The Royal Academies for Science and the Arts of Belgium RASAB

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

Contact Forum

Cell Signaling in Host-Microbe Interactions

Academy Palace, Brussels

November 18th 2014

History

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

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

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

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

Board

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

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

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

Activities

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

Advantages

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

Program

08.30 *Registration – Poster mounting*

09.00 *Welcome address*

09.10 **Paul Williams**, Centre for Biomolecular Sciences, University of Notttigham, UK

Quorum sensing & Pseudomonas aeruginosa: a tale of regulatory networks & multi-functional signal molecules

09.50 Short communication selected abstract *:* ***Van den Driessche Freija***

Screening of the NIHCC 1&2 to Identify Antibiotic Potentiators with Activity against Mature Bacterial Biofilms

10.05 **Michael Way,** Cancer Research UK London Research Institute, UK

How vaccinia virus (ab)uses the host cytoskeleton to promote its spread

10.45 Short communication selected abstract **Dourcy Mickael**

Murid herpesvirus 4 infection protects mice from the development of an anti-pneumovirus vaccine-induced TH2 immunopathology

11.00 *Coffee break and poster viewing*

11.30 **Vanessa Sperandio,** UT Southwestern Medical Center, Dallas, USA

Enterohemorrhagic E.coli (EHEC) sings: pour some sugar on me

12.10 Short communication selected abstract ***Renzi Francesco***

Linking growth and bacterial cell shape to host-glycan harvesting in C. canimorsus

12.30 *Lunch and poster viewing*

14.30 **Ari Helenius**, Institute of Biochemistry Zürich, CH

Uncoating of animal viruses

Short communications of selected abstracts

15.10 **Van Puyenbroeck Victor**: Signal peptide-binding drug inhibits cell surface expression of the CD4 receptor for HIV-1 attachment and entry

15.25 **Allais Liesbeth** The effect of chronic cigarette smoke exposure on the gut microbiome in healthy mice

15.40 **Jurenaite Dukas Milda** Acetyltransferase domain-toxins constitute a novel class of TA systems

15.55 **Kornelia Smalla,** Julius Kühn-Institut, Braunschweig, DE

Contribution of mobile genetic elements to bacterial adaptation and diversification in the rhizosphere

16:30 *General conclusions and presentation of best poster awards*

Sponsors

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Abstracts of invited lectures

Quorum sensing in Pseudomonas aeruginosa – a tale of regulatory networks and multi-functional signals

Paul Williams

Centre for Biomolecular Sciences, School of Life Sciences, University of Nottingham, Nottingham, U.K.

Bacteria employ quorum sensing (QS) systems for co-ordinating collective behaviours which depend on the production and actions of chemically diverse signal molecules to regulate for example, metabolism, motility, virulence and biofilm development. Multiple QS systems may be integrated with each other and within global transcriptional and post-transcriptional networks. QS signal molecules, although largely considered as effectors of QS-dependent gene expression are also emerging as multi-functional agents which influence life, development and death in single and mixed microbial populations in addition to their impact on host-pathogen interactions. In *Pseudomonas aeruginosa*, it is clear that there are at least seven different but overlapping QS pathways. Apart from the well characterized *las* and *rhl* QS systems that employ *N*-acylhomoserine lactones (AHLs) and the *pqs* system that employs 2-alkyl-4-quinolones (AQs), *P. aeruginosa* uses pyoverdine, unsaturated fatty acids and the '*gac*' signal to co-ordinate population-dependent gene expression. While the *las* system hierarchically controls both *rhl* and *pqs*, it has recently emerged that it is also responsible for regulating the biosynthesis of IQS, a thiazole signal molecule which links *las* to the phosphate stress response, *pqs* and *rhl* revealing the existence of a highly sophisticated QS regulatory network central to the adaptive lifestyle of *P. aeruginosa.* Furthermore, the central role of QS systems in the control virulence and biofilm development offers opportunities for developing novel anti-virulence agents for the treatment of *P. aeruginosa* infections.

How vaccinia virus (ab)uses the host cytoskeleton to promote its spread

Michael Way

London Research Institute, Cancer Research UK, London, UK

Viruses are obligate intracellular parasites that are critically dependent on their hosts to replicate and generate new progeny. To achieve this goal, viruses have evolved numerous elegant strategies to subvert and utilise the different cellular machineries and processes of their unwilling hosts. Moreover, they often accomplish this feat with a surprisingly limited number of proteins. Among the different systems of the cell, the cytoskeleton is often one of the first to be hijacked as it provides a convenient transport system for viruses to reach their site of replication with relative ease. At the latter stages of their replication cycle, the cytoskeleton also provides an efficient means for newly assembled viral progeny to reach the plasma membrane and leave the infected cell. Investigating exactly how viruses hijack and subvert their unwilling hosts offers a unique opportunity to obtain mechanistic insights into the regulation and function of a multitude of cellular processes. To this end, our lab uses a combination of quantitative imaging and biochemical approaches to study Vaccinia virus as a model system to interrogate the regulation and function of Src and Rho GTPase signalling networks, actin and microtubule-based transport as well as cell migration. I will discuss how studying Vaccinia has provided unprecedented insights into how a phosphotyrosine-based Nck, WIP and N-WASP signalling network activated by Src and Abl family kinases functions to stimulate Arp2/3 complex dependent actin polymerization to enhance viral spread.

EHEC sings: pour some sugar on me

Vanessa Sperandio

Depts. Of Microbiology and Biochemistry
UT Southwestern Medical Center, Dallas TX, USA

Gastrointestinal (GI) bacteria sense diverse environmental signals, including host hormones and nutrients, as cues for differential gene regulation and niche adaptation. Although the impact of carbon nutrition on the colonization of the gut by the microbiota has been extensively studied, the extent to which carbon sources affect the regulation of virulence factors by invading pathogens has not been fully defined. The enteric pathogen enterohemorrhagic *Escherichia coli* (EHEC) gages sugar sources as an important cue to regulate expression of its virulence genes. Specifically, this sugar dependent regulation fine tunes the expression of the locus of enterocyte effacement (LEE) pathogenicity island, which encodes for a type three secretion system, effectors, and an adhesin necessary for the formation of attaching and effacing (AE) lesions on enterocytes. Glycolytic environments inhibit the expression of the LEE genes. Conversely, growth within a gluconeogenic environment activates expression of these genes. Part of this sugar-dependent regulation is achieved through two transcription factors: KdpE and Cra. Cra and KdpE interact to optimally directly activate expression of the LEE genes in a metabolite dependent fashion. This sugar dependent regulation is key during infection of the mammalian host, given that a *kdpE* mutant is attenuated *in vivo*. Additionally, a novel two component signal transduction system, named FusKR (where FusK is a membrane bound histidine sensor kinase, and FusR a response regulator) that senses fucose, controls expression of the LEE genes. This fucose-sensing system is required for robust EHEC intestinal colonization. During growth in mucus, the glycophagic prominent member of the GI microbiota, *Bacteroides thetaiotaomicron,* supplies fucose to EHEC, modulating its virulence gene expression. Our findings suggest that EHEC uses fucose, a host-derived signal made available by the microbiota, to modulate EHEC virulence and metabolism, and suggest a new layer of complexity in the inter kingdom signaling that underlies EHEC pathogenicity

Uncoating of animal virus

Indranil Banerjee, Yasuyuki Miyake, Samuel Nobs, Christoph Schneider, Patrick Matthias, Manfred Kopf, Sarah Stauffer, Yohei Yamauchi, and ***Ari Helenius***

Institutes of Biochemistry and Molecular Health Sciences, ETH Zurich and the Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

When viruses enter cells, the capsids delivered into the cytosol must undergo disassembly to release the genome in replication or nuclear import competent form. Our studies with enveloped viruses have shown that uncoating can be quite a complicated process that depends on cellular cues as well as both viral and cellular proteins. The main focus in my talk will be the capsid of Influenza A virus, an enveloped virus where the capsid has a shell of matrix protein M1 covering 8 viral RNAs individually packed into helical ribonucleoprotein complexes (vRNPs). We have found that this particle undergoes pH- and potassium ion-mediated 'priming' already in early endosomes prior to hemagglutinin-mediated membrane fusion in late endosomes. Priming relies on the activation of M2-cation channels in the viral membrane and the conductance of protons and potassium ions. Exposure to these ions changes the conformation of M1 and renders the core uncoating-competent after penetration. Once in the cytosol, the 'primed' cores detach from the endosome surface, and M1 and vRNPs dissociate from each other. For this to take place, a variety of cytosolic cellular proteins are needed, especially elements of the cytoskeleton (microtubules and microfilaments) and molecular motors (dynein and myosin 10) normally involved in the handling of aggregated misfolded proteins i.e. so called 'aggresomes'. Binding of a cytoplasmic ubiquitin-binding protein, histone deacetylase 6 (HDAC6), to free ubiquitin chains present in the viral capsid plays a central role. Uncoating of the core thus constitutes a complex, multi-step process assisted by cellular factors.

Contribution of mobile genetic elements to bacterial adaptation and diversification in the rhizosphere

Sven Jechalke, Susanne Schreiter, Eman Nour, Eva Top and Kornelia Smalla*

Julius Kühn-Institut Braunschweig, Germany
** University of Idaho, Moscow, U.S.A.*

Plasmid mediated horizontal gene transfer (HGT) is assumed to be a major factor contributing to the rapid adaptation of bacterial communities to changing environmental conditions. HGT processes can be fostered by different stresses including various compounds such as antibiotics, heavy metals, and pesticides. The soil influenced by plants e.g. via root exudates and deposits – the so-called rhizosphere is considered to be a hot spot of HGT as high cell densities, metabolically active cells, and a diversity of chemical compounds might trigger not only the proliferation of existing populations carrying plasmids but also HGT.
We have studied the abundance and diversity of plasmids in the rhizosphere of maize and grass grown in manure treated soil by means of cultivation-independent methods (qPCR, amplicon sequencing, exogenous capture of plasmids). Our data showed that the presence of antibiotics in manure increased the abundance of rhizosphere bacterial populations carrying plasmids such as those belonging to the LowGC and the IncP-1 group as well as the transferability of those plasmids. All IncP-1 plasmids captured from the rhizosphere belong to the IncP-1 group and carry class 1 integrons with highly varying sizes of the gene cassette region. Thus, class 1 integrons were likely the reason for the enormous diversity of IncP-1 plasmids observed. Furthermore, a correlation between the abundance of class 1 integron carrying populations and antibiotics applied with manure was found. The data showed that anthropogenic pollutants foster the adaptation of bacterial communities in the rhizosphere.
In another study a unique experimental plot system with three soil types, which were exposed to identical weather conditions for more than ten years, was used to compare the effects of the plant species (lettuce and potato), the rhizosphere bacterial community composition, and the abundance of mobile genetic elements (IncP-1 plasmids, class 1 integrons). Pyrosequencing revealed that, despite of a soil type dependent diversity in the rhizosphere, several taxa such as *Sphingomonas*, *Pseudomonas*, *Variovorax*, *Burkholderia*, *Comamonas*, *Rhizobium,* and *Flavobacterium* were enriched in the rhizosphere of lettuce in all three soils. Quantitative real-time PCR data showed that class 1 integrons and broad host range plasmids belonging to the IncP-1 group were enriched in the rhizosphere bacterial community of lettuce in all three soils. Recently we were able to capture these IncP-1 plasmids from the rhizosphere of lettuce based on their ability to mobilize the IncQ plasmid pSM1890. This is the first example that bacterial populations carrying IncP-1 plasmids were enriched in response to root exudates and thus plant species dependent root exudates seem to represent a so far not considered factor modulating the abundance and diversity of plasmids in soils.

List of posters and Location

Abbreviations of topics:

V = Virology

- **VA = antivirals**
- **VM = molecular and immuno**

B = Bacteriology

- **BH = health**
- **BM = molecular**
- **BG = general and ecological**
- **BB = biofilms**

PF = Parasitology/Mycology

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18	*Abdelnabi Rana, Leen Delang, Pieter Leyssen, Johan Neyts* **The A239G mutation in motif A compensates for the lethal K159R mutation in the active site of the Coxsackievirus B3 RNA-dependent RNA polymerase and increases the susceptibility of this virus to the antiviral effect of T-705**	**VA**
19	*Allais Liesbeth, Frederiek-Maarten Kerckhof, Stephanie Verschuere, Ken Bracke, Rebecca De Smet, Debby Laukens, Pieter Van den Abbeele, Martine De Vos, Nico Boon, Guy Brusselle, Tom Van de Wiele, Claude A. Cuvelier* **The effect of chronic cigarette smoke exposure on the gut microbiome in healthy mice**	**BH**
20	*Bervoets Indra, Marjan De Mey, Daniel Charlier* **Use of sigma factor M from *Bacillus subtilis* in the development of an orthogonal expression system in *Escherichia coli.***	**BM**
21	*Brackman Gilles, Freija Van den Driessche, Tom Coenye* **How does the quorum sensing inhibitor hamamelitannin increase *Staphylococcus aureus* biofilm susceptibility towards glycopeptides**	**BB**
22	*Byloos Bo, Rob Van Houdt, Nico Boon and Natalie Leys* **The molecular mechanisms behind microbe-mineral interactions, on Earth and in Space**	**BG**
23	*Claes Sandra and Dominique Schols* **Use of Bio-Plex magnetic bead-based technology to evaluate CBAs induced stimulatory effects on HIV target cells**	**VM**

24	*Clais Sofie, Ellen Lanckacker, Gaëlle Boulet, Anne-Marie Lambeir, Ingrid De Meester, Peter Delputte, Louis Maes and Paul Cos* **Targeting the host-pathogen interaction: the role of DPPIV in the pathogenicity of *Porphyromonas gingivalis***	**BH**
25	*Condori Sandra Felice Mastroleo, Natalie Leys, & Ruddy Wattiez* **Cell-to-cell communication regulates biofilm formation in bacterium *Rhodospirillum rubrum* S1H**	**BB**
26	*de Araujo Nogueira Tatiane Cristina, Barbara Mertens, Graciela Andrei and Robert Snoeck* **NF-κB activity in parental and CDV-resistant cells after cidofovir treatment.**	**VA**
27	*De Schryver M., Leemans A., Heykers A., Maes L., Cos P., Delputte PL.* ***Characterization of antibody-induced internalization of human sialoadhesin.***	**BH**
28	*Deghorain M., N. Mine and L. Van Melderen* ***Exploring regulation and function of toxin-antitoxin systems within the SOS regulon in E. coli***	**BM**
29	*Deprez Karolien, Pieter Vanormelingen, Charlotte Crahay, Annick Wilmotte, John Day, Wim Vyverman* **PRESPHOTO – a project to improve the preservation of cyanobacteria and diatom cultures**	**BG**
30	*D'huys Thomas, Stephen J. Archibald, Dominique Schols* **Novel potent CXCR4 chemokine receptor antagonists as key manipulators of X4 HIV-1 entry and the CXCL12/CXCR4 axis**	**VA**
31	*Dingemans Jozef, Aurélie Crabbé, Rob van Houdt, Anne Malfroot, Pierre Cornelis* **Effect of shear stress on *Pseudomonas aeruginosa* isolated from the cystic fibrosis lung**	**BM**
32	*Dourcy Mickael, Bénédicte Machiels, Caroline Zeippen, Jérémy Dumoulin, Justine Javaux, Daniel Desmecht, Alain Vanderplasschen, Benjamin Dewals, Laurent Gillet* **Murid herpesvirus 4 infection protects mice from the development of an anti-pneumovirus vaccine-induced TH2 immunopathology**	**VM**
33	*Dumont Baptiste, Francesc Peris-Bondia, Marc Garcia-Garcera, Eduardo Rocha and Laurence Van Melderen* **Bacterial toxin-antitoxin systems: A reservoir of toxic domains for more sophisticated systems?**	**BM**
34	*Fauvart Maarten, Martijn Callens, Serge Beullens, Jan Michiels, Ellen Decaestecker* **Development of a Simple Animal Model to Study Chronic *Pseudomonas aeruginosa* Infections**	**BH**
35	*Férir Geoffrey, Sandra Claes, Stephanie C. Gordts and Dominique Schols* **HIV-1 and its resistance to peptidic carbohydrate-binding agents (CBAs)**	**VA**
36	*Francis Isolde M., Samuel Jourdan, Steven Fanara, Rosemary Loria, and Sébastien Rigali* **The Cellobiose Sensor CebR is the Gatekeeper of *Streptomyces scabies* Pathogenicity**	**BM**
37	*Francis Nayla, Katy Poncin, Charles Van der Henst, Jean-Jacques Letesson, Xavier De Bolle* **Investigating *Brucella abortus* cell cycle regulation in an infection model**	**BH**

38	Gde Sasmita Pande, Nguyen Thao Suong, Peter Bossier and Tom Defoirdt **The impact of the catecholamine stress hormones norepinephrine and dopamine on the virulence of aquaculture pathogenic *Vibrio campbellii***	**BH**
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44	*Grauwet Korneel, Cantoni Claudia, Thary Jacob, Steffi De Pelsmaeker, Christophe Claessen, Parodi Monica, De Maria Andrea, Moretta Lorenzo, Vitale Massimo, Favoreel Herman* **The pseudorabies US3 protein suppresses NK-mediated lysis of infected cells through interference with presentation of the CD300a ligand phosphatidylserine**	**VM**
45	*Hack Katrin, Jonathan Douxfils, Jean-Michel Dogné, Guy Cornelis* ***C. canimorsus* blocks coagulation by affecting the activity of Vitamin K dependent clotting factors**	**BH**
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54	*Lauber Frédéric, Pablo Manfredi, Francesco Renzi, Katrin Hack, Estelle Hess and Guy R. Cornelis* **A novel function for Polysaccharide Utilization Loci: Iron Capture System in *C. canimorsus***	**BM**
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57	*Liu Han, Alvaro Orell, Dominique Maes, Marleen van Wolferen, Ann-Christin Lindås, Sonja-Verena Albers, Daniel Charlier and Eveline Peeters* **BarR, an Lrp-type transcription factor in *Sulfolobus acidocaldarius*, regulates an aminotransferase gene in a β-alanine responsive manner**	**BM**
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59	*Marques Rafael Elias, Rebeca Rocha, Juliana del Sarto, Mauro Martins Teixeira* **Development of a novel St. Louis encephalitis mice model: Insight into disease pathogenesis**	**VM**
60	Masson Clement and Laurence Van Melderen **Regulation of *csrA* expression, a global regulator in *E.coli***	**BM**
61	*G.I. Matos, C.S. Junior, T.C. Oliva, D.F. Subtil, A.L. Chaves, M.T. Lutterbach, E.F. Sérvulo, S.N. Agathos and B. Stenuit* **Intrinsic Bioremediation of Soils Contaminated by Biodiesel and Diesel/Biodiesel Blends in Laboratory Microcosm Studies**	**BG**
62	*Mertens Barbara, Graciela Andrei, Robert Snoeck* **Mode of action of cidofovir against human papillomavirus positive and negative cells is not exclusively due to DNA damage.**	**VA**
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65	*Ong Thi Anh Phuong, Jean-Jacques Letesson, Xavier De Bolle* **Growth of *B. abortus* 2308 inside host cells**	**BH**
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67	*Pontes M, B. Devriendt, H. W. Favoreel* **Pseudorabies virus gE causes ERK1/2 activation in primary porcine T lymphocytes and subsequent cell aggregation and migration**	**VM**
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69	*Praet J., Ivan Meeus, Guy Smaghhe, Peter Vandamme* **The Gut Microbiota of Pollinators: an unknown and unexplored treasure chest of biodiversity**	**BH**
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71	*Renzi Francesco, Melanie Dol, Pablo Manfredi, Guy Cornelis* **Linking growth and bacterial cell shape to host-glycan harvesting in *C. canimorsus***	**BM**
72	*Schepens B, Sedeyn K, Vande Ginste L, De Baets S, Schotsaert M, Roose K, Houspie L, Van Ranst M, Gilbert B, van Rooijen N, Fiers W, Piedra P, Saelens X.* **Protection and mechanism of action of a novel human respiratory syncytial virus vaccine candidate based on the extracellular domain of small hydrophobic protein.**	**VM**
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84	*Vandecandelaere Ilse, Filip Van Nieuwerburgh, Dieter Deforce, Hans Nelis, Tom Coenye* **Mutual influence of *S. epidermidis* and *S. aureus* on gene expression in dual species biofilms**	**BB**
85	*Vanderlinden Evelien, Hugo Klaassen, Arnaud Marchand, Patrick Chaltin and Lieve Naesens* **Identification and mechanism of action of a novel influenza virus fusion inhibitor**	**VA**
86	Verhelst Judith, Jan Spitaels, Tine Ysenbaert, Walter Fiers and Xavier Saelens **Loop L4 in mouse Mx1 is crucial for antiviral activity against influenza A viruses.**	**VA**
87	*Vervaeke Peter, Sandra Liekens* **Dengue virus-induced apoptosis determines endothelial cell fate.**	**VM**
88	*Zeippen Caroline, Alain Vanderplasschen, Philip Stevenson, Laurent Gillet* **The envelope glycoprotein gp150 promotes sexual transmission of Murid herpesvirus 4.**	**VM**
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Poster Abstracts

The A239G mutation in motif A compensates for the lethal K159R mutation in the active site of the Coxsackievirus B3 RNA-dependent RNA polymerase and increases the susceptibility of this virus to the antiviral effect of T-705

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Favipiravir (T-705), is a broad-spectrum antiviral agent that was originally discovered as an inhibitor of influenza A virus replication. In the cell, T-705 is metabolized to its ribofuranosyl 5'-triphosphate form, which was shown to be a competitive inhibitor for the incorporation of ATP and GTP by the RNA-dependent RNA polymerase (RdRp). We recently demonstrated that T-705 also inhibits the *in vitro* replication of the chikungunya virus (CHIKV) and protects mice against CHIKV-induced mortality (Delang *et al*., J Antimicrob Chemother 2014). Low-level T-705-resistant CHIKV variants were selected. A K291R mutation in the F1 motif of the RdRp was shown to be responsible for the observed resistance to T-705. Interestingly, K291 is highly conserved in positive single-stranded RNA viruses.

