MICROBE INTERACTIONS

December 11th, 2009

House of the Academies
Hertogsstraat 1, Rue Ducale
Brussels

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PROGRAMME

08.30 Registration desk open – Poster installation

09.00 Welcome & Opening

In memoriam of the late Prof. Nicolas Glansdorff, honorary member of NCM
(Em. Prof. Dr. Raymond Cunin – VUB)

09.10 **Roy Goodacre,** School of Chemistry, University of Manchester, UK

"Investigating abiotic stresses on microbiological systems using metabolomics"

09.50 **Pierre-Olivier Vidalain**, Laboratoire de Génomique Virale et Vaccination, Institut Pasteur, France

"Comparative mapping of virus-host protein-protein interactions: some rules and many more questions"

10.30 Break

11.00 **Jeroen Raes,** Vrije Universiteit Brussel, Institute for Molecular Biology & Biotechnology, Belgium

"Investigating complete microbial communities using metagenomics from the oceans to the human body"

11.40 **Marc Van Ranst,** Rega Institute - Department of Microbiology and Immunology

"Pandemic Novel A/H1N1v/2009 Mexican Swine A-Flu: what's in a name ?"

12.20 General assembly BSM

12.45 Lunch – Poster viewing & poster discussion groups

Parallel session 1 – Virology

14.30 **Matthias Ackermann,** Institute of Virology, University of Zurich, Switzerland

"The case of ovine herpesvirus-2: Death penalty for missing classes in co-evolution?"

15.10 Short lecture (5) of selected abstracts

Parallel session 2 – Bacteriology

14.30 **Vincent Denef,** Department of Earth and Planetary Science, University of California Berkeley, USA

"Small changes, big deal? Linking genotype to the ecology of closely related bacteria in ecosystem context"

15.10 Short lecture (5) of selected abstracts

16.30 General conclusions and distribution of prizes of selected posters

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Poster awards

In memorian

Nicolas Glansdorff (1937–2009)

Founding member of the Belgian Society for Microbiology
Honorary member of the National Committee for Microbiology

A few months ago, Nicolas Glansdorff, accidentally passed away while hiking alone across Norwegian Lapland. Hiking was a pastime he had loved throughout his life, and he had a passionate interest in the Arctic region, having travelled from Alaska to Siberia. Nicolas was fond of places such as those where humans are confronted with the harshness of nature, and he especially enjoyed living this experience as a solitary hiker.

He was Professor Emeritus at the Vrije Universiteit Brussel. Originally trained as a botanist, he soon switched to bacterial genetics, and later to the microbiology of extreme environments. To his students, colleagues and friends, he was the essence of a learned man, one who inspired trust. With his charm and wit, in his teaching, he brought to life the mechanisms of evolution and theories on the origin of life. An expert at combining poetry and philosophy with extreme scientific rigor, he led his audience to profoundly contemplate the mechanisms that sustain life on our planet and possibly elsewhere! He will be deeply missed by microbiologists around the world.

ABSTRACTS OF INVITED LECTURES

Investigating abiotic stresses on microbiological systems using metabolomics

Felicity Currie[1], Emma Wharfe[1], Soyab Patel[1], Rick Dunn[2], David Broadhurst[1], Cate Winder[1], and Roy Goodacre[1,2].*

[1]School of Chemistry and [2]Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester, M1 7ND, UK

**Roy.Goodacre@manchester.ac.uk*

Human pharmaceuticals are readily detected in waste water treatment plants, rivers and estuaries. Whilst levels are not yet high enough to cause immediate harm to aquatic life, it is widely acknowledged that there is insufficient information available to determine whether exposure to low levels of these substances over long periods of time is having an impact on the microbial ecology of these environments. In order to investigate the effect on the metabolic potential of the microbial community we have been adopting a metabolomics approach using various analytical platforms including vibrational spectroscopic approaches for generating spatial metabolic fingerprints, gas chromatography-mass spectrometry (GC-MS) for metabolic profiling and direct infusion mass spectrometry (DIMS) for lipid profiling. Analysis of environmentally relevant microbes and algae will be presented. We shall show that Propranolol had significant effects on the lipid components of *Pseudomonas putida* cells [1], and in particular large changes in phospholipid head groups in order to maintain correct membrane fluidity (so called homeoviscous adaptation). In order to investigate this further, Fourier transform infrared (FT-IR) microspectroscopy was used to generate detailed metabolic fingerprinting maps from the alga *Micrasterias hardyi* [2]. These chemical maps revealed dramatic effects on the distribution of various chemical species throughout the algae. This illustrates the additional power of spatial metabolic fingerprinting for investigating abiotic stresses on complex biological species.

[1] Currie, F., Broadhurst, D.I., Dunn, W.B. & Goodacre, R. (2008) Discrimination of pharmaceutical exposure measured by GC-MS and FT-IR of *Pseudomonas putida* KT2440 (UWC1). Under review.

[2] Patel, S.A., Currie, F., Thakker, N. & Goodacre, R. (2008) Spatial metabolic fingerprinting using FT-IR spectroscopy: investigating abiotic stresses on *Micrasterias hardyi*. *Analyst* **133**, 1707-1713.

Comparative mapping of virus-host protein-protein interactions: some rules and many more questions

Pierre-Olivier Vidalain

Laboratoire de "Génomique Virale et Vaccination".
Institut Pasteur de Paris, 28 Rue du Docteur Roux, Paris, 75724 Cedex 15, France

Despite medical progress and vaccination, many infectious diseases have emerged or re-emerged since the second part of the twentieth century. The recent outbreak of Chikungunya virus in La Réunion illustrates the constant threat that represent emerging viruses, and the extreme limitations of our therapeutic arsenal. A better knowledge of viral protein properties and interactions with cellular components is a prerequisite for the identification of new drug targets. In a collaborative effort between Institut Pasteur (Paris) and IFR-128 BioSciences (Lyon), we have developed an integrated pipeline dedicated to the large-scale mapping of virus-host protein-protein interactions using high-throughput yeast two-hybrid (HT-Y2H) technology. The goal of the "Infectious Mapping Project" (I-MAP) is to identify potential drug targets through large-scale analysis of interactions between viral and cellular protein networks. First, a reference collection of viral encoding sequences (ORFs) is built into a versatile cloning system. In this setting, a single in vitro recombination reaction is sufficient to transfer any viral ORF from the collection into any expression vector and perform functional analysis of viral proteins using standardized methods. Then, large-scale mapping of virus-host protein-protein interactions is achieved using HT-Y2H technology. Interaction maps from several viruses of the same species, genus or family are combined and compared to eliminate background and identify cellular proteins, complexes or pathways that are either shared or specific viral targets. The most relevant virus-host interactions identified with this approach are further analyzed using functional in vitro assays that are currently under development in our labs. Finally, random libraries of constrained peptides and reverse two-hybrid technology are used to isolate interaction disrupting peptides and interaction defective alleles that should be instrumental to develop new antiviral lead compounds. Preliminary data and potential applications to *Paramyxoviridae* (measles virus, human parainfluenza virus type 3) and *Flaviviridae* (hepatitis C virus) will be discussed.

Investigating complete microbial communities using metagenomics from the oceans to the human body

Jeroen Raes

Vrije Universiteit Brussel, Institute for Molecular Biology & Biotechnology, Belgium

Metagenomics allows a large-scale, unbiased insight in the functional and phylogenetic composition of complex microbial communities. However, given the complexity of these data, computational analysis is a major bottleneck. Here, I will discuss our experiences in developing methods for the analysis of metagenomics data and their application in *eg.* the global ocean and human-associated communities (the human microbiome) such as the intestinal tract. These approaches should lead the way towards an 'eco-systems biology' approach to the study of microbial communities and provide great opportunities for the study of the role of commensals in human disease.

Pandemic Novel A/H1N1v/2009 Mexican Swine A-Flu: what's in a name?

Marc Van Ranst

Rega Institute - Department of Microbiology and Immunology, Leuven, Belgium

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Source : www.iph.fgov.be

Pandemic (H1N1) 2009 is a new influenza virus that has never circulated among humans before. After outbreaks in North America early in 2009, the virus spread rapidly around the world. Pandemic influenza is transmitted like seasonal influenza but people have virtually no immunity to it. Mitigating its effects is a public health priority.

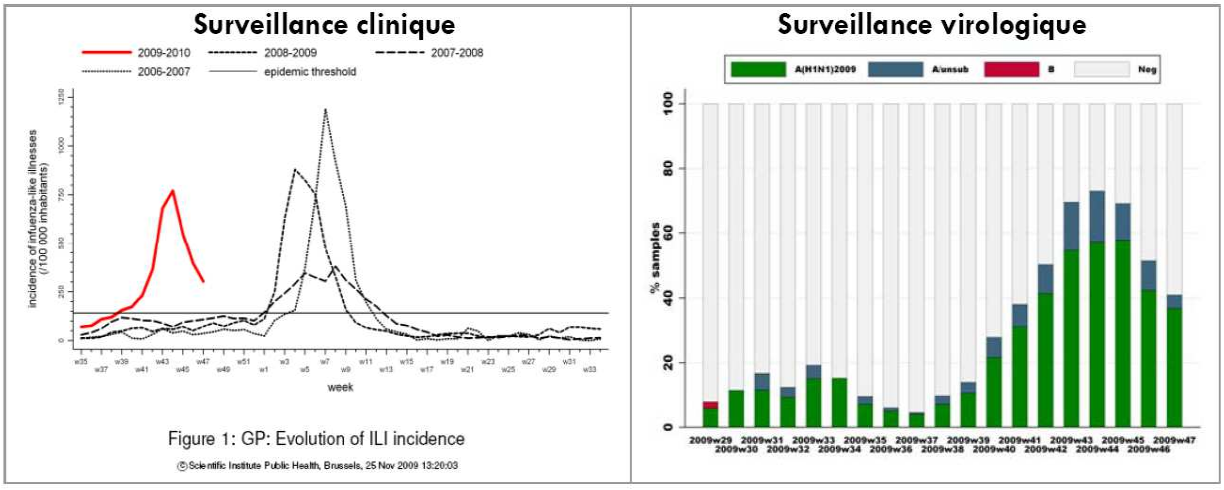

Figure 1: GP: Evolution of ILI incidence

General evolution: in Belgium, the influenza epidemic A(H1N1)2009 started week 40 (beginning of October 2009), progressed until week 44 (end of October) and diminished since week 45 until week 47. However, the criteria for an epidemic are still accomplished, and the virus circulates in the population. Consequently, the epidemic is not yet finished. Moreover, in October the epidemic developed in children, saving relatively well the adults. An important proportion of the population remains consequently susceptible to develop the disease. The hypothesis which explains the retardation might be All Saints Day holiday period (week 45). In addition, influenza prefers colder and dryer climate conditions, which is different from what we had last weeks.

The Case of Ovine Herpesvirus-2: Death Penalty for Missing Classes in Co-Evolution?

Mathias Ackermann

University of Zurich, Switzerland

Sheep-associated malignant catarrhal fever (SA-MCF) is a lethal disease of various ruminants, characterized by vasculitis, necrosis, and accumulation of activated, dysregulated cytotoxic lymphocytes in various tissues. Ovine gamma herpesvirus 2 (OvHV-2) is considered as the causative agent of SA-MCF. However, the pathogenic basis of the disease remains mysterious.

We hypothesized that the gene expression patterns of OvHV-2 and the relative abundances of host cell transcripts in lymphnodes may be used to identify pathways that may help to explain the pathogenesis of MCF. Therefore, viral and host cell gene expression patterns in lymph nodes of animals with MCF and healthy controls were analyzed by microarray. Two regions on the viral genome were transcriptionally active, one encoding a homologue to the latency-associated nuclear antigen (ORF73) of other gamma herpesviruses, the other with no predicted open reading frame. On the host's side, a vast number of transcripts related to inflammatory processes, lymphocyte activation, cell proliferation and apoptosis were detected at different abundances.

In my presentation, I will explain how these observations fit into the picture of our current understanding of the disease. Notably, it seems that participating or missing out in co-evolution may be an important factor in the process.

Small changes, big deal?
Linking genotype to the ecology of closely related bacteria in ecosystem context

Vincent Denef

University of California, Berkeley, USA

The availability of genome sequences for closely related microorganisms has at the same time clarified and complicated our view of species delineation. While 16S rRNA gene based classification generally corresponds to genomic and ecological differences, organisms grouped as one species often display both significant gene content variation as well as resource partitioning. In order to address the relationship between differences in gene content and sequence and ecological divergence in ecosystem context, we investigated links between genotype and ecology of two populations of *Leptospirillum* Group II bacteria in comprehensively characterized natural acidophilic biofilm communities. These populations share 99.7 % 16S rRNA gene sequence identity and 95 % average amino acid identity between their orthologs. One predominates during early colonization and the other typically proliferates in later successional stages, forming distinct tens to hundreds μm diameter patches. Absence of protein expression, measured via mass spectrometry-based community proteomics, of most population-specific gene content supports the argument that much of the laterally transferred gene pool found in closely related isolate genomes is of a transient, non-adaptive nature. Evolutionary signatures, and population-resolved expression patterns emphasize how sequence and expression variation of shared genes contributes to ecological divergence. As such, we highlight an interesting parallel to higher organisms, where evolution of gene expression has been suggested as an important factor in species differentiation. Ecological divergence between these two populations can be viewed as an example of *r*- vs. *K*-selection. The early colonizer proliferates optimally in the absence of competition from other organisms, thanks to adaptations that allow it to rapidly propagate in the AMD environment. Adaptations of the late colonizer allow its proliferation in conditions with high inter- and intra-specific competition for resources. Such distinct ecological strategies mediated by subtle genomic differences between closely related organisms, which are often found to co-exist in natural microbial systems, exemplify how fine-scale variation within ecological functional groups can have significant effects on community structure and functioning.

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M.L.S. (Medisch Labo Service) is specialised in the distribution of disposable laboratory material: plastic and glass products for collection, storage and analysis of samples. We herewith enumerate some important brands in our range: Plastiques Gosselin (Petri-dishes, containers, tubes, …), Vacuette (blood collection), TPP (Tissue Culture), Socorex (pipettes), Thermo Labsystems (Finnpipettes and tips), Knittel (microscope slides), Swann Morton (surgical scalpel blades), Treff (tips), R.A.L. (reactifs), hygienic products Cemex, Medica (gloves, aprons, food protection), disinfectants (Schumacher), RBS (soap and cleaning products for laboratory use), Biohit, Copan, Simport, Cellpath, National Scientific, …

M.L.S., established in 1986, is in full expansion and counts already 24 employees. This success is largely due to our excellent service, our wide range of products and the enthusiasm of our employees.

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LIST OF POSTERS

(alphabetic order of first author)

Virology:

V1 : Antivirals
V2 : DNA viruses
V3 : RNA viruses

Bacteriology:

B1 : Cell and Molecular Biology of Microbes
B2 : Environmental and Evolutionary Microbiology
B3 : Microbial Pathogenicity
B4 : Genes and Genomes
B5 : Physiology and Biochemistry

	A full-length infectious clone of *Beet soil-borne virus* indicates the dispensability of the RNA-2 for virus survival *in planta* and symptom expression on *Chenopodium quinoa* leaves	
32.	*Thierry De Baere, Eva Van Meervenne, Gerald Dupont, Sophie Bertrand* and *Marc Yde* **Comparison of two DNA-based typing techniques for the investigation of clonal relationship between *Listeria monocytogenes* isolates in Belgium.**	B2
33.	*M. De Barsy, C. Nicolas, A. Jamet, D. Filopon, G. Laloux, J.-J. Letesson and X. De Bolle* **ORFeome-based screening allowed identification of RicA, a Rab2-binding protein involved in intracellular trafficking control of *Brucella abortus***	B3
34.	*Tine De Burghgraeve, Suzanne Kaptein, Susan Obeid, Jan Paeshuyse, Maria Preobrazhenskaya, Michel Jacobs, Andrea Gamarnik and Johan Neyts* **An analogue of the antibiotic teicoplanin inhibits dengue virus replication in vitro**	V1
35.	*P. De Carvalho Maalouf, A. Lambion, A. De Wever, E. Verleyen, W. Vyverman and A. Wilmotte.* **Cyanobacterial molecular diversity and geographical distribution in microbial mats from Antarctic lakes**	B2
36.	*Haydée De Clippeleir, Siegfried E. Vlaeminck, Marta Carballa and Willy Verstraete* **Two actions to restore the microbial activity balance between aerobic and anoxic ammonium-oxidizing bacteria in a sequencing batch reactor**	B2
37.	*De Jonghe Valerie, Coorevits An, Marchand Sophie, De Vos Paul, Heyndrickx Marc* **Proteolytic spoilage of *Pseudomonas* species throughout the dairy chain.**	B2
38.	*L. Delang, I. Vliegen, P. Leyssen and J. Neyts.* **In vitro selection and characterization of hepatitis C virus replicons double or triple resistant to various non-nucleoside HCV polymerase inhibitors.**	V1
39.	*Delbrassinne Laurence, Andjelkovic Mirjana, Botteldoorn Nadine, Jacques Mahillon and Joris Van Loco.* **Comparison of different media (TSA, BHI, MYP, Nutrient Agar)by LC-MS/MS method for maximising production of cereulide**	B5
40.	*Sofie De Meyer, Bram Vekeman and Anne Willems* **Exploring Rhizobia in wild native legumes in Belgium**	B2
41.	*Armando De Palma, Pieter Leyssen, Hendrik Thibaut, Patrick Chaltin, Johan Neyts* **Antivirals for the Global Eradication of Polio**	V1
42.	*Nick De Regge, Nina Van Opdenbosch, Hans J. Nauwynck, Stacey Efstathiou and Herman W. Favoreel.* **Interferon Alpha Induces Establishment of Alphaherpesvirus Latency in Sensory Neurons *in vitro***	V2
43.	*Derous, V., Hernalsteens, J.-P. , Deboeck, F. and De Greve, H* **Reliable method for directed gene inactivation in wild type *Escherichia coli* strains**	B4
44.	*M. Deruelle, H.J. Nauwynck, and H.W. Favoreel* **Pseudorabies virus US3- and UL49.5-dependent and -independent downregulation of MHC I cell surface expression in different cell types**	V2
45.	*P. De Schryver, A. Kumar Sinha, P. Singh Kunwar, K. Baruah, N. Boon, W. Verstraete, G. De Boeck, and P. Bossier.* **The bacterial storage compound poly-β-hydroxybutyrate increases growth performance and intestinal microbial diversity in European sea bass juveniles (*Dicentrarchus labrax*)**	B2
46.	*Benoît Desguin, Nordine Bakouche, Eric Viaene, Damien Dandoy, Bernard Hallet, Phillipe Goffin and Pascal Hols* **Lactate racemization is positively controlled by LarR in *Lactobacillus plantarum***	B5
47.	*Benjamin Dewals, Françoise Myster, François Massart, Alain Vanderplasschen* ***Ex vivo* bioluminescent detection of *alcelaphine herpesvirus 1* infection during malignant catarrhal fever induced in rabbits**	V2
48.	*De Weirdt, R., Vermeulen, G., Possemiers, S., Van de Wiele, T., Verstraete, W.*	B5

	Glycerol metabolism by the human colonic microbiota	
49.	*Melissa Beth Duhaime, Antje Wichels, Jost Waldmann, Frank Oliver Glöckner* **Modular Evolution and Ecogenomics of Marine Pseudoalteromonas Phage H105/1**	V2
50.	*G. Duytschaever, G. Huys, L. Boulanger, K. De Boeck and P. Vandamme* **.** **Comparative study of predominant fecal microbiota of a group of cystic fibrosis children and healthy siblings**	B3
51.	*Nabil Abdullah El Aila, Inge Tency, Geert Claeys, Bart Saerens, Ellen De Backer, Marleen Temmerman, Rita Verhelst, Mario Vaneechoutte* **Genotyping of *Streptococcus agalactiae* (group B streptococci) isolated from vaginal and rectal swabs of women at 35-37 weeks of pregnancy**	B3
52.	*Nabil Abdullah El Aila, Inge Tency, Geert Claeys , Hans Verstraelen, Bart Saerens, Guido Lopes dos Santos Santiago, Ellen De Backer, Piet Cools , Marleen Temmerman, Rita Verhelst, Mario Vaneechoutte.* **Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora**	B3
53.	Karim El Bakkouri, Francis Descamps, Marina De Filette, Anouk Smet, Els Festjens, Walter Fiers, and Xavier Saelens. **Universal M2e-based Influenza A Vaccine: Fc Receptors and Alveolar Macrophages Mediate Protection**	V1
54.	*Sylvie François, Sarah Vidick, Michaël Sarlet, Pawel Koteja, Daniel Desmecht, Philip G. Stevenson, Alain Vanderplasschen and Laurent Gillet.* **Comparative study of Murid gammaherpesvirus 4 infection in mice and in its natural host, the bank voles**	V2
55.	*Damien Geeraerts, Nathalie Goeders, Régis Hallez, R. Leplae, Julien Guglielmini and Laurence Van Melderen.* **Detection and analysis of new toxin-antitoxin systems**	B5
56.	*Angélique Gobet, Simone Böer, Susan M. Huse, Justus E.E. van Beusekom, Mitchell L. Sogin, Antje Boetius, Alban Ramette* **Microbial ecological patterns revealed by the application of 454 Massively Parallel Tag Sequencing on temperate coastal sediments**	B2
57.	*Charlotte Grootaert, Barbara Vanhoecke, Tom Van De Wiele, Willy Verstraete and Marc Bracke.* **Host-microbiota interactions related to fat storage: gut bacteria modulate the fasting induced adipose factor (FIAF) in a Caco-2 cell line.**	B3
58.	*Annick C. Gryspeerdt, A.P. Vandekerckhove, G.R. Van de Walle and H.J. Nauwynck* **Differences between the replication of non-neurovirulent and neurovirulent EHV1 strains in the upper respiratory tract *in vivo***	V2
59.	*Aurore Hamels and Jean-Yves Matroule* **Characterization of the Type IV secretion system in *Caulobacter crescentus***	B1
60.	*Hardenne Sophie, Modrie Pauline, Beuls Elise and Mahillon Jacques.* **The onset of pAW63 plasmid conjugation is potentially controlled by a quorum sensing system**	B1
61.	*Hermant Pascale, Michiels Thomas.* **Study and characterization of a type I interferon: IFN-ε**	V1
62.	*K. Heylen, N. Botteldoor, B. Pochet, V. Real, K. Dierick.* **Trends in antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* from Belgian meat products, 2004-2008**	B3
63.	*Falk Hildebrand, Axel Meyer, Adam Eyre-Walker* **Evidence of Selection upon genomic GC-content in bacteria**	B4
64.	*Hollants Joke, Leliaert Frederik, De Clerck Olivier, and Willems Anne.* **Endosymbiotic bacteria within Bryopsis species (Bryopsidales, Chlorophyta): Naming the actors**	B2
65.	*B. Hoorelbeke, M. Renders, T. Oki, Y. Igarashi, A. Takahashi, H. Tanaka, D. Schols and J. Balzarini.*	V1

	The use of SPR technology in the unraveling of the mode of action of carbohydrate binding agents as anti-HIV leads	
66.	*Elham Hosseini, Charlotte Grootaert, Willy Verstraete, and Tom Van de Wiele* **Mannan oligosaccharides and guar gum substrates alter intestinal microbial composition and activity in a 5-stage colon simulator**	B2
67.	*Dana Huskens, Kurt Vermeire, Albert T Profy and Dominique Schols.* **PRO 2000, a broadly active anti-HIV sulfonated compound, inhibits HIV infection by multiple mechanisms.**	V1
68.	*Muhammad Saiful Islam, Pieter Monsieurs, Felice Masrtoleo and Natalie Leys* **Ionising radiation is not inducing hydrogen peroxide oxidative stress in *Cupriavidus metallidurans* CH34**	B1
69.	*Paul J. Janssen, Nicolas Morin, Pieter Monsieurs, Max Mergeay, and Natalie Leys* **The Complete Genome Sequence of the Edible Cyanobacterium *Arthrospira* (Spirulina) *platensis* PCC 8005**	B4
70.	*S. Jourdain, V. Sputael, X. Malaviolle, O. Denis, M. Struelens, L. Van Melderen, A. Vergison and P. Smeesters* **Dynamic epidemiology of naso-pharyngeal flora in nursery schools attendees**	B2
71.	*Morgane Kempenaers; Martine Roovers; Yamina Oudjama; Karolina L. Tkaczuk, Janusz M. Bujnicki and Louis Droogmans.* **Identification and preliminary characterisation of a new archaeal tRNA (m^1A) methyltransferase belonging to the SPOUT family**	B4
72.	*Marguerite Kreit, Sophie Paul and Thomas Michiels* **Lack of protection mediated by IFN-β in primary neurons against viral infection**	V1
73.	*Yannick Lara, Diana Menzel, Geoffrey Codd and Annick Wilmotte.* **Whole genome amplification of environmental colonies of the cyanobacterium *Woronichinia***	B2
74.	*Céline Lété, Bénédicte Machiels, Nicolas Markine-Goriaynoff, Minoru Fukuda, Alain Vanderplasschen and Laurent Gillet.* **Identification of a new inactive spliced variant of the β-1,6-N-Acetylglucosaminyl transferase encoded by Bovine Herpesvirus 4**	V2
75.	*Li Z., Derous V., Deboeck F., Bouckaert J. De Greve H. and Hernalsteens J.-P* **NAD auxotrophy of wild type uropathogenic *Escherichia coli* UTI89 by a single amino acid change in NadB**	B1
76.	*Bénédicte Machiels, Benjamin Dewals, Frédéric Minner, Alain Vanderplasschen and Laurent Gillet.* **Deletion of vFLIP impairs Bovine herpesvirus 4 latency and promotes lytic cycle activation**	V2
77.	*Loïs Maignien, John R. Parkes, Barry Cragg, Helge Niemann, Stephanie Coulon, Andrey AkhmetzHanov, Phil Weaver, Nico Boon* **Environmental Constraints On Microbial Activity And Community Structure in Marine Mud Volcanoes From The Gulf Of Cadiz**	B2
78.	*M.J. Mano, Z., R. Fernandez-Carazo, Annick Wilmotte* **Polyphasic study of the diversity of cyanobacterial communities in the Sör Rondane Mountains, Eastern Antarctica**	B2
79.	*Mehari Tesfazgi Mebrhatu, Chris Michiels, Ariel Lindner, François Taddei, Laurence Van Melderen and Abram Aertsen.* **The type III restriction modification system of *Salmonella* Typhimurium interferes with Mrr endonuclease activity**	B1
80.	*B. Michel, B. Leroy, V. Stalin Raj, F. Lieffrig, J. Mast, R. Wattiez, A. Vanderplasschen and B. Costes*. **The genome of cyprinid herpesvirus 3 encodes 40 proteins incorporated in mature virions**	V2
81.	*Kristel Mijnendonckx, Ann Provoost, Pieter Monsieurs, Natalie Leys, Max Mergeay, Jacques Mahillon and Rob Van Houdt.* **Identification, characterization and distribution of insertion sequence elements in *Cupriavidus metallidurans* CH34**	B4
82.	*Mirabella A., Godefroid M., De Bolle X. and Letesson J.-J.*	B3

	MucR regulates both flagellar genes expression and virulence in *Brucella melitensis* 16M	
83.	*Simone Musiu, Jan Paeshuyse, Philippe Lemey, Johan Neyts.* **Viral Genome Dynamics during Antiviral Resistance Selection: A First Glimpse into Viral Evolution**	V1
84.	*Naegels, E., Zhou, K., Vanoirbeek, K., Aertsen, A. and Michiels, C.W.* **Analysis of intragenic tandem repeats in *Escherichia coli***	B1
85.	*Phu Nguyen Le Minh and Daniel Charlier.* **Binding of RutR to the *carAB* operon of *Escherichia coli* K-12 and its interplay with PepA**	B1
86.	*Op de beeck A., Draps M.-L., Baurin S.,Timmerman D., Caillet-Fauquet P, Laub R.* **Active B19 virions production in hepatoblastoma and heparocarcinoma cell lines: amplification and genomic stability.**	V2
87.	*Eveline Peeters, Nuno Peixeiro, Marc Nadal, Patrick Forterre, Guennadi Sezonov, David Prangishvili and Daniel Charlier.* **Molecular mechanisms of regulation by Ss-LrpB, a transcriptional regulator from the archaeon *Sulfolobus solfataricus***	B5
88.	*Peeters K., Verscheure S., Verleyen E., Hodgson D, Willems A.* **Study of *Flavobacterium* strains isolated from Antarctic aquatic and terrestrial samples**	B2
89.	*Thomas Rolain and Pascal Hols.* **Study of the functional role of peptidoglycan hydrolases in *Lactobacillus plantarum***	B1
90.	*Roovers, M., Caillet, J., Tisné C., Bujnicki, J. and Droogmans, L.* **Characterization of the tRNA methyltransferase TrmK of *Bacillus subtilis***	B1
91.	*Ningning Song, Eveline Peeters, Amelia Vassart and Daniel Charlier* **High resolution contact probing of the DNA binding of SaLysM, a transcription regulator from the archaeon *Sulfolobus acidocaldarius*, involved in lysine biosynthesis regulation**	B5
92.	*Victor Stalin Raj, Benjamin Michel, Mickaël Vandecan, Jan Mast, Michaël Sarlet, Daniel Desmecht, François Lieffrig, Charles Melard, Alain Vanderplasschen and Bérénice Costes* **Removal of epidermal mucus enhances Cyprinid herpesvirus 3 entry through the skin of *Cyprinus carpio***	V2
93.	*Matthieu Terwagne, Julien Lemaire, Aurélie Mirabella, Chantal Deschamps, Xavier De Bolle and Jean-Jacques Letesson* **AiiD, identification of a quorum quenching enzyme which could contribute to the stealthy strategy of *Brucella melitensis* 16M**	B3
94.	*Hendrik Jan Thibaut, Lonneke van der Linden, Jan Paeshuyse, Armando De Palma, Leire Aguado, Frank Van Kuppeveld, María-Jesús Pérez-Pérez and Johan Neyts* **A novel 9-arylpurine acts as a selective inhibitor of *in vitro* enterovirus replication possibly by targeting virus encapsidation**	V1
95.	*Thierie Jacques and Penninckx Michel* **Secondary metabolites produced by complex bacterial flocs cultivated in continuous culture: a new Crabtree effect.**	B1
96.	*Johan Timmermans, Ethel Seyll, Pierre Englert, Aimable Rengero Lema, Sabrina Bousbata and Laurence Van Melderen* **Two proteins of unknown function, TldD and TldE, regulate central carbon metabolism in *E. coli***	B5
97.	*Wander Van Breedam, Hanne Van Gorp, Jiquan Q. Zhang, Paul R. Crocker, Peter L. Delputte and Hans J. Nauwynck* **The M/GP_5 glycoprotein complex of porcine reproductive and respiratory syndrome virus binds the sialoadhesin receptor in a sialic acid-dependent manner.**	V3
98.	*A. Vandekerckhove, A. Gryspeerdt, S. Glorieux, G.R. Van de Walle, H.J. Nauwynck* **Replication of non-neurovirulent versus neurovirulent equine herpesvirus type 1 strains: a study in equine respiratory mucosa explants**	V2
99.	*Pieter Van den Abbeele, Tom Van de Wiele, Willy Verstraete and Sam Possemiers*	B3

