BELGIAN SOCIETY FOR MICROBIOLOGY

Koninklijke Vlaamse Academie van België voor Wetenschappen en Kunsten

Vlaams contactforum

National Committee for Microbiology
of
The Royal Academies of Science
and the Arts of Belgium

Posttranscriptional regulation and epigenetics in microorganisms

Friday November 30th 2012

Academy Palace, Brussels

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History

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

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

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

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

Board

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

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

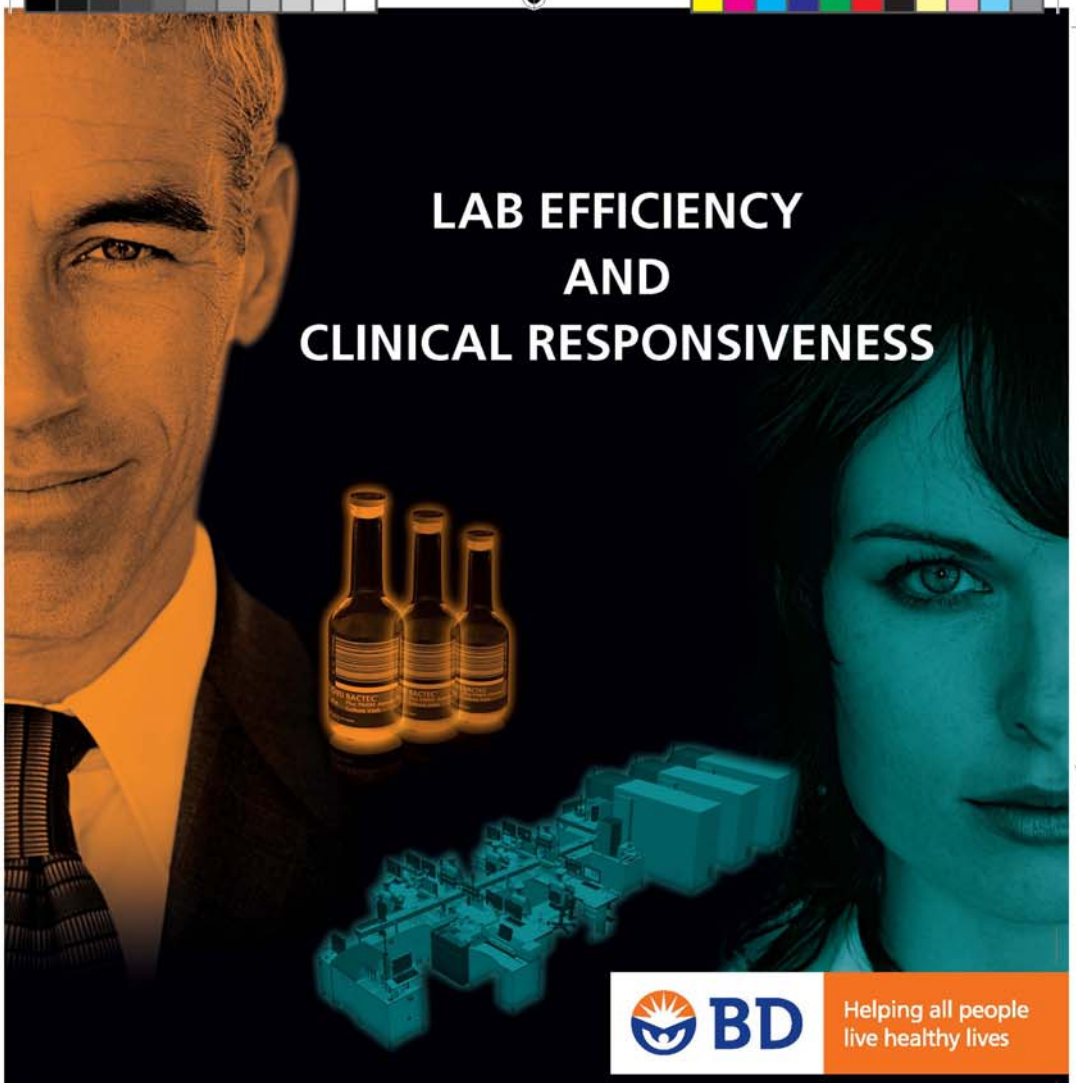
The other Council members are: Spiros Agathos (UCL), Abdelmounaaim Allaoui (ULB); Alfons Billiau (KU Leuven); Pierre Cornelis (VUB) and ASM ambassador, Paul Cos (UA), Herman Favoreel (UGent), Isabelle George, (ULB), David Gillan (UMons), Laurent Gillet (ULg), Natalie Leys (SCK-CEN), Max Mergeay (SCK-CEN),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



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Program

08:30 Registration desk open – Poster mounting

09:00 Welcome address

09:10 **Jörg Vogel,** Institute Molecular Infection Biology, University of Würzburg, DE
"New mechanisms of gene activation by small RNAs"

09:55 **Ben Berkhout,** Lab. of Experimental Virology, University of Amsterdam**,** NL
"HIV-1 and RNA"

10:40 Coffee break

11:20 **Susan Gottesman,** National Cancer Institute-Bethesda, USA
"Bacterial small RNAs as connectors for regulatory cascades"

12:05 **Martin J. Allday,** Virology, Div. Infectious Diseases, Imperial College London, UK
"Epigenetic reprogramming of host genes in viral and microbial pathogenesis"

13:00 Lunch – Poster viewing & poster discussion groups

	Parallel session 1 – Bacteriology	**Parallel session 2 – Virology**
14:30	**Stan Brouns,** Lab. Microbiology, Wageningen University, Wageningen, NL "RNA in defense: CRISPRs protect *prokaryotes against mobile genetic elements and bacteriophages"*	**Carine Van Lint,** Lab. Mol. Virology, ULB, Gosselies, BE *"Epigenetic control of Human Immunodeficiency Virus type 1 (HIV-1) postintegration latency: implications for therapeutic interventions"*
15:10	Short lectures (5) of selected abstracts	Short lectures (5) of selected abstracts

16:30 General conclusions and presentation of best poster awards

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Poster abstracts selected for oral presentation

Virology Session

15h15 *De Cock Aurélie, Thomas Michiels.* **Impact of cellular microRNA on Theiler's virus replication**

15h30 *Jacob Thary, Van den Broeke Céline, van Troys Marleen, Waterschoot Davy, Ampe Christophe and Favoreel Herman.* **The US3 kinase of pseudorabies virus leads to activation of the actin regulator cofilin to induce actin cytoskeleton changes**

15h45 *Machiels Bénédicte, Stevenson Philip, Vanderplasschen Alain, Gillet Laurent.* **Alternative splicing switches tropism of a gammaherpesvirus**

16h00 *Claes Sandra, Geoffrey Férir and Dominique Schols.* **The live cell image JuLI™ analyser as a useful tool for studying HIV cell-cell transmission**

16h15 *Vermeulen, B., Desmarets L., Dedeurwaerder, A., Olyslaegers, D., Dewerchin, H. and Nauwynck, H.* **The role of regulatory T cells during infection with feline infectious peritonitis virus**

Bacteriology Session

15h15 *Van Assche Elke, Hans Steenackers, Jos Vanderleyden.* **Interfering with regulatory RNAs in bacteria**

15h30 *Jové Thomas, Sterckx Y., Loris R. and Van Melderen L.* **Functional dissection of the antitoxin of a three-component type II toxin-antitoxin system**

15h45 *Renzi Francesco, Pablo Manfredi, Manuela Mally, Paul Jenö, Guy R. Cornelis.* **Deglycosylation of host glycoproteins by *C. canimorsus***

16h00 *Mijnendonckx Kristel, P. Monsieurs, N. Leys, J.Mahillon and R. Van Houdt.* **Dynamic genetic adaptation of *Cupriavidus metallidurans* in response to silver toxicity**

16h15 *Messiaen Anne-Sophie, Katrien Forier, Hans Nelis, Jo Demeester, Stefaan C. De Smeth, Kevin Braeckmans and Tom Coenye.* **Single particle tracking in *Burkholderia cepacia* complex biofilms as a tool to study transport of drug-loaded liposomes**

Abstracts of invited lectures

New mechanisms of gene activation by small RNAs

Jörg Vogel

Institute Molecular Infection Biology, University of Würzburg, DE

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While most small RNAs act as post-transcriptional repressors, some also activate gene expression at the mRNA level. Activation typically occurs through regulation of protein synthesis, by an 'anti-antisense mechanism' wherein the small RNA pairs with the 5' region of its target mRNA to liberate a sequestered ribosome binding site, which enhances translational initiation. I will report on a novel positive regulation by Hfq-dependent small RNA wherein stabilization of a *trans*-encoded mRNA is the primary mechanism of gene activation.

We have discovered that the glucose 6-P-responsive small RNA SgrS of *Salmonella* post-transcriptionally activates the synthesis of YigL, a phosphatase required to combat sugar stress. The activation of YigL involves a translation-independent mechanism of suboperonic gene expression control in which the small RNA selectively captures and stabilizes a decay intermediate of the dicistronic *pldB-yigL* mRNA. Intriguingly, the major cellular endoribonuclease RNase E which was previously known to function together with small RNAs to destroy mRNA targets is essential for this process of mRNA activation. The exploitation of and targeted interference with regular RNA turnover described here may constitute a general route for small RNAs to rapidly activate both coding and noncoding genes.

HIV-1 and RNA

Ben Berkhout

Lab. of Experimental Virology, University of Amsterdam, NL

We used deep sequencing technology to map HIV-1 derived small RNAs in infected T cells. This survey provided an in depth overview of possible virus-encoded miRNAs. Surprisingly, we also observed RNA signals from the minus-strand and propose a new mechanism for their generation.RNAi interference (RNAi) has been widely applied to silence genes of interest via the use of short hairpin RNAs (shRNAs). We developed a combinatorial RNAi therapy that prevents HIV-1 escape and thus should provide a durable antiviral effect. The translational track towards an effective gene therapy will be presented, including pre-clinical tests in the humanized mouse model.

ShRNAs are processed by the Dicer nuclease and end up in the Ago2 enzyme for mRNA silencing. We identified a novel shRNA design that triggers an alternative processing route that skips the Dicer step and directs the shRNA molecule directly to Ago2 for processing and subsequent RNAi silencing. We therefore termed these novel reagents AgoshRNAs, which may facilitate the future design of improved and more specific RNAi therapeutics.

Bacterial small RNAs as connectors for regulatory cascades

Susan Gottesman, *Nicholas De Lay, Daniel Schu, Aixia Zhang and Gisela Storz*

Center for Cancer Research, National Cancer Institute, and Eunice Shriver Institute for Child Health and Human Development, Bethesda, MD. USA

The largest class of small regulatory RNAs (sRNAs) in *E. coli* and related bacteria depend on the RNA chaperone Hfq and generally act by pairing to selected target mRNAs, changing both mRNA translation and stability. A single sRNA can act both positively and negatively on different targets, and a given target can be positively or negatively regulated by different sRNAs. The synthesis of the sRNAs is tightly regulated by classical transcriptional regulators, allowing the sRNAs to act as connections between multiple regulatory circuits. For instance, multiple sRNAs regulate bacterial motility. Two of these sRNAs are regulated by EnvZ and OmpR, reinforcing negative regulation by this two-component system. Another sRNA, ArcZ, is negatively regulated by a different two-component system, ArcB/ArcA, not previously described as participating in the regulation of motility.

Hfq helps to stabilize the sRNAs in vivo; in vitro it promotes rapid pairing between the sRNA and its mRNA targets, dependent upon Hfq binding sites on both sRNA and mRNA. In vivo Hfq function was examined using a series of mutations in the *hfq* gene; these were assayed for multiple sRNA:mRNA pairs. While some functions are required for all tested regulation, there are clear differences between how Hfq works in different cases. The results of these provide new insights into how regulation via sRNAs is carried out.

Epigenetic reprogramming of host genes in viral persistence and pathogenesis

Martin J. Allday

Virology, Div. Infectious Diseases, Imperial College London, UK

One of the key questions in the study of mammalian gene regulation is how epigenetic covalent modifications to histones and DNA are initiated and established. These stable, heritable, changes to chromatin are often associated with the repression or silencing of gene transcription and when deregulated can be involved in the development of human diseases such as cancer. The possible impact of extrinsic (environmental) factors, including infectious agents, on epigenetic regulation has attracted considerable recent interest. Here I will review some of the molecular mechanisms underpinning epigenetic marking of chromatin and briefly describe some viruses and bacteria known or thought to induce epigenetic changes in host cells, and how this might contribute to disease. I will then describe how Epstein-Barr virus (EBV) – a human tumour virus – harnesses the host polycomb group protein (PcG) system to epigenetically repress expression of tumour suppressor genes as an effective countermeasure to 'oncogenic stress' triggered by the early stages of infection. This is central to EBV's ability to infect resting B cells and establish latency – and persist life-long *in vivo*, but also contributes to the development of EBV-associated lymphomas.

CRISPR: small RNA guided DNA interference in prokaryotes

Stan J.J. Brouns,

Lab. Microbiology, Wageningen University, Wageningen, NL

An adaptive immune system in prokaryotes called CRISPR (clustered regularly interspaced short palindromic repeats) uses small guide RNAs to neutralize invading viruses and plasmids. In *Escherichia coli*, immunity depends on a ribonucleoprotein complex called Cascade. This protein complex recognizes double-stranded DNA from invaders by forming basepairs between the guide RNA and invader DNA, forming an R-loop. The structure of Cascade displays an unusual seahorse-shape that undergoes conformational changes when it binds target DNA. This recruits the effector nuclease Cas3 which progressively degrades the target DNA in an ATP-dependent manner, neutralizing the virus infection. Apart from giving insight into the function of Cascade, I will also show how viruses are able to escape immunity by point mutagenesis, and unveil how positive feedback from the targeting of an invader provides positive feedback that boosts the immune response.

References

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Swarts DC, Mosterd C, van Passel MW, Brouns SJ. CRISPR interference directs strand specific spacer acquisition. PLoS One. 2012;7(4)

Epigenetic control of Human Immunodeficiency Virus type 1 (HIV-1) postintegration latency: implications for therapeutic interventions

Carine Van Lint

Laboratory of Molecular Virology, University of Brussels (ULB), Gosselies, Belgium

The current antiretroviral therapy HAART is effective and life-prolonging but does not eradicate HIV-1 from infected patients. A reduction of HIV-1 RNA levels in the plasma in HAART-treated individuals to less than 50 copies/ml is frequently achieved but low-level viremia persists as detected by ultrasensitive assays. The sources of this persistent viremia are still not fully understood but could arise from ongoing cycles of residual viral replication and/or from the reactivation of viral expression from latently-infected cells. These latently-infected cells contain stably-integrated, transcriptionally-silent but replication-competent proviruses, thereby representing latent reservoirs of HIV-1. They are a permanent source for virus reactivation and could be responsible for the rebound of plasma viral load observed after HAART interruption.

HIV-1 transcriptional repression is crucial to the establishment and maintenance of postintegration latency. Several elements contribute to HIV-1 transcriptional repression including: 1) the site of integration and mechanisms of transcriptional interference, 2) the absence of crucial inducible host transcription factors, 3) the presence of transcriptional repressors, 4) the nucleosomal organization of the HIV-1 promoter, 5) the epigenetic control of the HIV-1 promoter (histone posttranslational modifications, such as histone acetylation and methylation, and DNA methylation), 6) the sequestration in an inactive form of the cellular Positive Transcription Elongation Factor b (P-TEFb), composed of cyclin-dependent kinase 9 (CDK9) and human cyclin T1, 7) the absence of the viral transactivator Tat, which promotes transcription via recruitment to the HIV-1 promoter of P-TEFb, histone-modifying enzymes and ATP-dependent chromatin-remodeling complexes required for nucleosomal disruption and transcriptional processivity. The involvement of these elements in postintegration latency depends on the status of activation and differentiation of the heterogeneous $CD4^+$ T cell populations hosting the HIV-1 reservoirs.

Further understanding of the epigenetic and non-epigenetic mechanisms regulating HIV-1 latency and reactivation from latency should help devise novel strategies to eliminate latent HIV-1 reservoirs or to restrict the latent pool to a size bearable by the host immune system.

Posters list, group and location

V1: DNA virures
V2: RNA viruses
B1: Bacteria, taxonomy, tools and methods
B2: Bacteria, biofilms and virulence
B3: Bacteria and regulation
B4: Bacteria and the environment

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16	*Badri Hanène, Ruddy Wattiez, Natalie Leys* **Effects of ionizing radiation on the photosynthetic cyanobacterium *Arthrospira sp.* PCC 8005, used for oxygen and food production in space**	**B3**
17	*Barbier T, Steinhauser-Durand M-C, Plovier H, Zuniga-Ripa A, Nicolas C, Bolten CJ, Wittman C, Moriyon-Uria I and Letesson J-J* **Unravelling the metabolic functionality of an intracellular pathogen, Brucella.**	**B2**
18	*Beaufay F., X. De Bolle & R. Hallez* **Metabolic control of asymmetric cell division in *Caulobacter crescentus***	**B3**
19	*Boutard Bérengère, Sophie Vankerckhove, Nicolas Markine-Goriaynoff, Azeddine Bentaib, Michaël Sarlet, Rudy Wattiez, Pierre Leprince, Daniel Desmecht, Grant McFadden, Alain Vanderplasschen and Laurent Gillet.* **Post-translational modification of the Myxoma virus chemokine-binding protein M-T7 by a virally encoded α-2,3-sialyltransferase.**	**V2**
20	*Brackman Gilles, Martijn Risseeuw, Shari Celen, Hans J. Nelis, Serge Van Calenbergh, Tom Coenye*. **Synthesis and evaluation of the quorum sensing inhibitory effect of substituted triazolyldihydrofuranones**	**B2**
21	*Cenens William, Mehari Mebrhatu, Angella Makumi, Rob Lavigne, Rob Van Houdt, François Taddei, Abram Aertsen.* **Looking into the carrier state of phage P22 in *Salmonella* Typhimurium**	**B1**
22	*Cherradi Y., L. Schiavolin, S. Moussa, L. Biskri, A.Allaoui, and A. Botteaux.* **Interplay between Rod and Gatekeeper in Controlling Substrate Specificity of the *Shigella* Type III Secretion System**	**B2**
23	*Claes Sandra, Geoffrey Férir and Dominique Schols.* **The live cell image JuLI™ analyser as a useful tool for studying HIV cell-cell transmission**	**V2**
24	*Clais Sofie, Wim Teughels, Marc Quirynen, Anne-Marie Lambeir, Gaëlle Boulet, Louis Maes and Paul Cos*. **Characterisation of clinical *Porphyromonas gingivalis* isolates to determine the significance of DPPIV as a virulence factor.**	**B2**
25	*Condori Sandra, Natalie Leys, Ruddy Wattiez. Felice Mastroleo.* **First insight into the cell-to-cell communication system of the life support bacterium *Rhodospirillum rubrum* S1H**	**B4**
26	*De Cock Aurélie, Thomas Michiels.* **Impact of cellular microRNA on Theiler's virus replication**	**V2**
27	*Dedeurwaerder Annelike, Lowiese M. Desmarets, Dominique A.J. Olyslaegers, Ben L.Vermeulen, Hannah L. Dewerchin, Hans J. Nauwynck.* **The role of accessory proteins in the replication of feline infectious peritonitis virus in peripheral blood monocytes**	**V2**
28	*Deghelt M., Sternon, J.-F., Letesson, J.-J. & De Bolle X.* **Made to invade.**	**B2**

	Characterization of the chromosomes replication stages of the infectious *Brucella abortus* cell type	
29	*De Maeyer Dries, Joris Renkens, Luc De Raedt, Kathleen Marchal.* **Network-based data integration for microbial systems biology**	**B1**
30	*Lowiese M.B. Desmarets, Sebastiaan Theuns, Dominique A.J. Olyslaegers, Annelike Dedeurwaerder, Ben L. Vermeulen, Hannah L. Dewerchin and Hans J. Nauwynck.* **Contrasting replication kinetics of feline enteric coronavirus and feline infectious peritonitis virus in newly established feline intestinal epithelial cell cultures**	**V2**
31	*Dingemans Jozef , Aurélie Crabbé, Rob van Houdt, Pierre Cornelis, Anne Malfroot.* ***Pseudomonas aeruginosa*** **adapts to the cystic fibrosis lung environment by acquiring iron via pyoverdine-independent mechanisms**	**B2**
32	*Elfaras Ameer & Pierre Cornelis***. The Ferripyochelin receptor, FptA, serves as a receptor for pyocin S5**	**B3**
33	*Férir Geoffrey , Anne Hänchen, Bart Hoorelbeke, Katrien O. François, Dana Huskens, Frank Dettner, Roderich D. Süssmuth and Dominique Schols.* **Feglymycin, a unique natural bacterial peptide isolated from *Streptomyces sp.* DSM11171, potently inhibits HIV replication by interfering with the CD4/gp120 interaction**	**V2**
34	*François Sylvie, Sarah Vidick, Mickaël Sarlet, Daniel Desmecht, Pierre-Vincent Drion, Philip G Stevenson, Alain Vanderplasschen and Laurent Gillet***. Illumination of Murid Herpesvirus 4 cycle reveals a sexual transmission route in laboratory mice**	**V1**
35	*Gennart Isabelle, Carole Mignion, Damien Coupeau, Sylvie Laurent, Denis Rasschaert, Benoît Muylkens.* **Role of DNA methylation during Marek's disease virus latency**	**V1**
36	*Ghosh Anirban, Mehari Tesfazgi Mebrhatu, Susana Rocha, Kristof Vanoirbeek, Johan Hofkens, and Abram Aertsen.* **Impact and and dynamics of the Mrr Type IV restriction endonuclease of *Escherichia coli***	**B4**
37	*Ghyselinck Jonas, Siva L. S. Velivelli, Kim Heylen, Eileen O'Herlihy, Paul De Vos, Barbara Doyle Prestwich.* **Bioprospecting in Potato Fields in the Central Andean Highlands: The Selection of Biocontrol Agents with Plant Growth-Promoting Properties**	**B1**
38	*Goeders Nathalie, Pierre Drèze and Laurence Van Melderen.* **Relaxed cleavage specificity within the RelE toxin family**	**B4**
39	*Hanot Mambres Delphine, Jean-Jacques Letesson, Eric Muraille.* **Characterisation of the relationship between *Brucella melitensis* and the protective immunity in a mouse model after an intranasal infection**	**B2**
40	*Hildebrand Falk, Anh Thi Loan Nguyen, Brigitta Brinkman, Roberto Garcia Yunta, Peter Vandenabeele, Adrian Liston and Jeroen Raes.* **Inflammation-associated enterotypes, host genotype, cage and interindividual effects drive gut microbiota variation in common laboratory mice**	**B2**
41	*Hoefman S, Vandamme P. , De Vos P. And Heylen K.* **Methanotrophic Resource Management: Rapid isolation, miniaturized characterization and long-term preservation of fast-growing methane-oxidizing bacteria**	**B1**
42	*Huskens Dana, Geoffrey Férir, Kenneth E. Palmer and Dominique Schols.* **Griffithsin, a lectin isolated from *Griffithsia sp.*, is endowed with potent and broad spectrum anti-HIV activity and has an outstanding safety profile**	**V2**