The Coxsackie B3 virus (CVB3) is 4 fold less sensitive to T-705 than CHIKV. Introduction of a K-to-R mutation at the corresponding position (K159R) in the F1 motif of the CVB3 RdRp resulted in a lethal phenotype. Thus the CVB3 RdRp has a different tolerance for mutations at this position than CHIKV. However, when the R159 CVB3 variant was transfected in Vero cells without antiviral pressure, a second mutation in the RdRp, i.e. A239G, emerged that was able to rescue the replication fitness of the R159 mutant. This amino acid position is located in motif A and is strictly conserved in the RdRp of all enteroviruses. In addition, both the reverse engineered CVB3 R159-G239 and G239 variant demonstrated to be more susceptible to the antiviral effect of T-705 than the wild-type virus. Interestingly, the CVB3 G239 mutant has been reported as a low RdRp fidelity variant (Gnädig NF *et al*., Proc Natl Acad Sci USA.2012). Therefore, the increased susceptibility of the double mutant and G239 variants to T-705 may be related to the increased rate of nucleotides misincorporation caused by A239G mutation.

The effect of chronic cigarette smoke exposure on the gut microbiome in healthy mice

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Background & Aim

Inflammatory Bowel Diseases (IBD) are complex multifactorial diseases characterized by an inappropriate host response to an altered commensal microbiota. The microbiome plays a crucial role in maintaining intestinal homeostasis. Disruption of this delicate balance leads to destabilisation of the gut immune system and aberrant immune responses against harmless antigens. Cigarette smoking is the best known environmental risk factor in IBD. Here, we studied the influence of 24 weeks of smoke exposure on the gut microbiome in conventional healthy mice.

Methods & Results

We analyzed the ileum, caecum and distal colon of smoke- and air-exposed C57BL/6 mice using denaturing gradient gel electrophoresis (DGGE) and Illumina Sequencing. Dendrograms applying the abundance-based Jaccard and Yue & Clayton's Theta index revealed a shift in the microbial community structure in the caecum and distal colon after chronic cigarette smoke exposure Furthermore, we performed an α-diversity analysis using the Inverse Simpson and Shannon index, which showed an increase in microbial diversity in the caecum and distal colon of smoke-exposed mice, however not in the ileum. β-diversity was evaluated by the sparse Partial Least Square Discriminant Analysis (sPLS-DA) method. In particular, we observed an increase of *Lachnospiraceae* spp. in the colon of smoke-exposed mice. Interestingly, qPCR analysis demonstrated a decrease over time of *Akkermansia muciniphila*, a mucolytic bacterium tightly associated with the mucus layer, in the faeces of smoke-exposed mice. Furthermore, staining of the sections with Alcian Blue/PAS to differentiate between acidic and neutral mucins and HID/Alcian Blue to differentiate between sulphated and sialylated mucins showed no differences in expression of the main mucin classes. However, the specific mucin expression pattern changed in smoke-exposed mice. Expression of MUC2 en MUC3, two types of mucins produced in the gut, increased in the ileum after cigarette smoke exposure. Expression of MUC4 increased in distal colon in response to smoking.

Conclusion

This is the first study to show a shift in the mucosa-adherent microbial population in response to cigarette smoke, and even reveals changes in specific species which might play role in the development of CD. In addition, we show changes in innate immune factors, in particular mucins, which are involved in the regulation of the gut bacterial population. We infer that the modulating role of chronic smoke exposure in IBD may be driven by the changes in microbiome composition, which are important factors in IBD development.

Use of sigma factor M from *Bacillus subtilis* in the development of an orthogonal expression system in *Escherichia coli.*

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Technological advances in synthetic biology, systems biology, and metabolic engineering have boosted applications of industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate regulatory mechanisms. Hence, fine-tuning the expression of these synthesis pathways in specific conditions is mandatory for successful production. However, whereas parts and tools have been developed for exponentially growing *Escherichia coli*, these are still lacking for non-growth related production despite clear advantages (in reduction of toxicity and competition, *etc*). To be able to adequately fine tune a multi-enzyme pathway under such stationary conditions, a new genetic circuit will be developed. This circuit consists of a heterologous sigma factor (σ) recognizing specific promoter sequences, which are not recognized by the native σ factors of *E. coli*. In combination a conditional constitutive promoter library linked to this specific σ factor will be constructed.

Twenty six native promoter sequences transcribed by seven different σ factors from *B. subtilis* were tested for their orthogonality in *E. coli*, by measuring the signal of a red-fluorescent protein engineered downstream of these promoters. On basis of these results we selected *B. subtilis* σ factor M (σ_M), an extracytoplasmatic function (ECF) factor, for further analysis. These ECF proteins offer a number of advantages as their divergence in sequence relative to most other sigma factors, their smaller size and differing consensus sequence. The expression and activity of σ_M will be tested in *E. coli* as well as the efficiency of transcription of *B. subtilis* promoters in *E. coli*. Therefore σ_M has to be able to compete with the natural occurring σ factors for the core *E. coli* RNA polymerase. To obtain a condition specific expression of the *B. subtilis* σ_M in *E. coli*, the corresponding gene can be cloned in the σ_S factor operon of *E. coli,* which is most abundantly expressed in stationary conditions. Combining all these elements should allow us to create an orthogonal genetic circuit that is able to transcribe specific genes under a specific condition (*i.e.* stationary phase) with a limited influence on the host cell's metabolism.

How does the quorum sensing inhibitor hamamelitannin increase *Staphylococcus aureus* biofilm susceptibility towards glycopeptides

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Background: Biofilm-associated infections caused by *Staphylococcus aureus* are often very difficult to treat and novel targets are needed to combat these infections. We have previously shown that the quorum sensing (QS) modulator 2',5-di-O-galloyl-D-hamamelose (hamamelitannin, HAM) increases the susceptibility of *S. aureus* biofilms towards vancomycin (VAN) *in vitro* as well as *in vivo*. However, the mechanism of action of HAM at the molecular level has not yet been elucidated.

Methods: Two parallel strategies were followed in order to gain insights in the way HAM affects QS. First, we evaluated the effect of HAM on the susceptibility of biofilms of *S. aureus* strains with mutations in the QS systems (e.g. *agrBCDA*, *trap*, *luxS*) or in genes involved in biofilm formation and virulence (e.g. *icaA*, *sarA*, *codY*). Secondly, using illumina sequencing we identified genes that were differentially expressed in untreated biofilms and biofilms treated with VAN alone or in combination with HAM. Results obtained with both strategies were further investigated using the appropriate tools.

Results: No loss in HAM activity was observed for most of the mutants. In contrast, HAM did not affect biofilm susceptibility of *S. aureus* strains with mutations in *agrA* or *trap* gene. This suggests that these genes are involved in mediating the activity of HAM. Using sequencing, we identified a large number of genes that were differentially regulated after treatment. Treatment with HAM (alone or in combination with VAN) resulted in a downregulation of genes involved in biosynthesis of lysine, glucosamine-6-phosphate and an upregulation of genes involved in glutamine consuming pathways. In addition, the upregulation of genes encoding virulence factors (e.g. *tst, hla, yent1, hlgB*) during VAN treatment was not observed when VAN was combined with HAM.

Conclusion: HAM reduces the upregulation of peptidoglycan biosynthesis normally observed after treatment with VAN. This possibly leads to the increased susceptibility of *S. aureus* biofilm cells towards VAN. Our results further indicate that combination therapy could positively affect morbidity since the upregulation of virulence factors observed for VAN treatment are not observed when VAN is combined with HAM.

The molecular mechanisms behind microbe-mineral interactions, on Earth and in Space

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Microbe-mineral interactions have become of interest for space exploration as microbes can biomine useful elements from regolith materials for use as nutrients in a life support system. Therefore, this research is aimed to identify the molecular mechanisms and assess the influence of space conditions on these microbe-mineral interactions on basalt, as a model for lunar-type material.

Survival and physiology of the bacterium *Cupriavidus metallidurans* CH34 was monitored over several months, in mineral water, with and without basalt, by plate counts, flow cytometry, ICP-MS and microscopy. To study the influence of micro-gravity on these interactions this setup was also send as a flight experiment onboard the Russian PHOTON-M4 capsule.

The results obtained from lab experiments show that CH34 was able to survive in mineral water, in the presence and absence of basalt. The viable cells concentration remained stable, but the cultivable fraction dropped to 10% after 3 months in water with and without basalt. Chemical analysis showed that in water without basalt the phosphate concentration declined. In the basalt containing water phosphate also declined but the concentration of copper, magnesium and calcium increased. CH34 also attached to the basalt rock and formed a biofilm. Preliminary results obtained from the space flight experiment indicate that more cells were alive, active, contained more ATP and fewer cells lost their membrane potential indicating a positive effect of the flight experiment on survival. Basalt had no negative effect on the survival of CH34 in the flight experiment. Cells in basalt containing water were more active compared to cells in water and to the ground experiment. Basalt may have enhanced thus the survival and viability of the cells in the space flight experimental conditions.

This will be further investigated by addition experiments and analysis to confirm this process, and also to elucidate the molecular systems behind. Additional physiological and molecular analysis is ongoing, to confirm these observations and to know more about the molecular processes behind.

Acknowledgments: This work is supported by the European Space Agency (ESA-PRODEX) and the Belgian Science Policy (Belspo) through the E-GEM/BIOROCK project

Use of Bio-Plex magnetic bead-based technology to evaluate CBAs induced stimulatory effects on HIV target cells

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Carbohydrate-binding agents (CBAs) or lectins can be found in different species such as prokaryotes, algae, plants and vertebrates. In this abstract, we mainly focused on Griffithsin (GRFT), Cyanovirin (CV-N) and Microvirin (MVN). These three CBAs demonstrated very potent and broad-spectrum anti-HIV-1 and anti-HIV-2 activity in multiple replication assays. The class of CBAs is considered to have several mechanisms of antiviral activity as they act as entry inhibitors but by interfering with HIV infection at 4 different pathways. CBAs can inhibit (*i*) infection with cell-free virus, (*ii*) the giant cell or syncytia formation between persistently HIV-infected T cells and non-infected T cells, (*iii*) the capture of HIV by DC-SIGN and their (*iv*) subsequent transmission to uninfected CD4+ target T cells. In addition, these CBAs can also create holes in the N-linked glycan shield of gp120 which can result in a lower viral fitness, increased HIV neutralization and cellular immune response.

For potential vaginal microbicidal applications, it is important that these CBAs do not induce stimulatory effects on the HIV CD4+ target cells as cellular activation can result in a higher susceptibility for viral infection. Therefore, it is very important to examine possible side-effects of microbicide candidates by measuring the induction of various cytokines/chemokines.

These measurements of cytokine and chemokine levels were performed by means of a multiplexed fluorescent microsphere immunoassay called the Bio-Plex system (Bio-Rad). This technique combines the principle of a sandwich immunoassay with the Luminex fluorescent bead-based technology, which allows the quantification of up to 100 different analytes simultaneously in one cell culture sample. Here, we measured the production of 27 different cytokines/chemokines by using the Bio-Plex Pro^{TM} Human Cytokine 27-Plex Assay. This kit determines the production of IL-1α, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, FGF, G-CSF, GM-CSF, IFN-ɣ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF, RANTES, TNF-α and VEGF in PBMCs that were precultured with different concentrations of CBAs. As a positive control, the mitogenic lectin PHA was used as this is known to result in an overall dramatic increase of multiple cytokines and chemokines.

Our data demonstrate that CV-N enhanced the production of a wide variety of inflammatory cytokines. MVN did also induce the production of various cytokines although this effect was much less pronounced as for CV-N. In contrast, GRFT had an outstanding safety and efficacy profile. In addition to its broad-spectrum anti-HIV-activity it stands out as a potential candidate for further microbicidal development.

Targeting the host-pathogen interaction: the role of DPPIV in the pathogenicity of *Porphyromonas gingivalis*

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To tackle the growing problem of antibiotic resistance, innovative antimicrobial agents targeting bacterial virulence factors are under development. *Porphyromonas gingivalis*, a member of the subgingival dental plaque microbiota, is a key pathogen in the development of periodontal diseases. It produces proteolytic enzymes that cause tissue destruction and immunomodulation. Dipeptidyl peptidase IV (DPPIV) is one of the proteases involved in the degradation of the connective tissue and might be an interesting target for virulence inhibition. However, its exact physiological and pathological functions are not yet fully understood.

This study aimed to elucidate the role of DPPIV as a virulence factor, by analysing its effect on *P. gingivalis* biofilm formation and IL-8 secretion by gingival epithelial cells using ELISA. In addition, the anti-virulence capacity of DPPIV-inhibitors was examined by assessing their effect on *P. gingivalis* growth, biofilm formation and *in vivo* pathogenicity, using a murine abscess model.

In vitro stimulation of gingival epithelial cells with recombinant DPPIV revealed no increase of IL-8 production. In contrast, our study revealed increased DPPIV activity for sessile bacteria compared to their planktonic counterparts, suggesting a role for DPPIV during biofilm formation. However, no effect of the selected DPPIV-inhibitors could be demonstrated on *P. gingivalis* growth, biofilm formation and *in vivo* pathogenicity.

In conclusion, this study indicates DPPIV as a target for virulence inhibition. Unfortunately, the applicability of DPPIV-inhibitors as an anti-virulence therapy could not be demonstrated. More inhibitors, acting selectively on *P. gingivalis* DPPIV should be evaluated to confirm these findings.

Cell-to-cell communication regulates biofilm formation in bacterium *Rhodospirillum rubrum* S1H

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MELiSSA (Micro-Ecological Life Support System Alternative) has been conceived as a 5 compartments microorganisms and higher plants recycling system for long haul space flights. *Rhodospirillum rubrum* S1H colonizes compartment II and grows under light anaerobic conditions (LAN) using acetate as carbon source (MELiSSA conditions). Previous work reported that continuous culture of the bacterium in a photobioreactor leads to thick biofilm formation, leading to bioreactor arrest. Since cell-to-cell communication (or quorum sensing) and biofilm formation are often closely linked our aim is to investigate the unknown quorum sensing (QS) system of *R. rubrum* S1H (wild type, WT) under MELiSSA relevant culture conditions. In this context we have constructed a mutant strain named M68 where the *rruI* gene was knocked out therefore M68 does not produce the acyl homoserine lactone signaling molecules, as shown by thin layer chromatography analysis. We have studied biofilm formation development of WT and M68 in a flow cell system under light microaerobic conditions in Melissa medium. Biofilms were stained for microscopic visualization (Nikon Eclipse Ti inverted fluorescence microscope) with LIVE/DEAD BacLight^{TM} Bacterial viability and Counting Kit (Life Technologies). In addition, we have compared the expression and proteomic profile of WT and M68 strains grown under MELiSSA conditions. Preliminary results have shown that WT formed an aggregation-like biofilm structure, similar as observed in *Serratia marcecens*. Unlike WT, M68 strain did not form biofilm under the conditions tested suggesting that biofilm is QS-regulated. Regarding transcriptomic results we found that 8% (326 genes) of the genome of M68 were statistically significant differentially expressed. Among the 8% differentially expressed genes, 97% (317) were found downregulated and 3% (9) upregulated. In addition, transcriptomic and proteomic results have shown that *R. rubrum* QS system regulates diverse biological processes. For instance, downregulation of reaction center coding genes and proteins *e.g. pufM*, *pufL* and *puhA* suggests that photosynthesis is indeed QS-regulated. In addition, carbon metabolism, energy generation and bacterial motility were also found to be QS-regulated.

In summary, *R. rubrum* has a cell-to-cell communication system based on acyl homoserine lactones signaling molecules and it regulates diverse biological processes under LAN conditions including biofilm formation. To our knowledge this is the first report where QS is linked to biofilm formation in *R. rubrum* S1H under light microaerobic conditions. Further flow cell experiments under MELiSSA conditions will help us to study biofilm formation and to test substances with known anti-biofouling properties.

NF-κB activity in parental and CDV-resistant cells after cidofovir treatment.

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Cidofovir (CDV) has been proven effective in treatment of human papillomaviruses (HPVs)-induced neoplasias. Recently, generated microarray data and IPA software analysis showed that the immune and inflammatory responses have been regulated by CDV treatment with several inflammation-associated signaling pathways being modulated in immortalized keratinocytes (HaCaT), $HPV16^+$ (SiHa) and $HPV18^+$ (HeLa) cervical carcinoma cells. In addition, those pathways seems to be altered in cells that have been selected for resistance to CDV.

NF-κB pathway is one of the well-known pathway that regulates both immune and inflammatory response among other physiologic processes. NF-κB activity drives chronic inflammation, which is essential to the development, maintenance, and progression of multiples diseases, including cancer. NF-κB has been linked to tumor promotion and progression, as well as to chemotherapy and radiotherapy resistance.

Here, we evaluated the activity of NF-κB transcription factor Rel A (p65) in nuclear extract of different cell lines, measured by TransAM NF-κB p65 kit. We assessed also the expression of p65 and the inhibitor of NF-κB (IκB-α) in both cytoplasm and nuclear extracts by Western Blot.

Nuclear and cytoplasmic fraction of HaCaT, SiHa, HeLa and primary human keratinocytes (PHKs) were obtained using a nuclear extract kit, after treatment for 3, 5 and 7 days with 50 μM CDV.

Additionally, nuclear and cytoplasmic fractions of $HaCaT_{parental}$, $SiHa_{parental}$ and $HeLa_{parental}$ cells previously selected for cidofovir-resistant ($HaCaT_{CDV}$, $SiHa_{CDV}$ and $HeLa_{CDV}$) were also extracted after CDV-treatment.

