BELGISCHE VERENIGING VOOR MICROBIOLOGIE
SOCIÉTÉ BELGE DE MICROBIOLOGIE
BELGIAN SOCIETY FOR MICROBIOLOGY

National Committee for Microbiology

ABSTRACT BOOK OF THE SYMPOSIUM

Contactforum

Crossroads of Microbiology and Informatics

Friday, December 3rd, 2004

Brussels

Under auspices of Académie royale des Sciences de Belgique,
Koninklijke Vlaamse Academie van België voor Wetenschappen, FNRS

SYMPOSIUM SPONSORS

Vlaams Kennis- en Cultuurforum

The Royal Academies for Sciences and the Art

Fonds National de la Recherche Scientifique

la Communauté
française de Belgique

D. Collen Research Foundation vzw
a non-profit organization for the advancement of biomedical sciences

AstraZeneca

gsk GlaxoSmithKline

bio TRADING BELGIUM B.V.B.A.

Programme

09.00 Welcome & Opening
Alfons Billiau
President BSM

09.15 Comparative genomics for reliable protein function prediction: application to the proto-mitochondrial proteome
Martijn A. Huynen,
Center for Molecular & Biomolecular Informatics – Nijmegen

10.00 *In silico* study of transcriptional networks in micro-organisms
Kathleen Marchal
Dept. Electrotechnics (ESAT) – Univ. Leuven

10.45 Break

11.15 Computational inference of alpha-proteobacterial genome evolution
Siv Andersson
Dept. Molecular Evolution – Univ. Uppsala

12.00 General Assembly BSM

12.10 Poster viewing

12.40 Lunch, followed by poster discussion groups

14.20 4 Short Communications

1. **Barcia-Macay M.,** S. Carryn, S. Lemaire, C. Seral, M.P. Mingeot-Leclercq, P.M. Tulkens & F. Van Bambeke. Intracellular models of infection to evaluate antibiotic activity.
2. **Chamekh Mustapha**, Armelle phalipon, Isabelle Salmon, Philippe Sansonetti, and Abdelmounaaïm Allaoui. Immunomodulation of the inflammation in shigellosis using recombinant IL-10 delivered by the *Shigella* type III secretion system
3. **Gevers Dirk**, Olena Vinnyk, Klaas Vandepoele, Cedric Simillion, Siv Andersson & Yves Van de Peer. Evolutionary and comparative genomics: gene and segmental duplication in bacterial genomes.
4. **Uyttebroek Maarten**, Philip Breugelmans, Mieke Janssen, Boris Joffe, Ulrich Karlson, Jose-Julio Ortega-Calvo, Leen Bastiaens, Annemie Ryngaert & Dirk Springael. Distribution of the *Mycobacterium* community among different size fractions of a polycyclic aromatic hydrocarbon contaminated soil

15.15 Computational Genomics: from *Aeropyrum* to *Yersinia*
Christos A. Ouzounis
European Bioinformatics Institute – Cambridge, UK

16.00 Exploring the RNA virus Universe
Alexander Gorbalenya
Center of Infectious Disease, Univ. Leiden

16.45 Concluding remarks

17.00 End of Symposium

ABSTRACTS OF

POSTERS AND SHORT COMMUNICATIONS

- List is ordered alphabetically
- Posters are grouped according to topics:
    1. Biodiversity, Evolution and Taxonomy
    2. Environmental Microbiology
    3. Medically oriented microbiology
    4. Biochemistry and Molecular Biology
    5. Bioinformatics
    6. Virology
- Poster abstracts selected for oral communication

1. **Barcia-Macay M.,** S. Carryn, S. Lemaire, C. Seral, M.P. Mingeot-Leclercq, P.M. Tulkens & F. Van Bambeke. Intracellular models of infection to evaluate antibiotic activity.
2. **Chamekh Mustapha**, Armelle phalipon, Isabelle Salmon, Philippe Sansonetti, and Abdelmounaaïm Allaoui. Immunomodulation of the inflammation in shigellosis using recombinant IL-10 delivered by the *Shigella* type III secretion system
3. **Gevers Dirk**, Olena Vinnyk, Klaas Vandepoele, Cedric Simillion, Siv Andersson & Yves Van de Peer. Evolutionary and comparative genomics: gene and segmental duplication in bacterial genomes.
4. **Uyttebroek Maarten**, Steven Vermeir & Dirk Springael. Microbial ecology of an acidic polycyclic aromatic hydrocarbon contaminated soil from a former gaswork manufacturing plant