43	*Ingelbrecht Ivan L, Francis Moonan and T Erik Mirkov.* **Identifying plant proteins involved in posttranscriptional gene regulation using HC-Pro, a viral suppressor of posttranscriptional gene silencing**	**B2**
44	*Jacob Thary, Van den Broeke Céline, van Troys Marleen, Waterschoot Davy, Ampe Christophe and Favoreel Herman.* **The US3 kinase of pseudorabies virus leads to activation of the actin regulator cofilin to induce actin cytoskeleton changes.**	**V1**
45	*Jové T., Sterckx Y., Loris R. and Van Melderen L.* **Functional dissection of the antitoxin of a three-component type II toxin-antitoxin system**	**B3**
46	*Kerstens Monique, Gaëlle Boulet[1], Sofie Clais, Tessa Horemans, Mario Hellings, Liesbeth Voeten, Isabel Pintelon, Peter Delputte, Louis Maes, Paul Cos.* **Quantification of Candida albicans by flow cytometry using TO-PRO®-3 iodide as a single-stain viability dye**	**B1**
47	*Kreit Marguerite and Michiels Thomas.* **Antiviral activity of a murine Apolipoprotein L**	**V2**
48	*Latif Bilal Muhammad, Vanderplasschen Alain, and Gillet Laurent.* **The NOD-like receptor homolog encoded by Murid Herpesvirus-4 orf63 is important but not essential for virus replication *in vitro***	**V1**
49	*Lété Céline, Bénédicte Machiels, Nicolas Markine-Goriaynoff, Minoru Fukuda, Alain Vanderplasschen and Laurent Gillet.* **Bovine herpesvirus 4 modulates its beta-1,6-N-acetylglucosaminyltransferase activity through alternative splicing**	**V1**
50	*Liu Han, Daniel Charlier, Eveline Peeters.* **Characterization of a beta-alanine responsive transcription regulator (BarR) from *Sulfolobus acidocaldarius***	**B3**
51	*Machiels Bénédicte, Stevenson Philip, Vanderplasschen Alain, Gillet Laurent.* **Alternative splicing switches tropism of a gammaherpesvirus**	**V1**
52	*Materne Philippe and Damien Hermand.* **Study of the role and regulation of the RNA Polymerase II carboxy-terminal domain phosphorylation in the induction of sexual differentiation in the yeast *S. pombe*.**	**B3**
53	*Meghraoui Alaeddine, Lionel Schiavolin and Abdelmounaaim Allaoui.* **Mutational analysis brings new insights into the multifunction of the Type III secretion needle tip protein IpaD of *Shigella flexneri***	**B2**
54	*Messiaen Anne-Sophie, Katrien Forier, Hans Nelis, Jo Demeester, Stefaan C. De Smeth, Kevin Braeckmans and Tom Coenye.* **Single particle tracking in *Burkholderia cepacia* complex biofilms as a tool to study transport of drug-loaded liposomes**	**B1**
55	*Gokhan Metan, Fatma Sariguzel, Bulent Sumerkan, Tanny van der Reijden, Lenie Dijkshoorn.* **Clonal diversity and high prevalence of OXA-58 among *Acinetobacter baumannii* isolates from blood cultures in a tertiary care centre in Turkey**	**B2**
56	*Michaux Charlotte, Axel Hartke, Nicolas Verneuil and Jean-Christophe Giard.* **CspR is a cold-shock RNA-binding protein involve in the long term survival and the virulence of *Enterococcus faecalis***	**B2**
57	*Mijnendonckx K., P. Monsieurs, N. Leys, J.Mahillon and R. Van Houdt.* **Dynamic genetic adaptation of *Cupriavidus metallidurans* in response to silver toxicity**	**B4**
58	*New Aaron M., Bram Cerulus, Kevin J. Verstrepen.* **Epigenetic inheritance in a simple gene circuit gives rise to "stochastic sensing"**	**B1**
59	*Nguyen Le Minh Phu, Daniel Charlier.* **The trigger enzyme PepA (aminopeptidase A) is a transcriptional repressor and induces positive**	**B3**

	supercoiling	
60	*Ooms Karen, Hanne Van Gorp, Inge Hoebeke, Hans Nauwynck and Peter Delputte.* **Porcine Sialoadhesin (CD169/Siglec-1) Is an Endocytic Receptor that Allows Targeted Delivery of Toxins and Antigens to Macrophages**	**V2**
61	*Peleg Anton Y., Anna de Breij, Mark D. Adams, Gustavo M. Cerqueira, Stefano Mocali, Marco Galardini, Peter H. Nibbering, Ashlee M. Earl, Doyle V. Ward, David L. Paterson, Harald Seifert, Lenie Dijkshoorn.* **The Success of Acinetobacter Species; Genetic, Metabolic and Virulence Attributes**	**B2**
62	*Renzi Francesco, Pablo Manfredi, Manuela Mally, Paul Jenö, Guy R. Cornelis.* **Deglycosylation of host glycoproteins by *C. canimorsus***	**B2**
63	*Ronneau S., F. Beaufay, X. De Bolle & R. Hallez.* **Molecular mechanism mediating the G1 block of *Caulobacter* swarmer cells in response to Nitrogen starvation**	**B3**
64	*Roosa S., B. Cornu, F. Van Vliet, R. Wattiez, D. Gillian, C. Vander Wauven.* **Resistant bacteria from metal-contaminated marine and river sediments**	**B4**
65	*Roovers Martine, Yamina Oudjama, Jonathan Somme, Marcus Fislage, Wim Versées, Janusz M. Bujnicki and Louis Droogmans.* **The open reading frame TTC1157 of *Thermus thermophilus* HB27 encodes the methyltransferase forming N^2-methylguanosine at position 6 in tRNA**	**B4**
66	*Rubin Sergio dC, Ivar Zekker, Chiara Destino, Enea G. Di Domenico.* **Post-transcriptional Control and Inductive Genetic Regulation in an 'almost' Prokaryotic cell**	**B3**
67	*Sanz Javier, Laurence Van Melderen.* **Characterization of AtaT toxin from the AtaR-AtaT toxin-antitoxin system: a new toxin family with an N-acetyltransferase domain**	**B3**
68	*Seyll E., S. Zedek S., A. Polomé, S. Bousbata, D. Schneider and L. Van Melderen.* **Experimental evolution with a global regulator mutant in *E. coli***	**B3**
69	*Schiavolin Lionel, Armelle Phalipon, Farida Nato, Philippe Sansonetti and Abdelmounaaïm Allaoui.* **Identification of monoclonal antibodies to the type III secretion needle tip IpaD that neutralize *Shigella* virulence both *in vitro* and *in vivo***	**B2**
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80	*Johan Vande Voorde, Federico Gago, Magdalena Slusarczyk, Christopher McGuigan, Sandra Liekens and Jan Balzarini.* **Optimizing antiviral and anticancer chemotherapy by the development of mycoplasma-insensitive prodrugs**	**V1**
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83	*Vairo SCT, W Van den Broeck, A Scagliarini, HJ Nauwynck.* **Replication characteristics of equine arteritis virus *08P178* in equine polarized upper respiratory tract mucosal explants**	**V2**
84	*Vassart Amelia, Eveline Peeters and Daniel Charlier.* **Regulating the glutamine synthase operon by Sa-Lrp, a glutamine-dependent Lrp-like regulator in *Sulfolobus acidocaldarius***	**B3**
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86	*Vercammen Ken, Qing Wei, Daniel Charlier and Pierre Cornelis.* **The PA4203 *Pseudomonas aeruginosa* LysR-type transcriptional regulator: determination of its regulon and of its DNA binding sites**	**B3**
87	*Vermeulen, B., Desmarets L., Dedeurwaerder, A., Olyslaegers, D., Dewerchin, H. and Nauwynck, H.* **The role of regulatory T cells during infection with feline infectious peritonitis virus**	**V2**
88	*Vidick Sarah, Leonor Palmeira, Beate Neumann, Christian Tischer, Rainer Pepperkok, Sylvie Francois, Alain Vanderplasschen, Laurent Gillet.* **Genome-wide RNAi screening identifies cellular genes involved in Murid Herpesvirus 4 cycle**	**V1**
89	*Wouters Katinka, Moors Hugo, Patrick Boven, Natalie Leys.* **Boom Clay pore water, home of a diverse microbial community**	**B4**
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91	*Yang Xiaoyun, Katrien Forier, Lennert Steukers, Kevin Braeckmans, Sarah Glorieux, Hans Nauwynck.* **Immobilization of Pseudorabies Virus in**	**V1**

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92	*Lumeng Ye, Sandra Cosijns, Sandra Matthijs, Steven Ballet, and Pierre Cornelis.* **Novel Antimicrobial Compounds from *Pseudomonas* Secondary Metabolites**	**B2**
93	*Zaluga J.,Heylen K., Van Vaerenbergh, Maes M. and De Vos P.* **Phylogenetic analysis and polyphasic characterization of *Clavibacter michiganensis* population isolated from tomato seeds**	**B1**

Poster Abstracts

Effects of ionizing radiation on the photosynthetic cyanobacterium *Arthrospira sp.* PCC 8005, used for oxygen and food production in space

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Arthrospira (Spirulina) is a edible photosynthetic filamentous cyanobacterium which is able to convert sunlight into cellular energy, to release oxygen from water, to fix carbon dioxide from air, and to remove nitrate from water. For these traits, the cyanobacterium *Arthrospira sp.* PCC 8005 was selected by the European Space Agency (ESA) as part of a life support system called MELiSSA aimed to recycle oxygen, water and food from waste in space capsules in the future. However, during long term manned spatial mission, the bacterium will be exposed to harmful cosmic radiation, and its high nutritive value and efficient oxygen production by photosynthesis may be jeopardized.
Our goal is to investigate in detail the response of *Arthrospira sp*. PCC 8005 to ionizing radiation.

After exposure to acute high doses of ^{60}Co gamma radiation, *Arthrospira* filaments were still able to restart photoautotrophic growth and proliferate normally, even after exposure to 6400 Gy the highest dose of gamma radiation tested. The assessment of the PSII quantum yield via chlorophyll fluorescence immediately after irradiation, showed that the photosystem activity decreased significantly from doses above 3200 Gy, but was still partially intact at 6400 Gy. Irradiated cells were still able to produce oxygen. At the molecular level, no significant impact of irradiation on DNA could be found based on our assessment of genomic DNA integrity on agarose gel electrophoresis immediately after irradiation. Using Inductively Coupled Plasma Mass Spectrometry, it was found that *Arthrospira sp.* PCC 8005 cells have relative high manganese to iron concentrations. It has been reported that radiation-resistant bacterial cells maintain high intracellular manganese and low iron levels, compared with radiation-sensitive bacteria. This high manganese content may protect their repair proteins to oxidative inactivation.

Our results thus show that *Arthrospira* sp. PCC8005 is highly tolerant to ^{60}Co gamma radiation without showing major cellular or molecular damage, and still capable of active photosynthetic growth and oxygen production after acute exposure to 6400 Gy. This suggests it is indeed a suitable candidate for life support in space flight.
Via additional DNA, RNA, protein and pigment analysis of the irradiated cells we are curently further investigating the exceptional radiation protective properties of this bacterium, which can be of interest for its use as human food supplement in space, and on Earth.

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Unravelling the metabolic functionality of an intracellular pathogen, *Brucella*.

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Brucella spp. are Gram negative coccobacilli responsible for chronic infections of animals and humans. During their co-evolution with their host, *Brucella* adapted to their preferential niches inside the organism and host cells. In contrast to the increasing knowledge on the molecular strategies used by this pathogen to be virulent, little is known on its metabolism closely linked though to the virulence of the bacteria. How these bacteria adapt their metabolism either *in vitro* or to the intra-host conditions and what are their nutritional needs during the infection? Two basic questions which remain however without answer.

According to genomic data and biochemical information, *Brucella* disposes of a complete pentose-phosphate pathway (PPP) and an incomplete classical glycolysis (Embden-Meyerhof-Parnas pathway, EMP), as these bacteria seem to lack phosphofructokinase. Moreover, *Brucellae* have the two genes coding for the enzymes of the Entner-Doudoroff pathway (gluconate-6-phosphate dehydratase and 2-keto-3-deoxygluconate aldolase), but no *in vitro* activity was found for the first enzyme (Robertson and McCullough, 1968). This is also true for several anaplerotic reactions. These information are useful to define the potential "architecture" of the central metabolic network of *Brucellae*, but they do not provide any evidence neither on how the network works nor on its adaptation potential.

The functionality of the central metabolism is currently investigated in *B. abortus 2308* and *B. suis* biovar 5. To do so, the growth of key metabolic mutants is characterized *in vitro* in a chemically defined medium supplemented with ^{12}C and/or ^{13}C carbon sources. The behavior of these mutants in cellular and murine models of infection is also analyzed in order to assess the relevancy of the different pathways during the infection process. In this manner, it has been demonstrated for the first time that the Entner-Doudoroff pathway is indeed active in *Brucella* but also critical for the bacteria to grow on glucose in defined medium. The importance of this pathway among others as well as the importance of glucose as a carbon source during infection is currently investigated.

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Metabolic control of asymmetric cell division in *Caulobacter crescentus*

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Cytokinesis is a critical cell cycle step tightly controlled by internal and environmental signals. In bacteria, FtsZ polymerizes as a Z-ring that recruits the division machinery at the future division site. As the earliest event of cytokinesis, FtsZ is a prime target for mechanisms regulating cell division processes. Here we show that *Caulobacter crescentus* uses a metabolic enzyme to coordinate asymmetric cell division with growth. Indeed, a NAD-specific glutamate dehydrogenase (GdhZ) was isolated as a physical partner of FtsZ in a yeast two-hybrid screen. Glutamate dehydrogenase catalyzes the conversion of glutamate into $NH4^+$ and α-ketoglutarate, thereby linking nitrogen and carbon cycles. We found that GdhZ co-localizes with FtsZ at midcell. Moreover, cytokinesis is strongly delayed in a Δ*gdhZ* mutant while the other cell cycle phases are not affected. Interestingly phenotypes displayed by the Δ*gdhZ* mutant can be modulated by the carbon source used by *C. crescentus.* We also showed that abundance of GdhZ varies according to the cell cycle phase and carbon source metabolized. Altogether, our results illustrate how bacteria can adapt to nutrient availability fluctuations by adjusting cell cycle parameters.

Post-translational modification of the Myxoma virus chemokine-binding protein M-T7 by a virally encoded α-2,3-sialyltransferase.

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Myxoma virus is a pathogenic Poxvirus that induces a lethal disease called myxomatosis in European rabbits. It is one of the very rare viruses that encodes an α-2,3-sialyltransferase that transfers sialic acid to glycoproteins and glycolipids. Very little information is available about the role played by this glycosyltransferase in the pathogenesis of the infection. Previous experiments showed that the enzyme, encoded by the M138L gene, is not essential for virus replication *in vitro* but is important in the *in vivo* pathogenesis of myxomatosis. The objective of this study was the identification of the viral and cellular proteins modified by the α-2,3-sialyltransferase. A two-dimensional differential gel electrophoresis revealed that a target of the enzyme is the viral chemokine-binding protein M-T7. This information was confirmed by western blots. Moreover, a mass spectrometry glycan analysis of purified M-T7 proteins revealed precisely the nature of the modifications introduced by the M138L gene product. As M-T7 is a known important virulence factor of the virus, the difference in M-T7 sialylation could therefore be responsible of the *in vivo* attenuation observed during the infection with the M138L knockout virus. In the future, these results could help us to better understand the pathogeny of myxomatosis in European rabbits. Moreover, they could also help us to decipher the importance of glycans in host-pathogens interactions.

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Synthesis and evaluation of the quorum sensing inhibitory effect of substituted triazolyldihydrofuranones

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Bacteria monitor their population density using communication systems, referred to as quorum sensing (QS). Several pathogenic bacteria use QS for the control of virulence, biofilm formation and maturation, and interfering with QS systems has been proposed as a novel strategy to combat bacterial infections. In the present study, we investigated whether substitution of the central amide by a triazol function would affect QS and biofilm formation. Acylhomoserine lactone (AHL) analogues in which the amide function was replaced by a triazol function were synthesised in two steps starting from alfa-bromo-gamma-butyrolacton. In addition, the acyl-chain length was varied in order to obtain more active inhibitors. Their effect on QS was evaluated using biosensor strains *E. coli* JB523 (responsive to C6-AHL) and *P. aeruginosa* QSIS2 (responsive to 3-oxo-C12-AHL). Modulation of biofilm formation was assessed using *B. cenocepacia* J2315 and *P. aeruginosa* PAO1. The compounds showed selectivity between two different AHL QS systems. Triazoldihydrofuranones, in which the acyl-side chain best resembled the acyl-chain of the native AHL molecule exhibited significant QS agonistic and antagonistic activities. Replacing the acyl-side chain by a phenyl-containing moiety resulted in active inhibitors of QS. The most active compounds showed biofilm inhibitory as well as biofilm eradicating activities in both test organisms. Several AHL analogues in which the amide function was replaced by a triazol function were capable of blocking C6-AHL-based QS, while only the short chain compounds were capable of activating QS in this system. In addition, only long acyl-chain compounds affected 3-oxo-C12-AHL-based QS. Altering the acyl-side chain by phenyl-compounds resulted in active modulators of QS in both systems. Finally, the most active QSI exhibited strong inhibitory and eradicating activities against *B. cenocepacia* and *P. aeruginosa* biofilms.

Looking into the carrier state of phage P22 in *Salmonella* Typhimurium

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The state in which the phage genome exists, nor as a stable lysogen nor as a lytic propagating phage, is often referred to as a pseudolysogenic- or phage carrier state. This specific state is proposed to be of high ecological importance in natural ecosystems as it would provide a stable phage-host interplay by which large phage populations can be maintained. However, the nature and physiological impact of the phage-carrier state so far remains obscure. By using genetics and cell biology with fluorescent markers, we aim to follow up the dynamics of cells and phages upon the infection of *Salmonella* Typhimurium with phage P22.

Presented by: **Cenens, William**

Interplay between rod and gatekeeper in controlling substrate specificity of the *Shigella* Type III secretion system

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Bacterial pathogens have evolved by developing sophisticated ways to invade and/or to subvert the defenses of their host by using the type III secretion apparatus (T3SA) to inject effector proteins into the eukaryotic cells cytoplasm. *Shigella*, the causative agent of shigellosis, which causes 1.1 million deaths each year, uses this apparatus to transport virulence effectors in a hierarchical and temporal manner. Mechanisms controlling the hierarchical addressing of needle subunits, translocators and effectors to the T3S apparatus (T3SA) are still poorly understood. In the *Shigella's mxiC* mutant, early and late effectors are constitutively secreted, indicating that the wild-type strain possesses a mechanism that sequesters effectors within the cytoplasm prior to T3S induction. The activation signal is transmitted to MxiC along the needle and the T3SA base. However, the molecules and molecular pathways involved in linking the needle and MxiC are not known. In the present study, we shed light on this mechanism by demonstrating, for the first time, a molecular interaction between MxiC and the rod component MxiI. Our results suggest that this interaction, in the absence of T3S induction, forms a plug at the T3SA entry gate leading to effectors sequestration within the bacterial cytoplasm. Upon T3S induction with Congo red (CR), we found that MxiI-MxiC complex dissociation facilitates the switch in secretion from translocators to effectors. Mutational analysis identified an $MxiC^{F206S}$ variant, unable to interact with MxiI, which exhibits a constitutive secretion phenotype although it remains responsive to CR induction. We also identified the $mxiI^{Q67A}$ mutant that secretes translocators but not effectors, a phenotype that was suppressed by co-expression of the $MxiC^{F206S}$ variant. We have also demonstrated the interaction between MxiI and MxiC homologous in various bacterial species and subsequently investigated its impact on Yop secretion by *Yersinia enterocolitica*. Lastly, MxiC was shown to regulate secretion of the translocators (IpaB and IpaC) by interacting with their common chaperone IpgC. In conclusion, the study presented here supports the existence of a widely conserved T3S mechanism that regulates the switch to effectors secretion.

The live cell image JuLI™ analyser as a useful tool for studying HIV cell-cell transmission

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HIV spreads by cell-free virions but also especially efficiently through cell-cell contacts. These cell-cell HIV transmission pathways have also been suggested as a mechanism of viral escape to neutralizing antibodies. High-mannose N-linked glycans on the viral envelope gp120 are specifically recognized by the class of carbohydrate-binding agents (CBAs). The CBA, griffithsin (GRFT), showed very potent and broad-spectrum anti-HIV-1 and anti- HIV-2 activity in viral replication assays and outstanding safety and efficacy profile.

Here, we evaluated the activity of GRFT in two different HIV cell-cell transmission models. The first model is mixing persistently HIV-1-infected T cells with non-infected $CD4^+$ target T cells and the second cellular viral transmission model is mixing HIV captured on DC-$SIGN^+$ cells and $CD4^+$ T cells. Pictures of the HIV-induced giant cell formations were generated in real time using the live cell (fluorescent and bright-field imaging) viewer JuLI™ (International Medical Products S.A. , Belgium). Cell cultures were also evaluated by multi-parameter flow cytometry to determine the percentages of $CD4^+$ target T cell destruction and p24 HIV-1 Ag ELISA for measuring viral replication.

