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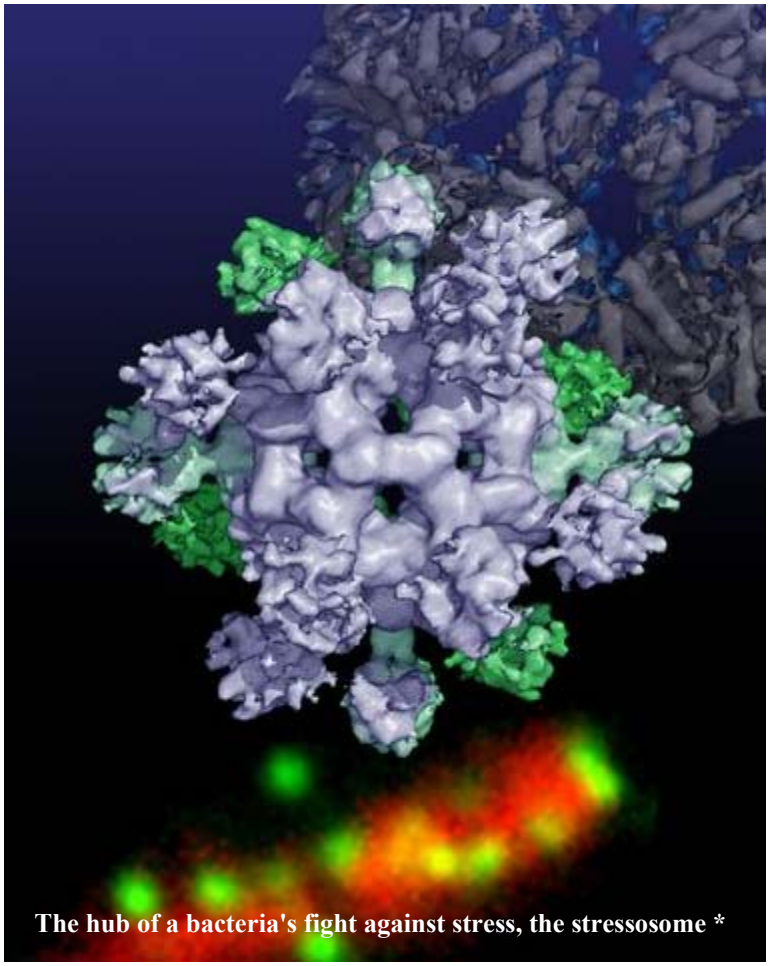
STRESS RESPONSES IN THE MICROBIAL WORLD



The hub of a bacteria's fight against stress, the stressosome *

Brussels, December 12, 2008

** With acknowledge for the figure to: Jon Marles-Wright, et al., Molecular Architecture of the "Stressosome," a Signal Integration and Transduction Hub. Science, 322, 92-6 (2008)*

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December 12th, 2008

House of the Academies
Hertogsstraat 1, Rue Ducale
Brussels

PROGRAMME

08.30 Registration desk open – Poster mounting

09.00 Welcome & Opening

09.10 **Mark Roberts,** Faculty of Veterinary Medicine, University of Glasgow, UK

"Salmonella survival in a stressful world"

09.50 **John Sinclair**, Department of Medicine, Cambridge University, UK

"Towards an understanding of the molecular basis of human cytomegalovirus latency"

10.30 Break

11.15 **Johan Thevelein,** Laboratory of Molecular Cell Biology, K.U.Leuven

"Nutrient control of general stress resistance in yeast"

11.55 Short communication (1) of selected poster *(see page 29)*

12.10 **Milton S. da Costa**, Dep. de Bioquímica Universidade de Coimbra, Portugal

"Compatible solutes in Thermophiles and Hyperthermophiles from the esoteric to the applied"

13.00 General Assembly BSM

Lunch – Poster viewing & poster discussion

Parallel session 1 – Virology

14.30 **Markus Heim,** Department of Research, University Hospital Basel, Basel, Switzerland

"Interferon alpha signaling in viral hepatitis"

15.15 Short communications (5) of selected posters *(sequence of speakers: see page 29)*

16.30 General conclusions and poster prize

Parallel session 2 – Bacteriology

14.30 **Jeremy S. Webb,** School of Biological Sciences, University of Southampton, UK

"Cellular differentiation in microbial biofilms"

15.15 Short communications (6) of selected posters *(sequence of speakers: see page 29)*

16.45 General conclusions and poster prize

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ABSTRACTS OF INVITED LECTURES

Salmonella survival in a stressful world

Mark Roberts, Institute of Comparative Medicine, Faculty of Veterinary Medicine
University of Glasgow, Glasgow UK.

Bacterial stress responses allow pathogens to perceive and adapt to deleterious environments inside and outside of their hosts. The surface of bacterial pathogens is a major target for the antibacterial defences of the host. In *Salmonella enterica* serotype Typhimurium and other enteric Gram negative pathogens a group of stress response systems known collectively as extracytoplasmic (or envelope) stress responses (ESRs) deal with perturbations to the cell envelope. There are at least five ESRs in *S.* Typhimurium, the most important for salmonella virulence is that controlled by the alternative sigma factor, RpoE (sigma E). The RpoE pathway is activated in salmonella in response to accumulation of outer membrane proteins in the periplasm. Under standard laboratory growth conditions RpoE is dispensable. However RpoE is essential for growth and survival of *S. typhimurium* during infection. RpoE is necessary for survival of *S. typhimurium* in macrophages where it provides protection against oxidative stress and antimicrobial peptides. Many RpoE regulated genes have now been identified and their roles and importance will be discussed.

Towards an understanding of the molecular basis of human cytomegalovirus latency

John Sinclair, Department of Medicine, University of Cambridge, UK

Latent carriage of virus, defined as the ability of virus to persist in the host in the absence of production of infectious virions, is an important mechanism for viral persistence in the face of potent host immune responses. Latency is a defining biological property of the herpesviruses, such as human cytomegalovirus (HCMV). One site of latency of HCMV is in cells of the myeloid lineage. Although viral genome is detectable in all myeloid cell types, viral lytic gene expression appears to be critically dependent on their state of differentiation: viral immediate early (IE) gene expression is silenced in CD34+ cells and monocytes but terminal differentiation to macrophages or dendritic cells results in the induction of viral IE gene expressionand reactivation from latency. This differentiation-dependent control of IE gene expression appears to be regulated by chromatin remodeling of HCMV major IE promoter (MIEP) Although our understanding of HCMV latency and reactivation in the myeloid lineage has increased substantially, a number of important questions remain unanswered. I will use experimental and natural latent myeloid cell models to describe our ongoing experiments to analyse latent replication of viral genomes, expression and function of viral latency-associated RNAs and the effects of latency and reactivation on cellular gene expression.

Nutrient control of general stress resistance in yeast

Johan M. Thevelein[1,2]

[1]*Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, Katholieke Universiteit Leuven and* [2]*Department of Molecular Microbiology, VIB; Kasteelpark Arenberg 31, B-3001 Leuven-Heverlee, Flanders, Belgium*

Yeast cells, like all microbial cells, are subject to rapidly fluctuating environmental conditions. This includes the availability and quality of nutrient sources and changes in physical conditions, such as high or low temperature, high osmolarity, oxidative stress, etc. Yeast cells display dramatic differences in their tolerance and response to stress conditions depending on their mode of growth: fermentative or respirative, as well as their stage in the life cycle. Cells that are rapidly growing on a fermentable sugar, like glucose, fructose or sucrose, have the lowest stress resistance. This is correlated with very low levels of the stress-protective sugar trehalose and very low expression levels of stress-response genes, like Hsp chaperone encoding genes. Yeast cells that grow with respiration on nonfermentable carbon sources, like glycerol or acetate, multiply more slowly and display higher stress resistance. Also stationary phase cells have higher stress resistance than multiplying cells. This is also true for fermenting cells that are starved for an essential nutrient, like nitrogen, phosphate or sulfate, and as a result accumulate in stationary phase. Also in these cases the level of stress tolerance correlates with the trehalose content and expression of stress-response genes, which are both much higher than in fermenting cells. Strikingly, when a fermentable sugar is added to respiring cells or a lacking essential nutrient, like nitrogen, phosphate or sulfate, is added to fermenting cells starved for such a nutrient, there is a rapid loss of general stress tolerance. This is in all cases associated with a very similar rapid mobilization of trehalose and disappearance of messenger RNA's of stress responsive genes. These observations indicate that yeast cells have sensing mechanisms for the detection of major nutrients that apparently activate a common signaling pathway triggering the molecular events underlying the rapid loss of general stress resistance. The cAMP-PKA pathway has been identified as a major player for nutrient control of stress tolerance. Fermentable sugars activate this pathway by increasing the cAMP level whereas amino acids, ammonium and phosphate trigger activation of PKA without using cAMP as a secondary messenger. Apparently, these nutrients activate sensing systems that make use of novel mechanisms for activation of PKA. The discovery of a G-protein coupled receptor for sugar sensing and transceptors for the other nutrients offers an explanation for this difference. The G-protein coupled receptor Gpr1 senses glucose and sucrose and activates adenylate cyclase via the Galpha protein Gpa2. Amino acids, on the other hand, are sensed by the Gap1 amino acid permease which fulfills a double role as amino acid transporter and receptor. The name of transceptor has been suggested for transporter-related receptors. Recent work has identified amino acid analogues that act as agonists for activation of the signaling function of Gap1 but are not transported by Gap1. Also competitive inhibitors of Gap1 transport have been identified that are unable to trigger signaling. These results indicate that signaling agonists have to induce a specific conformational change which may be part of but does not require the complete transport cycle. SCAM analysis has also revealed that the same amino acid binding site in Gap1 is used both for transport and for signaling with transported and nontransported agonists. Similar results were obtained for the Pho84 phosphate permease which functions both as a phosphate transporter and phosphate receptor for rapid activation of the PKA pathway in phosphate-starved fermenting cells. Ammonium sensing is performed mainly by the Mep2 ammonium transceptor. Other environmental variables such as heat shock also influence the PKA pathway. Heat shock triggers enhanced stress tolerance by inducing expression of stress-responsive genes and trehalose accumulation, counteracting the strong downregulating effect of a complete fermentable growth medium. How heat shock interferes with the cAMP-PKA pathway is not well understood. Also in other fungi, the cAMP-PKA pathway may play a role as integrator of environmental variables, such as the nutrient composition of the medium, and physiological properties such as the level of stress tolerance.

Compatible solutes in Thermophiles and Hyperthermophiles: from the esoteric to the applied

Milton S. da Costa and Nuno Empadinhas

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Many organisms that live at very high temperatures have been isolated from shallow marine and abyssal thermal environments, where the geothermal water may reach the salinity of the surrounding seawater. These organisms, like all other microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environments. The majority of microorganisms adjust osmotically by the selective accumulation of small molecular weight organic compounds. Thermophiles and hyperthermophiles accumulate a few compatible solutes that are also common in mesophilic bacteria and archaea, namely trehalose and glutamate and even the very rare glucosylglycerate (GG). However, the majority of the compatible solutes encountered in hyper/thermophiles are unique to these organisms. These compatible solutes include mannosylglycerate, di-myo-inositol-phosphate and the very rare compatible solutes di-glycerol-phosphate, di-mannosyl-di-myo-inositol-phosphate and mannosylglyceramide.
In recent years we have studied the synthesis of mannosylglycerate (MG) and trehalose in *Thermus thermophilus* (Phylum *Deinococcus*/*Thermus*), *Rubrobacter xylanophilus* (Phylum *Actinobacteria*) and *Persephonella marina* (Order *Aquificales*). The species of the genus *Thermus* have optimum growth temperatures that range between 70 and 75ºC and, with the exception of *Thermus thermophilus*, a maximum growth temperature below 80ºC. The species *Thermus thermophilus* have a maximum growth temperature of about 82 to 83ºC. This species is also capable of growing in media containing 3 to 5% NaCl. The strains of *T. thermophilus* accumulate primarily trehalose and lower levels of mannosylglycerate (MG) during osmotic adjustment. Recombinant mutants lacking the genes for the synthesis of trehalose, MG or both, result in a profound effect on the ability of organisms to grow in media containing NaCl. The synthesis of MG by *T. thermophilus* proceeds via a two step pathway catalyzed by mannosyl-phosphoglycerate synthase (MpgS) and mannosyl-phosphoglycerate phosphatase (MpgP) from GDP-mannose and 3-phosphoglycerate. These enzymes are very similar to those found in other hyper/thermophilic, however the MpgS and the MpgP from *R. xylanophilus* have little or no identity to the previous enzymes. The homologous enzymes of *R. xylanophilus* lead, depending of the substrate, to the synthesis of MG or GG. The thermophilic bacterium *P. marina*, on the other hand, is the only known thermophile to accumulate GG in response to salt stress and possesses genes that are also very different from the ones mentioned above. Moreover, this organism possesses two pathways for the synthesis of GG. The physiological relevance of MG and GG accumulation in these thermophilic bacteria and the evolution of MG and GG biosynthesis in prokaryotes are discussed. These studies led us to encounter a gene in the species of Mycobacterium that leads to the synthesis of GG that is bound to a polysaccharide and does not serve as a compatible solute.

Interferon Signaling In Viral Hepatitis

Markus Heim

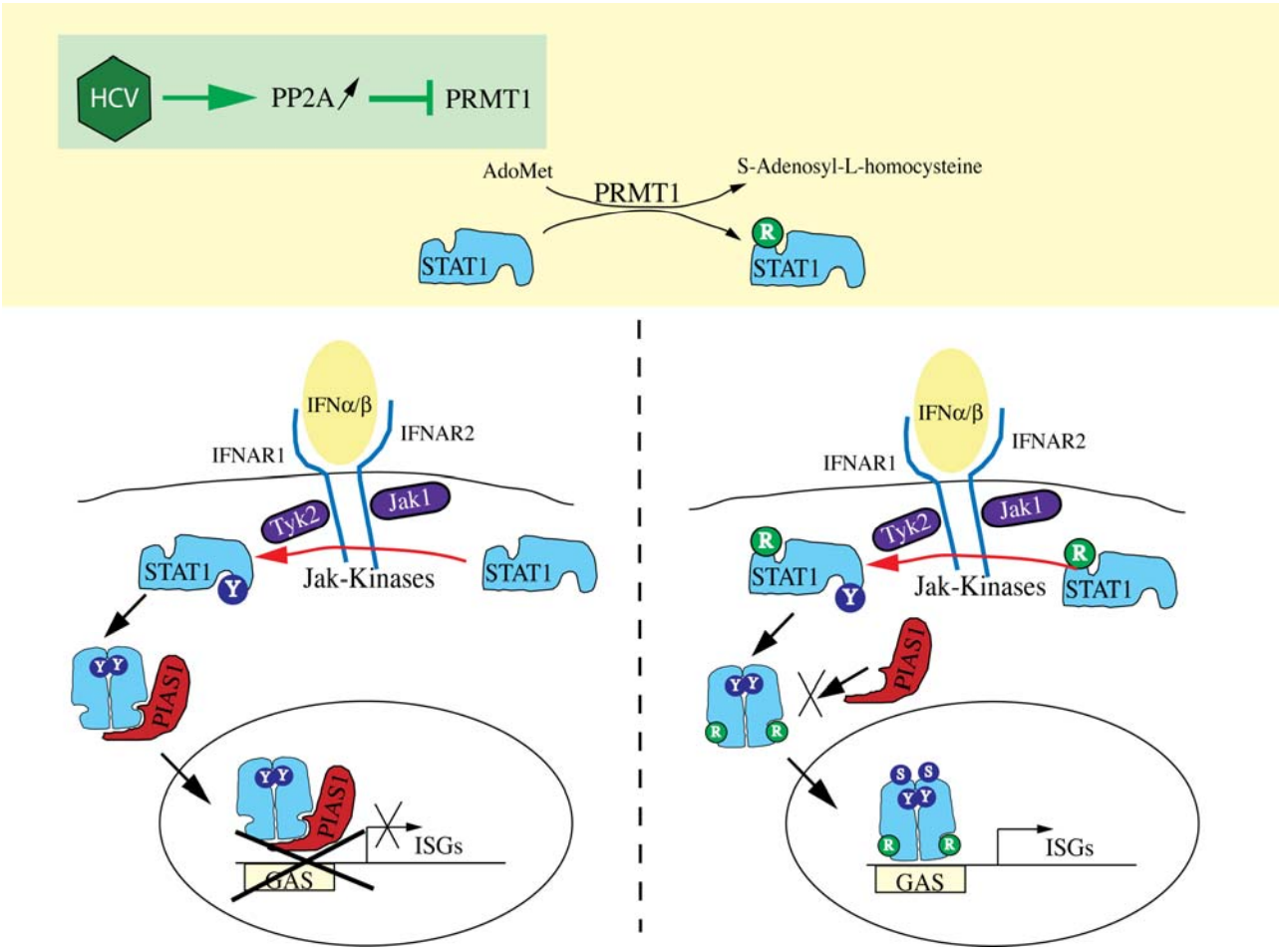
Department of Research, University Hospital Basel, Basel, Switzerland

Type I interferons (IFNs) are important and potent mediators of the early host response to viral infection, and are also used for the therapy of chronic hepatitis C (CHC). Using human osteasarcoma derived cell lines that allow the inducible expression of HCV proteins we could show that HCV protein expression inhibits IFNα signaling through the Jak-STAT pathway (1). The Jak-STAT pathway is the most important signal transduction pathway for IFNα, as has been shown by several studies with mice deficient in different components of the Jak-STAT pathway. STATs (signal transducers and activators of transcription) are activated at the IFN receptor by members of the Jak kinase family through phosphorylation of a single tyrosine residue. Phosphorylated STATs form dimers, translocate into the nucleus, and activate IFN target genes through binding to specific response elements in their promoters. Important negative regulators of IFNα signaling through the Jak-STAT pathway are member of the suppressor of cytokine signaling (SOCS) family, and protein inhibitor of activated STAT1 (PIAS1). SOCS family members inhibit STAT signaling at the level of the receptor kinase complex by binding either to the receptors or to the Jak kinases, and inhibiting the tyrosine phosphorylation of STATs. PIAS1 binds to tyrosine phosphorylated (activated) STAT1 and inhibits the binding of active STAT1 dimers to response elements in interferon stimulated genes (ISGs), predominantly to ISGs with weak STAT1 binding sites. The binding of STAT1 to PIAS1 is regulated by an other posttranslational modification of STAT1, namely arginine methylation, a process that is catalyzed by protein arginine methyl transferase 1 (PRMT1) (2).

Our analysis of IFNα signaling in cells expressing HCV proteins, in liver extracts from transgenic mice expressing HCV proteins in hepatocytes and in extracts from liver biopsies of patients with chronic hepatitis C consistently showed normal expression levels of the signaling components important for IFN signaling (Jak1, Tyk2, STAT1, STAT2, IRF9, SOCS1, SOCS3, PIAS1), and an intact and normal tyrosine phosphorylation of STAT1 and STAT2 (1, 3). However, electrophoretic mobility shift assays with nuclear extracts from HCV protein expressing cells or from liver biopsies of patients with chronic hepatitis C disclosed an impaired binding of STATs to their DNA response elements (1, 3). Further analysis of HCV interference with IFN signaling revealed a novel molecular mechanism of viral interference with the IFN system. HCV induces the over-expression of protein phosphatase 2A (PP2A), an important serine/threonine phosphatase involved in a wide range of cellular processes including cell cycle regulation, cell morphology, development, signal transduction, translation, apoptosis and stress response. PP2A is a heterotrimeric serine/threonine phosphatase consisting of a 36 kDa catalytic C subunit (PP2Ac), a 65 kDa structural A subunit and a variable regulatory B subunit. It is a very abundant protein that accounts for an estimated 0.3% to 1.0% of the total cellular proteins. Surprisingly, the induced over-expression of PP2A in cells inhibits IFNα signaling at the level of DNA binding of STATs while leaving intact the activation of STATs through tyrosine phosphorylation, a finding identical to the HCV situation (3).

How could PP2A inhibit IFNα signaling without disrupting the central tyrosine phosphorylation of STATs? Interestingly, biochemical experiments in vitro with purified PP2Ac and PRMT1 as well as functional experiments in cells (inhibition of PP2A with okadaic acid, siRNA knockdown of PP2Ac) revealed a novel function of PP2A. PP2A directly binds to and inhibits PRMT1 (4). PRMT1 is involved in the arginine methylation of STAT1 and, surprisingly, in the arginine methylation of the helicase domain of the HCV NS3. The inhibition of PRMT1 by PP2A results in a hypomethylation of STAT1 and NS3 helicase. The former is tightly bound by its inhibitor PIAS1, and can not bind to weak ISG promoter elements, whereas hypomethylated NS3 helicase is enzymatically more active (4). Both changes could favor viral replication.

In recent work we investigated how HCV infection could lead to PP2A over-expression. We found that the expression of HCV proteins in cells activates an endoplasmatic reticulum (ER) stress response. During this ER stress response, Ca^{2+} leaks from the ER into the cytoplasma, where it activates calcium/calmodulin-dependent protein kinase. The kinase phosphorylates and activates the transcription factor CREB. Phosphorylated CREB binds to CRE elements in the promoter of PP2Ac and stimulates the transcription of the gene (unpublished).
The consequences of HCV induced PP2A over-expression are summarized in the following figure, with the right panel showing normal signaling with methylated STAT1, and the left panel showing impaired signaling with unmethylated STAT1.



As depicted in the top panel of the figure, the methyl group donor for STAT1 methylation by PRMT1 is S-adenosylmethionine (AdoMet or SAMe), a compound that has been used to treat alcoholic liver disease and is available in many countries as a non-prescription drug. Consequently, we hypothesized that AdoMet could be used to correct the defects in INFα signaling induced by HCV. Indeed, pre-treatment of HCV protein expressing cells and of cells with HCV replicons with AdoMet corrected IFNα signaling and potentiated the inhibitory effects of IFNα on HCV replicons (5). An ongoing clinical study with previous non-responders to IFN-ribavirin combination therapy will clarify if the addition of AdoMet (and betaine) to a standard therapy with pegIFNα and ribavirin could improve the sustained response rate in these difficult to treat patients (ClinicalTrials.gov Identifier: NCT00310336).

The current standard therapy for CHC consists of a combination of pegylated interferon alpha (pegIFNα) and ribavirin. It achieves a sustained viral clearance in only 50-60% of patients. To learn more about molecular mechanisms underlying treatment failure in humans, we investigated IFN-induced signaling in paired liver biopsies collected from CHC patients before and after administration of pegIFNα (6). In patients with a rapid virological response to treatment, pegIFNα induced a strong upregulation of IFN-stimulated genes (ISGs). Non-responders had high expression levels of ISGs already before therapy.
Analysis of post-treatment biopsies of these patients revealed that pegIFNα did not induce expression of ISGs above the pre-treatment levels. In accordance with the ISG expression data, phosphorylation, DNA binding and nuclear localization of STAT1 indicated that the IFN signaling pathway in non-responsive patients is pre-activated and refractory to further stimulation. These findings support the concept that activation of the endogenous IFN system in CHC not only is ineffective in clearing the infection, but may also impede the response to therapy, most likely by inducing a refractory state of the IFN signaling pathway.

References:

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Cellular differentiation in microbial biofilms

Jeremy S. Webb

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Studies of biofilm-forming bacteria, including the medical pathogen *Pseudomonas aeruginosa*, have revealed that bacteria within biofilm microcolonies display considerable phenotypic variation. Characteristics of variant cells isolated from mature biofilms include enhanced biofilm formation and stress tolerance compared to early-stage biofilm bacteria. We have studied the genetics and physiology of these processes and found that bacteriophage Pf4, a prophage in the genome of *P. aeruginosa*, plays a significant role in phenotypic variation, biofilm development, and ecological adaptation of *P. aeruginosa*. The bacteriophage is also important for *P. aeruginosa* infection and together, these data suggest that bacteriophage may play more important roles in bacterial adaptation than previously suggested. We have also observed that accumulation of reactive oxygen species (ROS) in the interior of microcolonies triggers phenotypic variation and differentiation and among cells within biofilms formed by several gram negative bacteria. The predominant ROS in *P. aeruginosa* microcolonies was peroxynitrite. Add back of nitric oxide (NO), the precursor of stable peroxynitrite, to *P. aeruginosa* biofilms induces a shift from biofilm to planktonic cell physiology and greatly increases the sensitivity to antimicrobials. These phenotypic changes appear to occur through NO-mediated interference with cyclic-di-GMP signaling pathways which are known to regulate biofilm formation and dispersal in diverse bacteria. Thus new understanding of the mechanisms of differentiation and phenotypic variation within biofilms are revealing novel strategies for the manipulation of biofilms in medical and industrial settings.

LIST OF POSTERS

(alphabetic order of first author)

Poster classification in groups:

V= virology
B1= stress and regulation
B2= typing, diversity and ecology
B3= microbial pathogenicity
B4= cell and molecular biology of microbes
B5= physiology and biochemistry

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33	**Role of peptidoglycan modifications in *Lactobacillus casei* and *Lactobacillus plantarum*** *E. Bernard , P. Langella, P. Hols and M.-P. Chapot-Chartier*	B4
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35	**Identification of determinants involved in the ovoid shape of *Lactococcus lactis*.** *D. Blandine, M. Deghorain, S. Kulakauskas, P. Hols and B. Hallet*	B4
36	**Microbial activity is determined by the community composition and dynamics in a granular activated carbon reactor for drinking water production** *N. Boon, L. Wittebolle, Y. Wang, M. Marzorati, S. Velten, J. Traber, O. Köster and F. Hammes*	B2
37	**Absence of antibody-dependent complement-mediated lysis of FIPV-infected cells** *E. Cornelissen, H.L. Dewerchin, E. Van Hamme, L. Bossele and, H.J. Nauwynck*	V
38	**Spa32 interaction with the inner-membrane Spa40 component of the type III secretion system of *Shigella flexneri* is required for the control of the needle length by a molecular tape measure mechanism** *A. Botteaux, M. Sani, C. A. Kayath, E. J. Boekema, and A. Allaoui*	B4
39	**MxiC is secreted by and controls the substrate specificity of the *Shigella flexneri* type III secretion apparatus** *A. Botteaux, M.-P. Sory, L. Biskri, C. Parsot and A. Allaoui*	B4
40	**Functional analysis of the large cytoplasmic domain of *Shigella* Spa40**	B4

	in the assembly and the switch of substrate specificity of the type III secretion system *A. Botteaux, C.A. Kayath, M. Sani, A.L. Page, N. Jouihri, L. Biskri, E.J. Boekema, C. Parsot, and A. Allaoui*	
41	**Study of regulation mechanisms controlling competence in *Streptococcus thermophilus*** *C. Boutry, L. Fontaine, B. Delplace, E. Bernard, E. Guédon, and P. Hols*	**B4**
42	**The use of quorum sensing inhibitors to interfere with biofilm formation and development in *Burkholderia multivorans* and *Burkholderia cenocepacia.*** *G. Brackman, U. Hillaert, S. Van Calenbergh, H. J. Nelis and T. Coenye*	**B3**
43	**Identification of enzymes involved in linuron and 3,4-dichloroaniline degradation in the linuron-degrading β-*Proteobacterium variovorax* sp. WDL1: a proteomic approach** *P. Breugelmans, K. Bers, B. Leroy, R. Wattiez, R. De Mot and D. Springael*	**B5**
44	**Actvation of *argO*, the arginine exporter gene of *Escherichia coli*, by the transcriptional regulators ArgP and Lrp** *D. Charlier, M. Foulquié-Moreno, P.N. Le Minh and E. Peeters*	**B4**
45	***Pseudomonas aeruginosa* bacteriophage ϕKMV requires type IV pili and twitching motility for infection** *A. Chibeu, R. Lavigne, K. Hertveldt, P. Cornelis and G. Volkaert.*	**B3**
46	**Particular characteristics of the *in vitro* anti-hepatitis C virus activity of Debio-025, a non-immunosuppressive cyclophilin binding molecule.** *L. Coelmont, S. Kaptein, J. Paeshuyse, J.-M. Dumont, G. Vuagniaux and J. Neyts*	**V**
47	**Improvement of the identification of the *Pseudomonas syringae* group: a molecular approach** *A. Coorevits, E. Samyn, R. Ruiz de la Haba, B. Cottyn, A. Van Landschoot and P. De Vos*	**B2**
48	**PRRSV-infected macrophages are protected against MHC-I-restrictedcytotoxicity** *S. Costers, D.J. Lefebvre, P.L. Delputte and H.J. Nauwynck*	**V**
49	**The Synaptic Complex TnpI/IRS : Molecular Organisation and Assembly** *D. Dandoy, C. Galloy, D. Charlier and B. Hallet*	**B4**
50	**Diversity of rhizobia from Belgian leguminous plants: a molecular inventory** *S. De Meyer and A. Willems*	**B2**
51	**Multilocus sequence analysis using *pheS, rpoA* and *atpA* genes as a robust identification tool in the genus *Streptococcus*** *K. De Bruyne, S. Naser, C. Snauwaert, K. Lefebvre, and P. Vandamme*	**B2**
52	**Amplified Fragment Length Polymorphism (AFLP) analysis for *Arcobacter* identification and strain typing** *L. Debruyne, S. De Smet, K. Houf and P. Vandamme*	**B2**
53	**Biogenesis of outer membrane proteins in *Legionella pneumophila*** *E. De Buck, L. Vranckx and J. Anné*	**B3**
54	**Comparison of rodac plates and petrifilm™ to assess the microbial contamination of food-contract surfaces/ importance of additieves** *S. Deckers, J.G. Detry, C. Anceau and M. Sindic*	**B3**
55	**Sister chromosome cohesion in *E. coli*: cohesion with the FtsK activity domain** *M. Deghorain, C. Pages, J.-C. Meile and F. Cornet*	**B4**
56	**The quest for persistence genes in *Pseudomonas aeruginosa*** *V. De Groote, N. Verstraeten, M. Fauvart, C. Kint and J. Michiels*	**B3**