LIST OF POSTERS (ALPHABETIC ORDER OF FIRST AUTHOR)

	duplication in bacterial genomes	
35.	George I., Eyers L., Stenuit B., Agathos S. & El Fantroussi S. Impact of long-term contamination with 2,4,6-trinitrotoluene (TNT) on soil microbial communities	ENV
36.	Hachani Abderrahman, Giacomo Rossi, Claude Parso, Ménard Robert, Maria Lina Bernardini, and Abdelmounaaïm Allaoui. IpgB1 and IpgB2, two homologous proteins secreted by the type III secretion system, are synergistically required for both cell-to-cell spread and for the immunomodulation of the inflammation induced by *Shigella flexneri*	MOLGEN
37.	Hamonts K., I. Clijsters, W. Dejonghe, R. Lookman, B. Borremans, J. Bronders, L. Diels & D. Springael. Sediment biobarriers for chlorinated aliphatic hydrocarbons in groundwater reaching surface water	ENV
38.	Heylen Kim, Bram Vanparys, Nico Boon, W. Verstraete & Paul De Vos. Culture-dependent study on the diversity of the denitrifying microbial community in activated sludge	BIODIV
39.	Heyrman Jeroen and Paul De Vos. Novel *Bacillus* species from Drentse Grasslands	BIODIV
40.	Janssen Paul J, Anton J. Enright, Benjamin Audit, Ildefonso Cases, Leon Goldovsky, Nicola Harte, Dag Ahren, Victor Kunin & Christos A. Ouzounis. "CC"-COGENT: Two hundred completely sequenced and published genomes unified in one mySQL database	BIOINF
41.	Leplae Raphaël, Gipsi Lima Méndez, Shoshana Wodak & Ariane Toussaint. ACLAME: A Database for the Reticulate Classification of the Prokaryotic Mobilome	BIOINF
42.	Maertens J., S. Van Den Broeck, A. Bhagwat, G. Lequeux, M, De Mey & P.A. Vanrolleghem. Dynamic experiment design for identification of the lin-log kinetic parameters for the metabolic network of *Escherichia coli*	BIOINF
43.	Malhotra-Kumar Surbhi, A. Mazzariol, C. Lammens, J. Piessens, G. Cornaglia & H. Goossens. Unusual Resistance Patterns Related to Alterations in the *Erm*(A) Attenuator in Macrolide-Resistant (MAC-R) *Streptococcus pyogenes* (SPY)	MED
44.	Malhotra-Kumar Surbhi, Christine Lammens, Sabine Chapelle, Monique Wijdooghe, Jasper Piessens, Koen Van Herck & Herman Goossens. Molecular epidemiology of macrolide- and telithromycin-resistant group A *Streptococcu*s in Belgium, 1999-2003	MED
45.	Malhotra-Kumar Surbhi, C. Lammens, H. Bruylants, J. Piessens & H. Goossens. Multiplex PCR for Simultaneous Detection of Macrolide and Tetracycline Resistance Determinants in *S.* pyogenes (SPY) and *S. pneumoniae* (SPN).	MED
46.	Mastroleo Felice, Larissa Hendrickx, Sarah Baatout, Natalie Leys, Louis de Saint Georges, Ruddy Wattiez & Max Mergeay. Analysis on the effects of spaceflight on the photosynthetic bacterium *Rhodospirillum rubrum* ATCC25903 used in Life Support Systems	ENV
47.	Lima Mendez Gipsi, Raphael Leplae, Shoshana Wodak, Ariane Toussaint. Prophage Detection in Bacterial Genomes	BIOINF
48.	Jelle Mertens, Erik Smolders & Dirk Springael. Zinc tolerance and community structure of ammonia oxidizing bacteria in a long-term zinc contaminated soil	ENV
49.	Mesaros N., N. Caceres, F. Van Bambeke, M.P. Mingeot-Leclercq & P.M. Tulkens, Narcisa Mesaros. Quantification of *mexAB-oprM* and *mexXY-oprM* gene expression level in *Pseudomonas aeruginosa* by QC-RT-PCR	MED
50.	Monchy Sébastien, Daniel van der Lelie, Sean McCorkle, Safyih Taghavi, John Dunn and Max Mergeay. The four replicons of *Ralstonia metallidurans* CH34 revealed by IS reassembly	MOLGEN
51.	Monsieurs Pieter, Kathleen Marchal, Sigrid Dekeersmaecker, Gert Thijs, Jos Vanderleyden& Bart De Moor. Comparison of the PhoPQ regulon in *Escherichia coli* and *Salmonella typhimurium*	BIOINF
52.	Moreels David, Leen Bastiaens, Frans Ollevier, Roel Merckx, Dirk Springael & Ludo Diels. Effects of *in situ* conditions on MTBE biodegradation kinetics and microbial community composition during long-term enrichment	ENV
53.	Paul Sophie, Sophie Delhaye, Vincent van Pesch and Thomas Michiels. Immune evasion properties are shared by leader proteins of neurovirulent and persistent Theiler's virus strains.	VIR
54.	Schmoker C., Taton A., Balthasart P., Grubisic S., Waleron K. & Wilmotte A. Study of the cyanobacterial diversity and paleodiversity in microbial mats from Antarctic coastal lakes with molecular tools based on the rRNA gene sequences	BIODIV
55.	Scipioni A., Bourgot I., Ziant D., Czaplicki G., De Mol P., Lomba M., Daube G. & Thiry E. Genetic relationships between caliciviruses isolated in bovine species in Belgium, other bovine caliciviruses and human noroviruses bases on polymerase and capsid partial sequences	VIR