When mixing persistently HIV-1-infected T cells with non-infected $CD4^+$ target T cells, giant cells will appear after 8 h of co-culture and after 20-24 h the majority of the uninfected T cells are destroyed. This can be visualized in real time in a CO_2 cell incubator with the live cell image viewer JuLI™. In addition, $CD4^+$ target T cells can also be stained using green fluorescent CellVue® Jade (Polysciences Inc., Germany) according to manufacturer's guidelines to visualize the fusion of the $CD4^+$ target T cells. GRFT demonstrated to be an extreme potent inhibitor in the giant cell formation assay between HIV-infected T cells and non-infected $CD4^+$ target T cells. GRFT also inhibits profoundly viral capture and transmission through the DC-SIGN-mediated pathway and subsequent cellular destruction of the $CD4^+$ target T cells (See also Presentation Huskens et al., BSM 2012). These cell-cell HIV transmission models demonstrate the antiviral potency of GRFT and support further clinical investigation of GRFT in pre-exposure prophylaxis in the context of HIV transmission.

The JuLI™ viewer proved to be a simple and convenient operating cell analyzer and can be very useful in monitoring and evaluating compounds in HIV cell-cell transmission assays.

Characterisation of clinical *Porphyromonas gingivalis* isolates to determine the significance of DPPIV as a virulence factor.

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Resistance to antibiotics is an increasing concern for the public health. A possible innovative therapy to overcome this problem and which is being widely investigated, is the inhibition of virulence factors.

Porphyromonas gingivalis is a member of the subgingival plaque microbiota and a key pathogen in the development of adult periodontitis. This Gram-negative, anaerobic bacterium produces proteolytic enzymes that cause tissue destruction and modulate the immune system. Dipeptidyl peptidase IV (DPPIV) is a serine protease that removes X-Pro or X-Ala dipeptides from the N-terminal end of polypeptide chains. It is involved in the degradation of the connective tissue and might be an interesting target for virulence inhibition.

This study aims to correlate DPPIV-activity with biofilm formation and *in vivo* pathogenicity of clinical *P. gingivalis* isolates to evaluate DPPIV as a target for virulence inhibition.

A method to fractionate and isolate DPPIV was optimized to determine the DPPIV-activity of five clinical isolates with a different capsular type (K1-K5)[1,2] and three reference strains (W50, W83 and ATCC 33277). Their capacity to form biofilms in a microtiter plate was quantified after staining with 0,01% crystal violet. *In vivo* pathogenicity was evaluated by daily assessment of the formation and location of skin lesions in BALB/c mice after dorsal, subcutaneous injection of a bacterial suspension.

As stated in literature DPPIV is membrane-associated and consequently the highest activities were found in the membrane fractions. The capsular types K4 and K5 showed the highest DPPIV-activity while K1, K2, K3 and the reference strains showed 10 to 100 times lower activities. Moreover, DPPIV-activity was associated with the formation of *in vivo* abscesses. Only for K4 and K5 significant abscesses were obtained. ATCC 33277 showed the best capacity to form biofilms, followed by K4 and K5. Capsule type 3 and the reference strain W50 also formed biofilms but to a lesser extent.

In conclusion, our results suggest that DPPIV might be an interesting therapeutic target to inhibit the pathogenicity of *P. gingivalis*. Further research should focus on the evaluation of DPPIV-inhibitors in *P. gingivalis* infection models.

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First insight into the cell-to-cell communication system of the life support bacterium *Rhodospirillum rubrum* S1H

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MELiSSA (Micro-Ecological Life Support System Alternative) has been conceived as a micro-organisms and higher plants system based on bacterial waste conversion for long haul space flights. *Rhodospirillum rubrum* S1H colonizes compartment II and metabolizes mainly volatile fatty acids coming from compartment I, under light anaerobic conditions (LAN). Previous work reported that continuous culture of the bacterium in photobioreactor could lead to thick biofilm formation, ending up with a complete stop of the bioreactor. For other bacteria, it has been shown that biofilm formation is regulated by cell-to-cell communication or Quorum sensing (QS). QS is a mechanism where gram-negative bacteria can communicate with each other by producing and sensing certain concentration and types of *N*-acyl homoserine lactone (HSL). It allows the bacteria to jointly control the expression of target genes and mount a co-operative response (e.g biofilm maturation, regulation of virulence, bioluminescence etc).

The **aim** of this research is to further explore the QS system of *R. rubrum* S1H, specifically in MELiSSA relevant culture conditions, meaning in anaerobic conditions with light as energy source and acetate as carbon source.

Previous studies have shown that *R. rubrum* S1H possess a functional QS system that allows the production of HSLs in Sistrom medium (succinate as carbon source) under dark aerobic and light anaerobic conditions. Here we showed in addition, that *R. rubrum* S1H grown under MELiSSA conditions produces HSLs with acyl chains ranging from 4 to at least 12 carbons in whole-cell extracts. This profile is similar, but nevertheless different than the profiles obtained in the other culture conditions. Recently the genome of *R. rubrum* S1 (parent strain of *R. rubrum* S1H) has been sequenced. *R. rubrum* genome displays a LuxR/LuxI-type quorum-sensing system. This genomic structure will be compared to closely related species including *Rhodobacter sphaeroides* whose quorum sensing system was recently described. Based on these preliminary data the possible consequences for the MELiSSA system will be discussed.

To the best of our knowledge, the present report is the first to document the production of HSLs by *R. rubrum* S1H under light anaerobic conditions in Melissa medium (acetate as carbon source). Currently we are working on the construction of a knockout mutant unable to produce the HSLs signaling molecules that will help us to unravel the QS system of *R. rubrum*.

Impact of cellular microRNA on Theiler's virus replication

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Our study aims at testing the global impact of micro RNA (miRNA) on the replication of RNA viruses and more specifically on Theiler's virus.
To study the influence of miRNA on Theiler's virus replication, we generated a couple of cells that differed in their ability to synthetize miRNA. Dicer is a key enzyme involved in cellular miRNA biosynthesis. This enzyme cleaves the hairpin structure formed by miRNA precursors (pre-miRNA) to release short double stranded RNAs that are incorporated into the RISC complex and act to silence the target mRNA.

To generate Dicer-deficient cells, we started from mice carrying lox sequences around exons 22 and 23 of the *dicer* gene, which encode the catalytic site of the nuclease. Recombination of the lox sites mediated by the CRE recombinase of phage P1 is thus expected to trigger the inactivation of Dicer in such mice.
First, we crossed a female dicer $^{lox/lox}$ mouse with a male dicer $^{+/-}$ mouse and screened embryos for the *dicer* $^{lox/-}$ genotype. From such embryos, we obtained mouse embryonic fibroblast (MEF) cultures. Next, we immortalized one of these populations by expressing large T antigen of SV40 in the cells. Finally, we inactivated the remaining *dicer* allele by transduction of the cells with an adenoviral vector expressing the CRE recombinase. After a cloning step, we selected one cell line that lost the unique dicer allele.
In this way, we obtained equivalent cell lines that only differ by the expression of the *dicer* gene (*dicer* $^{flox/-}$ MEF and *dicer* $^{-/-}$ MEF). Inactivation of Dicer in *dicer* $^{-/-}$ MEF was confirmed by showing that the maturation of miR33 was deficient in these cells.

As an alternative approach to generate the use of adenovirus vectors, we constructed lentiviral vectors co-expressing CRE and mCherry. These vectors will be used to generate mixed populations of *dicer* $^{flox/-}$ and *dicer* $^{-/-}$ cells that can be distinguished by the expression of mCherry. This should allow testing the influence of miRNA in primary cells isolated from *dicer* $^{flox/-}$ mice.

We used *dicer* $^{flox/-}$ MEF and *dicer* $^{-/-}$ MEF to assess the influence of miRNA on TMEV replication. Vesicular stomatitis virus (VSV) was taken as a control as this virus was reported to be inhibited by cellular miRNA [J. Han *et al*, (2007) Immunity 27: 123-134]. Thus *dicer* $^{flox/-}$ MEF and *dicer* $^{-/-}$ MEF were infected with TMEV and VSV derivatives that express GFP and the fluorescence was followed by microscopy or FACS.
In a preliminary experiment, TMEV infected *dicer* $^{flox/-}$ cells as well as *dicer* $^{-/-}$ cells. In contrast, VSV replicated better in *dicer* $^{-/-}$ cells than in *dicer* $^{flox/-}$ cells. This suggests that, in contrast to VSV, TMEV replication was not affected by cellular miRNA. Further experiments are in progress to confirm this observation.

In conclusion, TMEV replication appears to be surprisingly insensitive to the presence of cellular microRNA. If this observation can be confirmed, the mechanisms of this resistance will be analyzed.

The role of accessory proteins in the replication of feline infectious peritonitis virus in peripheral blood monocytes

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The ability to productively infect monocytes/macrophages is the most important difference between the low virulent feline enteric coronavirus (FECV) and the lethal feline infectious peritonitis virus (FIPV). *In vitro*, the replication of FECV in peripheral blood monocytes always drops after 12 hours post inoculation, while FIPV sustains its replication in the monocytes from 45% of the cats. The accessory proteins of feline coronaviruses have been speculated to play a prominent role in virulence as deletions were found to be associated with attenuated viruses. Still, no functions have been ascribed to them. In order to investigate if the accessory proteins of FIPV are important for sustaining its replication in monocytes, replication kinetics were determined for FIPV 79-1146 and its deletion mutants, lacking either accessory protein open reading frame 3abc (FIPV-Δ3), 7ab (FIPV-Δ7) or both (FIPV-Δ3Δ7). Results showed that the deletion mutants FIPV-Δ7 and FIPV-Δ3Δ7 could not maintain their replication, which was in sharp contrast to wt-FIPV. FIPV-Δ3 could still sustain its replication, but the percentage of infected monocytes was always lower compared to wt-FIPV. In conclusion, this study showed that ORF7 is crucial for FIPV replication in monocytes/macrophages, giving an explanation for its importance *in vivo*, its role in the development of FIP and its conservation in field strains. The effect of an ORF3 deletion was less pronounced, indicating only a supportive role of ORF3 encoded proteins during the infection of the *in vivo* target cell by FIPVs.

Made to invade characterization of the chromosomes replication stages of the infectious *Brucella abortus* cell type

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Once entered into its host eukaryotic cell, the pathogenic gram-negative *Brucella abortus* 544 transits quiescently through different compartments before reaching its favorite proliferation niche composed of ER-derived vacuoles. While the factors directly involved in *Brucella*-host interactions are currently characterized, little is known about the basic processes of the bacterial cell cycle (growth, replication, division) and their control along the infection.

We previously identified a specific *Brucella* cell type during the first steps of the infection that seems to be generated once per cell cycle during the unipolar growth of the bacterium. Indeed, an old pole localized histidine kinase called PdhS is not observed in the small cell generated after the asymmetric division. The bacterium can only acquire a polar focus of PdhS after a certain time interpreted as a differentiation step. Remarkably, the PdhS-negative cell is the major cell type observed during the beginning of the infection suggesting that the bacteria could be blocked in a specific cell cycle stage before the differentiation event. We were wondering if those PdhS-negative cells were all standing in the B period (i.e. time between the cell constriction and the chromosomes replication). We thus investigated the replication mechanisms of the two circular chromosomes of *Brucella abortus* in order to generate reliable markers for the initiation of the S phase.

Different strategies were used in order to determine if the two circular chromosomes of *B. abortus* are temporally and spatially coordinated during *in vitro* cultures and infection. We first localized proteins involved in the segregation mechanisms of chromosome I (mCherry-ParB) and chromosome II (YFP-RepB). To our knowledge, we are the firsts to present the localization of a protein involved the Rep segregation machinery. By expressing mCherry-ParB and YFP-RepB in the presence of their putative binding sequences *parS* and *repS* in *E. coli*, we showed that those fusions are able to recognize their respective sequences that are located close to the origin of the *Brucella* chromosome I and II respectively. A representation of the distributions of mCherry-ParB and YFP-RepB in *Brucella abortus* 544 according to cell length shows that the distribution of mCherry-ParB is restricted to the cell poles while the YFP-RepB distribution oscillates more in the cytoplasmic region with the presence of two foci in larger cells. We also observed that the distance between two mCherry-ParB foci increases proportionally with the cell growth whereas the distance between two YFP-RepB foci is smaller than the distance between two mCherry-ParB foci. Those results indicate that YFP-RepB occupies more central regions than mCherry-ParB and seems to occupy the 1/4 and 3/4 zones of the cells. The intracellular distribution of the two chromosomes has also been confirmed by the localization of specific regions on the chromosomes such as the two origins and the two terminators.
The localization of ParB and RepB fusions during the different steps of the infection of *B. abortus* will also be statistically analyzed to determine if the PdhS-negative cells, during the beginning of the infection, are under replication or not. To confirm the results obtained during the infection with the labeling of the origins I and II, we will use BrdU which is an analog of thymidine that can be incorporated into newly synthesized DNA strands for bacteria under S phase. The presence or the absence of the BrdU labeling in the bacteria would indicate if they are replicating their genomes at different time post-infection.

Network-based data integration for microbial systems biology

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Increasingly omics data sets provide information on different molecular layers of the organisms regulatory networks. Data not only cover metabolic networks but increasingly also diverse signaling layers, including the transcriptional, posttranscriptional layers (Sanchez et al.). Integrating these molecular interactions and signaling layers into a single integrated network provides an intuitive way of representing our systems knowledge. Integrated networks also offer a mechanistic scaffold against which functional data can be interrogated or with which functional behavior can be predicted (Cloots et al.). In this work, we show how an integrated signaling network of the bacterial model organism *Escherichia coli* in combination with a dedicated path finding approach can be used to interpret in house generated omics data. We applied our network-based approach to study the mechanisms of acid resistance in E. coli by re-analyzing an expression dataset derived from 27 *E. coli* strains mutated in genes, known to be related to acid resistance.

Cloots L, Marchal K. (2011) Network-based functional modeling of genomics, transcriptomics and metabolism in bacteria, Curr Opin Microbiol, 14(5):599-607

Sánchez-Rodríguez A., Cloots L., Marchal K. (2012) Omics derived networks in bacteria. Curr Bioinform. In press.

Contrasting replication kinetics of feline enteric coronavirus and feline infectious peritonitis virus in newly established feline intestinal epithelial cell cultures

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Feline infectious peritonitis (FIP) is the most feared infectious cause of death in cats, induced by feline infectious peritonitis virus (FIPV). This coronavirus is a virulent mutant of the harmless, ubiquitous feline enteric coronavirus (FECV). Up till now, feline coronavirus (FCoV) research has been hampered by the lack of reliable *in vitro* models to perform comparative studies with FECV and FIPV and to cultivate and titrate field strains. Hence, it is still unclear why FECV and FIPV behave clinically and epidemiologically so different. In this study, infection and growth properties of FECV 79-1683 and FIPV 79-1146 were compared in primary cultures of ileocytes and colonocytes. FECV replicated efficiently in these cells, whereas FIPV did not. In addition, long-term cultures were derived from primary ileocytes and colonocytes by SV40-Tag- and human Telomerase Reverse Transcriptase (hTERT)-induced immortalization. Since comparable infection and growth characteristics were seen as in primary cultures, these cell lines can be considered as reliable models for elucidating the enteric pathogenesis of FCoVs. Interestingly, the continuous cultures were also sensitive to infection with field enteric strains, for which *in vitro* cultivation has never been achieved. In conclusion, a new reliable model for FCoV investigation and growth of enteric field strains was established. It was shown that only FECV fully replicates in intestinal epithelial cells, giving an explanation for the observation that FECV is the main pathotype circulation among cats.

Pseudomonas aeruginosa adapts to the cystic fibrosis lung environment by acquiring iron via pyoverdine-independent mechanisms

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It has been widely accepted that *Pseudomonas aeruginosa* uses the high-affinity iron(III)-chelating molecule pyoverdine to grow in aerobic environments in which the availability of iron (III) is low. This finding has been widely observed in environments like soil, water and iron-poor laboratory media. However, the mechanism by which *Pseudomonas aeruginosa* acquires iron in the cystic fibrosis lung environment is more complicated. Previous studies have shown that pyoverdine (as well as pyochelin) production were not present in sputum samples from a number of cystic fibrosis patients that were infected with *Pseudomonas aeruginosa* (Martin *et al*., 2011). In this study, we confirmed that about 30% of the strains that were isolated out of the cystic fibrosis sputum, did not produce detectable amounts of pyoverdine. Further, it has been observed that in a number of isolates, the *fpvB* gene, encoding the alternative type I ferripyoverdine receptor, harbored large deletions. These findings indicate that the pyoverdine system of iron(III)-uptake may play a less important role in *Pseudomonas aeruginosa* to acquire iron out of the cystic fibrosis lung and alternative iron-uptake systems can be used by this bacterium. Via Real-Time qPCR it was confirmed that the ferrous iron uptake system (represented by the *feoA* and *feoB* genes) and the Phu heme uptake system (represented by the *phuT* gene) are the two major systems that are used by *Pseudomonas aeruginosa* in the sputum of cystic fibrosis patients.

The Ferripyochelin receptor, FptA, serves as a receptor for pyocin S5

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Pyocins are proteins produced by some strains of *Pseudomonas aeruginosa* that are lethal for related strains of *P. aeruginosa*. They gain entry via specific receptors and they could be considered potential alternatives for antibiotics to combat bacteria during infection. The pyocin S5 gene (PA0985) has been cloned, the produced protein was expressed, purified, and the activity tested against the different *P. aeruginosa* strains having different types of ferripyoverdine receptors without showing any correlation with a specific ferripyoverdine receptor. To further identify the receptor, a transposon mutagenesis experiment using the miniTn*phoA*3 transposon was carried out. After pooling of the mutants and exposure to pyocin S5, several colonies growing in the killing zone and resistant to pyocin S5 were obtained and characterized. Sequencing of the neighboring DNA of these mutants revealed that many of them had lost the FptA receptor as a consequence of the transposon insertion in its gene, which indicates that pyocin S5 kills *P. aeruginosa* strains via the FptA ferripyochelin siderophore receptor. This hypothesis was confirmed by the complementation of the transposon mutant with the vector pME6000 containing the *P. aeruginosa fptA* gene. The complemented mutant exhibited a wild-type phenotype and became S5 sensitive again, confirming the involvement of FptA as S5 receptor.

To determine the receptor binding domain (RBD) of the pyocin S5, we constructed two hybrid pyocins with two different *N*-terminal regions from pyocin S5 (which could be the RBD) ligated to the *C*-terminal of pyocin S2 which contains the translocation and the DNase domains. Their killing abilities were investigated on a strain sensitive to both pyocins S2 and S5. These proteins showed killing activities only when the 2nd domain of S5 (aa from 150 to 300) was fused, indicating that the RBD of pyocin S5 is not located at the *N*-terminal of the protein as it is the case for the other S type pyocins.

Further, we tested the killing ability of pyocin S5 on some strains of *Burkholderia cenocepacia,* which produce a very similar FptA receptor, but the results showed that *B. cenocepacia* is unable to uptake pyocin S5.

Feglymycin, a unique natural bacterial peptide isolated from *Streptomyces sp.* DSM11171, potently inhibits HIV replication by interfering with the CD4/gp120 interaction

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Feglymycin (FGM) is a unique natural antibiotic peptide of 13 amino acids (MW, 1900.9 Da) originally isolated from *Streptomyces* sp. DSM11171 (1). It contains mainly unusual amino acids such as four 4-hydroxyphenylglycine (Hpg) and five 3,5-dihydroxyphenylglycine (Dpg). At present, it can be synthesized chemically by a sophisticated peptide synthesis strategy (2). X-ray crystallography showed that FGM could form a double stranded anti-parallel β-helical dimeric structure (3).

Here, we investigated its broad-spectrum anti-HIV activity profile, its mechanism of action and succeeded in generating *in vitro* HIV-1 FGM-resistant virus (4). FGM inhibited cell-free HIV replication, independent of viral co receptor use (CXCR4- or CCR5-using) and genetic subtype, in the lower μM range (EC_{50}: 0.5 – 6.7 μM). Antiviral activity was also observed in the HIV-1 cell-to-cell transfer experiments between persistently T cells and uninfected $CD4^+$ T cells (EC_{50}: 5.4 μM). In addition, FGM also inhibited the DC-SIGN-mediated viral transfer to uninfected $CD4^+$ T cells (EC_{50}: 2.6 μM). Surface plasmon resonance studies indicated a specific interaction with envelope gp120 of HIV-1 (X4 and R5), and virus binding studies showed that FGM acts as a CD4/gp120 binding inhibitor. Alanine-scan mutagenesis showed an important role for L-aspartic acid at position 13 in FGM in its anti-HIV activity. *In vitro* generated FGM-resistant HIV-1 IIIB virus showed 2 unique mutations in gp120 at positions I153L (V2 loop) and K457I (C5 region) and this virus was equally susceptible to various other viral binding/adsorption inhibitors such as T20 and AMD3100. Exceptions were noted with dextran sulfate (9-fold resistance) and cyclotriazadisulfonamide (>15-fold), 2 well-described compounds that interfere with the HIV entry process.

Overall, FGM is a unique and novel prototype bacterial lead peptide with potential for further development of more potent anti-HIV derivatives.

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Illumination of Murid Herpesvirus 4 cycle reveals a sexual transmission route in laboratory mice

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Transmission is a matter of life or death for pathogen lineages and can therefore be considered as the main motor of their evolution. Gammaherpesviruses are archetypal pathogenic persistent viruses which have evolved to be transmitted in presence of specific immune response. Identifying their mode of transmission and their mechanisms of immune evasion is therefore essential to develop prophylactic and therapeutic strategies against these infections. As the known human gammaherpesviruses, Epstein-Barr virus and Kaposi's Sarcoma-associated Herpesvirus, are host-specific and lack a convenient *in vivo* infection model, related animal gammaherpesviruses, such as Murid herpesvirus 4 (MuHV-4), are commonly used as general models of gammaherpesvirus infections *in vivo*. To date, it has however never been possible to monitor viral re-excretion or virus transmission of MuHV-4 in laboratory mice population. In this study, we have used the MHV-68 strain of MuHV-4 associated with global luciferase imaging to investigate potential re-excretion sites of this virus in laboratory mice. This allowed us to identify a genital re-excretion site of MuHV-4 following intranasal infection and latency establishment in female mice. This re-excretion occurred at the external border of the vagina and was dependent on the presence of oestrogens. However, MuHV-4 vaginal re-excretion was not associated with vertical transmission to the litter or with horizontal transmission to female mice. In contrast, we observed efficient virus transmission to naïve males after sexual contact. *In vivo* imaging allowed us to show that MuHV-4 firstly replicated in penis epithelium and *corpus cavernosum* before spread to draining lymph nodes and spleen. All together, those results revealed the first experimental transmission model for MuHV-4 in laboratory mice. In the future, this model could help us to better understand the biology of gammaherpesviruses and could also allow the development of strategies that could prevent the spread of these viruses in natural populations.