	The host selects mucosal and luminal teams of co-evolved, submissive gut microbiota	
100	*D. Vandenbosch, H. J. Nelis and T. Coenye.* ***In vitro* investigation of the activity of miconazole against biofilms of various *Candida* species**	B3
101	*Céline Van den Broeke, Maria Radu, Matthias Deruelle, Hans Nauwynck, Jonathan Chernoff & Herman W. Favoreel.* **Pseudorabies virus US3-mediated reorganization of the actin cytoskeleton is mediated by group A p21-activated kinases**	V2
102	*C. Van der Henst, F. Beaufays, B. Hallet, J-J. Letesson and X. De Bolle.* **Characterisation of PdhS, an essential and polarly localized histidine kinase in *B. abortus***	B5
103	*Vanderkelen, L., Goedgezelschap, J., Callewaert, L., Van Herreweghe, J., Aertsen, A. and Michiels, C.W.* **The periplasmic protein PliG bestows g-type lysozyme resistance in *Escherichia coli***	B3
104	*Johan Vande Voorde, Annelies Bronckaers, Sandra Liekens and Jan Balzarini* **Does the microenvironment shield tumors from chemotherapeutics?**	B3
105	*Hanne Van Gorp, Wander Van Breedam, Peter L. Delputte and Hans J. Nauwynck.* **The porcine reproductive and respiratory syndrome virus requires trafficking through CD163 positive early endosomes, but not late endosomes, for productive infection.**	V3
106	*Lies M. E. Vanhee, Wouter Meersseman, Katrien Lagrou, Hans J. Nelis and Tom Coenye* **Rapid and accurate quantification of viable *Candida* species in whole blood using immunomagnetic separation and solid-phase cytometry**	B3
107	*J.M. Van Herreweghe, L. Vanderkelen, L. Callewaert, A. Aertsen and C.W. Michiels* **Isolation of a bacterial inhibitor of the invertebrate type lysozyme**	B3
108	*Sophie Vankerckhove, Nicolas Markine-Goriaynoff, Azeddine Bentaib, Michaël Sarlet, Ruddy Wattiez, Pierre Leprice, Daniel Desmescht, Alain Vanderplasschenand Laurent Gillet.* **The α-2,3-sialyltransferase encoded by Myxoma Virus is not essential for virus replication *in vitro* but contributes to virulence *in vivo***	V2
109	*A. Vassart, E. Peeters and D. Charlier.* **L-glutamine dependent binding of the archaeal Lrp-like transcription regulator Sa-Lrp from the hyperthermoacidophile Su*lfolobus acidocaldarius* to different targets.**	B5
110	*Ines Verbaendert, Liesbeth Lebbe, Nico Boon, Paul De Vos* **Denitrification in *Bacillus* by yet unknown genes?**	B1
111	*Anne Vercammen, B. Vivijs, M. Hendrickx & C. W. Michiels* **Inactivation of bacterial spores in tomato sauce by high hydrostatic pressure**	B3
112	*Ken Vercammen and Pierre Cornelis* **Metagenome mining for the discovery of new antimicrobial molecules**	B4
113	*Joan Vermeiren, Tom Van de Wiele, Willy Verstraete, Pascal Boeckx, and Nico Boon* **Nitric Oxide Production by the Human Intestinal Microbiota by Dissimilatory Nitrate Reduction to Ammonium**	B5
114	*M.-A. Vitry, C. De Trez, J.-J. Letesson, E. Muraille.* **Identification of T cell subsets implicated in the resistance to *Brucella melitensis* infection in a mouse experimental model**	B3
115	*Siegfried E. Vlaeminck and Willy Verstraete.* **Did anammox introduce nitrate on earth?**	B5

Poster abstracts selected for oral presentation

Virology Session

15h15 Deletion of vFLIP impairs Bovine herpesvirus 4 latency and promotes lytic cycle activation
Bénédicte Machiels, Benjamin Dewals, Frédéric Minner, Alain Vanderplasschen and Laurent Gillet

15h30 The leader protein of Theiler's murine encephalomyelitis virus inhibits stress granule formation and eIF2α PKR-mediated phosphorylation
Fabian Borghese, Frédéric Sorgeloos and Thomas Michiels

15h45 Consistent antiviral activity of carbohydrate-binding agents against the 4 different serotypes of dengue virus
Marijke Alen, Suzanne J.F. Kaptein, Tine De Burghgraeve, Johan Neyts, Jan Balzarini, Dominique Schols

16h00 Interferon alpha induces establishment of alphaherpesvirus latency in sensory neurons *in vitro*
Nick De Regge, Nina Van Opdenbosch, Hans J. Nauwynck, Stacey Efstathiou and Herman W. Favoreel[1]

16h15 Universal M2e-based influenza A vaccine: Fc receptors and alveolar macrophages mediate protection
Karim El Bakkouri, Francis Descamps, Marina De Filette, Anouk Smet, Els Festjens, Walter Fiers, and Xavier Saelens

Bacteriology Session

15h15 Detection and analysis of new toxin-antitoxin systems
Damien Geeraerts, Nathalie Goeders, Régis Hallez, R. Leplae, Julien Guglielmini and Laurence Van Melderen

15h30 AiiD, identification of a quorum quenching enzyme which could contribute to the stealthy strategy of *Brucella melitensis* 16M
Matthieu Terwagne, Julien Lemaire, Aurélie Mirabella, Chantal Deschamps, Xavier De Bolle and Jean-Jacques Letesson

15h45 Isolation of a bacterial inhibitor of the invertebrate type lysozyme
J.M. Van Herreweghe, L. Vanderkelen, L. Callewaert, A. Aertsen and C.W. Michiels

16h00 Did anammox introduce nitrate on earth?
Siegfried E. Vlaeminck and Willy Verstraete

16h15 Molecular mechanisms of regulation by Ss-LrpB, a transcriptional regulator from the archaeon *Sulfolobus solfataricus*
Eveline Peeters, Nuno Peixeiro, Marc Nadal, Patrick Forterre, Guennadi Sezonov, David Prangishvili and Daniel Charlier

ABSTRACTS OF PARTICIPANTS

Consistent antiviral activity of carbohydrate-binding agents against the 4 different serotypes of dengue virus

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Dengue virus (DENV) is transmitted by the mosquito *Aedes Aegypti*. After a mosquito bite, the vector is deposited in the skin where dendritic cells (DC) are the first targets of DENV. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is present on DC and recognizes N-glycosylation sites on the viral envelope of several viruses such as human immunodeficiency virus (HIV) and DENV. The DENV E-protein is the glycoprotein on the surface of DENV and interacts with DC-SIGN.

Thus, DC-SIGN might be an important target for antiviral therapy, because it plays an initial role during transmission and infection. Carbohydrate-binding agents (CBAs) have been shown to prevent capture of HIV to DC-SIGN$^+$ cells (Balzarini et al., 2007). We evaluated CBAs against all four serotypes of DENV replication using monocytes-derived DC (MDDC), Raji and human astroglioma U87 cells transfected with DC-SIGN and the DC-SIGN related receptor called L-SIGN. The cells were infected with DENV in presence or absence of compounds and analyzed for DENV-infection by RT-QPCR and by specific anti-DENV mAbs by flow cytometry.

Our results indicate that DC-SIGN expression is needed to make the cells susceptible for DENV infection. Raji/DC-SIGN$^+$ and Raji/L-SIGN$^+$ cells are highly susceptible for DENV infection, in contrast to the Raji/0 cell line. Comparable results were obtained with U87 cells transfected with DC-SIGN and L-SIGN. We demonstrate that several CBAs (HHA, GNA and UDA) could inhibit DENV serotype 2 infection in Raji/DC-SIGN$^+$ cells dose-dependently with EC_{50} values ranging from 0.1 to 2.2 μM (Alen et al., 2009). When the cells were infected with the three other serotypes of DENV consistent antiviral activity data by the CBAs were obtained. Interestingly, the potency of CBAs against DENV in MDDC cultures, which highly express DC-SIGN after differentiation with IL-4 and GM-CSF, was markedly increased compared to the data obtained in DC-SIGN-transfected Raji cells (EC_{50}: 10-100 nM).

Our results demonstrate the importance of DC-SIGN/L-SIGN as attachment receptors for DENV. In addition, these interactions are inhibited by CBAs which exhibit a unique mechanism of antiviral action in inhibiting viral entry processes and could play an important role in the pathogenesis of dengue virus infection.

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Balzarini, J., Van Herrewege, Y., Vermeire, K., Vanham, G., Schols, D., 2007. Carbohydrate-binding agents efficiently prevent dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)-directed HIV-1 transmission to T lymphocytes. Mol Pharmacol 71, 3-11.

Co-residence of the Thuricin 7 biosynthesis cluster and other virulence loci on the newly characterized *Bacillus thuringiensis* plasmid, pGIA2405

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Bacillus thuringiensis is a spore forming Gram-positive bacterium, which is characterized by the production of two types of toxins during the sporulation, Cry and Cyt. These δ-endotoxins display a broad spectrum of entomopathogenic activities. *B. thuringiensis* is therefore used as bioinsecticide and represents the basis of commercial formulations used to control many agricultural pests and vectors of animal and human diseases. In addition to its insecticidal properties, *B. thuringiensis* produces several bacteriocins active on other microorganisms, mainly members of the *Bacillus cereus sensu lato* group.

B. thuringiensis strain BMG1.7 was isolated from the North forest of Tunisia in the region of Ain Draham. This strain displays several virulence activities. Southern hybridization experiments showed that genes coding for these activities are located on a large, ca. 300 kb plasmid, named pGIA2405. This plasmid was shown to harbour different entomopathogenic genetic determinants like the *cry1A* anti-Lepidoptera toxin gene or the *vip3A* gene coding for the Vegetative Insecticidal Protein. In addition, pGIA2405 contains both the zwittermicin A (*zmA*) biosynthesis gene coding for a natural aminopolyol antibiotic and the *zmA* genetic resistance determinant. ZmA has been shown to inhibit the growth of many prokaryotes and several lower eukaryotes, including phytopathogenic fungi.

B. thuringiensis strain BMG1.7 produces a bacteriocins, called thuricin 7, active on several *Bacillus* species, as well as on other Gram-positive pathogenic strains including *Listeria monocytogenes* and *Streptococcus pyogenes*. No thuricin 7 activity was detected on the Gram-negative or Lactic acid bacteria already tested. By analogy to the genetic organization of thuricin 17 from *B. thuringiensis* strain NEB17, the DNA sequence of the three thuricin 7 gene cluster has now been elucidated and compared to similar sequences observed in strain SF361 of *B. thuringiensis* and strain Q1 of *B. cereus*. Moreover, preliminary results have shown that, although the thuricin 7 genetic determinants are rather seldom, they are apparently only found in *B. thuringiensis* isolates, always plasmid-borne.

Role of peptidoglycan O-acetylation in *Lactobacillus plantarum*

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Peptidoglycan (PG) is a heteropolymer made of glycan chains composed of alternating N-acetyl-glucosamine (GlcNAc) and N-acetyl-muramic acid (MurNAc), which are cross-linked by short peptide chains. PG forms a network structure around the cell and ensures cellular integrity. In pathogens such as *Staphylococcus aureus* or *Listeria monocytogenes*, it was previously shown that PG structural modifications such as MurNAc O-acetylation or GlcNAc deacetylation, increase resistance to lysozyme and play a role in bacterial virulence (1, 2).

In contrast, in probiotic or commensal bacteria, the role of PG modifications was not previously studied. Here, we chose to analyze PG modifications in the commensal *Lactobacillus plantarum* WCFS1 strain.

We first analyzed the PG structure of these two selected strains. PG was digested with mutanolysin and the resulting muropeptides were separated by RP-HPLC and analyzed by mass spectrometry. We identified the following PG modifications: O-acetylation of MurNAc and GlcNAc, and amidations of the peptidic stems (glutamic acid and diaminopimelic acid).

Blast sequence homology search using *S. aureus oatA* (which encodes the O-acetyl-transferase responsible for MurNAc O-acetylation) as query, allowed us to identify two potential O-acetyltransferase encoding genes in *L. plantarum* (named *oatA* and *oatB)*. PG analysis showed disappearance of the O-acetylation of MurNAc in *oatA* mutant and the O-acetylation of GlcNAc in the *oatB* mutant. To our knowledge, this is the first identification of O-acetylation of GlcNAc in bacterial PG. Like in other bacterial species, MurNAc O-acetylation increased bacterial resistance to lysozyme whereas GlcNAc O-acetylation did not. Microscopic observation of the mutants showed morphological alterations suggesting a role of PG O-acetylation in bacterial cell morphogenesis.

The analysis of influence of PG O-acetylation on endogenous autolysin activities as well as on the immunomodulatory properties of the lactobacilli are currently in progress.

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The leader protein of Theiler's murine encephalomyelitis virus inhibits stress granule formation and eIF2α PKR-mediated phosphorylation

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Theiler's Murine Encephalomyelitis Virus (TMEV) is a member of the *Picornaviridae* family. The DA strain of this positive strand virus is able to establish a persistent infection of the central nervous system of the mouse, characterized by chronic demyelinating lesions similar to those found in multiple sclerosis (1). Disruption of the host immune response is critical for viral persistence. The leader (L) protein of TMEV plays a crucial role in this process, notably by blocking the expression of key cytokines and chemokines, such as type I interferons and RANTES (2).

Stress granules (SGs) are cytosolic granular structures appearing in cells exposed to different types of environmental stresses, including viral infection. Mainly composed of 48S pre-initiation complexes aggregates, they are thought to play a role in stress-induced cell translation inhibition by sequestering mRNAs and regulating their stability (3).

In this work, we have observed that SGs appear in cells infected with TMEV expressing a mutated L protein (2, 4), but not in cells infected with a wild-type virus (DA1 strain). Subsequently, we have shown that the L protein is able to inhibit stress granules formation induced by other stresses, such as oxidative stress. Taken together, our data demonstrate that TMEV triggers stress granules assembly, but that this process is inhibited by the L protein.

Inhibition of stress granule assembly constitutes a new intrinsic activity of the L protein.

Stress granules formation is known to be triggered by phosphorylation of the translation initiation factor eIF2α (5). We observed that L-mediated inhibition of stress granules formation correlates with a reduced phosphorylation level of eIF2α. Protein kinase R (PKR) is known to be activated by viral infections, leading to eIF2α phosphorylation and translational shutoff (6). L-mediated inhibition of eIF2α phosphorylation was readily detectable in $PKR^{+/+}$, but not in $PKR^{-/-}$ infected cells.

Taken together, these results suggest that L-mediated stress granules formation inhibition involves at least disruption of the PKR pathway.

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Effect of quorum sensing inhibitors on antimicrobial susceptibility of bacterial biofilms *in vitro* and *in vivo*

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It is generally accepted that quorum sensing (QS) affects bacterial biofilm formation, making it an attractive target for antimicrobial therapy. However, detailed information about the anti-biofilm effect of QS-inhibitors (QSI) is still lacking. In addition, whether these compounds, alone or in combination with conventional antimicrobial agents, are practically useful anti-biofilm agents remains to be determined. In the present study, we evaluated the anti-biofilm effect of several QSI (targetting the AHL, AI-2 or Gram positive QS system) and evaluated if disruption of QS-regulated biofilm formation has an effect on antimicrobial susceptibility of these biofilms. The effect of QSI on biofilm formation and antimicrobial susceptibility was examined for *Burkholderia cenocepacia*, *Burkholderia multivorans*, *Vibrio vulnificus*, *Vibrio anguillarum*, *Vibrio cholerae* and *Staphylococcus aureus* (including several MRSA strains) using crystal violet and resazurin staining, confocal laser scanning microscopy and standard plating techniques. Antibiotics tested included ciprofloxacin, clindamycin, doxycycline, tobramycin and vancomycin. The *in vivo* susceptibility was evaluated using a *C. elegans* modelsystem. Several QSI were shown to affect biofilm formation in the strains tested. For example, QSI targetting the AHL system decreased biofilm stability in *Burkholderia* spp. Similarly, QSI targetting the AI-2 system were found to decrease matrix-production in several *Vibrio* spp., while increasing biofilm formation in *Vibrio cholerae.* In addition, the effect on *in vitro* antimicrobial susceptibility was variable. No differences were observed on planktonic cells while an increased susceptibility was observed when biofilms were pretreated with QSI or when already formed biofilms were treated with a combination of QSI and antibiotics compared to antibiotic treatment alone. This was also observed *in vivo.*

Our data indicate that selected QSI only moderately affect biofilm formation and that this effect was species dependent. In addition, disruption of the QS system made some strains more susceptible towards antibiotic treatment, although the opposite was also observed. This supports the idea that a decrease in biofilm formation, although limited at first sight, may subsequently facilitate removal of bacteria by other means.

Differences in the dominant microbiota present on various growth media applied in fish analysis

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Microbiological (quality) control of fresh fish is necessary for the determination of the remaining shelf-life and important for food safety. The total viable count for microbiological fish quality is often performed on Plate Count Agar (PCA) (Oxoid) according to the International Standard Organisation (ISO 8552 and 17410). However, since it is known that PCA has some limits, the dominant microbiota of several fish species detected on various general growth media was studied.
A study based on 11 fish species during processing and storage on ice was performed. Fish samples were collected from a distribution centre of a large supermarket and were incubated during regular time intervals on different media, such as PCA, marine media (Marine Agar (Difco), Long and Hammer (Van Spreekens, 1974)) and Lyngby Iron Agar. After incubation, a replication technique was used to compare the colony growth on the different media. The colonies not growing on one of the media were collected, purified and DNA was extracted. The strains were clustered by rep-PCR analyses and identified based on partial 16S rRNA gene sequencing. A DGGE analysis based on the V3 region of the 16S rRNA gene was performed from plateswabs of the different media to visualise the differences in the observed microbiota.
Identification of the not growing colonies, indicate that several species important for fish quality and shelf-life, such as several specific spoilage organisms (eg. *Photobacterium* sp., *Shewanella* sp.) are not able to grow on all media.
The DGGE profiles show that the bacterial community growing on the various media clearly differs from each other. Especially the profiles obtained from PCA are different from those from the salt-containing (marine) media. The profiles between the marine media however are often similar. Internal markers are used to identify the presence of some important genera, however, this is not always possible. By running some pure strains, it is noticed that some species (eg. *Photobacterium* sp., *Pseudomonas* sp.) are visualised by several bands if using the 16S V3 region. This implies that a conclusion about eg. variation in number of species/genera cannot be made using this 16S V3-region.
We can however conclude that it needs to be considered that the use of only one medium (especially in the case of PCA) is in some cases not adequate for quality control of fish samples.

Application of molecular techniques to monitor the evolution of bacterial consortia composed of *Clostridium sp*. in a hydrogen producing bioreactor

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Our current dependence on fossil fuels as the primary energy source contributes to global climate change, environmental degradation and health problems. Hydrogen offers a tremendous potential as a clean, renewable energy currency and it is compatible with electrochemical and combustion processes for energy conversion without producing carbon – based emissions. Many microorganisms, especially photosynthetic as well as facultative and anaerobic bacteria have been reported to produce large amounts of hydrogen from soluble and insoluble biomass. *Clostridia,* being obligate anaerobes, are capable of biogas production during 'dark fermentation' of a wide range of carbohydrates.

In this ARC project, entitled Micro – H_2 we have focused on a new direction in bio – hydrogen production systems which is the use of mixed cultures of microorganisms (consortia). We expect that the combination of complementary metabolisms could significantly increase the efficiencies of mixed systems compared to monocultures. However, a few fundamental studies need to be carried out in order to investigate and improve the stability of microbial populations involved in the processes. It is now recognised that molecular microbial ecology tools provide the scientific basis to monitor the processes used in environmental biotechnology. To characterize the diversity of bacterial communities, quantitative techniques such as Real – Time Quantitative PCR and FISH (Fluorescence in situ hybridization) and semi – quantitative DGGE (Denaturing Gradient Gel Electrophoresis) have been optimized and applied on different bioreactor samples. This approach enabled for the temporal monitoring of the evolution of bacterial consortia, both in terms of species dominance and their metabolic activity. Molecular analysis of bacterial consortia allowed for careful examination of interactions between different bacterial species within a consortium, which is crucial in the stabilization of the hydrogen production process.

Widespread presence of the autoinducer-2 synthase LuxS in *Bifidobacteria*

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Bifidobacteria are high G+C, Gram-positive anaerobic bacteria. Due to their perceived positive contribution to the functionality of the human gastrointestinal tract, they have been investigated extensively. Furthermore, some strains are used as probiotics in functional foods. However, the exact underlying mechanisms responsible for these health-promoting properties are at present not entirely clear.

The aim of the present study was to determine whether the *luxS* gene (encoding for LuxS, the enzyme responsible for the synthesis of the autoinducer-2 (AI-2) quorum sensing molecule) is present in *Bifidobacteria*. In addition, we also determined the level of AI-2 production, using a *Vibrio harveyi* biosensor assay. AI-2 production may be of great importance because this feature has already been shown to affect the ability of other gut flora bacteria to attach to intestinal epithelial cells.

Our results show that all (22) strains tested induce bioluminescence in the biosensor, indicating the production of AI-2. Additional PCR experiments revealed the widespread presence of the gene encoding for the AI-2 synthase LuxS in *Bifidobacteria*. Whether the production of AI-2 by *Bifidobacteria* also affects their ability to attach to intestinal epithelial cells, and what the consequences for the functionality of the gut microbiota are, remains to be investigated.

GP4 of PRRSV contains a neutralizing epitope that is susceptible to immuno-selection *in vitro*

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GP4 of Lelystad virus (LV), the European PRRSV prototype strain, has been described to contain an immunodominant, neutralizing epitope in its amino-terminal domain[a]. This epitope coincides with the most variable region of GP4. Whether this variability results from immunological pressure exerted by neutralizing antibodies was never investigated. Therefore, the present study aimed to determine whether this neutralizing epitope on GP4 is sensitive to immuno-selection by antibodies *in vitro*.
For that purpose, LV GP4-specific monoclonal antibodies (mAb) were produced and characterized. Three mAb, directed against the neutralizing epitope on GP4, were selected. Cultivation of LV on macrophages or Marc-145 cells in the continuous presence of either of these 3 mAb resulted in the emergence of mAb-resistant LV within 5 passages. To determine whether the resistance to neutralization was mediated by amino acid (aa) substitutions in the neutralizing epitope of GP4, ORF 4 was sequenced. Comparing the GP4 aa sequence of the original LV and the GP4 aa sequences of the obtained mAb-resistant LV revealed aa substitutions within the GP4-neutralizing epitope. In conclusion, this study shows that the neutralizing epitope on GP4 is susceptible to immuno-selection by antibodies.

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The major portal of entry of koi herpesvirus in *cyprinus carpio* is the skin

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Koi herpesvirus (KHV), recently designated in the species *Cyprinid Herpesvirus 3,* is the causative agent of a lethal disease in koi and common carp. In the present study, we investigated the portal of entry of KHV in carp using bioluminescence imaging. Taking profit of the recent cloning of the KHV genome as a bacterial artificial chromosome (BAC), we produced a recombinant plasmid encoding a firefly luciferase (LUC) expression cassette inserted in the intergenic region between ORF 136 and ORF 137. Two viral strains were then reconstituted from the modified plasmid: the FL BAC 136 LUC excised strain and the FL BAC 136 LUC TK revertant strain encoding a disrupted and a wild-type thymidine kinase (TK) locus, respectively. *In vitro*, the two recombinant strains replicated comparably to the parental FL strain. The FL BAC 136 LUC TK revertant strain was shown *in vitro* to induce a bioluminescent signal allowing the detection of single positive cells as early as 24 hours post-infection; while *in vivo*, it induced KHV infection in carp that was indistinguishable from that induced by the parental FL strain. To identify the KHV portal of entry, carp were analyzed by bioluminescence imaging at different time post-infection with the FL BAC 136 LUC TK revertant strain. These analyses demonstrated that the skin of the fish, covering the fins and also the body, is the major portal of entry of KHV in carp. Finally, to further demonstrate the role of the skin as the KHV portal of entry, we constructed an original system nicknamed “U-tube” to perform per-cutaneous infection restricted to the posterior part of the fish. All the data obtained in the present study demonstrate that the skin and not the gills is the major portal of entry of KHV in carp.

A full-length infectious clone of *Beet soil-borne virus* indicates the dispensability of the RNA-2 for virus survival *in planta* and symptom expression on *Chenopodium quinoa* leaves

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Beet soil-borne virus (BSBV) is a *Pomovirus* transmitted to Chenopodiaceae by the protist *Polymyxa betae*, which is also the vector of the etiological agent of the rhizomania syndrome of sugar beet, the *Beet necrotic yellow vein virus* (BNYVV). Originally reported in Italy, rhizomania disease is now widespread in most countries where sugar beet is grown and BSBV is often found in beet infected by BNYVV. However, the pathogenicity of BSBV and its contribution to the rhizomania syndrome remain unclear, with opinions still divided on the matter. For a better understanding of the functionality and pathogenicity of BSBV, full-length cDNA clones have been constructed for the three genomic RNAs. With the aim of assessing their effectiveness and relative contribution to the virus house-keeping functions, transcripts were inoculated on *Chenopodium quinoa* and *Beta macrocarpa* leaves using five genome combinations. Both RNAs-1 (putative replicase) and -3 (putative movement proteins) proved to be essential for virus replication *in planta* and symptom production on *C. quinoa*, whereas RNA-2 (putative coat protein, CP, and a read-through domain, RT) was not. No symptoms were recorded on *B. macrocarpa*, but viral RNAs were detected. In both host plants, the 19 kDa CP was detected by western blotting as well as a 115 kDa protein corresponding to the CP-RT. Our study describing the first infectious full-length cDNA clone available for a beet *Pomovirus* provides therefore a useful tool for further investigating the pathogenicity of BSBV in the complex rhizomania syndrome, as well as its replication and infection mechanisms, and the potential viral interactions with other beny- and pomoviruses on both susceptible and rhizomania-resistant beets.

Comparison of two DNA-based typing techniques for the investigation of clonal relationship between *Listeria monocytogenes* isolates in Belgium.

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Introduction

Listeria monocytogenes is a Gram-positive food-borne pathogen causing listeriosis, a human infection with the highest overall mortality of all food-borne infections (20 à 30%). Listeriosis can range from clinically asymptomatic carriage to febrile gastroenteritis, severe mother-to-child infections (with abortion and preterm birth as possible outcome), septicaemia and central nervous system infections like encephalitis and meningitis. Given the severity of this organism, the National Reference Centre for Listeria (NRC *Listeria*) is collecting Belgian isolates from either human origin or from food monitoring projects conducted by the Federal Agency of Food Safety (FAVV-AFSCA).
Subtyping those isolates could reveal the relatedness of those isolates, facilitating outbreak control and epidemiological studies on the situation in Belgium.

Material and Methods:

Obtained *L. monocytogenes* strains were classified using serotyping by agglutination. This enables the subdivision of *L. monocytogenes* strains into 13 serotypes, with serotypes 4b and 1/2a as the most prevalent serotypes for clinical isolates and serotype 1/2a as the most predominant serotype for food isolates (68% in 2008). To investigate outbreaks and the overall epidemiology a more discriminative technique is needed.
This study will evaluate two DNA-typing techniques on the total group of strains: Pulsed Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing (MLST).
PFGE is the digestion of the total DNA with a restriction enzyme (*Asc*I and *Apa*I) followed by electrophoresis whereby the electric field is periodically switched among three directions, revealing a fingerprint for a specific strain. The obtained fingerprint can be compared to the fingerprints of other strains by visual analysis or by the use of a database containing previously encountered fingerprints.
MLST is the sequence analysis of 7 housekeeping genes followed by the comparison of the sequences to the MLST-*Listeria* database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Lmono.html) resulting in a sequence type (ST) number for that strain.
PFGE is an established typing technique, but has as disadvantages that it is time-consuming (analysis takes at least 4 days), interlaboratory comparison of obtained fingerprints is difficult and the technique is depending on the expertise of the technician. In contrast MLST is faster, is less technician-dependent and sequence data is highly exchangeable, however sequencing 7 genes is still expensive and is it as discriminative as PFGE?
For this study isolates from 2009 were investigated.