57	**Nitrifying membrane bioreactor as effective effluent polishing technique for instant 17α-ethinylestradiol removal** *B. De Gusseme, T. Hennebel, N. Boon and W. Verstraete*	**B5**
58	**Generation of PRRS virus susceptible cell lines by stable integration of recombinant, virus-specific receptors** *I. Delrue, H.J Nauwynck, H. Van Gorp and P.L. Delputte*	**V**
59	***Candida albicans* biofilm formation on modified polydimethylsiloxane substrates** *K. De Prijck, N. De Smet, E. Schacht, T. Coenye and H.J. Nelis*	**B3**
60	**Pseudorabies virus US3- and UL49.5-dependent and –independent alterations of MHC I cell surface expression in different cell types** *M. Deruelle, H.J. Nauwynck and H.W. Favoreel*	**V**
61	**Poly-β-hydroxybutyrate (PHB) in the feed of European sea bass induces increased growth performance combined with changes in the intestinal microbial community** *P. De Schryver, A.K. Sinha, P.S. Kunwar, K. Buruah, N. Boon, W. Verstraete, G. De Boeck and P. Bossier*	**B2**
62	**Cholesterol is important for the production of infectious pseudorabies virus** *A.S. Desplanques, N. Verheyen, H.J. Nauwynck, D. Vercauteren and H.W. Favoreel*	**V**
63	**Lack of transmission of low pathogenic avian influenza viruses between pigs and from pigs to ferrets** *A. De Vleeschauwer, D. Braeckmans, S. Van Poucke, F. Barbé and K. Van Reeth*	**V**
64	**A new internalization pathway revealed by an immune evasion strategy used by feline infectious peritonitis virus.** *H.L. Dewerchin, E. Cornelissen, E. Van Hamme, B. Verhasselt and H.J. Nauwynckx*	**V**
65	**Individualized, Degressive Maintenance Regimen using Fluconazole for Recurrent Vulvo-vaginal Candidiasis (ReCiDiF trial)** *G. Donders, G. Bellen, G. Byttebier, L. Verguts, P. Hinoul, R. Walckiers, M. Stalpaert, A. Vereecken and J. Van Eldere*	**B3**
66	**The Efficacy of a Single Oral Dose of 200 mg Pramiconazoleole in Vulvovaginal Yeast Infections: An Exploratory Phase IIa Trial** *G. Donders, J. Ausma, L. Wouters , G. Cauwenbergh , M. Borgers and D. Janssens*	**B3**
67	**Different types of abnormal vaginal flora as candidates for prophylactic treatment with probiotics** *G. Donders*	**B3**
68	**Molecular characterization of *Brucella abortus* differentiation** *D. Dotreppe, J.-J. Letesson and X. De Bolle*	**B4**
69	**Study of the predominant colon microbiota from cystic fibrosis patients** *G. Dutyschaever, G. Huys, L. Boulanger, C. De Boeck and P. Vandamme*	**B3**
70	***Acanthamoeba polyphaga:* a biological fitness of *Mycobacterium ulcerans*?** *M. Eddyani, E. Torrado, P. Stragier, J. Pedrosa, M.T. Silva and F. Portaels*	**B3**
71	**Oligopeptide transporters are essential for symbiotic nitrogen fixation and freeliving growth under stress conditions in *Rhizobium etli*** *M. Fauvart, M. Vercruysse, D. Bachaspatimayum, K. Braeken, M. Belete, K. Vos and J. Michiels*	**B5**
72	**GIL01 and relatives, tectiviral prophage dwellers in the *Bacillus cereus* group** *N. Fornelos and J. Mahillon*	**B5**

73	**Process technical oriented aspects of biological removal of 17α-ethinylestradiol in an aerated fixed bed reactor** *I. Forrez, M. Carballa, N. Boon and W. Verstraete*	**B5**
74	**Engineering *Escherichia coli* to improve L-carnitine production** *M.R. Foulquié Moreno, P. Arense, V. Bernal, M. Canovas and J.L. Iborra*	**B5**
75	**Differences in replication characteristics of pseudorabies virus strains in explants of porcine respiratory nasal mucosa** *S. Glorieux, H.W. Favoreel, G. Meesen, W. de vos, W. Van den Broeck and H.J. Nauwynck*	**V**
76	**Replication of equine herpes virus 1 in tissues of the upper respiratory tract during acute phase of infection in horses** *A.C. Gryspeerdt, A.P. Vandekerckhove and H.J. Nauwynck*	**V**
77	**Metabolites produced by *pseudomonas* sp. enable a gram positive bacterium to achieve extracellular electron transfer** *T. Hai Pham, N. Boon, P. Aelterman, P. Clauwaert, L. De Schamphelaire, L. Vanhaecke, K. De Maeyer, M. Höfte, W. Verstraete and K. Rabaey*	**B5**
78	**Endosymbiotic bacteria in siphonous green algae exploration of a partnership** *J. Hollants, F. Leliaert, O. De Clerck and A. Williams*	**B5**
79	**Oligofructose-insuline specifically upregulates bifidobacterium adolescentis and *B. longum*** *M. Joossens, V. De Preter, M. Cnockaert, G. Huys, P. Vandamme, P. Rutgeerts, K. Verbeke and S. Vermeire*	**B4**
80	**Impact of lipopolysaccharide and peptidoglycan modifications on bacterial virulence: functional analysis of five *Shigella flexneri* genes: *orf182, orf185, orf186, virK* and *ushA*.** *A. Kaoukab-Raji, L. Biskri and A. Allaoui*	**B3**
81	**Identification and preliminary characterisation of two new archaeal tRNA methyltransferases belonging to the SPOUT family.** *M. Kempenaers, M. Roovers, Y. Oudjama, D. Gigot, K.L. Tkaczuk, J.M. Bujnicki and L. Droogmans*	**B2**
82	**Class 1 integrons in ESBL carrying *Escherichia coli* from Kenyan Hospitals.** *J. Kiiru, S. Kariuki, B. Goddeeris and P. Buttaye*	**B3**
83	**β-lactamase inhibitor resistant *Escherichia coli* strains from Kenyan Hospitals: 1992-2008** *J. Kiiru, S. Kariuki, B. Goddeeris and P. Buttaye*	**B3**
84	**Phosphate starvation induce secretion of a putative lipoprotein, CC0170, via the type II secretion system of *Caulobacter crescentus*** *S. Le Blastier, A. Hamels and J.-Y. Matroule*	**B1**
85	**Unevenness under selective stress: a threaten for microbial communities functionality** *M. Marzorati, L. Wittebolle, L. Clement, A. Balloi, D. Daffonchio, W. Verstraete and N. Boon*	**B1**
86	**Bacteria, not archaea, restore nitrification in a zinc contaminated soil** *J. Mertens, K. Broos, S.A. Wakelin, G.A. Kowalchuk, D. Springael and E. Smolders*	**B2**
87	**Plasmid transfer among members of the *Bacillus cereus* group in food matrices** *P. Modrie, E. Beuls, S. Timmery, F. Hoton and J. Mahillon*	**B4**
88	**Heavy metal resistance in *Cupriavidus metallidurans*: towards the reconstruction of regulatory networks** *P. Monsieurs, A. Benotmane, S. Monchy, P. Janssens, R. Van Houdt, H. Moors, N. Leys and M. Mergeax*	**B1**

89	**Effects of hydrogen peroxide and ionising radiation on the physiology of *Arthrospira* sp. PCC8005** *N. Morin, S. Baatout, A. Wilmotte, L. Hendrickx, M. Mergeax and N. Leys*	**B1**
90	**Characterization of the antiviral activity of CF11001 against the bovine viral diarrhea virus.** *S. Musiu, J. Paeshuyse, J.-M. Chezal, H. Mittendorfer, G. Puerstinger, A. Gueiffier, O. Chavignon, J.-C. Teulade and J. Neyts*	**V**
91	**Resistance in *Candida albicans* biofilms: Investigating the expression of resistance genes in biofilms following treatment with antifungal agents** *H. Nailis, D. Vandenbosch, D. Deforce, H. Nelis and T. Coenye*	**B3**
92	**High-resolution contact probing of RutR, the regulator of pyrimidine utilization, binding to the *carAB* control region of *Escherichia coli* K-12** *P.N. Le Minh and D. Charlier*	**B1**
93	**Inhibition of Hepatitis C Virus Replication by Semisynthetic Derivatives of Aglycon Glycopeptide Antibiotics.** *S. Obeid, J. Paeshuyse, S.S. Printsevskayac, E.N. Olsufyevac, R. Bartenschlager, J. Balzarini, S.E. Solovievac, M N. Preobrazhenskaya and J. Neyts*	**V**
94	***In vivo* characterization of the physiological role of a transcriptional regulator from the archaeon *Sulfolobus solfataricus*** *E. Peeters, S. Albers, A. Vassart and D. Charlier*	**B1**
95	**Transcriptional responses in *Burkholderia cenocepacia* biofilms after exposure to chlorhexidine, H2O2 and NaOCl** *E. Peeters, A. Sass, E. Mahenthiralingam, H.J. Nelis and T. Coenye*	**B1**
96	**Exploring heterotrophic bacterial diversity of Antarctic samples through cultivation** *K. Peeters, E. Verleyen, D. Ertz, D. Hodgson and A. Willems*	**B2**
97	**Assessment of Micropollutant-induced Response in *Rhodospirillum rubrum* S1H using a Toxicogenomic Approach complemented with Physiological Analyses** *B. Pycke, L. Hendrickx, H. De Wever, P. Monsieurs, W. Verstraete, M. Mergeay and N. Leys*	**B1**
98	**Study of the functional role of peptidoglycan hydrolases in *Lactobacillus plantarum*** *T. Rolain and P. Hols*	**B4**
99	**Characterization of cupro-resistant Methylobacterium strains** *J. Scheers, A. Cubaka-Kabagal, B. Cornu, S. van Aelst, M. Mergeay, L. Droogmans, F. van Vliet, C. Vander Wauven, and N. Verbruggen*	**B1**
100	**Comparative analysis of Extended-spectrum-β-lactamase (ESBL)-carrying plasmids from different members of *Enterobacteriaceae* isolated from poultry, pigs and humans: evidence for a shared β-lactam resistance gene pool** *A. Smet, A. Martel, D. Persoons, J. Dewulf, M. Heyndrickx, A. Cloeckaert, K. Praud, G. Claeys, B. Catry, L. Herman, F. Haesebrouck, and P. Butaye*	**B3**
101	**Congenital cytomegalovirus infection in Flanders: presentation, methodology and first descriptive results of the Flemish CMV registry** *K. Smets and T. Vanduynslager*	**V**
102	**Pesticide-primed Soils as Supplement for On-farm Bioremediations systems to Improve Pesticide-Contaminated-Wastewater Treatment** *K. Sniegowski, D. Springael, J. Ryckeboer, P. Jaeken and P. Spanoghe*	**B2**
103	**Phenotypic, Transcriptome, and proteome analysis of a gentamicin-resistant small colony variant of *Pseudomonas aeruginosa*** *S. Tarighi, Q. Wei, C. Raine, M. Cámara, P. Williams, S. Haenen, B. Boerjan, A. Bogaerts, E. Vierstraete, P. Verleyen, L. Schoofs and P.*	**B3**

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104	**Characterization of CVB3 resistance to rupintrivir, a highly potent 3C protease inhibitor** *H.J. Thibaut, J. Mesters, K. Lanke, F. Van Kuppeveld, R. Hilgenfeld and J. Neyts*	**V**
105	**Mobile genetic elements and gene exchange among *Bacillus* spp. strains from the Antarctic Concordia Station and the International Space Station** *S. Timmery, X. Hu, O. Minet, R. Van Houdt, M. Mergeay and J. Mahillon*	**B2**
106	**MRSA associated with clinical and subclinical mastitis in Belgian cows and herds** *W. Vanderhaeghen, T. Cerpentier, C. Adriaensen, J. Vicca and P. Butaye*	**B3**
107	**Identification of a bacterial inhibitor of G-type lysozyme** *L. Vanderkelen, L. Callewaert, J. Van Herreweghe, A. Aertsen and C.W. Michiels*	**B5**
108	**Characterization of the VNTR region in a *Legionella pneumophila* collagen-like protein** *L. Vandersmissen, D.A. Coil, E. De Buck, E. Lammertyn and J. Anné*	**B3**
109	**Clathrin- and caveolae-independent Entry of feline infectious peritonitis virus in monocytes depends on dynamin** *E. Van Hamme, H.L. Dewerchin, E. Cornelissen, B. Verhasselt and H J. Nauwynck*	**V**
110	**Rapid detection and quantification of *Aspergillus fumigatus* in air using solidphase cytometry** *L.M.E. Vanhee, H.J. Nelis and T. Coenye*	**B2**
111	**Evaluation of a new experimental inactivated vaccine for the porcine reproductive and respiratory syndrome virus** *M. Vanhee, P.L. Delputte, I. Delrue, M.F. Geldof and H.J. Nauwynck*	**V**
112	**Isolation of a bacterial inhibitor of the invertebrate type lysozyme** *J.M. Van Herreweghe, L. Vanderkelen, L. Callewaert, A. Aertsen and C.W. Michiels*	**B5**
113	**Using Mild high-pressure shock to generate bacterial ghosts of *Escherichia coli*** *D. Vanlint, M.T. Mebrhatu, E. Ons, B.M. Goddeeris, C.W. Michiels and A. Aertsen*	**B5**
114	**Time to revise the unique role of the pig as a "mixing vessel" for influenza A viruses?** *S. Van Poucke and K. Van Reeth*	**V**
115	**To denitrify or not to denitrify? An issue in Gram-positive bacteria?** *I. Verbaendert, L. Lebbe, N. Boon and P. De Vos*	**B2**
116	**Decontamination of apple pieces in syrup by high hydrostatic pressure** *A. Vercammen, M. Hendrickx and C.W. Michiels*	**B3**
117	**Stress adaptation of the symbiotic soil bacterium *Rhizobium etli*: analysis of gene expression under non-growing conditions using a high-resolution tiling array.** *M. Vercruysse, M. Fauvart and J. Michiels*	**B1**
118	**Nitric oxide production by the human intestinal microbiota from nitrate** *J. Vermeiren, T. Van de Wiele, W. Verstraete, P. Boeckx and N. Boon*	**B2**
119	**Fluorescent *in situ* hybridization (FISH) to elucidate structure, function and diversity of granular biomass for the treatment of nitrogenous wastewater** *S.E. Vlaeminck, A. Terada, M. Carballa, N. Boon, B. Smets and W. Verstraete*	**B2**

120	**Comparative study of the genetic barrier and pathways towards resistance of selective inhibitors of HCV replication** *I. Vliegen, L. Delang and J. Neyts*	**V**
121	**Deletion of the vaccinia virus F13L gene results in a highly attenuated virus that mounts a protective immune response against lethal vaccinia virus challenge** *I. Vliegen, G. Yang, D. Hruby, R. Jordan and J. Neyts*	**V**
122	**Functional analysis of the *Legionella pneumophila* plasminogen activator** *L. Vranckx, E. De Buck and J. Anné*	**B3**
123	**Competing and socializing behaviour within a model bacterial community consisting of two different phenanthrene-degrading bacteria growing on phenanthrene-films in a model-ecosystem** *K. Wouters, E. Maes, J. Hofkens and D. Springael*	**B2**
124	**Simulation of the deep-sea biosphere by a continuous high-pressure bioreactor** *Y. Zhang, L. Maignien, W. Verstraete, J.-P. Henriet and N. Boon*	**B2**

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Poster abstracts selected for oral presentation

Virology Session

Morning session

11h55 Lack of transmission of low pathogenic avian influenza viruses between pigs and from pigs to ferrets
A. De Vleeschauwer, D. Braeckmans, S. Van Poucke, F. Barbé and K. Van Reeth

TOGETHER WITH:

Time to revise the unique role of the pig as a "mixing vessel" for influenza A viruses?
S. Van Poucke and K. Van Reeth

Afternoon session

15h15 Characterization of CVB3 resistance to rupintrivir, a highly potent 3C protease inhibitor
H.J. Thibaut, J. Mesters, K. Lanke, F. Van Kuppeveld, R. Hilgenfeld and J. Neyts

15h30 Cholesterol is important for the production of infectious pseudorabies virus
A. S. Desplanques, N. Verheyen, H.J. Nauwynck, D. Vercauteren and H.W. Favoreel

15h45 A new internalization pathway revealed by an immune evasion strategy used by feline infectious peritonitis virus.
H.L. Dewerchin, E. Cornelissen, E. Van Hamme, B. Verhasselt and H. J. Nauwynck

16h00 Congenital cytomegalovirus infection in Flanders: presentation, methodology and first descriptive results of the Flemish CMV registry
K. Smets and T. Vanduynslager

16h15 Clathrin- and caveolae-independent Entry of feline infectious peritonitis virus in monocytes depends on dynamin
E. Van Hamme, H.L. Dewerchin, E. Cornelissen, B. Verhasselt and H.J. Nauwynck

Bacteriology Session

Afternoon session

15h15 The quest for persistence genes in *Pseudomonas aeruginosa*
V. De Groote, N. Verstraeten, M. Fauvart, C. Kint and J. Michiels

15h30 Heavy metal resistance in *Cupriavidus metallidurans*: towards the reconstruction of regulatory networks
P. Monsieurs, A. Benotmane, S. Monchy, P. Janssen, R. Van Houdt, H. Moors, N. Leys and M. Mergeay

15h45 Unevenness under selective stress: a threaten for microbial communities functionality
M. Marzorati, L. Wittebolle, L. Clement, A. Balloi, D. Daffonchio, W. Verstraete and N. Boon

16h00 Molecular characterization of *Brucella abortus* differentiation
D. Dotreppe, J.-J. Letesson and X. De Bolle

16h15 Transcriptional responses in *Burkholderia cenocepacia* biofilms after exposure to chlorhexidine, H_2O_2 and NaOCl
E. Peeters, A. Sass, E. Mahenthiralingam, H. J. Nelis and T. Coenye

16h30 Different types of abnormal vaginal flora as candidates for prophylactic treatment with probiotics
G. Donders

ABSTRACTS OF PARTICIPANTS

Using P22 phage to dissect *Salmonella typhimurium* physiology

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Phage – bacteria interactions are more sophisticated than previously imagined. In fact, phages are expected to harbor a plethora of novel functions likely to participate in host manipulation and subversion. As evolutionary selection has shaped these functions into highly fine-tuned strategies to interfere with regular microbial physiology, phage – host interactions should be recognized as cutting edge 'dissection tools' that will help to increase the genetic and molecular resolution of bacterial physiology.

In this report, we have discovered a genetic response in the food-borne pathogen *Salmonella enterica* serovar Typhimurium LT2 that is specifically induced upon infection with the temperate phage P22. Interestingly, the observed interaction was absent both during pure lytic or lysogenic development, suggesting that another stage of the phage's life cycle is important for this phenomenon.

We were able to map the actual trigger of this physiological response to a ca. 400 bp region in the P22 genome. Although there is no annotation in this region, it contains a unique ORFan gene that bears limited or no homology with other known sequences. Deletion of this region in P22 correspondingly abolished the observed physiological response in *S.* Typhimurium upon infection.

Although bacterial phage infection has a long history of molecular and genetic study, still little is known about the actual mechanisms of interaction between the phage and its host. Nevertheless, such strategies could be highly instructive to better understand and interfere with the physiology of bacterial pathogens.

Insight into bacterial cellulose metabolism by functional metagenomics of Antarctic soil

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Metagenomics gives the possibility to access the genetic material of so-far uncultivated microorganisms and is routinely used to isolate new enzymes from environmental samples. Here, the mining of an Antarctic soil sample by functional metagenomics allowed the isolation of a cold-adapted protein (RBcel1) conferring carboxymethyl cellulose hydrolysis. The new enzyme was related to a glycosyl hydrolase family five (GH5)protein from *Pseudomonas stutzeri* (Pst_2494) and do not possess any carbohydrate binding-domain. The protein was produced and purified to homogeneity. The characterization of the cellulose hydrolysis reveals an endo-type of action producing cellobiose and cellotriose using carboxymethyl cellulose as substrate. No activity was observed using other polysaccharides as substrate. Moreover, studying pH and thermal dependence of the hydrolytic activity it appears that RBcel1 was active from pH 6 to 9 and remains significantly active when temperature decreases. In addition, RBcel1 characterization highlighted a cellulose production using cellobiose as substrate. Such polymerization was recorded in physiological conditions and results in the production of non-reticulated cellulose fibers. Although cellulose production by cellulase was previously recorded for industrial purposes, it is known that the last step in the bacterial cellulose synthesis pathway requires an endoglucanase (GH8). But, analyzing the *P. stutzeri*'s genome, the lack of a GH8 family related enzyme was revealed. Pst_2494 derived from *P. stutzeri* and RBcel1 obtained by functional metagenomics analysis of an Antarctic soil sample are the two first enzymes belonging to the GH5 family involved in bacterial cellulose production.

Role of peptidoglycan modifications in *Lactobacillus casei* and *Lactobacillus plantarum*

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

In contrast, in commensal or probiotic bacteria, the role of PG modifications was not previously studied. Here, we chose to analyze PG modifications in two lactobacilli representing the two phyla present in this group: the anti-inflammatory *Lactobacillus casei* BL23 strain and the commensal *Lactobacillus plantarum* WCFS1 strain.

We first analyzed the PG structure of the two selected strains. PG was digested with mutanolysin and the resulting muropeptides were separated by HPLC and analyzed by mass spectrometry. We identified the following PG modifications: O-acetylation in both strains, amidations of the peptidic stem (glutamic acid and diaminopimelic acid for *L. plantarum*; glutamic acid and aspartic acid for *L. casei*).

The gene homologous to *S. aureus oatA*, which encodes the O-acetyl-transferase responsible for MurNAc O-acetylation, was inactivated in both strains. PG analysis showed disappearance of the O-acetylated muropeptides in both mutants. Also, our results seem to indicate an effect of O-acetylation on PG synthesis and maturation.

In a future part, the influence of these modifications on endogenous autolysin activities as well as on the immunomodulatory properties of the bacteria will be investigated.

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Bacillus thuringiensis conjugation in simulated microgravity

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The *Bacillus cereus* group contains six genetically closely-related species, including *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus anthracis.* The phenotypical features that enable to discriminate these bacteria are mainly conferred by plasmids. Understanding the mechanisms of plasmid transfer among these bacteria, by conjugation and is very important, especially in extreme and/or confined environments such as space stations. This study focuses on the transfer behaviour of *B. cereus*/*B. thuringiensis* plasmids in various simulated microgravity conditions, aimed to mimic those encountered in space environments.

B. thuringiensis israelensis strains GBJ002 (pAW63, pUB110) and GBJ001 were used as donor and recipient bacteria, respectively. The mobilizable and conjugative plasmids were *Staphylococcus aureus* pUB110 and *B. thuringiensis* pAW63, respectively. Frequencies of conjugation were obtained in rich medium, at 30°C, without shaking. Magnetic levitation and Rotating Wall Vessel were used as devices to simulate microgravity. In order to better understand the transfer behaviours of the plasmids, kinetics of conjugation were also studied by plating bacteria every 10 minutes, during a period of 4 hours.

Transfer of the pAW63 conjugative plasmid was detected after 50 min of mating, followed 30 min later by the mobilizable plasmid, while the transconjugants carrying both plasmids were detected after 90 minutes. Maximum frequencies were reached after 3h. Clear differences in transfer frequencies were noticed for the conjugative pAW63 with regard to the different microgravity conditions: the conjugation frequencies observed under Magnetic Levitation were higher from those obtained in the Rotating Wall Vessel. On the contrary, only slight differences were observed for the mobilizable plasmid.

This study highlights the requirement for a first transfer of the conjugative plasmid before the migration of the mobilizable plasmid. It also shows that microgravity environments modify the performance of plasmid horizontal transfer by conjugation, and suggests different transfer behaviours between conjugative and mobilizable plasmids.

Identification of determinants involved in the ovoid shape of *Lactococcus lactis*.

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The morphogenesis of ovoid bacteria has for a long time been associated with the one of cocci. We can distinguish among cocci more elongated bacteria from the real round cells, commonly called "ovococci". In our laboratory, we're studying the determinants of the ovoid shape of the Gram-positive bacterium, *Lactococcus lactis*. The bacterial shape is mostly determined by the cell wall structure. In Gram-positive bacteria, the cell wall is mainly composed of peptidoglycan (PG), a polymer of glycan strands. The biosynthesis of PG occurs at two levels: the synthesis of the peptidoglycan precursor in the cytoplasm and its incorporation in the pre-existing PG layer by Penicillins Binging Proteins (PBPs).

In *Lc. lactis* the PG precursor is ended by D-ala-D-ala conferring sensitivity to vancomycine, an antibiotic inhibiting the insertion of the newly formed PG precursor in the pre-existing cell wall. It has been observed that the modification of D-ala-D-ala ending PG precursor in D-ala-D-lac alters the morphogenesis of *Lc. lactis* (M. Deghorain *et al.*, in preparation). The cells are round, form curved chains and are resistant to vancomycine. Interestingly, we observed an unexpected adaptation mechanism of the mutant strain to the newly formed precursor without any selective pressure (i.e. absence of vancomycin). This strain presents a phenotypic heterogeneity: the proportion of the population which is highly resistant to vancomycin increases along generations.

In a first part, we study the mechanism of adaptation of the precursor mutant strain. We tested the sensitivity of the "adapted" strain to beta-lactams affecting different PBPs. We observed an increased sensitivity to methicillin, a beta-lactam known to inhibiting the PBP(s) involved in cell division. This suggests alterations in the expression profile of the PBPs. Analysis of the PBP pattern is currently under investigation.

The second part of this work consists to develop tools for characterizing the morphogenesis process in *Lc. lactis*. Firstly, the morphology of *Lc. lactis* was analyzed after treatments with different beta-lactams. The addition of methicillin contributes to elongate the cells. Secondly, analyzes of PBPs mutants allow us to study the function of each PBPs in the morphogenesis. Additionally, these mutants were associated to antibiotic treatments. From these experiments, we showed that PBP2b has a key role in the elongation phase of *Lc. lactis*. In a closed future these tools will be combined with subcellular localizations of proteins involved in PG synthesis/degradation (MurG, PBPs, AcmA) and cytoskeleton elements (MreC, MreD, FtsZ).

Microbial activity is determined by the community composition and dynamics in a granular activated carbon reactor for drinking water production

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The quality of drinking water is ensured by hygienic barriers and filtration steps, such as ozonation and granular activated carbon (GAC) filtration. GAC filtration is essentially a microbial process that removes all biodegradable organic carbon from the ozonated water and ensures biological stability of the treated water. In this study, the start-up and maturation of an undisturbed pilot-scale GAC filter was monitored at 4 depths (10, 45, 80 and 115 cm) over a period of 6 months. New ecological tools (Marzorati *et al*, 2008), based on 16S rRNA gene PCR-DGGE (Boon *et al*, 2002), were linked to filter performance and microbial activity and showed that the stratification in a GAC reactor was of importance. At 10 cm, receiving the freshly ozonated water, microbial activity was inhibited by residual ozone. This was reflected in the microbial community where no changes were observed and the richness remained low. However, the GAC samples at 80-115 cm showed a 2-3 times higher richness then the 10-45cm samples. The highest biomass densities were observed at 45-80 cm, which corresponded with maximum removal of dissolved and assimilable organic carbon. Furthermore, the start-up period was clearly visible from Lorenz analysis: after two months, the community shifted to a more even organisation, which was accompanied by an apparent steady state condition. In conclusion, this study clearly shows that microbial community analysis can be linked to a good reactor performance. In the GAC filter sections with high activity, a high richness and evenness is needed for good performance. Moreover, these good performing microbial communities are 4 times more dynamic than the less performing communities.

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Absence of antibody-dependent complement-mediated lysis of FIPV-infected cells

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Feline infectious peritonitis (FIP) is a fatal chronic disease in cats caused by a coronavirus, feline infectious peritonitis virus (FIPV). Although virus-specific antibodies are present, the infected cat is not capable of overcoming the infection. Why and how the antibodies do not protect the cat is not fully understood. In general, virus-specific antibodies can help to resolve infection, not only by direct virus neutralization, but also by antibody-mediated lysis of infected cells. For many viruses, newly synthesized viral glycoproteins are incorporated in the plasma membrane, rendering the infected cells visible to the host humoral immune system. Virus-specific antibodies can then bind to the cell surface, which can result in antibody-dependent, complement-mediated cell lysis (ADCML).

In this study, the sensitivity of FIPV-infected cells to ADCML was determined by using an ADCML assay. Therefore, FIPV 79-1146 infected Crandell feline kidney (CrFK) cells and feline monocytes treated with vanadate or myosin light chain kinase (for inhibition of antibody induced internalization of surface expressed viral proteins) were incubated with virus-specific antibodies and feline complement. The percentage of dead infected cells was determined by immunofluorescence microscopy. It was shown that in the ADCML-assays the percentage of dead FIPV infected cells was not significantly different compared to the percentage of dead infected cells in the control (ADCML assay without virus-specific antibodies). For CrFK cells, 8 ± 6 % were killed in the ADCML assay compared to 11 ± 7 % in the control. For feline monocytes, 11 ± 8 % were killed in the ADCML assay compared to 14 ± 10 % in the control.