Role of DNA methylation during Marek's disease virus latency

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Marek's disease is a lymphoproliferative disease induced by the Marek's disease virus (MDV) infection. During latency, MDV, like other herpesviruses, expresses a limited subset of transcripts. These include two microRNA clusters, protein encoding genes, and long noncoding RNAs (telomerase RNA subunit and Latency Associated transcript). All of these latency products are involved in the maintenance of the latent state and/or in the virus induced-tumorigenesis. Epigenetic modifications (DNA methylation and post-translational histone modification) are more and more studied since these mechanisms were found to be essential constituent in the control of gene expression. In this study DNA methylation was assessed to determine if it regulates promoter activities and gene expression in an *in vitro* model of MDV latent infection. Three promoters encoding genes related with lytic (pp38 and ICP27) and latent (cluster microRNA-9/4) phases were selected and studied during latency phase observed in MDV transformed T lymphocytes. Firstly, these three promoters were analysed to determine DNA methylation pattern through Bisulfite Genomic Sequencing Assay. Pp38 promoter showed a high level of methylated cytosines in CpG dinucleotides while ICP27 and cluster microRNA-9/4 showed a low level of methylated cytosine in CpG. Secondly, ICP27 and cluster microRNA-9/4 promoters activity was evaluated by luciferase assay. Each promoter was tested in two forms: methylated and unmethylated form. Methylation induced a decrease in the promoter activity. Thirdly, pp38, ICP27 and miRNA-4 transcript levels were assessed after a 5-azacytidine treatment on MSB-1 cells. The treatment induced a sharp increase in the expression level of the three genes. Altogether, our data provide with a detailed analysis of the DNA methylation pattern on MDV genome during the latent infection. The luciferase assays and the transcriptional analysis showed strong association between the DNA methylation patterns and the promoter activities.

Impact and and dynamics of the Mrr Type IV restriction endonuclease of *Escherichia coli*

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Bacterial restriction-modification systems consist of a methyltransferase (MTase) that methylates specific sites of the chromosome to protect them from cleavage by its cognate restriction endonuclease (REase). However, a number of solitary MTases (e.g. Dam) exist that seem to have lost their cognate REase and have specialized into epigenetic regulators. Intriguingly, however, also solitary REases exist that have no cognate MTase and bear specificity for modified DNA. The model bacterium *Escherichia coli* has acquired a number of such solitary (or type IV) REases through lateral gene transfer, of which Mrr so far remained most cryptic. Upon investigating its cellular role, we found Mrr endonuclease activity to counteract the establishment of a class of laterally acquired MTases that can be involved in epigenetic regulation. Surprisingly, endonucleolytic activity of Mrr could also be elicited by exposing *E. coli* to hydrostatic pressure stress. A cell biological approach, however, indicates that both modes of activation are fundamentally different from each other.

Bioprospecting in potato fields in the Central Andean Highlands: the selection of biocontrol agents with plant growth-promoting properties

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The Central Andean Highlands are the center of origin of the potato plant (*Solanum tuberosum*). Ages of mutualism between potato plants and soil bacteria in this region support the hypothesis that Andean soils harbor interesting plant growth-promoting (PGP) bacteria. The aim of this study was to isolate rhizobacteria from Andean ecosystems, and to identify those with plant-growth promoting properties. The best performing isolates *in vitro* were determined. In total, 585 bacterial isolates were obtained from the rhizosphere of potato plants in eight fields in the Andes. All isolates were screened for broad spectrum pathogen suppression by analyzing antagonistic properties against *Phytophthora infestans* and *Rhizoctonia solani*. Mechanisms of antagonism were determined and isolates were tested for phosphate solubilization, ACC deaminase activity, NH_3- and IAA-production. Finally, the isolates were tested under growth room conditions for growth-promotion and *R. solani* suppression activities on potato plantlets. Performance was compared with the commercial strain *B. subtilis* FZB24® WG. Isolates were dereplicated with MALDI-TOF MS, and identified with 16S rRNA gene sequencing and Multi Locus Sequence Analysis. Results were used to discuss strain pathogenicity. Ten percent of the isolates were effective antagonists with 56 isolates showing potential for broad spectrum biocontrol. Further testing indicated production of IAA, ACC deaminase, NH_3, HCN and phosphate solubilization activity. Following inoculation of potato plants with the isolates *in vitro*, 23 were associated with plant growth-promotion and/or disease suppression. Ten isolates had a statistically significant impact on test parameters compared to the uninoculated control.

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Relaxed cleavage specificity within the RelE toxin family

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Type II bacterial toxin-antitoxin (TA) systems are usually composed of a toxin that targets an essential cellular process and an antitoxin that antagonizes the deleterious activity of the toxin. Type II toxins were shown to target diverse cellular processes such as DNA replication, translation, peptidoglycan synthesis and formation of cytoskeletal structures. However the majority of the toxins target translation, mainly by cleaving mRNAs although different molecular mechanisms were described.
The RelE/ParE toxin super-family is one of the most widespread and abundant in the prokaryotic kingdom. However most RelE-like toxins for which the molecular mechanism has been investigated in details in vivo belong to Escherichia coli K-12. In order to gain further insight into RelE toxins, six new RelE-like toxins from different phyla were experimentally validated in E. coli.
Five out of the 6 toxins belong to bona fide TA systems. Northern blots show that the 6 toxins cleave test-mRNAs in a translation-dependent manner. In addition, cleavage site analysis using primer extension demonstrates that although belonging to the same super-family, they have specific cleavage patterns with a slight preference for cleavage before purines. Furthermore, for 4 of them, the predominant cleavage sites occur at frequent codons used by their bacterial hosts, which might indicate an adaptation of activity towards their host.

Characterisation of the relationship between *Brucella melitensis* and the protective immunity in a mouse model after an intranasal infection

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Brucella bacteria are facultative intracellular Gram-negative coccobacilli that infect mammals, accidentally humans, and causing brucellosis, one of the most common zoonotic diseases worldwide. Brucellosis leads to abortion and infertility in animals and chronic debilitating disease in humans. Treatment with antibiotics is long, expensive and there is no safe and effective vaccine available to prevent human infection.
Bacteria are highly infectious by oral and aerosol route. In this thesis, we have thus developed an intranasal infectious model in the mouse using a *B. melitenis* strain expressing the mCherry fluorescent tracer. We have established kinetics of infection and analysed the spread of infection in lung, spleen and liver. Thanks to the use of genetically deficient mice we have demonstrated the role of TCD4 cells and the Th1 immune response to control the infection. In order to characterize the phenotype of infected cells, we have developed a new protocole facilitating the achievement of complete cryosections of lung. It allowed us to identify that the first infected cells are alveolar macrophages. We plan to characterize, by immune-histo-fluorescence, cellular recruitments into the lung during chronic infection and the phenotype of reservoir cells for bacteria. Study of deficient mice should allow us to identify essential elements of protective immune response. Finally, we have observed that a primo infection of *B. melitensis* partially protects mice against a challenge. We plan to analyse in details this phenomenon in situ in order to identify mechanisms responsible for this protection. This study will allow us to better understand the in vivo infectious cycle of these bacteria and could improve our ability to generate protective vaccines against *Brucella* and other intracellular bacteria.

Inflammation-associated enterotypes, host genotype, cage and interindividual effects drive gut microbiota variation in common laboratory mice

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Background: Confounder effects in murine gut microbiome models have had limited attention so far. Genetic backgrounds, inter-individual variation and environmental effects were previously assumed to have limited influence on the gut microbiota.

Methods: Here, we investigate the variability of the healthy mouse microbiota in five common lab mouse strains using 16S rDNA pyrosequencing. Results are analyzed with univariate statistics and numerical ecology methods, thus taking confounders into account.

Results: We find initial evidence for richness-driven, strain-independent murine enterotypes that show a striking resemblance to those in human. After enterotype stratification, we find that genotype, caging and inter-individual variation contribute on average 19%, 32% and 46%, respectively, to the variance in the murine gut microbiota composition. Genetic distance correlates positively to microbiota distance, so that genetically similar strains have more similar microbiota. However, specific environments can be linked to the presence of specific OTUs.

Conclusion: This study provides evidence that the two researched confounders can have a strong impact on study results. Therefore we suggest to control for these either in the study design or using statistical methods as described here. The detection of enterotypes in mice suggests a common ecological driver of differences among gut microbiota composition in mammals.

Methanotrophic resource management: rapid isolation, miniaturized characterization and long-term preservation of fast-growing methane-oxidizing bacteria

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INTRODUCTION. Methane-oxidizing bacteria (MOB) have a large potential as a microbial sink for the greenhouse gas methane as well as for biotechnological purposes. However, their application in biotechnology has so far been hampered, in part due to the relative slow growth rate of the available strains. To enhance the availability and to evaluate the applicability of fast-growing MOB, a series of procedures were optimized to allow efficient MOB isolation, purification, characterization and long-term preservation.

METHODS. Aerobic MOB were isolated and dereplicated by $(GTG)_5$ rep-PCR fingerprinting. Representative strains were identified by 16S rRNA gene sequencing. Growth rate and density (OD_{600nm}) were optimized for each strain by rapid medium optimization. Optimized conditions per strain were evaluated for enhanced methane oxidation activity by gas chromatography and protein measurements. Functional gene sequence analysis was performed to further characterize the strains. All strains were subsequently cryopreserved based on results of 10 MOB type strains applying fifteen preservation conditions.

RESULTS. 43 MOB were rapidly isolated by miniaturized extinction culturing. Seventeen representative strains were selected following dereplication. MOB were isolated from the three major types (Ia, Ib, II) and were identified as *Methylocystis* spp., *Methylosinus* sp. or *Methylococcaceae* sp., *Methylomonas* spp., several of which belonged to novel species. Miniaturized screening for growth rate and density allowed rapid medium optimization per strain. Strains cultivated in optimized media showed enhanced methane oxidation activity and grew at similar rates or faster than model MOB type strains at a variety of different temperatures. All fast-growing MOB were successfully cryopreserved with DMSO in a carbon-rich preservation medium and were deposited in a public culture collection.

CONCLUSION. Besides testing the few available model MOB strains, novel MOB can easily be isolated by rapid extinction culturing from enrichments mimicking the conditions of the desired potential application. Subsequently, miniaturized functional screening allows optimal strain selection after which the desired strains can be easily cryopreserved to ensure their availability during the development process.

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Griffithsin, a lectin isolated from *Griffithsia sp.,* is endowed with potent and broad spectrum anti-HIV activity and has an outstanding safety profile

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The most effective approach to halt the HIV epidemic will be establishing effective prevention methods including among others anti-HIV microbicides. Carbohydrate-binding agents (CBAs) are considered as potential microbicide candidates as they specifically interact with the glycans on the heavy glycosylated viral envelope of HIV and block viral entry into its target cells.

Griffithsin (GRFT), an algal lectin originally isolated from *Griffithsia sp.,* is able to inhibit *in vitro* multiple HIV transmission pathways. It has very potent and consistent activity against a wide variety of HIV-1 and HIV-2 strains and clinical isolates and subtypes with different co-receptor tropisms in peripheral blood mononuclear cells (PBMC) (IC_{50}: 0.043-0.63 nM). GRFT also inhibits syncytium formation between persistently HIV-1-infected T cells and uninfected $CD4^+$ T cells (IC_{50}: 0.087 ± 0.004 nM). In addition, it inhibits DC-SIGN-mediated HIV-1 capture (IC_{50}: 0.30 nM) and subsequent transmission to $CD4^+$ target T cells (IC_{50}: 0.27 nM). Long term passaging of HIV-1 exposed to dose-escalating concentrations of GRFT resulted in the selection of a mutant virus with 5 specific deleted high-mannose-type glycans in the envelope gp120 (N230, N234, N295, N386 and N448). The so-called broad neutralizing carbohydrate-specific 2G12 mAb completely lost anti-HIV activity against this GRFT resistant virus while GRFT was still active against a 2G12 mAb resistant virus (N295). For other antiviral lectins such as the banana lectin and cyanovirin-N, interactions of the lectins with unknown cellular proteins have been reported to induce undesirable side effects such as cellular activation. GRFT also binds to the surface of PBMC and various cell lines, however, this has no measurable effect on cell viability and it does not significantly upregulate markers of T cell activation or induce the secretion of inflammatory cytokines. Interestingly, GRFT retains its antiviral activity once bound to the surface of PBMC even if the cells are extensively washed before infection with HIV-1. The most plausible mechanism that might explain GRFT's retention of HIV-1 entry inhibition activity is that not all six carbohydrate binding sites are occupied when the lectin is docked on cell surface glycoproteins, leaving sites available for binding to the viral envelope glycoprotein. This unique mechanism of action may be beneficial as it increases the time period a microbicide remains effective after vaginal application. We believe these data are in strong support for clinical development of an HIV-1 microbicide containing GRFT as an active ingredient.

Suggested Reference:

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Identifying plant proteins involved in posttranscriptional gene regulation using HC-Pro, a viral suppressor of posttranscriptional gene silencing

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Sorghum mosaic virus (SrMV) is a single-stranded (-)RNA virus belonging to the family of the *Potyviridae*, genus potyvirus. Using a primer walking strategy we have determined the full genomic sequence of strain H of this virus which is 9,613 bp and encodes a single polyprotein of 3,077 amino acids. Sequence analysis showed frequent Single Nucleotide Polymorphisms (SNPs), often at the third, silent position of codons, ie synonymous SNPs, or non-synonymous SNPS resulting in conservative amino acid substitutions. Thus, the SrMV-H population examined was highly variable. HC-Pro is a well-known potyviral protein that suppresses RNA silencing in plants. We isolated P1-HC-Pro from SrMV-H and introduced the construct into a transgenic sugarcane line that was posttranscriptionally silenced for the SrMV coat protein. This resulted in reversal of gene silencing and accumulation of SrMV CP mRNA, confirming that SrMV P1/HC-Pro acts as a suppressor of RNA silencing in sugarcane, one of the most economically important crops worldwide. To investigate cellular proteins involved in RNA silencing and its suppression in sugarcane, HC-Pro was used as bait in a yeast-two-hybrid assay to screen a cDNA expression library constructed from a transgenic sugarcane line displaying RNA silencing. Yeast-two-hybrid screening identified several cellular proteins as interactors with HC-Pro. One of these proteins is a ca. 22 KDa protein that preferentially binds to ssRNA. *In vitro* binding assays such as pull-downs and far-western assays further confirmed that SrMV HC-Pro interacts with the 22 KDa protein. The involvement of these and other HC-Pro interacting sugarcane proteins will be presented.

The US3 kinase of pseudorabies virus leads to activation of the actin regulator cofilin to induce actin cytoskeleton changes

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Alphaherpesviruses constitute the largest subfamily of the herpesvirus family. In humans, they cause several diseases such as causes cold sores, genital lesions, keratitis, blindness, and encephalitis (herpes simplex virus), and chickenpox and shingles (varicella zoster virus). The closely related porcine alphaherpesvirus pseudorabies virus (PRV) is often used as a model organism to study general aspects of alphaherpesvirus biology (Pomeranz et al. 2005. Microbiol Mol Biol Rev).

The US3 kinase is a conserved alphaherpesviral serine/threonine kinase that, amongst other functions, causes alterations in the actin cytoskeleton such as the disassembly of actin stress fibers and formation of cell projections, associated with increased intercellular virus spread. The mechanisms used by US3 to exert these functions are poorly understood, however recently our research group identified the group A p21-activated kinases PAK1 and PAK2 as important effectors in the US3-mediated cytoskeletal rearrangements (Van den Broeke et al. 2009. PNAS).

We found that PRV NIA3 infection of porcine ST cells results in strong dephosphorylation of cofilin at S3. Interestingly, infection with an isogenic US3null virus did not cause cofilin dephosphorylation (whereas a US3rescue strain behaved like wild type NIA3). US3-mediated cofilin dephosphorylation was confirmed by US3 transfection assays. Cofilin dephosphorylation required an intact kinase domain of the US3 protein as infection or transfection assays in which the ATP binding domain of the US3 kinase was mutated did not result in cofilin dephosphorylation. Experiments using a PAK inhibitor, IPA-3, showed that the US3-mediated cofilin dephosphorylation depends on PAK activity. Importantly, using wild type or S3D (phosphomimetic) cofilin constructs showed that expression of S3D cofilin (which cannot be dephosphorylated) interferes with the ability of US3 to mediate its effects on the actin cytoskeleton.

In conclusion, the current data for the first time show that US3 of an alphaherpesvirus leads to cofilin dephosphorylation via cellular PAKs, and that cofilin dephosphorylation contributes to the ability of US3 to rearrange the actin cytoskeleton.

Functional dissection of the antitoxin of a three-component type II toxin-antitoxin system

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Type II toxin-antitoxin (TA) systems are small bacterial modules involved in the stability of mobile genetic elements and/or management of environmental stresses. They generally consist in a two-gene operon, the first gene encoding an unstable antitoxin and the second a stable toxin. The antitoxin binds to the toxin to inhibit its toxic activity. This complex also ensures negative autoregulation of the expression of both components. Today, 11 and 20 super-families of toxins and antitoxins respectively have been identified based on sequence similarities and ternary structure. We recently described an unusual three-components TA system *paaR2-paaA2-parE2* in which *paaA2* encodes a specific antitoxin PaaA2 (63-aa) that binds the ParE2 toxin to alleviate its toxicity. The PaaR2 protein is a transcriptional regulator that partially represses the transcription of this operon. To achieve full repression, the PaaA2-ParE2 complex is also needed despite the presence any recognizable DNA-binding domain on both proteins.
The objective of our work is to determine the different functional domains of the PaaA2 antitoxin. Canonical antitoxins are generally dimers containing two domains: a well-folded N-terminal moiety involved as a dimerization and DNA-binding domain (DBD) and the C-terminus, acting as a toxin-recognition element, which is intrinsically unstructured. From sequence alignments, it seems that PaaA2 is devoid of a DBD. Determination of the structure of PaaA2 in solution by NMR shows that it is a monomer consisting of two a-helices connected by a highly flexible linker. Based on this structure, we have initiated the dissection of the functionality of the different segments in the PaaA2 sequence. The first 15 aa form a disordered fragment which seems to be essential for the oligomerization of the ParE2-PaaA2 toxin-antitoxin complex, but not for antitoxicity. Indeed, the complex formed by the full-length PaaA2 and ParE2 is of 140 kDa while the complex formed by the PaaA2D15 and ParE2 is around 20 kDa. The shortest antitoxin fragment encompasses the entire first helix and a large region of the second helix (aa 13-57). The 13-52 fragment is not functional, suggesting that the 52-57 region contains key residues for ParE2 binding and neutralization. *In vivo* toxicity assays indicate that simultaneous substitutions of Phe53, Lys55 and Arg57 residues of this region alter the PaaA2 functionality while mutating each one of these residues separately has no effect.
In conclusion, we show that the small PaaA2 protein contains at least 2 functionally distinct regions: a C-terminal toxin-hampering part consisting of two helices and a N-terminal disordered moiety with a potential role in the oligomerization of the ParE2-PaaA2 toxin-antitoxin complex.

Quantification of *Candida albicans* by flow cytometry using TO-PRO®-3 iodide as a single-stain viability dye

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The Viable Plate Count (VPC) method to quantify micro-organisms is labor-intensive, time-consuming and only allows detection of organisms that readily grow on solid media. Flow cytometry (FCM) can be proposed as an alternative technique: it allows a fast and accurate detection of all micro-organisms [Davey (2011) Appl. Environ. Microbiol. 77: 5571-5576; Díaz *et al* (2010) Biochem. Eng. J. 48: 385-407].

In our study, we investigated a flow cytometric, single-stain approach for the quantification of viable *Candida albicans* cells.

C. albicans SC5314 was stained with TO-PRO®-3 iodide (TP3; Molecular Probes, OR, USA) and analyzed using a FACSCalibur flow cytometer (BD Biosciences, CA, USA). Confocal images of TP3-stained *C. albicans* demonstrated that the dye stains live and dead cell populations with a difference in fluorescence intensity. To assess the quantitative performance of the FCM technique, ten fivefold serial dilutions of a live, concentrated yeast suspension were analyzed by FCM and VPC.

After careful optimization of the staining parameters and the instrument and software settings, an excellent separation of viable and dead cells was achieved. A highly linear correlation (R^2 = 0.9984) was observed between the logarithm of the amount of Colony Forming Units (CFU) per ml determined by FCM and the logarithm of the CFU/ml obtained by the VPC over a concentration range of 4.20×10^2 to 2.42×10^8 CFU/ml. The 95 % confidence interval of the slope of the linear regression curve (0.9786 to 1.011) comprises 1, and the interval of the intercept (-0.07502 to 0.1114) equals 0, indicating a high accuracy with regard to the standard method. The limit of detection, determined as $3 \times \sigma_{blank}$, was established at 2.12×10^2 CFU/ml.

Our results show that the FCM quantification of viable cells using TP3 is a reliable alternative for the more time-consuming and labor-intensive VPC method. In comparison to other flow cytometric techniques, this mono-stain procedure is easy to apply and cost-effective: only one dye needs to be added. Moreover, the unique excitation/emission profile of TP3 allows the application of additional dyes to assess other cellular parameters with minimal spectral overlap.

Antiviral activity of a murine Apolipoprotein L

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Primary neurons readily responded to IFN-alpha/beta treatment by upregulating the expression of interferon stimulated genes (ISGs) such as Oasl2, Mx or STAT-1. However, in contrast to other cells, IFN-treated neurons failed to acquire a strong resistance to subsequent infection by Theiler's virus (TMEV) or vesicular stomatitis virus (VSV).

Microarray studies identified a set of 15 genes that were upregulated by IFN treatment in mouse embryonic fibroblasts (MEFs) and in L929 cells but that were not upregulated or much less expressed in IFN-treated primary neurons.

We expressed these genes using lentiviral vectors and screened them, by flow cytometry, for antiviral activity against a TMEV virus expressing the fluorescent GFP protein.

Using this strategy, we identified one gene that was induced by IFN in fibroblast but not in neurons and that displayed antiviral activity. This gene encodes Apolipoprotein L9b.

Apol9b is a 310 amino acid long protein that shares 97% identity with Apol9a. Both proteins belong to the murine Apolipoprotein L family and are encoded by the mouse chromosome 15.

Unlike other apolipoproteins, Apol9b and its human homolog ApoL6 (Hu et al., 2005) lack a predicted signal sequence and are thus likely not secreted.