Results and conclusion.

Because this study is currently ongoing, only preliminary results can be presented.
A group of clinical isolates, collected during the beginning of 2009 in East- and West-Flanders, are all expressing the same pulsotype using PFGE analysis. Comparison of those strains by MLST reveals that they all have the same ST-type. With this current data we can declare that both methods are useable for outbreak control.
For the total epidemiology study using the isolates of 2009, currently no conclusions could be made.

ORFeome-based screening allowed identification of RicA, a Rab2-binding protein involved in intracellular trafficking control of *Brucella abortus*

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Intracellular pathogenic bacteria selected several mechanisms allowing them to hijack host cellular functions such as cytoskeleton dynamics, vesicular trafficking or apoptosis.
Brucella spp. are Gram negative cocobacilli belonging to the alpha2 subdivision of Proteobacteria that regroups bacteria that have co-evolved with their animal or plant hosts, either in a pathogenic or symbiotic relationship. Bacteria of the genus *Brucella* are causative agents of brucellosis, a worldwide zoonosis that affects a wide variety of mammals including humans. These bacteria are facultative intracellular pathogens whose virulence relies on the capacity to enter and replicate in professional and non-professional phagocytes. Once internalized, *Brucella* resides in a membrane-bound compartment, the *Brucella* containing vacuole (BCV). It has been shown that the BCV interacts transiently and successively with early endosomes, late endosomes and lysosomes. Finally, BCV interacts with the endoplasmic reticulum (ER) and fuses with the ER to form an ER derived organelle that is permissive for its replication. Unlike other intracellular bacteria such as *Legionella pneumophila* or *Salmonella* spp., secreted effectors that may help to hijack cellular trafficking are still unknown.

We performed a high-throughput screen using yeast two hybrid system (Y2H) to detect interaction between all predicted proteins from *Brucella melitensis* and human phagosomal proteins, in order to identify bacterial effectors. A specific interaction between human Rab2 and bacterial RicA (Rab2 interacting conserved protein A) was identified. Rab2 is a small GTPase involved in ER to Golgi trafficking. A GST pull-down experiment confirmed this interaction and also showed that RicA interacts preferentially with a GDP bound form of Rab2. We also showed that 3Flag-RicA and RicA-3Flag fusions are secreted in culture medium by *Brucella abortus*. This secretion is not dependent on the previously identified type IV secretion system. Futhermore, a Δ*ricA* mutant proliferates faster inside HeLa cells and Δ*ricA* BCVs loose lysosomal marker (LAMP1) faster than wild-type strain, suggesting that RicA function could be to slow down trafficking to its replication niche.
To the best of our knowledge, RicA is the first reported effector of *Brucella* spp. involved in the control of vesicular trafficking.

An analogue of the antibiotic teicoplanin inhibits dengue virus replication *in vitro*

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The dengue virus is a mosquito-borne virus that belongs to the family of the *Flaviviridae;* it is endemic in (sub) tropical regions. Each year over 50 million people become infected with dengue of which about 250.000 to 500.000 develop severe and life-threatening conditions, i.e. dengue hemorrhagic fever and dengue shock syndrome. There is no vaccine, nor therapy available. We report on an analogue of the antibiotic teicoplanin LCTA-949 [devoid of antibacterial activity] that inhibits virus induced CPE in a dose dependent manner (EC_{50} of ~5µM). This finding is corroborated by quantification of viral RNA levels in culture supernatant by RT-qPCR (EC_{50} : 4.8µM). A selectivity index (50% effective concentration / 50% cytostatic concentration) of approximately 10 was calculated. Antiviral activity is also observed against other flaviviruses i.e. the yellow fever virus 17D and the Modoc virus as well as against the hepacivirus HCV. Time of addition experiments indicate that LCTA-949 inhibits early stages in the viral life cycle. This is corroborated by the fact that LCTA-949 lacks activity on DENV subgenomic replicon (that does not contain the structural genes) replication. Studies are currently ongoing to unravel the precise mechanism by which LCTA-949 inhibits DENV replication. Insight in the mechanism of action may also shed new light on the early stages of the DENV replication cycle.

Cyanobacterial molecular diversity and geographical distribution in microbial mats from Antarctic lakes

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Key words: Cyanobacteria, Antarctica, molecular diversity, biogeography, DGGE.

The coastal deglaciated areas of Antarctica hold lakes and other water bodies with a wide spectrum of limnological conditions. Aquatic habitats offer milder conditions to the microorganisms, which are the only permanent inhabitants of this glacial desert. Among them, cyanobacteria are the first photosynthetic colonisers, sometimes forming thick, cohesive and pigmented benthic mats.
In the frame of the BELSPO AMBIO project (Antarctic Microbial BIOdiversity, influence of geographical and ecological factors, www.ambio.ulg.ac.be), we have obtained benthic mat samples coming from lakes from various coastal regions of the continent (MERLIN 2007 and BELARE 2007 field campaigns and by collaborations).
We have investigated cyanobacterial molecular diversity *via* Polymerase Chain Reaction and Denaturating Gradient Gel Electrophoresis (PCR-DGGE) on a set of 80 samples. We couple the numerical analysis of the obtained band patterns with the phylogenetic analysis of the sequences, and by using multivariate analysis, we will assess the role of the ecological and geographical factors shaping the distribution and the diversity of cyanobacteria.
Preliminary results of the analysis of 13 samples seem to indicate that lakes separated by a small distance have different cyanobacterial communities, highlighting the importance of ecological factors. Fifty per cent of the obtained Operational Taxonomic Units (OTUs) are potentially endemic to Antarctica while others seem to have a global distribution.
The completed study will give us a wide scale view on the distribution and the diversity of cyanobacteria in two biogeographical zones: Continental Antarctica and Maritime Antarctica. Finally, our studies will supply new data and arguments for the ongoing "everything is everywhere, but the environment selects" debate concerning microbial biogeography.

Two actions to restore the microbial activity balance between aerobic and anoxic ammonium-oxidizing bacteria in a sequencing batch reactor

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Sequencing batch reactors (SBRs) have several advantages, such as a lower footprint and a higher flexibility, compared to biofilm based reactors, such as rotating biological contactors. However, the critical parameters for a fast start-up of the nitrogen removal by oxygen-limited autotrophic nitrification/denitrification (OLAND) in a SBR are not available. In this study, a low critical minimum settling velocity (0.7 m h^{-1}) and a low volumetric exchange ratio (25%) were found to be essential to ensure a fast start-up, in contrast to a high critical minimum settling velocity (2 m h^{-1}) and a high volumetric exchange ratio (40%), which yielded no successful start-up. Red and brown granules were detected in the OLAND SBR, which had similar sizes but different physical and microbial properties.

Besides the fast start-up and the efficient biomass retention, a good balance between the aerobic and anoxic ammonium oxidizers (AerAOB and AnAOB) is required for a successful OLAND process as well. A higher activity of the AerAOB in comparison to the AnAOB can result in nitrite accumulation in the reactor, which can inhibit the AnAOB activity. While in biofilm based reactors the microbial balance is equilibrated spontaneously due to the limited penetration depth of oxygen in the biofilm, the control of this microbial balance in SBRs is not straightforward. In this study the equilibration in aerobic and anoxic activity was represented by the nitrite accumulation rate ratio *(narr),* defined as the ratio of the net aerobic nitrite production rate to the anoxic nitrite consumption rate. To prevent nitrite accumulation, two effective actions were found to restore the microbial activity balance between AerAOB and AnAOB. A daily biomass washout at a critical minimum settling velocity of 5 m h^{-1} removed small aggregates rich in AerAOB activity (*narr* 4.1), and the inclusion of an anoxic phase enhanced the AnAOB to convert the excess nitrite.

This study showed that stable physicochemical conditions were needed in the OLAND SBR to obtain a competitive nitrogen removal rate of 1.1 g N L^{-1} d^{-1}.

Proteolytic spoilage of *Pseudomonas* species throughout the dairy chain

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Introduction Raw milk is stored under refrigerated conditions in the dairy chain (i.e. at the farm, during transport and at the dairy factory prior to heat treatment). Under these specific conditions, growth of psychrotolerant bacteria, especially *Pseudomonas*, is favoured. Even though these organisms are easily inactivated by various heat treatments, an important fraction of the spoilage enzymes (e.g. proteases, lipases) that they produce during growth, remains active because of their resistance to high temperatures. These enzymes can then cause spoilage and structural defects in pasteurized and UHT-treated milk(products). (1).

Methods Milk samples from different farms were collected from the farm bulk tank after the first milking episode and mixed together. To map the influence of refrigerated storage on the growth of *Pseudomonas*, the milk samples were incubated at temperatures simulating pre-processing conditions at two temperature extremes that represent optimal and suboptimal cooled storage conditions. Fourteen milk samples were collected for determination of total colony count and *Pseudomonas* count on PCA and CFC-agar respectively at regular time intervals during the six day simulation. Total bacterial DNA extraction from the raw milk was performed for cultivation-independent monitoring of microbiological outgrowth with DGGE. *Pseudomonas* strains were isolated from CFC-agar at 4 different time intervals: (i) at the beginning and (ii) ending of the simulation of farm bulk tank, (iii) at the ending of transport and (iv) of dairy factory. Isolates were characterised using BOX-PCR and identified using a polyphasic approach. Screening for proteolytic spoilage potential was performed with qualitative (screening on skim milk medium) and quantitative methods (TNBS-method (2)).

Results and discussion The lab-scale incubations revealed that an outgrowth of *Pseudomonas* is already visible at the farm level, and an even greater risk exists for a growth peak of 1-2 log colony forming units downstream in the chain if the cooling temperature increases. Species diversity is larger under suboptimal storage conditions, which was confirmed by the DGGE assay. This may have its effect on the proteolytic spoilage of raw milk stored under suboptimal storage conditions: two *Pseudomonas* groups with high proteolytic potential are able to grow out under these conditions much better than under optimal storage conditions. At the end of the dairy chain, less proteolytic groups start to dominate the *Pseudomonas* microbiota in the raw milk. Nevertheless, overall predominant groups of *Pseudomonas* species (tentatively identified as *P. gessardii*, and *P. fluorescens*-like) show to be vigorous proteolytic spoilers.

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In vitro selection and characterization of hepatitis C virus replicons double or triple resistant to various non-nucleoside HCV polymerase inhibitors

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Clinical studies with selective hepatitis C virus (HCV) inhibitors revealed that escape mutants may develop very rapidly. To prevent, delay or avoid the development of HCV resistance, combination therapies will most probably be necessary. Our aim was to determine the antiviral efficacy of various combinations of non-nucleoside polymerase inhibitors (that have a different allosteric binding site) and the barrier towards resistance development. Short-term antiviral combination assays were performed using Huh 5-2 replicon containing cells in a checkerboard format and were analyzed with the method of Prichard and Shipman. For resistance selection of mono- and double resistant replicons, a stepwise selection procedure (with Huh 9-13 replicons) was used. Triple resistant replicons were selected starting from a replicon that proved already resistant to thiophene carboxylic acid (TCA) and the benzofuran HCV-796. All pair wise combinations of non-nucleoside polymerase inhibitors resulted in an additive anti-HCV effect in short-term antiviral assays. Resistant replicons could be selected for three non-nucleoside polymerase inhibitors (TCA, benzimidazole JT-16 and benzofuran HCV-796) and for each pair wise combination. Triple resistant replicons could be selected for the following combinations: TCA + HCV-796 + VX-950 (protease inhibitor), TCA + HCV-796 + 2'-*C*-methylcytidine (nucleoside polymerase inhibitor) and TCA + HCV-796 + JT-16. Genotyping confirmed the presence of the in literature reported resistance mutations. Cross-resistance selection experiments revealed that combinations of non-nucleoside inhibitors (HCV-796 + TCA and HCV-796 + JT-16) at concentrations of 5-fold their EC_{50} (or higher) completely prevented the development of resistance, whereas mono-therapy with non-nucleoside inhibitors resulted readily in the development of drug resistance. Mono-, double and triple resistant replicons can thus be readily selected with a stepwise resistance selection protocol. The genotype of double and triple resistant replicons is largely the sum of the single resistance mutations. Our data further support the design of anti-HCV therapy based on combinations of non-nucleoside polymerase inhibitors.

Comparison of different media (TSA, BHI, MYP, Nutrient Agar) by LC-MS/MS method for maximising production of cereulide

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Introduction

Foodborne intoxications due to bacterial toxins are frequently reported. A lethal case caused by cereulide, the emetic toxin of *Bacillus cereus* has recently occurred (Dierick et al., 2005). This emetic toxin is pre-formed in food and is resistant to heat and normal food preparation methods. The use of a LC-MS (Liquid Chromatography Mass Spectrometry) may be a powerful analytical tool for the unambiguously identification and quantification of bacterial toxins in food such as cereulide. As a preliminary experiment, the cereulide production by *B. cereus* strain was evaluated on five different *media* (NA – Nutrient Agar, BHI - Brain Heart Infusion agar, MYP - Mannitol Egg Yolk Polymyxin agar, TSA - Tryptone Soy Agar for solid agar *media* and BHI broth for liquid *medium*). The aim of the study was to detect and compare the cereulide production in these *media* by LC-MS/MS technique with a goal to isolate cereulide.

Material and Method

B. cereus strain TIAC303 (Bacteriology Section, IPH), isolated from a lethal food poisoning, was grown in BHI broth (Oxoid) and on different agar plates (MYP, NA, BHI agar, TSA). All cultures were incubated at 30°C for 24h. In order to extract cereulide from solid *media*, one colony was picked from each agar plate, dissolved in 200µl methanol and subsequently boiled for 15min. The liquid *medium* was centrifuged, the pellet was dissolved in methanol, the supernatant was extracted by adding 1:1 volume of methanol and both were boiled for 15min. Cereulide content of each extract was analyzed by LC-MS as described by Haggblom et al. (2002). The LC-MS/MS analysis was performed on a LCQ Deca-XP Plus ion trap mass analyser (ThermoFinnigan, USA). For detection of cereulide, the m/z values for adduct ions 1170,5 (NH_4^+ adduct) and 1191,5 (K^+ adduct) were monitored.

Results

Cereulide was isolated and detected in all extracts from solid cultures but two of them, TSA and BHI in particular, were better *media* for cereulide production. The detection of mass ions 1170,5 and 1191,5 was possible in the extracts from the TSA and BHI *media*, while those of the MYP and NA *media* did not allow clear detection. On the other hand, cereulide extracted from liquid *medium* was partitioned. It was not detected in the supernatant but was present in the pellet.

Discussion

The developed LC-MS/MS method appears to be a useful cereulide-specific tool to analyze the impact of different factors on the production of cereulide. It was observed that the cereulide production and/or recovery were less efficient in the NA and MYP *media*. The absence of cereulide in the supernatant from the liquid culture underlines that the cereulide production is associated with the biomass. Further investigations should thus focus on the optimisation of the analytical LC-MS/MS method and purification of cereulide to be used in quantification in real food samples.

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Exploring Rhizobia in wild native legumes in Belgium

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Rhizobia are symbiotic bacteria capable of establishing root or stem nodules on various leguminous plants. An increasing number of nodule-forming bacteria have been reported, including bacteria from the alpha-, beta- and gammaproteobacteria (Chen et al., 2001; Chen et al., 2003; de Lajudie et al., 1994; de Lajudie et al., 1998; Jarvis et al., 1997; Jordan, 1982; Moulin et al., 2001; Rivas et al., 2002; Zakhia & de Lajudie, 2001). In Western Europe, relatively few native legume species have been studied in detail and therefore potentially a large part of the legume symbiont diversity remains to be discovered. In Belgium, legumes belong to 30 genera and comprise 113 plant species. They vary from annual and perennial herbs to shrubs and trees. In the present work, the diversity of bacteria isolated from root nodules from legumes in the area around Brakel (Belgium) was investigated. The sampling area comprised different habitat types, from nature reserves to industrial areas. Human activities are responsible for pollution and have a great influence on the plants. For example, in the more industrial habitats several times a year the area is sprayed with herbicides, such as Round up and Biofix.

The aim was to get a first insight in the diversity present in these wild Belgian leguminous plants and to compare the diversity in the different habitats and plants. A total of 439 samples were taken covering 28 different plant species. We used the rep-PCR technique as a first screening to reduce the number of isolates, and 16S rDNA partial gene sequencing as an initial identification tool. To gain insights in the nodulation, fixation capacity and host range of the strains, *NodC* and *NifH* were sequenced.

Our study revealed that two possible new species were found. Isolate B462 was identified as *Bradyrhizobium* sp. (97.3%) according to partial 16S rDNA sequencing and as *Bradyrhizobium cancariensis* (87.3%) according to *recA* sequencing. The strains B1209, B1215T11 and B1215T22 were identified as *Sinorhizobium morelense* (98.7%) according to partial 16S rDNA and as *Rhizobium gallicum* (88.8%) according to *recA*. Further investigation is necessary to reveal the true identity of the strains. Remarkable is the discovery of new associations between rhizobia and legumes, namely the presence of *Mesorhizobium* sp. in *Medicago lupulina*, the presence of *Rhizobium indigoferae* in *Trifolium campestre*, the presence of *M. caraganae* in *Securigera varia* and *Bradyrhizobium canariense* in *Robinia pseudoacacia*. Also noticeable is the rather small diversity in symbiotic genes present in the strains. This is most apparent when comparing the *recA* phylogeny and the *nodC*/*nifH* phylogeny. Our result did not reveal significant differences in nodule symbionts for the same plant species in different habitat types.

Future research will focus on broadening the sampling areas, hopefully revealing much more diversity in the nodule symbionts and interesting features of the association with native legumes.

Antivirals for the Global Eradication of Polio

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When the Global Polio Eradication Initiative (GPEI) was launched in 1988, endemic wild poliovirus transmission occurred in more than 125 nations with an estimated 350,000 persons, mostly children, developing paralytic disease each year. At this moment, only four countries remain with endemic poliovirus transmission. However, interruption of wildtype polio transmission –and the global eradication of polio- is still not within sight.
The GPEI relied exclusively on the oral live attenuated vaccine (OPV) and although generally safe, the OPV itself can cause paralysis in about 1 per 750,000 vaccinees (Vaccine-Associated Paralytic Poliomyelitis, VAPP). Moreover, polioviruses are excreted in the stool of healthy vaccinated individuals. These excreted viruses may circulate among non-immune persons in poorly vaccinated communities for years as circulating vaccine-derived polioviruses (cVDPVs). The current GPEI plan calls for discontinuation of OPV use once there is assurance that wild poliovirus transmission has been completely interrupted. At that time, the public health benefits of routine immunization with OPV will no longer outweigh the burden of disease due to VAPP and cVDPVs, and the use of OPV in routine immunization programs will cease globally.
In developing strategies to respond to and control poliovirus outbreaks during the final stages of eradication and in a post-eradication era, significant challenges present themselves. First, as VDPVs continue to circulate, there will be a high probability of outbreaks of paralytic disease in the first several years after OPV cessation. Second is the nature of the response to a polio outbreak post-OPV cessation. In this context, the subject of antiviral drugs for poliovirus was considered at a National Research Council (NRC) sponsored workshop. The workshop report, released in March 2006, concluded that the development of poliovirus antiviral drugs might be beneficial and probably essential to successfully eradicate polio.
In our laboratory, several inhibitors of polioviruses were identified with EC_{50} values in the nanomolar range. These inhibitors could serve as excellent starting points for the development of drugs to be used in the post-OPV era. Moreover, several new molecules with potent anti-poliovirus activity have recently been identified in a large screening effort for replication inhibitors.

Interferon Alpha Induces Establishment of Alphaherpesvirus Latency in Sensory Neurons *In Vitro*

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Alphaherpesviruses are a subfamily of the herpesviruses containing closely related human and animal pathogens, including human herpes simplex virus 1 (HSV-1; cold sores, corneal blindness, and encephalitis) and important animal viruses such as the porcine pseudorabies virus and bovine herpesvirus 1 (PRV and BoHV-1; respiratory symptoms, abortions, and/or neurological symptoms). The single most characteristic and enigmatic hallmark of herpesviruses is their ability to establish a lifelong latent infection in their host and to reactivate from this dormant state upon specific stimuli, which may lead to recurrent disease symptoms. Neurons of the trigeminal ganglion (TG) are the major target cells for establishment of latency for several alphaherpesviruses, including HSV-1, PRV, and BoHV-1. Although there is direct and indirect evidence to support the general concept that alphaherpesvirus latency and reactivation is based on a subtle interplay between virus, neurons and the immune system, it is not clear which immune components are of major importance for the establishment of latency. Consequently, natural latency of a wild type alphaherpesvirus has not been reproduced *in vitro*.

Here we show that interferon alpha (IFN-α) has the previously uncharacterized capacity to induce HSV-1 and PRV latency in porcine TG neurons cultured in an *in vitro* two-chamber model that enables a natural route of infection (De Regge et al, 2006, Vet Microbiol). IFN-α induced a stably suppressed HSV-1 and PRV infection in TG neurons *in vitro*. Subsequent treatment of neurons containing stably suppressed virus with forskolin, a known stimulus of alphaherpesvirus reactivation, resulted in reactivation of both viruses. The only abundant viral gene products transcribed during HSV and PRV latency *in vivo* are the latency associated transcripts (LATs). Infection of TG neurons with an HSV-1 mutant expressing LacZ under control of the LAT promoter showed activation of the LAT promoter and RT-PCR analysis confirmed that both HSV-1 and PRV express LATs during latency *in vitro*.

These data represent the first natural *in vitro* system of alphaherpesvirus latency, which will allow to mechanistically study the latency/reactivation cycle of wild type alphaherpesviruses and to screen for molecules that can interfere with latency/reactivation. Furthermore, the data point to a dual role for type I IFN during alphaherpesvirus infection. Type I IFNs have been shown before to be crucial to protect hosts from acute, often lethal, CNS-related alphaherpesvirus disease. Here, we show that type I IFNs at the same time may promote persistence of these viruses by stimulating latency establishment, thereby contributing to their lifelong infection.

Reliable method for directed gene inactivation in wild type *Escherichia coli* strains

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The entire genome sequence of the uropathogenic *Escherichia coli* model strain UTI89 (serotype O18:K1:H7), was recently determined (Chen *et al*., 2006). A function can be assigned to the relevant open reading frames, e.g. by characterizing the appropriate mutant strains. Methods for precise gene inactivation are important tools for studies in bacterial genetics. A number of allele replacement methods can be used to mutate bacterial genes. Datsenko and Wanner (2000) described an elegant method for the construction of deletion mutants in *E. coli* K-12 strains, based on homologous recombination mediated by the Red system of phage lambda. Derbise *et al*. (2003) described a PCR-based procedure, allowing the rapid deletion of chromosomal genes in *Yersinia*. We found that, although some mutants could be obtained, these methods did not work reliably in *E. coli* UTI89.

Here we report on the development and validation of an efficient method to inactivate specific genes in *E. coli* UTI89. This method allows the rapid and precise deletion of any given region - one gene or an entire operon - in *E. coli*. This is mediated by electroporation of a linear DNA fragment with about 500 bp sequences identical to the regions flanking the desired deletion. Red-mediated homologous recombination subsequently substitutes this region by the same P1-FRT-*cat*-FRT-P2 insert as in the mutants constructed by the protocol of Datsenko and Wanner (2000). This can be selected using the chloramphenicol resistance marker. Finally, it is possible to remove the *cat* gene by the expression of the FLP recombinase.

A UTI89Δ*lrhA*::Cm^R mutant was constructed, to prove that the described method is functional to construct mutants of one gene in *E. coli* UTI89. The LysR-type regulator LrhA (LysR homologue A), encoded by *lrhA*, is a regulator of genes involved in flagellation, motility, chemotaxis, type 1 fimbriae production and biofilm formation. The mutant showed the expected hypermotile and hyperagglutinating phenotype.

UTI89Δ*lacZYA*::Cm^R mutants, in which the whole *lac* operon was deleted, were also constructed, to prove that the described method is also functional to make larger deletions and to confirm its specificity.

In addition, we investigated whether the described method is also functional in other *E. coli* strains like the avian pathogenic *E. coli* O45 strain APEC1 (Vandemaele *et al.*, 2003). APEC1Δ*lrhA*::Cm^R and APEC1Δ*lacZYA*::Cm^R mutants were successfully constructed in the same way as the UTI89 mutants.

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Pseudorabies virus US3- and UL49.5-dependent and -independent downregulation of MHC I cell surface expression in different cell types

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Herpesviruses are large dsDNA viruses with an envelope. The alphaherpesvirus subfamily contains closely related pathogens of man and animal. Human alphaherpesviruses are the herpes simplex virus type 1 (HSV-1; cold sores) and type 2 (HSV-2; genital ulcerae), and the varicella zoster virus (VZV; chickenpox and shingles). The closely related porcine pseudorabies virus (PRV) is often used as a model pathogen to study alphaherpesviruses in general.

Many herpesviruses interfere in diverse ways with the major histocompatibility complex class I (MHC I)-mediated antigen processing pathway in order to limit elimination by cytotoxic T-lymphocytes. For the largest subgroup of the alphaherpesviruses, the varicelloviruses, two viral proteins have been reported to downregulate MHC I cell surface expression: UL49.5 for bovine herpesvirus type 1, equine herpesvirus type 1 and type 4, and PRV, and the conserved viral US3 serine/threonine protein kinase orthologue (ORF66) for VZV. However, for VZV, other, yet unknown and both US3- and UL49.5-independent MHC I downregulation mechanisms were also observed. Here, we report that the mechanisms by which PRV downregulates MHC I cell surface expression during infection are highly cell-type-dependent and not UL49.5-restricted. Indeed, in swine testicle (ST) cells, a kinase-active US3 was necessary but not sufficient to downregulate cell surface MHC I expression, whereas US3 was not required in porcine kidney PK-15 cells and porcine alveolar macrophages (PAM). Interestingly, MHC I downregulation was not (PAM, ST) or only partly (PK-15) dependent on UL49.5. As assessed by Western blot, the observed cell-type-dependent differences in MHC I downregulation mechanisms were not due to differential expression patterns of UL49.5 and US3 in the different cell-types used. Infection of PK-15 cells and PAM with either UV-inactivated PRV or with PRV in the presence of phosphono-acetic acid, an inhibitor of DNA synthesis and thus also of late viral protein synthesis, revealed that *de novo* synthesis of one or more early viral protein(s) is responsible for the observed MHC I downregulation in PK-15 cells and PAM.

In conclusion, we show that PRV reduces MHC I cell surface expression during infection in a cell-type-dependent manner, with variable roles for US3, UL49.5, and additional, yet unidentified early viral proteins.

The bacterial storage compound poly-β-hydroxybutyrate increases growth performance and intestinal microbial diversity in European sea bass juveniles (*Dicentrarchus labrax)*

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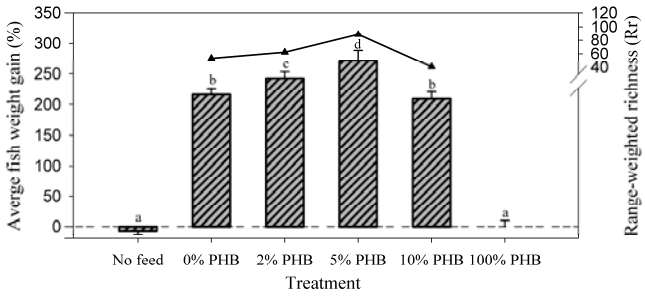
Poly-β-hydroxybutyrate (PHB) has a potential use as alternative anti-infective strategy for aquaculture rearing. However, its positive impact has only been reported for the aquaculture model organism *Artemia fransiscana.* In this research, the effects of (partially) replacing the feed of sea bass juveniles with PHB were investigated in relation to the growth performance and changes in the intestinal microbial community. During a 6 weeks trial period, sea bass juveniles were fed 6 different PHB treatments: a non-fed treatment and a 0%, 2%, 5%, 10% and 100% substitution of the normal feed (w/w) by PHB. At weekly intervals, several measurements including wet weight and intestinal pH were performed and samples of the gut microbial community were taken.

The fish treated with 0%, 2%, 5% and 10% PHB all survived well (ca. 90%, data not shown). For the non-fed sea bass the survival decreased to 40%, while feeding with 100% PHB resulted in a survival of 75%. The diets with 2% and 5% PHB resulted in a significant increase of the average fish weight gain to 243% and 271%, respectively, relative to 216% in the 0% PHB treatment (Fig 1). A PHB level of 10% resulted in a lower weight gain of 209%. Simultaneously, the range-weighted richness (Rr) of the gut microbiota, calculated from the DGGE patterns on day 42 in Figure 2, revealed a highly similar trend (Fig 1).

A trend of larger changes (= lower % similarity) in the microbial community at higher dietary PHB levels could be observed after 42 days of feeding (Fig 2). Finally, lower gut pH values were observed at higher PHB levels (data not shown).

PHB showed the ability to act as an energy source for survival in the absence of normal feed, confirming that PHB was degraded during gastrointestinal passage. The increasing shifts in the microbial community at higher PHB levels and the corresponding decreases in pH make it likely that microbial activity played a major role in the intestinal transformation of PHB. Based on the equal trends in average fish weight gain and range-weighted richness, a new parameter for the microbial diversity, it is hypothesized that the higher bacterial diversity was related to the increased growth performance. More research is currently being performed to evaluate host-microbial interactions by these biodiversity-functionality analysis parameters.