In conclusion, it can be stated that FIPV-infected cells with surface expressed viral proteins are protected against ADCML. It seems that, over time, FIPV has developed a variety of immune-evasion processes to escape humoral immune responses, namely the antibody dependent enhancement of infectivity (ADEI), the absence of viral proteins on the surface of infected cells by retention of the viral proteins in the cytoplasm or by antibody mediated internalization of surface-expressed viral proteins and the in this study described insensitivity to ADCML.

Spa32 interaction with the inner-membrane Spa40 component of the type III secretion system of *Shigella flexneri* is required for the control of the needle length by a molecular tape measure mechanism

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The effectors of enterocyte invasion by *Shigella* are dependent on a type III secretion system which contains a needle whose length average does not exceed 50 nm. Previously, we reported that Spa32 is required for needle length control as well as to switch substrate specificity from MxiH to Ipa proteins secretion. To identify functional domains of Spa32, eleven truncated variants were constructed and analysed for their capacity i) to control the needle's length ; ii) to secrete the Ipa proteins; and iii) to invade HeLa cells. Deletion at either the N-terminus or C-terminus affect Spa32 function in all cases, but Spa32 variants lacking internal residues 37-94 or 130-159 retained full Spa32 function. Similarly, a Spa32 variant obtained by inserting of the YscP's ruler domain retained Spa32 function although it programmed slightly elongated needles. Using the GST-pull-down assay, we show that residues 206 to 246 are required for Spa32 binding to the C-terminus of Spa40, an inner membrane protein required for Ipa proteins secretion. Our data clearly demonstrate that shortening Spa32 affects the length of the needle in a comparable manner to the *spa32* mutant, indicating that the control of needle length does not require a molecular ruler mechanism.

MxiC is secreted by and controls the substrate specificity of the *Shigella flexneri* type III secretion apparatus

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Many Gram-negative pathogenic bacteria use a type III secretion system (T3S) to interact with cells of their hosts. Mechanisms controlling the hierarchical addressing of needle subunits, translocators and effectors to the T3S apparatus (T3SA) are still poorly understood. We investigated the function of MxiC, the member of the YopN/InvE/SepL family in the *Shigella flexneri* T3S system. Inactivation of *mxiC* led specifically to a deregulated secretion of effectors (including IpaA, IpgD, IcsB, IpgB2, OspD1 and IpaHs), but not of translocators (IpaB and IpaC) and proteins controlling the T3SA structure or activity (Spa32 and IpaD). Expression of effector-encoding genes controlled by the activity of the T3SA and the transcription activator MxiE was increased in the *mxiC* mutant, as a consequence of the increased secretion of the MxiE anti-activator OspD1. MxiC is a T3SA substrate and its ability to be secreted is required for its function. By using copurification assays, we found that MxiC can associate with the Spa47 ATPase, which suggests that MxiC might prevent secretion of effectors by blocking the T3SA from the inside. Although with a ten-fold reduced efficiency compared to the wild-type strain, the *mxiC* mutant was still able to enter epithelial.

Functional analysis of the large cytoplasmic domain of *Shigella* Spa40 in the assembly and the switch of substrate specificity of the type III secretion system

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Type III secretion systems (T3SSs) are central virulence factors of many Gram-negative bacteria. Their overall morphology consists of a cytoplasmic region, an inner- and outer-membrane section and an extracellular needle whose length is regulated by Spa32 *in Shigella*. To search for *Shigella* interaction partners of Spa32, we used a two-hybrid screen in yeast and identified an interaction with the C-terminal domain encompassing residues 309-342 of Spa40, a member of the FlhB/YscU family. The latter was confirmed using GST-pull down assay which allowed the identification of additional molecular interactions between Spa40 and T3S components such as Spa33, Spa47, MxiN and MxiA. Inactivation of *spa40* abolished Ipas secretion and revealed a needless structure. We demonstrate by genetic and functional analyses that residues L310 and V320 of Spa40 are crucial for Ipas secretion and for Spa32 binding. We present evidence that Spa40's proteolysis at the conserved NPTH motif is not required for the assembly of the T3SA, but is however necessary for the switch to effector secretion. Furthermore, we show that the cleavage of Spa40 per-se is required for its interaction with Spa32, Spa33 and Spa47 but not with MxiA or MxiN. Taken together, our data suggest that the conformational change of Spa40 inside the bacterium is critical for substrate specificity switching.

Study of regulation mechanisms controlling competence in *Streptococcus thermophilus*

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Competence for natural transformation is a frequent property among pathogenic streptococci and is considered as a general mechanism for genome plasticity. In *S. pneumoniae*, competence is a transient event relying on an alarmone, ComC, that activates the ComDE two-component system. This results in the induction of *comX*, encoding an alternative sigma factor (σ^X). ComX is the central regulator of competence that activates all essential late genes required for DNA transformation. Recently, functional natural transformation was also shown in *S. thermophilus* LMG18311, a non-pathogenic streptococcus used in the dairy industry. However, this was achieved through the artificial expression of *comX* under control of a non-native promoter. Indeed, no functional homolog of the ComC/D/E system was found in *S. thermophilus*, and the mechanisms and natural growth conditions regulating *comX* expression and/or ComX stability still remain unknown. The aim of this study was to elucidate the regulation pathway underlying competence development in *S. thermophilus*.

A targeted mutagenesis strategy was followed to identify genes involved in the early steps of competence development. Targets were selected according to their implication in *comX* induction (*stkP*, *pppL*, *micAB*, *codY*, *hdiR*) or ComX degradation/stabilization (*spxB*, *stu0161*, *mecA*) in *S. pneumoniae* and *B. subtilis*. The effect of gene deletion on competence was studied at the functional and transcriptional level. For this latter purpose, disruption of target genes was performed in strains containing either a P_{comX}-*luxAB* or a P_{comGA}-*luxAB* fusion. Preliminary results suggest a post-translational regulation mechanism of competence development acting at the level of ComX. A random mutagenesis strategy is currently under progress to identify new specific regulators controlling *comX* expression in *S. thermophilus*.

The use of quorum sensing inhibitors to interfere with biofilm formation and development in *Burkholderia multivorans* and *Burkholderia cenocepacia*

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Burkholderia cepacia complex strains are opportunistic pathogens, causing life-threatening infections in CF patients. *Burkholderia cepacia* complex strains are resistant to many antimicrobial agents and commonly produce biofilms *in vitro* and *in vivo*. This contributes to their virulence and makes *Burkholderia* infections difficult to treat. Recently, the quorum sensing system of *Burkholderia* spp. has been found to affect their biofilm forming ability, making it an attractive target for antimicrobial therapy. In the present study we evaluated the anti-biofilm effect of several known quorum sensing inhibitors. The effect on *Burkholderia* spp. biofilm formation was examined using crystal violet, resazurin and SYTO9 staining as well as plating. Several compounds, when used in sub-inhibitory concentrations interfered with biofilm formation by *Burkholderia* spp. Our results suggest that the quorum sensing inhibitors do not interfere with the initial attachment but affect later stages of biofilm formation and detachment. In addition, several QS inhibitors had a considerable impact on biofilm structure. Our data indicate that several QS inhibitors had an effect on Bcc biofilm formation. These compounds may hold promise to treat Bcc biofilm related infections.

Identification of enzymes involved in linuron and 3,4-dichloroaniline degradation in the linuron-degrading β-Proteobacterium *Variovorax* sp. WDL1: a proteomic approach

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Phenylureas are commonly applied herbicides in agriculture and their extensive use has resulted into important environmental contamination. Members of the genus *Variovorax* are often isolated as the main linuron degraders from linuron-treated agricultural soils and seem to fulfill a crucial role in linuron degradation in soil. The main bacterial degradation route of linuron is initiated with a direct hydrolysis of the amide bond resulting in the metabolites 3,4-dichloroaniline (3,4-DCA) and *N,O*-dimethylhydroxylamine (*N,O*-DMHA). Further degradation of 3,4-DCA can proceed via different possible routes which include transformation of this product to catechol-based moieties. Knowledge about the catabolic genes and enzymes involved in linuron hydrolysis and further mineralization of 3,4-DCA is however scarce. We initiated research on the linuron/3,4-DCA catabolic genes and proteins of the linuron-degrading β-Proteobacterium *Variovorax* sp. WDL1 by means of a differential proteomic approach. Differential protein expression analysis of *Variovorax* sp. WDL1 grown in a heterotrophic medium in the presence and absence of linuron or 3,4-DCA was conducted using 2D-PAGE and selected up- and down-regulated proteins were identified with NanoLC-ESI-MS/MS. In the 3,4-DCA-supplemented culture, the up-regulation of several components of earlier described multicomponent aniline dioxygenases (AD) was observed. The different components of this putative (di)chloroaniline dioxygenase were similar to AD-like components of aniline-degrading Proteobacteria. The data indicated that possibly multiple versions of the AD complex are expressed. Unfortunately, several interesting protein spots which were up-regulated in the linuron- and/or 3,4-DCA-supplemented cultures compared to the control cultures did not show reliable or significant similarity to known protein functions. In both linuron- and 3,4-DCA-supplemented cultures, differential expression of stress-related proteins was observed. Currently, a similar analysis on *Variovorax* sp. WDL1 mutants impaired in linuron and/or 3,4-DCA degradation is performed to provide evidence on the participation of those proteins in linuron/3,4-DCA degradation. Moreover, we initiated the genetic identification of the AD-complex in strain WDL1.

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Actvation of *argO*, the arginine exporter gene of *Escherichia coli*, by the transcriptional regulators ArgP and Lrp

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High affinity import systems for amino acids are ubiquitously found in nature, also in microorganisms that can synthesize all twenty essential amino acids *de novo*. *E. coli* has even three uptake systems of the ABC-type for arginine. Their expression is controlled by ArgR, the transcriptional repressor of arginine biosynthesis (1). The underlying reason for this proliferation of import systems is most likely that uptake is energetically less demanding than *de novo* synthesis. Furthermore, imported amino acids can be either used for protein synthesis, or be catabolized and serve as a source of carbon, nitrogen, and/or energy. Export systems on the other hand may be used to avoid the accumulation of high and potentially toxic intracellular amino acid concentrations that might result from uptake or from an imbalanced metabolic overflow (2). Some amino acids like arginine and glutamate may also play an important role in pH homeostasis and osmoregulation. Therefore, amino acid import and export systems have to be controlled in an ingenious manner to avoid futile and energy demanding substrate cycling, and to ensure an appropriate response to continuously changing environmental conditions.

The *argO* (*yggA*) gene product of *E. coli* shows significant sequence similarity with LysE, the arginine and lysine exporter from *Corynebacterium glutamicum* (3, 4). Therefore, ArgO might play a similar role in *E. coli*. LysE was the first biochemically characterized amino acid exporter and the prototype of a growing superfamily of bacterial and archaeal efflux pumps for small metabolites (2).

Nandineni *et al*. (4) have shown that transcription of the *argO* gene is activated by ArgP (IciA), a transcriptional activator of the LysR family. ArgP binds to a 60 bp long target sequence partially overlapping the *argO* promoter (5). Arginine is not required for ArgP binding, but is required to stimulate transcription initiation. Lysine counteracts this effect (4, 5). Therefore, ArgO might be required to maintain an appropriate balance between the internal concentrations of arginine and lysine.

Here, we demonstrate that *argO* expression is also stimulated by the transcriptional regulator Lrp (Leucine responsive regulatory protein). *E. coli* Lrp is a global regulator and the prototype of a superfamily of prokaryotic regulators, also called feast/famine regulators (FFRP). We show that purified Lrp binds to the *argO* control region *in vitro*, thereby forming three complexes with a distinct migration velocity. *In vivo*, we show that Lrp stimulates *argO* expression in an L-leucine sensitive manner. Most interestingly, the activation by ArgP and Lrp appeared not to be independent but intertwined, exhibiting negative interferences. ArgP and Lrp act as competive activators, each one being more potent in the absence (knock-out) of the other, and ArgP being the most efficient one of the two. This observation strongly suggests negative interferences between ArgP and Lrp, likely due to binding of the two regulators to partially overlapping target sites in the *argO* control region.

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Pseudomonas aeruginosa bacteriophage ϕKMV requires type IV pili and twitching motility for infection

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Besides their use as fundamental tools in genetics, lytic phages are being studied as candidate antibacterial agents against pathogenic bacteria. This study focuses on the physical interaction process of lytic bacteriophage ϕKMV to its host *Pseudomonas aeruginosa* (Lavigne *et al*., 2003; Ceyssens *et al*., 2006). Specifically we aim at creating tools for the receptor analysis of ϕKMV and other lytic phages and seek to provide a streamlined platform for the isolation, identification and characterization of relevant phage receptors. In this manner, a pool of fully characterized phages ready for generation of phage cocktails can be created for use in combating *P. aeruginosa* infections.

The strategy adopted to identify the bacteriophage ϕKMV receptor involved the isolation of spontaneous phage ϕKMV resistant mutant *P. aeruginosa* strain, followed by the functional complementation of the mutant by mobilization of a cosmid library of *P. aeruginosa* ϕKMV sensitive wild type strain PAO1. A cosmid clone pRG4BG12 that complimented the phage ϕKMV resistant mutant PAO121Ar was isolated. The 26,401bp pRG4BG12 cosmid insert containing the entire *pilMNOPQ* gene cluster also re-established twitching motility in PAO121Ar therefore suggesting correlation between twitching motility and bacteriophage ϕKMV infectivity. This was further evidenced by resistance of bacteriophage ϕKMV to a *P. aeruginosa* PAO1 type IV pili defective *pilA* mutant. Hence the hypothesis that type IV pili are phage ϕKMV receptors.

Subcloning of cosmid pRG4BG12 and further complementation analysis suggests that a common regulatory mechanism and/or interaction between the *ponA* and *pilMNOPQ* gene products are essential for bacteriophage ϕKMV infectivity. A connection between fimbrial biosynthesis and cell wall growth and remodeling may explain complementation of ϕKMV sensitivity by both partial or entire *pilM* and *ponA* gene (Martin *et al*., 1995; Folster *et al*., 2007). Further on-going experimentation involving regulated expression and complementation of the divergently transcribed *pilM* and *ponA* genes in PAO121Ar will enable us to establish the cause of bacteriophage ϕKMV resistance.

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Particular characteristics of the *in vitro* anti-hepatitis C virus activity of Debio-025, a non-immunosuppressive cyclophilin binding molecule

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Debio-025 is a potent inhibitor of hepatitis C virus (HCV) replication [Hepatology, 43:761-70]. In phase I clinical studies monotherapy (dose 1200 mg BID of Debio-025) resulted in a mean maximal decrease in viral load of 3.6 $\log_{10}$ [Hepatology, 44: 4S1, 609A], whereas a reduction of 4.6 $\log_{10}$ was obtained in phase II studies where Debio-025 was combined with interferon [J Hepatol, 48: S2]. We here report on the particular characteristics of the *in vitro* anti-HCV activities of Debio-025. The combination of Debio-025 with either ribavirin (RBV) or STAT-C inhibitors [NS3 protease or NS5B (nucleoside and non-nucleoside) polymerase inhibitors] resulted in an additive antiviral activity in short term antiviral assays. Debio-025 has the unique ability to clear hepatoma cells from their HCV replicon when used alone or in combination with interferon and HCV polymerase and protease inhibitors. Debio-025, at concentrations that have been observed in human plasma (0.1 or 0.5 μM), was able to delay or prevent the development of resistance to HCV protease inhibitors as well as to nucleoside and non-nucleoside polymerase inhibitors. Debio-025 forms an attractive drug candidate for the treatment of HCV infections in combination with standard interferon-based treatment and treatments that directly target the HCV polymerase and/or protease.

Improvement of the identification of the *Pseudomonas syringae* group: a molecular approach

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The genus *Pseudomonas* accommodates a number of pathogenic species. The type species of the genus for instance, *Pseudomonas aeruginosa*, is frequently associated with infections of the urinary and respiratory tracts in humans. Certain species of *Pseudomonas* are well-known plant pathogens; *P. syringae* is frequently isolated from leaves showing yellowing lesions, and *P. marginalis* is a typical 'soft-rot' pathogen, infecting stems and shoots but rarely leaves. Because of the impact of these pathogenic species on human economy and health, an accurate identification is of utmost importance.
Unfortunately, taxonomy of the genus *Pseudomonas* is obscure with a lot of strains being misnamed, misclassified and/or poorly described. This is mainly due to the taxonomic history of *Pseudomonas* and inadequate identification tools. For a long time, all Gram-negative, rod-shaped, polar flagellated strictly respiratory bacteria were classified in the genus *Pseudomonas*. 16S rRNA gene sequencing revealed the heterogeneity of the genus, resulting in a major reorganisation that started some fifteen years ago and is still ongoing. Yet, clarification of the *Pseudomonas* taxonomy is hampered by the use of identification techniques with insufficient discriminatory power (e.g. API and Biolog), leading to false nomenclature.
The applicability of other than 16S rRNA conservative genes (e.g. *rpoB* gene) for taxonomic purposes is currently under investigation. This study reports on the simultaneous use of several conservative genes (among which *rpoB*, *atpA* and *glnA*) to reveal the taxonomic mix-up within the *Pseudomonas syringae* group. Use of the *rpoB* gene has already been described by Ait Tayeb *et al.* (2005) for identification purposes of pseudomonads and the *atpA* gene has already been used for phylogenetic analysis of vibrios and related species (Thompson *et al*., 2007), indicating the applicability of these genes in revealing the phylogeny of the *Pseudomonas syringae* group.

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PRRSV-infected macrophages are protected against MHC-I-restricted cytotoxicity

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Porcine reproductive and respiratory syndrome virus (PRRSV) mainly replicates in macrophages of lungs and lymphoid tissues. Clearance of PRRSV coincides with the appearance of neutralizing antibodies, but in some pigs, low levels of PRRSV replication are still found in lungs and lymphoid tissues in the presence of neutralizing antibodies. This indicates that other immune-mechanisms may be involved in the complete elimination of PRRSV at those replication sites. This study aimed to determine whether PRRSV-specific cytotoxic T-lymphocytes (CTL) are able to eliminate PRRSV-infected alveolar macrophages. Therefore, cytolytic assays were performed with PRRSV-infected alveolar macrophages as target cells and autologous PBMC as source of PRRSV-specific CTL. Alveolar macrophages were obtained by lung lavages of 3 anaesthetized PRRSV-free pigs, stored in N_2 until use, and inoculated *in vitro* with PRRSV Lelystad virus (LV). PBMC were isolated weekly from the same 3 pigs starting from 0 until 8 weeks post inoculation (wpi) with LV: half were used directly in CTL assays, half were restimulated *in vitro* with LV before use in CTL assays. Similar CTL assays with PRV-Begonia-infected alveolar macrophages and autologous PBMC, derived from 2 PRV-Begonia-inoculated pigs were performed for validation of the technique. Upon infection with PRRSV, the proportion of CTL gradually increased in freshly isolated PBMC until 7 wpi. However, freshly isolated PBMC poorly (not more than 7.5 %) and non-specifically lysed target cells. Restimulation of the PBMC with LV resulted in a weak proliferation of LV-specific CTL starting from 2 until 6 wpi, and in a stronger (values comparable to the previously mentioned PRV-begonia assays) proliferation at 7 and 8 wpi. However, LV-restimulated PBMC induced not more than 12.1 % lysis of autologous LV-infected macrophages and 10.6 % lysis of heterologous LV-infected macrophages. No significant reduction in lysis was observed when autologous LV-infected macrophages were incubated with anti-MHC-I antibodies prior to the CTL assays. In contrast, PRV-Begonia-restimulation of PBMC derived from PRV-Begonia-infected pigs induced maximum 35.1 % MHC-I-dependent lysis of autologous PRV-Begonia-infected macrophages. This study indicates that PRRSV-specific CTL, either derived directly from PBMC of PRRSV-infected pigs or generated by PRRSV-restimulation of PBMC, are unable to induce detectable levels of MHC-I-dependent cytotoxicity.

The Synaptic Complex TnpI/IRS : Molecular Organisation and Assembly

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The Tn*4430* transposon from *Bacillus thuringiensis* encodes a DNA site-specific recombination system to resolve cointegrate intermediates arising from its replicative mode of transposition. In contrast to other members of the Tn*3*-transposon family, the recombination reaction is catalysed by the TnpI protein, a member of the tyrosine recombinases family. The internal resolution site of Tn*4430* (IRS) contains a typical recombination core site (IR1-IR2) at which TnpI mediates strand exchange, and two additional TnpI binding motifs, DR1 and DR2, adjacent to the core. The DR1 and DR2 are dispensable accessory elements, the function of which is to prevent intermolecular recombination events by restricting recombination to sites that are present on the same DNA molecule.

Topological analysis of recombination products obtained with hybrid sites containing the *loxP* target site of Cre fused to the accessory motifs of the IRS suggests that the TnpI/IRS synaptic complex exhibits a specific organisation in which three negative DNA supercoils are trapped around the TnpI subunits. To confirm this observation, we have designed some experiments on which TnpI hybrid acts on knots made by Cre recombinase.

Bandshift experiments carried out with the full IRS reveal the formation of four complexes suggesting hierarchical and cooperative occupation of the recombination site by the TnpI recombination. The identity of these different complexes was established by *in situ* phenantroline-Cu footprinting experiments performed on the corresponding EMSA gel slices and by removing specific TnpI DNA binding motifs. Atomic force microscopy (AFM) was also used to visualise steps in TnpI/IRS complex assembly. Circular permutation experiments demonstrated that TnpI binding to the core and accessory motifs induce a DNA bent of 75° and 58° respectively. The phasing assay, in which we have modified the spacing between the two sites from 4 to 16 bp, show a phasing angle of 68°. A dynamic model for the assembly of TnpI/IRS recombination complex on the IRS will be presented.

Diversity of rhizobia from Belgian leguminous plants: a molecular inventory

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The family of leguminous plants is one of the major plant families in the world. Since the 19th century, the symbiosis between leguminous plants and certain bacteria, collectively called rhizobia, is well known. The plant supplies nutrition and a safe shelter for the bacteria. On the other hand the bacteria can fix nitrogen and deliver this to the plant, enabling it to grow well in nitrogen-limited conditions. This symbiosis is of agricultural (fertilizer, redevelopment of degraded soils) and economic (increased crop yield, food production) importance. Until recently, all known legume-nodulating bacteria belonged to genera of the Alphaproteobacteria. Research over the past years revealed that other bacteria are also capable of nodulating the roots of leguminous plants.

In contrast to rhizobial diversity in crop plants, symbiotic bacteria of wild legumes in Western Europe have not been extensively studied. In Belgium there are about 113 wild species divided into 2 subfamilies of the Fabaceae. The aim of our research is to perform a more systematic study of symbionts present in wild Belgian leguminous plants. The mapping of this micro-flora will allow us to see the effect of geographical location, soil, disturbed environments and pollution on the bacterial symbionts of these plants, to investigate the difference of wild and cultivated plants and to gain knowledge about introduced plant species.

Because surface sterilization of the nodule prior to isolation is essential, our first task was to select and optimize a protocol. To achieve this, different sterilization times and products were tested. The results showed that a sterilization with 3% NaClO during 3 minutes was the most successful.
Nodules selected from two very different habitats, namely from one disturbed habitat and a natural habitat were selected. These nodules were surface sterilized according to the optimized protocol and plated on YMA medium (Vincent, 1970). Different bacterial colonies were selected for DNA extraction by alkaline lysis. Because the number of strains is rather high, rep-PCR was used as a first screening. According to the outcome of the rep-PCR clustering, representatives of each cluster were identified by 16S rDNA sequencing. Depending on the identification obtained by 16S rDNA sequencing, other housekeeping genes will be selected and sequenced to optimize this identification.

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Multilocus sequence analysis using *pheS, rpoA* and *atpA* genes as a robust identification tool in the genus *Streptococcus*

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Multilocus sequence analysis (MLSA) is a polygenic technique applied for revealing taxonomic relationships at the inter- and intra-species level by comparing sequences from multiple bacterial housekeeping genes.

In this work, the applicability of MLSA was evaluated for identification of all validly named streptococci by using partial sequences of the genes encoding phenylalanyl-tRNA synthase (*pheS*), RNA polymerase alpha subunit (*rpoA*) and the alpha subunit of ATP synthase (*atpA*). The MLSA scheme was developed using sequence data from >150 type and reference strains representing all 71 validly named *Streptococcus* species and subspecies.

Sequence alignments and similarity analyses indicated that *pheS* and *atpA* provided interspecies gaps with at least 6% and 5% sequence divergence, and intraspecies variations up to 3% and 2%, respectively. The *rpoA* gene sequences revealed a lower resolution, with interspecies gaps typically exceeding 3% sequence divergence and intraspecies variations up to 1%, indicating a high degree of homogeneity within the *rpoA* gene. The use of concatenated sequences of the three genes offered a reliable identification system for all *Streptococcus* species.

Next to the full genus analysis, we focussed on the applicability of the MLSA scheme for the delineation of strains belonging to the clinically important *Streptococcus bovis* species group. For this purpose, 100 strains were subjected to MLSA. Sequence analysis allowed the correct species identification of the reference strains and was a valuable identification tool for other strains within this group.

Extension of this sequence-based identification system to other genera of the lactic acid bacteria provides a reliable, highly reproducible, low cost and rapid identification tool for identification of more than 250 species belonging to *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weissella*, *Lactococcus* and *Oenococcus*.

Amplified Fragment Length Polymorphism (AFLP) analysis for *Arcobacter* identification and strain typing

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In recent years there has been an increased interest in the genus *Arcobacter*, as some members of this genus may be food-borne pathogens. The genus comprises both free-living environmental species (e.g. *A. nitrofigilis*) and several animal-associated species, of which *Arcobacter butzleri* and *Arcobacter cryaerophilus* in particular have been implicated in human disease. Since the introduction of AFLP as a tool for bacterial taxonomy, almost 15 years ago, this technique has been used to study a diverse range of genera. This fingerprint technique is based on the selective PCR amplification of total genomic DNA restriction fragments, and can be used to differentiate strains below genus level. In the present study, a modified version of an AFLP scheme for the closely related genus *Campylobacter* was used for analysis of the genus *Arcobacter*. In brief, total genomic DNA was digested with a combination of two restriction enzymes (*Hind*III/*Hha*I). After restriction, adaptors were ligated to the restriction fragments, and selective amplification was performed using one selective nucleotide. Finally, fragments were separated by capillary electrophoresis. A total of 104 strains, representing all currently known *Arcobacter* species, were included in the analysis. Numerical analysis of the AFLP patterns yielded a single cluster per species, except for the *A. cryaerophilus* and *A. thereius* which formed two distinct clusters each. For *A. cryaerophilus*, this observation is in accordance with previous reports on the heterogeneity of this species. In conclusion, AFLP analysis is a valuable tool for identification, both at the species and strain level.

Biogenesis of outer membrane proteins in *Legionella pneumophila*

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Legionella pneumophila is a Gram-negative, facultative intracellular pathogen and the causative agent of Legionnaires' disease, a severe form of pneumonia. In Gram-negative bacteria the outer membrane is the site of contact between the cell and its environment. Therefore, outer membrane proteins (Omps) undoubtedly play an important role in the interaction of the bacterium with its environment, other bacteria or host cells. In *L. pneumophila* several Omps are characterized as important virulence factors, but the biogenesis of these Omps is not studied yet. Recently, a model for Omp biogenesis was proposed for *Neisseria meningitidis* with the protein Omp85 as a central player. Screening of the *L. pneumophila* genome resulted in the presence of a gene coding for the *N. meningitidis* Omp85 homologue. It was shown that *L. pneumophila* Omp85 is present in an outer membrane protein complex and that it interacts with Lpa, *L. pneumophila* plasminogen activator.

Comparison of rodac plates and petrifilm™ to assess the microbial contamination of food-contract surfaces/ importance of additieves

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Assessing the microbial contamination level of surfaces is critical in environments where high hygiene levels are required. This is typically the case in food and catering industries or in hospitals and medical appliances. On a routine base, RODAC plates and other techniques based on microbial transfer are generally used to quantify the microbial load of a surface. There are however still polemics on the limitations and performances of these techniques arising from the fact that the initial contamination level of a surface is never known and due to the difficulty of reproducing field conditions in laboratory environments. The present study brings further information in that direction.

Sister chromosome cohesion in *E. coli*: cohesion with the FtsK activity domain

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The *E. coli* chromosome is unique and circular. Its replication is bidirectional and initiates from a single origin, *ori*, to terminate in the *ter* region, diametrically opposite from *ori*. The *ter* region is of crucial importance for chromosome dynamics. It is the site of the last operations of sister chromosome separation: termination of replication, removal of the intercatenation links, and resolution of dimeric chromosomes by XerCD/*dif*-mediated recombination. Unlike the rest of the chromosome, the sister *ter* regions do not segregate rapidly after their replication but display a significant period of co-localization and do not separate until the onset of the division. This phenomenon is called "cohesion" by analogy to the chromosome cohesion observed in eukaryotes. While it is assumed that this cohesive period is required for the last steps of chromosome segregation, nothing is known about the genetic determinants and the mechanism of cohesion and of cohesion released in bacteria.

In the present work, we re-investigated in details the limits of the cohesive zone, i. e. showing the most delayed segregation, within the *ter* region. For this purpose, we used the ParB-GFP/*parS* Fluorescent Repressor-Operator System (FROS) previously developed by Austin and co-workers (Li *et al*., 2003, Nielsen *et al*., 2006).

This microscopy approach allowed us to determine the segregation timing of different fluorescent markers inserted in the *ter* region. The cohesive zone observed expanded on about 400kb centered on the *dif* recombination site.