We observed an increase in NF-κB activity in HaCaT after 5 days (1.7-fold; $p<0.01$) of CDV treatment and SiHa after 3 and 5 days of treatment (1.6 and 2.6-fold; $p<0.05$ and $p<0.01$, respectively). In HaCaT cells, activity of NF-κB was correlated to an increase in the expression of p65 in nucleus and a decrease of IκB-α in both cytoplasm and nucleus. Increased activity of NF-κB in SiHa cells, however, was related only to a decrease of IκB-α in both cytoplasm and nucleus. No changes in NF-κB activity or expression were observed in Hela cells or PHKs. Moreover, in both $HaCaT_{CDV}$ and $SiHa_{CDV}$, no further changes in p65 or IκB-α expression were observed resulting in no increase in NF-κB activity after CDV treatment.

Taking together, these data highlight the role of NF-κB on the treatment of neoplasias with CDV. The exact function of this transcription factor in the regulation of the immune and inflammatory response after CDV treatment and the role of NF-κB in resistance to CDV will be further investigated.

Characterization of antibody-induced internalization of human sialoadhesin.

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Sialoadhesin (Sn) is a member of the family of sialic acid-binding immunoglobulin-like lectins (Siglec) and exclusively expressed on a subset of resident tissue macrophages, but also on inflammatory monocytes and macrophages. The last decade, different researchers investigated the interaction between Siglecs and pathogens containing sialic acids. Different pathogens like HIV, *Campylobacter jejuni*, *Haemophilus influenzae* and porcine reproductive and respiratory syndrome virus (PRRSV) interact with Sn. Furthermore, for some pathogens, this interaction was shown to result in cellular uptake, suggesting that Sn is an internalization receptor. For most Siglecs, internalization pathways are known and amino acid motifs involved in internalization have been identified. For Sn however, the cytoplasmic tail does not contain a known internalization motif. In depth research was done for porcine Sn (pSn), which showed that antibody-induced internalization of pSn occurred through clathrin-mediated endocytosis. However, for human Sn (hSn) and murine Sn (mSn) there is less evidence that these are also internalization receptors. Furthermore, since the amino acid sequence of the cytoplasmic tail of Sn is variable between species, another internalization mechanism might be possible.

With this work, we aimed to investigate hSn internalization and identify the mechanism by which antibody-induced internalization occurs. Therefore, hSn transfected cells were incubated with a hSn-specific mAb at 37 °C for different times until 120 min. Afterwards, cells were fixed, permeabilized and stained with secondary labeled antibodies. Internalization was analyzed by confocal fluorescence microscopy. Internalization of hSn was shown to be comparable to that of pSn and reaches a maximum of internalization between 60 and 90 min. Mutants of Sn without cytoplasmic tail showed no or very limited internalization, suggesting that the signal mediating internalization is located in the cytoplasmic tail of hSn, similar to pSn and other Siglecs. To identify the internalization mechanism, different inhibitors and dominant-negative forms of cellular components involved in endocytic mechanisms were used. These results indicate that hSn is internalized via a clathrin-mediated process. Currently, similar experiments are being performed for mSn to analyze the internalization pathway. Furthermore, the intracellular trafficking upon antibody-induced internalization will be analyzed for both hSn and mSn.

Exploring regulation and function of toxin-antitoxin systems within the SOS regulon in *E. coli*

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Toxin-antitoxin (TA) systems are widespread in bacterial genomes. They consist in a toxin and its cognate antitoxin. Toxins and antitoxins form a tight complex in which toxins are inactive. These complexes also regulate expression of TAs at the transcriptional level. Toxins target essential physiological processes such as translation or DNA replication. Among TA systems encoded in the *E. coli* K-12 chromosome, five belong to the SOS regulon that is activated in response to DNA damages. The *dinJ-yafQ* and *yafNO* systems are type II systems, in which both the toxins and antitoxins are proteins. The three others (*symER*, *tisAB-istR and dinQ-agr*) belong to type I, in which antitoxins are small RNAs that inhibit toxin gene expression. The YafO, YafQ and SymR toxins are translation inhibitors; TisB and DinQ toxins are small inner membrane proteins. The precise roles of TAs within the SOS regulon remain to be established. In addition, whether TAs induction is a global response to stress conditions or whether it represents specific phenomenon involving specific TA systems in individual cells remains an open question. Our objective is to further understand the molecular mechanisms underlying TA activation as well as the consequence of activation on cell physiology, in particular within the SOS response.

Firstly, TAs-SOS regulation and activation in SOS conditions following mitomycin C treatment were analyzed at the population level, using transcriptional lacZ reporter fusions inserted at the *lacZ* locus. As expected, the p*symE* promoter was induced after 90 minutes of mitomycin C treatment. In contrast, in the conditions we tested, induction of the *dinJ-yafQ* and *yafNO* systems was very week and only detected in the strains deleted for the *dinJ-yafQ and yafNO* endogenous systems, respectively. Interestingly, the p*dinJ-yafQ* promoter was active both in the wild-type and *dinJ-yafQ* deletion strain with and without mitomycin C, indicating that this system is constitutively 'ON'. No activity was detected for the *tisAB* and *dinQ* transcriptional fusions, most likely due to post-transcriptional regulations. In order to further investigate regulation of these five TA-SOS systems both at population and single-cell levels, approaches based on fluorescent reporters (p*TA*-Ypet fusions inserted at the *lacZ* locus) combined with flow cytometry and microscopy analyzes are currently being developed. Preliminary results obtained with flow cytometry show that p*dinJ-yafQ* activity increases (about two times) upon SOS induction by ofloxacin treatment, in the wild-type background. This confirms that the *dinJ-yafQ* system is induced at low level during SOS response. Regulation of p*dinJ-yafQ* expression was further investigated at the single cell level by using fluorescence microscopy combined with quantitative image analysis using Microbetracker software. Fluorescence was heterogeneous within the population with a small number of cells very bright. Interestingly, heterogeneity of expression was still observed upon SOS induction. Gene expression fluctuations provide phenotypic variation within clonal population and can have consequences on the physiology of individual cells and on their ability to cope with stresses or rapid environmental changes. Further analyses will include characterization of other TAs-SOS systems as well as other TA systems that are not SOS-regulated. The fate of individual cells expressing TA systems at high (or low) levels will be investigated by time-lapse approaches. Cellular markers (live dead assay, PMF, nucleoid condensation,…) will be used as indicators of cell physiological state.

PRESPHOTO – a project to improve the preservation of cyanobacteria and diatom cultures

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The availability of biological material of guaranteed identity and quality in Biological Resource Centers is considered fundamental for scientific research and R&D, but depends heavily on adequate preservation methods. We present a new BRAIN-BE project on improving the preservation of two groups of photosynthetic microorganisms, cyanobacteria and diatoms, in two collections of the Belgian Co-ordinated Collections of Micro-organisms (BCCM). First, we will improve the cultivation success of diatoms from different habitats by testing different culture media. For organisms that keep resisting cultivation, we will develop DNA extraction and amplification, as well as morphological investigation based on single cells. Secondly, we will design and validate improved cryopreservation protocols for both diatoms and cyanobacteria, since cryopreservation is now the preferred method for the long-term storage of microalgal cultures. For that, the two-step cryopreservation method will be tested using several cryoprotectants and strains in different growth stages. Moreover, this will be compared with the encapsulation/dehydration method. For cyanobacteria, viability tests will be performed with vital dyes whereas diatoms' survival will be assessed by PAM fluorometry. In addition, genome resequencing will be applied to determine the impact of the cryopreservation protocol(s) on genomic stability. Finally, a genomic DNA bank will be constructed and validated. This is highly complementary to preservation as living strains, given that some users require only genomic DNA and it may not be feasible to preserve the global microalgal species diversity as living cultures.

Novel potent CXCR4 chemokine receptor antagonists as key manipulators of X4 HIV-1 entry and the CXCL12/CXCR4 axis

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The chemokine receptor CXCR4 is the main coreceptor in the entry process of T-tropic (X4) HIV-1 strains, and is, together with its unique ligand CXCL12, a major player in many human life-threatening diseases, such as various cancers, rheumatoid arthritis and asthma. Plerixafor (AMD3100, Mozobil), initially developed as an inhibitor of the HIV-1 coreceptor CXCR4, is currently the only CXCR4 antagonist approved for clinical use as a hematopoietic stem cell (HSC) mobilizing agent that disrupts the anchoring signal of stem cells in the bone marrow. It consists of two macrocyclic cyclam units linked by a phenyl ring and is thought to be a pro-drug that coordinates metal ions after administration in humans.

For this study, a wide range of novel bicyclams were designed based on the structure of the reference compound AMD3100. The antagonistic effects of the novel bicyclams on CXCR4-mediated cell entry of HIV-1 (NL4.3 strain) were evaluated *in vitro* in TZM-bl cells using a luciferase-based infection assay. IC_{50} values were determined in the subnanomolar to lower nanomolar range (0.03 – 17 nM). In addition, the activity of the newly developed compounds was measured in various cellular assays for their interference with other biological functions of CXCR4, such as (*i*) CXCL12-CXCR4 binding, (*ii*) intracellular calcium signaling via CXCR4, (*iii*) anti-CXCR4 mAb (clone 12G5) binding, (*iv*) chemotaxis of CXCR4 positive cells, and (*v*) CXCR4 endocytosis. The newly developed bicyclic compounds showed excellent inhibitory activity going to subnanomolar IC_{50} values.

In summary, various novel CXCR4 inhibitors were synthesized and evaluated for their potent anti-HIV-1 activity. The bicyclic compounds potently inhibited CXCL12-CXCR4 binding and CXCR4-dependent calcium signaling, chemotaxis and endocytosis in the lower nanomolar concentration range. This strong activity profile makes them interesting lead compounds for further development as potential inhibitors of HIV-1 entry, tumor cell growth, invasion and metastasis as well as promising molecules for stem cell mobilization. As such, CXCR4 antagonists may have multiple applications in a broad range of clinically important human diseases.

Effect of shear stress on *Pseudomonas aeruginosa* isolated from the cystic fibrosis lung

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Chronic infection of the lungs by *Pseudomonas aeruginosa* is the main cause of morbidity in cystic fibrosis (CF) patients. Switching from the planktonic to the biofilm lifestyle is the major trigger for this chronic infection. This switch in lifestyle can often be achieved by both genetic changes as well as total rewiring of regulatory networks distributed throughout the *P. aeruginosa* genome. Studies utilizing the "naïve" *P. aeruginosa* PAO1 reference strain in order to identify marker genes for biofilm formation in CF-mimicking conditions may therefore be less relevant. Previously, we have sequenced the genome of the transmissible Belgian epidemic strain CF_PA39. This strain was shown to be well adapted to the CF lung conditions and has been present in the UZ Brussel hospital environment for at least ten years. Furthermore, earlier experiments showed that culturing *P. aeruginosa* PAO1 in a low fluid shear environment, obtained by means of the Rotating Wall Vessel (RWV) technology, leads to the formation of a biofilm phenotype comparable to the phenotype observed in the cystic fibrosis lung. In this study, an RWV experiment was performed that closely resembled the *in vivo* situation by inoculating this adapted CF isolate in artificial sputum medium in the RWV either with or without two glass beads, simulating high and low fluid shear conditions, respectively. Via Scanning electron microscopy (SEM), it was shown that robust biofilms were formed in the low fluid shear conditions, whereas this was not observed in the high fluid shear conditions. Furthermore, an increased production of the quorum sensing molecules 3-oxo-C12-HSL and C4-HSL and the virulence factor elastase was observed in the high fluid shear versus low fluid shear conditions. In addition, RNA sequencing was performed to identify genes that were differentially expressed between both conditions and the expression of the most interesting genes was confirmed via qPCR. In the low fluid shear condition (resembling the biofilm lifestyle), genes involved in denitrification, tryptophane synthesis, choline metabolism, and alginate biosynthesis were up-regulated. In summary, these data indicate that biofilms of clinical *P. aeruginosa* isolates can be disrupted by increasing the fluid shear in conditions that are highly similar to those present in the CF lung environment.

Murid herpesvirus 4 infection protects mice from the development of an anti-pneumovirus vaccine-induced TH2 immunopathology

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Gammaherpesviruses are highly prevalent pathogens that establish lifelong latency. However, little is known about how these viruses imprint the immune system of their host. Here we used Murid herpesvirus 4 (MuHV-4) to investigate the impact of gammaherpesvirus infections on the development of an anti-pneumovirus vaccine-induced Th2-skewed immunopathology. Briefly, this respiratory hypersensitivity was induced in mice by a subcutaneous vaccination with formalin-inactivated antigens of pneumonia virus of mice (FI PVM) followed by an intranasal infection with wild-type PVM. We have observed that MuHV-4 infection, either before or after the FI PVM vaccination, prevented the development of the PVM-induced immunopathology while the protection against PVM infection was unaffected. This protective impact against the immunopathology was maintained over time and required pulmonary MuHV-4 replication. Altogether, these results open perspectives for vaccination against pneumoviruses and highlight that some so-called pathogens could be revealed in the end as beneficial for their host.

Bacterial toxin-antitoxin systems: A reservoir of toxic domains for more sophisticated systems?

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Bacterial type II toxin-antitoxin (TA) systems are small bicistronic modules encoding a toxic protein and its cognate antitoxin. These systems are widespread in bacterial genomes, however reasons of this evolutionary success are still unclear. We propose that TA systems might constitute a reservoir of toxic domains potentially recruited by polymorphic toxin systems such as contact-dependent growth inhibition (CDI) and Rhs systems.

CDI and Rhs systems are composed of large secreted proteins that possess extremely variable C-terminal domains (CdiA-CT/RhsA-CT). These domains are toxic but are antagonized by immunity proteins (CdiI and RhsI) which are encoded by genes located downstream of *cdiA* and *rhs*. Such systems are involved in inter-bacterial competition and virulence.

We recently identified and experimentally validated 2 novel families of type II TA systems whose toxins contain domains of unknown function. Interestingly, homologues of these toxic domains were detected in proteins of larger size that display classical features of CDI and Rhs systems. When isolated from the CDI/Rhs loci these domains are toxic upon overexpression in *E. coli* K-12, indicating that indeed, toxic domains might be shared by TA systems and more complex functions.

We are now undertaking a bioinformatics approach to expand our view of toxic domain shuffling. CdiA-CT and RhsA-CT domains will be collected and HMM profiles will be built. Bacterial genomes will be scrutinized for the presence of these domains under the canonical TA system organization (i.e. linked to a small ORF presenting a DNA binding domain). Selected toxic domains from both genetic contexts will be experimentally validated and characterized.

This work might reveal that small toxic domains are largely recruited by polymorphic toxin systems and may provide an explanation why TA systems are evolutionary successful.

Development of a Simple Animal Model to Study Chronic *Pseudomonas aeruginosa* Infections

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Chronic infectious disease is increasingly recognized as a major public health threat. The problem of antibiotic resistance makes the issue even more pressing. Consequently, the development of novel, more effective antimicrobial therapies is of utmost importance. While candidate antimicrobials are almost invariably identified by screening efforts for *in vitro* activity, this is often poorly reflective of *in vivo* effectiveness, contributing to high attrition rates in later stages of drug development. Validation in an *in vivo* infection model early in the process is likely to remedy this problem. However, animal models that enable the *in vivo* study of chronic bacterial infections are typically complex and generally involve higher animals such as mice. We therefore sought to develop a simple animal model that would nevertheless allow the study of a prolonged bacterial infection. As a case study, we selected the opportunistic pathogen *Pseudomonas aeruginosa*. This bacterium is best known for causing chronic lung infections in cystic fibrosis patients, but is also an important source of hospital-acquired infections and poses a danger to immunocompromised subjects. As a host we chose *Daphnia magna*, a species of aquatic crustaceans commonly known as water fleas. *D. magna* is easy to culture and can be readily visualized by microscopy thanks to a translucent exoskeleton. We infected *D. magna* juveniles with varying doses of a virulent strain of *P. aeruginosa*. While the highest infectious doses resulted in acute toxicity and complete mortality within 2 days after exposure, lower doses did not cause increased mortality compared to uninfected control populations up to 2 weeks after exposure. Mortality rates were significantly lower upon infection with an attenuated *P. aeruginosa* strain lacking two major quorum sensing systems. In addition, infection with the attenuated strain was less detrimental to host fecundity compared to the virulent strain. Concomitantly, grazing levels were lower after infection with the virulent strain compared to the attenuated strain. Infection with a *P. aeruginosa* strain expressing green and red fluorescent proteins allowed visualization of infection sites using fluorescence microscopy. Labeled bacteria were still readily identified 2 weeks after exposure, revealing a stable chronic infection. Together, our results strongly suggest that the simple *P. aeruginosa*-*D. magna* model is a suitable tool for studying chronic bacterial infections *in vivo* and support its further development for use in validation of candidate antimicrobials.

HIV-1 and its resistance to peptidic carbohydrate-binding agents (CBAs)

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The glycoproteins on the surfaces of enveloped viruses (such as human immunodeficiency virus [HIV], hepatitis C virus [HCV], Ebola virus,…), can be considered as a unique target for antiviral therapy. These glycoproteins play an important role in (i) viral infection/transmission and (ii) evading the immune system [1]. Carbohydrate-binding agents (CBAs) target these specific glycans, present on viral glycoproteins. It has been shown that long-term CBA pressure in cell culture results in mutant HIV-1 isolates with several (varying from one to twelve) N-linked glycan deletions on gp120. These viral resistance studies in different cell lines demonstrated that mainly high-mannose type glycans are deleted. Remarkably, the N241, N262 and N356 on gp120 were never found to be targeted after prolonged CBA exposure.

Here, we describe the mutation and (cross)-resistance profiles of nine mannose-specific [2G12 mAb, *Hippeastrum hybrid* agglutinin (HHA), *Galanthus nivalis* agglutinin (GNA), Actinohivin (AH), Cyanovirin-N (CV-N), Microvirin (MVN), Banana Lectin (BanLec), Griffithsin (GRFT), *Oscillatoria agardhii* agglutinin (OAA)] and two N-Acetylglucosamine (GlcNAc)-binding [*Urtica dioica* agglutinin (UDA), *Nicotiana tabacum* agglutinin (NICTABA)] generated CBA-resistant HIV-1 strains.

We observed that the human broad-neutralizing anti-carbohydrate binding mAb 2G12 became completely inactive against all the generated CBA-resistant HIV-1 clade B isolates. Different studies demonstrated also that CBAs could interfere with the binding of 2G12 mAb to gp120. As expected, all CBAs, with the exception of NICTABA, inhibited dose-dependently the binding of this mAb to gp120 in infected CD4+ target T cells.

The cross-resistance profiles are varying from low (<10-fold) to very high levels of resistance (>100-fold). Surprisingly, in some cases led certain glycan deletions on gp120 to increased sensitivity, and thus more potent IC_{50}-values, even between CBAs with different sugar specificities or binding moieties [e.g. α(1,3), α(1,2), α(1,6)-links]. Recent studies using CBAs against Ebola and influenza virus demonstrated also very promising results in non-topical formulations (e.g. intranasally or subcutaneously).

Overall, these data highlight their potential for prevention of novel viral infections (as topical microbicides) and antiviral therapy.