Fig. 1: Average fish weight gain of European sea bass juveniles (▨) and range weighted richness of the gut microbial community (—▲—). Different letters indicate significant differences for the average weight gain)



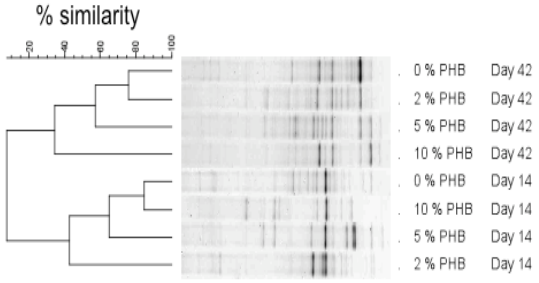


Fig. 2: DGGE band patterns based on the amplified bacterial DNA extracted from the gut of European sea bass juveniles

Lactate racemization is positively controlled by LarR in *Lactobacillus plantarum*

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Lactobacillus plantarum is a homo-fermentative lactic acid bacterium that produces lactate as final product of fermentation. Lactate is produced from pyruvate by L- and D-Lactate dehydrogenases (respectively L- and D-Ldh), that generate L-lactate and D-lactate, respectively. It was previously demonstrated that D-lactate is essential for cell-wall biosynthesis in *L. plantarum,* and that a second pathway for the production of D-lactate is operational in this species. This second pathway is based on lactate racemization, the Lar enzyme catalyzes the racemization of L- to D-lactate when L-lactate is more concentrated than D-lactate. The genes involved in Lar activity are clustered in two operons that form the *lar* locus. The first operon, *larA-E*, is thought to contain the Lar structural genes, while the second, *larR-O*, encodes a putative ABC transporter (*larMNO*) and a transcriptional regulator (*larR*). In this work, the function of LarR was investigated *in vivo* and *in vitro. In vivo,* we show that the deletion of the *larR* gene results in a complete loss of Lar activity. *In silico* analysis showed that LarR is a member of the PrfA group of the Crp-Fnr family. PrfA is involved in the pathogenesis of *Listeria monocytogenes* and binds to the palindromic sequence 5'-TTAACATTTGTTAA-3'. A similar sequence (86% of identity), 5'-TATACATTTGTTAA-3', can be found in the intergenic region between the two *lar* operons. *In vitro*, band shift experiments demonstrated that the purified LarR protein specifically binds to this sequence that we therefore named Lar-Box. Surprisingly, we found that both L- and D-lactate stimulate LarR binding *in vitro* to its operator sequence. As both molecules have an opposite effect on Lar activity *in vivo*, we propose a model in which L- and D-lactate allosterically modulate LarR activity by respectively stimulating and not stimulating transcription initiation by the RNA polymerase.

Ex vivo bioluminescent detection of *alcelaphine herpesvirus 1* infection during malignant catarrhal fever induced in rabbits

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Alcelaphine herpesvirus 1 (AlHV-1), carried by wildebeest asymptomatically, causes malignant catarrhal fever (WD-MCF) when cross-species transmitted to a variety of susceptible species of the *Artiodactyla* order. Experimentally, WD-MCF can be reproduced in rabbits. The lesions observed are very similar to those described in natural host species. Recently, we demonstrated that WD-MCF induced by AlHV-1 in rabbits is associated with the proliferation of $CD8^+$ cells supporting a latent type of infection. In the present study, we investigated whether the virus could be detected *ex vivo* in the tissues of rabbits developing WD-MCF. Taking advantage of the recent cloning of the AlHV-1 genome as a bacterial artificial chromosome (BAC), we produced a recombinant plasmid encoding a firefly luciferase (LUC) expression cassette inserted in a non-coding region of the AlHV-1 genome. *In vitro*, the reconstituted AlHV-1 LUC strain replicated comparably to the parental strain in permissive cells and was able to induce a bioluminescent signal. *In vivo*, rabbits infected with the AlHV-1 LUC strain developed WD-MCF similarly to the parental wild-type strain with hyperthermia, increased CD8/CD4 ratio and viral genomic charge over time in PBMC and in lymph nodes at time of death. To identify the presence of AlHV-1 infection *ex vivo*, various organs of infected rabbits developing WD-MCF were analysed by bioluminescent imaging. Luciferase activity could be detected macroscopically at the time of death in most of analyzed organs including lung, popliteal and mesenteric lymph nodes, spleen, liver, kidney and appendix. Infectious virus could be isolated following co-cultures of lymph node and permissive cells, and the isolated virus retained the ability to induce a bioluminescent signal. In conclusion, we produced an AlHV-1 LUC recombinant and we were able to detect the AlHV-1 infection *ex vivo* in many organs at the time of death.

Glycerol metabolism by the human colonic microbiota

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INTRODUCTION & AIM: Upon ingestion of dietary lipids, significant amounts of glycerol may reach the colonic microbiota intact. In an anaerobic environment glycerol is typically transformed into 1,3-propanediol, with 3-hydroxypropanal (3-HPA) as intermediate. In solution, 3-HPA is part of the HPA-system, also known as reuterin. Reuterin may have significant health-modulating effects that range from a broad antimicrobial activity to (geno)toxicity as it can chemically bind biological molecules in the gastrointestinal tract. Only a few species are known to have the tools for this fermentation process. Although all of them are numerously present in the gastrointestinal tract, the glycerol metabolism has barely been studied in mixed cultures of the human colonic microbiota.

MATERIALS & METHODS: Faecal samples were obtained from 10 healthy volunteers. Upon homogenization, yeast extract (7 g/L) was added to the inoculum resulting in a final dilution of 1 to 50. All faecal samples were either incubated without and in the presence of 140 mM glycerol and samples were collected regularly for quantitative analysis of SCFA, lactate, glycerol, HPA and 1,3-PDO. Qualitative changes in the total bacterial community were investigated using PCR-DGGE. For integrated data analysis Principal Component Analysis (PCA) was performed on metabolic parameters, microbial community parameters and on the combination of both.

RESULTS & DISCUSSION: For all data sets, PCA resulted in a clear separation of the treated and untreated incubations, demonstrating that glycerol addition significantly altered the faecal microbial metabolism and community composition. On the one hand, glycerol treatment resulted in a decreased concentration of branched SCFA, demonstrating that a more saccharolytic metabolism took place. On the other hand, microbial PCA showed a shift in the total bacterial community caused by glycerol addition. More specifically, while untreated samples were scattered on the plot, glycerol treated samples were grouped close together, implying a directional effect of glycerol to certain bacterial species in all faecal samples.
Among the treated incubations a variable metabolic response to the addition of glycerol was found. Metabolic PCA allowed to identify three groups of treated samples. One group was formed due to their common fast glycerol consumption, high 1,3-PDO yield and high acetate production. In contrast, a second group was characterized by very slow glycerol consumption, a low 1,3-PDO yield and low acetate production. The remaining five incubations had intermediate glycerol consumption and 1,3-PDO production. Remarkably, faecal samples with a slower glycerol consumption displayed an increased propionic acid and/or butyric acid concentration. We therefore hypothesized that glycerol reduction to 1,3-PDO competes with other hydrogen consuming reactions, such as propionate and butyrate production. A slow glycerol consumption rate can then be considered health promoting because of i) cholesterol lowering effects from propionate and ii) butyrate inducing apoptosis of colon cancer cells. The rate of glycerol consumption could thus play a role in the occurrence of obesity, associated cardiovascular diseases and colon cancer, implying that rapid glycerol fermentative persons should minimize their glycerol consumption.

Modular Evolution and Ecogenomics of Marine Pseudoalteromonas Phage H105/1

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Marine phages have an astounding global abundance and ecological impact. However, little knowledge is derived from their small genomes, which are often flooded with unknown proteins. We combine classical and traditional approaches to infer potential functional and ecological relevance of marine Pseudoalteromonas phage H105/1.

Functional modules of phage genomes are rampantly exchanged (Botstein 1980) and frequently swapped between phages infecting diverse hosts (Filée et al, 2006). Phage genomes can be thought of as veritable "concatenated metagenomes", with consecutive fragments having very dissimilar origins and evolutionary pasts. Tetranucleotide usage patterns, an approach often used to cluster sequence fragments originating from distinct organisms, i.e. metagenomes (Woyke et al, 2006; Andersson et al, 2008), were evaluated to differentiate and shed light on the evolutionary history of Phage H105/1 'functional modules'. We found the strongest bacterial tetranucleotide signature located in the 'Host Interaction Supermodule' (containing, e.g., single stranded binding proteins, repressors, transcriptional regulators, etc.). Conversely, a phage tetranucleotide signature dominates the 'Phage Structural Supermodule' (proteins of the virion). This likely reflects the evolutionary history of the modules.

Similarity searches of Phage H105/1 proteins were extended to environmental datasets (the Global Ocean Sampling dataset (Rusch, et al, 2008) and five marine viral metagenomes) missing from NCBI-nr and -env. Such an approach lends itself to "ecogenomic" interpretations (Kottmann et al, 2009), whereby insights about H105/1, i.e., its temperate replication strategy and estuarine origin, arise based on genomic and environmental patterns.

Among the traditional approaches, structural proteomics identified seven proteins of the virion, four of which were previously unknown. Furthermore, sequence homology to Phage H105/1 reveals a 'marine signature', in that of the >500 sequenced phages (~20 of which are marine), Phage H105/1 is most similar to other marine phages. Most notable is a MazG-like protein, which, based on previous observations, may maintain the metabolism of a nutrient-stressed host (Clokie et al. 2006) long enough for phage propagation. Nearly half of the phage proteins in the MazG Pfam family are marine, implicating a potentially important role for MazG in marine phage-host systems.

This integrated approach combines new and classical strategies to derive knowledge, in the form of evolutionary relationships and ecological inferences, from phage genomes that typically abound with unknown gene content.

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Comparative study of predominant fecal microbiota of a group of cystic fibrosis children and healthy siblings

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Background: The human gastrointestinal tract harbours a highly dynamic and complex microbial community which plays a key role in health and disease. Previous population fingerprinting research has indicated that the healthy microbiome is host specific and temporally stable. However, external factors such as diet and antibiotic treatment can alter its composition, contributing to several disorders. Patients suffering from cystic fibrosis (CF) are frequently treated with multiple antimicrobial agents to conquer infections caused by micro-organisms such as *Pseudomonas aeruginosa*. Although these antibiotic therapies are necessary to prevent further lung function decline, they may have some side effects such as triggering a state of dysbiosis in the gut. An improved knowledge of the bacterial diversity and the population dynamics of the CF-microbiome could enhance the development of alternative or supplementary therapies based on pro- and/or prebiotics.

Methods: This study aims to compare the predominant fecal microbiota in a group of CF infants with these of healthy siblings through a combination of culture-dependent and culture-independent techniques. One general and six selective media were selected to enumerate the predominant members of the fecal flora. Denaturing Gradient Gel Electrophoresis (DGGE) of 16S rDNA was applied to evaluate the community structure and to monitor the population dynamics. Statistical analysis was performed using SPSS software package version 17.0. 14 families each consisting of one CF-patient and one to two healthy siblings (age ranging from 10 months to 15 years old) were investigated.

Results: Where counts of lactic acid bacteria, *Bifidobacterium* sp., clostridia and counts on the general medium were higher for sibling samples, the enrichment for Enterobacteriaceae, *Veillonella* sp. and *Bacteroides-Prevotella* sp. exhibited higher CFU/g for CF samples. Analysis of samples collected at the first sampling point did not reveal significant differences between CF patients and siblings. Analysis of seven consecutive samples collected from one family over a 2y-period revealed significantly higher numbers of *Bacteroides-Prevotella* sp. and *Bifidobacterium* sp. for the healthy sibling. For both groups, average number of bands in DGGE fingerprints of fecal samples is comparable. However, fingerprint profiles of CF samples are less homogenous than those of samples of siblings without the CF-phenotype. Fingerprints obtained from cultured fractions are less complex than from total DNA extracts. The fecal microbiota of CF-patients is generally less stable in time (2y-period) compared to the healthy siblings.

Genotyping of *Streptococcus agalactiae* (group B streptococci) isolated from vaginal and rectal swabs of women at 35-37 weeks of pregnancy

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Background:
Group B streptococci (GBS), or *Streptococcus agalactiae*, are the leading bacterial cause of meningitis and bacterial sepsis in newborns. Here we compared different culture media for GBS detection and we compared the occurrence of different genotypes and serotypes of GBS isolates from the vagina and rectum.

Method:
Streptococcus agalactiae was cultured separately from both rectum and vagina, for a total of 150 pregnant women, i) directly onto Columbia CNA agar, or indirectly onto ii) Granada agar resp. iii) Columbia CNA agar, after overnight incubation in Lim broth.

Results
Thirty six women (24%) were colonized by GBS. Of these, 19 harbored GBS in both rectum and vagina, 9 only in the vagina and 8 exclusively in the rectum. The combination of Lim broth and subculture on Granada agar was the only culture method that detected all GBS positive women. Using RAPD-analysis, a total of 66 genotypes could be established among the 118 isolates from 32 women for which fingerprinting was carried out. Up to 4 different genotypes in total (rectal + vaginal) were found for 4 women, one woman carried 3 different genotypes vaginally and 14 women carried two 2 different genotypes vaginally. Only two subjects were found to carry strains with the same genotype, although the serotype of both of these strains was different.
Eighteen of the 19 subjects with GBS at both sites had at least one vaginal and one rectal isolate with the same genotype.
We report the presence of two to four different genotypes in 22 (61%) of the 36 GBS positive women and the presence of identical genotypes in both sites for all women but one.

Conclusion
The combination of Lim broth and subculture on Granada medium provide high sensitivity for GBS detection from vaginal and rectal swabs from pregnant women. We established a higher genotypic diversity per individual than other studies, with up to four different genotypes among a maximum of 6 isolates per individual picked. Still, 18 of the 19 women with GBS from both rectum and vagina had at least one isolate from each sampling site with the same genotype.

Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora

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Background
The vaginal microflora is important for maintaining vaginal health and preventing infections of the reproductive tract. The rectum has been suggested as the major source for the colonisation of the vaginal econiche.

Methods
To establish whether the rectum can serve as a possible bacterial reservoir for colonisation of the vaginal econiche, we cultured vaginal and rectal specimens from pregnant women at 35-37 weeks of gestation, identified the isolates to the species level with tRNA intergenic length polymorphism analysis (tDNA-PCR) and genotyped the isolates for those subjects from which the same species was isolated simultaneously vaginally and rectally, by RAPD-analysis.
One vaginal and one rectal swab were collected from a total of each of 132 pregnant women at 35-37 weeks of gestation. Swabs were cultured on Columbia CNA agar and MRS agar. For each subject 4 colonies were selected for each of both sites, i.e. 8 colonies in total.

Results
Among the 844 isolates that could be identified by tDNA-PCR, a total of 63 bacterial species were present, 9 (14%) only vaginally, 26 (41%) only rectally, and 28 (44%) in both vagina and rectum. A total of 121 (91.6%) of 132 vaginal samples and 51 (38.6%) of 132 rectal samples were positive for lactobacilli. *L. crispatus* was the most frequently isolated *Lactobacillus* species from the vagina (40% of the subjects were positive), followed by *L. jensenii* (32%), *L. gasseri* (30%) and *L. iners* (11%). *L. gasseri* was the most frequently isolated *Lactobacillus* species from the rectum (15%), followed by *L. jensenii* (12%), *L. crispatus* (11%) and *L. iners* (2%).
A total of 47 pregnant women carried the same species vaginally and rectally. This resulted in 50 vaginal/rectal pairs of the same species, for a total of eight different
species. For 34 of the 50 species pairs (68%), isolates with the same genotype were present vaginally and rectally and a high level of genotypic diversity within species per subject was also established.

Conclusion
It can be concluded that there is a certain degree of correspondence between the vaginal and rectal microflora, not only with regard to species composition but also with regard to strain identity between vaginal and rectal isolates.
These results support the hypothesis that the rectal microflora serves as a reservoir for colonisation of the vaginal econiche.

Universal M2e-based influenza A vaccine: Fc receptors and alveolar macrophages mediate protection

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The ectodomain of matrix protein 2 (M2e) of influenza A virus is an attractive target for an universal influenza A vaccine: the M2e sequence is highly conserved across influenza virus subtypes, and induced humoral anti-M2e immunity protects against a lethal influenza virus challenge in animal models. Clinical Phase I studies with M2e vaccine candidates were concluded recently. However, the *in vivo* mechanism of immune protection induced by M2e-carrier vaccination is unclear. Using passive immunization experiments in wild type, FcR $\gamma^{-/-}$, FcγRI$^{-/-}$, FcγRIII$^{-/-}$ and (FcγRI, FcγRIII)$^{-/-}$ mice, we here report that Fc receptors are essential for anti-M2e IgG-mediated immune protection. M2e-specific IgG1 isotype are shown to require functional FcγRIII for *in vivo* immune protection but other anti-M2e IgG isotypes can rescue FcγRIII$^{-/-}$ mice from a lethal challenge. Using a conditional cell depletion protocol, we also demonstrate that alveolar macrophages play a crucial role in humoral M2e-specific immune protection. We conclude that alveolar macrophages and Fc receptor-dependent elimination of influenza A virus infected cells are essential for protection by anti-M2e IgG.

Comparative study of Murid gammaherpesvirus 4 infection in mice and in its natural host, the bank voles

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Gammaherpesviruses are the archetypes of persistent viruses that have been identified in a range of animals from mice to man. They are host-range specific and establish persistent, productive infections of immunocompetent hosts. Thus, infected individuals simultaneously both elicit antiviral protective immune response and secrete infectious virions. The best studied gammaherpesviruses are Human herpesvirus 4 and Human herpesvirus 8. As these viruses have no well-established *in vivo* infection model, related animal gammaherpesviruses are an important source of information. We are studying Murid herpesvirus 4 (MuHV-4), a virus that has originally been isolated from bank voles (*Myodes glareolus*). Although MuHV-4 has not been isolated from house mice (*Mus musculus*), infection of inbred laboratory mouse strains is commonly accepted as a good model for studying gammaherpesviruses *in vivo*. It has however never been possible to monitor viral reexcretion and virus transmission in this species suggesting that this model could be imperfect. In this study, we therefore characterized MuHV-4 infection in its natural host, the bank voles, through classical virological methods but also through global luciferase imaging for an anatomical complete view of the infection. Results obtained show that, after intra-nasal infection, the natural route of infection is similar in mice and voles. Following nasal productive infection, the virus spreads to the lung where the infection is accompanied by massive cellular infiltrates. By opposition to extensive viral replication observed in mice, the different analyses indicated that the viral replication was ~1000 fold lower in bank voles. This lower replication did however not affect colonization of latency sites in superficial cervical lymph nodes and spleen as measured by real-time PCR quantification of viral genomes in these organs. In conclusion, this study revealed that MuHV-4 can experimentally infect bank voles, the supposed natural host, but with a lower replicative power. As, gammaherpesvirus epidemiology indicates that transmission correlates with the latent load, our results suggest that gammaherpesviruses may have evolved to infect their hosts without extensive lytic spread. In the future, establishment of experimental transmission in a population of *Myodes glareolus* should help us to better understand mechanisms used by gammaherpesviruses to evade immune response.

Detection and analysis of new toxin-antitoxin systems

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Type II toxin-antitoxin (TA) systems are highly represented in bacterial plasmids and chromosomes. They are generally composed of two small genes organised in an operon and encode a long-lived toxin and a short-lived antitoxin, respectively. Antitoxins protect the bacterium against the toxic activity of their cognate toxins by forming a tight protein-protein complex. Toxins target an essential cellular process. Their ectopic overexpression leads to growth inhibition and/or cell death. Two types of toxic activity have been identified so far. Toxins have been shown to inhibit either replication by poisoning the DNA-gyrase, an essential topoisomerase, or translation by mRNAs cleavage (directly or via translating ribosomes). Currently, TA systems are classified in 8 families based on the toxins sequence divergence. Each of the toxin families is associated with a specific antitoxin family. Nevertheless, many orphan toxin or antitoxin genes are present in bacterial chromosomes. We developed a bioinformatics procedure which considers that orphan genes are associated with potentially new antitoxin or toxin genes. This procedure allowed the identification of several thousands of new toxin families and antitoxin families (i.e. no similarity with known families). The functionality of both partners was assayed in *E. coli* through the expected toxicity of the toxin in the absence of its cognate antitoxin and through the ability of the antitoxin to relieve the other partner toxicity. We experimentally validated 23 novel antitoxins and 18 novel toxins belonging to 15 and 10 clusters, respectively. Preliminary characterisation of the toxins indicated that all of them inhibit methionine incorporation suggesting that they might all target translation or transcription. Our data also show that antitoxins from a given TA family are associated to several toxin families and *vice-versa*. All together, this questions the actual classification in families of TA systems that does not reflect the diversity and 'hybrid' nature of TA systems.

Microbial ecological patterns revealed by the application of 454 Massively Parallel Tag Sequencing on temperate coastal sediments

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Marine coastal sediments represent a place where the atmosphere, the continent, and the ocean interact. Hence, these sediments play a critical role in the recycling of carbon and nitrogen, and may concentrate microorganisms, nutrients and organic matter. Despite the importance of such ecosystems, the extent of microbial diversity and its response to environmental, spatial and temporal changes are still mostly unknown. Therefore, we applied high throughput 454 Massively Parallel Tag Sequencing (454 MPTS) to unveil microbial biodiversity and decipher ecological patterns in subtidal sands of the North Sea island Sylt. We obtained very high estimates of bacterial richness but rarefaction analyses revealed a richness of sand-associated bacterial communities still well underestimated and showed a very contrasted picture as compared to communities from the water column. The diversity also changed dramatically over few cm of sediment depth or between any two consecutive sampling times, with up to 70-80% of community turnover. To gain deeper ecological insights about the microbial diversity in sandy sediments, we thereafter applied multivariate analyses to extract the variation from diversity matrices and obtain ecological signals by including time, depth and several environmental parameters (e.g. pigments, nutrients, enzymes) in the analysis. Interestingly, a large part of the variation in community composition could be attributed to many biogeochemical parameters measured at the study site, and to specific shifts of the large majority of low-abundant bacterial types. In conclusion, our study suggests that 454 MPTS leads to an in-depth analysis of microbial community structure in its ecological context.

Host-microbiota interactions related to fat storage: gut bacteria modulate the fasting induced adipose factor (FIAF) in a Caco-2 cell line.

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Introduction

Obesity becomes more and more problematic in the present Western society. Recently, gut bacteria were shown to interfere with the mechanism of fat storage and synthesis in the human body. One example of this modulating effect is the repression of the fasting induced adipose factor (FIAF) gene in the gut epithelium by the intestinal microbiota (Bäckhed *et al.*, 2004; Bäckhed *et al.*, 2007). The FIAF protein, once released in the blood, adheres to lipases, thereby altering lipid levels in blood and adiposity (Mandard *et al.*, 2006). Hence, modulating the intestinal microbiota so that the FIAF production is less repressed, may provide an alternative strategy to prevent obesity development.

Material and methods

A FIAF producing colorectal cell line (Caco-2) was used to unravel the mechanism behind the modulating effect by different intestinal bacteria (10^9 log CFU/mL) towards FIAF production.

Results

In Caco-2 cells, FIAF protein forms with different molecular mass were present, and FIAF was secreted to the basal side of the polarized cell monolayer. A two hour incubation of the Caco-2 cells with *Clostridium perfringens* resulted in significantly lower cytoplasmatic FIAF levels. In contrast, treatment with *Lactobacillus brevis*, *Bifidobacterium breve* and *Enterococcus faecalis* stimulated FIAF secretion. No significant changes were observed with *Bacteroides thetaiotaomicron* and *Escherichia coli* incubation.

Conclusion

The Caco-2 cell line model offers a suitable *in vitro* methodology to study the interaction between the gut bacteria and the intestine in terms of FIAF production. In future research, different bacterial metabolites and bacterial cell fractions will be tested, in order to unravel the mechanism by which intestinal bacteria can modulate FIAF produced by the gut epithelium.

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Differences between the replication of non-neurovirulent and neurovirulent EHV1 strains in the upper respiratory tract in vivo

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Equine herpesvirus type 1 (EHV1) replicates in the respiratory tract of horses. Infected leucocytes transport virus throughout the body, where infection of endothelial cells of organs such as pregnant uterus or nervous system, results in abortion or nervous system disorders. Two types of EHV1 circulate in the field: neurovirulent and non-neurovirulent strains. To elucidate differences at the port of entry, we performed an *in vivo* study with non-neurovirulent EHV1 and compared results with a previous study, with neurovirulent EHV1. Six EHV-negative ponies were inoculated intranasally with $10^{6.5}$ $TCID_{50}$ of non-neurovirulent EHV1. At 1, 2, 3, 4, 5 and 7 days post inoculation (dpi), one pony was euthanized. Tissues were collected for titration and immunostainings. Number and size of EHV1-induced plaques were calculated, and individual EHV1-infected cells were quantified and characterised. Virus was recovered from nasal mucosa (2-7 dpi), nasopharynx (1-7 dpi), ethmoid (2-3 and 5-7 dpi), tubal/nasopharyngeal tonsils (2-7 dpi) and mandibular lymph nodes (4-7 dpi). Epithelial plaques were found in nasal mucosa and nasopharynx from 2 dpi. No changes were seen in plaque number and size at different dpi. Plaques did not cross basement membrane (BM), but individual infected cells were observed below BM from 2 dpi. Cells from the monocytic lineage (MØ) and T-lymphocytes were carrier cells of EHV1 in all tissues. Comparing this result with our previous study, we conclude that non-neurovirulent EHV1 displays differences when compared to neurovirulent EHV1: (i) both nasal mucosa and nasopharynx are important for replication of non-neurovirulent EHV1, whereas viral replication of neurovirulent EHV1 was mainly localized in nasal mucosa, (ii) the total number of individual EHV1-infected cells is 3-7 times lower with non-neurovirulent EHV1 and (iii) MØ and T-lymphocytes are equally important as carrier cells for non-neurovirulent EHV1, in contrast to neurovirulent EHV1, where almost all EHV1-infected immune cells were MØ.

Characterization of the Type IV secretion system in *Caulobacter crescentus*

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For a long time, bacteria have been mainly considered as planktonic, freely suspended cells. However, they are rarely found in a free-living phase but rather found in association with surfaces in a structure known as a biofilm. Biofilms contains proteins and DNA that may be secreted by the bacterial cells to communicate and to survive. Secretion is universal and essential for the survival and the development of all living organisms. Up to now, 7 secretion systems have been identified in gram negative bacteria. Among them, the Type IV secretion system (T4SS) is known to be involved in various biological processes such as genetic material exchange and proteins secretion. Therefore, we decided to study the potential role of the T4SS in a biofilm formed by the aquatic model bacteria *Caulobacter crescentus*. We identified a set of 8 genes, predicted to encode a T4SS, on the *C. crescentus* chromosome. A particular system was set up in our laboratory to perform a *C. crescentus in vitro* biofilm. Quantitative RT-PCR showed that these genes are constitutively expressed during the course of the cell cycle.
In order to characterize the T4SS, a deleted strain was created for all the genes encoding the T4SS. Morphology, motility and phages resistance were similar in both the wild type and the mutant strains suggesting that T4SS is not involved in cell morphogenesis. Interestingly, the *CB15Δt4ss* strain was unable to form biofilms indicating a potential role of T4SS in the development of such structures. Consistently, quantitative RT-PCR showed an increase of T4SS genes expression upon biofilm establishment. Comparison of protein secretion profiles between the wild type and *CB15Δt4ss* cell populations revealed a release of T4SS specific substrates in the same conditions.

The onset of pAW63 plasmid conjugation is potentially controlled by a quorum sensing system

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Background and objectives
Conjugative plasmids are commonly found in the *Bacillus cereus sensu lato* group. Among those plasmids, the pXO2-like element pAW63 from *Bacillus thuringiensis* harbours a putative RapD-PhrD system that might be involved in the regulation of conjugative transfer. This would be reminiscent of what has recently been reported for ICE*Bs*1, a conjugative transposon located on the chromosome of *Bacillus subtilis*, which was shown to use a quorum sensing system belonging to the Rap-Phr family to control its conjugative behaviour. The aim of this work was to study the influence of the RapD-PhrD system on pAW63 conjugation.

Methods
Several knockouts (KO) of genes involved in the Rap-Phr system were constructed and the effect of these mutations on the pAW63 conjugative behaviour was assessed through conjugation assays and kinetics.

Results
Mutants have been constructed and studied regarding their conjugative behaviour. Those mutants are: the RapD-PhrD double-KO mutant and pAW63-(RapD-PhrD)^{KO}, the pAW63-OppA^{KO} mutant, affected for the component of the transporter (OppA) that recognizes and binds the PhrD pentapeptide. The transfer frequency of those mutants is nearly identical to that of the wild-type plasmid. Kinetics experiments on the mutants indicated that the start of plasmid transfer is influenced by the knockout mutation. Transconjugants were detected after 90, 40 and 10 minutes, for pAW63, pAW63-OppA^{KO} and pAW63-(RapD-PhrD)^{KO} plasmid, respectively. The effect of PhrD-derived regulator peptide on these frequencies and on the "timing" of conjugation is under investigation.

Conclusions
Preliminary results strongly suggested that the RapD-PhrD-OppA system, and hence the cell density, might be involved in determining the time point at which mating is initiated while not interfering in the global transfer frequency.

Study and characterization of a type I interferon: IFN-ε

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Type I interferons (IFNs) are cytokines that exhibit antiproliferative, immunomodulatory and antiviral activities. The type I IFN family comprises different subtypes, of which IFN-α and IFN-β are the most well known. IFN-ε has been discovered more recently (1). IFNe was classified as a type I IFN on the basis of: i) overall protein sequence identity with other type I IFNs (25% identity with IFN-α), ii) conservation of cystein residues and motifs that are highly conserved among other type I IFNs, iii) scarce data suggesting antiviral activity. The IFN-ε gene is highly conserved in mammals. Yet, the role of this IFN is still poorly described (1-4).