This domain was found to be identical to the FtsK activity domain that was determined in our laboratory (Pages C. et al, unpublished). The FtsK DNA translocase is involved in the last steps of sister chromosomes separation and in dimeric chromosome resolution by activating the XerCD/*dif* recombination system.

Relocating the termination site of replication inside the FtsK domain did not significantly modify the cohesive domain. In contrast, cohesion was reduced in the absence of the C-terminal part of FtsK, showing that FtsK takes part in this phenomenon.

The role played by FtsK in sister *ter* regions cohesion is currently under investigation through the analysis of the cohesive domain in mutants deficient for different activities of FtsK and/ or for the XerCD recombinases.

Li Y et al.(2003) Mol Microbiol 50:825-34
Nielsen et al. (2006) Mol Microbiol 61 :383-93

The quest for persistence genes in *Pseudomonas aeruginosa*

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Background: *P. aeruginosa* is an opportunistic pathogen which causes severe infections among immunodeficient individuals. A fraction of the bacterial population, called persister cells, can tolerate high doses of antibiotics. Upon reinoculation, the persisters exhibit the same antibiotic susceptibility as the original population, indicating that they are not resistance mutants, but posses a phenotypic tolerance against antibiotics. Little is known about the mechanism of persistence. The objective of this study is to find factors that play a role in persistence at the genetic level.
Methods: Via insertional mutagenesis of the *P. aeruginosa* strain PA14 with a plasposon, a mutant library of 5000 mutants was constructed. After treatment with ofloxacin, the persister fraction of these mutants was compared to that of wild-type bacteria in a large scale screening procedure using a Bioscreen apparatus. After selection, the persistence phenotype was validated by plate counting and the mutant gene was identified by DNA sequencing.
Results: The screening procedure revealed 17 genes that play a significant role in the persistence of *P. aeruginosa,* either by lowering (10 mutants) or increasing the number of persisters (7 mutants) with a factor between 10 and 125. The identified genes belong to different functional groups including transcriptional regulators, transport systems and enzymes. The selected mutants were extensively phenotyped with regard to survival in other stress conditions (heat stress, UV stress and other antibiotics).
Conclusions: We have performed a high-throughput screening of a *P. aeruginosa* mutant library and have identified 17 mutants displaying increased or reduced persistence. The mutant genes were sequenced and the mutant strains phenotypically analysed.

Nitrifying membrane bioreactor as effective effluent polishing technique for instant 17α-ethinylestradiol removal

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The discovery of feminization of male fish and other aquatic organisms exposed to wastewater treatment plant (WWTP) effluents increased the concern about the fate of low-level concentrations of endocrine disrupting compounds (EDCs) in the environment. Particularly 17α-ethinylestradiol (EE2), the active compound of the contraceptive pill, is recalcitrant with a high endocrine disrupting potency. EE2 concentrations up to 106 ng L^{-1} are measured in WWTP effluents. Advanced wastewater treatment techniques include chlorination, ultraviolet photolysis, activated carbon or ozonisation but they can produce persistent and potentially dangerous byproducts or they represent a considerable cost for application to large wastewater streams. Therefore, increasing efforts are undertaken to develop alternative methods for WWTP effluent polishing.

In this study, the ability of a nitrifying sludge to biologically remove EE2 at low levels (ng - µg L^{-1}) was examined in both batch incubation tests as in a membrane bioreactor (MBR). After an adaptation period of 72 h, the microbial consortium was able to remove EE2 out of a synthetic wastewater at a rate of 97 ± 2 µg EE2 g VSS^{-1} d^{-1} in combination with a complete nitrification (initial concentrations of 750 µg EE2 L^{-1}, 52.5 mg NH_4^+-N L^{-1} and 0.75 g VSS L^{-1}). During incubation of the nitrifying biomass in actual WWTP effluent containing 1.5 mg of Total Ammonia Nitrogen (TAN) L^{-1} (Ossemeersen, Ghent, Belgium) an EE2 removal efficiency of 94% was achieved after 96 h (249 ± 1 µg EE2 g VSS^{-1} d^{-1}). Treatment of the synthetic wastewater with heat-inactivated biomass resulted in a removal of 21%, indication that the EE2 removal was not only due to sorption. Specific inhibition of the ammonia oxidizing bacteria (AOB) and pure culture experiments indicated that the AOB are potentially responsible for the first degradation step of the EE2 molecule whereas the heterotrophic bacteria present in the nitrifying inoculum play an important role in the subsequent removal of the metabolites.

Further application of the nitrifying inoculum in a plate MBR at the same VSS concentration resulted in a continuous removal of EE2 in combination with a complete nitrification. Variation of the EE2 loading rates ($B_{v,EE2}$) and the ammonium concentration in the synthetic influent, pointed out that an EE2 removal efficiency of 98% was possible, with a minimal ammonium concentration of 0.8 mg NH_4^+-N L^{-1} ($B_{v,EE2}$ = 29 µg EE2 L^{-1} d^{-1}, Hydraulic Retention Time (HRT) = 4 d). Using the same ammonium concentration, the MBR was used to treat a synthetic wastewater with an environmentally relevant EE2 concentration of 100 ng EE2 L^{-1}. Loading rates up to 250 ng EE2 L^{-1} d^{-1} could be achieved, resulting in an EE2 removal efficiency of 99% with a short HRT of 0.4 d.

This research gave rise to new perspectives on an alternative way to remove EDCs out of WWTP effluents. Application of commercially available highly active nitrifying consortia in an MBR can instantly and effectively remove estrogenic compounds at short HRT. Operation of the MBR at low VSS concentrations and cross-flow prevents biofouling of the plate membranes. Moreover, no addition of extra ammonium seemed necessary since actual WWTP effluent still contains 1.5 mg TAN L^{-1}. Without the need for excessive operation costs, this new feature for MBR technology can be very promising for effluent polishing to protect sensitive sites such as point discharges (e.g. WWTPs) near water intakes or near highly value ecological areas.

Generation of PRRS virus susceptible cell lines by stable integration of recombinant, virus-specific receptors

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Porcine reproductive and respiratory syndrome virus (PRRS virus; PRRSV), member of the family Arteriviridae, order Nidovirales, is the economically most important viral swine pathogen worldwide. In vivo, the virus infects a subpopulation of tissue macrophages. In vitro, PRRSV only replicates on primary pig macrophages (e.g. alveolar macrophages) or the African green monkey kidney derived cells, such as Marc-145. Currently, vaccine virus is produced on Marc-145 cells. However, since virus entry in Marc-145 cells is different compared to entry in primary macrophages, the possibility exists that specific epitopes associated with virus neutralization are lost during adaptation of the virus for growth on Marc-145 cells. Although virus production on primary macrophages would be ideal to avoid potential loss of neutralizing epitopes, these cells cannot be used because of batch variation, risk of contamination with other pathogens present in primary cells and increased costs. To avoid these problems, we aimed in this study to create cell lines that recombinantly express receptors that mediate PRRSV entry in macrophages, sialoadhesin (Sn) and CD163. We previously showed that non-permissive cells transiently transfected with Sn only sustained internalization, but not infection. Non-permissive cells transiently transfected with CD163 may allow infection depending on the cell type used, but co-expression of both sialoadhesin (Sn) and scavenger receptor CD163 is needed for efficient PRRSV infection. To construct a cell line co-expressing Sn and CD163, CHO-K1 cells were transfected with a plasmid containing the Sn cDNA and a geneticine resistance gene. After selection for geneticine resistance, cells were transfected with a plasmid containing the CD163 cDNA and a zeocin resistance gene, which allowed selection of cells expressing both Sn and CD163. Finally, 16 clones that co-expressed Sn and CD163 ($CHO^{Sn\text{-}CD163}$) were isolated. 10 clones in which 100% of the cells stably expressed Sn and CD163 were isolated, while the other 6 clones lost either Sn or CD163 receptor expression. After a first screening for susceptibility to PRRSV infection, three $CHO^{Sn\text{-}CD163}$ clones were selected that allow virus production up to the same level as the primary target cells. Currently, the characteristics of virus grown on these clones are further investigated.

Candida albicans biofilm formation on modified polydimethylsiloxane substrates

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Candida albicans biofilms are consortia of micro-organisms, encased in a self-produced extracellular polymer matrix and formed on (a)biotic surfaces such as on implanted medical devices (i.e. tracheoesophageal voice prostheses in laryngectomized patients). Biofilm development on the one-direction valve, particularly at the oesophageal side of this prosthesis, necessitates replacement of the device to avoid leakage of fluids into the respiratory tract. Eradication of biofilms is difficult since sessile cells show a decreased susceptibility to antifungals and disinfectants. In order to prolong the lifetime of a voice prosthesis, the silicone (polydimethylsiloxane) surface was modified by covalent binding of dimethylaminoethyl methacrylate, polyethylene imines, cationic peptides, polyarginine and polylysine homopolymers. The antibiofilm effect of the modified surfaces was estimated in a dynamic model system (Modified Robbins Device ([MRD]) by determining the number of sessile cells on silicone disks using the plate count method. Modification of PDMS by grafting of active moieties on the surface resulted in reduced biofilm formation in the MRD. However, the reduction in *C. albicans* biofilm biomass highly depended on the type of modification tested.

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

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

Many herpesviruses interfere in diverse ways with the MHC I antigen processing pathway to avoid or delay elimination by cytotoxic T lymphocytes. For the largest subgroup of the alphaherpesviruses, the varicelloviruses, two viral proteins have been described to interfere with MHC I antigen processing and cell surface expression. For different animal varicelloviruses, such as BoHV-1, PRV and EHV-1, but not for VZV, UL49.5 has been described to interfere with the transporter associated with antigen processing that is involved in MHC class I peptide loading (Koppers-Lalic et al., 2005, PNAS, Koppers-Lalic et al., 2008, PLoS Pathogens; Verweij et al., J. Immunol., 2008). For VZV, but up to now not for other alphaherpesviruses, the conserved viral US3 serine/threonine protein kinase orthologue (ORF66) has been described to mediate downregulation of MHC I from the cell surface via a largely unknown mechanism (Abendroth et al., 2001, JVI, Eisfeld et al., 2007, JVI). However, for VZV, other, unknown and both US3 and UL49.5 independent MHC I downregulation mechanisms were also observed (Eisfeld et al., 2007, JVI). In the current study, by flow cytometry, we found that the mechanism(s) by which PRV alters MHC I cell surface expression during infection is highly cell type dependent.

In porcine kidney PK-15 cells, PRV induced a strong (60-65%) downregulation of MHC I from the cell surface. Since a US3null PRV was not, and a UL49.5null PRV was only partially -but statistically significant- impaired in downregulating MHC I (30% less MHC I reduction compared to wild type (WT) PRV), other viral proteins than UL49.5 and US3 have to be involved in MHC I downregulation in these cells.
PRV infection of swine testicle (ST) cells resulted in a 35-45% cell surface MHC I reduction. Interestingly, both a US3null PRV and a PRV encoding a kinase-dead US3 showed no MHC I downregulation at all, indicating that the kinase activity of US3 is required for MHC I downregulation during PRV infection in ST cells. UL49.5null PRV infected ST cells showed a slightly higher but statistically not significant MHC I cell surface expression compared to WT PRV infected cells.
In porcine alveolar macrophages, a 40-50% cell surface MHC I downregulation was observed. A US3null PRV resulted in a similar MHC I downregulation as the WT PRV, while a UL49.5null PRV showed a slight (12%), but statistically significant, decrease in MHC I downregulating capacity. Thus, as in PK-15 cells, other viral proteins than UL49.5 and US3 have to be involved in MHC I downregulation in macrophages.
In conclusion, we report that in ST cells, the PRV mediated downregulation of cell surface MHC I is due to the kinase activity of US3. In PK15 cells and macrophages, PRV-induced downregulation of MHC I was only slightly dependent on UL49.5 and not dependent on US3, indicating that other, unidentified viral proteins are involved. Hence, the mechanisms by which PRV alters MHC I cell surface expression during infection are highly cell type dependent.

Poly-β-hydroxybutyrate (PHB) in the feed of European sea bass induces increased growth performance combined with changes in the intestinal microbial community

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The bacterial storage polymer poly-β-hydroxybutyrate (PHB) has the potential to be used as alternative anti-infective strategy for aquaculture rearing. However, until now its application has only been reported for the aquaculture model organism *Artemia fransiscana.* In this research, the effects of (partially) replacing the feed of sea bass juveniles with PHB (w/w) were investigated in relation to the growth performance and changes in the intestinal microbial community. During a six weeks trial period, 6 different treatments were assessed: a non-fed treatment and a 0% PHB, 2% PHB, 5% PHB, 10% PHB and 100% PHB treatment.

The PHB showed the ability to act as an energy source for survival in the absence of normal feed (100% PHB treatment, data not shown). This indicated that the PHB was degraded during gastrointestinal passage. The diets supplemented with 2% and 5% PHB resulted in a significant increase of the average weight gain to 243% and 271%, respectively, relative to the gain of 216% in the 0% PHB treatment (Fig. 1). Simultaneously, the presence of PHB in the gut induced significant changes in the intestinal microbial communities.

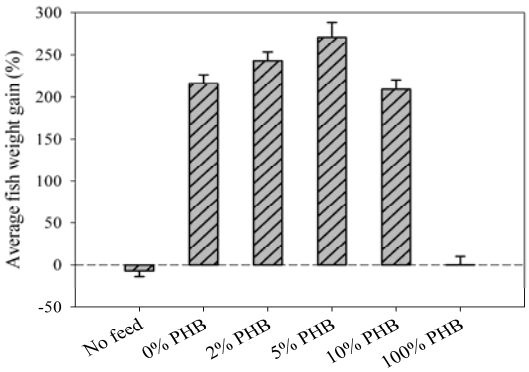

Fig. 1: Average fish weight gain of European sea bass juveniles after a 6 weeks feeding trial with diets containing different levels in PHB (n = 8)

In contrast to the samples from day 14, a trend of larger changes (= lower % similarity) in the microbial community at higher dietary PHB levels could be observed after 42 days of feeding (Fig 2.). The concurrent observation of lower gut pH values corresponding to this trend suggested an increased production of short chain fatty acids or PHB monomers/oligomers. The presence of PHB in the gut affected individual species as illustrated by the suppression of an unknown phylotype related to cynobacteria (frame 1, Fig. 2). A promoting effect was noted for *Methylobacterium* sp., a known PHB accumulator (frame 2, Fig. 2). The exact mechanisms behind the positive effects of PHB on the growth remain largely unclear up to date. Based on our results, it is hypothesized that microbial activity plays a major role in the intestinal transformation of PHB.

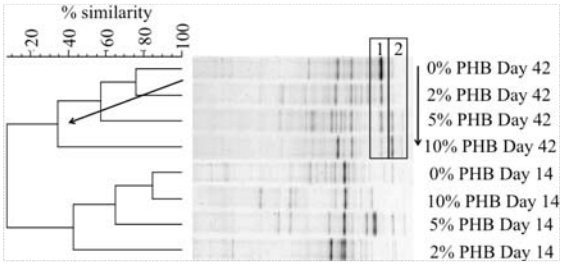

Fig. 2: DGGE band patterns based on the amplified bacterial DNA extracted from the gut of European sea bass juveniles. The diets represented are 0% PHB, 2% PHB, 5% PHB or 10% PHB (sampled on day 14 and day 42). On day 42, higher PHB levels corresponded to lower band pattern similarities.

Cholesterol is important for the production of infectious pseudorabies virus

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Lipid rafts are cholesterol-rich microdomains in the trans-Golgi network (TGN) and plasma membranes of eukaryotic cells. Depletion of cholesterol leads to disruption of lipid rafts. For different viruses like HIV, Ebola, measles, and influenza, cholesterol and/or lipid rafts have been demonstrated to be of importance in efficient virus maturation, budding and/or egress. Since these viruses generally bud at the plasma membrane, many of these studies were based on assessing the effect of disruption of cholesterol from the plasma membrane (using cyclodextrins) on infectious virus production.

Herpesvirus budding occurs at the trans-Golgi network, which is inaccessible for cyclodextrins, thereby complicating the assessment of an involvement of cholesterol and lipid rafts in herpesvirus maturation and egress. In the current study, we have assessed the involvement of cholesterol in maturation and egress of the alphaherpesvirus pseudorabies virus (PRV) by lowering intracellular levels of cholesterol using statins. Using this method, we have found that a less than 50% reduction in cellular cholesterol in swine kidney cells, which does not affect cell viability, results in > 95% reduction in extracellular virus titer at 8hpi. Addition of exogenous cholesterol to restore cholesterol levels restored the production of infectious PRV, indicating that the effect is specifically due to cholesterol modulation. Interestingly, the reduction in titer by cholesterol depletion is not due to a reduced intracellular production of structural viral proteins, as levels of envelope proteins gB and gE and tegument protein UL47 were unaffected. Flow cytometry indicated that intracellular transport of envelope proteins gB and gE was also unaffected by cholesterol depletion. These data indicate that cholesterol may serve an important structural function in infectious PRV virus particles. In support of this, we found that disruption of cholesterol from PRV virus particles using methyl-beta-cyclodextrin abolished their infectivity, which again could be restored by the addition of exogenous cholesterol.

In conclusion, our data point to an important role for cholesterol, production of infectious alphaherpesvirus particles. In addition, our data suggest that lowering cholesterol using statins and/or cyclodextrins may have potential in interfering with efficient herpesvirus replication.

Lack of transmission of low pathogenic avian influenza viruses between pigs and from pigs to ferrets

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Introduction: Pigs are susceptible to human and avian influenza (AI) viruses and they have been proposed to act as an intermediate host for the transmission of AI viruses from birds to humans. Despite this, wholly AI viruses generally do not transmit between pigs or from pigs to man, in nature. This study aimed to examine the capacity of different low pathogenic (LP) AI viruses to spread between pigs and from pigs to ferrets. Ferrets are susceptible to a wide range of influenza viruses and are frequently used as a model for humans in influenza research.

Materials and methods: Ninety-six 6-week-old pigs and 16 adult ferrets were used. All animals were influenza seronegative. An H1N1 and an H3N2 swine influenza virus (SIV) and 6 LPAI viruses of various hemagglutinin subtypes (see table 1) were used in 8 separate experiments. All viruses used, were previously shown to replicate in experimentally inoculated pigs and ferrets. In each experiment, 6 pigs were inoculated intranasally with 7,0 $\log_{10}$ EID_{50} of the respective virus. Two days later, 6 contact pigs were housed in direct contact with the inoculated pigs. At the same time 2 ferrets were placed in a wire cage in each pig stable, allowing aerogenic contact. All animals were monitored daily for clinical signs. Nasal swabs for virus titration were collected fom 0 until 10 days post-inoculation (DPI) or post-contact (DPC). Serum was collected from all animals at 0, 14 and 28 DPI or DPC and examined for antibodies against the homologous virus in an immunoperoxidase monolayer assay (IPMA). During the whole experiment the stable environment was maintained at a temperature of 20-22°C and a relative humidity of 50-70%, simulating field conditions.

Results:

Table 1. Transmission of influenza viruses from inoculated pigs to contact pigs and contact ferrets

Virus	Number of animals with					
	Inoculated pigs		Contact pigs		Contact ferrets	
	Excretion (peak)[1]	Antibodies (mean titre)	Excretion (peak)	Antibodies (mean titre)	Excretion (peak)	Antibodies (mean titre)
A/swine/Belgium/1/98 H1N1	6/6 (5,2)	6/6 (1024)	6/6 (6,0)	6/6 (4267)	2/2 (4,0)	2/2 (≥8192)
A/swine/Flanders/1/98 H3N2	6/6 (7,2)	6/6 (≥8192)	5/5 (7,0)	5/5 (≥8192)	2/2 (5,4)	2/2 (≥8192)
A/duck/Italy/1447/05 H1N1	6/6 (6,2)	6/6 (400)	0/6 (-)	0/6 (-)	0/2 (-)	0/2 (-)
A/mallard/Alberta/47/98 H4N1	6/6 (6,2)	5/5[2] (973)	0/6 (-)	0/5[2] (-)	0/2 (-)	0/2 (-)
A/duck/Belgium/06936/05 H4N6	6/6 (6,0)	6/6 (3072)	0/6 (-)	0/6 (-)	0/2 (-)	0/2 (-)
A/mallard/Italy/3401/05 H5N1	1/6 (4,2)	6/6 (437)	0/6 (-)	0/6 (-)	0/2 (-)	0/2 (-)
A/chicken/Belgium/150/99 H5N2	5/6 (6,2)	6/6 (2410)	0/6 (-)	0/6 (-)	0/2 (-)	1/2 (128)
A/chicken/Italy/1067/V99 H7N1	5/6 (5,9)	6/6 (1068)	0/6 (-)	2/6 (288)	0/2 (-)	0/2 (-)

[1]: virus titres are expressed as $\log_{10}$ EID_{50}/100mg; [2]: one pig died before 14 DPI or DPC; -: not applicable

Discussion: Our data indicate that the examined LPAI viruses transmit inefficiently between pigs and generally fail to transmit from pigs to ferrets. From the 6 LPAI viruses tested in this study, only 1 spread to a very limited degree between pigs and 1 from pigs to ferrets. These findings are in agreement with reports of failure of HPAI viruses to spread between pigs or ferrets, and suggest a minimal risk of transmission of LPAI viruses via pigs to humans. The reason for the lack of transmission of LPAI viruses between pigs and from pigs to ferrets remains obscure. Virus titres in nasal swabs of LPAI inoculated pigs were generally lower and more variable than those of the SIV inoculated pigs. The lower virus dose to which the contact animals were exposed may be an important factor for the lack of transmission. Serological evidence of virus spread to contact pigs and ferrets was found only with the H7N1 and H5N2 isolate respectively, but no virus excretion was detected in any of the contact animals. However, virus excretion titres of the H7N1 inoculated pigs were similar to those of pigs inoculated with the other LPAI viruses. Our results are in line with the assumption that AI viruses have to undergo genetic changes to adapt to a mammalian host and to spread efficiently between mammals. It is noteworthy in this regard that both the H5N2 and H7N1 viruses were isolated from chickens, while the 4 other LPAI viruses examined were isolated from wild ducks. It is possible therefore that chicken viruses are more adapted to replication in mammals than the viruses from wild ducks. However, further research is needed to examine this possibility. We now have a suitable model to examine the transmission capacity of AI viruses with well defined genetic differences between pigs and from pigs to ferrets.

A new internalization pathway revealed by an immune evasion strategy used by feline infectious peritonitis virus

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Infection with feline infectious peritonitis (FIPV), a feline coronavirus, leads frequently to death in spite of a strong humoral immune response. In previous work, we reported that infected monocytes, the in vivo target cells of FIPV, express viral proteins in their plasma membranes. These proteins are quickly internalized upon binding of antibodies. As the cell surface is cleared from viral proteins, internalization might offer protection against antibody-dependent cell lysis. Here, the internalization and subsequent trafficking of the antigen-antibody complexes were characterized using biochemical, cell biological and genetic approaches. Internalization occurred through a clathrin- and caveolae-independent pathway that did not require dynamin, rafts, actin nor rho-GTPases. These findings indicate that the viral antigen-antibody complexes were not internalized through any of the previously described pathways. The experiments further showed that internalization and trafficking was dependent on microtubules but did not require actin polymerization. In fact, the cortical actin network formed a barrier that slowed down internalization and that had to be overcome by moving or disintegrating actin filaments. With co-localization stainings, it was found that myosin 2a, 2b, 5a, 7a, 9b and 10 were not involved in the internalization or subsequent trafficking of the antigen-antibody complexes. However, myosin 1 and 6 co-localized with the internalizing complexes during passage through the cortical actin and might thus be involved in the required actin reorganization. One minute after internalization started, vesicles had passed the cortical actin, co-localized with microtubules and association with myosin 6 was lost. The vesicles were further transported over the microtubules and accumulated at the microtubule organizing center after 10 to 30 minutes. During transport over microtubules, the vesicles were associated with myosin 1 and a small actin tail indicating that actin, myosin 1 and microtubules cooperated during intracellular trafficking. This study characterizes what is probably a new internalization pathway into monocytes, confirming once more the complexity of endocytic processes.

Individualized, Degressive Maintenance Regimen using Fluconazole for Recurrent Vulvo-vaginal Candidiasis (ReCiDiF trial)

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Introduction. Although many women with recurrent vulvovaginal candidiasis (RVC) initially benefit from prophylactic intermittent treatment with antimycotics, most of them relapse after cessation of therapy and often they return to the pre-treatment recurrence rate.

Aim. Demonstrate the efficacy and safety of an individualised, degressive, prophylactic regimen in 136 women with RVC.

Methods. After an induction dose of 600 mg fluconazole during the first week, 117 women started maintenance therapy, 200mg fluconazole weekly for 2 months, followed by 200mg biweekly for 4 months and 200mg monthly for 6 months, according to their individual response to therapy. All women were tested for recurrences monthly with wet mount microscopy and vaginal culture during the first 6 months and bi-monthly during the next 6 months. Patients were only allowed to move on to the next level of maintenance therapy, if they were symptom-free and also microscopy and culture negative.

Results. Of the women successfully cured after the induction phase, 101 (90%) were disease-free after 6 months of maintenance therapy with this degressive regimen and 80 (77%) after 1 year. The weekly incidence of the first clinical relapse was 0.5% during any period of the maintenance phase, and the rate of all new relapses including evidence of mycological or microscopic colonisation was 1% per week. Women suffering several relapses (poor responders) had experienced more relapses before entering the study compared to the optimal responders (OR 4.9 (1.8-13.7) p=0.002), suffered from the disease for a longer period of time (6.5 vs 3.7 years, p=0.06) and harboured significantly more *Candida* non-albicans during maintenance therapy (p=0.001). No serious side effects were noted.

Conclusion. Individualised, degressive, prophylactic maintenance therapy with oral fluconazole is an efficient treatment regimen to prevent clinical relapses in women with RVC.

The Efficacy of a Single Oral Dose of 200 mg Pramiconazoleole in Vulvovaginal Yeast Infections: An Exploratory Phase IIa Trial

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Introduction. Pramiconazole (R126638) is a novel azole with potent antifungal activity against yeasts, dermatophytes and many other fungal species.

Aim. Evaluate the efficacy and tolerance of a single oral dose of 200 mg pramiconazole in acute and recurrent vulvovaginal yeast infections.

Patients and methods. Thirty two patients (15 acute and 17 recurrent cases) were KOH microscopy and culture-positive at inclusion and this population was evaluated for mycological and clinical outcome. All evaluations were performed before, at 1 week and 1 month after treatment. Several individual signs and symptoms (edema, erythema, excoriation, pruritus, burning and irritation) were evaluated as well.

Results. Clinical cure was 53% at 1 week and 66% at 1 month. Mycological eradication was obtained in 88% at 1 week, whereas at 1 month 75% of the patients at 1 month were still culture-negative. Effects in both acute and recurrent cases appeared to be similar for mycological cure. The composite sign and symptom score (sum of scores for edema, erythema, excoriation pruritus, burning and irritation) had a median value of 7.5 (range 2–17) at inclusion. At 1 week this value was reduced to 1.0 (range 0–8) and at 1 month a further reduction to 0 (range 0–11) was seen. P-values compared to baseline at both follow-up visits were <0.001. The drug was well tolerated and the reported adverse events were rare and minimal.

Conclusion. The results of this trial indicate that pramiconazole possesses properties that warrant further clinical studies in a larger number of patients with acute and recurrent vulvovaginal yeast infection to confirm its efficacy and tolerability.

Different types of abnormal vaginal flora as candidates for prophylactic treatment with probiotics

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Most of the patients suffering from recurrent vaginal infections are tired of repetitive treatment courses with antibiotics, as they keep having recurrences, experience side effects, or, most dramatic of all, worsening of their symptoms. Treatment of bacterial vaginosis with metronidazole, or clindamycin, and candida with fungostatic azole therapy usually enables alleviation of their symptoms, but in most cases, the condition comes back necessitating ever repeating courses of antibiotics or antimycotics to be taken. Therefore, the idea of replacing the failing vaginal resistance system by potent lactobacilli that could protect the vagina against foreign invaders or own potential pathogens became very popular over time. Sadly enough, some conditions are more prone to be treated successfully, while others are not responding to antimicrobial treatment at all. In this presentation we overview the different subtypes of abnormal vaginal flora (AVF) that may benefit from treatment with probiotics to increase and maintain a endogenous vaginal resistance: recurrent bacterial vaginosis (BV), which has an intermediate from between normal and full blown BV, called partial BV, aerobic vaginitis (AV) and even certain types of recurrent candidiasis. Lactobacillary grades (LBG's, see above) are the basis for a composite score of AV to which the following four variables were added: 1) proportional number of leukocytes, 2) presence of toxic leukocytes, 3) presence of parabasal epithelial cells and 4) type of background flora. The use of this AV criterion enables us to divide the flora in a more detailed and comprehensive way, avoiding undefined and unclear categories. AV and partial BV may be important in pregnancy and not respond well to classical treatment such as metronidazole. Also in recurrent candidiasis, and maybe trichomoniasis, the reintroduction of health-maintaining lactobacilli may be beneficial.

Molecular characterization of *Brucella abortus* differentiation

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For some time already, the scientific community agrees that cellular differentiation is an inherent characteristic of the eukaryotic and prokaryotic worlds. In this context, *Caulobacter crescentus* is considered as a model of prokaryotic differentiation. Its phylogenetic relationship, within the family of α-proteobacteria, with the genus *Brucella* allows to understand with *a priori* the molecular mechanisms involved in the asymmetry and the differentiation. In our laboratory, we showed that an histidine kinase named PdhS and a fumarate hydratase called FumC are localized at the old pole of the bacterium. This suggests that a series of proteins of different functions could be associated with one or both bacterial poles, either old or new. These hypotheses imply that after the division, a differential segregation of functions would take place, suggesting a functional asymmetry and thus a differentiation in *B. abortus*.