Quantitative RT-PCR and microarray data show that ApoL9a and b are constitutively expressed in the liver and, to a lesser extent, in the intestine. Type-I IFN or type-III IFN triggered ApoL9b upregulation in many organs. Expression of Apol9b was also upregulated in the central nervous system of mice infected with the DA strain of Theiler's virus.

We observed that Apol9b had a dose-dependent protective effect on TMEV infection. Neurons transduced to express Apol9b displayed increased resistance toward TMEV infection. TMRE staining suggested that this activity did not correlate with sensitization of the cells to apoptosis. The antiviral effect of ApoL9b was virus-dependent as it was more pronounced against the neurovirulent GDVII TMEV strain than against the persistent DA strain and even weaker against VSV.

In conclusion, we identified Apol9b as novel ISG that displays antiviral activity. The lack of expression of this protein in primary neurons after IFN treatment might contribute to the surprisingly high susceptibility of these cells to viral infection.

Ref : Liu Z, Lu H, Jiang Z, Pastuszyn A, Hu CA; Mol Cancer Res. 2005 Jan;3(1):21-31; "Apolipoprotein l6, a novel proapoptotic Bcl-2 homology 3-only protein, induces mitochondria-mediated apoptosis in cancer cells."

The NOD-like receptor homolog encoded by murid herpesvirus-4 ORF63 is important but not essential for virus replication *in vitro*

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The role of the innate immunity is central in the immune system of the body. Therefore, pathogens have had to develop strategies in order to evade recognition and clearance by innate immune effectors. The immune response to viral infections is determined by a complex interplay between the pathogen and the host through their molecular effectors. This is particularly true for persistent viruses such as gammaherpesviruses. While evasion of the adaptive immune response by these viruses is well described, less is known about how they evade innate immunity inside the infected cells. Interestingly, a recent study showed that the open reading frame 63 (ORF63) of the Kaposi's Sarcoma Associated Herpesvirus (KSHV) can halt NOD-Like Receptor (NLR) recognition and inflammasome activation *in vitro*. However, because KSHV has no well-established *in vivo* infection model, the consequences of this observation on the *in vivo* lifecycle of KSHV are still unknown. As ORF-63 is a tegument protein which is conserved among all gammaherpesvirinae, related animal viruses such as Murid herpesvirus 4 (MuHV-4) allow us to tackle the same fundamental questions in a more accessible form. In the present study, we firstly performed a computational analysis of the protein encoded by MuHV-4 ORF63. This revealed that this protein shares homology with KSHV ORF63 and mouse NLRP1. Secondly, ORF63 expression as a fusion protein with the enhanced green fluorescent protein (eGFP) showed that this protein is mainly localized in the cytoplasm of the cell. Finally, to define the role that ORF63 plays in gammaherpesvirus infections, we disrupted its coding sequence. Interestingly, despite a severe growth deficit, MuHV-4 lacking ORF63 was still viable. In the future, complete understanding of the functional significance of ORF63 in biological cycle of MuHV-4 could not only allow better understanding of the virus cycle but could also help us to define new anti-viral targets to fight these infections.

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-mucine 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 showed in this study that two different messenger RNAs are encoded by the Bo17 gene. The first one corresponds to the entire coding sequence of the Bo17 gene. Surprisingly, the second results from the splicing of a 138 pb intron. Analysis of different homologous sequences showed that, compared to cellular sequences, only Bo17 gene presents the consensus sites for this 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. By using an *in vitro* assay, we showed that the spliced form of Bo17 is not anymore active and could therefore regulate enzymatic activity. Finally, recombinant strains expressing only the long or the short form of Bo17 showed that BoHV-4 could use alternative splicing 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 enzymatic activity in the cell. This new regulatory mechanism could have implication in viral immune evasion but also more generally in cellular biology.

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Characterization of a beta-alanine responsive transcription regulator (BarR) from *Sulfolobus acidocaldarius*

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Sulfolobus is a model organism of the aerobic hyperthermoacidophilic crenarchaeota that develops optimally around 80°C and pH 2-3. For the fitness of *Sulfolobus*, it is crucial that the organism is able to respond and adapt to environmental changes, essentially by regulation of gene expression. BarR is a novel beta-alanine responsive DNA-binding protein, which was first identified in *Sulfolobus tokodaii* (E. Peeters, unpublished work from this laboratory). In bacteria, beta-alanine is a precursor of pantothenate that itself is further used for the biosynthesis of CoenzymeA and acetyl-CoA. Little is known about the synthesis of beta-alanine and its further conversion in archaea. BarR has a homolog in *S. acidocaldarius* (Saci_2136), which is better amenable to genetic analysis.

Saci_2136 encodes a 152-amino-acid protein. As overexpressed in inclusion bodies, BarR was refolded and purified on His-tag column in the presence of imidazole and 400 mM L-arginine to get active (correctly folded) protein. *In vitro* binding assays (Electrophoretic Mobility Shift Assay, EMSA) revealed that stable DNA-protein complexes were formed with the intergenic region of Saci_2136 - Saci_2137 (K_D~ 200 nM). Interestingly, DNA-protein complexes were not formed with neither of the two single promoter regions (Saci_2136 and of Saci_2137), indicating that BarR may regulate the Saci_2136 gene and its target in a coordinated manner.

In gel copper-phenanthroline footprinting assays revealed three possible binding sites for BarR in the intergenic region. One site locates in the Saci_2137 promoter region while the other two locate in the Saci_2136 promoter region. The BRE and TATA box promoter elements are located between the latter two binding sites. Addition of beta-alanine as the effector molecule for BarR binding indicates 50% binding inhibition (IC_{50}) at approximately 4 μM, which is slightly lower than that of its homolog in *Sulfolobus tokodaii*.

In vivo gene expression was assayed by qRT-PCT with RNA extracted from *S. acidocaldarius* cells grown with and without beta-alanine. The results revealed a significant up-regulation of Saci_2136 (3-fold) and a strong up-regulation of its target (Saci_2137; 190-fold), indicating that BarR acts as a transcriptional repressor and that this repression is alleviated in the presence of beta-alanine. Considering that beta-alanine is a precursor of CoA, which is a crucial intermediate of the central metabolism, we presume that the Saci_2136 gene may be involved in the regulation of central metabolism in *Sulfolobus acidocaldarius*.

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Alternative splicing switches tropism of a gammaherpesvirus

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Herpesviruses have complex lifecycles that involve infection of various cell types. Interestingly, some of herpesvirus are able to route infection by the use of different cellular receptors and different viral glycoprotein complexes. Thus, Epstein-Barr virus appears to use gp42 – Human leukocyte antigen class II interaction as a switch of cell tropism. Rhadinoviruses do not encode any gp42 homolog and the mechanisms that regulate cell tropism are less clear for them. In this study, we showed that Bovine Herpesvirus 4, a rhadinovirus closely related to Kaposi's Sarcoma associated herpesvirus (KSHV), could orientate tropism of progeny virions by using alternative splicing of its Bo10 gene. Indeed, recombinant viral strains expressing one or the other transcript showed different infectivity patterns depending of the presence of glycoaminoglycans at the cell surface. Moreover, we showed that the proportion of the two transcripts changed between the different cell types. As a similar gene organization is observed in the KSHV K8.1 homologous gene, we hypothesized that this mechanism could be conserved in different rhadinoviruses.

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Study of the role and regulation of the RNA Polymerase II carboxy-terminal domain phosphorylation in the induction of sexual differentiation in the yeast *S. pombe*.

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When nutrients are available, the fission yeast *Schizosaccharomyces pombe* (*S. pombe*) undergoes vegetative growth. In contrast, when nutrients (usually a nitrogen or a carbon source) become limiting, cells of opposite mating type (h^+ and h^-) initiate sexual differentiation, which consists of mating and meiosis (Egel 1973). Remarkably, the induction of this switch solely relies on the Ste11 transcription factor whose expression is tightly controlled by a myriad of interconnected pathways. (For review see: Yamamoto 1996).

Nucleosome is the basic unit of chromatin. It is formed one heterodimer of histones H2A, H2B and a tetramer of histones H3 and H4. The nucleosome is wrapped around 147 bp of DNA (Luger, Mader et al. 1997). The histones can be displaced, evicted or covalently modified. Many enzymes are involved in those active processes (For review see: Li, Carey et al. 2007).

The RNA Polymerase II (Pol II) is a macromolecular complex composed of 12 subunits. The largest subunit, Rpb1, harbours an unusual Carboxy-Terminal Domain (CTD) composed of the heptad YSPTSPS repeated 29 times in *S. pombe*. The CTD sequence is highly conserved among species but the number of repetitions varies on a wide range depending of the complexity of the organism. During gene transcription, the CTD follows opposite serines 2 and 5 phosphorylation patterns with S5P peaking near the promoter and S2P peaking closer to the 3□ end of the transcribed region. This combination of phosphorylation both enforces and restricts the recruitment of proteins required for mRNA maturation, including capping enzymes, poly-adenylation factors, splicing enzyme or chromatin remodellers (For review see: Palancade and Bensaude 2003; Zhang, Rodriguez-Molina et al. 2012).

Surprisingly, the absence of S2P has only minor impacts on genome-wide expression but is critical for sexual differentiation in fission yeast. Indeed, in a S2A mutant (where every serine in position 2 is replaced by an alanine) or in a Δ*lsk1* (a deletion mutant for the main kinase of serine 2) strain, the expression of *ste11* is severely impaired, which results in almost sterile strains. This lowered expression is correlated with a markedly reduced PolII occupancy along the *ste11* locus (Coudreuse, van Bakel et al. 2010).

These results led us to investigate the state of the chromatin onto *ste11* in S2 mutants. The role of chromatin remodellers has also been investigated in the context of sexual differentiation.

Those data provide for the first time a connection between CTD phosphorylation and nucleosome occupancy.

Mutational analysis brings new insights into the multifunction of the Type III secretion needle tip protein IpaD of *Shigella flexneri*

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Shigella is the causative agent of shigellosis, a diarrhoeal disease responsible of about 1 million deaths per year. This Gram-negative bacterium infects colonic epithelial cells and possesses mechanisms that hijack their physiology through secretion of effector proteins. The virulence of *Shigella* is associated with a type III secretion system (T3SS) composed of a cytoplasmic bulb, a basal body, and an extracellular needle. The needle tip is capped by the protein IpaD which regulates secretion of T3 effectors and inserts a pore made of translocators IpaB and IpaC into host cell membrane thereby allowing injection of proteins exported through the needle canal. To better understand the function of IpaD in the virulence of *Shigella*, we substituted residues conserved among IpaD homologous proteins. We generated thirteen punctual mutations: Y149A, Y153A, T161D, Q165L, K189A, K205A, W226A, Y276A, Y301A, N305A, V312A, V314D, V319D and tested their impact on the ability of *Shigella* **i)** to control the T3S activity, **ii)** to insert the translocation pore, and **iii)** to invade cultured cells *in vitro*. Phenotypical analysis revealed three classes of mutants. The first class behaved similarly to the wild type strain; the second class abolished secretion control without impairing bacterial entry into cells; while the third class increased bacterial virulence by provoking a loss of translocators secretion control. Interestingly, the second class of mutants, designated constitutive secretion variants, allowed us to discriminate between IpaD functions in terms of secretion control, translocon insertion and cell entry. The third class of mutants, designated hypervirulent variants, is characterized by **i)** a constitutive secretion of translocators, **ii)** an early translocon formation, and **iii)** a rapid penetration within cells. To further understand the hypervirulent phenotype, we subsequently performed a translocon insertion kinetics assay which revealed an early pore formation particularly spectacular with mutation Q165L. Moreover, we observed an altered surface localization of IpaB in this mutant which reflects both its early release and rapid insertion into the host cell membrane. Altogether, our findings bring new insights into how IpaD takes part to several key virulence steps of *Shigella* and provide new evidence of how these numerous functions are inter-connected.

Single particle tracking in Burkholderia cepacia complex biofilms as a tool to study transport of drug-loaded liposomes

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In cystic fibrosis patients acute lung infections or exacerbations by *Pseudomonas aeruginosa* or *Burkholderia cepacia* complex are often treated with high doses of intravenously administered antibiotics, combined with the daily use of nebulized tobramycin to prevent chronic lung infections. Once chronic lung infections are established, eradication of these pathogens is very difficult. This is due to the biofilm mode of growth of pathogenic *P. aeruginosa* and *B. cepacia* complex strains. Sessile cells are resistant to most antibiotics, partly because of the latter's limited diffusion into the biofilm.

In the present study, we investigated whether a liposomal tobramycin formulation can deliver a high dose of antibiotics closely to the bacterial cells and increase the success rate of the treatment. In order to determine suitable physic-chemical characteristics of the liposomal formulation, we performed a single particle tracking study of the transport of positive and negative 200 nm fluorescent polystyrene model nanospheres in *Burkholderia cepacia* complex biofilms.

Negative particles penetrated into the biofilm and were then immobilized in cell clusters, while positively charged particles hardly penetrated into the biofilm but adhered to threadlike structures in the extracellular matrix. These threadlike structures are believed to consist of DNA as they disappeared when biofilms were grown in the presence of recombinant human DNase. Positively charged particles added to biofilms grown in the presence of rhDNase showed higher mobility. Some of these particles were able to penetrate deeply into the biofilm where they were immobilized in cell clusters.

Our data indicate that negative particles are directed towards cell clusters, which means that negatively charged nanomedicines could be suitable candidates to deliver antibiotics to the bacterial clusters. Positively charged nanomedicines appear only promising when they are administered in combination with rhDNase therapy.

Currently tobramycin loaded liposomes (both positively and negatively charged) are being produced to compare the activity of drug-loaded liposomes with free tobramycin against *in vitro* grown Bcc biofilms.

Clonal diversity and high prevalence of OXA-58 among *Acinetobacter baumannii* isolates from blood cultures in a tertiary care centre in Turkey

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Genotypic diversity, antimicrobial susceptibilty, and presence of OXA-genes were assessed in 100 nosocomial *Acinetobacter* strains from a tertiary-care hospital, Turkey. Ninety-eight isolates were identified by AFLP library identification to *A. baumannii*. Furthermore, the isolates were divided into 30 AFLP clusters and single strains at a similarity cut-off level of 90%, the defined strain level. Most of these clusters grouped together in larger clusters at a lower similarity level, indicating diversification beyond the strain level. At a similarity level of 80%, the *A. baumannii* isolates were allocated to eight clusters of multiple isolates (A, C, D, E, G, H, J, L) and 3 single isolates (B, F, I). Comparison of the isolates to those of the Leiden AFLP database revealed that the large cluster H (41 isolates) corresponded to a tentative novel international clone previously identified both by AFLP and MLST (CC15). Clusters D and E grouped with European (EU) clone II isolates, and cluster J with those of EU clone I. Clusters A, C, G, and L could not be identified to any international clone. MLST of selected isolates of the major clusters corroborated the clone allocation by AFLP, except for the tested cluster A isolate which was identified to CC2 (EU clone II). Carbapenem resistance of 75 *A. baumannii* isolates was associated with the $bla_{\text{OXA-58-like}}$ gene or $bla_{\text{OXA-51-like}}$ with IS*Aba1* upstream. Altogether, 99% of the *Acinetobacter* isolates were multidrug resistant (MDR) and 77% extensively drug resistant (XDR). The findings show that multiple strains and clones MDR and XDR *A. baumannii* are endemic in the hospital.

CspR is a cold-shock RNA-binding protein involve in the long term survival and the virulence of *Enterococcus faecalis*

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Since the last ten years, several studies point out the important role of posttranscriptional gene regulation in bacterial behaviour, adaptation to environmental changes as well as in virulence. Such process involves small non-coding RNAs and RNA-binding proteins whose most studied actor is Hfq. However this protein is lacking in numerous bacterial species including *Streptococci* and *Enterococci* and we were then interested by knowing whether other protein may act as RNA-interacting molecules**.** By coprecipitation, we identified RNA-binding proteins in the Gram-positive opportunistic pathogen *Enterococcus faecalis* known to be deficient of the RNA chaperone Hfq. We particularly characterized one belonging to the Cold-shock protein (Csp) family (Ef2925) renamed CspR for Cold-shock protein RNA-binding. Compared to the wild-type strain, the *ΔcspR* mutant was less virulent in an insect infection model (*Galleria mellonella*), exhibited decreased persistence in mouse kidneys and lower survival inside peritoneal macrophages. As expected, we found that *ΔcspR* mutant strain was more impaired in its growth than the parental strain in cold condition and in its long term survival under nutrient starvation. All these phenotypes were restored after complementation of the *ΔcspR* mutant. In addition, Western blot analysis showed that CspR was overexpressed under cold-shock condition as well as in stationary phase. Since CspR may act as RNA chaperone, putative targets were identified using global proteomic approach completed with transcriptomic assays. These studies revealed that 19 proteins were differentially expressed in the *ΔcspR* strain (9 upregulated, 10 downregulated) and that CspR mainly acted at post-transcriptional level. These data highlights for the first time the role of the RNA-binding protein CspR as a regulator in *E. faecalis* and its requirement in stress response and virulence in this important human pathogen.

Dynamic genetic adaptation of *Cupriavidus metallidurans* in response to silver toxicity

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The *Cupriavidus metallidurans* species is characterized by multiple metal resistances and is often isolated from industrial sites linked to mining-, metallurgical-, and chemical industries. Next to this, *C. metallidurans* strains were isolated from non-industrial environments such as different spacecraft-related environments, or even as the causative agent of an invasive human infection and from patients with cystic fibrosis.

Here, we investigated more in detail the genetic adaptation of *Cupriavidus metallidurans* to toxic silver concentrations. The resistance mechanisms involved as well as the genetics causing the increased resistance were examined.

Mutants of *C. metallidurans* type strain CH34, AE104 (plasmidless derivative of CH34) and NA4 (isolated from the potable water of the ISS), displaying increased resistance to silver occur naturally at low frequency after exposure to silver. Whole-genome gene expression profiling and Illumina resequencing of multiple independent mutants indicated that the uncharacterized small protein MmmQ and the two-component regulatory system AgrRS are involved. The MmmQ protein is one of a group of 19 homologous proteins in *C. metallidurans* CH34 that is apparently only found in the *Cupriavidus* and *Ralstonia* genera. Transcriptional expression of several of the associated genes is induced when *C. metallidurans* CH34 is exposed to lethal concentrations of metals. Deletion of *mmmQ* resulted in the loss of the increased silver resistance, which could be restored by complementation with a plasmid containing *mmmQ*. The AgrRS two-component system, more specifically *agrS,* was affected either by insertion of different endogenous Insertion Sequence (IS) elements or by point mutation. Furthermore, a phylogenetic footprinting approach of the *agrR* promoter region predicted a regulatory motif that resembles the regulatory motif found in the *mmmQ* promoter as well as in other promoters from metal resistance genes. Therefore, the AgrR transcription factor not only regulates its own dedicated system, but also affects other metal resistance regions containing a similar transcription factor binding site in their promoter region.

These results show the involvement of a novel protein in the interaction with silver. In addition, two-component regulatory systems in *C. metallidurans* affect other genes besides their own system, thereby, regulating key genes involved in adaptation to high concentrations of metals (such as silver).

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Epigenetic inheritance in a simple gene circuit gives rise to „stochastic sensing“

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Microbes must decide whether or not to reprogram their genes to respond to environmental changes. For example, it can sometimes pay for cells to use carbon catabolite repression to preferentially consume glucose over other carbon sources in the environment because glucose offers high growth rates. On the other hand, this gene-regulatory strategy can leave cells ill-prepared for the depletion of glucose, for example giving rise to the lag phase observed in diauxic shift conditions [Monod (1941)]. At the other extreme from sensing strategies are bet-hedging-like gene circuits that, regardless of the current environment, stochastically generate variability to ensure that some fraction of the population is prepared for different conditions. By paying a cost in favorable environments, bet-hedging can increase fitness in the long run. The evolutionarily optimal strategy – sensing and signaling or stochastic switching – depends upon how reliably the environment changes. Stochastic switching is best suited for spontaneously changing, unpredictable environments, whereas sensing is beneficial in stable, predictable conditions [Kussel and Leibler (2005) Science; Antonini et. al. (2012) PLoS Comp Biol].

Here we have observed that the budding yeast *S. cerevisiae* can implement a strategy that is intermediate between sensing and switching. Using an experimental evolution strategy, we evolved many different mutants to have short lag phases in environments that suddenly change from glucose to maltose. Further analysis revealed that these carbon catabolite mutants also have short lag phases in traditional diauxic shift conditions, where glucose is gradually depleted and cultures transition to maltose fermentation. The shorter lag phases arise because the mutants have lost preference for glucose, allowing them to activate their maltose utilization (*MAL*) genes in anticipation of glucose depletion. This gene activation proceeds by a surprising epigenetic mechanism, whereby daughter cells inherit the expression state of the *MAL* genes from their mothers, giving rise to “memory” that can persist for more than 12 generations. We observe that cells within growing populations can switch between OFF and ON states, characteristic of a stochastic switching strategy. On the other hand, maltose must be present in the medium to induce this phenomenon, and in this regard it appears to be a sensing mechanism. Given that stochastic transitions between uninduced and induced *MAL* states allows sensing of maltose in the environment, we have termed this phenomenon “stochastic sensing”. We believe that other positive-feedback driven nutrient assimilation pathways like the *MAL* system can act as basic sensing tools, allowing microbes to increase long-term fitness in changing environments.

The trigger enzyme PepA (aminopeptidase A) is a transcriptional repressor and induces positive supercoiling

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The inherent topological constraints of the DNA double-helix structure bear many implications for biological functions involving DNA transactions such as DNA replication and transcription, which require a transient unwinding of the DNA helix. The expression of many genes is regulated at the level of transcription, sometimes involving mechanisms that modulate the DNA conformation. Here we demonstarte that the multifunctional trigger enzyme Aminopeptidase A (PepA) represses transcription initiation at the *car*P1 promoter of the carbamoylphosphate synthase (CPSase) operon of *Escherichia coli* and induces a positive supercoil upon *car*P1 operator binding.