We cloned the murine and human IFN-ε genes. However, when these IFNs were expressed by transient transfection in 293T cells, we could not demonstrate any obvious antiviral activity in the cell supernatant, contrary to IFN- α or IFN-β that demonstrated high antiviral activities when produced in parallel (> 10 000 units/ml). Accordingly, supernatents from cells expressing muIFN-ε induced a very low expression of interferon stimulated genes (ISGs) in responding cells.

A plasmid expressing flagged murine IFN-ε was generated. After transfection in 293T cells, IFN-ε expression was readily detectable in the intracellular fraction, by western blot or immunofluorescence. However, no IFN-ε was recovered from the cell supernatant, suggesting a blockade in the secretion.
On western blot muIFN-ε migrated at a higher molecular weight than expected. This observation, added to the lack of antiviral activity, led us to suspect a miscleavage of the signal sequence.

We thus compared the migration profiles of intracellular IFN-ε (and IFN- α as a control) with corresponding IFNs expressed without signal sequence. Wilde-type (wt) IFN-α migrated at the same molecular weight as IFN- α without signal sequence, showing efficient signal sequence cleavage in expressing cells. On the contrary, wt IFN-ε migrated at a higher molecular weight than IFN-ε without signal sequence, which confirmed inefficient signal sequence cleavage.
When IFN-ε signal sequence was replaced by that of limitin, another murine type-I IFN, cleavage of the signal sequence was partly restored. Yet, antiviral activity was not restored in the supernatants of cells transfected with plasmids expressing IFN-ε, suggesting that other steps might be blocked in the secretion of this IFN.

These results raise many questions about the actual role of the IFN-ε and its mechanism of action. We showed that the muIFN-ε signal sequence is not well processed in the cell lines that we used. It might be that the secretion of this protein requires specific chaperones which are only present in some cell types.

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Trends in antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* from Belgian meat products, 2004-2008

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Introduction

Campylobacter is the most common cause of bacterial gastroenteritis in humans in Belgium. The consumption of undercooked poultry meat (and pork) represents the main mode of contamination. The potential for transfer of antimicrobial resistance from enteric zoonotic bacteria of food animals to the human population is a cause of concern. Therefore, trends in antibiotic resistance of *Campylobacter* isolates from food, sampled in the framework of the national zoonoses monitoring plan, were analyzed.

Material & Methods

A total of 1266 *Campylobacter* isolates (459 *C. coli* and 807 *C. jejuni*) from pork or poultry meat and meat products, isolated between 2004 and 2008, were screened for their antibiotic resistance. MICs were determined for ampicilline, ciprofloxacin, gentamycin, erythromycin, nalidixic acid and tetracycline using the E®-test strips. EUCAST epidemiological cut-off values were used to assess resistance.

Results & Discussion

Generally, clear upward or downward trends in resistance (or susceptibility) per antibiotic, organism and/or matrix were difficult to observe. However, a significant increase in gentamycin resistance was observed in 2008 for *C. coli*, both in pork (22.86%) and poultry meat (10.47%), and *C. jejuni* in poultry meat (19.81%) compared to all other previous years. Also, erythromycin resistance for *C. jejuni* from poultry meat is clearly higher in 2008 than before (7.99%). In addition, multi-resistance, i.e. resistance to four or more antibiotics, also significantly increased in 2008 compared to previous years. For *C. coli,* 14.29% of isolates from pork and 37.21% from poultry meat were multi-resistant, with CIP-ERY-NAL-TET and AMP-CIP-NAL-TET as dominant resistance profile respectively. For *C. jejuni*, 19.81% of isolates from poultry meat were multi-resistant, also with AMP-CIP-NAL-TET as dominant resistance profiles. It should be noted that variation and bias can be introduced in the data set due to possible yearly changes of applied national sampling plans.

These data from national sampling and monitoring plans demonstrate that antibiotic resistance in *Campylobacter* in food has not diminished over the recent years. On the contrary, new increases were clearly observed for gentamycin and erythromycin. These upward trends can have consequences for treatment of *Campylobacter* infection in humans and clearly underlines the continuous importance of food animals as resistance reservoir.

Evidence of Selection upon genomic GC-content in bacteria

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The genomic GC-content is the proportion of guanine and cytosine bases in a genome. It varies in bacteria dramatically from less than 30% to more than 70%. This is rather surprising, because under a simple neutral model, one would expect equal frequencies of A, G, C and T. Thus there must be forces acting upon the GC content.
Even though this topic has been extensively investigated over the past decades, the major forces that shape base composition still remain unclear. A debate between a selection-based and neutralist model of GC content evolution is emerging, that is far from concluded. The neutralist view is represented by the widely accepted hypothesis of directional mutation pressure (DMP). DMP states that base composition is changing neutrally due to nucleotide mutations being biased either towards AT → GC or GC → AT. Contrasting this hypothesis are several studies, that showed single species having DMP-inconsistent mutational patterns.
Here we test the DMP hypothesis by examining patterns of synonymous polymorphism using datasets from more than 160 eubacterial species. We find a large excess of synonymous GC → AT mutations over AT → GC mutations segregating in all but the most AT-rich bacteria. We show that the excess of GC → AT mutations is inconsistent with mutation bias, since it would imply that most GC-rich bacteria should be declining in GC-content; such a pattern would be unsustainable. We also show that the patterns are not to due to translational selection or biased gene conversion. We therefore conclude that there is selection to increase synonymous GC-content in many species. Since synonymous GC-content is highly correlated to genomic GC-content, we further conclude that there is selection on genomic base composition in many bacteria.

Endosymbiotic bacteria within Bryopsis species (*Bryopsidales, Chlorophyta*): Naming the actors

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Microbial symbionts have been well documented in animals and plants, but they are also commonly found in association with various algal groups. In the marine green alga *Bryopsis* the endosymbiotic bacteria even seem to be present in every stage of the life cycle, suggesting vertical transmission of the endosymbionts[1]. This indicates an ancient association among host and symbiont, rather than a recent opportunistic and non-specific relationship. Although this remarkable algal-bacterial partnership was already noticed in the early 1970s, no research has been performed to explore the physiological nature and specificity of the endosymbiosis. Therefore this research focuses on the identity and diversity of endosymbiotic bacteria within *Bryopsis* species. To identify the bacterial partner, epiphytes were chemically and enzymatically removed from *Bryopsis* plants from diverse geographical regions. Incubation of sterilized *Bryopsis* samples on Marine Agar plates showed no bacterial growth. Moreover, staining of the sterilized thalli with DAPI revealed the absence of nearly all bacterial fluorescence on the surface of the algae. The algal cells themselves were not lysed, suggesting the endophytic bacteria might still be present within the *Bryopsis* plants after sterilization. Subsequently, the different samples were submitted to a range of molecular techniques such as 16S rDNA PCR, cloning, DGGE and DNA sequencing. The phylogenetic analysis revealed that only a small fraction of clones carried bacterial sequences, in contrast to the majority of the clones which had the *Bryopsis* 16S rRNA chloroplast gene inserted. The obtained bacterial sequences covered just five different species: (i) two diverse species belonging to the Rhizobiales, (ii) a Bacteroidetes bacterium (iii) a bacterium in tight phylogenetic alliance with *Labrenzia alba*, and (iv) a species closely related to the *Rickettsia* symbiont of a marine organism. All five bacteria are present in several *Bryopsis* plants from various regions. Not only does this indicate that these endophytic bacteria are preserved within *Bryopsis* species, it also suggests that they might be actual endosymbionts with a significant function. The occurrence of bacteria belonging to the Bacteroidetes, Rhizobiales and Rickettsiales, with well-known symbiotic features, validates this hypothesis. Attempts are being made to visualize the endosymbionts with electron microscopy and fluorescent probes to confirm their identification and location inside the algal host.

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The use of SPR technology in the unraveling of the mode of action of carbohydrate binding agents as anti-HIV leads

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Carbohydrate-binding agents (CBAs) may qualify as potential inhibitors of HIV infection (1). A variety of CBAs such as the prokaryotic cyanovirin (CV-N) and the plant lectins *Hippeastrum* hybrid agglutinin (HHA) and *Galanthus nivalis* agglutinin (GNA) have been described to interact with the glycans on HIV gp120 and inhibit viral entry (2). Here we will focus on two CBAs: Pradimicin S (PRM-S), an antifungal non-peptidic benzonaphtacenequinone (molecular weight of 948 Da) (3) and a 12.5 kDa protein, Actinohivin (AH) produced by the actinomycete *Longispora albida*, of which the amino acid sequence contains a highly conserved triple tandem repeat (4).

PRM-S and AH specifically interact with gp120 HIV-1 and inhibit the virus entry process. They also efficiently block giant cell formation in co-cultures of HUT-78/HIV-1 and Sup T1 cells and DC-SIGN-mediated virus transmission. The test compounds inhibit various X4 and R5 HIV-1 clinical isolates. Prolonged exposure of HIV-1-infected CEM cell cultures with PRM-S and AH selects for mutant HIV-1 strains containing N-glycosylation site deletions (preferably resulting in high-mannose-type glycan deletions) in gp120. Both CBAs have a high genetic barrier, since at least several N-glycosylation site deletions in gp120 are required to afford a significant level of drug resistance. PRM-S and Actinohivin both interact with gp120 immobilized on a BIAcore sensorchip with an affinity constant in the higher and lower nanomolar range, respectively. Kinetic analysis of the interactions between these CBAs and HIV-1 gp120 point to biphasic binding kinetics, revealing an initial fast off-rate (α-phase) followed by a slower dissociation rate (ß-phase). The ß-phase off-rate values for AH were markedly lower if gp120 expressed in insect cells was used than when mammalian Chinese hamster ovary (CHO) expressed gp120 was used. SPR-analysis reveals pronounced affinity of PRM-S and AH for the transmembrane envelope gp41 as well. The gp120 binding of both CBAs can efficiently be inhibited by α(1,2) man oligomers. Finally, the CBAs don't induce cytokines and chemokines in peripheral blood mononuclear cells.

PRM-S and Actinohivin are interesting carbohydrate binding agents that efficiently bind gp120 and inhibit the HIV entry process and DC-SIGN-mediated virus transmission. SPR data suggests that PRM-S and AH interact with gp120 through preferential binding to high-mannose-type N-glycans. The current study points to PRM-S and Actinohivin as promising candidates to be further investigated preclinically as topical microbicides in anti-HIV therapy.

Key words CBA – HIV – gp120 - SPR – glycans – pradimicin - actinohivin

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Mannan oligosaccharides and guar gum substrates alter intestinal microbial composition and activity in a 5-stage colon simulator

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Introduction Increased interest in indigestible fibers has occurred in recent years due to their beneficial effect on the gastrointestinal microbiota in terms of both metabolic activity and microbial composition. A shift toward increased short chain fatty acids production, as the major end products of fermented fibers with a substantial role in host metabolism has been shown. Moreover there is evidence on the shift of the intestinal microbiota towards more beneficial microbial groups in several studies (Makelainen et al. 2007; Van De Wiele et al. 2007)

Methodology Two simulators of the human intestinal microbial ecosystem (SHIME) were operated in parallel to examine the effect of two indigestible substrates: manno-oligosaccharides and guar gum oligosaccharides. This Twin-SHIME reactor was running over 10 weeks including stabilization (2 weeks), basal (2 weeks), treatment (3 weeks), and washout period (2 weeks). The two substrates were added individually to the normal reactor feed in 3-g/l concentration. Colon compartments of the Twin-SHIME were inoculated with human fecal sample, originating from a healthy 23 year old volunteer without antibiotic treatment in the past 6 months. Samples were regularly taken and analyzed for SCFA, enzymatic activity, and detection of microbial groups of interest using plate counts, DGGE and Q-PCR.

Results On one hand, the mannan oligosaccharide containing substrate resulted in a shift toward more SCFA production in ascending colon during treatment period. Furthermore, at the end of the SHIME-run, both selective plate counts and Q-PCR data showed a 0.5 log increase of *Bifidobacterium* spp. in the proximal colon compartments. On the other hand, degradation of the guar gum oligosaccharide containing substrate resulted in a specific increased butyrate production in the ascending colon compartment. In parallel with this observation, there was also a significant increase in *Roseburia spp.*, a known butyrate producer in the human intestine. We also found an increased enzyme activity of ß–glucosidase, ß-galactosidase, and ß-glucoronidase due to the addition of both substrates.

Conclusion As shown in this study, the Twin-SHIME model provides a suitable *in vitro* model to study both the modulation of microbial community and metabolites of two substrates in parallel. Both substrates have their own prebiotic potency, which is indicated by the changes in microbial community and activity towards more healthy condition.

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PRO 2000, a broadly active anti-HIV sulfonated compound, inhibits HIV infection by multiple mechanisms.

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PRO 2000 is a polyanionic compound under development as a topical antimicrobial gel for the potential prevention of HIV-1 transmission. PRO 2000 is a synthetic naphthalene polymer which is documented to bind to HIV-1 gp120 and interferes with virus binding to $CD4^+$ T cells. However, PRO 2000 appeared to be more active (~10-fold) in inhibiting the infection of CXCR4-using (X4) viruses (IC_{50}: 1.9 µg/ml) compared to the CCR5-using (R5) viruses in $CD4^+$ T-cells (IC_{50}: 20.8 µg/ml).
Therefore, we investigated the interaction of PRO 2000 with cellular receptors with special attention for its interactions with the HIV-1 cellular coreceptors CXCR4 and CCR5. Peripheral blood mononuclear cells (PBMCs) were incubated with PRO 2000 and flow cytometric assays demonstrated that PRO 2000 dose-dependently interfered with the binding of several anti-CD4 mAbs and anti-CXCR4 mAbs, whereas minor or no effects were observed on DC-SIGN and on CCR5. Further investigation showed that PRO 2000 has CXCR4 antagonistic properties as it inhibits the binding of a fluorescent-labeled chemokine CXCL12 to $CXCR4^+$ T-cells with an IC_{50} of 2.2 µg/ml. In addition, the compound inhibited the CXCL12-induced signal transduction (IC_{50}: 19.9 µg/ml), CXCR4 internalization (IC_{50}: 9.5 µg/ml) and chemotaxis in PBMCs (IC_{50}: 6.7 µg/ml). These CXCR4 antagonistic properties of PRO 2000 are a novel and potential additional mechanism of action that could explain the observation that PRO 2000 is more active against X4 viruses than R5 viruses.
In addition, we also examined potential cellular activation and inflammatory properties of PRO 2000 in PBMCs. Although PRO 2000 had minor effects on the induction of the cellular activation markers CD25, CD69 and HLA-DR on T cells, the compound enhanced significantly the production of a small number of cytokines/chemokines (especially IL-1ra, IL-8 and surprisingly the CCR5 ligand MIP-1β) as determined by the Bio-Plex human cytokine 27-plex assay system. In conclusion, our data suggest that although PRO 2000 is a virus attachment inhibitor by interfering with the gp120-CD4 interaction, it can have additional important mechanisms of antiviral activity.

Ionising radiation is not inducing hydrogen peroxide oxidative stress in *Cupriavidus metallidurans* CH34

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Introduction. *Cupriavidus metallidurans* CH34 is a bacterium with a unique spectrum of metal tolerance, and has potential applications for remediation of metal and radionuclide contaminated water and soil, or radionuclide biosensor development. In this study, the susceptibility and specific response of *Cupriavidus metallidurans* CH34 to ionizing radiation was characterised, and we compared the response to those of chemical oxidative agents such as hydrogen peroxide and metals.

Methods. The dose response curves of CH34 were determined for Co^{60} γ-radiation and H_2O_2 by counting CFU after exposure. The bacterium CH34 was exposed for short times (< 1 generation time) to sub lethal doses of γ-radiation and H_2O_2. The global transcriptome profiles of both conditions were compared with those of the control cells, and the existing profiles of 16 different metal exposures.

Results. Acute exposure to γ-radiation and H_2O_2 modulated (up and down) 727 and 97 genes, respectively. Many genes modulated by γ-radiation or H_2O_2 were also found modulated by heavy metals induction in our previous studies. The partial similarities with response to metals, points toward a substantial degree of similarity of ROS (reactive oxygen species) induced damage as well as common cellular strategies of ROS management under ionizing radiation and hydrogen peroxide, and metal stress. Up regulation by H_2O_2 showed comparatively higher overlap of genes with those by Se, As and Cd; whereas up regulation by γ-radiation had comparatively higher overlaps with those by Cs, Sr and Mn. Only few genes were commonly modulated by both γ-radiation and H_2O_2. Acute exposure to low dose λ-radiation modulated the up regulation of genes related to transcription and translation, indicating cellular need for synthesis of damaged proteins. Genes related to DNA damage and nucleotide biosynthesis were not as much unregulated as expected, which indicated that the damages done by radiation were rather random in nature and not specifically directed to DNA molecules. On the other hand, two *copQ*-like genes, which were unregulated with Cd, Pb and Se, were highly over expressed in response to H_2O_2, which indicates a possible common role of these hypothetical genes to combat these stresses. Oxygenase genes related to aromatic ring degradation were notably down regulated in H_2O_2 induction, which probably indicates a critical need to reduce direct O_2- mediated redox reactions in this pathway at the rise of ROS level in the cell.

Conclusion. It could be concluded that the modes of damage and consequential cell response in *Cupriavidus metallidurans* CH34 caused by γ-radiation and those by H_2O_2 were very different, which have led to significantly different responses. Gamma radiation, at sub lethal doses, does not induce DNA damage and has other modes of action than generating hydrogen peroxide ROS in CH34. The CH34 has, probably through its metal reduction systems in general and some special proteins in particular, a high capacity to deal with hydrogen peroxide stress. The impact of ionising radiation on protein damage and the potential role of Fe: Mg content in enzymes is further investigated. In addition, mutants inactivated in key processes are constructed and under investigation.

Keywords: *Cupriavidus metallidurans* CH34, γ-radiation, H_2O_2, oxidative agents

The Complete Genome Sequence of the Edible Cyanobacterium *Arthrospira* (Spirulina) *platensis* PCC 8005

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Arthrospira species are filamentous non-heterocystous cyanobacteria that typically reside in alkaline lakes rich in carbonates and high in pH and salinity. They have a long history of human consumption, possibly as far back as the 9th century. Their global production today in the commercial form ("Spirulina") is estimated at 3000 ton a year. These bacteria were classified by the US Food and Drug Administration as "generally regarded as safe". Besides the interest in *Arhrospira* species as a foodstock and for biomass production, there is now also a growing interest in their ability to produce hydrogen as a clean energy source.

Whole-genome shotgun sequencing of strain PCC 8005 was performed using 454 pyrosequencing technology (amounting to 400,000 reads) supplemented with Sanger sequencing (up to 96,000 longer reads), leading to a final assembly in 16 contigs representing 6,279,260 bases with an average G+C content of 44.7 %. The PCC 8005 genome is most likely organised in a single replicon without current evidence for plasmids. These contigs were processed by the MaGe annotation platform predicting 5,856 protein-coding sequences (CDSs) and 176 genes encoding RNA. Currently, 4,958 CDSs have been manually validated.

The nutritional value of *Arthrospira* sp. PCC 8005 was confirmed through the presence of biosynthetic pathway genes enabling the organism to produce important vitamins (*e.g.* thiamine, panthotenate, pyridoxine, folic acid, cobalamin, and biotin), β-carotene, and the essential fatty acids linoleic acid (LA-18:2$^{\Delta 9,\Delta 12}$) and γ-linolenic acid (GLA-18:3$^{\Delta 6,\Delta 9,\Delta 12}$) constituting, respectively, 17% and 27% of total fatty acid. Genome data on PCC 8005 indicate the absence of phycotoxin or hepatotoxin biosynthesis pathways. However, not all toxin biosynthesis pathways in cyanobacteria are yet fully characterized and we recommend continued chemical analysis of mass-cultivated PCC 8005 and related *Arthrospira* species.

At least 15% of the PCC 8005 genome is repetitive in nature, with a total of 300 kb of sequence repeated in a wide variety of tandem sequences. It contains a high number of insertion sequences (ISs) with nearly 200 full length transposase genes (*tnpA*), six group II introns, and four CRISPR elements. Compared to other cyanobacterial genomes, PCC8005 displayed the highest overall synteny (i.e. a conserved order of sets of homologous genes) with *Arthrospira maxima* CS-328 having around 85% of its genes in conserved gene clusters (syntons), followed by *Lyngbya* sp. PCC 8106 and *Trichodesmium erythraeum* IMS101, with approx. 37% of their genes in syntons.

The PCC 8005 genome contains a *nthPAB* locus encoding the two subunits of nitrile hydratase (NHase) and a NHase activator protein NthP enabling strain PCC 8005 to utilise nitriles (R-C≡N) as the sole source of nitrogen.

Hydrogen production in *PCC 8005* occurs under anaerobic conditions in the dark and in the light and is made possible via a soluble bidirectional hydrogenase encoded by the *hox* locus.

The availability of the full genome sequence of this fascinating cyanobacterium allows us to predict metabolic pathways, and zoom in on particular features that are of relevance to its use in the MELiSSA lifesupport system for the recycling of human waste to water, oxygen, and food, during long-haul space explorations.

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Dynamic epidemiology of naso-pharyngeal flora in nursery schools attendees

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Background and aims A cohort study in healthy children,3 to 6 years old was conduced in Brussels over 2 school years to determine naso-pharyngeal carriage rates and antimicrobial resistance of 5 different bacterial species: *S. pneumoniae, S. aureus, H. influenzae, M. catharralis* and *S. pyogenes.*

Methods Antibiotic usage and epidemiological data's were collected. Three sequential naso-pharyngeal aspirates were performed in autumn, winter and spring. Identification of the 5 bacterial species was performed as recommended by CLSI. Antibiotics resistance profiles were determined by disc diffusion. MICs of resistant pneumococci were determined by E-test. *S. pneumoniae* serotyping was carried out using the Quellung reaction.

Results 333 healthy children (median age 4.4 years) were included. 19.5% children had received at least one dose of PCV7. Carriage rate was 43.6% for *S. pneumoniae*, 34.7% for *S. aureus*, 60.7% for *H. influenzae*, 41.9% for *M. catarrhalis* and 3.2% for *S. pyogenes*. Simultanous colonisation with *S. pneumoniae* and *H. influenzae* in 32.2% of the aspirates. *S. pneumoniae* and *S. aureus* were present together in 14.6%. The most frequent *S. pneumoniae* serotypes were, in decreasing order: 6B, 19F, 23F, 6A, 19A, 23A, 11, 3, 15 and 35. Among our 362 pneumococcus isolates, 10.7% were penicillin non susceptible, 24% were erythromycin resistant, 7.1% were penicillin and erythromycin resistant. 5.2% were MRSA.
25% *H. influenzae* and *M. catarrhalis* were beta-lactamase producers.

Conclusions These data provide an overview of naso-pharyngeal flora in healthy children in Brussels. Our carriage rates are high. Antibiotic resistance rates are globally comparable to clinical isolates.

Identification and preliminary characterisation of a new archaeal tRNA (m^1A) methyltransferase belonging to the SPOUT family

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Transfer RNA (tRNA) possess a large number of modified nucleosides that are posttranscriptionaly formed by diverse enzymes. Among the nucleoside modifications, base and ribose methylations are by far the most frequently encountered. They are catalysed by methyltransferases (MTases), of wich the majority uses S-Adenosyl-L-methionine as methyl donor. From a structural point of view, two tRNA specific MTases classes exist. The MTases of class I possess a Rossmann-like fold, and are therefore named Rossmann fold MTases (RFM). The second class of tRNA MTases, is the SPOUT family of MTases, characterised by the presence of a deep topological knot at the C-terminal part of the protein.

The modified nucleoside 1-methyladenosine (m^1A) is found at four positions, 9-14-22 and 58 in tRNA molecules, and is also formed at position 57, as an intermediate in the biosynthesis of 1-methylinosine (m^1I). Only the MTases acting at position 22 and 58 have been identified and characterised to date.

The tRNA (m^1A22) MTase TrmK was first identified and characterised in the bacteria *Bacillus subtilis*. It belongs to the RFM familly of MTases, and is unrelated to the second characterised m^1A MTase, the TrmI MTase. This MTase was characterised in the extreme thermophilic bacteria *Thermus thermophilus* were it displays site specificity, modifying position 58 of tRNA. In the hyperthermophilic archaea *Pyrococcus abyssi*, TrmI displays region specificity, modifying both position 57 and 58, m^1A57 being further modified in m^1I57.

Aiming to complete the knowledge of m^1A MTases, we undertook the identification of the two still unknown (m^1A9) and (m^1A14) MTases. A bioinformatic study combining folding recognition and phylogenomics lead us to identify an ORF from *Sulfolobus acidocaldarius* that encodes a putative tRNA MTase. Here, we show that this enzyme is the m^1A MTase acting at position 9 of tRNA. Contrary to the previously characterised m^1A MTases, this latter enzyme belongs to the SPOUT-MTase family.

Lack of protection mediated by IFN-β in primary neurons against viral infection

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Type I interferons (also called IFNα/β) are a family of antiviral cytokines crucial for organisms survival upon viral infections. Previous work performed in our laboratory showed that some neurons were able to produce type I IFNs and to respond to these cytokines in vivo, thus taking part in the innate immune response (1). This prompted us to analyze in more depth the IFN response, in vitro, in primary mouse neurons. Neurons responded well to IFN-β treatment by upregulating the expression of a number of interferon stimulated genes (ISGs). However, in contrast to other cell types such as embryonic fibroblasts, neurones that were treated with IFN-β remained highly susceptible to viral infection in spite of the strong transcriptional activation of the ISGs.

We investigated if the lack of resistance of IFN-treated neurons could result from a blockade of mRNA translation after stimulation with IFN. This is unlikely since viral RNA also needs translation to complete the virus replication cycle. Moreover, we observed that luciferase expression driven by the Mx gene promoter (responsive to IFN) yielded a level of luciferase activity that correlated with the level of transcripts, measured by quantitative RT-PCR.

Next, we analyzed whether the lack of resistance of IFN-treated neurons to viral infection could result from a neuron-specific alteration in the spectrum of ISGs induced by IFN treatment. Therefore, we used microarray technology to compare the genes that were upregulated by IFN-β in neurons versus embryonic fibroblasts derived from the same animals. A preliminary analysis of the data fails to reveal major differences in the spectrum of upregulated ISGs. Further analysis of the data is in progress.

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Whole Genome Amplification of environmental colonies of the cyanobacterium *Woronichinia*

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Cyanobacteria from the genus *Woronichinia* are ubiquitous bloom-forming cyanobacteria in temperate regions. Though there are reports of high hepatotoxin concentrations in such blooms, the microcystin could be produced by another cyanobacterial taxon which is present in lower abundances. Thus, there is yet no evidence about the toxicity of *Woronichinia*. So far, strains of *Woronichinia naegliana* were isolated only two times according to the literature (Rajanemi et al., 2005; Willame et al., 2006) and the isolates were not toxic. However, the cultures were quickly lost, illustrating the difficulties in obtaining and keeping strains of this genus. Therefore, there is an important lack of knowledge and molecular data.

As an alternative, we propose to analyze genotypes of environmental *Woronichinia* directly isolated under a binocular from a fresh sample. Recently, other authors have carried out genotypic analysis of single colonies of *Microcystis* and single filaments of *Planktothrix* directly isolated from the environment. However, the low DNA content of one single colony limits the number of PCR reactions that can be carried out. Therefore, we have developed a new approach using a Whole Genome Amplification with *Phi29* polymerase to enable the Multi Locus Sequences Analysis of a single colony combined with an ELISA assay to detect microcystins.

For the first time, we have simultaneously obtained the sequences of *rbcLX* and rRNA-ITS from 4 single colonies of the genus *Woronichinia* (identified by microscopy).

The *mcyE* gene involved in microcytins biosynthesis was detected by PCR in one single colony. The PCR fragment was cloned and sequenced. Translated aminoacid sequence was 50% identical to a ketoacyl protein coded by the genome of *Microcystis* NIES-843. This data is not a proof of the presence of a microcystin biosynthesis operon. Therefore, as microcystins and many other secondary metabolites are encoded by a combination of non-ribosomal peptide synthases (NRPS) and polyketide synthetases (PKS), we have used cyanobacterial specific primers to amplify respectively A-domains and KS regions. NRPS A-domain region and PKS KS region were simultaneously detected in 4 colonies. Then, two clone libraries (A-domain region and KS region) were constructed from the same single colony. Analysis of translated sequences of the A-domain showed high identities with cyanopeptolin gene clusters (71.3% to 98.5%).

Our results suggest, for the first time, the potential production of secondary metabolites by the genus *Woronichinia.* This approach allows to work with a small amount of DNA, and represents a concrete answer to the lack of data on non-cultivable or difficult to isolate cyanobacteria.

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Identification of a new inactive spliced variant of the β-1,6-N-Acetylglucosaminylransferase encoded by Bovine Herpesvirus 4

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The Bo17 gene of bovine herpesvirus 4 (BoHV-4) is the only virus gene known to date that encodes a homologue of the cellular core 2 β-1,6-*N*-acetylglucosaminyltransferase-mucine type (C2GnT-M) (1). The nucleotide sequence of the Bo17 gene has 95% identity with the cellular sequence that it comes from (2). By opposition to what is observed for the cellular gene, we however showed in this study that 2 different messenger RNAs are encoded by the Bo17 gene. The first one corresponds to the entire coding sequence of the Bo17 gene. Surprisingly, the second results from the splicing of a 138pb intron. Analysis of different homologous sequences showed that only Bo17 gene presents the consensus sites for this splicing and that these sites are conserved in all the BoHV-4 strains known to date. This splicing does not change the reading frame of the protein and antibodies generated against Bo17 C-terminus showed that the two forms of Bo17 are expressed in BoHV-4 infected cells. By using an *in vitro* assay, we showed that the spliced form of Bo17 is not anymore active and could therefore regulate enzymatic activity. Finally, in order to study the potential functional consequences of Bo17 splicing on the BoHV-4 infection biology, we constructed recombinant viruses expressing only the long or the short form of Bo17 and characterized these different mutants *in vitro* and *in vivo*. In the future, this work should help us to understand the functional importance of Bo17 gene in the biology of BoHV-4 infection.