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The Cellobiose Sensor CebR is the Gatekeeper of *Streptomyces scabies* Pathogenicity

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Scab lesions on root and tuber crops are mainly caused by phytopathogenic *Streptomyces* species amongst which *Streptomyces scabies* had become the model species to unveil the genetic traits to adopt a pathogenic life style. The pathogenicity of *S. scabies* is strictly associated with the levels of production of the phytotoxin thaxtomin which function as a potent and non- discriminative plant cellulose synthesis inhibitor. If genome sequencing and mining efforts have been able to propose an almost complete map of genetic loci associated with plant virulence, the molecular mechanisms by which *S. scabies* interpret environmental signals into a decision to trigger the pathogenicity machinery are still poorly understood. In order to coordinate the sensing of plant material and the triggering of the phytotoxin, we show here that evolution included binding sites for the cellulose utilisation repressor, CebR, within the thaxtomin biosynthetic cluster. Once inside the cytoplasm, cellobiose and cellotriose inhibit the DNA-binding ability of CebR leading to an increased expression of thaxtomin biosynthetic and regulatory genes. Deletion of *cebR* results in constitutive thaxtomin A production and hypervirulence of *S. scabies*. We showed here that a plant pathogen which phytotoxin - thaxtomin - targets the plant cellulose synthase machinery has enrolled a regulator devoted to primary metabolism and nutritive functions – the cellulose utilization regulator - to trigger the production of its phytotoxin. The occurrence of CebR-binding sites in thaxtomin biosynthetic clusters in *S. turgidiscabies* and *S. acidiscabies* suggests that CebR is a master switch of virulence in other phytopathogenic streptomycetes.

Investigating *Brucella abortus* cell cycle regulation in an infection model

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Bacteria of the genus *Brucellae* are facultative intracellular pathogens of mammals, and humans are accidental hosts. They can invade both non-professional and professional phagocytes and hijack the endocytic pathway of their host cell in order to avoid killing in phagolysosomes. Their vacuole maturates in an ER-derived compartment allowing their growth and division. *Brucella*'s intracellular trafficking has been most studied in HeLa cells. Recent results published by our lab show that the intracellular trafficking of *B. abortus* in HeLa cells harbours two distinct phases: an early non-proliferative phase during which bacteria block their cell cycle (absence of growth and chromosome replication) and a late proliferative phase. The first phase lasts between 6 and 8 hours. As early as 15 minutes post-infection (PI), 73% of intracellular bacteria are in G1 phase, whereas bacteria with one copy of their genome constitute only 19 to 26% of a population grown in rich medium. These data show that a subset of *Brucella* (bacteria in G1 phase) is preferentially internalized, suggesting a link between cell cycle regulation and virulence.

Brucella abortus is part of the alphaproteobacteria, to which belongs the model organism *Caulobacter crescentus*. The cell cycle of this bacterium has been thoroughly studied during the last decades. A phosphorelay has been identified as regulating many processes involved in cell cycle progression such as growth, chromosome replication, polar morphogenesis etc. This phosphorylation cascade involves an essential response regulator and transcription factor called CtrA. At the top of this cascade is a two-component system (TCS) involving two histidine kinases, PleC and DivJ, and a single-domain response regulator, DivK. In *Brucella abortus*, we find homologs of these molecular actors, however their role has not been investigated yet. The goals of this project are to investigate the PleC/DivJ-DivK TCS and to identify CtrA target genes.

B. abortus possesses a third histidine kinase homologous to PleC and DivJ, called PdhS. We purified PdhS and DivK and showed that PdhS can phosphorylate DivK on a conserved aspartate residue, as replacing this residue by an alanine abolishes the phosphotransfer between these two proteins.

In *C. crescentus*, CtrA is known to bind a consensus sequence, TTAA(N7)TTAAC. Using RSATools, we screened the intergenic regions of *B. abortus* genome for the presence of this sequence. We generated a list of putative CtrA target genes. We then performed a ChIP-seq assay (in collaboration with E. Biondi's lab, Lille) and were able to fish out some interesting targets. We decided to further investigate the promoter activity of *ccrM* and *repABC*. CcrM is a DNA methyltransferase known to be cell cycle regulated in *C. crescentus* and to be involved in the regulation of gene expression by methylating target promoters. CcrM is produced at the end of the S phase and it methylates the replicated hemimethylated DNA in predivisional cells. The *repABC* operon is involved in *B. abortus* chromosome II replication and segregation. In order to follow the activity of these promoters, we fused each of them to a gene coding for an unstable GFP. In bacteria grown in rich medium, p_{ccrM} shows a maximal activity in predivisional bacteria. In a HeLa cell infection model, p_{ccrM} activity increases during the proliferative phase. The p_{repABC} activity shows an opposite profile to p_{ccrM} in rich culture medium: it peaks in growing bacteria, probably in bacteria at the S phase. During infection, p_{repABC} activity increases significantly between 8 and 12h PI, which correlates with the onset of the cell cycle.

The impact of the catecholamine stress hormones norepinephrine and dopamine on the virulence of aquaculture pathogenic *Vibrio campbellii*

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Aquaculture is defined as the cultivation of aquatic animals and plants in natural or controlled marine, brackish water or freshwater environments. As the fastest growing food producing sector, aquaculture plays an important role in food security and economic development. Along with the massive development of the aquaculture industry worldwide, this sector still faces important challenges with respect to controlling infectious diseases. The frequent use of antibiotics in attempts to control problems caused by pathogenic bacteria has led to the development of antibiotic resistance, and as a consequence, new strategies to control bacterial infections in aquaculture animals are urgently needed.

Host stress has been known for a long time to influence the outcome of host-microbe interactions, and this has been associated with a decreased activity of the host defense system. In this study, we investigated the impact of catecholamine stress hormones on growth and virulence factor production of aquaculture pathogenic *Vibrio campbellii*. Both norepinephrine and dopamine (at 100 lM) significantly induced growth in media containing serum. The compounds also increased swimming motility of the tested strains, whereas they had no effect on caseinase, chitinase, and hemolysin activities. Further, antagonists for eukaryotic catecholamine receptors were able to neutralize some of the effects of the catecholamines. Indeed, the dopaminergic receptor antagonist chlorpromazine neutralized the effect of dopamine, and the α-adrenergic receptor antagonists phentolamine neutralized the effect of norepinephrine. Finally, pretreatment of pathogenic *V. campbellii* with catecholamines significantly increased its virulence toward larvae of the commercially important giant freshwater prawn *Macrobrachium rosenbergii*.

In summary, our results show that – similar to enteric pathogens – catecholamines can also increase the virulence of aquatic pathogens such as *V. campbellii*.

Keyword: host-microbe interaction; microbial endocrinology; vibriosis

Cupriavidus necator B9, a new metal resistant strain isolated from a medieval archeological site in Verdun (France)

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Remains of a well-preserved medieval foundry were excavated by archaeologists in 2013 in the city of Verdun (Meuse Valley, Northern France). Three to four ancient workshops specialized in brass and copper alloys were found with an activity between 13th c. to 16th c. Furnaces, crucibles, moulds and metal residues were identified in several places. Levels of Cu, Zn and Pb reached 20 000, 7000 and 6000 mg kg^{-1} (dw), respectively, in several soil horizons. In parallel to archaeological investigations, samples of soil were prepared for microbiological analyses. Using selective media three *Cupriavidus necator* (formerly *Ralstonia eutropha or C. eutrophus*) strains were isolated and confirmed by 16S rRNA sequencing. One of those strains, strain B9, differred from *C. necator* N1 and *R. eutropha* H16 on several aspects. For instance, strain B9 was not able of autotrophic growth but was copper resistant (up to 1.2 mM). The strain features a plasmid that differs in size in comparison to the one observed in *R. eutropha* H16. Strain B9 was therefore characterized through genomic sequencing and a total of 516 contigs were obtained (Illumina sequencing, N50=25246 pb). Preliminary analyses of the contigs confirm the absence of *cbb* genes necessary for autotrophy and confirm the presence of various *cop* genes likely related to its copper resistance (*cop SRABCDIJGF*) and that are fully syntenic with a region of *C. metallidurans* plasmid pMOL30 carrying various metal resistance genes.

ATP-dependent Lon protease overexpression specifically activates a subset of type II TA systems in E. coli

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Type II toxin-antitoxin systems (TA) are small operons encoding a toxic protein and its cognate antitoxin protein. These TA modules are widespread in bacteria and archaea and have been involved in physiological processes such as stress response, persistence or virulence.

In steady-state conditions, a cytoplasmic toxin reservoir is present under the form of hetero-complexes composed of antitoxin and toxin proteins. These protein complexes also act as transcriptional repressors by binding specific operator sites located in the corresponding promoter. Currently, antitoxin degradation by ATP-dependent proteases is thought to be the trigger for TA system activation. Antitoxin degradation leads to toxin release from the complex, which can in turn act on its target. Despite the different functions assigned to TA systems in the literature, little is known about the control and the specificity of activation. In Escherichia coli K-12, most antitoxins are considered as being Lon substrates despite the lack of direct experimental data. One can ask how TA systems could perform different functions if all the antitoxins are degraded by the same protease. Furthermore, by investigating the toxicity due to the overexpression of the Lon ATP-dependent protease in *E. coli*, our lab previously showed that only one system (yefM-yoeB) out of the 5 known at that time was activated under these conditions, showing that TA systems are not equivalent.

As new TA systems have been identified since then, we tested 5 additional systems under the same conditions. Interestingly, we showed that a second system, mqsRA, is specifically activated by the Lon protease in our experimental set-up. Triggering of both the yefM-yoeB and mqsRA systems contributes to the toxicity mediated by Lon overexpression. The yefM-yoeB system appears to be the major system contributing to Lon-dependent lethality as its deletion partially restores viability while the single deletion of mqsRA has only a small effect on Lon-mediated toxicity. Interestingly, the YoeB toxin contributes to growth inhibition in Lon overexpression conditions by affecting translation. The contribution of mqsRA appears to rely on the MqsA antitoxin. As this antitoxin has been shown to regulate gene expression, including that of several global regulators, activation of this system might affect expression of other genes. Following Lon proteolysis of MqsA, these genes could be de-repressed and affect *E. coli* physiology. Why these two systems are specifically activated in our experimental set-up is currently under investigation. In summary, our data suggest that the 10 tested *E. coli* type II TA systems are not redundant and that Lon specifically triggers the yefM-yoeB and the mqsRA systems.

E. coli persister cells rely on their physiological state and metabolism

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Persistence is defined by a phenotypic switch occurring at very low frequency ($\approx 10^{-6}$) and generating a sub-population tolerant to antibiotics. After removal of the antibiotic, persistent bacteria are able to regrow. Persistence is therefore thought to be responsible for chronic infections (e. g. *Pseudomonas aeruginosa* in cystic fibrosis patients) and could play a key role in the survival of biofilms to antimicrobials. Despite these observations, the molecular mechanisms underlying persistence remain unclear. Initially, it has been suggested that persistent bacteria could enter a dormant state in which they would be protected against antibiotics. However, recent publications have shown that dormancy is not necessary nor sufficient for bacterial persistence.

The aim of this study is to shed some light on the molecular mechanisms by which phenotypic variability is generated in bacteria, allowing them notably to switch from an antibiotic susceptible state to a persistent state. In a first approach, we identified the main experimental parameters affecting *E. coli* persistence in our experimental conditions (aeration rate, overnight culture duration, culture medium, carbon source, etc.). We then set up a standard and reproducible method for measuring persistence by optimizing these parameters so as to minimize measurements variability. Establishing a reproducible method was indeed of major importance, knowing that the persistence phenomenon is highly sensitive to the experimental conditions and that the field lacks a standard method for measuring persistent rates. Using this standard method, we are now investigating persister cells genetics and metabolism. On the one hand, a genetic approach is used, in order to determine the genetic bases of persistence and on the other hand, a metabolic approach based on fluorescent biosensors and transcriptional fusions is used, in order to determine which metabolic pathways are activated/shut down in persister cells. Finally, different genetic and metabolic conditions have been found to increase persistent rate from 10 to 10000 fold in *E. coli*. These conditions will be used to allow fluorescent microscopic analyses of persister cells at the single cell level, which is extremely difficult to realise in normal conditions because of the low frequency of persistence ($\approx 10^{-6}$).

NICTABA and UDA, two GlcNAc-binding CBAs with unique antiviral activity profiles

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Many viruses affecting human health use the host cell machinery to assemble glycans on their surface, which are important for viral entry and escape from the host immune system. Glycans are bound by natural ligands called lectins that have differential binding preferences for them. Targeting viral glycans is a promising antiviral strategy since there are multiple glycosylated sites on the virus shield. In addition, emergence of resistance to such anti-carbohydrate therapies will most likely involve the removal of multiple sugar residues thereby exposing the surface of the virus to host-derived neutralizing antibodies.

The tobacco plant, *Nicotiana tabacum*, produces a unique lectin in the leaves that is endowed with antiviral activity. This study aimed to assess, in various cellular assays, the antiviral properties of this novel N-acetylglucosamine (GlcNAc)-binding carbohydrate-binding agent (CBA) called NICTABA and compared it with UDA, another GlcNAc-binding lectin isolated from stinging nettle (Urtica dioica L.) rhizomes. Surface plasmon resonance studies were performed to study the sugar specificity and the interactions of both lectins with the envelope glycoproteins of HIV-1.

This GlcNAc-binding lectin exhibited broad-spectrum activity against several families of enveloped viruses including influenza A subtype H1N1 and H3N2 (50% inhibitory concentration (IC_{50}): 30 and 18 nM, respectively), influenza B (IC_{50}: 10 nM), herpes simplex virus (HSV)-1 and HSV-2 (IC_{50}: 260 and 52 nM, respectively) and human immunodeficiency virus (HIV). The IC_{50} of NICTABA for various HIV-1 strains and clinical isolates and HIV-2 strains evaluated in PBMCs ranged from 5 to 30 nM. NICTABA also inhibited syncytium formation between persistently HIV-1-infected T-cells and uninfected $CD4^+$ T lymphocytes (IC_{50}: 320 nM) and prevented DC-SIGN-mediated HIV-1 transmission to $CD4^+$ target T lymphocytes (IC_{50}: 140 nM). However, unlike many other antiviral CBAs described sofar (*e.g.*, HHA, GNA, CVN, and UDA), NICTABA did not block HIV-1 capture to DC-$SIGN^+$ cells (IC_{50}: >1300 nM) and it did not interfere with the binding of the anti-carbohydrate human mAb (clone 2G12) to gp120. Surface plasmon resonance studies for HIV-1 envelope glycoproteins showed that the affinity of NICTABA for gp120 and gp41 was also in the low- to (sub)nanomolar range. In addition, NICTABA binding to gp120 was prevented in the presence of a (GlcNAc)3 trimer but not of mannose trimers. NICTABA displayed no antiviral activity to non-enveloped viruses such as coxsackie virus and reovirus (IC_{50}: > 2600 nM).

Since CBAs possess a high genetic barrier for viral resistance development and NICTABA's broad antiviral activity profile, this CBA may therefore qualify as a potential antiviral candidate for further preclinical studies aimed at targeting the entry and replication of enveloped viruses such as HSV, HIV and influenza virus.

Identification and characterization of Raf-1-NS5A interaction during HCV replication

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Worldwide an estimated 170 million people are chronically infected with HCV (WHO) and thus at high risk for developing fatal liver disease. Current standard therapy for the treatment of HCV infection is (pegylated) interferon-α combined with ribavirin and one of the protease inhibitors, Telaprevir or Boceprevir in genotype 1 patients or the polymerase inhibitor sofosbuvir for genotype 1-4 HCV infected patients. Despite recent breakthroughs the development of antiviral therapy for the treatment of HCV infections, not all cases can be treated (because of resistance development and/or genotype specificity).

We employ an approach to identify potentially druggable protein-protein interactions within the virus-host interactome. These interactions may form a potential target for specific HCV inhibitors yet to be discovered. A crucial protein-protein interaction involved in the HCV life cycle is between NS5A and Raf-1 kinase. Several truncated forms of the viral NS5A are expressed for pull down assays. We show here the interaction between NS5A domain 3 and full length Raf-1. The binding area of NS5A shall be further confined to the minimal structure of amino acids required for binding. Subsequently, the identification of the interaction sites of these crucial host factors should allow to develop potent pangenotypic antivirals with high genetic barrier to development of resistance.

The pseudorabies US3 protein suppresses NK-mediated lysis of infected cells through interference with presentation of the CD300a ligand phosphatidylserine

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Natural killer (NK) cells are key players in the innate response to viral infections. Killing of target cells by NK cells is regulated by a complex sum of signals received through activating/inhibiting receptors on the NK cell surface.

Here, we report that porcine SK cells infected with a porcine alphaherpesvirus pseudorabies virus (PRV) variant that lacks the viral kinase US3 (US3null PRV) are more susceptible to lysis by primary porcine NK cells than cells infected with wild type PRV (wt PRV), indicating a protective role of US3. Flow cytometric studies in primary porcine epithelial cells and SK cells show that the absence of US3 during PRV infection causes a reduced exposure of phosphatidylserine (PS) at the cell surface. Interestingly, PS is a known ligand for the inhibiting NK receptor CD300a in man (Borrego et al., 2012). Using the CD300a antibody IT144 in a redirected cytotoxicity assay using P815 cells and porcine NK cells, indicated that CD300a may also serve as an inhibitory receptor in porcine NK cells. To investigate whether the differences in PS exposure in cells infected with wt or US3null PRV translated in differences in CD300a binding efficiency, binding assay with recombinant CD300a were performed on primary porcine epithelial cells and SK cells. Cells infected with wt PRV showed a markedly increased binding of CD300a compared to mock-infected cells, while CD300a binding was not increased in cells infected with US3null PRV. We have shown before that PRV US3 triggers activation of group I p21 activated kinases (PAKs), central regulators of Rho GTPase signaling (Van den Broeke, 2009). To investigate a potential role of group I PAKs in our observations, PRV wt infected cells were treated with IPA-3, an inhibitor of group I PAKs. IPA-3 substantially decreased PS exposure on PRV wt infected cell surface, and almost completely abolished CD300a binding.

In conclusion, we demonstrate a novel function for the PRV US3 protein. Expression of US3 during infection suppresses NK cell-mediated lysis of infected cells, results in increased exposure of PS at the cell surface and in increased binding of the inhibitory NK receptor CD300a. The porcine CD300a is shown to be an inhibitory NK receptor in porcine NK cells, suggesting its involvement in the observed US3-mediated protection against NK cells.

C. canimorsus blocks coagulation by affecting the activity of Vitamin K dependent clotting factors

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Capnocytophaga canimorsus is a Gram-negative bacterium belonging to the normal oral flora of dogs. The bacterium causes rare but severe infections in humans that have been bitten or licked. Patients frequently develop severe sepsis with disseminated intravascular coagulation (DIC). In many cases, hemorrhagic skin lesions on limbs, abdomen or on the face, also around the puncture site have been described.

Several bacteria interact with coagulation factors or platelets. Considering this as well as the frequency of bleeding abnormalities in *C. canimorsus* infection we were interested if *C. canimorsus* interferes with coagulation.

We therefore incubated normal pooled plasma (NPP) with *C. canimorsus* strain 5 (*Cc*5*)* and monitored thrombin generation by a calibrated automated thrombogram (CAT) assay. Additionally, clotting times of *Cc*5 treated NPP were measured. The activity of individual coagulation factors in *Cc*5 treated plasma was assessed by means of factor depleted plasmas.

We observed that *Cc*5 blocked thrombin generation in a dose dependent manner. *E. coli* MG1655 and *Y. enterocolitica* E40 which were used as controls did not have any impact. Clotting times of NPP incubated with *Cc*5 were significantly increased, but pre-treatment of *Cc*5 with the irreversible serine protease inhibitor AEBSF prior to incubation in NPP, completely abolished this increase.

Amongst the panel of clotting factors tested, decreased activity was observed for FX, FIX, FII and FVII, which are Vitamin K dependent (VKD) factors and all share a similar light chain (LC) structure. Interestingly, incubation of purified FX with *Cc*5 resulted in LC cleavage, which could be blocked by addition of AEBSF.

To summarize, our results show that there is a proteolytic mechanism by which *Cc*5 inhibits coagulation and by which the LC of FX is degraded. Since all VKD factors tested are affected it is thinkable that LC cleavage might contribute to the prolonged clotting times.