PepA is involved in the control of two distinct DNA transaction processes: transcriptional control of the *car*P1 promoter and site-specific resolution of ColE1-type plasmid multimers (Charlier *et al*. (1995) J. Mol. Biol. 250: 392-406; Stirling *et al*. (1989) EMBO J. 8: 1623-1627]). Both processes require communication at a distance. Previously we have shown by Atomic Force Microscopy that the *car*P1 operator DNA is foreshortened by ~ 235 bp through wrapping around one PepA hexamer [Nguyen Le Minh *et al* (2010) Nucleic Acids Res. 37: 1463-1476]. Here we proceeded to study the topological aspects of the PepA-DNA complex in the context of plasmid topoisomers and artificial DNA minicircles bearing the *car*P1 operator region. Minicircles produced by ligase in the presence of PepA are represented mainly by two populations of molecules both displaying a higher migrating velocity on polyacrylamide than that of the major population of minicircles ligated in the absence of PepA. A volley of restriction reactions and topological assays with Type IA, IB and II topoisomerases revealed that the major population produced in the absence of PepA corresponds to the relaxed form of the minicircles, whereas the binding of PepA prior to ligation promotes the formation of two types of minicircles with a linking number different from that of the relaxed cirles: (i) a trapped positive supercoil topoisomer and (ii) a noded topoisomer. Separation of plasmid topoisomers by agarose gel electrophoresis in the presence or absence of the intercalating agent chloroquine confirmed that PepA binding induces positive supercoiling

Single-round *in vitro* transcription assays with purified PepA and *E. coli* RNA polymerase saturated with σ^{70}-factor clearly demonstrated that PepA specifically inhibits transcription initiation at the *car*P1 promoter. PepA is therefore a repressor in its own right and not a mere architectural element of the regulatory process. These results bring forward insights into the working of PepA in modulation of *carAB* expression.

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Porcine sialoadhesin (CD169/Siglec-1) is an endocytic receptor that allows targeted delivery of toxins and antigens to macrophages

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Sialoadhesin (Sn) is exclusively expressed on specific subpopulations of macrophages. Since Sn-positive macrophages are involved in inflammatory autoimmune diseases, such as multiple sclerosis, and potentially in the generation of immune responses, targeted delivery of drugs, toxins or antigens via Sn-specific immunoconjugates may prove a useful therapeutic strategy. Originally, Sn was characterized as a lymphocyte adhesion molecule, though recently its involvement in internalization of sialic acid carrying pathogens was shown, suggesting that Sn is an endocytic receptor. In our study, we show that porcine Sn-specific antibodies and F(ab')2 fragments trigger Sn internalization, both in primary porcine macrophages and in cells expressing recombinant porcine Sn. Using chemical inhibitors, double immunofluorescence stainings and dominant-negative constructs, porcine Sn internalization was shown to be clathrin- and Eps15-dependent and to result in targeting to early endosomes but not lysosomes. Besides characterizing the Sn endocytosis mechanism, two Sn-specific immunoconjugates were evaluated. We observed that porcine Sn-specific immunotoxins efficiently kill Sn-expressing macrophages. Furthermore, porcine Sn-specific albumin immunoconjugates were shown to be internalized in macrophages and immunization with these immunoconjugates resulted in a rapid and robust induction of albumin-specific antibodies, this compared to immunization with albumin alone. Together, these data expand Sn functionality and show that it can function as an endocytic receptor, a feature that cannot only be misused by sialic acid carrying pathogens, but that may also be used for specific targeting of toxins or antigens to Sn-expressing macrophages.

The success of *Acinetobacter* species; genetic, metabolic and virulence attributes

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An understanding of why certain *Acinetobacter* species are more successful in causing nosocomial infections, transmission and epidemic spread in healthcare institutions compared with other species is lacking. We used genomic, phenotypic and virulence studies to identify differences between *Acinetobacter* species. Fourteen strains representing nine species were examined. Genomic analysis of six strains showed that the *A. baumannii* core genome contains many genes important for diverse metabolism and survival in the host. Most of the *A. baumannii* core genes were also present in one or more of the less clinically successful species. In contrast, when the accessory genome of an individual *A. baumannii* strain was compared to a strain of a less successful species (*A. calcoaceticus* RUH2202), many operons with putative virulence function were found to be present only in the *A. baumannii* strain, including the *csu* operon, the acinetobactin chromosomal cluster, and bacterial defence mechanisms. Phenotype microarray analysis showed that compared to *A. calcoaceticus* (RUH2202), *A. baumannii* ATCC 19606^T was able to utilise nitrogen sources more effectively and was more tolerant to pH, osmotic and antimicrobial stress. Virulence differences were also observed, with *A. baumannii* ATCC 19606^T, *A. pittii* SH024, and *A. nosocomialis* RUH2624 persisting and forming larger biofilms on human skin than *A. calcoaceticus*. *A. baumannii* ATCC 19606^T and *A. pittii* SH024 were also able to survive in a murine thigh infection model, whereas the other two species were eradicated. The current study provides important insights into the elucidation of differences in clinical relevance among *Acinetobacter* species.

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Deglycosylation of host glycoproteins by *C. canimorsus*

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Capnocytophaga canimorsus is a Gram-negative bacterium which lives as a commensal in dog's oral cavity. Infection upon scratches or bites can cause fatal septicaemia in humans. *C. canimorsus* is able to feed on cultured mammalian cells, including phagocytes by harvesting glycan moieties from host cells. Analysis of the genome of *C. canimorsus 5*, a human strain isolated from a fatal septicaemia, revealed the presence of 13 different putative polysaccharide utilization loci (PULs) [Martens *et al.*, *J Biol Chem*., 2009; 284(37):24673-7; Manfredi *et al., Mol Microbiol.*, 2011; 81(4):1050-60]. Systematic knockout of the 13 PULs revealed that 6 PULs are involved in growth during cell culture infections with most dramatic effect observed for PUL5 [Manfredi *et al., Mol Microbiol.*, 2011; 81(4):1050-60] . Here, we show that *C. canimorsus 5* has the capacity to deglycosylate N-linked glycoproteins as human IgG and fetuin and we analyze the mechanism. We show that deglycosylation is achieved by a large complex spanning the outer membrane and consisting of the Gpd proteins and sialidase SiaC. GpdD-G-E-F are surface-exposed outer membrane lipoproteins. GpdD-E-F contribute to the binding of glycoproteins at the bacterial surface while GpdG is a β-endo-glycosidase cleaving the N-linked oligosaccharide after the first GlcNAc residue. GpdC, resembling a TonB-dependent OM transporter is presumed to import the oligosaccharide into the periplasm after its cleavage. SiaC is an outer membrane-anchored lipoprotein oriented towards the periplasm. It interacts with GpdC and removes the terminal sialic acid residue of the oligosaccharide. Finally, degradation of the oligosaccharide proceeds sequentially from the desialylated non reducing end by periplasmic exoglycosidases. This work represents the first characterization of a bacterial complex specialized in deglycosylation of N-linked host glycoproteins [Renzi *et al.*, *PLoS Pathog.,* 2011; 7(6):e1002118].

Molecular mechanism mediating the G1 block of *Caulobacter* swarmer cells in response to Nitrogen starvation

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The cell cycle consists of a highly coordinated sequence of events culminating in cell division. Unlike rapidly growing bacteria that initiate DNA synthesis before ending cytokinesis, *Caulobacter crescentus* initiates DNA replication only once per cell cycle. Moreover, the swarmer progeny first enters in a G1 phase during which it persists as a chemotactically active swimming cell before replicating its DNA. Presence of nitrogen is required for the G1-to-S transition so much that swarmer cells remain blocked in G1 in a nitrogen-limited environment. In many organisms, the uridylyltransferase GlnD is known to activate proteins from the PII superfamily by uridylylation when nitrogen becomes limited. Once urydylylated, PII-UMP will then activate, directly or indirectly, proteins required to compensate for the nitrogen starvation. One of these proteins is the glutamine synthetase (GS) which condenses ammonia (NH_4^+) and glutamate (Glu) to form glutamine (Gln). In this work, we characterized at the genetic level the GlnD pathway in *C. crescentus*. We show that (i) this regulatory pathway participates to the G1 block encountered by *C. crescentus* during nitrogen starvation by modulating the glutamine synthetase activity; (ii) Gln is synthetized by 3 paralogous GS tightly controlled; (iii) the intracellular pool of Gln is the main signal of nitrogen availibility in *Caulobacter* swarmer cells. We are currently investigating the molecular details of this Nitrogen checkpoint.

Resistant bacteria from metal-contaminated marine and river sediments

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In this study, bacteria were isolated from sediments heavily contaminated with metallic ions and aromatic compounds in two locations: (i) the river Deûle in the North of France, which is exposed to effluents from the metallurgic plant MetalEurop, (ii) the Belgian Continental Plate, off Ostend.

The strains were isolated in the presence of different metallic ions. The isolates were identified on the basis of molecular markers, mainly the 16s rRNA gene. In fresh water sediments, most isolates were Gram-negative, the genus *Pseudomonas* constituting the prevalent group. On the contrary, marine sediments yielded mainly Gram-positive isolates with the genus *Bacillus* being the most frequent. The predominant genera in the collection were: *Pseudomonas*, *Bacillus*, *Klebsiella*, *Enterobacter* and *Mycobacterium*. These genera are well known to include species degrading organic matter, often harbouring plasmids which enable them to thrive on aromatic compounds.

Most isolates were resistant to more than one metallic ion. Resistances to As, Pb, Ni and Cu were the most frequent. Contrary to Gram-positive isolates, more than 90% of the Gram-negative isolates were also resistant to Zn, particularly *Pseudomonas* isolates.

The open reading frame TTC1157 of *Thermus thermophilus* HB27 encodes the methyltransferase forming N^2-methylguanosine at position 6 in tRNA.

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Transfer RNA (tRNA) maturation is a complex process. Primary tRNA transcripts undergo several processing steps: nucleotide sequences are removed from the 3' and 5' ends of the primary transcript, the CCA triplet is added to the 3' end and in some cases an intron is removed by a specific splicing mechanism. The final step in tRNA processing is the modification of some nucleosides by for instance methylation, deamination or reduction. The role of these modifications is poorly understood, but some have been shown to be important in the decoding properties of tRNA. Methylation is the far most widespread base or ribose modification. Methyltransferases (MTases) catalyse these methylations, and most of them use S-Adenosylmethionine as methyldonor. The large majority of the tRNA specific MTase adopts a Rossmann-like fold.

One of the methylated nucleosides found in tRNA is N^2-methylguanosine (m^2G). This modification is commonly found in eukarial and archaeal tRNAs, mostly at positions 10 and 26. The corresponding MTases have been characterized in yeast and *Pyrococcus*, model organisms for respectively eukarya and archaea. In bacteria however, m^2G is remarkably scarce. It is not encountered in tRNAs of *Enterobacteriacaea*. In *Thermus thermophilus*, a thermophilic bacterium with a growth optimum of 70°C, m^2G is present at position 6 of $tRNA^{Phe}$. We have identified the MTase catalysing this methylation reaction. The enzyme has been fully characterized and its corresponding gene has been interrupted by a Km^R cassette. Finally, this *Thermus* MTase is compared with its archaeal counterpart in *Pyrococcus furiosus*.

Post-transcriptional Control and Inductive Genetic Regulation in an 'almost' Prokaryotic cell

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Eukaryotic unicellular protozoan *Trypanosome cruzi* share transcriptional genetic mechanism to prokaryotic domain. Genes encoding proteins normally are transcribed in polycistronic units. The mature mRNA molecule is derived by adding the splice leader (SL) to the polycistronic 5 'end and to the poly-A at the 3' end transcript, in a trans–splicing process. In addition *T.cruzi* does not has RNA interference process. By stable transfections, two vectors containing T7-RNA-Pol + NEO and the exogenous gene of interest (Luciferase or Red Fluorescent Protein-RFP) under T7 promoter followed by cloning cells, we have generated an transcript controllable recombinant trypanosome, which can regulate the gene expression by dose-dependent induction with tetracycline. The transfected protozoan resulted in 20000 times higher of Luciferase activity when induced with tetracycline and compared to the wild type cells, and 200 times more compared to the basal levels observed in transfected non-induced cells. The transfects expressing RFP are induced in a time-dependent manner and show 100 times more number of fluorescent cells compared to the wild type and 10 times more compared to the basal levels observed in transfected non-induced cells. In addition the controllable induced protozoans showed a bright red fluorescence distributed throughout the cell and grew similar when compared to wild type cells. These results indicate that the expression of different dependent-time rates of gene expression in *T.cruzi* could be related to similar post-transcriptional control than prokaryotic microbes, suggesting the closer genetic evolution of protozoa to prokaryotic domain also at this level. Beyond that, this development in trypanosome genetic engineering and artificial genetic expression offers for the first time open possibilities and importance of protozoans in synthetic biology and biotechnology.

Characterization of AtaT toxin from the AtaR-AtaT toxin-antitoxin system: a new toxin family with an N-acetyltransferase domain

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Toxin-antitoxin (TA) systems are abundant in the bacterial genomes. Their diversity and their biological function are still not well established. Our laboratory is interested in type II systems that are composed of a toxic protein and a specific antitoxin protein inhibiting the toxic activity of the toxin. Toxins from type II systems characterized until now target three different cellular processes: replication, translations and peptidoglycan synthesis. Quite surprisingly, a vast majority of them inhibits translation. Recently, our laboratory has developed a bioinformatics approach that allowed the identification of novel toxins devoid of similarities with known toxin families and associated with potential antitoxin. One of these hypothetical systems is the *ataR-ataT* system of *Escherichia coli* O157:H7. Interestingly, the AtaT toxin contains a characteristic domain belonging to the GNAT family (GCN5-related N-acetyltransferase), which indicates potentially a novel toxin activity. The acetyltransferase activity was supposed to be an uncommon posttranslational modification in prokaryotic world. However recent studies have shown that a high number of proteins are acetylated, especially Nε-lysine acetylated. In this work we validated the *ataR-ataT* system as a TA gene pair and show that overexpression of AtaT inhibits translation. The molecular mechanisms underlying this inhibition are under investigation.

Experimental evolution with a global regulator mutant in *E. coli*

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The small CsrA protein and its homologs act as global regulators in bacteria. CsrA modulates gene expression at the post-transcriptional level either positively or negatively by affecting mRNA stability of target genes. In *E. coli*, CsrA regulates central carbon metabolism, mobility and biofilm formation. Mutants of *csrA* have been obtained in *E. coli* by transposon insertion at the 5'-end of the gene leading to a residual activity of the truncated protein.

In this work, we have shown that a complete deletion of the *csrA* gene leads to rapid selection of compensatory mutants suggesting that this gene is essential for growth. We thus performed experimental evolution of *csrA* deletion mutants in the uropathogenic *E. coli* CFT073 strain. Five independent *csrA* mutant populations were grown during 8 days in LB medium and diluted every 24 hours. Our data show a rapid selection of compensatory mutants, observed by a change of colony morphology on LB plates. After six days, these compensatory mutants totally outcompete the original *csrA* mutant in the five populations and are phenotypically stable. Characterization of these mutants by whole genome sequencing and proteomics is in progress.

The phenotype of the deletion mutants is strongly altered as compared to the wild-type strain, notably their generation time is increased and they show a strong aggregation phenotype in liquid medium, compatible to increased biofilm formation. In addition, RT-qPCR data indicate that these mutants show a constitutive induction of the hexose-phosphate stress response (*sgrS*) and a strong increase in *pgaA* expression.

In conclusion, the *csrA* deletion mutants suffer from metabolic stress and rapidly accumulate compensatory mutations, indicative of a high selective pressure against mutation in such global regulator.

Identification of monoclonal antibodies to the type III secretion needle tip IpaD that neutralize *Shigella* virulence both *in vitro* and *in vivo*

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Shigella spp. are Gram-negative pathogens responsible for bacillary dysentery in humans. The disease is characterized by an acute inflammation of the colonic mucosa and relies on the expression of a type III secretion (T3S) system. The T3SS functions as a molecular syringe that injects effector proteins into host cell across a translocation pore to hijack cellular pathways. The T3SS needle tip consists of IpaD and IpaB proteins that control both secretion and translocation of effectors. We previously reported that polyclonal antibodies directed against the last 200 residues of IpaD are able to neutralize entry of *Shigella* into host cell. In the present study, eight mouse monoclonal antibodies (MAbs) to *Shigella flexneri* 5a IpaD were generated and characterized *in vitro* and *in vivo*. We identified a first group of MAbs (A5-3, B8-2, E5-4 and H15-15) that bind conformational epitopes comprising residues 151 and 210. All, except MAb A5-3, neutralized T3S-mediated virulence *in vitro* by reducing red blood cell lysis (translocon insertion) and the entry into host cell. The second group of MAbs (A8-1 and C20-1) recognized conformational epitopes located between residues 181 and 270 but partially reduced RBC lysis and cell invasion. Besides, two other MAbs that bind linear epitopes (residues 221-240 and 301-332) were not protective *in vitro*. As IpaD is a key player in the invasive phenotype of all virulent *Shigella* spp., we subsequently determined cross-protection *in vitro* of group I and II MAbs against *S. flexneri* 5a *ipaD*-null strain expressing IpaD from representative *Shigella* species. We observed a strong protection to *S. dysenteriae* type I and *S. flexneri* 2a, but only a weak protection to *S. sonnei* and *S. boydii* 2. Lastly, we used the newborn mice model to assess the protective capacity of three selected MAbs (A8-1, B8-2 and E5-4). Our results clearly demonstrated that only group I MAbs (B8-2 and E5-4) are protective *in vivo*. All together, our results contribute to the identification of a valuable target that could takes place in the formulation of a *Shigella* vaccine.

LysM, a transcriptional regulator of the Lrp-family, regulates amino acid biosynthesis and transport in *Sulfolobus solfataricus*

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LysM is an Lrp-like transcriptional regulator from *S. solfataricus* that was previously shown to bind to the control region of the *lysWXJK* operon, the sole known target of LysM (Brinkman *et al* (2002) J. Biol. Chem. 277: 29537-29549).

To identify all LysM binding sites in the genome of *S. solfataricus* we have performed a LysM-specific nanobody-based chromatin immunoprecipitation assay coupled to array hybridization (ChIP-chip). The utilization of a LyM-specific nanobody avoided the need for overexpressing or epitope-tagging the transcription factor. Consequently, the genomic association profile was determined at homogenous expression levels of the regulator. In total 72 novel ChIP enriched regions (chers) with an enrichment of at least four-fold were identified. In each cher the best LysM binding site was predicted using an energy-based position weight matrix determined from a saturation mutagenesis of the LysM consensus binding site. These results were validated for a subset of selected target sites with *in vitro* binding assays, electrophoretic mobility shift assays (EMSAs) and in gel cupper-phenanthroline footprinting. High affinity targets were found in both intergenic control regions and in ORF's, and both classes comprise sites that are efficiently bound *in vitro* and *in vivo*, whereas others are only bound efficiently *in vivo*.

Various LysM binding sites are positioned in close proximity to promoter sequences. Transcription units, of which the expression is potentially influenced by LysM binding include: amino acid metabolism, central metabolism, transport, CRISPR immunity system, translation and hypothetical proteins. *In vitro* binding assays demonstrated high affinity binding of LysM to the control region of *leuA*-2 (2-isopropylmalate synthase), *gltB* (glutamate synthase) and Sso1906 and Sso2043, both encoding amino acid transporter related proteins. Binding of LysM to all these targets was decreased in the presence of lysine, and lysine supplementation to the growth medium resulted in a reduction of the corresponding gene expression levels, as demonstrated with qRT-PCR. Therefore, it appears that LysM is a much more versatile regulator than previously thought and that LysM stimulates the expression of all target genes when the intracellular lysine concentration is low.

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Alphaherpesviruses use different finely tuned mechanisms to overcome the basement membrane barrier

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During primary contact with susceptible hosts, microorganisms face an array of barriers that thwart their invasion process. Passage through the basement membrane (BM), a 50-100 nm thick barrier underlying epithelia and endothelia, is a prerequisite for successful host invasion. Such passage allows pathogens to reach nerve endings or blood vessels in the stroma and to facilitate spread to internal organs. During evolution, pathogens have developed various mechanisms to breach the BM. Some microorganisms have developed independent mechanisms, others hijack host cells that are able to transverse the BM (e.g. leukocytes). However, mechanisms underpinning binding to, breakdown of and passage of the BM are generally better understood for bacteria and fungi than for viruses. Many alphaherpesviruses use the epithelium of the respiratory and/or genital tract as a preferential site for primary replication. They gain access to the stroma for spread in the host by yet unidentified mechanisms. We investigated and compared how pseudorabies virus (PRV), equine herpesvirus 1 (EHV-1), bovine herpesvirus 1 (BoHV-1) and human herpesvirus 1 (HSV-1) cross the BM. We used *in vitro* models consisting of excised mucosae as tools for visualization, identification and characterization of putative invasion mechanisms. PRV, BoHV-1 and HSV-1 exhibited a plaquewise spread across the BM. Whereas PRV and BoHV-1 plaques showed a distinct penetration of the BM encompassing the entire radius of the viral plaque, HSV-1 plaques showed a more localized area of BM penetration. An unidentified trypsin-like serine protease was found to be involved in PRV-BM crossing. Viral crossing of the BM was not observed for EHV-1, where plaques remain confined to the epithelium. Interestingly, EHV-1 developed another system to invade. It hitchhikes across the BM by using local immune cells as Trojan horses. Further elucidation of these aspects will be instrumental to attain a better control of herpesvirus mediated diseases.

Characterization of prokaryotes in environmental samples from terrestrial Antarctica: comparison of DNA extraction procedures

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In continental Antarctica with its extremely harsh environmental conditions, where ecosystems are dominated by microorganisms and the food webs are relatively simple [Ellis-Evans JC. 1996. Biodiv. Conserv. 5:1395-1431], the main primary source of organic carbon (and energy) and fixed nitrogen for microbial communities is generally thought to be photosynthesis by Cyanobacteria [Taton A et al. 2003. Appl. Environ. Microbiol. 69:5157-5169]. However, there are sites where this group is less abundant in the microbial communities [Yergeau E et al. 2007a. Environ. Microbiol. 9:2670-2682]. Therefore, in these sites, alternative mechanisms for CO_2 fixation and solar energy exploitation may exist, providing the required energy for nitrogen fixation.

In this study, the diversity of several alternative mechanisms will be explored by studying the presence and diversity of key genes for these processes, using PCR clone libraries. The first and most important step to provide an image of the diversity present in a sample is the DNA extraction method. A diversity has been reported in literature and therefore we set up a comparative study to asses which DNA extraction method most accurately maps the bacterial community present in the samples. Seven extraction methods, with and without modifications, were tested. The methods which hardly yielded any DNA were excluded from subsequent steps. Of the remaining DNA extracts, the V3 region of the 16S rRNA gene and part of the pufM gene were amplified by PCR. A subsequent DGGE step provided a picture of the diversity of microbes present in the DNA extracted with each method.