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NAD auxotrophy of wild type uropathogenic *Escherichia coli* UTI89 by a single amino acid change in NadB

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Background: Nicotinamide adenine dinucleotide (NAD) is indispensable for all living organisms. The *nadA* and *nadB* genes are required for the synthesis of quinolinate, an intermediate of NAD synthesis. *Shigella flexneri* strains typically carry mutations inactivating *nadA* and/or *nadB*. In *S. flexneri*, *nadA* and *nadB* were defined as anti-virulence loci, because expression of active *nadA* and *nadB* led to a severe reduction of virulence (Prunier *et al*., 2007a; Prunier *et al*., 2007b). Many *E. coli* O18:K1 isolates are also NAD auxotrophic (Achtman *et al*., 1983). In the present study, the molecular basis of the NAD auxotrophy of the uropathogenic *E. coli* O18:K1:H7 model strain UTI89 (Mulvey *et al*., 2001), isolated from an acute cystitis patient, was determined. In addition, the influence of this NAD auxotrophy on the pathogenicity of UTI89 was investigated in the murine ascending infection model.

Methods: The auxotrophy of UTI89 was identified by growing the bacteria on Minimal A agar plates supplement with vitamins, amino acids and nucleic acid bases. The complementation assay of UTI89 was performed by testing the growth of UTI89 on Minimal A medium, after introduction of the corresponding genes of the prototrophic *E. coli* K-12 strain MG1655. Point mutations of the gene were obtained by overlap PCR. Pathogenicity tests in the mouse ascending infection model were performed by mixed infection, followed by plating of bladder homogenates on the appropriate selective media.

Results: *E. coli* UTI89 is an NAD auxotroph. Addition of the metabolite quinolinate allows its growth on Minimal A medium, suggesting a mutation in the *nadB* gene. A prototrophic derivative of UTI89 was obtained by P1*vir*-mediated transduction of the wild type gene from *E. coli* K-12. Transposon mutagenesis and sequencing of the *nadB* and *nadA* genes of this transductant showed that the *nadB* gene of *E. coli* K-12 is present and is necessary for *de novo* synthesis of NAD. The auxotrophy of UTI89 was also complemented by the introduction of a plasmid carrying the wild type *nadB* gene of *E. coli* K-12. There are four amino acid differences between the NadB proteins of *E. coli* strains K-12 and UTI89. Each of these was introduced in the *nadB* gene of the complementing plasmid. Only the plasmid harboring the Ala28Val mutation did not allow growth of UTI89 on minimal medium. In the murine ascending infection model, UTI89 and a spontaneous prototrophic *nadB*$^+$ revertant were equally pathogenic.

Conclusions: (i) The pathogenicity of *E. coli* UTI89 in the mouse model is not influenced by the NAD auxotrophy, which is different from *Shigella,* even though *E. coli* and *Shigella* are close relatives. (ii) Mutation of the 28th amino acid of NadB, from alanine to valine, causes the NAD auxotrophy of wild type *E. coli* UTI89.

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Deletion of vFLIP impairs Bovine herpesvirus 4 latency and promotes lytic cycle activation.

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Among the cluster of genes expressed during gammaherpesvirus latency, the ORF71 encoding a vFLIP was shown to be essential for the survival of some *Human Herpesvirus-8* (HHV-8) infected cells *in vitro*. Even if different *in vitro* studies have investigated vFLIP signalling pathways, the significance of these processes still remains unknown in *in vivo* infections. As *Bovine herpesvirus 4* (BoHV-4), a HHV-8 phylogenetically related *Rhadinovirus*, represents the most accessible model for studying the role of a vFLIP *in vivo*, we studied the function of this protein in the biology of BoHV-4 infection. First, sequence analysis and protein modelling demonstrated a strong conservation between BoHV-4 vFLIP proteins and highlighted close similarities between HHV-8 and BoHV-4 vFLIPs. After a classical characterization of the ORF71 transcription, a BoHV-4 strain deleted for ORF71 and his revertant were produced to study the functional importance of vFLIP in the biology of viral infection. Although *in vitro* experiments performed on permissive cells did not reveal any difference between the recombinant strains, a "latency like" culture model based on a bovine macrophage cell line showed that deletion enhances viral replication and thus impairs viral persistence in these cells. These results suggested that BoHV-4 vFLIP could control the switch between latent and replicative cycle. Therefore, the recombinant viruses were finally compared *in vivo*. Surprisingly, infection of rabbits by the ORF71deleted strain revealed a stronger antibody response generated against viral antigen suggesting that BoHV-4 vFLIP could downregulate expression of lytic genes and subsequently minimize the antibody response. All together, these results show that BoHV-4 vFLIP could play a role in latency establishment *in vitro* and *in vivo*. In the future, this model could help us to unravel the mechanisms of gammaherpesvirus latency and to understand the relation between early latency establishment and the evasion of antibody response.

Environmental constraints on microbial activity and community structure in marine mud volcanoes from the Gulf of Cadiz

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Marine mud volcanoes are major escape pathways for methane and hydrocarbons present in deep marine sediments, which fuel the development of complex chemosynthetic microbial communities at the sediment-seawater interface. In this study, four different marine mud volcanoes (MV's) from the Gulf of Cadiz (East Atlantic) were studied to compare microbial activities, and associated microbial diversity in relation to MV structure. Sulphate reduction (SR), anaerobic oxidation of methane (AOM) and methanogenesis (MG) were measured using radio-labelled substrates immediately upon sediment recovery, and diversity analysis was carried by means of 16s rRNA gene libraries and (CARD-) FISH direct microscopic observations. Methane and sulphate gradients associated with anaerobic oxidation of methane (AOM) where present in these sediments except at Mercator MV, where dissolution of gypsum ($CaSO_4$) possibly maintained high sulphate concentration along the entire core. At Mercator, Darwin, Carlos Ribeiro (CRMV) and Captain Aryutinov (CAMV) MV's, maximum AOM activity ranged from 0,7 to 260 $nmol.cm^{-3}.d^{-1}$. Low activities were measured at Mercator MV, where high salt concentration (up to 10 times seawater concentration) may inhibit AOM. Highest activities were measured at Darwin MV in a discrete AOM near-surface hot-spot. The comparison between different stations at each MV showed that AOM activity display considerable variability correlated with mud volcano structure. In contrast, AOM depth and amplitude seemed relatively stable in time at CAMV at an annual scale. Archaeal and bacterial 16s rRNA gene clone libraries revealed that AOM communities differed greatly in between these three ecosystems with mud volcanoes structure, habitat complexity, and salinity apparently exerting selective pressure on AOM community structure.

Polyphasic study of the diversity of cyanobacterial communities in the Sör Rondane Mountains, Eastern Antarctica

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During austral summer 2007-08, the construction of the new Belgian "Princess Elisabeth" research station has started near the Sör Rondane Mountains (Eastern Antarctica). It is a continental region with air temperatures always below zero. This pristine area has not yet been studied at the biological level.

Samples were taken in several locations during the austral summers 2007 and 2009 (BELSPO projects ANTAR-IMPACT and BELDIVA). A polyphasic approach was used to study the cyanobacterial diversity. It included a molecular study using 16S rRNA gene sequences, an isolation and characterization of cyanobacterial strains, and a microscopic observation of environmental samples.

Cyanobacteria were mostly associated with gravel, and in some cases, with rocks, whereas lichens were dominant on rock surfaces. The morphological identifications and the molecular analysis showed the presence of a relatively high biodiversity in this hostile area, with at least 17 taxa of cyanobacteria: *Anabaena* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Coleodesmium* sp., *Cyanothece aeruginosa*, *Gloeocapsa* sp., *Leptolyngbya* sp., *Leptolyngbya antarctica, Microcoleus sociatus, Nostoc commune, Nostoc* sp., *Phormidium* sp., *Phormidium autumnale*, *Phormidium priestleyi, Stigonema* sp., *Synechococcus* sp. and *Tolypothrix* sp.. Three morphotypes were not present in the molecular data: *Aphanocapsa* sp., *Gloeocapsa* sp. and *Stigonema* sp.. In contrast, we obtained sequences of Anabaena sp., Microcoleus sociatus. and Synechococcus sp. which were not observed by microscopical analysis. After comparison with the Genbank database, 5 sequences remained unidentified, so called 'uncultured'.

These results show the importance of using a polyphasic approach to estimate the diversity of cyanobacterial communities. This region of the Sör Rondane Mountains harbors relatively diverse cyanobacterial communities. Their richness is comparable to the one in non polar regions, in spite of permanently negative values of air temperature and high UV exposure.

The type III restriction modification system of *Salmonella* Typhimurium interferes with Mrr endonuclease activity

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The Mrr protein of *E. coli* is a cryptic type IV restriction endonuclease, which has specificity for methylated DNA and is located in a laterally acquired element called the 'Immigration Control Region'. Although overexpression of Mrr is completely harmless in *E. coli*, its heterologous expression in *S.* Typhimurium yields distinct translucent colonies. After closer examination, we found that prophage induction and concomitant cell lysis were responsible for this translucency, and we could demonstrate that expression of the *E. coli* Mrr protein caused DNA damage in *S.* Typhimurium. Through the use of genetic techniques, we were able to identify the type III restriction modification (RM) system in *S.* Typhimurium as the sole responsible determinant for sensitivity to the *E. coli* Mrr protein. In fact, deleting this RM system conferred Mrr resistance in *S.* Typhimurium, while expressing the corresponding methyltransferase rendered *E. coli* sensitive to its own Mrr protein. It is noteworthy that *S.* Typhimurium itself codes for an inactive Mrr homolog, possibly because of its evolutionary antagonism with the endogenous type III RM system.

The genome of cyprinid herpesvirus 3 encodes 40 proteins incorporated in mature virions

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Koi herpesvirus, also known as cyprinid herpesvirus 3 (CyHV-3), is the etiological agent of an emerging and mortal disease in common and koi carp. CyHV-3 virions present the characteristic morphology of other members of the order *Herpesvirales*, being composed of an envelope, a capsid containing the genome, and a tegument. In the present study, we identified CyHV-3 structural proteins and the corresponding encoding genes using liquid chromatography tandem mass spectrometry based proteomic approaches. In addition, exponentially modified protein abundance index (emPAI) analyses were used to estimate the relative abundance of identified proteins in CyHV-3 virions. These analyses resulted in the identification of 40 structural proteins that were classified based on bioinformatic analyses as capsid (3), envelope (13), tegument (2) and unclassified (22) structural proteins. Finally, a search for host proteins in purified CyHV-3 virions indicated the potential incorporation of up to 18 distinct cellular proteins. The identification of the proteins incorporated in CyHV-3 virions and the determination of the viral genes encoding these proteins are key milestones for further fundamental and applied research on this virus.

Identification, characterization and distribution of insertion sequence elements in *Cupriavidus metallidurans* CH34

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Cupriavidus metallidurans CH34 (formerly *Ralstonia metallidurans*), isolated from metallurgical sediments in Belgium, has been studied in detail for its resistance towards multiple heavy metals, its potential to accept and express foreign genes, and its applicability in environmental biotechnology. Recently, CH34 is also being used as a model in microbial space research (De Boever *et al.*, 2007; Leys *et al.*, 2009). Most of these heavy metal resistance determinants appeared to be carried by the two megaplasmids namely pMOL28 and pMOL30 (Mergeay *et al.*, 2009; Monchy *et al.*, 2007). With the unraveling of its genomic sequence, it became possible to completely scrutinize its genetic content even outside the bias of selectable markers. This revealed a remarkable difference between the number and diversity of genes related to mobile genetic elements in strain CH34 compared to related strains from the genera *Cupriavidus* and *Ralstonia* (Van Houdt *et al.*, 2009). The latter study primarily focused on genomic islands in strain CH34. Here we performed a detailed and comprehensive survey of the insertion sequence (IS) elements in *C. metallidurans* CH34. A set of 21 IS elements was found, counting in total for 57 copies dispersed over chromosome 1 (30), chromosome 2 (19), pMOL28 (3) and pMOL30 (5). The number of copies ranged from 1 (e.g. for IS*Rme6*, *9, 10, 12, 17, 18, 19* and *20*) to 9 (IS*1088*) and 10 (IS*Rme3*). The 21 IS elements could be divided into 10 different families. The two largest families were IS*3* and IS*30* with respectively 18 and 13 copies, while the IS*66* family had only one member (IS*Rme19*). At least 18 copies on chromosome 1, 2 on pMOL28, and all 5 on pMOL30 were identified inside previously described putative genomic islands (Mergeay *et al.*, 2009; Monchy *et al.*, 2007; Van Houdt *et al.*, 2009). The elements IS*Rme5* and IS*1071* were putatively involved in the recruitment of the genes for hydrogenotrophy and CO_2 fixation. Furthermore, mutants unable to grow on H_2 and CO_2 appeared to have lost these genes by IS*1071*-mediated excision.

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MucR regulates both flagellar genes expression and virulence in *Brucella melitensis* 16M

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Successful establishment of infection by bacterial pathogens requires fine-tuning regulatory systems like Quorum Sensing (QS) and signal transduction through two-component regulators. *Brucella* spp., as facultative intracellular pathogens, invade and replicate within host cells. To achieve their successful extracellular/intracellular life transition, *Brucella* spp. modulate their metabolism and the expression of surface-associated virulence factors. Phylogenetically very close to *Sinorhizobium meliloti, Brucella melitensis* could share common molecular mechanisms for host-bacterium interactions.

Among QS-regulated target genes in *B. melitensis*, we found an homologue of *mucR*, coding for a transcriptional regulator firstly reported as regulator for exopolysaccharide (EPS) production. Recently, another function for MucR was also described in *S. meliloti* : MucR represses the expression of some flagellar genes. To investigate the role of MucR in *B. melitensis*, we constructed a deletion mutant for *mucR* in *B. melitensis* and started its phenotypic characterization. Even if described as non-motile, *Brucella* spp. possess several flagellar genes which are expressed only in the early log phase of growth in rich medium. Using specific antibody against FlgE (hook monomer) and FliC (flagellin), we looked for FlgE and FliC expression during growth curve in the mutant strain and found expression of these flagellar proteins even in stationary phase. These data suggest that MucR could be a repressor of flagellar genes expression in *B. melitensis* as in *S. meliloti*. Moreover, we observed a strong attenuation of Δ*mucR* strain in both cellular and animal models of infection suggesting an important role for MucR and most likely for genes under its control in the virulence of *B. melitensis*.

Knowing that *B. melitensis* produce an EPS under the QS control, we are now asking if the regulator MucR could also play a role in the regulation of EPS production in *B. melitensis.* Furthermore, looking for target genes of MucR by transcriptomic analysis should allow a better understanding of the role of MucR in the regulatory system and virulence in *Brucella melitensis* 16M.

Viral genome dynamics during antiviral resistance selection: a first glimpse into viral evolution

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The bovine viral diarrhea virus (BVDV) is an enveloped (+)RNA virus belonging to the family of the *Flaviviridae*, genus pestivirus. The nature of its RNA-dependent RNA polymerase (RdRp) renders its genome prone to the accumulation of mutations. Hence the virus population exists as a complex and dynamic mutant distribution i.e. a quasispecies [Domingo & Gomez (2007) Virus Res 127: 131-150]. The quasispecies enable rapid adaptation of the virus to any perturbation in its natural environment. Also during antiviral therapy, rapid selection of drug resistant virus is facilitated by the existence of the virus as a quasispecies rather than a defined genomic sequence. Most, if not all of the currently know pestiviral RdRp inhibitors target a 7 Å region between F224 and E291 within the finger domain of the enzyme. Here we describe how the non-structural 5B coding region (that encoded for the RdRp) of the viral genome evolves during *in vitro* antiviral resistance selection. To this end we employed a panel of selective inhibitors of BVDV replication i.e. LZ37 [Paeshuyse et al. (2009) Antiviral Res. 82: 141-147], AG110 [Paeshuyse *et al* (2007) J. Virol. 81: 11046-11053] and BPIP [Paeshuyse *et al* (2006) J. Virol. 80: 1469-160]. Resistant virus was selected (against each compound) by serially passaging the virus in the presence of increasing concentrations of inhibitor for 25 times. The entire NS5B gene was sequenced. Furthermore the sequence flexibility of the polymerase region F224-E291 was analysed. It was observed that for AG110 that different resistance mutation can be obtained during independent parallel resistance selection. The results provide a first glimpse into patterns of viral evolution during selective antiviral pressure exerted by specific pestiviral polymerase inhibitors. These results illustrate the genome plasticity of pestiviruses at the level of the drug binding pocket within a defined region [F224-E291] of the polymerase.

Analysis of intragenic tandem repeats in *Escherichia coli*

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Background: Tandemly repeated DNA sequences have previously been recognized in both inter- and intragenic regions of eukaryotes. Such regions typically define hypervariable loci as they are prone to slipped strand mispairing or recombination events that result in expansions or contractions in the number of repeats. While the instability of intergenic tandem repeats is often exploited for genotyping, intragenic tandem repeats that keep the open reading frame intact upon expansion of contraction are less well studied. Nevertheless, they are believed to play an important role in the rapid adaptation to environmental stresses. The aim of this study was therefore to investigate the presence and variability of intragenic tandem repeats in *Escherichia coli*.

Objectives: To investigate the presence and variability of intragenic tandem repeats in *Escherichia coli*.

Methods: First, an *in silico* screen was performed to search for coding intragenic tandem repeats in the sequenced genomes of different *E. coli* strains. Subsequently, the repeat variation of some loci of interest was determined for a large set of natural *E. coli* isolates (n = 113) by PCR amplification, gel electrophoresis and sequence analysis of the repeat region.

Results and conclusion: By comparing the currently sequenced *E. coli* genomes, we have detected several loci that display a variable number of intragenic coding repeats between different strains, providing a first indication of their evolutionary instability. These loci include *ftsK*, *tolA* and *rsxC*, which are involved in cell division, membrane stability and the response to oxidative stress, respectively. Subsequently, primer sets flanking the repeats of these loci were designed, and used to examine their variability in a large set of *E. coli* isolates from different origins. While the distinct variability at these loci could be confirmed, no obvious correlation could be observed between the number of repeats at a given locus and the evolutionary or environmental relatedness of the corresponding strains.

Binding of RutR to the *carAB* operon of *Escherichia coli* K-12 and its interplay with PepA

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RutR (b1013, ycdC) is a member of the TetR family of transcriptional regulators and is the transcriptional activator of the *rutABCDEFG* operon (b1012), encoding a novel pathway for pyrimidine utilization discovered in *E. coli* (1, 2). RutR consists of a N-terminal DNA-binding domain with a helix-turn-helix motif, and a C-terminal ligand-binding domain (1). Shimada *et al*. (2) identified twenty RutR binding sites in the *E. coli* genome, among which the *carAB* operon, and established a 16 bp palindromic consensus sequence (TTGACCAnnTGGTCAA) (2, 3). The RutR box in the *car*P1 control region was identified far upstream of the promoter and overlapping the binding site for aminopeptidase PepA, the key architectural element that plays a crucial role in pyrimidine and purine-specific regulation of *carAB* transcription through remodeling of the *car*P1 control region by DNA wrapping.

We established a high-resolution contact map of the RutR-*car*P1 operator for both backbone and base-specific contact. To this end, we used phosphate ethylation interference, depurination, depyrimidation, and premethylation binding interference, and groove specific ligand bindings. The results indicate that RutR binds to two successive major groove segments and the intervening minor groove, all aligned on once face of the helix. Nearly all the nucleotides of the 16 bp of RutR binding box contribute to complex formation, but to a variable extent. The DNA sequence specificity was further analyzed by saturation mutagenesis of several highly conserved positions of the RutR box. The most remarkable revelation of these experiments is the complete abolishment of RutR binding to a mutant of position -4/+4 of the binding site where the wt base pairs G·C/C·G were replaced by T·A/A·T, whereas the replacement by A·T/T·A displays a much milder effect. Further analysis with substitution of C·G by A·U reveals that the C^5 methyl group of a thymine residue at position 4 of the top strand and position -4 of the bottom strand strongly interferes with RutR binding, likely by exerting a steric hindrance on the establishment of one or more nearby contacts. Furthermore, we demonstrate that uracil is the physiologically relevant ligand that reduces RutR binding, whereas thymine is not. Finally, we analyze the PepA-RutR interplay in *car*P1 regulation by a combination of DNA-binding, single-round *in vitro* trancription transcription and *in vivo* reporter gene expression studies. We demonstrate that PepA inhibits transcription initiation at *car*P1 *in vitro* and that RutR acts as a uracil-sensitive anti-repressor *in vivo*. However, in a pure *in vitro* system, RutR is unable to counteract the PepA-mediated inhibition of transcription initiator and does not inhibit PepA binding. Therefore, we may conclude that RutR antagonizes pyrimidine-specific repression of *car*P1 in a uracil-sensitive manner, but this is not simply by inhibition of PepA binding.

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Active B19 virions production in hepatoblastoma and heparocarcinoma cell lines : amplification and genomic stability

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Human parvovirus B19 virus is the most commonly responsible for mild disease including erythema infectiosum in childhood and arthropathy in young adult. B19 virus can also cause more severe disease, such a transient aplastic crisis in patients suffering from chronic hemolytic disorders and fetal infection during pregnancy that can lead to spontaneous abortion, fetal hydrops, or fetal death. B19 is a frequent contaminant of blood and plasma-derived medicinal products and transmission of this virus has been shown to occur through the administration of contaminated products. Inactivation of the virus is difficult and as a consequence, manufacturers of blood products have to implement screening measures to reduce the load of parvovirus B19 in manufacturing plasma pools by the use of quantitative NAT (EMEA and FDA regulations).

We have developed different assays to measure parvovirus B19 infectivity and neutralization activity based on erythroid cell *(Caillet-Fauquet et al, J Virol Meth 2004)* and two hepatoblastoma and hepatocarcinoma cell lines (HepG2 and HuH7) *(Caillet-Fauquet et al, Transfusion 2004)*. The model is relevant as several successive runs of progeny are successfully obtained. Briefly, subconfluent cells are infected at a low multiplicity of infection or m.o.i (0.1) and 48 hours post-infection, the viral progeny in the cellular supernatant is measured by quantification of the viral DNA with commercial quantitative B19 PCR tests (Lightcycler, Roche). The B19 amplification is efficiently prevented by preincubation of the virus with antibodies raised against the capsid VP proteins or with IVIG. In proper conditions, these systems allow an amplification of the viral population up to 10^7 times as measured by quantitative PCR. Our cell culture assay is very sensitive since an input of 0.1 international unit (IU) gives a viral production (demonstrating that 1 IU is more than 1 infectious virus) while the most sensitive PCR commercial quantitative B19 PCR test, LightCycler-Parvovirus B19 quantification kit, requires at least 10 IU/ml to detect a specific signal. Different genotype 1 B19 strains (WHO strain and blood-donnor units) efficiently multiply in hepatoblastoma and hepatocarcinoma cell lines and up to 5 successive runs leading to an efficient production. The viral progeny of the fifth run was sequenced and found identical to the inoculum of the first run, demonstrating the genomic stability of the B19 produced in this infectivity test.

Molecular mechanisms of regulation by Ss-LrpB, a transcriptional regulator from the archaeon *Sulfolobus solfataricus*

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Transcription in Archaea resembles closely the eukaryotic process, with a basal transcription apparatus minimally composed of TATA-binding protein (TBP), transcription factor B (TFB) and a RNA polymerase (RNAP) resembling the eukaryotic RNA polymerase II. In contrast, most archaeal transcription regulators are predicted to resemble their bacterial counterparts. Only a limited number of archaeal transcription regulators have been studied, most of which belong to the bacterial/archaeal Lrp (Leucine-responsive Regulatory Protein) family of regulators. However, regulatory mechanisms and effects of archaeal Lrp-like regulators are very poorly characterized. Here, we study the function of the Lrp-like regulator Ss-LrpB of *Sulfolobus solfataricus*, a hyperthermoacidophilic archaeon. Previously, we have identified four target promoters of the regulator, namely the promoters of *Ss-lrpB* itself (autoregulation), and of the adjacently located pyruvate ferredoxin oxidoreductase (*porDAB*) operon and two permease genes. *In vitro* binding to all promoter regions has been extensively studied and gene expression of the three non-autogenously regulated target genes is lower in an *Ss-lrpB* gene disruption mutant strain as compared to the isogenic WT strain, indicating that Ss-LrpB may activate transcription.
Regulatory effects exerted by Ss-LrpB were further analyzed by using a *Sulfolobus in vitro* transcription system. Transcription at all four target promoters was clearly stimulated upon addition of recombinant Ss-LrpB at low concentrations. However, major qualitative and quantitative differences were observed for the various target promoters in function of the Ss-LrpB concentration. The stimulatory effect was much stronger for *porDAB* than for the two transporter genes, and transcription increased constantly with increasing Ss-LrpB concentration. In contrast, the stimulatory effect on initiation at the permease promoters and the own promoter observed at low Ss-LrpB concentrations was reversed into a specific repression at higher Ss-LrpB concentrations. These results clearly demonstrate that Ss-LrpB is a dual regulator, able to 'switch' its function between an activator and a repressor in a concentration-dependent manner. Crucial elements determining the regulatory outcome are the binding site organization, the position of the regulatory protein relative to the promoter, cooperativity in the binding and Ss-LrpB induced DNA wrapping, as observed with the own control region. DNA wrapping was shown by a topological assay to result in a positive supercoil and is assumed to be a major determinant of repression.

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Study of *Flavobacterium* strains isolated from Antarctic aquatic and terrestrial samples

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The microbial diversity on Antarctica is largely under-explored however these baseline data are necessary to observe future changes in biodiversity and taxonomic composition due to ecosystem change and/or human introduction. As part of the AMBIO-project that aims to explore bacterial distribution patterns in Antarctica, nine samples, both terrestrial and aquatic, from different regions were investigated. Isolations were made using several conditions and strains were then subjected to rep-PCR fingerprinting as a fast screening to eliminate duplicate isolates. Cluster analysis of fingerprint patterns using BioNumerics software revealed a number of clusters (cut-off level 80%) of similar strains and a number of separate isolates. Representatives were used in partial 16S rRNA gene sequencing to obtain a first approximate identification. The results show a large diversity, distributed over the major phylogenetic groups and only little overlap between the samples.

We focussed on the genus *Flavobacterium* because several of the isolated clusters and strains that were found in this genus show low similarity values with neighbouring sequences in the EMBL-database and thus may represent new species. The 16S rRNA gene sequence was completed and fatty acid analysis as well as some phenotypic tests (API® -20E, API®-20NE and a selection of biochemical growth and degradation tests) were performed. The obtained 16S rRNA gene dendrogram shows that some reference species are very closely related to each other. This makes it difficult to use the 16S rRNA gene to discriminate between potential new species and reference strains. Therefore a housekeeping gene, *gyrB* is being sequenced and, if required DNA-DNA hybridizations will be carried out, to establish whether these isolates can be described as new species.

Study of the functional role of peptidoglycan hydrolases in *Lactobacillus plantarum*

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Cell wall is an essential element for the survival of bacteria. Besides its role of protection against different stresses and attacks coming from outside of the cell, it is also conferring shape to bacteria.

In Gram positive bacteria, peptidoglycan (PG) is a major compound of the cell wall. This polymer is constituted of glycan strands, composed of alternating N-acetyl-glucosamine and N-acetyl-muramic acid, that are crosslinked by short peptides to form a rigid network around the cell. Bacteria produce a variety of enzymes able to degrade PG. They are called peptidoglycan hydrolases (PGH) or autolysins when they target their own PG. These enzymes were shown to play a major role in different processes such as cell separation or cell-wall turnover and are divided into 5 major families depending on the activity of their catalytic domain.

Thanks to the fact that the genome of *Lactobacillus plantarum* was sequenced and annotated, we were able to perform an *in silico* analysis in order to identify genes predicted to encode PGH. 12 genes encoding putative PGH from the 5 families of PGH were found: Acm2 (Lp_2645), Lp_3093, Acm1 (Lp_1138), and Lys (Lp_1158) belong to the muramidase-glucosaminidase family. LytH (Lp_1982) is a member of the N-acetylmuramoyl-L-alanine amidase family. Lp_3421, Lp_2162, Lp_2520, and Lp_1242 belong to the family of NlpC/p60 endopeptidases. Finally, Lp_0302, Lp_3014 and Lp-3015 are putative lytic transglycosylases. The aim of this study is to investigate and characterize the functional role of these PGH in *L. plantarum* NCIMB8826.

Using the *cre-lox* based system for multiple gene deletions, we performed single gene inactivation of the 12 identified PGH in order to study the phenotype of resulting mutant strains. The results obtained so far showed that at least two of the 12 single PGH mutants (Acm2 and the putative Lp_3421 endopeptidase) displayed a morphological defect in *L. plantarum*: *acm2* mutant showed a defect in the separation of sister cells during division and *lp_3421* inactivation strongly affected growth and shape of bacteria.

In a future work, multiple deletions of PGH belonging to the same family or displaying structural similarities will be performed in order to investigate their mutual role. In addition, purification and enzymatic characterization of Acm2 and Lp_3421 are underway.