Inhibition of coagulation could promote bacterial dissemination. In addition it is possible that, by blocking coagulation, *Cc*5 could aggravate DIC associated bleeding or contribute to the skin hemorrhage that is often observed.

Bridging metabolism and cell envelope homeostasis in *E.coli*

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In order to adapt to their ever-changing environments, bacteria are able to sense and respond accordingly to a wide variety of stress. This remarkable capacity relies on complex regulatory networks composed of sensors and regulators, which modulate gene expression. The CsrA protein (Carbon Storage Regulator A) is a global regulator that controls carbon fluxes and group behaviors such as biofilm formation and quorum sensing. It acts at the post transcriptional level by regulating positively or negatively RNA-tagert stability. We recently showed that a mutant of *E. coli* deleted for the *csrA* gene suffers from envelope stress. Cell envelope is the first line target of physicochemical injuries that might arise from the environment. It also supports fundamental processes such as energy production, nutrient acquisition and environment surveillance. Bacteria have evolved complex envelope stress responses (ESR) to preserve envelope homeostasis. We have shown that two ESR are constitutively induced in the *ΔcsrA* mutant, one monitoring the integrity of the outer membrane and the other the inner membrane integrity. Investigating the origin of this second stress, we found that the proton motive force (PMF) is lower and the amount of reactive oxygen species (ROS) is higher in the *ΔcsrA* mutant. Our main hypothesis is that there is a link between the metabolic consequences of *csrA* deletion and envelope stress. In order to unravel this link, we will extend our genetic approach and analyze mutant phenotypes by a series of experiments such as gene expression analysis, PMF evaluation, ROS quantification, scanning electron microscopy observations. In addition, to be able to formulate robust hypotheses discriminating direct and indirect effects of *csrA* deletion, we compiled a list of CsrA potential targets based on several published works. We will characterize potential gene candidates, preferentially encoding functions related to carbon metabolism or envelope biogenesis or maintenance.

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

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RSV is responsible for 64 million infections/year, with young children and elderly risking the development of severe bronchiolitis. Besides this severe acute pathology, RSV is also linked with chronic pulmonary problems, like asthma and recurrent wheezing. Because there is a link between the pathology and the host immune response, and because macrophages are, besides DC, prominent cells of the lung immune system, research groups have studied the role of macrophages during RSV infection. Interestingly, macrophages may not only play a role in RSV induced pathology, but also appear to be permissive for RSV infection in specific conditions. The obtained results however are often inconclusive and sometimes contradictory. A possible explanation is that diverse types of macrophages and macrophage cell lines were used in different studies. The aim of this study was therefore to evaluate the susceptibility of different types of macrophage cell lines for RSV infection.

The murine macrophage cell lines MH-S, RAW 264.7 and J774 were infected with the prototype RSV strain A2 and infectivity was compared to RSV infection of the epithelial type cell line HEP-2, which is traditionally used for *in vitro* RSV propagation and functional studies. Cells were inoculated with RSV and incubated at 37°C. To have a general idea of both RSV entry and infection, cells were respectively fixed at 2 and 24h post inoculation. Cells were subsequently permeabilized and RSV antigens were visualized by staining with a goat polyclonal anti-RSV serum followed by an AF 488-labelled donkey-anti-goat conjugate. As a control, mock-infected cells were used.

Fluorescent microscopy analysis showed that both MH-S (2%) and RAW 264.7 (0,4%) were infected, showing clear staining of RSV-antigens in the cytoplasm of the cells 24h p.i. This staining was clearly more intense compared to the cells that were stained at 2h p.i., indicating that new RSV antigens were synthesized and that virus was replicating. Surprisingly, J774 cells showed no clear positive signal of RSV antigens at 24h p.i.

Further analysis of MH-S and RAW 264.7 also revealed differences, since there are no RSV-antigens expressed on the surface of RAW 264.7 cells in contrast to MH-S cells. This suggests that RSV knows an abortive infection in RAW 264.7 cells, where the cell is indeed infected but where this does not result in the production of new virus particles. This assumption was explored with HEP-2 cells which were inoculated with supernatants of infected RAW 264.7 cells and MH-S cells, collected 24 and 72h p.i. The percentage of infected HEP-2 cells varied from 1,5 to 5% if it was inoculated with supernatants of MH-S from 24 or 72h p.i. This in contrast to HEP-2 cells inoculated with supernatants of RAW 264.7 cells, where the percentage of infected cells varied between 1.4 and 1.2%. In conclusion, we have shown that the RSV infection with the A2 strain varies among mouse macrophage cell lines.

A Structured Annotation Frame for the Transposable Phages, the Saltoviridae.

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ViralZone (http://viralzone.expasy.org, Hulo et al. 2011, NAR, Jan;39 Database issue:D576-82; Epub 2010 Oct 14), is a freely accessible web resource, which aims at providing a comprehensive resource bridging together textbook knowledge on viruses with genomic and proteomic viral sequences. In an effort to structure the annotation of phage proteins, a set of paradigm bacteriophage genomes has been defined in the frame of ViralZone. This reference set allows for a coverage of the diverse molecular mechanisms driving these viruses life cycles. Enterobacteriophage Mu is the best studied and paradigm member of the Saltoviridae, a newly proposed group of viruses among the Caudovirales. Phage Mu-encoded proteins have been annotated in detail in UniProtKB. The various steps involved in the phage lytic and lysogenic cycles have been illustrated in the general frame of viral biological processes (http://viralzone.expasy.org/all_by_species/507.html), and linked to a controlled vocabulary describing all replication steps. All this information should allow for the future robust annotation of all Saltoviridae.

Acetyltransferase domain-toxins constitute a novel class of TA systems

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Toxin-antitoxin (TA) systems are small genetic loci widespread in bacterial and archaeal genomes. Due to their addictive properties TA genes prevent loss of mobile genetic elements. Chromosome-encoded TA systems are proposed to act as bacterial stress response elements owing to their ability to rapidly stop growth [Van Melderen (2010) Curr Opin Microbiol 13:781-5]. With a few exceptions a majority of currently known toxins target translation through various mechanisms, most commonly by degrading mRNAs [Guglielmini & Van Melderen (2011) Mob Genet Elements, 1(4):283-90]. We have discovered a novel family of toxins that possess an acetyltransferase domain. GNAT (GCN5-related N-acetyltransferase) domain is widely spread throughout all kingdoms of life and is present in proteins acetylating wide range of targets (Vetting *et al* (2005)Arch Biochem Biophys Biophys 433 (1): 212-26). We have shown that small GNAT domain proteins found in pair with RHH-domain antitoxin constitute TA systems. As examples we have studied such systems from enterohemorrhagic *E. coli* O157:H7 strain and *Vibrio cholerae* O1 biovar El Tor N16961. We have shown that both of these bacteria encode two independent functional RHH-GNAT systems. Similarly to classical type-II TA systems toxin and antitoxin form physical complex which prevents toxicity. When expressed alone GNAT-domain toxins strongly inhibit translation, without causing RNA degradation and therefore exhibit yet undescribed mechanism of toxicity. We have introduced mutations in predicted active site and Coenzyme-A binding pocket, which rendered toxin inactive suggesting that acetylation is necessary for toxicity. We are screening multi-copy libraries and naturally occurring mutants to find the target(s) of this novel family of acetyltransferase toxins.

Altered Desferrioxamine-mediated Iron Utilization is a Common Trait of *bald* Mutants of *Streptomyces coelicolor*

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Streptomyces coelicolor is an important model organism for developmental studies of filamentous GC-rich actinobacteria. The genetic characterization of mutants of *S. coelicolor* blocked at the vegetative mycelium stage, the so-called bald (*bld*) mutants that are unable to erect spore-forming aerial hyphae, has opened the way to discovering the molecular basis of development in actinomycetes. Desferrioxamine (DFO) production and import of ferrioxamines (FO; iron-complexed DFO) are key to triggering morphogenesis of *S. coelicolor* and we show here that growth of *S. coelicolor* on the reference medium for *Streptomyces* developmental studies is fully dependent on DFO biosynthesis. UPLC-ESI-MS analysis revealed that all *bld* mutants tested are affected in DFO biosynthesis, with *bldA*, *bldJ*, and *ptsH* mutants severely impaired in DFO production, while *bldF*, *bldK*, *crr* and *ptsI* mutants overproduce DFO. Morphogenesis of *bldK* and *bldJ* mutants was partially recovered by supplying exogenous iron. Transcript analysis showed that the *bldJ* mutant is impaired in expression of genes involved in the uptake of FO, whereas transcription of genes involved in both DFO biosynthesis and FO uptake is increased in *bldK* mutants. Our study allows proposing altered DFO production and/or FO uptake as a novel phenotypic marker of many *S. coelicolor bld* mutants, and highlights the role of siderophores and iron acquisition in morphological development of actinomycetes.

Increased pDC-mediated interferon response triggered by a pseudorabies virus vaccine strain point to pDC evasion mechanisms in wild type virus

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Despite their social and economical importance, different attempts to construct successful vaccines against Herpes Simplex Virus (HSV) type 1 and type 2 have failed. Pseudorabies virus (PRV) is a porcine alphaherpesvirus that is closely related to HSV. Efficient vaccines have been generated against PRV. One particularly successful vaccine is the attenuated PRV Bartha strain (Pomeranz et al. 2005. Microbiol Mol Biol Rev; Bartha. 1961. Magy Allatorv Lapja). The PRV Bartha vaccine has been generated by sequential in vitro passage of PRV and has played an important role in the eradication of PRV from most West-European countries, but the reasons underlying its efficacy are poorly understood.

Plasmacytoid dendritic cells (pDC) are the most potent producers of Type I interferon (TI-IFN) upon viral infection. TI-IFN has potent antiviral effects, is crucial to activate both the innate and adaptive immune system and is known to be a powerful adjuvant (Schuster et al. 2011. Adv Virol).

Here we report that porcine ST cells infected with the vaccine strain Bartha trigger a substantially higher TI-IFN production in primary porcine pDC compared to cells infected with the wild type PRV strain Becker. Compared to the wild type PRV genome, PRV Bartha contains a deletion encompassing four viral genes (US2, gE, gI, US9). Using PRV Becker mutant strains that harbored deletions in one or all of these genes (to mimic the deletion in PRV Bartha), we found that this deletion is involved in, but is not sufficient, to trigger the strong TI-IFN response by pDC. Particularly, infection of ST cells with a PRV Becker strain with a deletion in the genes encoding the gE/gI complex resulted in a noticeable increase in TI-IFN response by pDC compared to ST cells infected with wild type PRV Becker. However, deletion of US2 and/or US9 did not affect TI-IFN levels. This suggests a previously unidentified role for gE/gI in suppressing TI-IFN responses by pDC.

More recently, it has been shown that the PRV Bartha genome contains additional point mutations, including four unique point mutations in the gene encoding viral glycoprotein gB (Szpara et al. 2011. PLoS Pathog). Interestingly, here, we show that ST cells infected with Bartha PRV show reduced cell surface expression (but not total protein expression) of PRV gB. In addition, we show that ST cells infected with a gBnull mutant PRV trigger substantially increased TI-IFN responses by pDC compared to ST cells infected with wild type virus. Hence, our results point to previously uncharacterized pDC evasion functions of the gB and gE/gI glycoproteins of PRV. Our data may help to explain the potent immune response generated by the PRV Bartha vaccine and may possibly contribute to the rational design of improved vaccines against other alphaherpesviruses, including HSV1&2.

Structure-based development of *Plasmodium* hypoxanthine-guanine phosphoribosyltransferase inhibitors: a proof of concept study

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The malarial parasites *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) are purine auxotrophs that rely on the salvage of host purines for their survival and growth. A critical enzyme in this salvage pathway, hypoxanthine guanine phosphoribosyltransferase (HGPRT), is considered a prime target for antimalarial therapy. Our collaboration between the University of Queensland, the Institute of Organic Chemistry and Biochemistry (Prague) and the Rega institute (KU Leuven), is focussed on rational development of novel acyclic nucleoside phosphonates (ANPs) that are structural analogues of the HGPRT nucleotide reaction products IMP and GMP. Based on available HGPRT crystal structures, ANP derivatives with a second phosphonate group attached (bisANPs) were designed. These compounds proved to be particularly strong HGPRT inhibitors with K_i values as low as 30 nM, and displayed antimalarial activity in *Pf*-infected erythrocytes with IC_{50} values as low as 3.8 µM (Keough et al. 2013). However, since the *Plasmodium* parasite possesses several enzymes that could possibly serve as the target for inhibition by the (bis)ANPs, either directly or after metabolic conversion, it remained to be established whether the observed antiparasitic effect indeed results from HGPRT inhibition. We here present a target validation assay to assess, in a cellular environment, the inhibitory effect of (bis)ANPs towards HGPRT. This method complements the enzymatic assays (in which purified HGPRT enzymes are studied in a cell-free environment), and the *Plasmodium* cell culture assay (that involves replication of the whole *Pf* parasite). First, we created adenoviral (Ad) vectors containing the cDNA sequences encoding human, *Pf* or *Pv*HGPRT. Transduction of these Ad vectors into HGPRT-deficient human 1306 cells generated high HGPRT expression levels, as estimated by a tritium release assay with [2,8-^{3}H]hypoxantine (Balzarini and De Clercq, 1992). Several (bis)ANPs were shown to inhibit the HGPRT reaction by the human or *Pv* enzyme. Our novel assay allows to validate *Plasmodium* HGPRT inhibitors in cell culture and will be instrumental to guide further development of this new class of antimalarial drugs.

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

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Herpesvirus virions consist of four morphologically distinct structures: DNA core, capsid, tegument and envelope. The tegument consists of about 20 different viral proteins and plays diverse roles in the virus lifecycle, including: capsid transport during entry and egress; regulation of transcription and translation; viral DNA replication; viral assembly and immune modulation. Most of these functions have been defined in alphaherpesviruses. Interestingly, a recent study identified a potential role in immune evasion for the tegument protein encoded by ORF63 of gammaherpesviruses. However, this study did not involve the construction of ORF63 knockout strain and the significance of these results in the viral lifecycle remains unknown. In this project, we wanted therefore to define the functional importance of ORF63 expression during Murid Herpesvirus 4 (MuHV-4) infection *in vitro* and *in vivo*. We showed that a lack of the ORF63 in MuHV-4 was associated with a severe viral growth deficit both *in vitro* and *in vivo*. The latter deficit was mainly associated with a defect during the viral lytic cycle in the lung but did not appear to be due to a reduced ability to establish latency. On a functional point of view, inhibition of caspase-1 or NLRP3 inflammasome did not restore the growth of the ORF63 deficient mutant suggesting that the observed deficit was not associated with the immune evasion mechanism identified previously. Moreover, this growth deficit was also not associated with a defect in virion egress from the infected cells. In contrast, it appeared that the entry process of MuHV-4 virions was deeply affected by the absence of ORF63. Indeed, MuHV-4 ORF63 deficient mutants failed to address most of their capsids to the nucleus, suggesting that ORF63 plays a role in capsid movement along the microtubule network. Altogether, this study provides new insights into the mechanisms used by gammaherpesvirus capsids to reach the nucleus during entry.

A novel function for Polysaccharide Utilization Loci: Iron Capture System in *C. canimorsus*

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Capnocytophaga canimorsus, a dog mouth commensal and a member of the *Bacteroidetes* phylum, causes rare but often fatal septicemia in humans that have been in contact with a dog [Bobo & Newton (1976) Am J Clin Pathol 65:564-569]. Here, we show that *C. canimorsus* strains isolated from human infections grow readily in heat inactivated human serum and that this property depends on a typical polysaccharide utilization locus (PUL), namely *PUL3* in strain *Cc5*. PULs are a hallmark of *Bacteroidetes* and they encode various products including surface protein complexes that capture and process polysaccharides or glycoproteins. The archetype system is the *Bacteroides thetaiotaomicron* Sus system, devoted to starch utilization [Shipman et al (1999) J Bacteriol 181:7206-7211; Shipman et al (2000) J Bacteriol 182:5365-5372]. Unexpectedly, *PUL3* conferred the capacity to acquire iron from serotransferrin and this capacity required each of the seven encoded proteins, indicating that a whole Sus-like machinery is acting as an iron capture system (ICS), a new and unexpected function for a Sus-like machinery. No siderophore could be detected in the culture supernatant of *C. canimorsus*, suggesting that the Sus-like machinery captures iron directly from serotransferrin. Interestingly, hemoglobin and hemin could not serve as iron source while lactoferrin did, indicating that the range of the *PUL3* encoded proteins is not restricted exclusively to serotransferrin. Moreover, the genes of the ICS were found in the genomes of several opportunistic pathogens from the *Capnocytophaga* and *Prevotella* genera, in different isolates of the severe poultry pathogen *Riemerella anatipestifer* and in strains of *Bacteroides fragilis* and *Odoribacter splanchnicus* isolated from human infections. Thus, this study describes a new type of iron acquisition system that evolved in *Bacteroidetes* from a polysaccharide utilization machinery and could represent an important virulence factor in this group.

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

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Similar to several other viruses, RSV expresses viral proteins on the surface of infected cells, which can be detected and targeted by RSV-specific antibodies. Previous studies have shown that upon binding of polyclonal RSV-specific antibodies to RSV antigens expressed on the surface of infected HEp-2 cells, internalization may occur of these RSV antigen-antibody complexes, presumably by a clathrin-dependent mechanism.

For further elucidation of the viral protein(s) involved in these processes and confirmation of the mechanism of internalization, cells transfected with the RSV F protein were used, instead of infected cells, to study antibody-induced internalization. Transfected cells were incubated with polyclonal RSV-specific antibodies at 37°C during different times (0 min up to 120 min), followed by fixation, permeabilization and staining with secondary labeled antibodies. Analysis of the cells was performed by confocal fluorescence microscopy. Addition of polyclonal RSV-specific antibodies resulted in a clear uptake of viral protein-antibody complexes in a time-dependent manner. This observation indicates that at least the RSV F protein is involved in antibody-induced internalization of RSV proteins at the surface of infected cells. The process was rapid and stagnated between 60 and 90 minutes after the addition of antibodies. By using RSV-infected cells and F-specific monoclonal antibodies, the involvement of the RSV F protein in this internalization process was further confirmed in the context of RSV infection. Upon internalization, complexes of viral proteins and antibodies were transported towards a perinuclear region resembling the lysosomal compartment. Also a clear reduction in surface expression was observed and confirmed by flow cytometric analysis of RSV infected cells. Experiments with specific inhibitors of different endocytic pathways and co-transfection with dominant negative proteins indicate that this process of antibody-induced internalization of the RSV F protein is clathrin dependent. Currently, experiments are ongoing to analyze whether internalization of the viral protein-antibody complexes interferes with antibody-dependent complement-mediated lysis.

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

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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-mucin type (C2GnT-M). The nucleotide sequence of the Bo17 gene has 95% identity with the cellular sequence from which it was acquired. However, by opposition to what is observed for the cellular gene, we show in this study that two different transcripts are encoded by the Bo17 gene. The first one corresponds to the entire coding sequence of the Bo17 gene and, surprisingly, the second results from the splicing of a 138 bp intron. Analysis of different homologous sequences revealed that, compared to cellular sequences, only Bo17 gene presents the consensus sites for splicing and that these sites are conserved in all the BoHV-4 strains identified 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. However, by using an *in vitro* assay, we showed that the spliced form of Bo17 is not anymore active. In order to test the role of these two forms in the viral lifecycle, recombinant strains expressing only the long or the short form of Bo17 were constructed. Interestingly, we showed that BoHV-4 could use alternative splicing in order to modulate the cellular C2GnT-M activity. We postulate that the relative abundance of active/inactive forms of pBo17 in Golgi oligomeric complexes may define the level of C2GnT-M activity in the cell. This new regulatory mechanism could have implication in viral immune evasion but also more generally in cellular biology.