It is with the aim of characterizing the differentiation in *B. abortus* that we began a project to identify on one hand markers of poles (either new, young or old) and on the other hand functions associated with the poles. To do it, we benefited from the availability in the laboratory of an ORFeome of *Brucella melitensis* (phylogenetically close to *B. abortus*) in which the various ORFs are cloned in a donor vector. The first stage was to clone the ORFeome by pools of 46-48 clones in a destination vector allowing a translation fusion between the various ORFs and the coding sequence (*cds*) *yfp*. Finally, we transformed pools in the *B. abortus* 544 *pdhS-cfp* strain constructed previously. This one produces the PdhS-CFP fusion from a construct integrated into the *pdhS* locus, obtained by the replacement of the *pdhS cds* by a *pdhS-cfp* allele. This strain allows to mark the old pole of the bacterium and to quickly determine the nature of the pole marked by the Protein-YFP fusion.

On 68 observed pools, we selected 8, for which a substantial proportion of bacteria showed polar foci. Until now, we identified five fusion proteins presenting polar foci. These proteins are BMEI1434, BMEI1198, ExbB, NtrB and BvrR. Two proteins particularly drew our attention, the hypothetical protein BMEI1434 and the conserved protein ExbB. We observed that the BMEI1434-YFP fusion protein localizes almost always of the opposite pole compared to PdhS-CFP, so it could be use as a young pole marker. The ExbB-YFP fusion protein presents a bipolar localization in the majority of cells. We also observe in certain cells several fluorescent foci. The ExbB protein is involved in the active transport through the outer membrane (for example for the acquisition of the iron) which is essential for the survival of the pathogenic bacteria. ExbB forms a complex localized in the inner membrane, with TonB and ExbD proteins. This complex interacts with outer membrane TonB-dependent receptors, each specific to a ligand for example a siderophore. Such data suggest that the bacterial poles would be privileged sites for the active transport through the TonB-dependent receptors.

Study of the predominant colon microbiota from cystic fibrosis patients

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Cystic fibrosis (CF) is a hereditary disorder in humans that mainly affects the respiratory and digestive systems causing progressive disability. CF patients suffer from chronic inflammation and respiratory infections caused by micro-organisms such as *Pseudomonas aeruginosa* and members of the *Burkholderia cepacia* complex which are the main causes of morbidity and mortality in this population. Because of chronic or recurrent lung infections, CF patients consume an unusually high amount of multiple antimicrobial agents. This antimicrobial therapy affects their general well-being and must have detrimental effect on the stability and composition of the intestinal flora, thus provoking an additional burden on their health.

The present study aims to characterize the predominant members of the intestinal microflora of a group of cystic fibrosis patients and to compare these with the flora of siblings without the cystic fibrosis phenotype.

To explore the gastrointestinal microflora of our patients, we have chosen a polyphasic approach, including both culture-dependent and culture-independent techniques. We have started to grow the predominant members of the human colon microflora (*Enterobacteriaceae,* lactic acid bacteria, clostridia, bifidobacteria, *Veillonella* sp., *Bacteroides* sp. and *Prevotella* sp.) using 6 selective culture media and one non-selective medium for colon bacteria. Per medium, serial dilutions were made on agar plates in triplicate and anaerobically incubated at 37°C up to 72h. After 24h, 48h and 72h of incubation, the colony forming units (CFU) were counted. As expected, the results of the plate counts mainly show a higher number of CFU for the sibling samples compared to the samples of the CF patients. Finally, after three days of incubation, the cells were harvested and pellets were stored at -20°C for further analysis. Subsequently DNA was extracted directly from the samples and from the cultivable fractions, followed by amplification of the hypervariable V3 region of the 16S rRNA gen. These PCR-products were finally evaluated on Denaturing Gradient Gelelectrophoresis. Analysis of the DGGE fingerprint profiles revealed complex microbial communities which differed between the investigated subjects. On the other hand, visual interpretation of the fingerprints indicates the presence of subject-specific bacterial populations within each subject.

Acanthamoeba polyphaga: a biological fitness of *Mycobacterium ulcerans*?

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Buruli ulcer, or *Mycobacterium ulcerans* disease, severely affects skin and bone. Countries in which the disease is endemic are predominantly in the tropics. *M. ulcerans* produces a necrotising and immunosuppressive polyketide toxin called mycolactone which is responsible for the virulence of the bacteria. The reservoir of *M. ulcerans* is linked to aquatic environments with some evidence on the involvement of insects. Recently, amoebae have been suggested to be involved in the reservoir as well. Virulent *M. ulcerans*, like other pathogenic mycobacteria, has a phase of intracellular growth within macrophages.
Protozoa have previously been proposed as a "biological fitness" or "training ground" for several microorganisms such as *Legionella pneumophila*, *Cryptococcus neoformans* and *M. avium*.

To test the hypothesis that the phagotrophic amoeba, *Acanthamoeba polyphaga*, serves as a "biological fitness" for *M. ulcerans, M. ulcerans* strains with different virulence levels were passaged inside *A. polyphaga* and later injected in mouse foot pads (MFP). The delay before a swelling appeared in a MFP after injection was a measure for virulence. The strains were tested for the presence of mycolactone producing genes MUP038 and repA before and after amoeba and mouse passage.

In the mouse model of infection, African strains were more virulent than Asian, Latin-American and Australian strains of *M. ulcerans*. Also in Buruli ulcer patients in Africa, lesions are more serious and extended compared to Australia, Asia and Latin-America.
The strains ITM5114 and ITM842 are considered not virulent since they never cause MFP swelling when injected directly. These strains were isolated more than 40 years ago and have been subcultured repeatedly which probably led to the loss of some genes on the plasmid responsible for the production of mycolactone. Indeed, ITM5114 is negative for both repA and MUP038. ITM842, however, does contain both genes. Both non-virulent strains only caused a swelling in MFP when they had been previously passaged in *A. polyphaga*. ITM5114 became positive for repA after amoeba passage and after subsequent mouse passage both repA and MUP038 were present.
The virulent strains ITM98-912 and ITM03-216 caused a swelling in almost all MFP whether previously passaged in *A. polyphaga* or not. They were always positive for MUP038 and repA.

The interaction with *A. polyphaga* could be responsible for the restored virulence. An increased virulence after amoeba-passage has previously been observed for *M. avium* as well. However, the mechanism of this increasing virulence is not known. An explanation would be that the ITM5114 and ITM842 cultures consist of a mixture of a majority of nonvirulent and a minority of virulent bacilli. The minority of virulent bacilli would have a selective advantage inside amoebae and would then be able to cause MFP swelling. Another explanation would be that there is an increased gene expression after amoeba passage.
In this context it is relevant to note that intracellular pathogens possess genes that allow their entry and growth within host cells. Several genes that have been shown to be over-expressed by *M. avium* after being ingested by macrophages or amoeba were found to be also present in the *M. ulcerans* genome. Further studies are required to investigate the mechanisms of increasing virulence in amoebae.

Oligopeptide transporters are essential for symbiotic nitrogen fixation and free-living growth under stress conditions in *Rhizobium etli*

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Rhizobium etli is a Gram-negative root-colonising soil bacterium capable of fixing nitrogen while living in symbiosis with its leguminous host *Phaseolus vulgaris*. An efficient symbiosis requires an extensive molecular communication between plant and bacteria. Although a lot of research has been devoted to the initial steps of the interaction, little is known about the signals that are important during the late steps of the symbiosis. In search of novel symbiotic genes, a genome-wide screening for *R. etli* symbiotic mutants was performed, revealing two oligopeptide ABC transporters (*opp* and *opt*) involved in the uptake of oligopeptides. Plants nodulated by *R. etli* strains containing mutations in the *opp* and/or *opt* genes showed a significant reduction in symbiotic nitrogen fixation activity. Further phenotypic analysis of the *opp* and *opt* mutants revealed a difference in antibiotic resistance compared to the wild type and a decreased osmotolerance, demonstrating the importance of both oligopeptide transporters in stress resistance during free-living growth.

GIL01 and relatives, tectiviral prophage dwellers in the *Bacillus cereus* group

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pGIL01 is a 15 kb linear prophage hosted by *Bacillus thuringiensis* serovar *israelensis*, an entomopathogen broadly used to control insect pests. pGIL01 belongs to the family of *Tectiviridae* (from *tectus*, meaning covered), which mainly distinguishes itself from other phage groups by the presence of a double layered lipid membrane beneath the capsid coat. Despite an apparent rarity, it appears that tectiviruses display a wide distribution by infecting Gram-negative (PRD1 and siblings) as well as Gram-positive (Bam35, pGIL01, pGIL16, pBClin15 and AP50) bacteria. Yet, little is known about physiological conditions and molecular interactions taking place between tectiviruses and their respective hosts. While PRD1 and its siblings are all lytic phages that kill the host upon infection, pGIL01 and its relatives are able to enter a quiescent state in which detrimental functions for lytic growth are tightly repressed.

The present project was undertaken with the twin aims of characterizing pGIL01 and defining the tight relationship that prophage and host display from lysogeny on to lytic induction. The isolation of spontaneous GIL01 clear plaque mutants highlighted at least two putative genes implicated in prophage regulation. A series of experiments confirmed the role of their gene products in the lytic switch and most relevant, the direct action of stress inducible host determinants in phage proliferation has been demonstrated. Similarly to the lambdoid family of phages, pGIL01 and its relatives are induced by DNA damaging treatments such as UV radiation and mitomycin C. However, pGIL01 does not code for a typical repressor that is inactivated in response to stress but is instead regulated by the global SOS response repressor LexA. LexA and phage encoded factors work in concert to efficiently repress lytic growth in a first place, and to operate the transition from the quiescent mode to lytic propagation in response to stress signals.

These results show the remarkable degree to which prophage and bacterial chromosomes have co-evolved and thus emphasize the pertinence of further exploring these as yet unknown phage systems.

Process technical oriented aspects of biological removal of 17α-ethinylestradiol in an aerated fixed bed reactor

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Conventional wastewater treatment plants (WWTPs) tend to partially remove recalcitrant chemicals, such as pharmaceuticals. Among these, the synthetic estrogen 17α-ethinylestradiol (EE2) is of great environmental concern. In this work a continuously aerated submerged fixed bed bioreactor was used for the biological removal of EE2 at μg L^{-1} levels. Removal efficiencies higher than 96% were obtained at a hydraulic retention time (HRT) of 4.3 days and a volumetric loading rate (B_v) of 11 μg EE2 L^{-1} d^{-1}. Increasing the B_v up to 40 and 143 μg EE2 L^{-1} d^{-1} led to slightly lower removal efficiencies, 81 and 74%, respectively. Nitrification was confirmed to be the main biological mechanism involved in EE2 removal. Most interestingly, the elimination of EE2 was not affected by the absence of ammonium in the feed, suggesting ammonia-oxidizing bacteria (AOB) were able to maintain their population density and their activity, even after several months of starvation.
The concept of an aerated submerged fixed bed bioreactor, capable of removing estrogens in a sustainable and biological way, shows great potential as an effluent polishing step for existing WWTPs.

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Engineering *Escherichia coli* to improve L-carnitine production

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The carnitine metabolism is inducible in the presence of crotonobetaine under anaerobic conditions and is repressed by several environmental stimuli, such as the presence of oxygen and PTS sugar. Therefore, the aim of this work was to investigate the effects of two single-gene knock-in mutations on the carnitine metabolism in *E. coli* under aerobic conditions and grown in a medium rich in glucose. These effectors repress the levels of the carnitine metabolism enzymes by combining and affecting the global regulatory proteins FNR and CRP. The targets were the promoters of *caiF* (the transcriptional activator of the *caiTABCDE* operon) and the *caiTABCDE* operon itself, both of which were removed and replaced by other constitutive promoters (p8 and p37). These artificial promoters were inserted barring the binding sites for the transcriptional regulators FNR and CRP. The carnitine production was measured in batch reactor to study the expression of the genes belonging to the carnitine metabolism. The elimination of the FNR regulatory region together with the new promoter allowed the carnitine production under aerobic conditions. This result was shown both in the constitutive promoter of *caiF* and in the constitutive promoter of the *caiTABCDE* operon.

Differences in replication characteristics of pseudorabies virus strains in explants of porcine respiratory nasal mucosa

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Before the 1960-70s, Aujeszky's disease occurred sporadically in pigs and was restricted to central nervous disorders in a low number of animals. During the 1960-70s, much more outbreaks were reported, with a concommitant change in clinical pattern. Upper respiratory tract problems were prominent and accompanied by severe general symptoms. The proportion of piglets with neurological problems increased and abortion was appearing. It has been postulated that the virulence of the virus increased in time. Characteristics of virus replication were investigated using a recently developed *in vitro* model of porcine respiratory nasal mucosa in order to find the basis for these differences in virulence. Different strains from the European continent, Becker (1961), NS374 (1971), 75V19 (1975), 89V87 (1989) and 00V72 (2000), and two Irish strains, NIA1 (1962) and NIA3 (1971), were taken into account. The number of primary infectious centers (I) and plaques (P) were quantified at 12 and 36hpi respectively in order to analyse the number of cells that were primary infected and the capacity to spread. I values of Becker and NIA1 were comparable and lower than for the other strains. P was lower than I for Becker, NS374, 75V19, 89V87 and 00V72. Becker and NS374 showed a much lower P/I compared to 75V19, 89V87 and 00V72. NIA strains showed a higher P compared to I but a similar P/I. In order to compare the invasive capacity of the different strains in the respiratory mucosa in the depth, plaque depths underneath the basement membrane barrier were measured at 24 and 36hpi. Depths underneath the basement membrane were much lower for Becker compared to the other strains.

In conclusion, Becker and NIA1 were demonstrated to form a lower number of primary infectious centers compared to the other strains. Becker and NS374 showed a lower capacity to form plaques from infected cells compared to the other strains. In addition, Becker also had a reduced invasive capacity in the depth.

Replication of equine herpes virus 1 in tissues of the upper respiratory tract during acute phase of infection in horses

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Equine herpesvirus type 1 (EHV1), a member of the *Alphaherpesvirinae*, is a highly prevalent equine pathogen. The upper respiratory tract is the primary replication site of EHV1. Subsequently, the virus spreads in infected blood leukocytes to internal organs, where it replicates. Internal replication causes lesions and symptoms such as abortion, neonatal foal death and nervous system disorders. During an outbreak many cases may occur, resulting in important economic and emotional losses. How the virus invades through the different layers of the respiratory mucosa and submucosa is largely unknown and formed the basis for the present study. Six EHV-negative ponies were inoculated intranasally with $10^{6.5}$ $TCID_{50}$ of the neuropathogenic EHV1 strain 03P37. At 1, 2, 3, 4, 5 and 7 days post inoculation (dpi), one pony was euthanized. Tissues were collected from different sites of the upper respiratory tract for virus titration and immunostainings. The number and size of EHV1-induced plaques were calculated, using the software program ImageJ. Individual EHV1-infected cells were quantified and characterised, using monoclonal antibodies DH59B (macrophage), HT23A (T-lymphocyte) and 1.9/3.2 (B-lymphocyte). Virus was recovered from nasal septum (2-7 dpi), nasopharynx (2-7 dpi), mandibular lymph nodes (3-5 dpi), retropharyngeal lymph nodes (2 and 4 dpi) and tubal / nasopharyngeal tonsils (3, 4 and 7 dpi). Plaques in the epithelium appeared from 2 dpi. No significant changes were seen in plaque number and size at the different days pi. Plaques did not cross the basement membrane, but individual infected cells were observed below the basement membrane from 1dpi. Macrophages (13-100 %) were the most important carrier cell of the virus in all analysed tissues, followed by T-lymphocytes (0-26 %). In conclusion, EHV1 infects the epithelium of the upper respiratory tract in a restricted way and crosses the basement membrane using individual infected leucocytes, mainly macrophages.

Metabolites produced by *pseudomonas* sp. enable a gram positive bacterium to achieve extracellular electron transfer

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Previous studies revealed the abundance of *Pseudomonas* sp. in the microbial community of a microbial fuel cell (MFC). These bacteria can transfer electrons to the electrode via self-produced phenazine-based mediators. A MFC fed with acetate where several *Pseudomonas* sp. were present was found to be rich in a Gram positive bacterium, identified as *Brevibacillus* sp. PTH1. Remarkably, MFCs operated with only the *Brevibacillus* strain in their anodes had poor electricity generation. Upon replacement of the anodic aqueous part of *Brevibacillus* containing MFCs with the cell free anodic supernatants of MFCs operated with *Pseudomonas* sp. CMR12a, a strain producing considerable amounts of phenazine-1-carboxamide (PCN) and biosurfactants, the electricity generation was improved significantly. Supernatants of *Pseudomonas* sp. CMR12a_Reg, a regulatory mutant lacking the ability to produce PCN, had no similar improvement effect. Purified PCN, together with rhamnolipids as biosurfactants (1 $mg.L^{-1}$), could clearly improve electricity generation by *Brevibacillus* sp. PTH1, as well as enable this bacterium to oxidize acetate with concomitant reduction of ferric iron, supplied as goethite (FeOOH). When added alone, PCN had no observable effects on *Brevibacillus'* electron transfer. This work demonstrates that metabolites produced by *Pseudomonas* sp. enable Gram positive bacteria to achieve extracellular electron transfer. Possibly, this bacterial interaction is a key process in the anodic electron transfer of a MFC, enabling *Brevibacillus* sp. PTH1 to achieve its dominance.

Endosymbiotic bacteria in siphonous green algae exploration of a partnership

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Endosymbiosis is an important driving force behind eukaryotic evolution and led to important key innovations such as the acquisition of mitochondria and chloroplasts. Many algae still host endosymbiotic bacteria which play an important role in various metabolic functions. In the siphonous green alga *Bryopsis* bacteria are present in every phase of the life cycle, indicating an ancient association among host and symbiont, rather than a recent opportunistic and non-specific relationship. To identify the bacterial partner, epiphytes were mechanically removed from *Bryopsis* spp. by a combination of vortexing and the use of glass beads. Cultured epiphytes were sequenced after an initial screening by Rep-PCR and identified as *Alteromonas, Halomonas, Marinobacter, Pseudoalteromonas and Sulfitobacter* spp. Subsequently, different *Bryopsis* samples – cleaned as well as possible – were submitted to a range of molecular techniques such as 16S PCR, cloning, DGGE and RFLP. The clones with bacterial sequences fell apart in different clusters, a representative number of which were sequenced and identified as being *Acinetobacter, Flavobacterium, Glaciecola, Mycoplasma, Planctomyces, Pseudonocardia* en *Roseobacter* spp. In order to examine whether the identified bacteria are in fact endosymbionts, we tried to visualize them by the use of FISH with the universal bacterial probe EUB338. The probe assay was optimized on pure bacterial cultures, both Gram-positives and Gram-negatives. We opted to perform FISH on *Bryopsis* gametes which do not have a cell wall and also contain the endosymbionts. Gametogenesis was effectively generated *in vitro* and the gametes went through different fixation steps, prior to the actual hybridization. Currently the fixation is being optimized.

Oligofructose-insuline specifically upregulates bifidobacterium adolescentis and *B. longum*

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INTRODUCTION: Prebiotics are food ingredients which are not hydrolysed by the human digestive enzymes in the gastrointestinal tract and which have beneficial effects on the host by selectively stimulating the growth and/or activity of bacteria which may improve the host health.

AIMS & METHODS: To study if the predominant faecal microbiota of healthy volunteers was altered after oligofructose-inuline (OF-IN) intake, and to characterize which bacterial species are responsible for this effect using molecular techniques. The composition of the predominant faecal microbiota of 17 healthy volunteers before and after four weeks of OF-IN intake was studied using a culture-independent molecular approach, PCR-DGGE (Denaturing Gradient Gel Electrophoresis). DGGE bands that differed significantly were purified and sequenced. The corresponding bacteria were identified through BLAST analysis and were subsequently quantified using real time PCR. Real time PCR was performed on total bacterial DNA from faecal samples using the LightCycler system I (Roche, Mannheim, Germany). The total number of bifidobacteria was quantified using group-specific primers. Real time results were statistically analysed using a one-sided paired T-test.

RESULTS: Two bands on the PCR-DGGE profiles increased significantly before and after OF-IN intake (p= 7 x10-15 and p= 3 x10-13). After purification and sequencing, the corresponding bacteria were identified as *Bifidobacterium longum* (Identities= 140/140, Gaps= 0/140) and Bifidobacterium adolescentis (Identities= 144/144, Gaps= 0/144), respectively. There was a significant increase (13%) of the total number of bifidobacteria after OF-IN intake (p= 5 x10-6). The median log10 number of B. adolescentis increased from 9.21 before to 9.73 after OF-IN intake.

CONCLUSION: When comparing the predominant faecal microbiota of 17 healthy volunteers before and after four weeks of OF-IN intake, a highly significant increase of two bifidobacteria was observed, confirming its bifidogenic effect. Further investigation of the banding pattern of the predominant microbiota, enabled us to specify this effect to *B. adolescentis* and *B. longum*. For *B. adolescentis* the increase was confirmed using real-time RT-PCR. Since bifidobacteria are known to be less present in gut inflammatory disorders as Crohn's disease, further studies using OF-IN as an adjuvant treatment in this patient population are suggested.

Impact of lipopolysaccharide and peptidoglycan modifications on bacterial virulence: functional analysis of five *Shigella flexneri* genes: *orf182, orf185, orf186, virK* and *ushA*

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Shigella flexneri is a Gram-negative pathogenic bacterium that causes bacillary dysentery, a disease responsible for one million deaths per year in developing nations. After ingestion, *S. flexneri* travels to the colon where it induces macrophages apoptosis and invades epithelial cells. The required virulence genes are located on a large virulence plasmid (pWR100) encoding a type III secretion system (T3SS). Here, we investigated the virulence function of 5 uncharacterised plasmidic genes; *orf182*, *orf185*, *orf186*, *virK* and *ushA*. Proteins sequence alignment reveals that the product of these genes could be implied either in the modification of the PGN/LPS. *Shigella* genome contains two copies of the *ushA* gene; one located on pWR100 and the second on the chromosome. Here, we knock out (KO) the 6 genes and studied the phenotype of generated mutants both *in vitro* and *in vivo*. We show, *in vitro,* that mutation of *orf182*, *orf185*, *orf186*, affect the entry into HeLa cells, whereas, individual or double *ushA* mutation exhibit wild-type phenotype. In contrast to what has been reported in literature, *virK* mutation does not affect bacteria spread between cells. Next, we evaluated, *in vivo*, the capacity of our mutants to induce keratoconjunctivitis in Guinea-pig. We found that only strains mutated in *orf182*, *orf185*, *orf186* and *virK* affect the wild-type virulence property. VirK homologous protein in *Salmonella* is required for bacterial survival within macrophage. Thus, we monitored the behaviour of our mutants (including *virK*) within macrophages. Our preliminary data support a possible role of some of studied genes in bacterial survival and suggest that modification of LPS or PGN promotes bacteria resistance within the precarious intracellular macrophages environment. Future work will address the molecular mechanisms involving the products of studied genes. In conclusion, we bring here some new finding related to the role of the PGN and LPS in bacterial virulence. The achievement of this work will exceed the framework of *Shigella* in regard to the high conservation of the studied genes among several other pathogenic bacteria.

Identification and preliminary characterisation of two new archaeal tRNA methyltransferases belonging to the SPOUT family

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Chemicaly modified nucleosides have been found in almost all types of cellular RNAs, but the largest number and the greatest variety are found in transfer RNA (tRNA) (McCloskey and Crain, 1998). These modifications are post-transcriptionnaly added on nucleosides by modification enzymes during the complex processus of tRNA maturation. The chemical nature of these modifications is very diverse, going from simple chemical alteration like base or ribose methylation, base isomerisation, reduction, thiolation or deamination, to the formation of hypermodified nucleosides. The functions of these modified nucleosides are not well known, but it seems that modifications outside the anticodon region are involved in maintenance of the structural integrity of tRNA, while modifications in the anticodon region play a direct role in increasing translational efficacity and fidelity (Kowalak, 1994 ; Giégé, 1998). Among the naturally occuring nucleosides modifications, base and ribomethylations are by far the most frequently encountered. Most of them are formed by S-adenosyl-L-methionine (AdoMet)-dependent methyltransferases (MTases) (Schubert, 2003).

We have undertaken a large-scale project aiming at characterisation of a complete set of RNA modification enzymes of model organisms belonging to the domains of bacteria and archaea. Using bioinformatic tools, we have identified two open reading frames (ORFs) in the genomes of *Thermococcus kodakaraensis* and *Sulfolobus solfataricus* encoding putative new tRNA MTases of the SPOUT family. This class of MTases is characterised by the presence of a deep knot in the polypeptide chain (Tkaczuk, 2007). The two putatives MTases have been produced in *E.coli* as recombinant His-tagged proteins and were purified by immobilized metal-ion affinity chromatography. The enzymatic activity of the two proteins was tested using either unfractionated *E.coli* tRNA or synthetic transcripts of archaeal tRNA genes as substrates and [^{14}C]AdoMet as methyl donnor. Our results show that both proteins display tRNA MTase activity. The nature of the methylated nucleosides formed by the enzymes and the biochemical characterization of the enzymes is now under way.

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Class 1 integrons in ESBL carrying *Escherichia coli* from Kenyan Hospitals

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Introduction. Antibiotic resistance is an ever growing problem in the world. The situation in developing countries is also evolving towards increased resistance to all classes of antibiotics. Resistance to cephalosporins, mediated by extended spectrum ß-lactamases (ESBL), is also becoming increasing worldwide. Resistance is disseminated by several mobile genetic elements that include plasmids and transposons. Integrons are known to sequester antibiotic resistance gene cassettes and are normally localised on such mobile genetic elements in which case, their ability to transfer resistance genes horizontally within and between species is highly facilitated. However, little is known about ESBLs and the role integrons play in the spread of antibiotic resistance genes in developing countries including Kenya. We therefore investigated the prevalence of ESBLs and the integron content in *Escherichia coli* isolated from patients in Kenya from 1992 to 2008.

Methods. We determined the susceptibility of 730 *E. coli*, isolated from different kinds of infections in different hospitals around the country. A panel of 21 antibiotics representing various classes of ß-lactam antibiotics, quinolones, aminoglycosides, tetracyclines, chloramphenicol and nitrofurantoin was utilised for these susceptibility tests. ESBL positive strains were screened for ß-lactamases using PCRs targeting CTX-M group, TEM group and SHV group using previously published methods. Strains that produced ESBL enzymes were also screened for the class 1 integron using published PCR methods. The class 1 integrons (integrase genes and the antibiotic resistance cassettes) were characterised by using a combination of sequencing techniques, and PCR mapping.

Results. Thirty-six out of 730 *E. coli* strains showed an ESBL profile. Twenty three (64%) of these were positive for integron class 1 by PCR. A total of 12 (33%) tested positive for group 1 CTX-M-ESBL, while 5 (14%) were positive for group 2 CTXM-ESBL. The CTX-M gene was localised in the integron class 1 in 15 (88%) of these strains. The SHV gene was detected in 13 (36%) of ESBL-producers. Only 2 and 3 isolates were positive for class 2 and 3 integrons respectively. The aminoglycoside modifying enzyme (AME) *aac(6')-Ib-cr* that confers resistance to fluoroquinolones and aminoglycosides was localised within the class 1 integron in 12 (33%) of the ESBL positive isolates. Preliminary sequencing results suggest that the *dhfrVII* gene encoding resistance trimethoprim is also localised within this integron.

Conclusions. The occurrence of mobile genetic elements harbouring a wide range of antibiotic resistance genes in ESBL positive strains posses a great challenge in the management of infections in resource-poor setups. This kind of genetic environment offers a great potential for the spread of multiple antibiotic resistances leading to therapeutic failure and increased mortality and morbidity. There is therefore a need to put up surveillance programs to gain insight in the situation of the spread of these resistances.

β-lactamase inhibitor resistant *Escherichia coli* strains from Kenyan Hospitals: 1992-2008

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Background. Production of β-lactamase enzymes is the most common mode of resistance to β-lactam antibiotics in gram negative bacteria. Over the past 10 years, inhibitors of these ß-lactamases, such as clavulanic acid, tazobactam and sulbactam, have been used extensively to combat bacteria producing Extended Spectrum β-Lactamases (ESBLs). ESBL enzymes hydrolyse all penicilins and most of the newer β-lactam antibiotics such as cephalosporins but are incapable of hydrolysing cefoxitin and are inhibited by clavulanic acid and tazobactam. In recent years however, inhibitor-insensitive bacteria have been reported increasingly from developed countries but rarely from Sub-Saharan Africa. Therefore we investigated the prevalence of E. *coli*, isolated in Kenyan hospitals that are not susceptible to neither to the inhibition of clavulanate nor to tazobactam.

Methods. A total of 730 *E. coli* from 11 hospitals in Kenya accumulated continuously from 1992 to 2008 were investigated. Among these, 508 non-duplicate strains were selected for further characterization. The sources of the selected strains were as follows: blood (109), urinary tract infections (172), bloody diarrhoea (187) and sputum (30). Disc diffusion method was used to determine the resistance profile for β-lactam antibiotics. Based on their susceptibility profiles, strains were grouped into various phenotypes including ESBL producers, ampC producers, and the inhibitor resistant phenotype (IRP). Representative strains of each phenotype were selected for the detection of the β-lactamase-encoding genes known to confer inhibitor-resistance phenotype such as TEM and SHV and for integrons belonging to class 1, 2, and 3, and for transposon Tn21 using published PCR methods.