In conclusion, different extraction methods yield highly diverse amounts of DNA and higher yield does not always go together with higher diversity.

Transposable Mu-like phages in Firmicutes: New instances of divergence generating retroelements.

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First traced as a mutator agent during Hrf/F$^-$ crosses, *E.coli* bacteriophage Mu was isolated 60 years ago. It is the best-characterized transposable phage and remains a paradigm for the study of transpositional recombination. Among the most promiscuous genome re-organizers, Myoviridae and Siphoviridae transposable phages have so far been found, with slightly different genome organization, which infect or reside as prophages in Gram- α, β, γ, δ and ε proteobacteria.

Using the sequences of the most conserved proteins encoded by transposable (pro)phages I searched recently sequenced Firmicutes genomes for candidate transposable prophages. Two different complete Mu-like prophages, SglyMu-1 and 2, were identified in *Syntrophobotulus glycolicus* DSM 8271. Related prophages were also found in partially assembled genomic sequences of other Firmicutes and newly sequenced Proteobacteria genomes.

SglyMu-1 appears to carry a host variation system related to the DGR tropism switching retroelements first characterized in *Bordetella* phages BPP-1, BIP-1 and BMP-1. Transposable phages are thus thriving among Firmicutes and can harness either of two host variation systems, the fiber genes inversion and reverse transcriptase-mediated site-directed, adenine-specific mutagenesis.

Involvement of toxin-antitoxin modules in tolerance of *Burkholderia cenocepacia*

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Burkholderia cenocepacia is an opportunistic pathogen that can cause severe lung infections in cystic fibrosis patients. Infections are often difficult to treat because of its innate resistance and its capacity to form biofilms. Biofilms play an important role in the recalcitrance of infections due to their tolerance. Tolerance is an ability to survive antibiotic treatment without expressing a resistance mechanism. Although the molecular basis of tolerance is still largely unknown, toxin-antitoxin modules (TA) are thought to play a role. The toxin (T) is a protein that inhibits an important cellular function such as translation or replication and which can form an inactive complex with an antitoxin (AT). Bactericidal antibiotics kill cells by corrupting the target functions. Inhibition of these functions would prevent antibiotics from corrupting their targets and would give rise to tolerant persister cells. In the present study we wanted to investigate whether these TA modules play a role in *B. cenocepacia* biofilm tolerance towards different antibiotics.

Using bioinformatics tools, 17 pairs of genes were identified as possible TA modules in the genome of *B. cenocepacia* J2315. Based on microarray data, which indicated that these genes were upregulated in biofilms treated with tobramycin, RNA of treated and untreated *B. cenocepacia* J2315 biofilms and planktonic cultures was extracted and analyzed by qPCR. The expression of these TA modules in treated sessile and planktonic cultures was compared to that in untreated cultures. Toxin overexpression mutants with a rhamnose inducible promoter were constructed and evaluated for growth, resistance, biofilm formation and response to antibiotics.

Generally the toxins showed a higher basal expression in sessile cells than in planktonic cells, but the expression patterns were similar for most T-AT pairs. Four toxins were upregulated in all conditions tested, whereas only one toxin was systematically downregulated. Operons showing similarity with *mazEF*, *relBE* or *mqsRA* and five other TA modules were significantly upregulated in biofilms and planktonic cultures after treatment with tobramycin but not after treatment with ciprofloxacin. None of the operons was upregulated in both conditions after treatment with ciprofloxacin. Others were only upregulated in sessile or in planktonic cultures, which indicates that planktonic and sessile cells may have different mechanisms to regulate the relative amounts of T/AT.

Overexpression of toxins had different effects on growth, biofilm formation and the number of surviving cells after treatment. For example, overexpression of *RelE* or *MazF* resulted in a prolonged lag phase and more cells surviving antibiotic treatment. These results indicate that various TA modules may play a role in tolerance and persistence but that their exact contribution depends on the mode of growth and the antibiotic used.

Interfering with regulatory RNAs in bacteria

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In eukaryotes, RNA interference (RNAi) is routinely used as a powerful experimental technique to silence gene expression. This technique employs the cellular mechanisms involved in the degradation of targets by mimicking naturally occurring small interfering RNAs (siRNAs) or microRNAs (miRNAs). In that way, there can be attenuation of translation or mRNA degradation to silence any gene of interest. This technique is extended to prokaryotes, where RNA interference comprises of RNA molecules that cover the Shine-Dalgarno sequence or the start codon within the ribosome binding region to prevent the translation of the mRNA of interest, based on the traditional working mechanism of bacterial small non-coding regulatory RNAs (sRNAs). Another technique in the miRNA field in eukaryotes uses antisense oligonucleotides (antimiRs) for miRNA inhibition. These antimiRs sequester the miRNA and thus compete with cellular target mRNAs leading to functional inhibition of the miRNA and derepression of the direct targets. We wondered whether the same technique can be extended to prokaryotes as well. To interfere with the sRNAs, an interesting characteristic of these molecules can be used. They have a conserved target binding region that is often essential and sufficient for target repression by the particular sRNA. We studied the possibility of interfering with this regulatory region via antisense oligonucleotides and thereby interfering with the function of these sRNAs and the whole pathway in which these important gene expression regulators are involved. The sRNA we used to test this hypothesis is CsrB, one of the sRNA regulators of the RNA binding protein CsrA. CsrA is a global regulator that has an influence on multiple different pathways like carbon storage regulation and biofilm formation. Our results show that it is indeed possible to influence CsrB regulating pathways by expressing RNA molecules that are complementary to these target binding regions. Moreover, dependent on which bases are included in the antisense construct, the impact on the CsrB regulating pathways varies. This makes it a promising sequence specific tool to interfere with sRNAs and indirectly with their target mRNAs.

Protease secretion of *Staphylococcus epidermidis* isolated from biofilms on endotracheal tube biofilms and the effect on *Staphylococcus aureus* Mu50 biofilms

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Staphylococcus epidermidis is part of the human skin flora. However, it is also an opportunistic, nosocomial pathogen causing a variety of infections. The major virulence factors of *S. epidermidis* are its ability to form biofilms on indwelling medical devices and its increasing resistance towards a whole range of antibiotics. *S. epidermidis* also produces an extracellular serine protease (Esp) which inhibits *Staphylococcus aureus* biofilm formation. In the present study, we investigated *S. epidermidis* obtained from biofilms on endotracheal tubes (ET) of 20 mechanically ventilated patients. In total, 33 *S. epidermidis* isolates were obtained from ET biofilms. Extracellular protease production by *S. epidermidis* isolates was determined by the azocaseine assay. 97% (32/33) of ET *S. epidermidis* isolates produced extracellular proteases. Specific protease inhibitors were used to determine the types of the secreted proteases and we observed that most isolates produced a mixture of serine, cysteine and metalloproteases. The effect of extracellular protease production of *S. epidermidis* isolates on *S. aureus* Mu50 biofilms was investigated and a significant reduction in biomass of *S. aureus* Mu50 biofilms was observed. In addition, viability of *S. aureus* Mu50 biofilms treated with supernatant (SN) of protease positive *S. epidermidis* isolates was decreased. Confocal laser scanning microscopy showed that *S. aureus* Mu50 biofilms treated with the SN of protease positive *S. epidermidis* isolates were less mature and thinner than non treated ones. A *C. elegans* infection model was used to elucidate if *S. epidermidis* isolates protect *C. elegans* against a *S. aureus* Mu50 infection. Indeed, an increased survival was observed if *C. elegans* were co-infected with *S. aureus* Mu50 and protease positive *S. epidermidis* isolates compared to an infection with *S. aureus* Mu50 alone. We also investigated the relation between the production of extracellular proteases by *S. epidermidis* and the presence of *S. aureus* in ET biofilms. We observed that, in ET biofilms from which no *S. aureus* was isolated, *S. epidermidis* isolates produced relatively higher amounts of extracellular proteases compared to ET biofilms from which both *S. epidermidis* and *S. aureus* isolates were obtained. Hence, our data suggest that there is a relation between the production of extracellular proteases by *S. epidermidis* and the absence of *S. aureus* in ET biofilms.

Contribution of stress-activated Insertion Sequences to metal resistance

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Genetic rearrangements caused by horizontal gene transfer and mobile genetic elements (MGEs) play a significant role in bacterial evolution. It is hypothesized that mobility of these MGEs is enhanced when the host is faced with environmental challenges. Our aim is to scrutinize the mechanisms and contribution of stress-activated MGEs to genome plasticity and concomitant adaptability of the host cell to environmental factors, such as radiation, temperature, pressure and toxic metal concentrations. In this study, *Cupriavidus metallidurans* AE126, a derivative of *C. metallidurans* CH34 cured of the plasmid carrying the most important determinants for zinc resistance (i.e. pMOL30), was selected as model organism. The possible role of Insertion Sequence (IS) elements in the adaptive behavior of *C. metallidurans* AE126 to toxic zinc concentrations was scrutinized.

It was found that strain AE126 could readily acquire increased resistance to zinc. This phenotype was at least partly caused by transposition of IS elements in the regulatory regions of the *cnr* (cobalt-nickel) resistance system on plasmid pMOL28. Cnr is a RND-family (resistance, nodulation and cell division) metal efflux pump comprising three components that span the complete cell wall and mediate cation/proton antiporter efflux. Resistance to cobalt and nickel is mediated by an intricate coordination between the regulatory locus, *cnrYXH* and the structural locus, *cnrCBA*. Detailed genetic analysis of this adaptation process revealed two zinc resistant AE126 mutant populations. The number of mutants obtained after the first two days of incubation in the presence of toxic zinc concentrations displayed a high variance-to-mean ratio, indicating that these mutants incurred spontaneous mutations that arose during cell divisions prior to the challenge with zinc. The second population that emerged in the ensuing days, however, displayed a significantly decreased variance-to-mean ratio, suggesting zinc-induced mutagenesis might have played a role in the creation of these mutants. Interestingly, the majority of the latter mutants were found to contain IS insertions in the *cnrYXH* locus, and likely overexpress the CnrCBA efflux pump due to inactivation of the CnrY or CnrX anti-sigma factor. Our current results therefore suggest zinc-stress induced mobility of IS elements, and the identification of the elements involved is subject of future research.

Temperature-induced mutagenesis and mortality in *Cupriavidus metallidurans* CH34

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The facultatively hydrogenotrophic *C. metallidurans* CH34 was isolated from metallurgical sediments in Belgium in 1976 and has been studied ever since for its resistance towards multiple heavy metals, its potential to accept and express foreign genes and, its applicability in environmental biotechnology. CH34 is well-equipped to overcome environmental stresses as it harbors a high amount of various mobile genetic elements, including 21 different insertion sequence (IS) elements, 11 genomic islands, 5 transposons, and 2 plasmids. It was also observed that growth of *C. metallidurans* CH34 at 37°C in rich media leads to high mortality, with ca. 10^{-5} surviving cells compared to growth at non-restrictive temperature (30°C). This effect is called 'temperature-induced mutagenesis and mortality' (TIMM), since survivors typically incur different mutations (e.g. loss of autotrophic growth, auxotrophy) and an increased redistribution of IS elements.

The aim of this study is further investigating this TIMM mutation phenomenon of *C. metallidurans* strains at the molecular level. The first question which was addressed was whether this TIMM phenotype is universal to the species or strain specific. Secondly, the influence of the growth medium was evaluated. The third question which was addressed was whether the mutations facilitating survival at 37°C are actually induced by exposure to 37°C (i.e. stress-induced) or rather predate this exposure (i.e. spontaneous).

It was shown that not all *C. metallidurans* strains display TIMM and analysis of 22 strains indicated that 5 strains escaped this phenomenon. Remarkably, TIMM is only induced at 37°C when *C. metallidurans* CH34 is incubated in rich media (e.g. Lysogeny Broth but not in minimal medium unless it was supplemented with 0,5% casamino acids). The role of each amino acid in TIMM is currently being examined. A fluctuation test was performed to distinguish between the spontaneous or stress-induced mutation hypotheses. Interestingly, the results revealed two different populations of survivors emerging at 37°C. The number of mutants obtained after the first two days of incubation at 37°C displayed a high variance-to-mean ratio, indicating they reflected spontaneous mutations incurred prior to growth at 37°C. The second population that emerged in the ensuing days, however, displayed a significantly decreased variance-to-mean ratio, suggesting that temperature-induced mutagenesis might have played a role in the creation of these mutants. A question which still remains is whether the mutations facilitating survival at 37°C target the same loci in both populations.

Our current results show that the TIMM phenotype is strain and medium specific, and is the result of both pre-existing as well as stress-specific induced mutagenesis events. The role of amino acids in this phenotype and whether the mutations facilitating survival at 37°C target the same loci in both populations will be further investigated. In a next phase we will identify genes responsible for the TIMM phenotype.

Bacterial persister cells as a strategy to evade extinction by antibiotic therapy: the epigenetic nature of persistence

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Bacteria possess an inherent strategy, namely persistence, to evade extinction by antibiotics used to treat infectious diseases. Almost any isogenic bacterial population contains a small fraction of specialized "persister cells" that can survive exposure to high doses of antibiotics. These cells are present before the event of disaster and, as such, are an insurance against unpredictable stress situations. Persisters do not divide during exposure to unfavorable conditions. However, when conditions improve, surviving persisters can resuscitate and give rise to a new population exhibiting the same antibiotic susceptibility as the original population. These findings show that persister cells are in fact not resistant mutants, but rather possess a phenotypic tolerance against antibiotics while being genetically identical to the bulk of the sensitive cells in the population. The presence of persister cells in biofilms significantly contributes to the clinical relevance of this phenotype in chronic infections as persisters within biofilms are shielded from the immune response. Recently, it was shown that persister cells are a likely cause of the chronic nature of *Pseudomonas aeruginosa* infections in cystic fibrosis and of *Candida albicans* infections in immunocompromised cancer patients. Moreover the fraction of persister cells was shown to increase over time during the infection as a result of prolonged antibiotic treatment. As such, persisters pose a serious threat to future antibacterial therapy of chronic infections.

Recent research has shown that many cellular pathways underlie bacterial persistence, yet little is known about epigenetic mechanisms involved in this complex phenotype. Moreover, although the fraction of persister cells within an isogenic population is assumed to be genetically determined, it is becoming increasingly clear that one strain can produce different amounts of persister cells in different environments. In this study, we aim to examine environments and treatment regimens selective for a high/low persistence phenotype. Elucidating the cause of these alterations will allow us to identify both genetic and epigenetic mechanisms affecting persistence. In this way, new targets to eradicate persisters or to reduce their formation can be identified, ultimately leading to new treatment regimens to prevent the formation of persisters or to limit the selection for highly persistent strains.

Optimizing antiviral and anticancer chemotherapy by the development of mycoplasma-insensitive prodrugs

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Mycoplasmas are bacteria that are characterized by the total lack of a cell wall and a strongly reduced genome. They are not able to synthesize pyrimidine and purine nucleosides *de novo* but instead rely on the expression of salvage enzymes to recuperate nucleic acid building blocks from the environment. Several studies have reported a high mycoplasma colonization rate in human tumor tissue and mycoplasmas are known to be the cause of several secondary infections in immunocompromised individuals such as patients suffering from AIDS. The treatment of cancer and many viral infections is largely based on the use of nucleoside-derived therapeutics which are often subject to inactivation by catabolic nucleo(s)(t)ide-metabolizing enzymes. We found that the cytostatic/antiviral activity of several clinically relevant drugs is severely compromised in the presence of a mycoplasma infection and that the multisubstrate mycoplasma-encoded pyrimidine nucleoside phosphorylase (PyNP) is responsible for the metabolic inactivation of these drugs. *Mycoplasma hyorhinis* PyNP was expressed as a recombinant protein and its kinetic properties were studied. The protein showed a high affinity for the phosphorolysis of various uridine- and deoxyuridine-based drugs, explaining their decreased cytostatic activity in the presence of a mycoplasma infection. We designed and synthesized a novel prodrug of the anti-cancer agent 5-fluoro-2'-deoxyuridine (floxuridine) and found it to be insensitive to inactivation by mycoplasmas. In contrast to the parent compound, the prodrug NUC-3073 retains its cytostatic activity in mycoplasma-infected tumor cell cultures. The development and assessment of such prodrugs may therefore be instrumental in the rational optimization of nucleoside-based antiviral/cancer chemotherapy in mycoplasma-infected individuals.

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HIV-1 Vpr is Nα-terminally acetylated: a functional exploration.

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The HIV-1 Vpr protein is a 14 kDa accessory protein and late viral product. Although Vpr is not required for efficient replication in dividing cells, Vpr has many functions. Vpr is believed to participate in the docking of the HIV-1 pre-integration complex to the nucleus and to facilitate its transport to the core. Vpr also induces cell cycle arrest in the G2 phase, favoring transcription from the HIV-1 LTR and is able to transactivate it. Other features of the Vpr protein are its incorporation into HIV particles and its presence in the serum of infected patients, where it can induce similar effects in penetrated bystander cells. Moreover, increasing evidence suggests its role in viral pathogenesis.

The N-terminal amino acid sequence of the HIV-1, SIVmac and SIVcpz Vpr proteins is fully conserved. Given the nature of the second amino acid, Vpr is expected to be Nα-terminally acetylated by the human NatB complex. Nα-terminal acetylation is one of the most common protein modifications in eukaryotes occurring in about 80%-90% of all human soluble proteins and is believed to affect protein stability, degradation and function. We confirmed Nα-terminal acetylation of Vpr by the N-terminal COFRADIC technique in infected Jurkat cells (unpublished results) and set out to determine possible functions of this highly conserved co-translational protein modification.

The HIV-1 NL4.3 wild-type Vpr protein, was cloned into a retroviral expression vector allowing detection and quantification by the independent co-expression of a reporter gene (Human Nerve Growth Factor Receptor). Point mutations were introduced abolishing Nα-terminal acetylation partly or completely. Functional protein testing of Vpr wild-type and mutant proteins was conducted on transduced Jurkat E6.1 cells or JLTRG cells, a reporter cell line expressing eGFP under the control of the HIV-1 LTR. Protein stability/expression, induction of G2 arrest, protein localization and transactivation capacities were evaluated. In parallel, the Vpr protein of a NL4.3 HIV-1 reporter virus was similarly mutated. Mutations in the N-terminal part of Vpr inevitably results in mutation of the C-terminal EDRW domain of Vif. Extra controls were taken along, such as Vif complementation experiments, a Vif deletion mutant and a Vif mutant resulting in the expression of a truncated Vif protein, deleted for the sequence overlapping with the Vpr open reading frame. Vpr WT and mutant stability/expression was evaluated within the HIV context, as well as its incorporation into viral particles. Furthermore, infectivity of these point mutants was evaluated in primary cells, after complementation of the viral producer cells, and in cell lines, and replication was monitored in Vif permissive cell lines only.

Results show protein function is unaffected by the point mutations in the Vpr protein, even though protein stability seemed to be affected. In the context of a functional replication competent reporter HIV-1 virus, the Vpr mutants also show a lower stability/expression in infected cells which results in the diminished incorporation of Vpr mutants into the HIV particles. Despite this, and after Vif complementation, infection rates were comparable between all conditions. Also, during replication we could not demonstrate any difference between conditions.

Our results suggest Nα-terminal acetylation of the HIV-1 Vpr protein is not essential or required for the functions tested, but affects the protein's stability or expression within infected cells. Tests to confirm this hypothesis are currently ongoing.

A well-timed trigger for *Salmonella* biofilm formation: the Hfq-dependent switch

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It has been assumed that the most abundant life style of bacteria is not planktonic but sessile growth, namely within biofilms. Biofilms are structured, multicellular communities offering a protected environment to the bacteria. The importance of bacterial biofilms is reflected by the fact that about 80 % of bacterial infections can be linked to biofilms, pinpointing the importance of this phenotype for pathogens such as *Salmonella*. Regulation of *Salmonella* biofilm formation is complex, involving many actors at the transcriptional level. However, there is a growing awareness that post-transcriptional regulators come in the act as well. The best described and most abundant class of non-coding RNAs (sRNAs) act by direct base pairing with their mRNA-targets, thereby influencing mRNA stability, processing, and/or translation. These interactions are facilitated by the chaperone Hfq. We have noticed that a *Salmonella* Hfq mutant, which is severely affected in post-transcriptional regulation, is unable to form mature biofilms (Kint *et al*. (2010) *BMC Microbiol* 10:276). This indicates the importance of post-transcriptional control for biofilm formation.

Based on this observation, we performed a comparative phenotypic analysis of *S.* Typhimurium wild-type and *hfq* knockout mutant during initial biofilm formation. Confocal microscopy shows that the *hfq* mutant has a completely altered 3D-multicellular biofilm phenotype. By analyzing the distribution of planktonic versus biofilm cell counts in function of time during initial attachment, we pinpointed a specific time point 'T' at which the biofilm formation of the *hfq* mutant started differentiating from the wildtype structure. We continued with gene expression measurements of key regulatory genes in the biofilm regulatory network. At the crucial time point 'T', a specific branch within the complex regulation network stays silent in the mutant, while it gets switched on in wildtype *Salmonella*.

Since the global effect of sRNA-regulators and Hfq on bacterial gene expression is broad and many different genes are affected, we were wondering whether this Hfq-dependent switch is a result of a polygenic effect, for which a diverse set of genes are essential, or could be specifically explained by its effects on the few genes within the identified branch. Therefore we tried to complement the down-regulated branch of genes in an *hfq* mutant strain. Our result show that the pronounced effect of an *hfq* mutant on the biofilm phenotype can probably be largely explained to post-transcriptional control of only a limited set of genes from the complex biofilm regulatory network.