BarR, an Lrp-type transcription factor in *Sulfolobus acidocaldarius*, regulates an aminotransferase gene in a β-alanine responsive manner

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In microorganisms, β-alanine is not incorporated in proteins but important for CoA biosynthesis. However, little is known about the β-alanine degradation pathway or its regulation in archaea. In this degradation pathway, β-alanine and pyruvate are conversed into L-alanine and malonate semialdehyde via transamination, followed by the formation of energized NADH and acetyl-CoA in the second step. BarR is a novel Lrp-like transcription factor (TF) that we recently identified and characterized. It is the first Lrp-like TFreported that has a non-proteinogenic amino acid ligand. BarR is conserved in *Sulfolobus acidocaldarius* and *Sulfolobus tokodaii* and is located in a divergent operon with a gene predicted to encode β-alanine aminotransferase. In-frame deletion of *barR* resulted in a reduced exponential growth rate in the presence of β-alanine. Gene expression (qPCR) and promoter activity assays demonstrated that BarR activates the expression of the adjacent aminotransferase gene only withβ-alanine supplementation. In contrast, auto-activation proved to be β-alanine independent. Heterologously produced BarR displays octamericstate in solution and forms a single complex by interacting with binding sites in the 170-bp long intergenic region separating the divergently transcribed genes. ChIP assay followed by qPCR revealed that BarR is binding *in vivo* to the same intergenic region. *In vitro*, DNA binding is specifically responsive to β-alanine and site-mutant analyses indicated that β-alanine directly interacts with the ligand-binding pocket. Altogether, this work contributes to the growing body of evidence that in archaea, Lrp-like TFs have physiological roles that go beyond the regulation of α-amino acid metabolism.

Related publication:

Liu H., Orell A., Maes D., van Wolferen M., Lindås A.C., Bernander R.,*et al*. (**2014**)BarR, an Lrp-type transcription factor in *Sulfolobus acidocaldarius*, regulates an aminotransferase gene in a β-alanine responsive manner. *Molecular Microbiology*: doi:10.1111/mmi.12583.

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Allergic asthma increases the susceptibility of mice to *Brucella melitensis* intranasal infection

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Brucella spp. are facultative intracellular bacterial pathogens responsible for brucellosis, a worldwide zoonosis that causes abortion in domestic animals and chronic febrile disease associated with serious complications in humans. There is currently no approved vaccine against human brucellosis and antibiotic therapy is long and costly.

The protection against *Brucella melitensis* in an experimental mice model requires IL-12 dependent IFN-α-producing $CD4^+$ T_H1 cells that mediate activation of microbicidal functions of infected macrophages and dendritic cells.

Identification of key factors regulating host resistance to *Brucella* or other pathogens is classically performed in immunologically healthy animals. This approach is highly reductionist and ignores that hosts frequently contract numerous pathologies throughout their life. Asthma is considered as a T_H2 mediated immunopathology induced by numerous environmental factors such as dust mite allergens. Its occurrence has increased significantly since the 1970s. In 2011, 235–300 million people were diagnosed with asthma. In order to characterize the impact of allergic asthma on the course of *Brucella* infection in mice, we induced asthma by repeated intranasal injection with house dust mites extract before intranasale infection with *Brucella melitensis*.

Our results demonstrate that allergic asthma decreases the ability of the lungs of wild type BALB/c mice to control *B. melitensis* growth. Asthma increases significantly the CFU bacterial count in lungs of astmatic mice but not in the liver and the spleen as compared with control mice. This suceptibility is not observed in STAT-6 deficient mice (deficient for T_H2 response). We thus hypothesize that asthma induced pulmonary T_H2 response that inhibits locally the efficient T_H1 immune response against *Brucella.* These results suggest a possible higher persistence of *Brucella melitensis* in the lungs of infected patients and open a new area of research concerning the factors favouring *Brucella* infection.

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Development of a novel St. Louis encephalitis mice model: Insight into disease pathogenesis

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St. Louis encephalitis virus (SLEV) is a causative agent of human and veterinary encephalitis in the western hemisphere. SLEV is included in the *Flavivirus* genus together with important human pathogens such as *Dengue virus* and *West Nile virus*. At the onset of encephalitis, mortality rates can reach 30% and the possibility of neurological sequelae is significant. Although St. Louis encephalitis pathogenesis is poorly understood, misguided or excessive inflammatory responses in the central nervous system are thought to contribute to disease development. In order to investigate the immune/ inflammatory responses to SLEV *in vivo*, we developed an experimental model of St. Louis encephalitis in mice. Intracranial inoculation of our SLEV strain (isolated from a Brazilian symptomatic patient) in adult wild-type C57BL/6 mice caused death in approximately 7 days, in an inoculum-dependent fashion. Viral load assessment by plaque assay and RT-qPCR indicated that SLEV replicates extensively in the brain, yet SLEV could not be detected in the periphery. Interestingly, viral load in brain reach peak values at 7 days post-infection (p.i.), concomitant with mice death. We observed that SLEV replication in mice brains induced pro-inflammatory cytokine and chemokine production, markedly IFN-γ, CXCL1 and CCL5, measured by ELISA. Flow cytometry data showed that SLEV infection led to an intense leukocyte recruitment to the brain, composed mainly by neutrophils and lymphocytes and corroborating chemokine production. Excessive cytokine production and leukocyte recruitment to the infected brain is consistent with encephalitis and is associated to brain tissue damage, which is evident at day 7 p.i. Thus, we considered that the peak of disease in this model is at 7 days p.i., when infection, inflammation and tissue damage lead to mice death. Finally, SLEV infection in mice caused behavioral alterations comparable to neurological alterations observed in human encephalitis, such as paralysis and reduction in neuropsychological scores, assessed with the SHIRPA test. In summary, our experimental SLEV infection model resembles important aspects of human St. Louis encephalitis and could contribute to the understanding of this disease and others caused by related flaviviruses. More important, we conclude that inflammation is a major component in experimental St Louis encephalitis and likely contributes to disease pathogenesis rather than protection.

Regulation of *csrA* expression, a global regulator in *E.coli*

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Global regulators play major roles in response to a variety of intracellular and extracellular signals, allowing bacteria to adapt to environmental changes. Lack of global regulators generally leads to strong defects. We are interested in CsrA, a post-transcriptional global regulator acting by binding target mRNAs and modulating their translation. This regulator is important for cell growth and regulates central carbon metabolism and social behavior pathways such as glycolysis, glycogenogenesis, motility and biofilm formation. An *E. coli csrA* deleted strain grows slowly, is hyper-adherent and shows loss of envelope homeostasis.

Transcriptional regulation of *csrA* expression remains largely unknown although it was shown that Sigma 70 and Sigma 38 (RpoS) directly regulate *csrA* transcription. At the post-translational level, activity of CsrA is regulated by 2 major sRNAs, *csrB* and *csrC*, which bind CsrA and sequester it away from its targets. *csrB* and *csrC* expression is itself regulated by the BarA/UvrY two-component system (TCS).

As CsrA is involved in various pathways that are dependent on environmental conditions, we set up a screen using a p*csrA::lacZ* transcriptional fusion to identify whether known TCS are involved in *csrA* expression regulation. Preliminary data indicated that *csrA* expression is positively regulated by *envZ/ompR*, a TCS sensing the osmolarity. Bioinformatics analysis indicated that this regulation might be direct since potential OmpR binding sites are detected in the *csrA* promoter region. Further experiments such as EMSA and mutagenesis will be carried out to verify the direct regulation of *csrA* expression by this TCS.

Intrinsic Bioremediation of Soils Contaminated by Biodiesel and Diesel/Biodiesel Blends in Laboratory Microcosm Studies

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The gradual introduction of biodiesel in the Brazilian energy landscape has primarily occurred through its blending with diesel (e.g., B20 (20% biodiesel) and B5 (5% biodiesel) formulations). Because B20 and lower-level blends generally do not require engine modifications, their use as transportation fuel is increasing in the Brazilian distribution networks. However, the environmental fate and ecotoxicological impacts of low-level biodiesel blends and pure biodiesel (B100) are poorly understood. Particularly, we need to define the ecotoxicological-safety endpoints and to decipher the mechanisms of biodegradation in environmental sites intentionally or accidentally contaminated by biodiesel blends. In this work, intrinsic biodegradation activities of native soil microbial communities were assessed in laboratory microcosms using closed reactor columns containing clay loam soil (20% humidity) contaminated with biodiesel (EXPB100) and a low-level blend (EXPB5) (10% w/v). Laboratory microcosms were incubated at 25-28 °C for 60 days and samples were collected at regular interval times at different column depths. Dehydrogenase assays using triphenyltetrazolium chloride (TTC) method, soil ecotoxicological tests using red worms (*Eisenia fetida*) and quantification of total cultivable fungi and heterotrophic bacteria (aerobic and anaerobic) have been carried out over the course of the incubation. Dehydrogenase activity is considered a suitable indicator of microbial degradation of (bio)diesel and significantly increased in both microcosms compared to the pristine soil with the highest activity rates in the bottom of the reactors after 60 days of incubation. In addition, the results showed a significant ecotoxicity decrease of soils contaminated with pure biodiesel compared to those spiked with the B5 blend after 60 days of incubation. Results from quantification of cultivable bacteria and fungi indicated a decrease of fungal biomass and an increase of aerobic and anaerobic bacterial biomass in both B100 and B5-contaminated soil column incubations. These first findings suggest that biodegradation of biodiesel and its blends catalyzed by native soil microorganisms occurred in soil columns and natural attenuation of biodiesel-contaminated soils can contribute to establish (bio)remediation strategies. Chemical analyses using GC-FID and UPLC-UV-MS will provide new insights into the biodegradation profiles of B100 and B5 in laboratory microcosms. Targeted sequencing of 16S rRNA and ITS genes using illumina platforms will also decipher the bacterial and fungal microbial community structure and dynamics in soil microcosms contaminated by pure biodiesel and biodiesel blends.

Mode of action of cidofovir against human papillomavirus positive and negative cells is not exclusively due to DNA damage.

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Human papillomavirus (HPV) can induce both benign and malignant lesions. It is involved in almost all cervix carcinoma and in an increasing amount of head and neck cancers. Cidofovir (CDV) was found to be efficacious in the treatment of HPV related hyperplasias. It has been shown that inhibition of cell proliferation contributes to the effects of CDV and has been associated with apoptosis induction, cell cycle arrest and increased levels of tumor suppressor proteins. Evaluation of the effect of CDV by means of microarray suggested that CDV causes DNA damage in HPV^+ tumor cells. Moreover, we found that CDV incorporates into genomic DNA.

In this study, we evaluated whether a correlation exists between DNA incorporation, DNA damage and the anti-proliferative effects caused by CDV. This study will provide a better insight into the way CDV is capable of killing HPV^+ tumor cells, without harming normal cells. Moreover it will allow us to determine the potential use of this drug for the treatment of neoplasias other than those related to HPV.

In the present work HPV^+ and HPV^- cells of both cervix carcinoma and head and neck squamous cell carcinoma (HNSCC) were included. As a control, we compared with primary human keratinocytes (PHKs), human embryonic lymphocytes (HEL) and primary epithelial tonsil cells (PET). The anti-proliferative effect is expressed as the concentration needed to inhibit cell growth by 50% (CC_{50}). Cells were counted with a Coulter counter. CDV drug metabolism and drug incorporation of CDV into genomic DNA was studied by use of radiolabeled [5-^{3}H]-CDV. CDV metabolites were separated by HPLC. DNA damage was evaluated by a flow cytometry assay using a double staining with propidium iodide and anti-gamma-H2AX antibody, a selective marker for double stranded DNA breaks.

Our results showed that the levels of intracellular CDV metabolites (CDV-monophosphate, CDV-diphosphate and CDV-phosphocholine) were much higher in PHKs and PET cells compared to HPV^+ and HPV^- tumor cells. However, the amount of CDV incorporated in tumor cells was higher compared to normal cells. CDV-diphosphate is the active form of CDV and the only metabolite that can be incorporated into genomic DNA. We could prove at the protein level that cidofovir causes double stranded DNA breaks in each phase of the cell cycle. The percentage of cells with DNA damage was higher in the tumor cells compared to the normal cells.

CC_{50} values, CDV incorporation and DNA damage were compared to investigate whether a correlation exists between them. We observed a correlation between DNA incorporation and DNA damage but a correlation between CC_{50} values and CDV incorporation or CC_{50} values and DNA damage was not present. This indicates that DNA damage is caused by incorporation of CDV into genomic DNA, but it also means that DNA damage is not the only strategy of CDV to kill cancer cells.

We have evidence, from our microarray data, that the selectivity of CDV for cancer cells could be associated, at least in part, with a higher CDV incorporation into cancer cell lines and a lack of DNA repair in the cancer cells in comparison to primary cells. This last hypothesis is currently being tested on the protein level.

Link between PTS^{Ntr} of *Brucella abortus* and its central metabolism

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Brucella is a gram-negative intracellular bacterium belonging to the α2-proteobacteria. It is the etiological agent of brucellosis that generates a worldwide zoonosis, causing abortion and infertility in animals. It can also infect human causing a disease, called Malta fever with flu-like symptoms. If not treated, it can lead to a chronic infection with severe complications.

To proliferate, bacteria need to get carbon sources from their environment and the phosphotransferase system (PTS) contributed to this goal. Discovered first in *E. coli*, it forms a phospho-relay, ending with the entry and concomitant phosphorylation of a sugar. It possesses regulating functions in carbon metabolism with, for instance, its well-known implication in carbon catabolism repression. *Brucella abortus* possesses a paralogous system called Nitrogen PTS (PTS^{Ntr}), acting the same way except that no sugar entry occurs. This suggests that it can be implicated in metabolism regulation process only. Starting from the phosphoenolpyruvate, the phosphoryl group is successively transferred on histidine residues of Enzyme I (EI^{Ntr}) (coding by the *ptsP* gene), then to the NPR (*ptsO*) protein and finally to EIIA^{Ntr} (*ptsN*) or EIIA^{MAN} (*ptsM*).

We were interested in the identification of a link between central metabolism and the *B. abortus* PTS^{Ntr}. In this goal, we have generated the knockout mutant of those 4 protagonists and studied their bacterial growth behavior on media containing different single carbon source. We found that *B. abortus* was mainly able to grow on sugar entering the pentose phosphate pathway. Interestingly, compared with the wild type strain, the *ptsP* (EI^{Ntr}), *ptsO* (NPR) and *ptsN* (EIIA^{MAN}) mutants shows a growth defect on those sugars while the *ptsM* (EIIA^{Ntr}) mutant growths better. Moreover, point mutation study on NPR protein shows that phosphorylation state of the histidine residue seems to be the main actor controlling growth behavior. All these results suggest a role of the *Brucella* PTS^{Ntr} in the regulation of central metabolism.

Bioinformatics Algorithms to get accurate 16S rRNA high throughput sequencing data.

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The revolution in new DNA sequencing technologies has led to an explosion of possible applications, including microbial biodiversity studies by bacterial 16S rDNA amplicon sequencing. However all sequencing technology platforms suffer from the presence of erroneous sequences: (i) chimera, i.e. artificial (non-biological) sequences introduced by the PCR reaction during sample preparation, and (ii) sequencing errors produced by the sequencing platform itself. As such, there is a need for efficient algorithms to remove those erroneous sequences in order to be able to accurately assess the microbial diversity.

First, a machine learning method called CATCh (Combining Algorithms to Track Chimeras) is developed which is able to integrate the output of existing chimera detection tools into a new more powerful method. Via a comparative study with other chimera detection tools, CATCh was shown to outperform all other tools, thereby predicting up to 9% more chimera than could be obtained with the best individual tool. Second, NoDe (Noise Detector) was introduced as an algorithm to correct existing 454 pyrosequencing errors, thereby decreasing the number of reads and nucleotides that are disregarded by the current state-of-the-art denoising algorithms. NoDe was benchmarked against state-of-the-art denoising algorithms, thereby outperforming all other existing denoising tools in reduction of the error rate (reduction of 75%), and decrease in computational costs (15 times faster than the best individual tool). Third, as the 454 pyrosequencing platform is in many microbial diversity assessments replaced with the more cost-effective Illumina MiSeq technology, the IPED (Illumina Paired End Denoiser) algorithm was developed to handle error correction in Illumina MiSeq sequencing data as the first tool in the field. It uses an artificial intelligence-based classifier trained to identify Illumina's error and remove them, reducing the error rate by 73%.

The combined effect of chimera removal had a positive effect on the clustering of reads in operational taxonomic units, with an almost perfect correlation between the number of clusters and the number of species present in the mock communities. Indeed, when applying our improved pipeline introducing CATCh and NoDe on a 454 pyrosequencing dataset obtained from a mock community, our pipeline could reduce the number of OTUs to 28 (i.e. close to the real number of 18 species present in the 454 pyrosequencing mock community). In contrast, running the straightforward pipeline without our algorithms included would inflate the number of OTUs to 98. Similarly, when tested on Illumina MiSeq sequencing data obtained for a mock community, using a pipeline integrating CATCh and IPED, the number of OTUs returned was 33 (i.e. close to the real number of 21 species present in the Illumina MiSeq mock community), while a much higher number of 86 OTUs was obtained using the default mothur pipeline.

Conclusively, Introducing CATCh and denoising algorithms (NoDe or IPED) into 454 pyrosequencing or Illumina sequencing analysis increases the overall reliability of 16S rRNA amplicon sequencing data. Our algorithms are freely available, via our website: http://science.sckcen.be/en/Institutes/EHS/MCB/MIC/Bioinformatics/ and can easily be integrated into other 16S rRNA data analysis pipelines (e.g. mothur).

Growth of *B. abortus* 2308 inside host cells

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The characterization of the trafficking of intracellular *Brucella* strains inside host cells is incomplete yet and many interesting mechanisms still need to be studied and discovered. It is possible to follow the growth of single bacteria inside infected cells by labelling bacteria with Texas-Red Succinimidyl Ester (TRSE) since the unipolar growth generates new non-labelled parts on the surface of *Brucella.* In addition, the replication and segregation of replication origins of both chromosomes, named *oriI* and *oriII*, could be observed by using fluorescent fusions to ParB and RepB, putatively binding to *parS* and *repS* sequences located close to *oriI* and *oriII*, respectively. Besides, it was revealed that replication and segregation of *oriI* always occurs before *oriII* in tested conditions. Hence a ParB fusion is sufficient to monitor G1 phase or the initiation of S phase. By using these methods and tools, in previous studies we have found that 80% of the intracellular *Brucella abortus* block their growth and their cell cycle at the G1 stage during the first 6 hours post infection (PI) and resume their growth and replication at 8 hours PI in HeLa cells. It would be important to continue to expand the discoveries of intracellular growth lifestyles of *B. abortus* in the other cell types such as murine RAW264.7 macrophages, bone marrow-derived macrophages (BMDM) and trophoblasts which are more relevant to the natural infections. Our results of growth of *B. abortus* in RAW264.7 macrophages showed the same growth trend as these bacteria behaved in HeLa cells. In RAW264.7 macrophages, near 60% of *B. abortus* arrest their growth during the first 4 hours PI and resume their growth after 4-6 hours PI. In BMDM, only a fraction of 17% of *B. abortus* is able to resume growth after 4h PI. In addition, at later times PI in BMDM such as 8h and 10h PI, there are more than 40% of *B. abortus* still not growing. These suggest that the more agressive behaviour of BMDM against *B. abortus*, compared to HeLa cells and RAW264.7 macrophages, the larger number of bacteria limit the resuming of growth. In all tested cell types, human trophoblasts are best environments for *B. abortus* growing and proliferating, which are near 70% of bacteria in JEG-3 and 30% of bacteria in BeWo start resuming their growth after 2h PI. Therefore, it is very interesting to discover the cell-cycle situations of *B. abortus* at the early time point such as 15 minutes post infection in BeWo and JEG-3.