Results. A total of 259 (57%) and 187 (37%) of the 508 strains were resistant to amoxicillin-clavulanic acid (augmentin) and to piperacillin-tazobactam (TZP) respectively. While 33 (6%) of the isolates had an ESBL phenotype, 129 (25%) had the combined IRP-ESBL phenotype. The IRP phenotype (without combination with either ESBL or ampC phenotype) was observed in 96 (6%) of all isolates while 45 (9%) were ampC producers. Resistance percentages to augmentin and to TZP were highest among isolates from urine (76% and 55% respectively) and those from bloody diarrhoea cases (50% and 31%, respectively). Using PCR methods, 36 (78%) strains were positive for the TEM genes and 19 (46%) for the SHV gene among ESBL producers while only 3(6%) of the strains exhibiting ampC phenotype were positive for the CMY2 gene. Preliminary sequencing results show that TEM-125 in the most common gene among strains exhibiting the inhibitor resistant phenotype.

Conclusions. The use of a ß-lactam in combination with β-lactamase inhibitors has been the best choice for treating gram negative infections caused by ESBL-producing strains due to their inhibitory effect on β-lactamases. Such inhibitor-containing antibiotics are also affordable and easily available. However, the emergence of inhibitor-resistant strains will pose a great therapeutic challenge in resource poor setups. It is therefore important to step up surveillance of the emergence and spread of such strains.

Phosphate starvation induce secretion of a putative lipoprotein, CC0170, via the type II secretion system of *Caulobacter crescentus*

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A complete set of the 12 genes (*gsp*C to *gsp*N), coding for the type II secretion system (T2SS), are consecutingly located in the *Caulobacter crescentus* chromosome. Deletion of complete sequence (genes between *gspC* and *gspN* inclusive) results in impaired secretion of a putative lipoprotein CC0170. Although the cellular function of CC0170 is still unknown, this protein is specifically produced and secreted during phosphate starvation. Three different CC0170 isoforms with different molecular weights were detected. The largest CC0170 was found in the cellular fraction of the T2SS deletion strain, whereas the smallest was found in the cultivation supernatant of the wild type strain. These observations suggest the occurrence of a T2SS-dependent maturation process of CC0170. Interestingly the amount of GspL, which is a membrane-bound component of the T2SS, was not affected by the decrease in phosphate concentration. Whereas CC0170 secretion seems to directly depend on its concentration, it is not regulated by the production of T2SS components.

Unevenness under selective stress: a threaten for microbial communities functionality

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Considering the current global biodiversity crisis, the biodiversity-stability relationship and the effect of biodiversity on ecosystem functioning have become a major focus in ecology. Biodiversity concept consists of species richness and evenness. While previous studies have mainly focused on how a higher level of richness is related to a greater stability of specific ecosystem properties, the relationship between functionality and evenness has not been so far deeply investigated. In this study, we aimed to demonstrate that provided a good level of functional redundancy within microbial populations, the relative degree of evenness plays an important role in conserving a given functionality on short terms under perturbed conditions.

To test the relation between community evenness and functionality, we used model communities composed by 18 functional species of denitrifying bacteria spread over four different phyla and characterized by different physiological properties. The 18 species of denitrifiers were assembled to form 1260 microcosms with the same richness, but different levels of evenness. The microcosms were incubated for 20 h under three distinct conditions: no stress (control), low temperature (non selective stress) and high salt concentration (selective stress). Lorenz curves and relative Gini coefficient have been used as a mean to visually depict the structure of the microbial communities and its degree of evenness. A model was created to assess the interactions of specific parameters on the ecosystem functionality. We found a very significant effect from the factor stress and its interactions with other variables, indicating that the type of stress had a strong impact on the functionality. The effect of evenness on functionality was modelled by a quadratic effect of the Gini coefficient. The denitrification activity of microbial communities was positively correlated with the degree of evenness when a selective stress was applied and, to a lesser extent, also in absence of stress. On the contrary, at low temperature, which disfavours all species to nearly the same extent, the decrease of the functionality was independent from the community evenness.

In conclusion we showed that, on short terms, evenness plays a key role in preserving communities' functional stability, even with a considerably high degree of species richness. To quickly respond to selective stress, a community requires an even distribution among the redundant functional members of the community. In other words, when in a microbial community the ecosystem function strongly depends on few dominant species, the functional stability is threatened upon environmental fluctuations.

Bacteria, not archaea, restore nitrification in a zinc contaminated soil

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The biological ammonia oxidation, the rate limiting step of the nitrification, has been long-time thought to be mediated by a discrete clade of β- and γ-proteobacteria ('AOB'). Recently ammonia-oxidizing Crenarchaeota ('AOA') have been identified and proposed to be the dominant ammonia-oxidizing micro-organisms in soil, undermining the unique role of AOB. We aim to elucidate the dynamics of AOB versus AOA and their relative contribution to soil ammonia oxidation as a response to Zn stress as an environmental perturbation. Soils, contaminated with increasing $ZnSO_4$ concentrations, were sampled on an ongoing field study in Spalding (Australia) immediately after Zn contamination and after 1 and 2 years of exposure. The AOB and AOA *amoA* gene number, *amoA* transcript number and *amoA* gene diversity was monitored over time and related to the ammonia-oxidizing activity. Initially, ammonia oxidation was completely inhibited at 33.7 mmol Zn/kg, and both AOB and AOA *amoA* gene numbers and transcriptional activities were reduced. The ammonia oxidation process in fields which had received this contamination level was, however, fully restored within 2 years, concomitant with a restoration of the AOB *amoA* gene number and AOB *amoA* transcript number to levels of the non-contaminated soil and with the development of a zinc-tolerant AOB community with a composition different from the original community. In contrast, no resilience in AOA *amoA* gene numbers and activity took place. These findings demonstrate that the recovery of the nitrification under Zn-stressed soil conditions was coupled to the restoration of a Zn tolerant AOB, and not AOA, community.

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Plasmid transfer among members of the *Bacillus cereus* group in food matrices

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The food pathogen *Bacillus cereus* and the biopesticide *Bacillus thuringiensis* are genetically closely related and belong to the *B. cereus sensu lato* group. Differences in their virulence spectra are mainly conferred by plasmids that often display self-transfer capabilities. Food safety concerns have stressed the potential for plasmid transfer among members of the *B. cereus* group. In this study, the potential for plasmid exchange between species from *B. cereus* and *B. thuringiensis* was assessed using a molecular model.

Bi-parental and tri-parental mating experiments were performed using the *B. thuringiensis* conjugative plasmids pAW63, a 72-kb element resembling the *B. anthracis* virulence plasmid pXO2, and the non-conjugative *Staphylococcus aureus* plasmid pUB110. Different transfer modes (conjugation and mobilization) were studied in various liquid foodstuffs (milk, skimmed milk, soymilk, and rice milk), using both *B. thuringiensis* and *B. cereus* strains as donor and recipient partners. For all the experiments, the observed frequencies were compared to those obtained in standard LB medium. Plasmid transfers were studied at 30°C, during 4 hours without shaking, and were estimated as the number of transconjugants per recipient cell.

The frequencies of pAW63 conjugation and pUB110 mobilization occurred at detectable levels in the five tested media, and were globally increased in food matrices, most notably in full-cream milk, skimmed milk and soya milk. Conjugation kinetics revealed that higher frequencies obtained in foodstuffs compared to those observed in LB could be explained by an earlier onset of conjugation and a longer “mating time”. Furthermore, the ability of an emetic strain of *B. cereus* to function either as donor or recipient for pAW63, in bi-parental mating with *B. thuringiensis*, was shown to occur, albeit at frequencies 100-fold less efficient than in standard conditions.

Heavy metal resistance in *Cupriavidus metallidurans*: towards the reconstruction of regulatory networks

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Micro-organisms are deployed in bioremediation strategies to clean up environments that are contaminated with organic pollutants, pharmaceutical products, or toxic metals. The soil bacterium *Cupriavidus metallidurans* CH34 shows great promise in light of these technologies since it contains a significantly high number of metal-resistance genes. Recently, the two plasmids pMOL28 and pMOL30 were fully annotated and various heavy metal resistance gene clusters were identified. Thanks to progress in the annotation efforts directed to the two chromosomes and the availability of whole-genome expression data, it now has become feasible to investigate the full genomic stress response of *C. metallidurans* CH34 when challenged to different heavy metals. Our ultimate goal is the full reconstruction of regulatory networks enabling CH34 to overcome metal toxicity.

We have performed a large number of microarray experiments to follow the whole-genome transcriptomic response of *C. metallidurans* CH34 when exposed to heavy metals such as zinc, copper, cadmium, and lead. Obtained data suggest that the total number of genes that are up- or down-regulated relates to the level of toxicity exerted by the heavy metal e.g. it appears that the more toxic a heavy metal is for *C. metallidurans* CH34, the more severe the transcriptional response.

In addition, genome-wide expression experiments also show that there is multiple cross-talk at transcriptional level between the different heavy metal responses. Different clusters of co-expressed genes can be identified showing similar expression profiles when exposed to varying combinations of heavy metals. Some genes were only switched on by one particular metal while others were activated by different metals. This suggests that *C. metallidurans* CH34 reacts to (heavy) metal toxicity at two separate transcriptional levels corresponding to an initial general metal-related stress response and a secondary metal-specific response.

Our hypothesis is that the complex transcriptional response can only be mediated by the joined action of different transcription factors (TFs). These regulatory proteins bind DNA target sites (i.e. regulatory motifs) near the initiation site of transcription and either assist or repress RNA polymerase activity. According to the genome's annotation, *C. metallidurans* CH34 possesses several hundreds of such regulatory proteins, many of which have a distinct specificity and function. We are currently identifying those regulatory motifs related to heavy metal response in order to fully understand the regulatory network underlying this complex transcriptomic response.

Effects of hydrogen peroxide and ionising radiation on the physiology of *Arthrospira* sp. PCC8005

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At the time being, space research is entering a new era, as numerous projects are expected for the next twenty years, including the implementation of a lunar base or the organization of a human trip to Mars. Nevertheless, in order to attend such ambitious research programs, the development of life support systems has become a necessity in order to ensure the safety of Man in space by providing him with oxygen, food, water and recycling his wastes. Among the different projects actually under development, the MELiSSA (Micro-Ecological Life Support System Alternative) project of the European Space Agency presents the particularity of combining bacteria, higher plants and photosynthetic microorganisms to process the waste, recreate a breathable atmosphere and produce biomass and water.

Due to its efficient oxygen production, along with its valuable nutritional properties, the cyanobacterium *Arthrospira* sp. PCC8005 has been selected as oxygen producer and complementary food source in the MELiSSA recycling loop. As a life support system organism, *Arthrospira* will hence undergo different stresses induced by the environmental conditions encountered in space. Among these conditions, oxidative stresses (including cosmic radiations, UV or high light) might have an important effect on the metabolism of this photosynthetic organism.

We tested the evolution of different physiological parameters of *Arthrospira* (viability, membrane potential and chlorophyll autofluorescence) by flow cytometry after exposure to oxidative stresses. As a preliminary experiment, some samples were analyzed after a pulse exposition of one hour to different concentrations of hydrogen peroxide (H_2O_2). Further investigations have been performed to assess the effects of a direct exposure to ionising radiations on *Arthrospira*. The radiation doses measured within the International Space Station (ISS) have been simulated at the Belgian Nuclear Research Center, using gamma and neutron sources. Cultures of *Arthrospira* have been exposed to a dose equivalent to a stay of respectively 10 and 100 days aboard the ISS.

A response curve of *Arthrospira* in function of H_2O_2 concentration was determined, and highlighted a certain range of resistance of this organism to oxidative stress. Furthermore, flow cytometry analysis showed no significant modification of the viability, membrane potential or chlorophyll autofluorescence of *Arthrospira* after exposure to gamma and neutron irradiations. Growth of the samples during and after exposure to radiations followed a normal pattern. However, significant changes appeared in morphology, with an apparent increase of the mean size of the cyanobacterial filaments for the higher dose of radiation.

Arthrospira showed not to be highly sensitive to oxidative stresses, both in terms of viability and physiology, but ionising radiation may affect morphology. Additional experiments are on their way to confirm and extend these results. A special attention will be given to unravel the mechanisms controlling the morphological changes, as cell shape is an important parameter of the photosynthetic efficiency.

Characterization of the antiviral activity of CF11001 against the bovine viral diarrhea virus

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We identified and characterized the antiviral activity of CF11001 against the bovine viral diarrhea virus (BVDV). The 50% effective concentration (EC_{50}) for inhibition of BVDV induced cytopathic effect formation is 9 $\pm$ 1 μM. This observation was further corroborated by monitoring the anti-BVDV activity using RT-qPCR, immunofluorescence and a virus yield assay. No toxicity could be observed at the highest concentration tested (CC_{50} >100 μM) resulting in a selectivity index (CC_{50}/EC_{50}) of ~11. CF1001 is inactive against the classical swine fever virus (pestivirus), the hepatitis C virus, a selection of unrelated DNA (i.e. herpesvirus, vaccinia virus & vesicular stomatitis virus) and RNA (i.e. respiratory syncytial virus, coxsackie virus B4, parainfluenza-3 virus, Reovirus-1, Sindbis virus, Punta Toro virus) viruses. Furthermore, CF11001 displayed similar activity against AG110-resistant virus (Paeshuyse *et al*, 2007 J.Virol. 81:11046-11053) which carries the E291G mutation in its RNA-dependent RNA polymerase (RdRp) gene, BPIP-resistant (F224S mutation in RdRp) virus (Paeshuyse *et al*, 2006 J. Virol. 80: 149-60) and the LZ37-resistant (F224Y mutation in RdRp) virus as to wild-type virus. The lack of cross-resistance may indicate that CF11001 inhibits the replication of BVDV by yet an unknown mechanism. Detailed time-of-(drug)-addition experiments together with selection, genotypic and phenotypic characterization of drug-resistant mutants will further contribute to the elucidation of this, potentially novel, mechanism by which CF11001 exerts its anti-BVDV activity.

Resistance in *Candida albicans* biofilms: Investigating the expression of resistance genes in biofilms following treatment with antifungal agents

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Candida albicans (*C. albicans*) is a commensal in the oral cavity and gastro-intestinal tract of humans. This fungus can become a pathogen and form biofilms on medical devices and human tissues. Biofilms are complex three-dimensional structures of yeast cells and filaments, embedded in a self-produced extracellular matrix. Biofilm-related infections are difficult to treat since sessile cells are highly resistant towards antifungal agents including fluconazole and amphotericin B. However, the molecular mechanisms responsible for biofilm resistance remain to be elucidated.

The main goal of this study was to investigate the expression of genes associated with resistance in *in vitro* grown biofilms after treatment with antifungal agents using Reverse Transcriptase quantitative PCR (RT-qPCR). Resistance genes investigated in this study are genes encoding efflux pumps and genes involved in ergosterol and ß-1,6-glucan biosynthesis. Furthermore, we evaluated the antimicrobial effect of fluconazole (1000 µg/ml: 8000x MIC) and amphotericin B (32 µg/ml: 250x MIC) on young and mature biofilms.

Our data show that amphotericin B had a fungistatic effect on both young and mature biofilms. Nevertheless, treatment of biofilms with amphotericin B still resulted in the survival of a small fraction of cells (persisters). Treatment of biofilms with amphotericin B resulted in an up-regulation of genes involved in ß-1,6-glucan biosynthesis, suggesting changes in the cell wall composition. It is important to note that even after removal of this antifungal agent these genes remained up-regulated. On the other hand, fluconazole had a fungistatic effect on young biofilms and no effect on mature biofilms. Exposure of biofilms to this antifungal agent induced an up-regulation of genes involved in ergosterol biosynthesis, which may result in higher levels of ergosterol and alternative sterols in the membrane of sessile cells. However, the expression levels of *ERG* genes immediately decreased after removal of fluconazole.

In conclusion, our data indicate that *in vitro* grown biofilms are highly resistant to antifungal agents. Furthermore, *C. albicans* biofilms show an immediate and controlled, yet transient, transcriptional response in the presence of antifungal agents, which probably contributes to biofilm resistance.

High-resolution contact probing of RutR, the regulator of pyrimidine utilization, binding to the *carAB* control region of *Escherichia coli* K-12

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RutR (YcdC) is a homodimeric transcription regulator of the TetR family. It consists of a N-terminal DNA-binding domain with a helix-turn-helix motif, and a C-terminal ligand-binding domain (1). *rutR* is adjacent to and regulates the b1012 operon (renamed *rutABCDEFG*), recently shown to encode a novel pathway for pyrimidine utilization (1). Using genomic SELEX and ChIP, twenty RutR binding sites were identified in the *E. coli* genome and a 16 bp palindromic consensus sequence was established (TTGACCAnnTGGTCAA) (2, 3). Six binding sites are within control regions of genes and operons involved in autoregulation, degradation of pyrimidines and purines, transport of glutamate, synthesis of glutamine, and the *carAB* operon, encoding carbamoyl phosphate synthase, involved in the biosynthesis of arginine and pyrimidines. *In vitro* binding of RutR to the *carAB* control region was nearly abolished in the presence of uracil or thymine. Therefore, RutR was proposed to be a uracil/thymine-sensing master regulator of pyrimidine and purine utilization. Curiously, fourteen RutR binding sites are within coding regions, but they do not appear to affect gene expression.
Shimada *et al*. (2) identified a RutR box in the *car*P1 control region, far upstream of the promoter and overlapping the binding site for PepA, the major architectural element in *car*P1 regulation. However, the molecular details of RutR-operator binding have not been analyzed and the regulatory mechanism is not known.
To establish a high-resolution map of the RutR-*car*P1 operator contacts we performed various *in vitro* binding experiments. Mobility shift assays with purified RutR, in the presence of non-specific competitor DNA, indicated that RutR binds specifically to the *car*P1 operator with an apparent equilibrium dissociation constant of approximately 20 nM. RutR binding was inhibited by 50% with 25 μM uracil and completely abolished with 250 μM. In contrast, thymine at 250 μM had only a minor inhibitory effect. In gel footprinting of the RutR-*car*P1 complex with the 1,10-phenanthroline-copper ion revealed protection of a 28 nt long stretch that comprises the proposed 16 bp RutR box and slightly extends on either side. To reveal base-specific contacts, we performed various premodification binding interference experiments. The results indicate that RutR binds to two successive major groove segments and the intervening minor groove, all aligned on once face of the helix. Nearly all the nucleotides of the 16 bp RutR box contribute to complex formation, but to a variable extent. The removal or modification of either one of four guanine residues (G11, G12 of the top strand, and the symmetrically positioned G5 and G6 of the bottom strand), and of the highly conserved thymine residues T13 (top strand) and T4 (bottom strand) nearly abolished RutR binding. In contrast, the removal of the highly conserved adenine residue A4 (top strand) of the consensus had a rather moderate effect. Circular pemutation assays indicated a modest RutR induced bending of the *car*P1 operator by 37°.
Binding experiments with purified RutR and PepA strongly suggest that PepA can bind to preformed RutR-operator complexes. Therefore, it appears unlikely that RutR would act as a uracil/thymine sensitive activator of *car*P1 activity by inhibiting the binding of PepA, as suggested previously by Shimada *et al*. (2).

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Inhibition of Hepatitis C Virus Replication by Semisynthetic Derivatives of Aglycon Glycopeptide Antibiotics

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Glycopeptide antibiotics (teicoplanin and eremomycin) are used as antibacterial agents. Several semisynthetic hydrophobic derivatives of glycopeptides antibiotics exert, in contrast to their natural counterparts, *in vitro* anti-HIV and anti-coronavirus activity. We here report on the anti-HCV activity of hydrophobic teicoplanin and eremomycin derivatives. Aglycon of teicoplanin LCTA-949 resulted in the most selective anti-HCV activity in different replicon systems with a selectivity index ~25. LCTA-949 also inhibited replication of the HCV_{cc} in dose dependent manner. The combination of selective HCV inhibitors with LCTA-949 results overall in an additive antiviral activity. Efficient clearance of cell-containing replicon from HCV RNA was determined after 4 successive passages under LCTA-949 selection. LCTA-949 had no inhibitory effect on the replication of the related bovine viral diarrhea virus (genus pestivirus) in MDBK cells. Semisynthetic hydrophobic derivatives of aglycon glycopeptides antibiotics may thus be an interesting route to explore as potential HCV inhibitors.

In vivo characterization of the physiological role of a transcriptional regulator from the archaeon *Sulfolobus solfataricus*

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Transcription in archaea resembles closely the eukaryotic process but is regulated by bacteria-like regulators. Only a limited number of archaeal transcription regulators have been studied, most of which belong to the bacterial/archaeal Lrp family of regulators. The physiological role of most characterized archaeal Lrp-like regulators remains elusive. Here, we unravel the function of the Lrp-like Ss-LrpB of *Sulfolobus solfataricus*, a hyperthermoacidophilic archaeon.
Ss-LrpB binds the control region of its own gene, which indicates an autoregulation (1). Based on the identified binding sites in the *Ss-lrpB* control region, the DNA-binding sequence specificity of the regulator was studied extensively (2). Using this information, the *S. solfataricus* P2 genome was scanned for the occurrence of other potential regulator target sites. Several potential binding sites were found in promoter regions of genes encoding proteins with different functions. This list includes an operon encoding pyruvate ferredoxin oxidoreductase (*porDAB*) and two genes encoding putative permeases. *In vitro* binding studies of Ss-LrpB demonstrated cooperative binding at three target sites in the *porDAB* control region. In contrast, at low Ss-LrpB concentrations, binding to the control regions of the two permease genes occurs at a single binding site relatively far upstream and extends further downstream at higher protein concentrations. We constructed an *Ss-lrpB* gene disruption mutant, which is the first report of a mutant of an archaeal *lrp*-like gene. Transcription of the *porDAB* and permease genes was lower in an *Ss-lrpB* gene disruption mutant. Therefore, Ss-LrpB functions as an activator for all these promoters, although the binding relative to the promoter and possibly also the corresponding molecular mechanism of gene regulation appears to be different for the permease genes and for *porDAB*.

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Transcriptional responses in *Burkholderia cenocepacia* biofilms after exposure to chlorhexidine, H_2O_2 and NaOCl

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Burkholderia cepacia complex bacteria are opportunistic pathogens that can cause severe respiratory tract infections in cystic fibrosis patients. Because of the high resistance of these organisms to various antibiotics, treatment of infected patients is particularly difficult. Thorough cleaning and disinfection of respiratory equipment and environmental surfaces is recommended in multiple infection control guidelines to prevent acquisition of these bacteria. Biofilm formation has been described for multiple *B. cepacia* complex strains and these communities of sessile cells often show an increased resistance to antimicrobial agents compared to their planktonic counterparts. In fact, various disinfectants fall short of completely removing the highly resistant *B. cepacia* complex sessile cells. The goal of the present study was to perform a microarray analysis of the transcriptional responses of sessile *B. cenocepacia* LMG 16556 cells after exposure to chlorhexidine (CHX), H_2O_2 and NaOCl, using custom made Agilent microarrays.

The exposure of the *B. cenocepacia* LMG 16656 biofilm (formed after 4h adhesion and 20h biofilm formation) to CHX for 15 min resulted in an up-regulation of the transcription of 582 genes and a down-regulation of the transcription of 285 genes (2-fold difference; $p<0.05$). Amongst the up-regulated genes, six belong to two RND-operons; one of these tripartite efflux pumps shows a strong similarity with the MexCD-OprJ multidrug efflux pump, which is involved in the CHX resistance in *Pseudomonas aeruginosa*. This treatment also resulted in the up-regulation of the transcription of a large number of membrane-related genes, which is probably a result of the membrane damage caused by this disinfectant.

The exposure of the *B. cenocepacia* biofilms to H_2O_2 (30 min) and NaOCl (5 min) resulted in an up-regulation of the transcription of 380 and 452 genes, respectively; for 238 and 402 genes, respectively, the transcription was down-regulated. Many of the up-regulated genes are involved in oxidative stress responses, e.g. two genes which code for alkyl hydroperoxide reductases (AhpC and AhpF) and two which code for organic hydroperoxide resistance (Ohr) proteins. The transcription of genes encoding an RNA polymerase sigma-32 factor and the RecA protein were also clearly up-regulated. In addition, treatment of the sessile cells with H_2O_2 resulted in the up-regulation of the transcription of multiple genes encoding (putative) catalases, a non-heme chloroperoxidase, an OsmC-like protein (generally involved in oxidative stress responses) and an OsmB-like lipoprotein (usually involved in general stress responses). Treatment with H_2O_2 also resulted in the up-regulation of multiple phage-related genes (BCAS 0539-BCAS 0554; BcepMu16-BcepMu1).

Our initial results show the increased transcription of several genes in sessile *B. cenocepacia* cells in response to treatment with CHX, H_2O_2 and NaOCl. Further experiments including qPCR using both wild-type and mutant strains, will be performed to confirm these transcriptional responses.

Exploring heterotrophic bacterial diversity of Antarctic samples through cultivation

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The microbial diversity on Antarctica is largely under-explored. As part of the AMBIO-project that aims to explore the factors affecting bacterial distribution patterns in Antarctica, nine samples from different regions were investigated. They include two samples from the Belgian Base site at Utsteinen: BB50 from a gravel and green microbial/algal mat from the nunatak and BB115 from a black mat on gravel and rock debris from a frozen lake on the south side of the nunatak Utsteinen; two samples from the Trans-Antarctic Mountains: TM2 from a cyanobacterial mat at the bottom of Forlidas Pond (Pensacola Mountains) and TM4 from Lundström Lake (Shackleton Mountains); a littoral sample PQ1 from Pourquoi-Pas Lake (Pourquoi-Pas Island); samples LA3 (Langhovde Peninsula), SK5 (Skarvsness Peninsula) and WO10 (West Ongul Island) from three lakes at Lützow-Holm Bay, Syowa and sample SO6 from the Schirmacher Oasis.

Samples were investigated with a culture-dependent approach. Dilution series were plated on four media (MA, R2A, R2A/10 and PYGV) and incubated at three temperatures (4, 15 and 20ºC). Different types of colonies were picked up for all conditions. As a fast way of screening the isolates, the genotypic typing method rep-PCR fingerprinting was used. Cluster analysis of fingerprint patterns using Bionumerics software revealed a number of clusters (cut-off level 80%) of similar strains and a number of separate isolates. Representatives of each rep-cluster and the separate isolates were used in partial 16S rDNA sequencing to obtain a first approximate identification.

For the different samples, between 338 (BB115) and 669 (WO10) isolates were obtained and purified. Rep-PCR fingerprinting analysis resulted in 33 (LA3) to 101 (BB50) clusters and 54 (BB115) to 151 (PQ1) separate isolates (excluding samples WO10 and SO6 for which the analysis is not finished yet).

The preliminary results of the 16S rDNA sequencing show a large diversity, distributed over the major phylogenetic groups. The BB samples and TM4 are dominated by isolates of the class of *Actinobacteria*, most TM2 isolates belong to the *Firmicutes* class. The PQ1 and LA3 samples are dominated by the class of *Bacteroidetes* and the most SK5 isolates belong to the class of *Alphaproteobacteria*. Other phylogenetic groups were found in smaller amounts.

Despite the large diversity and the fact that some samples originate from the same general area, only little overlap between the samples was observed. Some of the isolated clusters show low similarity values with neighbouring sequences in the EMBL-database and may represent new species or even new genera.

Assessment of Micropollutant-induced Response in *Rhodospirillum rubrum* S1H using a Toxicogenomic Approach complemented with Physiological Analyses

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The Micro-Ecological Life Support System Alternative (MELiSSA) is a bacteria-driven recycling system that was devised in order to treat organic waste, regenerate the atmosphere, purify water, and provide food during long-haul manned space missions. The MELiSSA consists of four microbial compartments (bioreactors), each of which is responsible for a specific task in the degradation of waste or production of edible biomass and oxygen. The importance of the tasks provided by MELiSSA during such voyages requires the system to be fully controllable on the level of metabolic transformations, but insights into the biomass' vitality are also required. It is therefore crucial to identify and study potential stressors *a priori*, which will ultimately allow the development of sensors for assessing the physiological state of the MELiSSA bacteria *in flight*. The most obvious spaceflight-related stressors are microgravity and cosmic radiation; but due to the nature of the system, an intrinsic issue arises, namely the discharge of micropollutants, *e.g.* steroid hormones, pharmaceuticals, and personal care products, in the system. Because these molecules solicit specific biological effects in humans, they should not be allowed to accumulate in the life support system. What is more, some of the compounds could interfere with proper functioning of the bacterial compartments, which could also adversely affect the crew since they rely on the numerous functions of the system. Triclosan is a chlorinated biphenylether commonly used in personal care products, e.g. toothpaste, soap and deodorants, and has been chosen as a model micropollutant in this study.

The goal of this research was to determine the sensitivity of the MELiSSA carbon-mineralising bacterium *Rhodospirillum rubrum* S1H to triclosan via modelling of the growth kinetics, and to assess the transcriptomic and physiologic response of the bacterium using microarray and flow cytometry analyses, respectively, to identify stress marker-genes for the stress-detection sensor.