Characterization of the tRNA methyltransferase TrmK of *Bacillus subtilis*

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The TrmK methyltransferase (MTase) from *Bacillus subtilis* catalyses the formation of m^1A at position 22 of tRNA (1). Among the 19 sequenced tRNAs of *B. subtilis* only 5 possess m^1A at position 22. These tRNAs have all a long extra arm.
The TrmK enzyme displays a Rossman fold-like structure, typical for classI MTases. A model of the enzyme was constructed. Based on this model three critical acidic residues (D25, E50 and D78) were predicted to be important for S-adenosylmethionine (S-AdoMet) binding. The residues M96, Q121 and Y153 were predicted to play an important role in tRNA binding and/or catalysis. Mutation analyses were carried out. All these residues were mutated into an alanine. All the mutant TrmK enzymes were purified to homogeneity and their activity measured using total tRNA of *E. coli* as a substrate, since *E. coli* tRNAs lack m^1A22.
The E50A enzyme showed a total loss of activity. It could suggest its predominant importance in S-AdoMet binding. The enzyme variants D25A and D78A showed a three and six-fold reduction in kcat, respectively. Kinetics and S-AdoMet binding assays should confirm whether this reduced activity relies on a diminished interaction between S-AdoMet with the enzyme. The kcat of two other mutants (M96A and Y153A) are largely affected. The TrmK residue Y153 is located in the MTase motifVIII. By analogy to DNA MTases this residue might be important in stabilisation of the A22 base in the tRNA substrate. The 15 fold reduction in activity of the M96A variant suggests, as predicted, that the length of the hydrophobic side chain is important for base stabilization as well. The activity of the Q121A mutant is reduced, although to a lesser extent than the two previous ones. Based on the model a tRNA binding role is predicted for Q121. Binding experiments should confirm this hypothesis.
For an overall understanding of the catalytic mechanism of the *B. subtilis* TrmK enzyme, the 3D structure should be elucidated. Crytals of the TrmK enzyme have been obtained.

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High resolution contact probing of the DNA binding of SaLysM, a transcription regulator from the archaeon *Sulfolobus acidocaldarius*, involved in lysine biosynthesis regulation

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Generally, plants and bacteria synthesize lysine via the diaminopimelate (DAP) pathway and fungi via the α-aminoadipate (AAA) pathway. However, thermophilic bacteria such as *Themus thermophilus* also utilize AAA instead of DAP, but via a modified AAA pathway (KOBASHI ET AL, 1999). This modified AAA pathway appears to be present also in hyperthermoacidophilic archaea belonging to the genus Sulfolobus. In Sulfolobus, the genes responsible for the second part of the pathway are organized into two subsequent operons, lysYZM and lysWXJK. The last gene in the first operon is lysM, encoding a transcription regulator belonging to the bacterial/archaeal Lrp (Leucine-responsive Regulatory Protein) family. In Sulfolobus solfataricus, it has been demonstrated that LysM binds the control region of the second operon and it is suggested that LysM is an activator of the lysine biosynthetic genes in a lysine-dependent fashion (BRINKMAN ET AL, 2002).

Here, we study the DNA binding of the LysM regulator from *Sulfolobus acidocaldarius* (SaLysM), which has 76 % sequence identity with its homolog from S. solfataricus. The SalysM gene was heterologously overexpressed in Escherichia coli with a C-terminal hexa-histidine tag. This allowed purification with affinity chromatography, resulting in electrophoretically pure SaLysM. Electrophoretic mobility shift assays (EMSAs) demonstrated that SaLysM specifically binds the control region of lysWXJK and that the protein binds with a high affinity to a previously determined LysM consensus binding site (Yokoyama et al., 2009). The addition of arginine at 0.5mM had hardly any effect on complex formation but lysine at the same concentration increased the affinity of LysM for a 47 bp duplex bearing the consensus LysM box .'In gel' Cu phenantroline footprinting allowed us to determine the position of the high-affinity SaLysM binding site, which is located just upstream of the lysWXJK promoter. This binding site also overlaps the 3' end of the open reading frame of lysM. Its location suggests a potential activation of the lysWXJK operon and possibly an autoregulation by affecting lysM termination. The interaction between SaLysM and its binding site has been further analyzed by applying high resolution contact probing techniques. Missing contact probing and premethylation binding interference were used to identify the bases that are important for efficient binding of SaLysM. The results indicate that SaLysM protects a 21 bp long stretch against degradation and interacts with two major groove segments and the intervening minor groove segment, all aligned on one face of the helix. Circular permutation assays indicate a SaLysM-induced DNA bending with an average bending angle of 42°. The sequence-specific contribution of each base pair of the 17 bp long consensus binding site to the interaction is presently studied in more detail by analyzing SaLysM binding to a set of 24 substitution mutants (saturation mutagenesis by introduction of symmetrical substitutions at positions 1 to 8 in both half-sites of the binding site).

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Removal of epidermal mucus enhances Cyprinid herpesvirus 3 entry through the skin of *Cyprinus carpio*

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Koi herpesvirus, recently designated in the species Cyprinid Herpesvirus 3 (CyHV-3), is the causative agent of a lethal disease in Koi carp (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio carpio*). Very recently, we demonstrated using a CyHV-3 recombinant strain expressing luciferase and in vivo bioluminescence imaging that the major portal entry is the skin of the fish. Here, we investigated the effect of epidermal mucus removal and progressive skin abrasion on CyHV-3 entry in fish. Our results demonstrate that mucus removal and moderate skin abrasion enhance CyHV-3 infection of carp. Electron microscopic examination of skin explants infected ex vivo revealed that these skin treatments enhanced the binding of CyHV-3 to the upper cell layer of the skin. All together, the results of the present study suggest that skin lesions could enhance the susceptibility of carp to CyHV-3.

AiiD, identification of a quorum quenching enzyme which could contribute to the stealthy strategy of *Brucella melitensis* 16M

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Successfull establishment of persistent infection by *Brucella* spp. is mostly linked to its ability to survive and replicate in host phagocytic cells while remaining discrete and modulating the host immune response. In our laboratory, *Brucella melitensis* 16M has been reported as the first identified intracellular pathogen having a quorum sensing (QS) system based on the synthesis and the detection of N-dodecanoyl homoserine lactone (C12-HSL). Through the QS-related transcriptional regulator VjbR, C12-HSL has been shown to regulate the production of surface proteins crucial for *Brucella* virulence as VirB proteins (assembling the type IV secretion system (T4SS) essential for *Brucella* to reach its intracellular replicative niche), flagellar proteins (involved in the establishment of long lasting infection) and some outer membrane proteins (like Omp25 which is involved in the regulation of TNFα secretion by infected cells).

Up to now, most studies on *Brucella* QS were based on the addition of exogenous synthetic C12-HSL during vegetative growth or at different steps of a cell infection. To get further insight into the role of C12-HSL in *Brucella*, we used a tool allowing its *in situ* detection at single-bacteria-level. In this system, acyl-homoserine lactones (AHL) activation of the *Pseudomonas aeruginosa* QS-related regulator LasR induces expression of *gfp*(ASV) coding for an unstable version of the green-fluorescent protein. That enabled us to confirm that *Brucella melitensis* 16M produce AHL during *in vitro* growth. Moreover, we could demonstrate for the first time, that AHL are produced by *Brucella melitensis* 16M during its intracellular trafficking in murine macrophage-like cells RAW264.7. AHL synthesis seems to take place until 24h post-infection (p.i.). Then, an important decrease in intrabacterial AHL concentration has been observed at 48h p.i. suggesting a negative regulation of AHL production.

In this context, we identified in *Brucella* a gene (BMEII0211-0212) homologous to the *aiiD* gene coding for an AHL acylase able to remove the acyl chain of AHL in *Ralstonia* spp. We could show that *Brucella* AiiD is a quorum quenching enzyme able to degrade a broad range of synthetic AHLs. Moreover we propose that AiiD plays a major role in the negative regulation of AHL production seen during cell infection. Indeed, the use of the *gfp*(ASV)-based AHL-detection system in a *B.melitensis* 16M strain deleted for *aiiD* (Δ*aiiD*) highlighted a greater AHL concentration in this strain compared to the wild type (wt) strain all along the infection process. No such difference was seen during vegetative growth. This observation is consistent with the fact that wt and Δ*aiiD* strains have the same *in vitro* production profile of VirB8 or FlgE, two proteins encoded by VjbR-regulated genes and known to be repressed by C12-HSL. Until now, we were not able to show any difference in the Δ*aiiD* strain replication ability in cells, compared to the wt. The absence of an attenuation of the virulence for the Δ*aiiD* in a model of cell infection despite its greater AHL content suggests that this concentration is not sufficient to impair *virB* operon expression and T4SS function.

The use of Δ*aiiD* in a mouse model of infection revealed remarkable characteristics of this strain. No difference in the virulence between Δ*aiiD* and wild type strains was observed 5 days p.i. Anyway, after 4 weeks, the Δ*aiiD* were recovered from spleens at a significantly higher level than the wt (1,5log $P<0,05$). At the same time, mice infected with Δ*aiiD* showed a higher splenomegaly with a 2,5-fold heavier spleen on average. Remarkably, histopathological analysis showed that this exacerbated splenomegaly was correlated with drastic changes in splenic architecture reflecting an important inflammatory reaction. Such data suggest that the regulation of C12-HSL production by the quorum quenching acylase AiiD during infection could contribute to the stealthy strategy of *Brucella*.

A novel 9-arylpurine acts as a selective inhibitor of *in vitro* enterovirus replication possibly by targeting virus encapsidation

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Enteroviruses (family of the Picornaviridae) are implicated in a wide spectrum of illnesses ranging from mild respiratory syndromes, herpangina, hand–foot–mouth-syndrome and common cold to potentially life-threatening disorders such as pancreatitis, myocarditis, meningitis, encephalitis and exacerbations of COPD and asthma. A number of 9-arylpurines have been identified as selective inhibitors of the replication of various enteroviruses. A representative example of this series of compounds is 9-(3-acetylphenyl)-6-chloropurine [TP219] that emerged as one of the most potent congeners in this series. The antiviral activity against Coxsackievirus B3 [CVB3] of TP219 was further assessed by (i) CPE assays, (ii) virus yield reduction assays, (iii) real-time quantitative PCR, (iv) bioluminescence and (v) antigen detection. Also potential effects of the compound on the accumulation of viral intra- and extracellular (+)ssRNA and on polyprotein processing were determined. Above described experiments revealed that TP219 probably acts at a stage which coincides with the correct encapsidation of the virion. To study whether newly formed assembly intermediates could be detected, we optimized and implemented so called SILAC-experiments in which untreated and treated infected cell cultures were analyzed and compared using nanoLC-MS/MS. The use of electron microscopy should be enabling us to visually detect any morphological or structural changes in treated versus untreated infected cell cultures. Drug-resistant variants were selected that were at least a 10-fold less susceptible to TP219 than the wild-type virus. TP219 did not prove cross-resistant to other classes of enterovirus inhibitors (including 3A, 2C and a 3D inhibitor). Genotyping of the drug-resistant variants revealed that several mutations, located in genes encoding for structural and non-structural proteins, may be responsible for the drug-resistant phenotype. To study whether or not individual mutations are sufficient to confer resistance, either single mutations or multiple mutations are being introduced in the wild-type genome. Further genotypic and phenotypic characterization of drug-resistant mutants will help to further understand the mechanism how TP219 is involved in the virus encapsidation and how this compound exerts its anti-enterovirus activity.

Secondary metabolites produced by complex bacterial flocs cultivated in continuous culture: a new Crabtree effect

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Free (isolated) bacteria represent only a negligible part of the bacterial population in the aquatic natural (or semi-natural) environments. A great majority of these micro-organisms (more than 90%) are living in the form of aggregates, biofilms or flocs. It is generally assumed that these structures are ecologically advantageous for the individuals. Ecological and public health importance of microbial aggregates and related forms has stimulated in the last ten years a growing number of studies on the subject (O'Toole et al, 2000). For example, flocs are structures dominating microflora in the wastewater plants. They consist in clusters of complex organic substances, of glutinous aspect, sheltering multiple colonies of various bacterial species, aerobic, anaerobic, or both. Small channels cross the flocs and ensure circulation of dissolved substances according to simple diffusion and passive transport. The arrival of the nutrients and the departure of the excreted products are therefore ensured according to one or the other of these mechanisms, or both. This is obviously true for dissolved gases (O_2, CO_2 …). Flocs of a wastewater purification plant were cultivated in a chemostat during two years (Bensaid, 2000). This resulted in flocs composed of stable bacterial consortia of at least 11 species, aerobes and/or anaerobes, of which 7 accounted for at least 99% of the microbial biomass. Coherence and robustness of these consortia were described in Thierie *et al.* (1999). The consortia, which were cultivated without pH and sterility control, under an oxygenation higher than 40% of saturation, displayed constant respective percentage of the species during time and at various dilution rates (D = inlet flow/volume of the reactor). It was assumed that the principal types of inter-specific relations also remained invariant under these conditions. The resultant flocs showed thus some "pluricellular" characteristics, presenting some coordination and co-operation between species, which differentiates a true consortium from a simple "mixture" of independent bacteria. Two outstanding observations concerning the metabolic activity of the flocs were made during this study: (i) a capacity of homeostasis and (ii) occurrence of a Crabtree effect. Adapted and stable flocs were in fact able to produce secondary metabolites, for example butyric acid at constant rate at the stationary state, independently of the of dilution rates with $\Pi^{m}_{BUT} = 0.012 \pm 0.001$ gBUT/ (Lxh). The precise mechanism of this "protected" production is not fully elucidated, but quite probably the producing subpopulations are in the "core" of the floc, more or less protected from the environmental influences of the reactor. The other effect is still more surprising, since the whole consortium was able to show a Crabtree effect, with acid acetic production. The effect we observed was however different from the well-known "acetate switch", also compared to a Crabtree effect (Wolfe, 2005). Two major differences that we observed were on the one hand, a nearly constant production of acetate with the dilution rate (and not a simple peak) and a reduction of the floc biomass, as opposed to what happens classically in the "acetate switch". A comparison with a mathematical model developed previously for describing the Crabtree effect in *S. cerevisiae* (Thierie, 2004) showed that the mechanism concerned in the floc could be analogous with that present in the yeast. Production of secondary metabolites in a controlled way in a continuous culture, without taking care about sterility and with cheap nutrients, should constitute obviously an industrial advantage in term of cost and rate. Development of a desired consortium remains evidently a central point to elucidate for each desired application.

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Two proteins of unknown function, TldD and TldE, regulate central carbon metabolism in *E. coli*

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Global regulators allow rapid and coordinated responses to environmental changes and/or modifications inside the bacterial cell. Global regulators act at every level of gene expression control (transcription, translation and protein stability). In E. coli, the global regulator CsrA (carbon storage regulator A) regulates central carbon fluxes, mobility and biofilm formation. CsrA acts at the post-transcriptional level by affecting the stability of its target mRNAs. We recently showed that the csrA gene is essential for growth on glycolytic carbon sources due to an imbalance of carbon fluxes. Our data suggests that the protein-encoded by the tldD and tldE genes interfere with CsrA activity by affecting its stability. These genes are widely distributed in the bacterial world and encode proteins of unknown functions.

To unravel the function(s) of TldD and TldE in bacterial physiology, the proteomes of E. coli strains deleted for csrA, tldD and tldE in various combinations and in various growth conditions were compared. Differential gene expression was observed upon growth in LB medium: the expression level of 20 proteins varied in the tldD tldE strain, 18 in the csrA mutant and 64 proteins in the csrA tldD tldE as compared to the wild-type strain. Proteins were identified by mass spectrometry. Interestingly, most of them are involved in carbon or amino acid metabolism. The tldD tldE mutant presented a 10-fold increase of the Crr protein as compared to the wild-type strain. The crr gene encodes the $EIIA^{glc}$ enzyme which is part of the PTS system (phosphoenolpyruvate:phosphotransferase systems) involved in glucose uptake and phosphorylation. In the absence of glucose, Crr is phosphorylated and activates adenylate cyclase which induces cAMP production. In the presence of glucose, Crr is not phosphorylated and inhibits uptake of other carbon sources (catabolite repression). Preliminary results suggest that CsrA, TldD and TldE regulate negatively cAMP synthesis and that TldD and TldE control the expression of the crr gene. We propose that CsrA-dependent regulation of cAMP production is linked to the s-called 'hexose-phosphate stress' (accumulation of glucose-6-phosphate and/or fructose-6-phosphate in the cell). This hypothesis is currently being tested.

The M/GP$_5$ glycoprotein complex of porcine reproductive and respiratory syndrome virus binds the sialoadhesin receptor in a sialic acid-dependent manner

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The porcine reproductive and respiratory syndrome virus (PRRSV) is a major treath to swine health worldwide. The virus infects specific subsets of porcine macrophages, central players in the immune system, and can persist in animals for extended periods of time due to a hampered immunity. The macrophage-specific lectin sialoadhesin is a crucial virus receptor on macrophages and although its role in PRRSV infection is well documented, its viral counterparts have remained elusive. The objective of this study was to identify PRRSV ligands for the sialoadhesin receptor and to characterize their interaction with sialoadhesin.

A soluble form of sialoadhesin was constructed by fusing the 4 N-terminal domains of sialoadhesin to the Fc- and hinge-region of human IgG1. This recombinant receptor displayed a similar binding functionality as wild type sialoadhesin, as it showed sialic acid- and PRRSV-binding activity. Using the soluble receptor, the M/GP$_5$ glycoprotein complex of PRRSV was identified as a ligand for sialoadhesin. Interestingly, this ligand-receptor interaction was found to be critically dependent on sialic acids lining the virion surface. These data represent a major breakthrough in the understanding of the role of PRRSV proteins in viral entry and pave the way for the development of a new generation of PRRSV vaccines capable of inducing an immunity that specifically blocks the interaction between viral M/GP$_5$ and sialoadhesin.

Replication of non-neurovirulent versus neurovirulent equine herpesvirus type 1 strains: a study in equine respiratory mucosa explants

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The subfamily of the *Alphaherpesvirinae* contains several respiratory viruses of human and veterinary importance including equine herpesvirus type 1 (EHV1). EHV1 is a major pathogen in horses and can cause respiratory disease, abortion and nervous system disorders. The mucosal surface of the respiratory tract is the common port of entry for respiratory viruses. In the present study, we examined viral replication of EHV1 in equine respiratory mucosa explants. Equine explants were incubated on the one hand with the non-neuropathogenic EHV1 isolate 97P70 and on the other hand with the neuropathogenic EHV1 isolate 03P37. At several time points post inoculation (pi), virus plaque volumes and latitudes in the epithelium were measured and penetration of the virus through the basement membrane (BM) was inspected. For both isolates, plaque formation could be observed in the epithelium starting from 24 hpi and an increase in plaque volume and latitude was observed over time. Interestingly, plaques never crossed the BM. In addition, single EHV1-infected cells were observed below the BM at 36 hpi for 97P70 and at 24 hpi for 03P37. For 97P70, these cells were mainly $CD5^+$ T-lymphocytes, while for 03P37 EHV1-infected cells below BM were mainly cells from the monocyte lineage. These results on differences in replication kinetics and identity of EHV1-infected cells will be further substantiated by repeating the experiments on explants derived from the same horse, to exclude possible inter-horse variability. In addition, to evaluate whether these differences can be attributed to the single polymorph nucleotide (SNP) associated with neuropathogenicity, we will also include parental Ab4, N752 mutant and D752 revertant recombinant viruses and parental NY03, D752 mutant and N752 revertant recombinant viruses.

The host selects mucosal and luminal teams of co-evolved, submissive gut microbiota

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The human body confronts microbes in its gastrointestinal tract with a multitude of barriers, by imposing selective physical conditions. Moreover, the epithelium is regularly replaced and mucus secretions result in a protective layer trapping immune molecules, which allows the host to particularly select the microbiota of this mucus layer. The host-microbiota interaction is characterized by reciprocal adaptation and benefits and reveals existence of an Evolutionary Stable Strategy (ESS). If host or microbiota deviate from this well-established strategy, negative interactions follow which ultimately lower their fitness. The central hypothesis of our recent review (3) is therefore that the host selects its microbiota, particularly those occurring very close to its epithelium i.e. the Mucosa Associated Microbial Community (MAMC). Recent assumptions about the submissive nature of microbiota are extended by distinguishing truly submissive and opportunistically submissive commensals, occurring respectively very close and relatively distant from the epithelium. The mucosal and luminal communities overlap in the very outside of the mucus layer, where host defence molecules are diluted. Using human microbiota associated rats, we showed that prebiotics which strongly modify the mucosal microbiota, also strongly increase host defence in terms of mucin secretions. On the other hand, prebiotics which maintain or even fortify the mucosal microbiota induce much less host defence. We further showed that *Faecalibacterium prausnitzii* might act as a peacekeeper at the mucosal interface by calming down the host responses towards microbiota. Moreover, *Akkermansia muciniphila* was identified as an indicator organism for host-microbe interactions since its activity in faeces was positively correlated with mucin secretions by the host.
We hypothesize that besides between host and microbiota, an ESS also evolved within the intestinal microbial community, meaning that microbiota act as a team and always prefer their familiar teammates. This could explain why microbial communities tend to return to their initial composition, even after severe disturbances. The outer MAMC could serve as an inoculum to restore previously existing ESS. Finally, when the host is unable to keep its microbiota submissive, health problems can occur. Previously submissive commensals become renegade and persistently cause health problems like inflammatory bowel disease (1) or obesity(2).

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In vitro investigation of the activity of miconazole against biofilms of various *Candida* species

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Biofilms formed by *Candida* species consist of a dense network of cells, hyphae and pseudohyphae embedded in an extracellular matrix. These biofilms are highly resistant against antifungal agents. Azoles have a fungistatic effect based on the inhibition of the enzyme 14α-demethylase in the ergosterol biosynthesis. Previous research showed that miconazole (an imidazole) has also fungicidal activity associated with the induction of ROS (reactive oxygen species). In the present study the fungicidal activity of miconazole against *in vitro* grown *Candida* biofilms was investigated. Furthermore, the relationship with the production of ROS was examined.

Biofilms of ten *Candida albicans* strains and five other *Candida* species were grown for 24 h on silicone disks. The effect of miconazole (5 mM) on these biofilms was investigated by determine the number of CFU by plating. The level of ROS induction in planktonic and sessile cells was determined using a fluorometric assay with DCFHDA (2',7'-dichlorofluorescein diacetate). The MIC (minimal inhibitory concentration) of miconazole was determined according to the EUCAST protocol. All experiments were performed in the absence and presence of ascorbic acid (10 mM), a quencher of ROS activity.

Miconazole showed a significant ($p < 0.05$) fungicidal effect against mature *Candida* biofilms (reductions ranging from 89.3% to 99.1%). Furthermore, miconazole strongly induced ROS production both in planktonic and sessile cells. The addition of ascorbic acid to miconazole-treated planktonic *Candida* cells drastically reduced ROS production for all strains. A simultaneous decrease in susceptibility to miconazole was observed for most (10) strains. In contrast, the significant quenching of ROS after addition of ascorbic acid to *Candida* biofilms did not lead to a reduction of the fungicidal activity of miconazole.

In conclusion, the fungicidal activity of miconazole against *Candida* biofilms may be of importance in the treatment of biofilm-related *Candida* infections. An increased ROS production was observed during miconazole treatment, however this was not directly related to the fungicidal activity of miconazole against *Candida* biofilms.

Pseudorabies virus US3-mediated reorganization of the actin cytoskeleton is mediated by group A p21-activated kinases

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The pseudorabies virus (PRV) is a member of the alphaherpesvirus subfamily of the herpesviruses, and is often used as a model for this family in general (Pomeranz et al., 2005, Microbiol Mol Biol Rev). The alphaherpesvirus subfamily contains closely related viruses of man and animal, including the human herpes simplex virus type 1 and type 2 and varicella zoster virus and different animal pathogens, including PRV in pigs, equine herpesvirus 1 in horses, bovine herpesvirus 1 in cattle, and Marek's disease virus in poultry.

The US3 protein is a viral serine/threonine kinase that is conserved among all members of the *Alphaherpesvirinae*. The US3 protein of different alphaherpesviruses (PRV, MDV, HSV-2) causes rearrangements of the actin cytoskeleton, such as the disassembly of actin stress fibers and/or formation of cell projections (Murata et al., 2000, Genes Cells, Van Minnebruggen et al., 2003, JVI, Calton et al., 2004, Virus Genes, Schumacher et al., 2005, JVI, Favoreel et al., 2005, PNAS, Van den Broeke et al., 2009, Virology).

For pseudorabies virus (PRV), these actin alterations have been associated with increased intercellular virus spread (Favoreel et al., 2005, PNAS). Here, we find that inhibiting group A p21-activated kinases (PAK), which are key regulators in Cdc42/Rac1 Rho GTPase signaling pathways, using the group A PAK-specific inhibitor IPA-3 or the PAK inhibitory peptide (PID) impairs PRV US3-mediated actin alterations. Using $PAK1^{-/-}$ and $PAK2^{-/-}$ mouse embryo fibroblasts (MEFs), we show that PRV US3-mediated stress fiber disassembly requires PAK2, whereas PRV US3-mediated cell projection formation mainly is mediated by PAK1, also indicating that PAK1 and PAK2 can have different biological effects on the organization of the actin cytoskeleton. Infection of MEF with wild PRV, but not with US3null PRV, and transfection of MEF with wild type PRV US3, but not with kinase-dead US3, resulted in phosphorylation of group A PAKs on a critical, activation-associated threonine residue. Phosphorylation assays showed that PRV US3 is able to directly bind and phosphorylate both PAK1 and PAK2. Lack of group A PAKs in MEFs was correlated with inefficient virus spread in vitro, and lack of PAK2 was correlated with reduced virus egress.

Thus, PRV US3 induces its effect on the actin cytoskeleton via group A PAKs and group A PAKs are involved in efficient spread of PRV in vitro.

Characterisation of PdhS, an essential and polarly localized histidine kinase in *B. abortus*

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During development, one cell undergoing asymmetric division is able to give rise to different sibling cells despite an identical inherited genetic material. The asymmetric dividing bacterium *Caulobacter crescentus*, an alpha-proteobacterium, is a model for the understanding of how the specific localization pattern of molecular determinants is involved in cell cycle control and the generation of asymmetry in prokaryotes. In this free living bacterium, a well characterized molecular network is involved in the asymmetric division process, i.e. two histidine kinases (HK) named PleC and DivJ are responsible for the phosphorylation state of their cognate monodomain response regulator (RR) DivK. In our laboratory, we are focusing on an other alpha-proteobacterium dividing asymmetrically called *Brucella abortus*, a facultative intracellular pathogen responsible for a worldwilde zoonosis. After each division, two sibling cells are generated, a small cell and a large cell. In *B. abortus* genome, genes coding for HK PleC and DivJ and RR DivK homologs are present. Interestingly, a third HK, homologous to both PleC and DivJ, is also found in *B. abortus* genome. This HK, named PdhS (for PleC/DivJ Homologous Sensor), interacts with DivK, suggesting that it plays a role in the phosphorylation pathway responsible for cell cycle control and asymmetry generation in *B. abortus*. Moreover, a strain overexpressing *pdhS* gene ($pdhS^{+++}$) displays several morphological defects such as multipolar cell and mini cells, suggesting that PdhS is indeed involved in cell cycle control. Consistently, *pdhS* and *divK* genes are essential, in contrast to *pleC* and *divJ*, suggesting that PdhS is mainly involved in DivK control *in vivo*. Time lapse microscopy showed that PdhS is located at the old pole of the large cell. We observed that the small cell acquires PdhS at its old pole with a delay after division.

In this context, we searched for conditionnal alleles of *pdhS*. Using allelic replacement of chromosomal *pdhS* by mutated *pdhS* alleles, we obtained thermosensitive strains of *B. abortus* that are able to grow at 37°C (permissive temperature) but not at 41°C (restrictive temperature). Complementation assay with wild type *pdhS* carried on a plasmid showed that the thermosensitive phenotype was indeed due to mutations in *pdhS* gene ($pdhS^{ts}$ alleles). Interestingly, spontaneous supressor strains were obtained at the restrictive temperature. An approach to sequence the whole genome of three supressor strains and a thermosenstive strain as reference is now initiated to identify mutations responsible for the supression of the thermosensitive phenotype at restrictive temperature. At restrictive temperature, the $pdhS^{ts}$ strains do not display detectable morphological defects. This observation prompted us to consider this thermosensitive phenotype at 41°C as a PdhS loss of function while the phenotype of aberrant morphologies (AM) in a $pdhS^{+++}$ overexpression context at 37°C is considered as a PdhS gain of function i.e. impairing the cell cycle of *B. abortus*. The localization study of a PdhS-YFP fusion in the AM cells by fluorescent microscopy associated with time lapse microscopy showed that only the pole in front of PdhS could present two new growing poles, suggesting that PdhS stimulates the growth at this particular site. Furthermore, these observations also highlighted the fact that the pole labelled by PdhS is systematically a static pole while the other moves together with the bacterium growth, arguing for a unipolar growth for *B. abortus*. In an infection context, after being phagocyted by its host cell, this bacterium do not replicate until it reaches its replication site i.e. the endoplasmic reticulum (ER) of its host cell (8 to10 hours post infection) where it begins to replicate. Preliminary results show that PdhS is not detected during the traffic inside the host cell. But, at 24 hours post infection, when *B. abortus* reaches the ER and replicates, PdhS is localized again at one pole of *B. abortus* in approximately one half of the bacteria. Theses observation strongly suggest a link between cell cycle regulation, asymmetry and infection process of *B. abortus*.