Replication characteristics of equine arteritis virus *08P178* in equine polarized upper respiratory tract mucosal explants

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The upper respiratory tract mucosa represents the first line of defense, which has to be overcome by pathogens before invading the host. Considering the economical and ethical aspects involved in using experimental animals for pathogenesis studies, respiratory mucosal explants in which the three-dimensional architecture of the tissue are preserved, are ideal alternatives. Up till now, different respiratory mucosal explant cultures have been developed. However, none of them could be inoculated with pathogens solely at the epithelium side. In the present study, equine nasal and nasopharyngeal mucosal explants were embedded in agarose (3%) leaving the epithelium side free to allow apical inoculation. Morphometric analysis of epithelium, basement membrane and connective tissue did not show degenerative changes during 72 h of cultivation. The number of apoptotic cells in the epithelial and connective tissue slightly increased during time. The impermeability of agarose to virus particles was demonstrated by absence of streptavidin-labeled microspheres (40nm), lack of infection at the lateral and bottom sides of the mucosa and absence of RK13-EAV-positive cells seeded underneath the agarose layer in which inoculated explants were embedded. After validation, the agarose model was used to investigate equine arteritis virus (EAV) *08P178* replication characteristics and leukocytes tropism in respiratory mucosa. Nasal and nasopharyngeal agarose embedded mucosae of three horses were incubated for 1h with 1ml of medium containing $10^{7.6}$ tissue culture infectious dose ($TCID_{50}$) of EAV *08P178*. At 24, 48 and 72 hpi an immunofluorescence double staining for EAV nucleocapsid (N) protein and alternatively $CD172a^+$ cells (monocyte lineage), $CD3^+$ cells (pan T lymphocyte marker) or IgM^+ cells (B lymphocytes) was performed. The number of EAV-positive cells increased in time with a mean of 68 in $8mm^2$ of nasal mucosa and 225 in $8mm^2$ of nasopharyngeal mucosa at 72hpi. In particular, 74, 76 and 54 antigen-positive cells/$8mm^2$ were counted in nasal explants and 201, 254 and 220 EAV-positive cells/$8mm^2$ were quantified in nasopharyngeal explants of horse I, II and III, respectively. Specifically, in nasal mucosa, 22, 34 and 27% of EAV-positive cells were identified as CD172a+ at 24, 48 and 72hpi, respectively. The percentage of EAV infected cells positive for CD3 increased linearly from 10 (24hpi) to 19% (48 and 72hpi) during the experiment. In the nasopharyngeal mucosal explants, the percentage of EAV infected cells positive for CD172a increased from 22 (24hpi) to 26 (48hpi) and 45% (72hpi) over time, while the percentage of EAV infected CD3 cells reached 22% at 24hpi and decreased to 15% afterwards (48 and 72hpi). At 24hpi, EAV-positive cells were mainly localized in the upper layers of both nasal and nasopharyngeal mucosae and were scattered all over the connective tissue at later time points. In nasal explants were mainly detected clusters of positive cells whereas in the nasopharynx mainly individual positive cells. In general, individual EAV-infected cells were mostly found under the basement membrane with sporadic presence in between epithelial cells. In addition, in nasopharyngeal explants individual EAV-positive cells were frequently localized in the parafollicular area. Viral antigen-positive cells were not found at the cutting edges of the agarose embedded explants. Intracellularly, viral antigens were seen in the cytoplasm, in the form of small dots but mostly in the form of large masses occupying most of the cytoplasm. This study validates the usefulness of a polarized mucosal explant system and shows that mucosal $CD172a^+$ myeloid cells and $CD3^+$ T lymphocytes may represent an important target cells for EAV.

Regulating the glutamine synthase operon by Sa-Lrp, a glutamine-dependent Lrp-like regulator in *Sulfolobus acidocaldarius*

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Microorganisms need to adapt quickly to environmental and nutritional changes in order to maintain their fitness. These adaptations are mainly driven by regulation of gene expression. The family of the Lrp-like (Leucine-responsive Regulatory Protein) transcriptional regulators is one of the most abundant families of transcriptional regulators in archaea and bacteria. These regulators are either specific or global and may have different effects, depending on the targets and/or the presence of a suitable cofactor. Bacterial Lrp-like proteins are generally involved in the regulation of the amino acid metabolism, but the archaeal regulators are also involved in central metabolic processes.

Sa-Lrp binds to the control region of its own gene with a higher binding affinity in the presence of L-glutamine. This effect is specific since none of the other amino acids affected the DNA-binding affinity. Moreover, glutamine also enhances the *in vitro* binding specificity of Sa-Lrp approximately 13 times. Several potential target genes of Sa-Lrp were investigated by *in vitro* binding (EMSAs). Because L-glutamine plays a crucial role in the *in vitro* binding, target genes involved in nitrogen metabolism were chosen for EMSAs. One of the Sa-Lrp targets is the control region of glutamate synthase (GOGAT).

In order to gain further insight into the binding regions of Sa-Lrp to the glutamate synthase promoter, the *in situ* phenantroline-Cu footprinting technique was applied. Two discrete regions were protected upon binding of Sa-Lrp. These are approximately 20 base pairs long AT-rich regions separated by a short GC-stretch of 4 base pairs. To identify critical points of contact between Sa-Lrp and its target DNA, base removal (missing contact) interference techniques were applied.

To examine the DNA deformations induced by Sa-Lrp binding circular permutation assay and atomic force microscopy (AFM) were performed. With the circular permutation assay a major Sa-Lrp induced bending of about 86° was calculated. AFM was used to further visualize Sa-Lrp induced DNA deformations of the glutamine-bound Sa-Lrp-*gltB* operator complexes. These results demonstrate a considerable condensation of the *gltB* operator DNA upon Sa-Lrp binding, which strongly suggests that the DNA is wrapped around the Sa-Lrp protein.

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Long-term preservation of key players in the nitrogen and carbon cycle

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Introduction: Long-term preservation of key players of the nitrogen and carbon cycle has proven difficult, making them unavailable for the scientific community and limiting further research. While most do not survive freeze-drying, some can be cryopreserved successfully, but usually only for short periods only. Because of scarce data from the literature in combination with focus on glycerol as cryoprotecting agent (CPA) a suitable (and ideally universal) preservation protocol is lacking.

Methods: Five different CPA, the cryoprotective effect of carbon and concentration of CPA were tested for cryopreservation in either liquid nitrogen or -80°C. Scoring of viability (live-dead flow cytometry), growth (OD measurements, most-probable number analysis and plating), or specific activity was used to evaluate preservation success.

Results: Viability and growth recovery experiments of representatives of ten different methanotrophic species after cryopreservation for a one-year period consistently demonstrated the best results for dimethyl sulfoxide (DMSO) as CPA or a combination with 1% trehalose in tenfold diluted trypticase soy broth (TT). The latter condition even allowed complete recovery in viability and growth, while other conditions clearly induced a viable but non-culturable state (VBNC). Activity and growth recovery after cryopreservation of six nitrite-oxidizing bacteria (NOB) belonging to six different species further demonstrated that carbon compounds can successfully be used as sole CPA, but also showed that the choice of carbon compound and optimatised percentage of DMSO are strain-dependent. However, the success of DMSO + TT as combined CPA was indeed confirmed for these NOBs, as well as for several ammonium oxidizing (AOB) strains and (single cell) enrichment cultures of anaerobic AMOs and nitrite-dependent anaerobic MOBs.

Conclusion: Long-term preservation of fastidious key players of nitrogen and carbon cycle is possible and should be in depth tested further. Our results so far suggest that 5% DMSO (v/v) alone or combined with Tryptic Soy Broth may be good candidates as universally applicable cryoprotective agents.

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The PA4203 *Pseudomonas aeruginosa* LysR-type transcriptional regulator: determination of its regulon and of its DNA binding sites

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The opportunistic pathogen *Pseudomonas aeruginosa* has a large genome (6.2 Mb) with 5,500 ORFs. Ten percent (~500 genes) of the genes encode transcriptional regulators, which reflect the high adaptability of this bacterium to changing environments. Among the different classes of regulators, the LysR regulators (LTTR, LysR type of transcriptional regulators), of which PA4203 is a member, form the largest class. Generally, these regulators control the transcription of neighboring genes by binding DNA via an N-terminal HTH motif and via the interaction with a ligand at the C-terminal end of the protein. PA4203 is a LTTR and is flanked by the PA4202 gene, which is transcribed in the opposite orientation, and by the PA4204 gene downstream, which is transcribed in the same orientation as PA4203. The function of PA4204 (*ppgL*) is known: it is involved in the conversion of a toxic gluconolactone to gluconate. PpgL (periplasmic gluconolactonase) is therefore a detoxification gene ("house cleaning gene"). The function of PA4202 is not known, but it encodes for a nitropropane monooxygenase which is known to be involved in the oxidative denitrification of toxic nitroalkanes to aldehydes and nitrite, suggesting that this gene is also capable in detoxifying the cell. We know from *lux*-fusion, microarray, qRT-PCR and ChIP-ChIP data that PA4203 is negatively autoregulating itself, directly repressing the expression of the PA4202 and PA4204 genes and (indirectly) repressing *hem*O (heme oxygenase) and PA1225 (NADPH dehydrogenase). However, the majority of the genes under control of PA4203 have shown to be (indirectly) up-regulated, including *pvdA* (involved in pyoverdine synthesis).

The direct regulation of PA4203 on its own promotor region, and those of the PA4202 and PA4204 genes, has still to be determined. However, recent electrophoretic mobility shift assays (EMSA's) of the 4203 protein to its own promotor region showed that the protein binds to it without the need of a cofactor. Adding gluconate as a cofactor inhibited the binding of the 4203 protein to its own promotor region, which means that in the presence of the cofactor, transcription of the 4203 regulated genes is de-repressed.

Phenotypical characterization revealed an increased swimming (flagellae) and swarming (type IV pili + flagellae) of the PAO1Δ4203 and PAO1Δ4204 mutant compared to the PAO1 WT. This observation was more profound on the iron-depleted CAA medium for the PAO1Δ4203. We also observed a decreased twitching motility for the PAO1Δ4204 mutant, indicating the overproduction of the biosurfactant rhamnolipid, indicating the involvement of the PA4204 gene in biofilm formation. Measurement of the pyoverdine production confirmed microarray and qRT-PCR data: pyoverdine production was decreased in the PAO1Δ4203 mutant compared to PAO1 WT.

Since PA4203 is repressing the PA4202 and PA4204 genes, which are involved in detoxifying the cell, and PA4204 produces to more rhamnolipids, this could indicate a role for PA4203 in biofilm formation and maintenance.

The role of regulatory T cells during infection with feline infectious peritonitis virus

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Feline infectious peritonitis virus (FIPV), a coronavirus with a near 100% fatality in cats, is thought to be controlled by a strong cell-mediated immunity. Regulatory T cells (Tregs), are a subset of lymphocytes that can suppress the mounting immune response during a normal immune reaction by direct interaction with effector T cells and the production of cytokines (IL-10 and TGF-β). Tregs have been implicated in many autoimmune diseases such as multiple sclerosis and Crohn's disease as well as in many chronic virus infections such as Epstein-Barr virus, herpes simplex virus and both human and feline immunodeficiency virus infections. The typical chronic infection and immunopathogenesis of FIPV suggests that Tregs might not be functioning correctly during infection. In the present study, Tregs were quantified in blood, mesenteric lymph node (mLN), spleen, kidney and mesentery of FIPV-infected cats as well as in control cats. Immunofluorescence staining were performed using monoclonal antibodies against CD3, CD4, CD8, CD25 and Foxp3 to detect regulatory cells. Flow cytometrical analysis revealed that classical Tregs (CD4+CD25+Foxp3+) were drastically depleted from the blood, mLN and spleen in FIP cats when compared to healthy cats. Additionally, other regulatory T cells (CD4+CD25-Foxp3+ and CD3+CD8+Foxp3+) were also found to be depleted from the immunological compartments. Inflamed tissue however, did not shown any differences in Treg numbers. These results indicate that during a FIPV infection, the number of Foxp3 regulatory cells is drastically lowered in the blood, mLN and spleen. This reduction was not due to migration to lymphoid organs nor to sites of infection and will contribute substantially to the unchecked overwhelming immune response and inflammatory state that follows FIPV infection and eventually causes the death of infected cats. Additional Treg functionality assays will further elucidate the role of Tregs in the immunopathology of FIPV infection and determine if Tregs can be of therapeutic use.

Genome-wide RNAi screening identifies cellular genes involved in Murid Herpesvirus 4 cycle

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Gammaherpesviruses are archetypes of persistent viruses that have been identified in a range of animals from mice to man. They are host-range specific and establish lifelong latency of immunocompetent hosts. Most of the gammaherpesvirinae members are associated with neoplastic diseases. For example, the best studied gammaherpesviruses are Human herpesvirus 4 and 8 that are respectively associated with Burkitt's lymphoma and Kaposi's sarcoma. By opposition to its human counterparts, Murid herpesvirus-4 (MuHV-4) is able to replicate to high titers in cultured cells and is therefore an excellent candidate for studying gammaherpesvirus cycle. RNA interference (RNAi) is a natural process that cells use to turn down, or silence, the activity of specific genes by inhibition of mRNAs. The selective and robust effect of RNAi on gene expression makes it a valuable research tool in cell culture because synthetic dsRNA introduced into cells can induce suppression of specific genes. RNAi may also be used for large-scale screens that systematically shut down each gene in the cell. Here, we describe the use of high troughput screening by reverse transfection of cells of 17 820 small interfering RNAs (siRNA), which, combined to fluorimetry explored 5940 human genes (Ambion silencer druggable genome). The data were analysed for each siRNA (3/gene) and the results were obtained by bioinformatics. 67 genes appeared to be potentially involved in MuHV-4 cycle and were specifically retested. Among these genes, protein encoded by 1 gene in particular recently emerged as membrane trafficking protein and could therefore be involved in cellular trafficking of viral particles.

Boom Clay pore water, home of a diverse microbial community

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SCK•CEN
Environment, Health and Safety
Molecular and Cellular Biology - Microbiology

The Boom Clay layer located at 230 m depth under the Mol site of SCK•CEN (Belgium) is presently investigated as potential host formation for the disposal of high-level nuclear waste. Using the HADES underground laboratory of SCK•CEN in this clay layer (Fig. 1), Boom clay and Clay pore water (BCPW), has been studied in this regard for over two decades. A reference composition for synthetic clay water has been derived earlier, mainly based on geochemical analysis of interstitial water sampled from different layers within the Boom clay. In this study, **the possibility of biological factors, microbes, interacting with future radioactive waste in Boom Clay is addressed.**

Similar to the previous characterization of the 'average' BCPW chemical composition, the **primary aim** of the presented microbiological study was to determine a representative BCPW bacterial community which can a.o. be used in laboratory studies. **Secondly**, the *in situ* activity and the metabolic properties of members of this community were addressed, aiming to assess their survival and proliferation chances in repository conditions.

In a **first approach** to address microbial presence, scanning electron microscopy (SEM) was performed and total microbial DNA of the community was extracted from ten BCPW samples from different clay layers. By polymerase chain reaction (PCR) on the highly conserved bacterial 16S rRNA genes in this DNA pool and subsequent sequencing and bio-informatic analysis, operational taxonomic units (OTUs) could be assigned to the bacterial community. In a **second approach**, microbial activity and metabolic capacity in BCPW samples was assessed by analysis of intracellular adenosine triphosphate (ATP) and cultivation in relevant, anaerobic media by most probable number technique (MPN). In a **third approach**, individual microbial strains were isolated, propagated and identified, in order to evaluate specific properties of cultivated subpopulations.

Based on SEM and DNA analysis, it became clear a large diversity of microbes were present and abundant in the clay pore water. **A core bacterial community (CBC)** of Boom Clay pore water samples was characterized, representing six bacterial phyla present in all BCPW samples. A combination of BCPW from three piezometer filters was selected as a representative microbial community inoculum for future lab scale experiments. This microbial community was proven to be not merely present, but also alive and active. Even without the addition of extra substrates for growth, microbial **activity** was indicated in sampled BCPW. Moreover, the bacteria were clearly capable of **proliferation** on a range of growth substrates..

The omnipresence of such a diverse and *in situ* active microbial community in Boom Clay pore water samples is surprising. Microbial **contamination** during piezometer installation and survival of introduced species during several years in stringent conditions are therefore considered quite credible. On the other hand, the indicated diversity of strict anaerobic micro-organisms with specific properties like sulphate reduction and sporulation invites speculations that **indigenous** micro-organisms, living in the clay since many centuries, will account for at least part of the observed viable community. The interaction of this clay microbial community with the waste disposal facility and sensor systems installed, and/or the radioactive waste itself, now needs to be further investigated.

Design of multiplex assays for molecular subtyping of pathogens with the Luminex xMAP technology

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Multiplex assays are a powerful tool for molecular subtyping of pathogens, which is crucial for rapid diagnosis, surveillance and identification and containment of outbreaks. The Luminex xMAP technology allows detection of up to 500 different analytes per sample in a high-throughput format through a liquid bead suspension array. Different types of Luminex assays can be envisaged. Each assay has its advantages and limitations, some of which are related to the inherent design of the primers and probes used. Software that predicts melting temperatures, hybridization structures and specificity for different types of oligonucleotides, and thereby simulating the multiplex assays *in silico* can facilitate the design of multiplex assays by reducing time and cost of experimentation.

We have compared two commercial software packages for the development and *in silico* simulation of a ligation dependent amplification (LDA) assay and a direct hybridization assay for the subtyping of a pathogen. The main findings, limitations and challenges will be discussed.

Immobilization of Pseudorabies Virus in Porcine Respiratory Mucus Revealed by Single Particle Tracking

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Pseudorabies virus (PRV) initially replicates in the porcine respiratory tract. It easily invades mucosae and submucosae for subsequent spread throughout the body via blood vessels and nervous system. In this context, it developed ingenious processes to overcome different barriers such as epithelial cells, which possess strong intercellular junctions, and the basement membrane. Another important but often overlooked barrier is the mucus layer which coats the surface of mucosae and blocks the access of PRV to epithelial cells. However, little is known about how the invading PRV particles interact with porcine respiratory mucus. We therefore measured the barrier properties of porcine respiratory mucus, and investigated the mobility of nanoparticles including PRV in this mucus. We developed an in vitro model utilizing single particle tracking. Firstly, pore size formed by the mucus elements was evaluated polyethylene glycol coupled (PEGylated) nanoparticles and atomic force microscop. Secondly, the mobility of PRV, negative, positive and PEGylated nanoparticles in the mucus was examined. We discovered that pore size of porcine respiratory mucus ranged from 80 to 1500 nm, with an average diameter of 455±240 nm. PRV (ζ potential: -31.8±1.5 mV) experienced a severe obstruction in porcine respiratory mucus, diffusing 59-fold more slowly than in water. Diffusion of PRV in mucus was found to be substantially reduced, similar as the negative (-49.8±0.6 mV) and positive (36.7±1.1 mV) nanoparticles, and it was 30-fold lower than that of nearly neutral PEGylated nanoparticles (-9.62±0.8 mV). These findings clearly show that mobility of PRV was significantly hindered in porcine respiratory mucus, and the inhibition was not due to steric obstructions.

Novel Antimicrobial Compounds from *Pseudomonas* Secondary Metabolites

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Pseudomonas aeruginosa and *Staphylococcus aureus* are two major pathogens involved in an array of infections (cystic fibrosis, post-surgery). By acquiring antibiotic resistance these pathogens easily outdate our conventional antibiotic stock. Thus it is crucial to find more antimicrobial molecules which possess novel structures and unique inhibitory mechanisms. Fluorescent *Pseudomonads* are known to produce a remarkable array of secondary metabolites. There are non-ribosomal peptides with antimicrobial, surfactant, or siderophore activities, and polyketides such as mupirocin, active against *S. aureus*.

In this research, one orphan gene cluster, which is responsible for the synthesis of an anti-*S. aureus* molecule was found in a *P. fluorescens* type strain. By comparing the HPLC profiles of culture extractions, 2 fractions in hydrophobic part were isolated from the culture of wild type strain. Both of the 2 fractions have anti-*S. aureus* activity. By investigating its production under different conditions, we found that this molecule is produced under nutrition limitation. We hypothesize that this molecule could give the bacterium a competitive edge in extreme nutrition-low environments.

One environmental isolate of *Pseudomonas* spp. strain W15Oct28 was found to produce a novel type of pyoverdine, a broad spectrum antimicrobial compound and a biosurfactant. The productions of both pyoverdine and the antimicrobial molecule are iron regulated. Several pyoverdine negative mutants obtained by plasposon mutagenesis also lost their antimicrobial activity, which indicates that the production of the two compounds shares one common biosynthesis step, while, the biosurfactant is produced independently. LC-MS results pointed out that this biosurfactant is very similar to putisolvin. The pyoverdine is hydrophilic and has a brown color, while the antimicrobial compound is hydrophobic and gives a white powder. LC-MS analysis indicated the molecular weights of pyoverdine and the antimicrobial compound are 1625 and 1395, respectively. The study of the structure of this novel antimicrobial molecule and its inhibitory mechanism is ongoing.

Phylogenetic analysis and polyphasic characterization of *Clavibacter michiganensis* population isolated from tomato seeds.

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The bacterial genus *Clavibacter* includes five subspecies, all of which are plant pathogens of specific hosts and most of them belong to quarantine or q-alert organisms. *Clavibacter* belongs to pathogens of economically important crops and causes serious economic damage. Losses are due to actual yield reduction and also due to statutory measures taken to eliminate the disease. Moreover, despite many attempts to breed resistant varieties no resistant plant cultivars are known. In this PhD we focus on a pathogenic to tomato *C. michiganensis* subsp. *michiganensis* (Cmm) which is considered one of the most destructive bacterial diseases of this crop. Despite efforts to prevent seed contamination, new introductions are regularly detected in Europe, associated with new regions of tomato seed production. It seems as if the expanding diversity of Cmm also challenges the limited host range**.**

Clavibacter michiganensis – like strains isolated from tomato seed are phenotypically similar to genuine Cmm in the common semi-selective media and are identified as Cmm in the customary tests but are nonpathogenic to tomato. They formed a separate cluster in *gyrB* sequence analysis and MALDI-TOF mass spectrometry. Their presence prevents clear judgment on the health status of the seeds. As their function, nature and economic impact are unclear we aimed to investigate and compare them to genuine Cmm.

Eleven nonpathogenic strains isolated from tomato seeds together with a selection of genuine Cmm strains were subjected to analysis of core genes *dnaA* and *gyrB*, together with detection of virulence/pathogenicity factors i.e. *cel*A, *tom*A, *ppa*A, *chp*C and *pat*-1. Virulence genes present in Cmm's could also be detected in some of the look-alikes. DNA-DNA hybridization and sequence analysis of *gyrB* and *dnaA* proved that they belong to the *Clavibacter michiganensis* species and can be unambiguously separated from Cmm. Evidence of the non-pathogenic nature of the Cm look-alikes was obtained in pathogenicity tests in tomato plantlets. Leaf spots or wilting could not be induced after local or systemic inoculation. Tomato stems were not colonized nor there was evidence of survival in tomato stems. Most of the look-alikes contained only one plasmid and showed no cellulase activity on M9CMC plates. The BOX patterns displayed a higher diversity among nonpathogenic strains than in the genuine Cmm collection. We speculate that tomato seeds likely represent a larger reservoir of unexplored *Clavibacter* diversity.

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