Our results indicate that chemotrophic *Rhodospirillum rubrum* is sensitive to triclosan-induced inhibition, and that the minimal inhibitory concentration is in the same order of magnitude as for other bacteria reported in literature (*e.g. Escherichia coli* ATCC8739, *Staphylococcus aureus* RN4220). Upon exposure to MELiSSA-relevant concentrations (10-25 µg/L), triclosan solicited an important increase in lag phase, but not on the generation time. It was determined with flow cytometry that during the prolonged lag phase, triclosan induced a rapid decrease in viability. Via microarray analyses, we were able to identify a number of concentration-dependent defensive pathways that were induced by the bacteria to circumvent growth inhibition. When exposed to 10 µg/L triclosan (where triclosan caused only a minimal effect on the lag phase), *R. rubrum* mainly induced efflux pumps. Whereas, at 25 µg/L, *R. rubrum* activated genes coding for additional efflux pumps (a.o. a MexAB-OprM homolog) and general stress response proteins (a.o. phage shock proteins), but also a cluster of five hypothetical genes encoding for small proteins of unknown function that are unique to *R. rubrum*.

These results offered a clear perspective on the sensitivity of *Rhodospirillum rubrum* to the organic micropollutant, and gave insights into the defensive pathways the bacterium activates upon exposure to the detrimental organic compound. What is more, the in-depth analysis of the gene expression profiles not only allowed the selection of candidate marker genes that can be used in the development of a stress-detection sensor, but also allowed the identification of a novel triclosan-responsive gene cluster encoding small uncharacterised proteins that are unique to *R. rubrum*.

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

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

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

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

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

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

Characterization of cupro-resistant *Methylobacterium* strains

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Members of the *Methylobacterium* genus are ubiquitous: they have been detected in soil, dust, freshwater, lake sediments and in the air. Plants represent one of their major habitats where they can thrive as epiphytic or endophytic colonizers. Facultative methylotrophy is their main metabolic characteristic.

We have recently isolated *Methylobacterium* strains from soil and plant samples collected in Katanga (RDC) at different sites with high concentrations of heavy metals, particularly copper. The strains were either endophytic in Cu-, Co-hyperresistant plant species, or isolated from the soil surrounding the roots. The *Methylobacterium* strains belong to several species, and are characterized by a high-level of resistance to cupric ions and other metallic ions.

Most of the resistant bacteria belonging to other genera isolated from the same samples seem to rely on a Cop mechanism, homologous to the one described in the model organisms *Cupriavidus metallidurans* (Mergeay et al., 2003; Monchy et al., 2006) and *Pseudomonas syringae* (Cha and Cooksey, 1991; Cooksey, 1993). Remarkably, copper-resistant *Methylobacterium* strains do not possess CopA, the core protein of the mechanism. Nevertheless some of them seem to possess genes encoding other copper-induced proteins from *C. metallidurans*. The function of these putative Cop proteins, as well as their role in the resistance to copper and/or other heavy metals is under investigation.

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Comparative analysis of Extended-spectrum-β-lactamase (ESBL)-carrying plasmids from different members of *Enterobacteriaceae* isolated from poultry, pigs and humans: evidence for a shared β-lactam resistance gene pool

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The location, genetic environment and transfer possibilities of three plasmid-borne extended-spectrum-β-lactamases (ESBLs), *bla*$_{\text{TEM-52}}$, *bla*$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-15}}$, present in 14 members of *Enterobacteriaceae* from humans, broilers and pigs and the relatedness of these ESBL-carrying plasmids were studied. Plasmid transfer experiments were carried out and the size and incompatibility group (Inc) of each ESBL-carrying plasmid were determined. Restriction fragment length polymorphism (RFLP) was used to characterise the plasmids. The genetic organisation of the *bla* genes was investigated by PCR mapping and sequencing. *E. coli* transconjugants were obtained for all selected isolates. Plasmid analysis revealed different ca. 150 kb ESBL-carrying plasmid for all isolates. The *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{TEM-52}}$, and *bla*$_{\text{CTX-M-15}}$-carrying plasmids belonged to IncHI2, IncI1 and IncI1, respectively. All *bla*$_{\text{TEM-52}}$-carrying plasmids showed the same RFLP fingerprint and the same genetic environment around the *bla*$_{\text{TEM-52}}$ gene suggesting that this is a rather stable plasmid. Our results suggest the clonal spread of a *bla*$_{\text{CTX-M-2}}$-carrying multi-resistant plasmid among *E. coli* and *Salmonella enterica* isolates from broilers and pigs. The plasmid originating from an *E. coli* strain isolated from humans differed in RFLP fingerprint pattern from the other *bla*$_{\text{CTX-M-2}}$-carrying plasmids. Similarly, the *bla*$_{\text{CTX-M-15}}$-carrying plasmids showed the same RFLP fingerprint pattern except for the plasmid originating from a human *E. coli* strain. Contrary to the *bla*$_{\text{CTX-M-2}}$ gene the genetic environment around the *bla*$_{\text{CTX-M-15}}$ gene was identical for all human, porcine and broiler isolates. These data show the emergence and horizontal spread of several plasmid-borne ESBLs in different members of *Enterobacteriaceae* isolated from humans, poultry and pigs in Belgium.

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Congenital cytomegalovirus infection in Flanders: presentation, methodology and first descriptive results of the Flemish CMV registry

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Introduction Currently, congenital cytomegalovirus (cCMV) infection receives considerable interest in the context of the availability of a specific antiviral therapy (ganciclovir). Data on cCMV infection in Flanders, however, are fragmentary. Therefore, a working group of the Flemish Society of Pediatrics, consisting of neonatologists, general pediatricians and otorhinolaryngologists from different university- and general hospitals and institutions in Flanders, decided to start up a registry of patients with cCMV infection.
Methodology Since January 1st 2007 data are collected prospectively on a voluntary basis with written informed consent from the parents, approval of the Ethics Committees and registration at the Privacy Commission. Our first aim is to include all neonates who present with cCMV in the participating 7 of 8 hospitals in Flanders with a neonatal intensive care unit. The registry is split in 3 parts. The first part consists of data on diagnosis and initial working-out, including imaging of the central nervous system, hearing tests and ophthalmologic evaluation. The second part includes data on treatment with ganciclovir for the treated baby's. The third part consists of data on neurological, audiological and ophthalmological follow-up, which will continue until the patient reaches the age of 6 years.
Results on diagnosis Since opening of the registry, 60 babies are included until now. In 95% of babies, the diagnosis was confirmed within the first 2 weeks of life. In 4 babies (age 2 to 3 months; 2 presenting with hearing loss, one with prolonged jaundice, and one with abnormal central nervous system imaging after referral for subdural hemorrhage) the diagnosis of cCMV was confirmed retrospectively by PCR on Guthrie dried blood spot. In 63% of cases it was maternal seroconversion that gave occasion to neonatal diagnosis. About half of seroconversions took place in the first trimester of pregnancy. In 41% of pregnancies amniocenthesis for PCR was performed, 3/4 of which were positive. In all cases neonatal diagnosis is confirmed by viral isolation in urine. In 38% of patients additional CMV-PCR in blood was peformed: 3/4 were PCR-positive. In 63% of patients CMV antibody titers were determined in blood: while all babies were IgG positive, only 1/3 were IgM positive. Cerebrospinal fluid examination is rarely done: only 4 neonates underwent spinal tap for CMV-PCR; in only one of them CMV-PCR was positive.
Results on treatment 7 children were treated with ganciclovir. In two children treatment had to be temporarily interrupted because of profound neutropenia. In 1 patient treatment had to be stopped prematurily for technical reasons.
Results on follow-up Two patients have profound hearing loss, necessitating a hearing aid in one and a cochlear implant in the other. The first baby was asymptomatic at birth and diagnosis was made at the age of 2 months after referral for a failed Algo hearing screening test. The latter baby was born with the full-blown clinical picture of cCMV (jaundice, hepatosplenomegaly, petechiae, thrombocytopenia), only mild ventriculomegaly on brain MRI and profound hearing loss on BERA (55 dBnHL right and >100 dBnHL left). This baby's hearing deteriorated despite treatment with ganciclovir.
Conclusion We present for the first time the Flemish CMV registry and its (still very incomplete) data about the circumstances of diagnosis in the newborn, data on treatment, and long term outcome. It is our aim to gradually recrute more hospitals for cooperation, in order to get a more representative picture of our cCMV population in Flanders.

Pesticide-primed Soils as Supplement for On-farm Bioremediations systems to Improve Pesticide-Contaminated-Wastewater Treatment

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Pesticide point contamination caused by the handling of pesticides at cleaning and filling places on the farms, can be limited by the installation of on-farm biofilter systems, in which biodegradation and sorption of the pesticides are the main removal processes for treating the pesticide-contaminated wastewater. Despite the high efficiency of these systems, the more mobile and persistent active compounds still hinder the effectiveness. The efficacy can be improved by increasing the biodegradation activity in the systems by introducing appropriate pesticide-degrading bacteria. Different studies show that lab-cultivated inoculants are not recommended because of their poor survival ability in a new biotope. In addition, although soils from pesticide-treated fields often contain the capacity to degrade/mineralize the pesticide, the responsible micro-organisms can often not be cultivated. Therefore, this study investigates, as an alternative for bioaugmentation with pure cultures, if soils showing pesticide-degrading activity are advisable for stimulating biodegradation in Bioremediations systems. The soils qualified for this strategy are long-term contaminated soils or pesticide-primed soils.

Lab-scale bioaugmentation experiments were performed to test the survival capacity and the degrading ability of pesticide degrading micro-organisms inoculated along with the soil when the biofilter is subjected to successional stress conditions (e.g. sudden pesticide supply-stop, a cold period, dryness and the presence of pesticide cocktails) which are expected to occur in real systems. Additionally, the minimal amount of soil necessary to accomplish increased biodegradation activity was determined. Linuron was used as a model-pesticide. Soils long-term treated with linuron where used as pesticide-primed soil. The change in pesticide degrading activity of the lab-scale Bioremediations systems, inoculated with a pesticide-primed soil or a reference soil without pesticide-degrading activity, was monitored by means of ^{14}C-mineralisation experiments performed with samples regularly taken from the Bioremediations systems.

The results from the mineralization experiments show that the Bioremediations systems inoculated with the pesticide-primed soil directly gained the ability of degrading this pesticide which was not the case for the biofilter containing the soil without degrading capacity. The degrading activity was maintained for more than a year and application of the pesticide even resulted into increased activity. These results were accomplished with 50vol.% and with only 5 vol.% pesticide-primed soil. Subjecting the Bioremediations systems to a cold period and the presence of a pesticide cocktail did not have any effect on the degrading capacity. Stopping the pesticide supply and a drought period caused no damage to the activity. However, the degrading population did decrease but could reestablish when the standard conditions returned. In contrast, the biofilter inoculated with the non-primed soil developed a pesticide-degrading activity only after an extended period of pesticide supply and this activity showed less resistance towards stress periods.

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Phenotypic, Transcriptome, and proteome analysis of a gentamicin-resistant small colony variant of *Pseudomonas aeruginosa*

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Small colony variants (SCVs) are slow-growing bacteria with distinctive phenotypic and pathogenic traits. SCVs are often less susceptible to antibiotics and can cause latent or recurrent infections. During sub-culturing of *P. aeruginosa* on gentamicin, one SCV was isolated, which showed resistance to the antibiotic, a delayed lag-phase in LB or in casamino acid medium, and a defective motility. It also produced low levels of C4-acyl-homoserine lactone and of the 2-alkyl-4-quinolones signal quorum sensing molecules (AQs) compared to the wild-type. Virulence factors, such as the siderophore pyoverdine, elastase and the redox-active phenazine pigment pyocyanin were also reduced. On excised Roman lettuce (*Lactuca sativa*) leaves the SCV caused strongly reduced necrosis compared to the wild-type. The SCV caused also reduced mortality in *Drosophila*. A proteome comparison revealed a highly reduced production of the extracellular chitinase ChiC and the flagellar capping protein FliD. Conversely, production of the two-component response regulator PhoP was much higher in the SCV. The transcriptome analysis revealed a down-regulation of 74 genes, including those for the synthesis of AQs, and other QS-regulated genes such as the elastase gene *lasA* or phenazine biosynthesis genes, in agreement with the phenotypic characterization results. Hundred sixty genes were up-regulated in the SCV mutant, among them the antibiotic resistance genes *mexR*, *mexY*, *mexX*, and *mexZ* and LPS modification genes PA3552-PA3559, and the *phoP* and *oprH* genes, confirming the proteome results.

Characterization of CVB3 resistance to rupintrivir, a highly potent 3C protease inhibitor

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Enteroviruses (family of the *Picornaviridae*) are implicated in a wide spectrum of illnesses, ranging from mild respiratory syndromes, herpangina, hand–foot–mouth-syndrome and common cold, to life-threatening disorders such as exacerbations of COPD and asthma, pancreatitis, myocarditis, meningitis and encephalitis. The 3C serine protease ($3C^{pro}$) of picornaviruses has a crucial role in mediating viral replication by carrying out most of the proteolytic processing of the viral polyprotein and is an attractive molecular target for therapeutic intervention. AG7088/rupintrivir (De Palma, *et al.*) is a potent inhibitor of the picornavirus $3C^{pro}$ and possesses broad-spectrum anti-enteroviral activity. To identify the precise interactions of this molecule with the $3C^{pro}$, three independent AG7088-resistant enterovirus [Coxsackie virus B3 (CVB3)] clones were selected by serial culturing of the virus in increasing concentrations of the drug. Genotyping of these resistant viruses resulted in the identification of two amino acid mutations, N126Y substitution alone or in combination with T68A. To assess the precise contribution of the T68A and/or N126Y mutation(s) to the drug resistant phenotype, these mutations were reintroduced, either alone or combined into the full-length viral cDNA clone, by means of site-directed mutagenesis. All mutants replicated as efficiently as the wildtype virus in the absence of drug. Introduction of the T68A mutation resulted in about a two-fold reduction in AG7088 sensitivity. Viruses that carry the N126Y or T68A_N126Y mutation are about five-fold less susceptible to AG7088 than the wildtype virus. The three mutants produced smaller plaques than the wild-type virus, in particular mutant T68A_N126Y produced markedly smaller plaques than the wildtype virus. The mutations were introduced in the crystal structure of the CBV3 $3C^{pro}$, revealing that mutations Thr68Ala or Asn126Tyr affect the integrity and position of the beta-hairpin in the substrate specifity pocket 2 and thus explains the altered sensitivity of CVB3 to AG7088.

Mobile genetic elements and gene exchange among *Bacillus* spp. strains from the Antarctic Concordia Station and the International Space Station

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During space exploration, bacterial contamination and horizontal gene transfer can have critical consequences, especially in the case of dissemination and/or re-assortment of virulence and antibiotic resistance genes among pathogenic or opportunistic bacteria such as *Staphylococcus aureus* or *Bacillus* spp. It is therefore crucial to determine the microbial risk incurred by people confronted to these confined environments. In order to assess these risks, a survey of the natural bacterial gene pool (i.e. plasmid abundance, diversity and transfer activity) associated with the Bacilli microflora has been carried out in the remote and confined environments of the Antarctic Concordia Station and the International Space Station (ISS).

A collection of *Bacillus* spp. isolates from both stations was first analysed for the presence of extra-chromosomal molecules using appropriate *large plasmid gel electrophoresis*. Detection of resident conjugative plasmids was then performed using tri-parental mating which consists in identifying a conjugative plasmid through its ability to mobilize a small plasmid from a helper strain into a recipient strain. When present in the Bacilli isolates, the resident conjugative plasmids were then analysed for their ability to (retro-)mobilize other plasmids. The presence of plasmid replicons related to the *Bacillus anthracis* virulent plasmids pXO1 and pXO2 was also assessed by PCR.

A large variety of plasmid profiles were found in the *Bacillus* spp. strains analysed. Among the eighty strains tested, two *Bacillus cereus* strains were shown to carry a conjugative element able to (retro-)mobilize the small plasmids pUB110 and pBC16 (originating from *S. aureus* and *B. cereus*, respectively) at frequencies of about 10^{-6} transconjugants per recipient. Moreover, six Bacilli strains were shown to receive either or both of the *Bacillus thuringiensis* conjugative plasmids pXO16 (350 kb) and pAW63 (72 kb), and the pUB110 and pBC16 mobilizable plasmids. Finally, eight strains were found to harbour a pXO1-like *repS* replicon, while a single strain contained the pXO2-like *repA* gene. Interestingly, no pXO2-like transfer (*tra*) genes could found associated with the *repA*-like replicon.

MRSA associated with clinical and subclinical mastitis in Belgian cows and herds

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Staphylococcus aureus is a major pathogen causing clinical and subclinical mastitis in cows and heifers. Resistant *S. aureus* strains are prevalent, but methicillin-resistance, caused by the presence of the *mecA*-gene in a genomic island (SCC*mec*), appears to be relatively rare. After the first isolation of a methicillin-resistant *S. aureus* (MRSA) from a mastitic cow in 1972, only a few recent studies have reported of sporadic isolation of MRSA in mastitis. The first case was clearly shown to be of human origin. In most other cases the origin was unclear.

In 2005 a first report on an animal reservoir of MRSA was published. These Animal-Associated MRSA (AA-MRSA) show multiple resistance profiles and are typically non-typable by PFGE with *Sma*I restriction. They all belong to one clonal complex, CC398. There is some variability in *spa*-types. SCC*mec*-types are mainly IVa and V. AA-MRSA were shown to be able to colonise numerous animal species and humans, originating from several European countries, Canada and Singapore. Despite this wide dissemination among species and countries, to our knowledge AA-MRSA has been found only once in bovine mastitis (Monecke et al., 2007). In the Netherlands, a significant association was found between human AA-MRSA carriage and cattle farming, but it was not clear whether this was caused by a reservoir of AA-MRSA in cows.

We present the first data describing the occurrence of MRSA among Belgian S*taphylococcus aureus* isolated from cases of clinical and subclinical mastitis. We analysed 118 successive non duplicate isolates of *S. aureus*, originating from diagnostic milk samples of subclinical and clinical mastitis. Presence of the *mecA*-gene was investigated by a triplex PCR (Maes et al., 2002). All MRSA strains were typed by MLST, *spa* and SCC*mec*, following published protocols. They were also tested for antimicrobial resistance with the disk diffusion method, according to the CLSI.

We also investigated the prevalence of MRSA at herd-level in Belgium. All lactating cows from 4 herds (1 located in the Netherlands, 3 in Belgium), proven to be MRSA positive in the first study, were sampled at quarter level, using a standardised method. Also a randomly chosen herd was included. The same day, milk samples were inoculated on agar plates containing 5% sheep blood and MRSA-ID plates (Biomérieux, France). Pure colonies showing the typical morphology for *S. aureus* on the MRSA-ID plates were tested by the triplex PCR. Strains confirmed as MRSA were then further typed by MLST, *spa* and SCC*mec*, and susceptibility was tested.

Among the 118 isolates from the first study, 11 (9.3%) were found to be MRSA. They were all ST398 and belonged to the closely related *spa*-types t011 and t567. One SCC*mec*-type could not be determined, the others had type IVa or V. The percentages of MRSA positive cows for herds 1, 2, 3, 4 and the control herd were 6.3, 7.4, 3.9, 0 and 0 respectively. They were all ST398, t011 or t567 and had SCC*mec* type IVa or V. The strains were multi-resistant.

In conclusion, prevalence of MRSA approximately is 10% in the studied sample. This is a higher prevalence than any ever reported. Moreover, after isolation in Belgian pigs and poultry, AA-MRSA also appears to have colonised cattle and, even more warring, seems to be implicated in disease. Since β-lactams are the antibiotics most used in the treatment of mastitis, this may cause severe treatment problems in the near future. More investigation is needed to better estimate the prevalence of AA-MRSA in mastitis and to estimate the burden for animal and human health.

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Identification of a bacterial inhibitor of G-type lysozyme

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INTRODUCTION: Lysozymes are hydrolytic enzymes that cleave the β-(1,4) glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine in peptidoglycan, which is a unique and essential component of the bacterial cell wall. Based on amino acid similarity, these enzymes are classified into several types, such as chicken type (C-type), phage type (T4-type), invertebrate type (I-type) and goose type (G-type). In view of the widespread occurrence of lysozymes and their prominent role in the innate immune system of both vertebrates and invertebrates, it is not surprising that bacteria have developed several lines of defence against this bactericidal enzyme. Recently, it was demonstrated that some bacteria are able to produce specific lysozyme inhibitory proteins. Until now, however, only inhibitors of C-type lysozyme have been identified [1,2], while the existence of inhibitors against other types of lysozyme could only be anticipated. Here, we report the existence and identification of a novel type of lysozyme inhibitor against G-type lysozyme in *Escherichia coli*.

MATERIALS AND METHODS:
Osmotic shock: Periplasmic extracts were isolated by cold osmotic shock as described by Callewaert *et al*. [1].
Lysozyme inhibition assay: Lysozyme inhibitory activity was measured in a turbidity assay as previously described [1], but using *Yersinia enterocolitica* cell walls as a substrate
Chromatography: Weak cation exchange using a CM Sepharose Fast Flow resin was performed on an ÄKTA-FPLC (Amersham Pharmacia Biotech) at room temperature. The retained molecules were eluted with a lineair gradient of 0 to 100% 1.0 M KCl in 50 mM K^+ phosphate (pH 7.0) buffer. Size exclusion chromatography was performed on a Superdex 75 HR 10/30 column using a 10 mM K^+ phosphate (pH 7.0) buffer with 0.15 M NaCl.
Construction of mutants: A knock-out mutant in the inhibitor gene (*pliG*) was constructed using the standard one step inactivation protocol of Datsenko and Wanner [3].

RESULTS: Periplasmic extracts of several bacteria were previously screened for inhibitory activity against G-type lysozyme. Interestingly, the extract of *E. coli ivy*::Cm *mliC*::Kan [2], which has knock-out mutations of both its C-type lysozyme inhibitor genes, still exhibited inhibitory activity against G-type lysozyme. In order to isolate and identify this novel inhibitor, 150 ml of the periplasmic extract was subjected to cation exchange. The active fractions were pooled, lyophilized, and dissolved in 200µl MilliQ water. Finally, size exclusion chromatography was performed and an absorption peak of 2.9 mAU corresponded with a single peak of inhibitory activity. These isolation steps were repeated 5 times and the corresponding fractions of the active peak were pooled together. After lyophilisation, the active sample was subjected to tandem mass spectrometry to identify the inhibitor protein and its corresponding gene. Because of its GEWL inhibitory activity we named the protein PliG (**P**eriplasmic **l**ysozyme **i**nhibitor of **G**-type lysozyme). Correspondingly, an *E. coli pliG::Kan* mutant displayed a strong reduction of G-type lysozyme inhibitory activity in periplasmic extracts. Interestingly, an iterative search for sequences similar to the mature PliG protein using Psi-Blast revealed homologs in other *E. coli* strains, but also in *Salmonella* and *Shigella*, and even in some cyanobacteria, β-proteobacteria, and α-proteobacteria.

DISCUSSION: We have identified a novel type of bacterial lysozyme inhibitor in *E. coli*, PliG, which is the first proteinaceous inhibitor to be characterized against G-type lysozymes. When comparing amino acid sequences, this inhibitor is significantly different from the two earlier discovered C-type inhibitors of *E. coli*. Interestingly, this indicates that *E. coli* harbours at least three different inhibitors against two types of lysozyme. As both C-and G- type lysozymes are present in all vertebrates, it can be anticipated that the corresponding lysozyme inhibitors play an important role in bacteria-host interactions.

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Characterization of the VNTR region in a *Legionella pneumophila* collagen-like protein

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Legionella pneumophila is a Gram-negative facultative intracellular pathogen that can cause a severe pneumonia, known as legionellosis. This bacterium is found worldwide in freshwater systems where it mainly resides in biofilms and replicates in protozoa. Upon human infection, *Legionella* replicates in human macrophages.
The *Legionella* genome encodes a number of eukaryotic-like proteins and motifs that modulate the host cellular functions in favor of the pathogenic bacteria. We identified a gene (*lpg 2644*) encoding a protein (Lcl) with significant homology with eukaryotic collagen. Additionally, there is a significant homology between Lcl and SclB of *Streptococcus pyogenes*, for which the collagen-like structure and function have already been proven. Analysis of the *lcl*-gene showed a clear variation in repeat number within different *Legionella pneumophila* strains. Recently, the existence of genes with intragenic tandem repeats was shown in the genome of *L. pneumophila*. The hypothesis is that this variation in intragenic repeats could be a mechanism for the bacteria to evade the host defence mechanism.

We describe the distribution in repeat copy number within *Legionella pneumophila* serotype I strains of different origin (clinical, environmental, hot springs). Furthermore, we studied the influence of RecA on the observed variability.

To get a better insight in the host-parasite interaction and evasion of the human immune system, it is important to investigate the possible role of this collagen-like protein and to elucidate the influence of repeat variation on this function.

Clathrin- and caveolae-independent Entry of feline infectious peritonitis virus in monocytes depends on dynamin

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Feline infectious peritonitis virus (FIPV) is a coronavirus belonging to phylogenetic group 1. The virus causes a lethal chronic disease in cats, that is characterized by granulomatous lesions at the serosae of different organs. Like several phylogenetic group 1 coronaviruses, FIPV enters its target cells, feline monocytes, via endocytosis. The pathway of entry was characterized by evaluating the effect of chemical inhibitors and/or expression of dominant-negative (DN) proteins on the percentage of internalized virions per cell and on the percentage of infected cells. Further, co-localization studies were performed to determine the involvement of certain cellular internalization proteins. FIPV is not internalized through a clathrin-mediated pathway as chlorpromazine, amantadine and DN eps15 did not influence virus uptake and FIPV did not co-localize with clathrin. The caveolae-mediated pathway could be excluded based on the inability of genistein and DN caveolin-1 to inhibit virus uptake and lack of co-localization between FIPV and caveolin-1. Dynamin inhibitory peptide and DN dynamin effectively inhibited virus entry. The inhibitor strongly reduced uptake to 20.3±1.1 % of uptake in untreated cells. In the presence of DN dynamin, uptake was 58.7±3.9 % relative to uptake in untransduced cells. Internalization of FIPV was slightly reduced to 85.0±1.4 %, respectively 87.4±6.1 % of internalization in control cells by the sterol-binding drugs nystatin and methyl-β-cyclodextrin. Rho GTPases were inhibited by *Clostridium difficile* toxin B, but no effect was observed on the internalization of FIPV. These results were confirmed with infection studies showing that infection was not influenced by chlorpromazine, amantadine and genistein but was significantly reduced by dynamin inhibition and nystatin. In conclusion, these results indicate that FIPV enters monocytes through a clathrin- and caveolae-independent pathway that strongly depends on dynamin and is slightly sensitive to cholesterol depletion.

Rapid detection and quantification of *Aspergillus fumigatus* in air using solid-phase cytometry

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A. fumigatus is a ubiquitous fungus causing severe infections such as aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis in immunocompromised patients. Monitoring of the number of *A. fumigatus* spores in the air inhaled by patients is crucial for infection control. In the present study, a new and rapid technique for the quantification of *A. fumigatus*, based on solid phase cytometry and immunofluoresent labelling, was developed. Air samples were collected by impaction on a water soluble polymer which was subsequently dissolved. A part of the sample was filtered and microcolonies were allowed to form on the filter for 18 hours at 47°C. Subsequentely, labelling with a monoclonal anti-aspergillus antibody and tyramide signal amplification was used to detect the microcolonies with the aid of a solid phase cytometer (ChemScan *RDI*). The detected spots were microscopically validated using an epifluorescence microscope. The specificity and sensitivity of the assay were evaluated by testing pure cultures of 40 *A. fumigatus* strains, 12 other *Aspergillus* species, 14 different *Penicillium* species and 14 other filamentous fungi. All *A. fumigatus* strains yielded labelled microcolonies, which confirmed the sensitivity of the assay. Only *Rhizopus stolonifer* and *Paecilomyces varotii* were labelled with the antibody and were able to form microcolonies at 47°C. These fungi, however, could be discriminated from *A. fumigatus* based on morphology. Comparison with traditional culture-based methods indicated that our novel approach is a rapid and reliable alternative with a high dynamic range.

Evaluation of a new experimental inactivated vaccine for the porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive single stranded RNA virus that belongs to the family of the *Arteriviridae* in the order of the *Nidovirales*. PRRSV can cause severe reproductive failure in sows, characterized by late term abortions, early farrowings, stillbirths and the birth of weak piglets, and is associated with the porcine respiratory disease complex in combination with secondary infections. The virus is present in a majority of swine producing countries around the world and gives rise to enormous economic losses in the swine industry. Virus-neutralizing (VN) antibodies against PRRSV protect against viremia, virus replication in lungs, transplacental spreading of the virus and reproductive failure. However, VN antibodies only appear in low amounts around 4 weeks or more after PRRSV infection, which is in contrast with the fast appearance of high amounts of non-neutralizing antibodies. Attenuated as well as inactivated PRRSV vaccines are frequently used in the field. Attenuated vaccines induce VN antibodies and protect against viremia and virus-induced respiratory and reproductive disorders. However, the protective immune response induced by attenuated PRRSV vaccines is mostly strain specific, as these vaccines do not always sufficiently protect against virus strains that are genetically different from the vaccine virus strain. Besides, there are major concerns about the safety of attenuated PRRSV vaccines as the vaccine virus on itself can cause viremia and can spread transplacentally with the risk of reverting to virulence. Inactivated vaccines on the other hand are safe, but commercially available inactivated PRRSV vaccines do not induce VN antibodies and do not sufficiently protect against viremia.