The periplasmic protein PliG bestows g-type lysozyme resistance in *Escherichia coli*

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INTRODUCTION: Lysozymes hydrolyse the peptidoglycan wall of the bacterial cell, resulting in cell lysis. In the animal kingdom the main lysozyme families are chicken (c-), goose (g-) and invertebrate (i-) type lysozyme. As a response to the widespread natural occurrence of lysozyme and its important role in the innate immune system of animals, some bacteria have developed lysozyme inhibitors to evade lysozyme mediated lysis. Three different inhibitors have been reported in *E. coli,* of which two are specific for c-type lysozyme (Ivy, Inhibitor of vertebrate lysozyme; MliC, membrane bound lysozyme inhibitor of c-type lysozyme), and one specific for g-type lysozyme (PliG, periplasmic lysozyme inhibitor of g-type lysozyme)[1]. Both Ivy and MliC confer c-type lysozyme tolerance in *E. coli*, supporting a role of these inhibitors in bacteria-host interactions [1,2,3]. Here we report the protective role of PliG in *E. coli* against salmon g-type lysozyme (SL).

MATERIAL AND METHODS:
Strains: *E. coli* MG1655 was used as wild-type from which the following mutants were constructed: *E. coli* Δ*pliG*, *E. coli tolA*::Kan, *E. coli tolA*::Kan Δ*pliG*, *E. coli tolA*::Kan araBAD::*pliG*.
Viability reduction: Exponential phase (OD_{600}=0,3) cells of *E. coli* and *E. coli* Δ*pliG* grown at 37°C in Luria Bertani Broth (LB) were harvested by centrifugation (4000 x g, 5 min), washed and resuspended in an equivalent volume of 10 mM potassium phosphate buffer (PPB) pH 7.0 without and with 1mM EDTA and/or salmon lysozyme (15 µg/ml). The three strains with a *tolA* background were grown as described above but 0,1% arabinose was additionally added for induction of the *pliG* gene under control of the araBAD promotor. After harvesting, the cells were washed and resuspended in 10 mM PPB pH 7.0 with or without 18 µg/ml salmon g-type lysozyme (SL). After 24h of incubation at 22°C, the viability reduction was determined by plate counts on LB.
Growth inhibition: Overnight cultures of *E. coli* and *E. coli* Δ*pliG* were diluted (1/100) in fresh LB and OD_{600} was followed at 30°C in a microplate reader. After 2 h 1mM EDTA and/or 150 µg/ml SL was added. A similar experiment was conducted with the 3 strains with *tol*A background, but in this case 37,5µg/ml and 3,75µg/ml of SL were added at time '0'.

RESULTS: A major challenge to test lysozyme sensivity of gram-negative bacteria is the presence of an outer membrane layer that is impermeable to lysozyme. Therefore, to investigate whether PliG contributes to g-type lysozyme tolerance, *E. coli* was rendered sensitive to lysozyme either by using EDTA as outer membrane permeabilisator or by introducing a *tol*A mutation that increases outer membrane permeability.Wildtype *E. coli* survived the treatement with both EDTA and SL but *E. coli* Δ*pliG* showed almost a 300-fold inactivation in this situation. When EDTA and SL was added to growing *E. coli* Δ*pliG*, growth was retarded compared to *E. coli* wildtype cells in the same conditions. Similar results were found in the growth experiments with *E. coli tolA*::Kan and *E. coli tolA*::Kan Δ*pliG*. Furthermore, overexpression of the inhibitor in *E. coli tolA*::kan *araBAD*::*pliG* increased significantly the number of survivors after treatement with SL compared to *E. coli tolA*::Kan and *E. coli tolA*::Kan Δ*pliG*. These results demonstrate that PliG can effectively protect bacterial cells against g-type lysozyme.

DISCUSSION: Using *pliG* knock-out strains and overexpressor strain, we were able to demonstrate that, PliG contributes to g-type lysozyme tolerance in *E. coli*, under conditions where the outer membrane is permeabilized. Together with earlier similar findings for the c-type lysozyme inhibitors Ivy and MliC [1,2,3], these results strengthen the evidence that bacteria have evolved several specific lysozyme inhibitors as virulence and/or as colonization factors in pathogenic or commensal relationships with animal hosts.

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Does the microenvironment shield tumors from chemotherapeutics?

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Mycoplasmas (class Mollicutes) are the smallest self-replicating bacteria. They mainly cause asymptomatic infections in humans and are abundantly found in human tumors, probably due to the high nutrient turnover and active metabolism of cancer tissue. Compared to other bacteria mycoplasmas are characterized by a strong genome reduction (as small as 600 kbp) and have therefore lost many metabolic activities such as the *de novo* synthesis of nucleosides. As a result these prokaryotes encode for a wide variety of nucleoside-metabolizing "salvaging" enzymes (e.g. dCTPase, dUTPase, dCMP deaminase, thymidine and uridine phosphorylase, …) that may also interfere with the efficacy of nucleoside-analogues used in cancer chemotherapy (Liekens *et al*., 2009). We have recently shown that the presence of *Mycoplasma hyorhinis* in tumor cell cultures dramatically (20-150-fold) reduces the cytostatic activity of nucleoside analogues, such as 5-fluoro-2'-deoxyuridine and 5-trifluorothymidine (Bronckaers *et al.*, 2008). We provided evidence that the mycoplasma-encoded catabolic enzyme thymidine phosphorylase (TP), catalyzing the conversion of thymidine into thymine and 2-deoxyribose-1-phosphate, is responsible for this phenomenon.

In this study we demonstrate that mycoplasma infection also significantly reduces the cytostatic activity of the drug gemcitabine (dFdC; 2',2'-difluorodeoxycytidine), currently used as a treatment for non-small cell lung, bladder and pancreatic cancer. After transport into the cell, gemcitabine is phosphorylated in three successive steps to generate gemcitabine-triphosphate (dFdCTP) which will then be incorporated into the cellular DNA, causing death of the tumor cells. The rate limiting step in this process is catalyzed by the enzyme deoxycytidine kinase (dCK).
M. hyorhinis infection of osteosarcoma (OST.TK$^-$) and 3 different breast carcinoma (MDA-MB-231, FM3A and MCF-7) cell lines resulted in a 10- to 70-fold reduction in the cytostatic activity of gemcitabine. The cytostatic activity of this cytidine analogue could be fully restored either by the administration of a TP inhibitor or by adding thymidine. We hypothesize that these observations can be at least partially explained by decreased thymidine triphosphate (dTTP) levels due to mycoplasma-encoded TP-expression. Decreased dTTP pools may lower dCK activity, the key enzyme in the activation (phosphorylation) of gemcitabine. By means of flow cytometry we showed that gemcitabine causes MCF-7 cell cycle arrest in the S-phase at a concentration of 0.2 μM. In contrast, a 25-fold higher concentration of gemcitabine was needed to cause a similar effect in *M. hyorhinis*-infected MCF-7 (MCF-7/HYOR) cells. Using radiolabeled gemcitabine, the incorporation of its active metabolite was compared in MCF-7 versus MCF-7/HYOR DNA and in FM3A versus FM3A/HYOR DNA. Depending on the concentration of gemcitabine up to 170- and 100-fold reduction of gemcitabine-triphosphate incorporation was observed in MCF-7/HYOR and FM3A/HYOR DNA, respectively.

In conclusion, our results show that the cytostatic potency of gemcitabine in different human and murine tumor cell lines is drastically decreased upon mycoplasma infection. Our current findings suggest that mycoplasma-encoded TP may play a key role in the inactivation of different classes of chemotherapeutics including gemcitabine. We thus hypothesize that cancer chemotherapy with certain nucleoside analogues could be significantly improved by the co-administration of a mycoplasma-specific antibiotic or specific inhibitor of mycoplasma-encoded enzymes.

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The porcine reproductive and respiratory syndrome virus requires trafficking through CD163 positive early endosomes, but not late endosomes, for productive infection

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The porcine reproductive and respiratory syndrome virus (PRRSV) has a restricted tropism for subsets of differentiated porcine macrophages. To date, two macrophage-specific molecules have been described to be involved during PRRSV entry in macrophages, namely sialoadhesin and CD163. Incubation of macrophages with sialoadhesin- or CD163-specific antibodies showed that sialoadhesin functions as PRRSV attachment and internalization receptor. CD163 was shown not to function as an attachment receptor but rather during a later step in PRRSV entry. Confocal analysis of PRRSV entry in non-permissive cells expressing only sialoadhesin showed PRRSV internalization but not uncoating. In contrast, when both sialoadhesin and CD163 were expressed, PRRSV was uncoated upon internalization resulting in productive infection, indicating a role for CD163 in PRRSV uncoating. Although the relative wealth of information regarding the initial steps of infection, questions remain. In this study, we aimed to identify the endocytic compartments needed for productive PRRSV infection. Furthermore, colocalization between PRRSV and sialoadhesin and CD163 along the endocytic pathway was investigated to determine their specific site of action. Using dominant-negative Rab5 and Rab7 mutants, it is shown that upon internalization, PRRSV enters early endosomes but does not continue through the endocytic pathway up to late endosomes. This was confirmed via colocalization experiments visualizing PRRSV and markers for different compartments of the endocytic pathway. Furthermore, it was shown that PRRSV colocalizes with its attachment and internalization receptor sialoadhesin on the cell surface and beneath the plasma membrane, while CD163 and PRRSV only meet in early endosomes, supporting the described roles of both molecules.

Rapid and accurate quantification of viable *Candida* species in whole blood using immunomagnetic separation and solid-phase cytometry

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Candida species are a common source of nosocomial bloodstream infections in critically ill patients. Therefore, rapid quantification and identification of *Candida albicans* and other pathogenic yeasts is crucial for the early initiation of adequate antifungal therapy. The sensitivity of the traditional diagnostic procedure based on blood culture is variable and it usually takes two to four days before growth of *Candida* species is detected. We developed a rapid method for the quantification of *Candida* species in blood, combining immunomagnetic separation with solid-phase cytometry using viability and FISH (*C. albicans*) labelling. In a first step, *Candida* cells present in a whole blood sample (max. 15 ml) are magnetically labelled with a primary anti-*Candida* FITC conjugated antibody and a secondary anti-FITC Microbead conjugated antibody. Subsequently, *Candida* cells are separated using the MACS technology (Miltenyi Biotec). The obtained suspension is filtered and the retained cells are labelled using FISH (leading to red fluorescence of *C. albicans*) and ChemChrome V6 (which allows for the green fluorescent labelling of all viable cells). Finally, the membrane filter is scanned by a solid-phase cytometer and each detected cell is microscopically inspected for green and red fluorescence. Upon analysis of spiked blood samples, our method was shown to be sensitive and specific with a low detection limit (1 cell/ml of blood). For the analysis of clinical samples (14 so far), our method proved more sensitive than culture and identification results were in agreement. Additionally, numerous mixed infections were identified and an extremely high number of cells was twice associated with an endovascular source of infection.

Isolation of a bacterial inhibitor of the invertebrate type lysozyme

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Background: Lysozymes play an important role in the innate immune system of many organisms, due to their capability to hydrolyse the peptidoglycan layer in the bacterial cell wall. Bacteria on the other hand have developed several barriers against this bactericidal enzyme. In Gram-negative bacteria, one of these barriers was recently discovered to be the production of periplasmic or membrane bound proteinaceous lysozyme inhibitors. Thus far, two different families of inhibitors have been described that are specific for chicken (C)-type lysozyme. Although invertebrate (I-) type lysozymes have been less studied than their C-type counterparts, their phylogenetic distribution, enzymatic and bactericidal activity suggests an equally important role in antibacterial defense and possibly in other functions.

Objectives: To screen for bacterial inhibitors of Invertebrate (I)-type lysozyme, and to characterize their biological function.

Methods: Periplasmic extracts from a range of Gram-negative bacteria were isolated by cold osmotic shock. Inhibition of recombinantly produced I-type lysozyme (from the marine bivalve *Tapes japonica*) was measured using the standard *Micrococcus* cell suspension assay. Potential inhibitors were purified by affinity chromatography with I-type lysozyme as a ligand, and identified by tandem mass spectrometry. Specificity and affinity for I-type lysozyme was examined by Surface Plasmon Resonance. The influence of a bacterial I-type lysozyme inhibitor, identified in *Aeromonas hydrophila*, on the sensitivity of this bacterium towards I-type lysozyme was analysed by comparing the survival of a wild type and inhibitor knockout strain, upon the combined treatment with I-type lysozyme and lactoferrin (a natural outer membrane permeabilizing protein).

Results and conclusion: We have isolated and identified a **P**eriplasmic **l**ysozyme **i**nhibitor of **I**-type lysozyme (PliI) from *Aeromonas hydrophila.* Homologues of PliI occur in a range of proteobacterial species, of which several have a pathogenic or symbiotic relationship to invertebrate organisms. PliI has a dedicated affinity for I-type lysozyme, and does not inhibit C- or G (goose)-type lysozymes. Knockout of *pliI* rendered *A. hydrophila* more sensitive to challenge with *T. japonica* lysozyme in the presence of lactoferrin as an outer membrane permeabilizer. Preliminary observations using Gfp-tagged bacteria suggest that PliI promotes survival of *A. hydrophila* in the gastrointestinal tract of *C. elegans.* We hypothesize that PliI, and lysozyme inhibitors in general, play an important role in bacteria-host interactions, and could be interesting potential targets for antibacterial drug development.

The α-2,3-sialyltransferase encoded by Myxoma Virus is not essential for virus replication *in vitro* but contributes to virulence *in vivo*

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Myxoma virus is a pathogenic Poxvirus that induces a lethal disease called Myxomatosis in European rabbits. Myxomatosis is characterized by fulminating lesions at the primary site of inoculation, followed by rapid dissemination to internal organs, production of external secondary lesions and bacterial superinfection. Myxoma virus is one of the very rare viruses that encodes an α-2,3-sialyltransferase that transfers sialic acid from CMP-Sia to glycoproteins and glycolipids. Very little information is available about the role played by this glycosyltransferase in the biology of the infection and in the pathogenesis. Here, we report the construction of two recombinant Myxoma virus strains deleted for the M138L gene encoding the α-2,3-sialyltransferase and a derived revertant strain. The highly virulent Lausanne strain of Myxoma virus was used as parental strain. After a classical *in vitro* characterization that did not reveal any difference between the recombinant strains and the wild-type strain, the virulence of the three recombinant strains was compared to the parental strain by intradermal inoculation of rabbits. Our data show that the M138L deleted strains are attenuated *in vivo* in comparison with the revertant and the parental strains. All but one rabbits inoculated with the deleted strains survived the infection while all rabbits infected with the parental and the revertant strains died within 9 days post-infection. Moreover, rabbits infected with the deleted strains survived to a subsequent challenge with the parental strain. Histological analysis of tissue materials from the primary site of infection revealed that by opposition to wild-type parental and revertant strains, an intense heterophils infiltration was observed all over the dermis at day 4 post-infection in deleted strains infected rabbits and a widespread mononuclear cell infiltrate was present at day 9 post-infection. All together, these results demonstrate therefore that, although non essential, the α-2,3-sialyltransferase is a virulence factor for Myxoma virus pathogenesis in the European rabbit. In the future, this work should help us to understand how the α-2,3-sialyltransferase could regulate the host's immune response to infection.

L-glutamine dependent binding of the archaeal Lrp-like transcription regulator Sa-Lrp from the hyperthermoacidophile *Sulfolobus acidocaldarius* to different targets

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Members of the Lrp (Leucine-responsive Regulatory Protein) family of transcriptional regulatory proteins are widely distributed among archaea and bacteria. These transcriptional regulators are either specific activators or repressors, or global regulators with different effects, depending on the targets and/or the presence of a suitable cofactor. Lrp-like regulators consist of an N-terminal DNA-binding domain with a helix-turn-helix motive and a C-terminal RAM domain with an αβ-sandwich fold, responsible for cofactor-binding and oligomerization. Bacterial Lrp-like proteins are generally involved in the control of amino acid metabolisms. Archaeal genomes encode several Lrp-like regulators, but generally their physiological role and cofactor are not known.

Sa-Lrp is a Lrp-like transcriptional regulator of the hyperthermoacidophilic *Sulfolobus acidocaldarius.* Previously, this regulator was shown to bind to the control region of its own gene in the absence of cofactor(s) (ENORU-ETA *et al.*, 2000).

Recently, cofactor predictions of Lrp-like proteins were made based on the identity of amino acid residues predicted to be involved in the formation of the cofactor binding pocket (KAWASHIMA *et al.*, 2008). Sa-Lrp was thereby predicted to bind with L-glutamine.

Here, we analyze the effect of amino acids as potential cofactors in the binding of Sa-Lrp to the control region of its own gene. Sa-Lrp showed a strong increase in binding affinity in the presence of 10 mM L-glutamine. This effect is specific since none of the other amino acids tested affected the DNA-binding affinity. Binding assays performed in the presence of various concentrations of L-glutamine indicate that a concentration of 2 mM is sufficient to fully stimulate the DNA binding. Binding assays performed in the presence of increasing concentrations of non-specific competitor-DNA and binding to a bacterial control region DNA indicated that this binding is sequence-specific.

Considering the fact that L-glutamine plays an important role in the *in vitro* binding of Sa-Lrp to the control region of its own gene, *in vitro* binding analysis were made with the control regions of other potential target genes. These genes were selected based on their role in nitrogen metabolism. Binding of Sa-Lrp to the control region of 3 glutamine synthetases, glutamate synthase (GOGAT) and glutamate dehydrogenase showed strong binding in the presence of L-glutamine.

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Denitrification in *Bacillus* by yet unknown genes?

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Background:
The information on denitrification in Gram-positive bacteria, like *Bacillus*, is rather limited and often only partially complete. However, the ecological relevance of Gram-positive denitrification in e.g. soils could be very important. Most available primer sets targeting the key denitrification genes, *nir* and *nor*, are designed on the basis of gene sequences derived from closely related Gram-negative laboratory strains.

Objectives:
1. Phenotypic investigation of the *distribution of the denitrification trait* in the Gram-positive genus *Bacillus*.
2. Investigation of the *distribution of the key denitrification genes* (*nir* and *nor*) in the *Bacillus* strains that phenotypically denitrify

Methods:
1. We have screened 92 of the ± 150 validly described type strains of *Bacillus* for their capacity to denitrify. Subsequently, within several selected species a diverse set of strains was analyzed. This analysis comprised nitrate and nitrite reduction tests with the Griess reagent confirmed with gas chromatographic measurements of N_2O.
2. The phenotypically denitrifying *Bacillus* strains were then further investigated with 17 currently existing primer sets for the amplification of *nir* and *nor* genes.

Conclusions:
For the phenotypic tests data show an unexpected high prevalence of the denitrification trait within *Bacillus*. However, the 17 primer sets for *nir* and *nor* did not allow for the straightforward detection of these key denitrification genes, despite optimization of the different PCR set-ups. Therefore, the current molecular approaches to study the gene diversity in environmental samples seems to be highly biased and special attention is needed interpreting PCR based analysis to study denitrification. In conclusion: the design of novel primers targeting the Gram-positive diversity of denitrifiers is of major importance to re-assess the complete diversity of denitrifiers.

Inactivation of bacterial spores in tomato sauce by high hydrostatic pressure

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Introduction: Bacterial sporeformers, like *Alicyclobacillus acidoterrestris* and *Bacillus coagulans,* can cause flat-sour type of spoilage in heat-processed tomato products. *B. coagulans* is moderately acidophilic, being able to grow down to a pH of about 4.0, while *A. acidoterrestris* can even grow down to a pH of 2.5. Since the spores of both organisms are both heat and acid resistant, they can survive the commercial heating process applied to tomato products.

Purpose: The objective of this work was to study the germination and inactivation of *A. acidoterrestris* and *B. coagulans* spores in tomato sauce by high hydrostatic pressure treatment at temperatures up to 60°C.

Methods: Spore suspensions of *A. acidoterrestris* ATCC49025 and *B. coagulans* ATCC7050 (approximately 10^7 CFU ml^{-1}) in citric acid (0,02 M, pH 3.5 - 5.0) or tomato sauce (pH 4.2 or 5.0) were subjected to pressure treatments at 100 to 800 MPa (5 - 60°C, 10 minutes). After processing, spore inactivation and germination was determined by plating the suspensions either directly, or after an additional heat treatment to kill the germinated spores. All experiments were conducted in triplicate and data are expressed as mean values ± standard errors of mean.

Results: In general, little or no inactivation of *A. acidoterrestris* spores after a HHP treatment (100 - 800 MPa, 10 min) was noticed at all temperatures tested. However, treatment at low pressures (< 400 MPa, optimum of about 200 MPa) caused germination of *A. acidoterrestris* spores. The proportion of germinated spores increased with increasing process temperature, up to 99 %. Spores of *B. coagulans* also germinated upon high pressure treatment, but showed more germination as pressure increased.

Significance: The data show that germination of *A. acidoterrestris* and *B. coagulans* spores can be induced by high hydrostatic pressure treatment in an acidic environment. In contrast, spores of non-acidophiles, like *B. subtilis,* can not be pressure-germinated in acidic conditions. We anticipate that it will be possible to inactivate *A. acidoterrestris* and *B. coagulans* spores by applying a two-step process consisting of a mild high pressure treatment to induce germination, followed by a mild heat treatment or a more elevated pressure treatment to kill the germinated spores.

Metagenome mining for the discovery of new antimicrobial molecules

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Over decades human activity is destroying the environment, without knowing the consequences it brings along. One of the major consequences/problems is the rise in antibiotic resistance of microorganisms, inclusive in nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa* making the treatment of these infections problematic. Indeed, the decreasing number of new antibiotics and the increase in antibiotic resistance are threatening. Furthermore, natural occurring environments are known to be a reservoir for antibiotic molecules and antibiotic resistance genes. The screening of such environments offers the potential to find new antibiotic molecules and their corresponding or even new resistance mechanisms. But, in order to achieve this goal we have to overcome the non-cultivability of the majority microorganisms, since only 1% of the microbial population is cultivable. Recently, a new approach has been developed, named metagenomics. Metagenomics is the sequence-based and function-based analysis of microbial populations on the basis of environmental DNA without the need of any cultivation. In this way it can explain who is there, who is doing what and why they are doing it without the need of cultivation.
A lot of metagenomic projects on different environmental habitats already allowed the discovery of new species, industrial applicable enzymes and antimicrobial molecules. In this study, a metagenomic library from the polluted freshwater river the Zenne was constructed. It allowed us the observation of an unexpected high microbial diversity, with an estimation of approximate 195 different species, and resistance to several antibiotic classes and metals. This included resistance to the metals cadmium, mercury, chromate and antibiotics tetracycline, erythromycin and beta-lactams. The resistance to the latter revealed a new beta-lactamase and the resistance to the metal cadmium could be assigned to *Polynucleobacter neccesarius*.
This unexpected high microbial diversity and the resistance to several antibiotics and metals make the polluted Zenne river a potentially interesting source for metagenome mining for the finding of new antimicrobial molecules active against *S. aureus* or *P. aeruginosa*.

Nitric oxide production by the human intestinal microbiota by dissimilatory nitrate reduction to ammonium

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The free radical nitric oxide (NO) is an important signaling molecule in the gastrointestinal tract. Epithelial cells are known to produce NO through oxidation of L-arginine. Besides, gut microorganisms are also capable of producing NO. However, the exact mechanism of NO production by the gut microorganisms is unknown. Microbial NO production was examined under *in vitro* conditions simulating the gastrointestinal ecosystem using L-arginine or nitrate as substrates. L-arginine did not influence the microbial NO production. However, NO concentrations in the order of 90 ng NO-N per L feed medium were produced by the fecal microbiota from trace levels of nitrate (110 µg NO_3^--N/L). ^{15}N tracer experiments showed that nitrate was mainly reduced to ammonium by the dissimilatory nitrate reduction to ammonium (DNRA) pathway. To our knowledge, this is the first study showing that gastrointestinal microbiota can generate substantial amounts of NO by DNRA and not by the generally accepted denitrification or L-arginine pathway. Previously, a bacterial origin for the production of NO in the rectum of patients with Inflammatory Bowel Disease (IBD) was proposed. In these studies, no NO was found in the rectum of healthy volunteers but around 40 ng NO-N/L was measured in the rectum of patients with active IBD. Moreover, external addition of NO has been shown to alter the mucosal barrier function and to have an effect on the metabolism of colonocytes. As it was strongly suggested that several constitutive and/or inducible defense systems exist in mammalian cells that neutralize the damaging effects of NO, the high rectal levels of NO in IBD patients and the low levels in healthy persons might indicate that the expression of these defense mechanisms is ineffective or that there is a disequilibrium in the microbial NO production in IBD patients. Further work is needed to elucidate the exact role between NO produced by the gastrointestinal microbiota and host cells of healthy and diseased persons.

Identification of T cell subsets implicated in the resistance to *Brucella melitensis* infection in a mouse experimental model

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Objectives:
Brucella organisms are facultative intracellular Gram-negative coccobacilli that cause brucellosis in humans and animals. Acute human brucellosis is characterized by undulating fever, which may result in chronic disease with serious clinical manifestations. Despite progress in mouse models of brucellosis, much remains unknown regarding cellular components of the innate and adaptive immune responses induced by *Brucella* infection.
The focus of this study was to determine which lymphocyte subsets are implicated in the immune response against *B. melitensis* infection.

Methods:
C57BL/6 or BALB/c wild type and deficient mice were injected intra-peritoneally with $4x10^4$ CFU of *B. melitensis* strain 16M in 500 µl of PBS. Bacterial growth in vivo was evaluated by plating serial dilutions of spleen homogenates on 2YT.

Results:
Resistance and susceptibility to *Brucella* infection has been previously associated to T helper 1 (Th1) and Th2 response, respectively. However, this paradigm is mainly derived from experiments using neutralising antibodies. Some studies have reported that the susceptibility of BALB/c mice compared to C57BL/6 mice could be associated with the development of Th2 response that might inhibit the differenciation of Th1 cells. In addition, the importance of the recently described Th17 subset has never been analysed during *Brucella* infection. In order to gain insight into the role of Th subset in *Brucella* control, we compared the susceptibility of IL-$4^{-/-}$, IL-$12p35^{-/-}$ and IL-$17R^{-/-}$ C57BL/6 and IL-$4^{-/-}$ BALB/c mice. Results show that IL-$12p35^{-/-}$ mice displayed an enhanced bacterial count at early (5 days) and later time (28 days) post infection when compared to wild type mice. Surprisingly, we did not find any negative role of IL-4 in *Brucella* control in both C57BL/6 and BALB/c mice. Moreover, the absence of IL-4 led to an increased bacterial count 5 days post infection. IL-$17R^{-/-}$ and wild type mice presented similar bacterial growth.

Conclusions:
Taken together, our results confirm the predominant role of IL-12-mediated Th1 response in the control of *Brucella* infection and suggest that Th2 response is not so unfavourable to the host.

Did anammox introduce nitrate on earth?

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In the Archaean eon, from around 3.8 to 2.5 10^9 years ago, life in the oceans was subject to a major change since oxygenic phototrophic micro-organisms introduced oxygen in an almost fully reduced environment. Before that point, it is likely that nitrogen fixation, mineralization of organic nitrogen to ammonium and ammonium assimilation were the only microbiological nitrogen conversions.

With the advent of oxygen, the aerobic oxidation of ammonium to nitrite (nitritation) could evolve, introducing for the first time nitrite in the biosphere. From the presence of nitrite, the anabolic possibility of assimilatory nitrite reduction to ammonium arose, as well as three novel catabolic conversions, i.e. nitratation, denitritation and anammox. Nitratation comprises the aerobic oxidation of nitrite to nitrate, which could evolve above the oxycline in the combined presence of oxygen and nitrite. In the anoxic zone below the oxycline, electron donors such as organic molecules or sulfide could be found to reduce nitrite subsequently to nitric oxide, nitrous oxide and nitrogen gas (denitritation). In the same zone, the combined presence of nitrite and ammonium could give rise to anoxic ammonium oxidation to nitrogen gas (anammox), a pathway oxidizing also around 20% of the nitrite to nitrate. The appearance of these processes had two major implications for Archaean biological nitrogen availability. Firstly, from the existence of denitritation or anammox on, dissolved nitrogen compounds were for the first time converted to gas entering the atmosphere and hence resulting for the first time in a closed nitrogen cycle. Secondly, from the existence of nitratation or anammox on, nitrate was for the first time introduced in the biosphere. In the presented work, we focussed on the latter event and dedicated an important role to the anammox process.

Various arguments indicate that anammox emerged earlier than nitratation. Anammox is performed by anoxic ammonia-oxidizing bacteria (AnAOB), a deep-branching group within the Planctomycetes phylum. Some peculiar Planctomycetes features such as the cell compartmentization and the peptidoglycan-lacking cell wall insinuate a very early development of this phylum. Further, if nitritation in the Archaean ocean was mainly performed by aerobic ammonia-oxidizing bacteria (AerAOB), as in the extant nitrogen cycle, two facts suggest the late emergence of nitrite-oxidizing bacteria (NOB) who perform nitratation. A first fact is the large phylogenetic distance between AerAOB and NOB. Since AerAOB were the first to produce the substrate of the NOB, the latter came out a long time after the AerAOB. A second fact is that AerAOB generally have a higher affinity for oxygen than NOB, indicating that the NOB could only thrive when the oxygen levels in the Archaean oceans had increased significantly.

The consequences of the introduction of nitrate in the environment were doubtlessly enormous, and gave rise to the appearance of many assimilatory and dissimilatory nitrate reduction pathways. Assimilation of nitrate is in the extant nitrogen cycle for instance a widespread capability among heterotrophic marine bacteria and algae. Nitrate respiration (denitratation) is nowadays a trait which allows a huge variety of bacteria to gain energy in the absence of oxygen.

Overall, from an evolutionary physiological and phylogenetic point of view it makes sense that anammox was the first process to introduce nitrate on earth, a step which had tremendous consequences on the biodiversity in the Archaean oceans.