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Comprehensive analysis of the genomic localization of bacterial toxin-antitoxin systems

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Bacterial toxin-antitoxin (TA) systems are composed of a stable toxin and an unstable antitoxin. These modules were originally detected on plasmids in the 80's. In this context, TA systems are involved in plasmid addiction. This phenomenon relies on differential stability of the toxin and antitoxin components. When plasmid-free daughter-cells are produced, antitoxin molecules are degraded. Toxins are then free to act on their targets and this lead to growth inhibition and eventually cell death. Therefore, the cells are addicted to the presence of the TA systems. These systems are widely spread in bacterial and archeal genomes. Although a variety of functions have been assigned to chromosomally-encoded systems, their biological roles are still unclear or limited to particular cases, making the 'selfish' behaviour an attractive hypothesis to explain the evolutionary success of TA systems. Type II TA systems are thought to invade bacterial genomes through horizontal gene transfer but it still remains unclear. Are these systems mainly present in mobile elements? Are the TA genes always part of TA systems or some of them can exist without the presence of its partner?

We have performed an exhaustive search for proteins belonging to TA systems (type II to V) and a comprehensive analysis of their distribution, genomic organization and context in order to answer these questions. Using the known and experimentally tested TA systems we have searched for similar proteins in all the bacterial, viral and plasmidic genomes available in the NCBI database using hmm profiles. We have determined if toxin and antitoxin ORFs co-localize and look for the presence of signature of mobile elements in the neighbouring genes.

The primary results of this work are that each TA family has a particular distribution, which different antitoxins can be associated to the same toxin, as it has been seen in other studies, and in some cases toxin or antitoxins can appear without known antitoxin or toxin, respectively. Genomic distribution of some families is coherent with 'selfish' behaviour while others are more easily explained by acquisition of different roles in bacterial physiology throughout evolution. This might thus reveal novel putative functions and/or TA integrations in host regulatory networks. In addition, this work highlighted potential novel toxins or antitoxins that will be experimentally validated in our lab.

Pseudorabies virus gE causes ERK1/2 activation in primary porcine T lymphocytes and subsequent cell aggregation and migration

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Alphaherpesviruses are the largest subfamily of the Herpesviruses. This subfamily contains closely related pathogens, including the herpes simplex virus 1 and 2 (HSV -1 and HSV-2), and varicella zoster virus (VZV) in man. Another member of the alphaherpesvirus subfamily is the porcine pseudorabies virus (PRV) that is often used as a model to study general features of alphaherpesvirus biology (Pomeranz, 2005, MMBR).

We previously demonstrated that Pseudorabies virus (PRV) activates the MAP kinase Erk 1/2 in Jurkat T-cells. Activation of Erk 1/2 was dependent on the extracellular domain of viral glycoprotein gE, revealing a new role for gE during alphaherpesvirus infection.

To corroborate our findings in a porcine system, which is the natural host of PRV, we used primary T lymphocytes isolated from porcine peripheral blood mononuclear cells. The isolated T lymphocytes were co-cultured with gE-expressing porcine epithelial cells. In line with our previous observations in Jurkat T-cells, gE caused a rapid and transient activation of Erk 1/2 in primary T lymphocytes.

In a next step, we wanted to address potential biological consequences of gE-mediated Erk 1/2 activation in T lymphocytes. One of the consequences of Erk 1/2 signaling in T lymphocytes described in literature is T lymphocyte aggregation, which is indicative for T cell activation (Layseca-Espinosa et al., 2003, JLB). At 24h post inoculation (hpi), wild type (WT) PRV caused a substantial increase in primary porcine T lymphocyte aggregation, leading to the formation of large cell aggregates when compared to mock-infected cells. An isogenic gEnull PRV variant also induced some cell aggregation, this was significantly less pronounced compared to wild type PRV. Importantly, addition of U0126, an inhibitor of Erk 1/2 phosphorylation, drastically reduced WT PRV-induced T lymphocyte aggregation levels to mock levels. Cellular aggregation often correlates with cell migration (Layseca-Espinosa et al., 2003; Jevnikar et al., 2008). Using a Transwell migration system, we found that at 24 hpi, WT PRV led to a significantly increased migration of primary porcine T lymphocytes when compared to mock-infected cells. The gEnull PRV variant caused a less pronounced migration, whilst U0126 almost abrogated WT PRV-induced migration. Collection of migrated T lymphocytes and co-culture of these cells with porcine ST cells resulted in obvious virus plaques in ST cells, indicating that migrating T lymphocytes are able to transmit PRV to other susceptible cells.

Taken together, our results indicate a potential new role for gE in viral spread, where gE-mediated ERK 1/2 activation triggers PRV-carrying T lymphocytes to migrate and possibly infect other cells susceptible to PRV replication.

Membrane targeting during Type III protein secretion

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The Type III secretion system (T3SS) is used by many Gram-negative pathogenic bacteria to deliver effector proteins directly into eukaryotic host cells. The T3SS machinery exports virulence factors from the cytoplasm through both the inner and the outer membrane to the external milieu. The proteins that are being secreted through this unique nanomachine are essential for the pathogenicity of the bacteria (1). Although T3SS is common in Gram-negative bacteria, little is known about its function and the precise pathway that proteins follow towards their secretion.

To address this, we performed a comprehensive complexome analysis using Size exclusion chromatography and/ or Native PAGE coupled to protein identification through high resolution MS, from which we have extracted information about potential protein complexes that are formed during protein secretion through T3SS. Specific complexes were validated, using affinity purification under native conditions, SEC, N- PAGE analysis and Knock-out mutant strains (2).

To gain more structural insight, we focused on one protein complex as a model, that of the translocator EspA and its chaperone CesAB (3, 4). First, we monitored EspA and its interaction with CesAB, in the bacterial cytosol and we characterized them using NMR, Isothermal titration Calorimetry (ITC) and Multi Angle Light Scattering (MALS) (4). CesAB is self-regulated so as to prevent unspecific protein interaction. It forms an unstable homo-dimer that mimics the hetero-interactions it forms with its substrate, EspA. The CesAB-EspA heterodimer, is significantly more stable and maintains EspA unfolded, translocation competent and prevents its oligomerization and aggregation in the cytoplasm (4).

Once the CesAB-EspA complex is formed in the cytosol, CesAB can interact with the ATPase of the system EscN. As determined using in-solution NMR and biochemical assays, this interaction is EspA- dependant. Conformational changes that occur on CesAB upon binding to EspA, lead to the exposure of an EscN targeting signal (5).

Additional regions of CesAB are required for membrane targeting of the protein complex and delivery of EspA to the translocation pathway for complete translocation of the secretory protein (6).

Our data reveal dynamic complexes between T3SS chaperones and secreted proteins and shed light in the molecular mechanisms that mediate their targeting to the T3S translocase

The Gut Microbiota of Pollinators: an unknown and unexplored Treasure Chest of Biodiversity

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Bumblebees are important pollinators of tomatoes, sweet pepper and many other commercial crops and wild plants. However there is currently great concern about their worldwide decline and that of other pollinators like butterflies and hoverflies [1]. These declines may have a detrimental economical impact and may create an instable ecosystem. The gut microbiota of bumblebees consist of few and very specific bacterial species [2], [3] and include *Snodgrassella alvi*, *Gilliamella apicola* and *Bifidobacterium bombi*. These symbiotic gut bacteria may contribute to the health of bees by helping with the digestion of pollen, the detoxification of compounds and the pathogen inhibition [4]. Therefore an inventarization of the cultivable bacteria in the gut of bumblebees and of their functionality is being made.

We will present polyphasic taxonomic and whole genome sequence data reporting the presence of novel bacteria belonging to the genera *Gilliamella, Bifidobacterium*, *Leuconostoc* and *Lactobacillus,* and to the family *Acetobacteraceae.*

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Low concentrations of cereulide, the emetic *Bacillus cereus* toxin, alters cell metabolism in differentiated intestinal Caco-2 cells

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Cereulide is a heat-, protease- and pH stable toxin produced by *Bacillus cereus*. Relatively high doses of the cereulide (8 µg/kg body weight) cause nausea and gastric discomfort, and acute doses of cereulide have lethal consequences. Recent research demonstrated a wide-spread prevalence of relatively low concentrations of cereulide in rice and pasta dishes. However, the impact of a continuous exposure of the human body to low doses of cereulide is currently unknown. Therefore, in this research, we investigate the effect of low (sub-emetic) concentrations of cereulide on the behavior of intestinal cells – the first exposure site after ingestion – using the Caco-2 cell line.

Toxicity assays showed that undifferentiated Caco-2 cells were more sensitive towards low doses of toxins than differentiated cells. Low concentrations of cereulide caused cell release from the differentiated monolayer and increased lactate levels in the cell culture medium. Proteomic data showed that cereulide at concentration of 1 ng/mL led towards a significant decrease in energy managing and H_2O_2 detoxification proteins and to an increase in cell death markers.

We conclude that sub-emetic doses of cereulide may induce altered enterocyte metabolism and epithelium integrity, and may hence affect human health in general.

This work was supported by the Research Foundation – Flanders (FWO – Vlaanderen) in the framework of postdoctoral mandate of Dr. Andreja Rajkovic. Authors wish to thank Federation of European Microbiological societies, FEMS, for the exchange scholarship provided to Ana Butorac.

Linking growth and bacterial cell shape to host-glycan harvesting in *C. canimorsus*

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Capnocytophaga canimorsus are gliding Gram-negative bacteria from the *Bacteroidetes* phylum that are part of the normal flora of dogs which upon scratches or bites can infect humans and cause fatal septicemia [Bobo & Newton (1976) *Am. jour. of clin. path.* 65(4):564-569].
We found that *C. canimorsus* feed on cultured mammalian cells by harvesting glycan moieties from host surface glycoproteins via the Gpd proteins complex encoded by the Polysaccharide Utilization Locus 5 (*PUL5*). Mutation of *PUL5* reduces bacterial growth, which can be restored to *wt* levels only by addition of aminosugars such as GlcNAc. We propose a model for *N*-linked glycan harvesting where the surface-exposed GpdCDGEF complex captures the glycan moieties of glycoproteins which are then cleaved by GpdG, an endo- *β*-*N*-acetylglucosaminidase, and internalized by the GpdC porin. Once in the periplasm, a sialidase (SiaC) removes terminal sialic acids and the oligosaccharide is sequentially processed by exoglycosidases and the monosaccharides, including GlcNAc, would be liberated [Renzi *et al.* (2011) *PLoS pathogens* 7(6):e1002118].

C. canimorsus is also able to obtain aminosugars from salivary mucin via the *PUL9* encoded Muc surface complex. This consist of two putative mucin-binding proteins, a protease which cleaves mucin into glycopeptides and a porin which transports them into the periplasm where SiaC removes terminal sialic acids and a sulfatase and a *β*-galactosidase further process the mucin *O*-glycans. Mucin rescues the growth of a *PUL5* mutant but not of a double *PUL5*-*PUL9* mutant, indicating that the Muc complex provides *C. canimorsus* with aminosugars.

The need of several systems to harvest host glycans is linked to the inability of *Cc* to synthesize GlcNAc due to the lack of two key enzymes, GlmU and GlmM, which lead to the conversion of glucosamine into GlcNAc. Heterologous expression of these enzymes in *PUL5* mutant bacteria restored the growth defect to *wt* levels, making growth independent from cells deglycosylation. Starvation for GlcNAc affects the synthesis of neo peptidoglycan monomers and leads to the formation of abnormal cell shapes and growth arrest.

We propose that *C. canimorsus* lost the capacity to synthesize GlcNAc after it adapted to the dog mouth and could retrieve GlcNAc from mucin and from *N*-glycoproteins.

A phosphoproteome analysis at the host-Respiratory syncytial virus interphase

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Human respiratory syncytial virus (HRSV) is an enveloped, negative single stranded RNA virus belonging to the *Paramyxoviridae* family and a common human respiratory pathogen. Although an HRSV infection is commonly associated with only mild respiratory symptoms, very young children and the elderly can be at risk for developing (severe) bronchiolitis or pneumonia. By the age of 2 years, almost all children have been infected with HRSV at least once, requiring hospitalization in 1-2% of the cases. Despite 60 years of research and the importance of this pathogen, no licensed HRSV vaccine or antiviral therapy are available. Only a humanized monoclonal antibody (Palivizumab, Synagis®) is used prophylactically in high risk infants. At present, very little is known about the cellular factors that counteract or are needed for HRSV infections. Just recently, cell surface expressed nucleolin was identified as the cellular HRSV receptor (Tayyari *et al,* 2011, nature medicine), illustrating how little is still known on the interplay between the host cell and HRSV. In this project we are investigating kinase-controlled signaling pathways of the host that either enhance or counteract HRSV infections. We believe that elucidating these pathways will help to understand the infection process in more detail and help to discover novel therapeutic targets.

To investigate kinase controlled signaling pathways, we first performed a siRNA based knockdown screen of the complete human kinome (719 genes) in A549 cells. Replication of HRSV-A2 was determined by staining of HRSV plaques and validated afterwards by quantifying released HRSV virions in a kinetics setting. In a second approach that we are currently setting up, we want to determine and quantify changes in protein phosphorylation following HRSV infection by phospho-proteomics. TiO_2 enriched phosphorylated peptides from mock and HRSV infected A549 cells will be compared by label-free proteomics.

Knockdown of 2 kinases resulted in reduced HRSV replication and this reduction was evident after knockdown with different siRNAs targeting these kinases. This suggests that these kinases play an important role during a HRSV infection and that the observed reductions in HRSV replication are the result of the specific knockdown of these 2 kinases and not due to off-target silencing. A MTS based metabolic activity assay excluded the possibility that the reduced HRSV replication after knockdown of these 2 kinases was due to a general decrease in metabolic activity. We also confirmed that a HRSV-Nucleoprotein siRNA (ALN-RSV01) clearly abolished HRSV replication. Currently we are trying to elucidate at which stage of the HRSV infection cycle these kinases might play a role.

Experimental evolution with a global regulator mutant in *Escherichia coli*

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CsrA is a global regulator and the main player of the carbon storage regulator (Csr) network, a well-conserved regulatory network in the bacterial world. CsrA is involved in regulation of many physiological processes, including pathways of central carbon metabolism, biofilm formation, motility and virulence in pathogenic species. CsrA acts at the post-transcriptional level by binding specific sequences on its target mRNAs, leading to mRNA destabilization or stabilization. The majority of studies were analyzing a *csrA* mutant of *E. coli* K-12 encoding a truncated form of the CsrA protein, retaining residual activity.

This work aims at further characterize the roles of CsrA by deleting the entire *csrA* gene in a recently isolated strain, the uropathogenic *E. coli* CFT073 strain. Deletion of *csrA* leads to a marked growth defect, indicating that this gene, although not essential, is primordial for growth. We performed experimental evolution of *csrA* deletion mutants. Compensatory mutants totally outcompete the original *csrA* deletion mutant after six days of culture, indicating that the applied selective pressures are strong. The *ΔcsrA* and three *ΔcsrA* evolved mutants were extensively analyzed by combining molecular techniques such as genetics, microscopy and use of fluorescent reporters, and global approaches, including comparative proteomics and whole genome sequencing.

Our data indicate that *csrA* deletion strongly affects central metabolism and energy status, constituting an endogenous metabolic stress that, in turn, induces specific stress responses. This work illustrates the interconnection of multiple regulation networks for responding to specific conditions and demonstrates the flexibility of metabolic network to compensate for genetic perturbations in *E. coli*.

Protection and mechanism of action of a novel human respiratory syncytial virus vaccine candidate based on the extracellular domain of small hydrophobic protein.

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Infections with human respiratory syncytial virus (HRSV) occur globally in all age groups and can have devastating consequences in young infants. We demonstrate that a vaccine based on the extracellular domain (SHe) of the small hydrophobic (SH) protein of HRSV, reduced viral replication in challenged laboratory mice and in cotton rats. We show that this suppression of viral replication can be transferred by serum and depends on a functional IgG receptor compartment with a major contribution of FcγRI and FcγRIII. Using a conditional cell depletion method, we provide evidence that alveolar macrophages are involved in the protection by SHe-specific antibodies. HRSV-infected cells abundantly express SH on the cell surface and are likely the prime target of the humoral immune response elicited by SHe-based vaccination. Finally, natural infection of humans and experimental infection of mice or cotton rats does not induce a strong immune response against HRSV SHe. Using SHe as a vaccine antigen induces immune protection against HRSV by a mechanism that differs from the natural immune response and from other HRSV vaccination strategies explored to date. Hence, HRSV vaccine candidates that aim at inducing protective neutralizing antibodies or T-cell responses could be complemented with a SHe-based antigen to further improve immune protection.

Development of an *in vitro* onychomycosis platform for the evaluation of topical antifungal activity.

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Although current systemic therapies for onychomycosis have high cure rates compared to topical treatment, drug interactions and side effects limit their use in immunocompromised and diabetic patients. Given the increasing prevalence of these patient groups, new topical therapies are of great importance. As few *in vitro* models are available to easily predict the effectiveness of potential topical antifungals, we developed a novel evaluation platform for onychomycosis drugs, with terbinafine, itraconazole, fluconazole and amorolfine as reference drugs. The platform encompasses *in vitro* antifungal susceptibility testing and a drug permeability evaluation through bovine hoof slices using Franz diffusion cells. Based on the typical agar diffusion test, a novel onychomycosis diffusion model was developed to assess antifungal activity against *Trichophyton mentagrophytes*, directly after diffusion through the hooves.

From all the reference drugs tested, terbinafine, itraconazole and amorolfine demonstrated the highest activity against *T. mentagrophytes*. However, only small amounts of terbinafine and amorolfine actually penetrated the bovine hoof slices. As low diffusion rates are difficult to correlate with antifungal activity, the novel agar diffusion model was used to directly evaluate the antimicrobial efficacy of topical antifungals. In contrast to permeation studies, results from this novel diffusion model are easy to interpret, demonstrating clear growth inhibition with low variability for both terbinafine and amorolfine. Such model may be of additional value in the search for new topical antifungal therapies against onychomycosis.

Brucella suis and *Brucella abortus* use distinct molecular components for adhesion to host cells

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Bacteria from the *Brucella* genus are closely related intracellular pathogens responsible for brucellosis, a worldwide anthropo-zoonotic disease.

Adhesion and invasion are thought to be critical steps for *de novo* infection by *Brucella* spp, however little is known about the mechanisms allowing *Brucella* spp. to adhere to and invade host cells. So far, several publications have highlighted the importance of interactions between *Brucella* and components of host cell extracellular matrix for infection (Castañeda-Roldán *et al*. 2004). More specifically, it was shown in *Brucella suis* that the deletion of either one of the three genes coding for the monomeric autotransporter *bmaC,* the trimeric autotransporter *btaE*, and the trimeric autotransporter *btaF* severely impacted the adherence and thus infectiosity of *B. suis* in HeLa cells (Posadas *et al.* 2012, Ruiz-Ranwez *et al*. 2013).

Here, we report that as opposed to *B. suis*, the deletion of *bmaC*, *btaE* and *btaF* either separately or combined in a triple deletion strain does not alter the infectiosity of *Brucella abortus* 2308 in HeLa cells and RAW 264.7 macrophages, thus implying that adherence mechanisms are not shared among these closely related bacterial species. We therefore suggest that B. abortus 2308 is using other molecular components to stimulate their adherence and/or invasion in host cells.