Recently, a strategy was developed to select appropriate techniques for controled inactivation of PRRSV *in vitro*, concerning complete inactivation of the virus on the one hand and conservation of antigenicity on the other hand (patent application GB0811276.5). Based on this strategy, an experimental inactivated PRRSV vaccine was developed and its efficacy was evaluated *in vivo* in PRRSV-negative piglets. It was shown that vaccination with this vaccine induced virus-specific antibodies, and strongly primed the VN antibody response, in contrast to a commercial European-type PRRSV vaccine that neither induced, nor primed VN antibodies. In addition, vaccination with the experimental vaccine resulted in a stronger reduction of viremia after infection compared to the commercial inactivated vaccine. By testing different adjuvants in combination with the inactivated virus, the immunogenicity of the vaccine was enhanced, resulting in a stronger induction of VN antibodies, and further reduction of the viremia after infection. This strategy offers new perspectives for the worldwide control of PRRS.

Isolation of a bacterial inhibitor of the invertebrate type lysozyme

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Introduction: Lysozymes are a family of enzymes (E.C. 3.2.1.17) capable of hydrolyzing the peptidoglycan layer, a unique and essential polymer in the bacterial cell wall. Due to their anti-bacterial action, lysozymes play an important role in the innate immune system of many organisms and/or contribute to the digestion of bacteria in organisms feeding on them. In the animal kingdom three major different lysozyme types are found. The best known is the C (Chicken)-type which, together with G (Goose)-type lysozymes, is referred to as the vertebrate lysozymes. The I (Invertebrate)-type lysozymes, however, can only be found in invertebrate organisms.
Throughout evolution bacteria seem to have developed several barriers against lysozyme attack. In some Gram-positive bacteria, subtle peptidoglycan modifications provide protection against C-type lysozyme, while some Gram-negative bacteria appear to produce periplasmic or membrane bound proteinaceous **lysozyme inhibitors**. Up till now, however, such inhibitors were only found for C-type lysozyme. Therefore, this study aimed to search for bacterial I-type lysozyme inhibitors, using the lysozyme of the marine bivalve *Tapes japonica* (TjL) as a representative of the I-type lysozymes.

Results and discussion: Since most of the currently known lysozyme inhibitors are all localised in the periplasmic space, periplasmic extracts of 30 Gram-negative bacteria were screened for their ability to inhibit the TjL. During this screening a distinct inhibitory activity of this I-type lysozyme was found in the extract of *Aeromonas hydrophila* ATCC7966, suggesting that this bacterium might encode a potential I-type lysozyme inhibitor. It should be noted that no inhibitory activity could be found in the extracts of *Escherichia coli* and *Salmonella* Typhimurium, indicating that the known inhibitors of C-type lysozyme in these bacteria are unable to inhibit I-type lysozyme.

The potential I-type lysozyme inhibitor from *Aeromonas hydrophila* was purified by affinity chromatography using a TjL-affinity column, and could subsequently be identified by tandem mass spectrometry. As such, the inhibitor was found to be a hypothetical protein of 15 kDa with a predicted signal peptide, corresponding with its cellular localisation. This first inhibitor of I-type lysozyme was designated PliI-Ah (**P**eriplasmic **L**ysozyme **i**nhibitor of the **I**-type lysozyme from ***A****eromonas* ***h****ydrophila*).

In order to determine its cross reactivity, the ability of purified PliI-Ah to inhibit C- or G-type lysozyme was subsequently examined. This analysis clearly pointed out that even a molar excess of PliI-ah is unable to inhibit C- nor G-type lysozyme, and that PliI-Ah is a dedicated I-type lysozyme inhibitor.

Conclusions: We have isolated and characterised the first bacterial inhibitor against I-type lysoyme. Moreover, this inhibitor proved not active against C- or G-type lysozyme, and its amino acid sequence differs significantly from the two earlier discovered classes of lysozyme inhibitors. Given the ubiquity of lysozymes in nature on the one hand, and the increasing number of different bacterial lysozyme inhibitors on the other, we hypothesize that these inhibitors could play an important role in bacteria-host interactions. In future work, the influence of PliI-Ah on the interaction of *A. hydrophila* with I-type lysozyme producing hosts like *Caenorhabditis elegans* and *Hirudo medicinalis* will be investigated.

Using Mild high-pressure shock to generate bacterial ghosts of *Escherichia coli*

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Introduction

In the context of vaccine development, bacterial ghosts are inert cells that retain the capacity to activate the host immune system, and that can be used as vaccine or adjuvant for subunit or DNA vaccines. In this paper we examine a new type of mechanism to generate inactive bacterial ghosts of virulent pathogenic *Escherichia coli* (APEC), based on high hydrostatic pressure (HP) treatment of bacteria. We have recently discovered that *Escherichia coli* K12 can be dramatically sensitised to a mild, sublethal HP shock when the copy number of its *mrr* locus is increased [1]. This gene codes for an endogenous restriction enzyme that can apparently be activated by HP, and in this work we would like to examine whether this activation can be used to make bacterial ghosts of APEC strains. We also demonstrate that the generated inactive bacterial ghosts of these APEC strains by HP do not show any microscopically visible structural damage.

Material and methods

In this study we used virulent avian pathogenic *Escherichia coli* (APEC) strains APEC1 (O45) and CH2 (078) [2] and transformed them with plasmid pAA810 or pAA812, encoding a wild-type or compromised *mrr* locus, respectively [1]. Liquid cultures of APEC1 and CH2 with the appropriate plasmids were aerobically grown overnight at 37°C in Luria Bertani (LB) broth. Stationary cultures of these strains were diluted 100-fold in fresh pre-warmed LB media and incubated up to an optical density (OD_{600}) of 0.6 [1]. Next, 200 µl of these suspensions were heat sealed in a sterile polyethylene bag after exclusion of air bubbles and subjected to a pressure between 100 and 250 MPa for 15 minutes at 20°C. After HP treatment, pressurized and non-pressurized samples were serially diluted in potassium phosphate buffer (10 mM; pH 7.0). Alternatively, they were placed on a microscopy glass slide and examined by phase-contrast microscopy at 1000 X magnification with a DMLB microscope equipped with a DC200 digital camera (Leica, Wetzlar, Germany).

Results

It was shown that both APEC1 and CH2 strains become hypersensitive to a 100 MPa treatment in the presence of functional K12 Mrr, leading to an inactivation of the population of > 99 %. Moreover, cells in the presence of the control plasmid (pAA812) were unaffected by this HP treatment. To obtain a similar inactivation in the absence of K12 Mrr, cultures containing pAA812 had to be subjected to pressures of 200 MPa, in the case of APEC1, or even higher, in the case of CH2. Interestingly, when the cellular integrity of the treated strains was examined by phase contrast microscopy, it became evident that cells killed by activation of the Mrr nuclease at 100 MPa failed to show any microscopically visible structural differences in comparison with untreated cells. In contrast, when cells harbouring the control plasmid with an inactive *mrr* gene (pAA812) were inactivated to a similar extend by HP alone (200 MPa), they showed a granular cytoplasm, which is typically an indication of cellular leakage and proteins aggregation.

Discussion

In this study we have shown that activation of the cryptic Mrr protein of *E. coli* K12 by sublethal HP shock also takes place in APEC strains. As a result, we were able to construct “bacterial ghosts” of these strains, which are intact but non-reproductive cells that can be useful in vaccination strategies. Indeed, APEC cells that were sensitised to mild HP treatment by overexpressing K12 Mrr displayed an almost complete inactivation without any visual signs of cellular deterioration, indicating they can be readily recognised by the immune system. Since Mrr is a restriction endonuclease it can be assumed that the genomic DNA in the cells is degraded, leaving the remainder of the cell still intact but unable to reproduce. How HP molecularly activates Mrr, however, is still elusive [1]. Future studies will examine the capacity of HP generated bacterial ghosts to activate the host immune system *in vivo*.

Time to revise the unique role of the pig as a "mixing vessel" for influenza A viruses?

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Introduction: Pigs are traditionally considered as intermediate hosts for the transmission of avian influenza viruses to humans or for the generation of reassortants between avian and human influenza viruses with pandemic potential. This hypothesis was based on the fact that pigs are susceptible to both human and avian influenza viruses. Another argument was the demonstration in the porcine trachea of sialic acid receptors with both α2,3- and α2,6-linkages to galactose, which are preferred by avian and human influenza viruses respectively. Today an increasing number of arguments indicate that the unique role of the pig as an intermediate host or mixing vessel might have been overstated in the past. Most important, studies on influenza virus receptor expression in the porcine airways have been limited to the trachea and the two types of receptors were recently also found in human tissues. In addition there are few comparative studies of the replication of swine, human and avian influenza viruses in the pig. Therefore it was our aim to study receptor expression in both the upper (nose and trachea) and lower (bronchi and lung) porcine respiratory tract and to correlate these results with the susceptibility for avian influenza viruses.

Materials and methods: We established *ex vivo* cultures of the porcine nasal mucosa, trachea, bronchi and lung based on the liquid-interface principle. Using these explants, we assessed the virus yields of 3 porcine, 2 human and 5 low pathogenic avian influenza viruses in the supernatant fluid at 0, 24 and 48 hours post inoculation. Additionally we performed lectin histochemistry to obtain semi-quantitative information on the distribution of α2,3- and α2,6-receptor variants. The Sambucus nigra agglutinin (SNA) and the Maackia amurensis agglutinin (MAA) preferentially bind α2,6- and α2,3-receptors respectively.

Results: For the first time we showed a very distinct distribution pattern of the receptor variants at different levels of the porcine respiratory tract (overview see table 1). The porcine influenza viruses replicated obviously better than the avian viruses in the nasal, tracheal and bronchial explants, while the differences with avian and human influenza virus titres were minimal in the lung explants. Interestingly the replication of avian influenza viruses was clearly hampered in the tissues of the upper respiratory tract, increased in the bronchi and peaked in the lung explants.

Conclusions: In our in vitro systems of the porcine respiratory tract we confirmed the results from previous experimental infection studies in pigs, showing a low replication efficiency of avian influenza viruses in the upper respiratory tract and a similar replication capacity to human and porcine influenza viruses in the lungs. Generally this was in accordance with the absent to rare expression of α2,3-receptors in the upper respiratory tract. Considering the overall low proportion of α2,3- versus α2,6-linkages in the upper respiratory tract the chance for the simultaneous infection of one cell by an avian and human virus that is required for reassortment, is rather low. But by far the most important finding is that the distribution pattern of the virus receptors in the pig is almost identical to that in humans. This means that there are no molecular arguments to assume that pigs are more sensitive to avian influenza viruses than humans.

Table 1: Summary of the binding intensities of SNA, MAA I and MAA II lectins in the porcine respiratory *ex vivo* cultures.

		SNA	MAA I(*)	MAA II(*)
Nasal explants	Epithelium	++	-	+/-
	Glands	+/-	+	+
Tracheal organ cultures	Epithelium	++	-	+/-
	Glands	+	+/-	-
Bronchial organ cultures	Epithelium	++	-	+/-
	Glands	++	+/-	-
Lung explants	Bronchioles	++	-	+
	Alveolae	+	-	+

-: no binding, +/-: rare binding, +: moderate binding, ++: abundant binding
(*): for this lectin 2 isoforms are available

To denitrify or not to denitrify? An issue in Gram-positive bacteria?

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Little reliable information is available on the distribution of denitrification amongst bacteria in general and amongst Gram-positive bacteria in particular. One of the major shortcomings concerns the inaccuracy of existing data regarding the nitrogen reduction characteristics in novel bacterial species descriptions. In those , denitrification is often reported as the result of miniaturized test panels, such as API galleries, which are based on dye reactions without really measuring the gaseous compounds of the processes. Moreover, generally, only 'anaerobic growth' and 'nitrate reduced to nitrite' are mentioned, clearly not referring to the true nature of denitrification.

It has been known for decades that the genus *Bacillus* has denitrifying representatives (e.g. *B. licheniformis*). However, despite their great potential for wastewater treatment and their great impact in food industries, members of *Bacillus* are neglected for their impact in the denitrification process. Consequently, the prevalence of the denitrification capacity within the genus *Bacillus* is largely unknown. It is to be expected that further in-depth phenotypic and genotypic research of *Bacillus* strains will reveal the real prevalence of denitrification in this genus pointing to so far unknown denitrifying *Bacillus* species, as will be the case for other Gram-positive taxa.

Therefore, we have phenotypically screened 87 type strains of the 146 present valid species of *Bacillus* for their capacity to denitrify. Subsequently, within several selected species, a diverse set of strains was analyzed (96 isolates in total). Phenotypical analysis consisted of nitrate and nitrite reduction tests in conjunction with gas chromatographic analysis of N_2O production (acetylene inhibition technique). Preliminary data showed an unexpected high prevalence of the denitrification trait within *Bacillus*. However, the present knowledge of denitrification genes did not allow the straightforward detection of the genes that are involved in the *Bacillus* denitrification capacities.

Decontamination of apple pieces in syrup by high hydrostatic pressure

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Introduction: The low pH of fruit products typically restricts spoilage microflora to acid-tolerant micro-organisms like yeasts. Nevertheless, the acidity does not prevent the presence of potential pathogens that can survive for several weeks in the fruit product and cause a risk for human health upon ingestion. Moreover, in preparations using diced fruit the microbial load inside the fruit might increase as the skin microflora is transferred to the flesh[1]. High Hydrostatic Pressure (HHP) processing for the preservation of foodstuffs has been proposed as a viable alternative to conventional heat treatment[2]. While HHP can readily inactivate microflora, an outspoken advantage of this technology is that it has only minimal impact on the nutritional and organoleptic quality of foods[3]. The objective of the current study was to investigate the effect of HHP processing on the inactivation of *Candida lipolytica* and *Escherichia coli* inoculated on diced apples in syrup. **Material And Methods:** Apples (cultivar Jonagold) were washed with tap water and 70% ethanol. Next, the apples were sterily cut into cubes (ca. 0.5x0.5x1cm). Stationary phase cells of *C. lipolytica* LMM02.68 or *E. coli* LMM1010, a pressure-resistant derivative of strain MG1655[4], were suspended at approximately 10^6 - 10^7 CFU/ml in different sugar solutions (0, 12.5 and 25% D-glucose, pH 3.5) containing 0.15% ascorbic acid to prevent browning. Subsequently, 1 ml of this suspension was added to 1 gram of apples. This product was pressurized at 25 and 40°C (10 min). Inactivation was determined by plate counts on OGYE and LB for *C. lipolytica* and *E. coli*, respectively. All experiments were conducted in duplicate and results are presented as mean ± standard deviation. **Results and Discussion: 1. Inactivation of *C. lipolytica* LMM02.68 on apple pieces in syrup by HHP** The pressure-resistance of this yeast was determined at pressures between 200 and 500 MPa (10 min) at 25 and 40°C. At 25°C the viability of *C. lipolytica* LMM 02.68 started to decrease significantly at 300 MPa, and a complete inactivation (ca. 6.7 log cycles) was obtained at 400 MPa. Increasing the processing temperature to 40°C considerably increased the sensitivity of *C. lipolytica*, shifting the threshold value for inactivation to 200 MPa, and the value for complete inactivation to 300 MPa. Interestingly, a protective effect of sugar was noticed at both 25 and 40°C. Indeed, at 300 MPa (25°C) the 5.0 log reduction in the absence of added sugar diminished to 1.6 and 0.4 log reduction in the presence of 12.5 and 25% glucose, respectively. Similarly, the 4.4 log reduction obtained at 200 MPa (40°C) was reduced to 0.7 log reduction and no reduction in the presence of 12.5 and 25% glucose, respectively. **2. Inactivation of *E. coli* LMM1010 on apple pieces in syrup by HHP** The pressure-resistance of this strain was determined in apple pieces in acidified syrups with different concentrations of D-glucose at pressures between 200 and 650 MPa (10 minutes) and temperatures of 25 and 40°C. The threshold value for the reduction of *E. coli* LMM1010 in the absence of sugar was 300 MPa at 25°C, while complete inactivation (ca. 6.8 log reduction) of this pathogen was observed at 400 MPa. The addition of 12.5 or 25% glucose shifted complete inactivation of LMM1010 to 500 and 600 MPa, respectively. Increasing the processing temperature to 40°C concomitantly increased the HHP sensitivity of *E. coli* LMM1010. At this temperature, complete inactivation was observed at 300 MPa in the absence of glucose, and at 400 MPa in the presence of 12.5 or 25% glucose. **Conclusion:** The effect of HHP processing on the inactivation of *C. lipolytica* LMM02.68 and the HHP resistant *E. coli* LMM1010 in diced apples in acidified glucose syrup was examined. Although the presence of 12.5 or 25% glucose significantly reduces the sensitivity of both organisms to HHP, a > 6-D reduction could be obtained for both species at 600 MPa using a processing temperature of 25°C, and at 400 MPa when the processing temperature was increased to 40°C. Our results indicate that the application of HHP can be a useful tool to ascertain both the microbiological stability and safety of this fruit product. **Acknowledgments** This research was conducted within the framework of a Flanders' Food (Brussels, Belgium) project.

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Stress adaptation of the symbiotic soil bacterium *Rhizobium etli*: analysis of gene expression under non-growing conditions using a high-resolution tiling array

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Bacteria rarely live in environments that permit long episodes of exponential growth and therefore spend most of their time in a nutrient-limited or stress induced stationary phase. *Rhizobium etli* is a nitrogen-fixing micro-symbiont of the common bean plant *Phaseolus vulgaris* and primarily exists in a non-growing state, both in the soil and the plant. Sophisticated regulatory networks allow bacteria to sense and respond to a variety of environmental stresses to rapidly adjust their cellular physiology for survival. An important global regulatory system is the stringent response, mediated by the alarmone guanosine tetraphosphate (ppGpp), that is activated in response to many unfavorable growth conditions. In order to understand how *R. etli* maintains viability during non-growing conditions, we studied the gene expression using a custom design tiling array that represents the entire genome by overlapping 60mer probes.

By comparing the gene expression in exponential and stationary phase of the wild type and a *rel* mutant, unable to produce the alarmone, ppGpp–dependent and –independent genes were identified during the different free-living growth phases. Surprisingly ppGpp regulates many genes not only in the stationary phase but in the exponential phase as well, including genes associated with cell motility and signal transduction. During the stationary phase there is a general downregulation of most genes necessary for growth and an upregulation of genes required for survival such as genes associated with carbohydrate metabolism, posttranslational modification and protein turnover. Most genes necessary for replication, recombination and repair are alarmone-independent.

A comparison of gene expressions during stationary phase and symbiosis revealed several common transcriptional regulators, suggesting a general role of these transcription factors in non-growing conditions. As expected, genes associated with nitrogen fixation are highly upregulated during symbiosis. Surprisingly, many nodulation genes are still expressed in the nodules.

To conclude, using a high-resolution tiling array, we have determined differential gene expression of *R. etli* during the transition of fast growth to growth arrest in both free-living and symbiotic conditions.

Nitric oxide production by the human intestinal microbiota from nitrate

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Inflammation of the gut wall triggers the production of the free radical NO in the intestinal mucosa. Besides eukaryotic cells, some micro-organisms are also capable of producing NO. We investigated whether intestinal bacteria can produce NO by the following mechanisms: NO from nitrate as intermediate in the denitrification pathway, L-arginine as substrate for NO synthase enzymes (both mammalian and prokaryotic forms are identified) and chemical reduction of nitrite at pH<5. To exclude eukaryotic NO production, NO production was studied under *in vitro* conditions mimicking the gastrointestinal tract. High concentrations of NO (45 ppbv NO/mL medium) were produced under gastrointestinal conditions in feed medium with trace levels of nitrate. Nitrate addition increased NO production significantly (145,000 ppbv/mL medium) during non-buffered incubations (pH 4) but the increase was less significant when the pH was buffered (pH 6) (70 ppbv/mL medium), suggesting a partial chemical production from nitrite. L-arginine supplementation did not increase the NO concentrations. Hence, we conclude that NO production by the microbiota under gastrointestinal conditions is correlated to the concentrations of nitrate added to the medium. Even trace concentrations of nitrate can be reduced to high concentrations of NO by the microbiota. Elucidating the microbial NO production in the gut and the interaction with intestinal cells might bring us closer to understanding the role of gut microbiota in certain diseases such as chronic auto-immune diseases of the gastrointestinal tract with unknown etiology.

Fluorescent *in situ* hybridization (FISH) to elucidate structure, function and diversity of granular biomass for the treatment of nitrogenous wastewater

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The combined application of aerobic and anoxic ammonium-oxidizing bacteria (respectively AerAOB and AnAOB) is a cost efficient and sustainable way to remove nitrogen from wastewater. Granular biomass with a mean diameter of 1.8 mm was obtained from an ammonium fed bioreactor and fluorescent *in situ* hybridization (FISH) was applied to elucidate biofilm structure, function, and bacterial diversity. Thin cross-sections revealed that the granules were bordered with a highly active, narrow AerAOB layer. Large clusters of densely packed AnAOB were heterogeneously spread over the anoxic granule core and broke through internal parallel AerAOB 'bands', which were residual structures from the inoculum biofilm. Calcofluor white staining showed the presence of extracellular beta-polysaccharides in the AerAOB zones. With 36% AerAOB and 22% AnAOB, the eubacterial biovolume consisted mainly of AerAOB. Given a steep oxygen gradient however, only the outer layer AerAOB could metabolize, so that the specific granular AnAOB activity exceeded the AerAOB activity. The rest of the biovolume consisted of Alpha-proteobacteria, Gamma-proteobacteria, Chloroflexi and Bacteroidetes. A specific metabolic interaction was suggested by the juxtaposition of filamentous Chloroflexi and AnAOB. In conclusion, the oxygen gradient resulted in a multilayered arrangement of autotrophic AerAOB and AnAOB, interlarded with heterotrophs living from autotrophs' metabolites or decay products.

Comparative study of the genetic barrier and pathways towards resistance of selective inhibitors of HCV replication

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Potent and selective inhibitors of HCV replication, including NS3 serine protease inhibitors, nucleoside and non-nucleoside polymerase inhibitors and cyclophylin binding compounds (eg Debio-025) have been developed in recent years. Drug resistant HCV variants have been obtained for most classes of these antiviral drugs; each molecule has a particular genetic barrier. We report on a comparative study in which the genetic barrier to drug resistance of a selection of reference compounds is evaluated employing a number of resistance selection protocols. The NS3 protease inhibitors (VX-950, BILN-2061), the nucleoside polymerase inhibitors (2'-*C*-methylcytidine, 4'-azidocytidine), the non-nucleoside polymerase inhibitors (HCV-796, thiophene carboxylic acid, JT-16, and two benzothiadiazines) as well as Debio-025 were included in this study. For a number of molecules (BILN-2061, HCV-796, thiophene carboxylic acid, benzothiadiazines) drug resistant variants were readily selected when wild-type replicons containing cells were directly cultured in the presence of high drug concentrations (25- to 125-fold the EC_{50} value). Resistance to Debio-025 could only be selected following a lengthy stepwise selection procedure. For 4'-azidocytidine an initial round of selective pressure with low drug concentrations (5-fold the EC_{50} value) was required to "prime" replicons to become resistant to 25-fold the EC_{50} value. For each drug and passage in the selection process the pheno- and genotype of the drug-resistant replicon was determined. These data provide important insights in the various pathways leading to HCV drug resistance and allow a direct comparison of the genetic barrier of various (classes of) HCV drugs.

Deletion of the vaccinia virus F13L gene results in a highly attenuated virus that mounts a protective immune response against lethal vaccinia virus challenge

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F13L encodes the envelope protein p37 required for production of extracellular vaccinia virus. We demonstrate that a F13L (p37) deleted vaccinia virus recombinant (Vac-ΔF13L) is replication-competent in cell culture but that this virus produces smaller plaques than the wild-type vaccinia (Western Reserve vaccinia). Vac-ΔF13L proved, when inoculated either intravenously or intracutaneously in both immunocompetent and immunodeficient (athymic nude or SCID) mice, to be severely attenuated. Intravenous or intracutaneous inoculation of immunocompetent mice with the ΔF13L virus efficiently protected against a subsequent intravenous, intracutanous or intranasal challenge with vaccinia WR (Western Reserve). This observed protection was corroborated by the observation that the Vac-ΔF13L induced a humoral immune response against vaccinia following either intravenous or intracutaneous challenge. In conclusion, F13L deleted vaccinia viruses may have the potential to be developed as smallpox vaccines.

Functional analysis of the *Legionella pneumophila* plasminogen activator

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Legionella pneumophila is a Gram-negative bacterium that is found in fresh water environments, but this human pathogen can also resides in man-made water systems. People typically become infected with *L. pneumophila* upon inhalation of contaminated aerosols generated by for example whirlpools, cooling towers and air-conditioning systems. If the host immune system is impaired, adhesion to and infection of alveolar macrophages can lead to Legionnaires' disease, a severe form of a possibly fatal pneumonia. To get a better insight in the pathogenesis of *L. pneumophila* knowledge of its virulence factors is very important. Based on their localization at the boundary of the bacterial cell and its environment, outer-membrane proteins (Omps) are important determinants for interaction of bacteria with their host cell. Looking for *L. pneumophila* Omps potentially involved in virulence, we identified Lpa, an outer membrane protein belonging to the class of omptins, a family of surface proteins that exhibit differing virulence-associated functions.

Omptins have a specific requirement for lipopolysaccharides present on the outer membrane of Gram-negative bacteria. On the one hand omptins need binding with LPS to obtain an active conformation, but on the other hand omptins are sterically hindered by long O-polysaccharide chains present in smooth-type LPS. We show that the activity of Lpa might also be sterically hindered by long O-antigen repeats.

We have already shown that Lpa can interfere with the plasminogen/plasmin cascade in the human body by activating plasminogen into the protease plasmin. Host proteolytic cascades are tightly controlled by activators and inhibitors. In this poster we show that *Legionella* can overcome the inhibition of the generated plasmin by α_2-antiplasmin by degrading this plasmin inhibitor. This could be an important feature for *Legionella* to create a localized, uncontrolled proteolysis. In this way *L. pneumophila* may be able to penetrate tissue barriers in order to colonize the whole lung tissue and to spread to the rest of the body.

At the moment the relevance of the plasminogen activation for *L. pneumophila* virulence is further investigated.

Competing and socializing behaviour within a model bacterial community consisting of two different phenanthrene-degrading bacteria growing on phenanthrene-films in a model-ecosystem

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Polycyclic Aromatic Hydrocarbons (PAHs) are a class of hydrophobic organic soil contaminants comprising health risks. Biodegradation by endogenous bacterial populations is considered to be the most important route for removal of PAHs from the environment. These bacterial populations seem well adapted to the oligotrophic conditions in PAH-contaminated soils, and are likely to have ways of enhancing the low bioavailability of PAHs. However, in soil, different types and species of PAH-degading bacteria exist with apparently similar PAH-catabolic capacities, suggesting that that they are competitors of each other. On the other hand, it can be hypothesized that different bacteria occupy different niches governed by the local micro-scale environmental characteristics. However, interspecies interactions of PAH-degrading bacteria at the micro-scale and their influence on PAH degradation are still poorly understood. Two key players in PAH degradation in soil are members of the *Pseudomonas* and *Sphingomonas* genus.

In this study, the competing and/or socializing behaviour of a duospecies consortium consisting of the phenanthrene degrading strains *Pseudomonas putida* OUS82 and *Sphingomonas sp*. LH128 was monitored in a batch conditions in which phenanthrene was provided as a film floating in liquid minimal medium. Triplicate samples were taken during a period of 20 days to monitor phenanthrene degradation by High Performance Liquid Chromatography (HPLC) and bacterial cell number by Helber counting. The structure and composition of mono- and duospecies microcolonies and/or biofilms growing on the phenanthrene films were studied by Confocal Scanning Laser Microscopy (CSLM), using *gfp* and *ds-red* labeled derivatives of the strains.

Large variations were recorded in HPLC results, bacterial counts and CSLM images, reflecting the complexicity of even this simple batch system. The high variability among replicas can be explained by the presence of different microniches, causing a different bacterial growth and PAH degradation in apparent similar conditions.
Eventhough, after selection of the most reoccurring CSLM images and averaging of HPLC results, general trends were observed. It appears that LH128 is the better phenanthrene degrader and biofilm developer - in the mono-species as well as the duospecies situation - although this strains has shown previously to be the slowest grower on regular minimal or rich medium. The presence of OUS82 initially seems to retard phenanthrene degradation by LH128. After initial socializing or tolerance in this duospecies case, LH128 starts to outcompete OUS82, as shown by CLSM imaging and bacterial counts. As shown for LH128, forming biofilms on PAHs seems to be a way to promote PAH bioavailability, resulting in a more efficient degradation and an advantage when competing with OUS82.

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Simulation of the deep-sea biosphere by a continuous high-pressure bioreactor

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The global methane budget needs to be well studied, because methane is an important greenhouse and fuel gas. In ocean system Anaerobic Oxidation of Methane (AOM) followed by carbonate precipitation has a significant effect on the climate regulation, since this process avoids large methane emissions to the atmosphere and fixes carbon dioxide into carbonate structures. However the main difficulty to study AOM is that the consortia involved have extremely long doubling time (2-7 months) at ambient or low pressures. Therefore we designed and constructed a unique continuous high-pressure bioreactor. The reactor can reach pressure up to 100 bars, representing a depth of 1000 m below sea level. The reactor headspace can be filled with 100% methane, thus increasing substrate concentration (partial methane pressure), and therefore the microbial community obtains increased amounts of energy gained from the reaction. By the help of this high pressure reactor system, we are also able to study the effect of environmental factors on AOM activity and microbial diversity. Captain Arutyunov Mud Volcano (Gulf of Cadiz) sediment has been used as biomass resource and different molecular techniques (DGGE, cloning library, FISH) have been applied to examine the microbial community structure.