Nitrogen assimilation and dissimilatory nitrate reduction processes in *Bacillus azotoformans* LMG 9581^T

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Genome analyses of the soil organism *Bacillus azotoformans* LMG 9581^T, a phenotypically denitrifying strain, surprisingly revealed genes encoding all necessary enzymes for denitrification and dissimilatory nitrate reduction to ammonium (DNRA), previously thought mutually exclusive pathways. In addition, it is devoid of the assimilatory nitrate and nitrite reductase genes (no *nirBD*), suggesting that DNRA might serve nitrogen assimilation. However, it also lacks an ammonium-transporter gene (AmtB-type). So we suppose that ammonium in the media can't be utilized and DNRA, if functional, is only used for energy conservation and not for assimilation. To confirm this, growth experiments were performed both anaerobically and aerobically in various growth media with excess of carbon compared to nitrate (10mM), to stimulate DNRA over denitrifiaction, and with/without 4.6mM ammonium. No obvious ammonium decrease was observed with both aerobic and anaerobic growth, which confirms that the organism does not contain an ammonium transporter and thus cannot use ammonium in the media as nitrogen source. For indication of two possible dissimilatory nitrate reduction processes, DNRA and denitrification, we also further checked N equilibration in anaerobic growth experiments by colorimetric determination and gas chromatography. Results shows that, no ammonium is produced during anaerobic growth and all nitrate was converted to nitrous oxide, thus no indication for DNRA proceeding in *B. azotoformans* is obtained under experimental conditions. Moreover, yeast extract proved pivotal and unreplacable for both aerobic and anaerobic growth in mineral media. We assume that yeast extract not only works as an organic nitrogen source, but also works as a crucial factor for growth.

Prodiginines, secondary metabolites associated with the programmed cell death of *Streptomyces coelicolor*

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Prodiginines (PdGs or prodigiosin-like pigments) are natural red-pigmented compounds produced by a series of microorgansims, with *Streptomyces coelicolor* and *Serratia marcescens* as best studied model species. PdGs are promising molecules in medicine due to their immunosuppressive and anticancer activities besides to their previously described antibacterial, antifungal, antiprotozoal, antihelmintic, antiviral, and antimalarial properties. Numerous studies describe the role and the molecular targets of PdGs in eukaryotic cancerous cell lines. In light of the known anti-proliferative properties of PdGs, we questioned whether these molecules could display similar activities and, in consequence, would be associated with the programmed cell death (PCD) process of the producing microorganism.

S coelicolor is known to undergo several rounds of PCD during its complex life cycle and produces PdGs, namely undecylprodigiosin and streptorubin B, in a conditional and developmental manner prior to the sporulation process. By monitoring the Red Auto Fluorescence (RAF) of PdGs, we show that PdGs production localizes in the dying filaments of a confluent culture. Furthermore, we observed that a mutant unable to produce PdGs (Δ*redD*) showed a higher proportion of viable filaments compared to the wild type strain (WT). This PdGs –deficient mutant also accumulated more intracellular total proteins, RNA, and DNA than the parental strain *S. coelicolor* M145 suggesting that PdGs participate in the destruction of all macromolecules. Our results suggest that PdGs participate in the PCD process of *S. coelicolor*.

Screening of the NIHCC 1&2 to Identify Antibiotic Potentiators with Activity against Mature Bacterial Biofilms

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New therapies to treat resistant micro-organisms are urgently needed. However, development of antibiotics with new mechanisms of action is a risky business, taking several years of investigation and expensive clinical trials with a low rate of success.

A promising strategy to overcome these problems and fill the antibiotic pipeline, is drug repurposing or repositioning, in which existing drugs are used to treat diseases they were not initially developed for.

In this study we evaluated which compounds of the NIHCC 1 & 2 library could increase the susceptibility of *Burkholderia cenocepacia* biofilms towards the antibiotic tobramycin. Biofilms are cell communities embedded in a highly hydrated self-produced extracellular matrix, showing a higher tolerance and resistance towards antibiotics compared to their planktonic counterparts.

Mature *B. cenocepacia* biofilms were treated during 24 hours with 100 µM of each compound together with 512 µg/ml tobramycin. The anti-biofilm effect was subsequently determined using resazurin staining.

64 out of 727 compounds investigated showed a decrease of 90% or more in the fluorescence signal compared to the fluorescence signal generated by biofilms that were treated with tobramycin alone. These compounds could be divided into 6 groups: anti-infective agents (13), antidepressants (14), anti-psychotics (11), anti-allergic agents (5), and a miscellaneous group (21).

The most promising compounds displayed a broad potentiator effect. They enhanced the effect on several antibiotics against biofilms of both Gram-negative and Gram-positive bacteria. These *in vitro* results were confirmed in *in vivo* experiments using the invertebrate model *Galleria mellonella*. Altogether these results indicate that our compounds show great potential as potentiators of antibiotics.

Comparison of Illumina MiSeq and Ion Torrent PGM next generation sequencing for influenza A virus quasispecies analysis

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Determining the composition of the influenza viral quasispecies requires an accurate and sensitive sequencing technique and analysis pipeline. We compared the suitability of two benchtop next-generation sequencers: the Illumina MiSeq sequencing-by-synthesis and the Ion Torrent PGM semiconductor sequencing technique. We first validated the accuracy and sensitivity of both sequencers using plasmid DNA and spiking-in experiments of mutant plasmid. Illumina MiSeq sequencing reads were one and a half times more accurate than those of the Ion Torrent PGM. To evaluate the suitability of the two techniques for determining the quasispecies composition of influenza A virus, we generated plasmid-derived PR8 virus. We optimized an RT-PCR protocol to obtain uniform coverage of all eight genomic RNA segments. Sequence analysis of these amplicons revealed that more sequencing errors (mostly indels) were generated by the Ion Torrent PGM. Based on RT-PCR and intrinsic sequencing errors, we found that the detection limit for reliable recognition of variants in the viral genome required a frequency of 0.5% or higher. Most of the variants in the PR8 genome were present in hemagglutinin, and these mutations were detected by both sequencers. Our approach underlines the power and limitations of two commonly used next-generation sequencers for the analysis of influenza gene diversity.

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Characterizing the biofilm phenotype in *Staphylococcus* spp. from central venous catheters using two *in vitro* models

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Objective: The *in vitro* characterization of the biofilm-forming capacity of clinical isolates on central venous catheters (CVC) is often based on static models, although dynamic models might be more relevant. The biofilm-forming capacity of *Staphylococcus* clinical isolates was compared using both model systems.

Methods: Biofilms of 40 clinical isolates (*S. aureus*, *S. epidermidis*, *S. hominis*, *S. warneri* and *S. capitis*) from intensive care unit CVC's were cultured in microtiter plates (static) or the microfluidic Bioflux™ system (dynamic) and were quantified using crystal violet and SYTO9 respectively. Tryptone soy broth was used in both models while the substrate (polystyrene vs. glass) and the incubation time (48h vs. 17h) varied. Using the strong biofilm-forming *S. aureus* ATCC 6538 as reference (static: OD_{570nm} = 0.69±0.16; dynamic: % area = 32.5±0.7), OD-values or covered areas >75% of reference were categorized as strong, and <25% of reference were defined as weak biofilm-formers.

Results: Using the static model, 8 strains were categorized as strong (OD 0.51-0.81) and 11 strains as weak (OD 0.08-0.17) biofilm-formers, compared to 3 strong (% area 24.8-36.9) and 35 weak (% area = 0-4.1) in the dynamic model. All strong biofilm-formers in the static model were weak biofilm-formers in the Bioflux system, while 2 of the weak biofilm-forming strains proved strong biofilm-formers using the Bioflux.

Conclusion: *In vitro* biofilm formation is strongly model dependent, warranting appropriate standardization to enable more accurate comparisons of the biofilm phenotype of clinical isolates.

Funding: BOF-GOA grant 25624 - IWT

Biological applications of solid-state nanopores

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In the past two decades, nanopores have been studied intensely for their potential as DNA-sequencing devices that would enable affordable personalized medicine [Wanunu (2012) Phys. Life Rev. 9: 125-158]. In this pursuit, other applications have been uncovered with less stringent requirements: applications that do not need single base-pair resolution. These include DNA detection and sizing [Storm *et al* (2005) Nano Letters 5: 1193-1197], as well as the determination of dose-response curves for antigen-antibody interactions [Han *et al* (2008) Anal. Chem. 80: 4651-4658]. Even more extensive studies have been performed to characterize protein-DNA interactions, including nanopore force spectroscopy, i.e., the study of the voltage-dependent dissociation of a complex at a nanopore that is too small to allow for translocation of that complex. This enabled the determination of binding and kinetic constants of ExoI to ssDNA within a biological α-hemolysin pore [Hornblower *et al* (2007) Nat. Methods 4: 315-317]. In this work, we show that short DNA oligonucleotides can be detected with solid-state nanopores, paving the way towards the determination of binding and kinetic constants of protein-DNA interactions and biomolecular interactions in general, as solid-state nanopores are adjustable in size.

Signal peptide-binding drug inhibits cell surface expression of the CD4 receptor for HIV-1 attachment and entry

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Human immunodeficiency virus (HIV) uses the human CD4 protein (hCD4) as the primary surface receptor for attachment and infection of host cells such as $CD4^+$ Th-lymphocytes. This hCD4 receptor dependency can be exploited in an antiviral strategy: removal of the CD4 receptor from the cell surface will prevent HIV infection of target cells and viral replication. In eukaryotic cells, surface expression of most transmembrane proteins is dependent on the presence of a hydrophobic N-terminal signal peptide (SP) on nascent proteins. It facilitates targeting of the nascent proteins to the Sec61 translocon, a universally conserved protein-conducting channel in the ER-membrane, and subsequent insertion of the chain for translocation. Despite their common function, signal peptides have diverse primary sequences. Thus, drugs that recognise unique signal peptide sequences could be exploited to inhibit ER translocation and cell surface expression of selected proteins. Previously, the small-molecule macrocycle CADA was identified as an antiviral drug with broad spectrum anti-HIV activity. It acts as a highly selective hCD4 expression down-modulator. Here we show that CADA inhibits hCD4 biogenesis by preventing co-translational translocation of hCD4 to the ER lumen, both in cell culture and in a cell-free *in vitro* translation/translocation system. The activity of CADA maps to the signal peptide of hCD4 which represents the minimal sequence required for full CADA sensitivity. Importantly, we could show direct binding between this SP and CADA through suface plasmon resonance (SPR). Furthermore, translocation inhibition by CADA causes the precursor protein to be routed to the cytosol for degradation. These findings demonstrate that a synthetic, cell-permeable small-molecule such as CADA can act as a signal peptide-binding drug to regulate the expression of specific target proteins by selective and reversible inhibition of protein translocation.

Mutual influence of *S. epidermidis* and *S. aureus* on gene expression in dual species biofilms

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The effect of the presence of *S. epidermidis* ET-024 on the gene expression of *S. aureus* Mu50 (and *vice versa*) was investigated by performing RNA-Seq of dual species biofilms and by comparing the expression levels to those observed in single species biofilms.

RNA-Seq data showed that, in dual species biofilms, *S. epidermidis* genes encoding resistance to erythromycin, oxacillin and tobramycin were significantly upregulated. In agreement with this, single and dual species biofilms were treated with oxacillin and we observed that significantly more *S. epidermidis* cells survived treatment in dual species biofilms. However, no differences between single and dual species biofilms were observed for tobramycin and erythromycin.

Analysis of the RNA-Seq data also revealed that, for both *S. epidermidis* and *S. aureus*, the seven genes encoding the urease enzyme were downregulated in dual species biofilms. qPCR experiments using strain-specific primers confirmed this downregulation. Also, there was significantly less urease activity in dual species biofilms compared to single species biofilms. As literature data showed that there is link between urease activity and pH, we are investigating the changes in pH and urease activity in single and dual species biofilms of *S. epidermidis* and *S. aureus* as function of time.

Compared to single species biofilms, there was significantly less expression of genes (of both *S. epidermidis* and *S. aureus*) encoding proteins that are involved in metabolic processes such as citric acid cycle and ATP production. These results suggest that, in dual species biofilms, less metabolically active cells are present compared to single species biofilms. Currently, we are determining the number of metabolically active cells in single and dual species biofilms by resazurin-based viability staining and solid-phase cytometry.

Identification and mechanism of action of a novel influenza virus fusion inhibitor

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Introduction: The influenza virus hemagglutinin (HA) plays a crucial role in fusion between the viral and endosomal membranes during influenza virus entry. This fusion process can be blocked by compounds interfering with the acid-induced conformational change of HA.

Methods: After identification of the anti-influenza virus activity of CIM07 in MDCK cells, its mechanism of action was unraveled by performing time-of-addition studies, entry assays using confocal microscopy and HA-mediated fusion (i.e. syncytium formation and tryptic digestion) assays. Finally, CIM07-resistant mutants were selected and characterized.

Results: In Madin-Darby canine kidney (MDCK) cells infected with influenza virus A/H3N2, the 50% effective concentration of CIM07 was 0.20 μM, as determined by microscopy of the viral cytopathic effect and MTS cell viability assay. The concentration causing minimal alterations in cell morphology was ≥25 μM. Similar activity was seen for other A/H3N2 strains, but no activity was noted for influenza A/H1N1 and B viruses. In time-of-addition studies, CIM07 lost activity when added 1 hour or later post infection, showing that CIM07 inhibits an early step in virus replication. CIM07 was proven to completely prevent the nuclear entry of influenza virus, based on confocal microscopy with anti-nucleoprotein staining. The inhibitory effect of CIM07 on low pH-induced syncytium formation in HA-expressing cells indicated that CIM07 blocks HA-mediated membrane fusion. Definite proof for this mode of action was provided by the tryptic digestion assay, in which CIM07 prevented the acid-induced conversion of HA to its trypsin-sensitive conformation. CIM07-resistant mutants, selected after three passages in 0.37-10 μM CIM07, were plaque-purified. Phenotypic and genotypic characterization of these resistant viruses is currently ongoing.

Discussion: CIM07 represents an excellent tool to further delineate the structural changes during the low pH-induced HA refolding process. The resistance mutations allow to identify its binding pocket and design new CIM07 derivatives with improved activity.

Loop L4 in mouse Mx1 is crucial for antiviral activity against influenza A viruses.

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The interferon-induced *Mx1* gene is an important part of the mammalian defense against influenza viruses. Mouse Mx1 inhibits influenza virus replication and transcription by suppressing the polymerase activity of viral ribonucleoproteins (vRNPs). We previously showed that *Mus musculus* Mx1 interacts with the main component of these vRNPs, *i.e.* nucleoprotein (NP). Here, we show that binding of mouse Mx1 to NP depends on loop L4 of the Mx1 protein and that deletion of this loop abolishes its antiviral activity against influenza A viruses. To identify other regions in Mx1 that are important for inhibiting influenza A virus infection, we compared the anti-influenza activity of Mx1 from *M. musculus* A2G with its ortholog from *Mus spretus*. We found that the antiviral activity of *M. spretus* Mx1 against PR8 virus was less potent than that of *M. musculus* Mx1. However, both Mx1 proteins inhibited avian-type influenza virus replication equally well. Sequence comparison of *M. musculus* with *M. spretus* Mx1 revealed 25 amino acid differences, 2 of which are present in loop L4. Replacing one of these residues in *M. spretus* Mx1 by the corresponding residue of A2G Mx1 increased the *in vitro* antiviral activity against PR8. Our results indicate that loop L4 of mouse Mx1 is an important determinant of antiviral activity and viral target recognition. Our findings suggest that Mx proteins from different mammals use a common mechanism to recognize influenza A viruses.

Keywords: Mx1, Influenza A Virus, Nucleoprotein, Dynamin-like GTPase, Myxovirus resistance

Dengue virus-induced apoptosis determines endothelial cell fate.

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Dengue virus (DENV) is one of the most important emerging viruses with more than 2.5 billion people at risk of getting infected. No specific antivirals or vaccines are available, and treatment relies on supportive care of the symptoms. The clinical disease spectrum ranges from the self-limiting dengue fever (DF) to the more severe and life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which are characterized by thrombocytopenia, increased vascular permeability and subsequent plasma leakage. Dendritic cells, monocytes and macrophages are considered to be the primary target cells of DENV *in vivo*, but other cell types, including endothelial cells (ECs), may be infected by DENV as well. Apoptotic microvascular ECs have been found in pulmonary and intestinal tissues from Cuban fatal DENV cases (Limonta *et al. J. Clin. Virol.* 2007) and EC apoptosis may provide an explanation for the vascular leakage in these tissues.

Here we describe that human macro- and microvascular primary endothelial cells and endothelial cell lines are permissive for DENV infection with all four serotypes (DENV 1-4) *in vitro*. Throughout the infection process, the cells were investigated microscopically for morphological changes. DENV infection induced a cytopathic effect (CPE), which became apparent after 24 h (DENV-4), 48 h (DENV-2) or 72 h post-infection (DENV-1 and -3). Cell cycle analysis revealed an increase in the sub-G1 population (indicative of apoptosis) in infected ECs when compared to uninfected control cells at different times post-infection. The presence of apoptotic ECs was further confirmed using Annexin V-FITC/propidium iodide staining and flow cytometry. Experiments are ongoing to elucidate the apoptotic pathways induced by DENV infection in ECs and to investigate the contribution of apoptosis to DENV-enhanced endothelial permeability.

The envelope glycoprotein gp150 promotes sexual transmission of Murid herpesvirus 4.

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Gammaherpesviruses are important pathogens in human and veterinary medicine. During co-evolution with their hosts, they developed many strategies allowing them to shed infectious particles in presence of immune response. Understanding these strategies is likely to be important to control infection. Interestingly, we recently observed that Murid herpesvirus 4 (MuHV-4), a gammaherpesvirus infecting laboratory mice, could be sexually transmitted between mice. This model offers therefore the opportunity to test the importance of immune evasion mechanisms during transmission. One of these mechanisms could rely on the glycoprotein 150 (gp150) which seems to be used as an immunogenic decoy that could limit virus neutralization. Indeed, while gp150 is the main target of antibodies raised against MuHV-4, anti-gp150 antibodies are not neutralizing but instead enhance Fc-receptor dependent infection *in vitro*. Furthermore, gp150 could form a glycan shield at the surface of the virion that limits neutralization and could promote the release of infectious particles from infected cells. In this study, we tested therefore the importance of gp150 in the context of MuHV-4 sexual transmission. Briefly, female mice were infected with strains expressing luciferase and deficient or not for the expression of gp150. At different times post-infection, these mice were imaged with an *in vivo* imaging system to follow the lytic infection. Finally, at the moment of lytic replication in the genital tract, infected females were mated with naïve males to compare the capacity of transmission of the two strains. Our results show that, while the gp150- strain has no deficit in infection or in genital excretion compared to the wild-type strain, the gp150- strain displays a major deficit of sexual transmission. Interestingly, this deficit does not result from an increased sensitivity to antibody neutralisation but seems to reflect a release deficit of virions from vaginal epithelial cells. Altogether, our results show that, while gp150 is not required for efficient dissemination and maintenance of MuHV-4 within its host, it is essential for efficient transmission, at least by the sexual route.

Host-pathogen interaction as a potential target for development of antivirals: Role of SUMOylation in DENV life cycle.

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Dengue fever (DF), which can progress into dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), forms a global health problem. Epidemiological studies suggests that annually up to 390 million people become infected of which 96 million become severely sick and half of million people develop DSS/DHF leading to over 22 000 deaths each year [1]. There is neither vaccine nor antiviral drug. Conjugation of small ubiquitin-like modifier (SUMO) to proteins (SUMOylation) [which regulates multiple biological systems by altering the functions and fates of a large number of proteins [2,3] and it is directly involved in the replication of flaviviruses [4,5]. We investigated the role of SUMOylation E2 ligase (Ubc9) in DENV replication and type I IFN signaling. We demonstrate using a yeast two hybrid technique that Ubc9 interacts with the flaviviral nonstructural 4b (NS4b) protein and identified non-interacting mutants. We assessed antiviral activity of available SUMOylation inhibitors.

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