56.	Soetaert W., P. Vanrolleghem, M. De Mey, J. Maertens & E. Vandamme. Metabolic Modelling and Engineering of Micro-Organisms (M^2EMO)	**BIOINF**
57.	Sommereyns Caroline & Thomas Michiels. Characterization of the murine beta interferon N-glycosylation	**MOLGEN**
58.	Stakenborg T., Jo Vicca, Rita Verhelst, Patrick Butaye, Dominiek Maes, Anne Naessens, Jos Bogaert, Geert Claeys, Catharine De Ganck, Freddy Haesebrouck & Mario Vaneechoutte. Evaluation of tDNA-PCR for the identification of *Mollicutes*	**BIODIV**
59.	Stakenborg Tim, Jo Vicca, Patrick Butaye, Dominiek Maes, Aart de Krui, Johan Peeters, Freddy Haesebrouck. Characterization of *in vivo* acquired resistance of *Mycoplasma hyopneumoniae* to macrolides and lincosamides	**MED**
60.	Steurbaut S., Rombaut B. & R. Vrijsen. Virus subtype dependent clearance or persistence of Theiler's murine encephalomyelitis in RAW264.7 macrophages	**VIR**
61.	Thiry E., Schynts F., Muylkens B & Meurens F. Safety issues of recombination between glycoprotein E deleted marker vaccine and wildtype strains of infectious bovine rhinotracheitis virus	**VIR**
62.	Thys Bert, David Franco, Harsh Pathak, Craig Cameron, Eckard Wimmer, Aniko V. Paul & Bart Rombaut. Stimulation of poliovirus synthesis in a HeLa cell-free *in vitro* translation-RNA replication system by viral protein $3CD^{PRO}$	**VIR**
63.	Uyttebroek Maarten, Philip Breugelmans, Mieke Janssen, Boris Joffe, Ulrich Karlson, Jose-Julio Ortega-Calvo, Leen Bastiaens, Annemie Ryngaert & Dirk Springael. Distribution of the *Mycobacterium* community among different size fractions of a polycyclic aromatic hydrocarbon contaminated soil	**ENV**
64.	Uyttebroek Maarten, Steven Vermeir & Dirk Springael. Microbial ecology of an acidic polycyclic aromatic hydrocarbon contaminated soil from a former gaswork manufacturing plant	**ENV**
65.	Van Coillie E., Swiecicka I, Heyndrickx M , Mahillon J & Dierick K. Typing and toxigenic profile of *Bacillus cereus* strains isolated from a fatal food intoxication in Belgium	**MED**
66.	Van der Auwera Géraldine & Jacques Mahillon. Conjugative plasmid pAW63 from *Bacillus thuringiensis* and its relation to the *Bacillus cereus sensu lato* gene pool	**MOLGEN**
67.	Vankerckhoven V., T. Van Autgaerden, C. Vael, C. Lammens, S. Chapelle, R. Rossi, D. Jabes, & H. Goossens. Multiplex PCR for the Detection of *asa1*, *gelE*, *cylA*, *esp* and *hyl* genes in European Hospital Isolates of *Enterococcus faecium*	**MED**
68.	Vankerckhoven V., T. Van Autgaerden, G. Huys, M. Vancanneyt, J. Swings & H. Goossens. Inventory and identification of lactic acid bacteria used as probiotics	**BIODIV**
69.	Van Minnebruggen Geert, Herman W. Favoreel & Hans J. Nauwynck. Internalization of pseudorabies gB is mediated by an interaction between the YQRL motif in its cytoplasmic domain and the clathrin associated AP-2 adaptor complex	**VIR**
70.	Van Nooten T., B. Borremans, J. Dries, K. Vanbroekhoven and L. Bastiaens & D. Springael. Characterization of the microbial ecology in the surroundings of an iron barrier	**BIODIV**
71.	Vanparys Bram, Kim Heylen, Nico Boon, W. Verstraete & Paul De Vos. Microbial diversity study of a nitrifying enrichment culture	**BIODIV**
72.	Werbrouck H., Herman L., Botteldoorn N., Grijspeerdt K., Rijpens N., Van Damme J. & Van Coillie E. Expression of virulence genes by *Listeria monocytogenes* strains of different origin and interactions with different human cell types	**MED**

ABSTRACTS

ORAL COMMUNICATIONS

Comparative genomics for reliable protein function prediction: application to the proto-mitochondrial proteome

Martijn A. Huynen

Center for Molecular & Biomolecular Informatics – Nijmegen

The accumulating wealth of genomes and other types of genomics data gives us the opportunity both to predict protein function as well as to trace its evolution at a genomic scale. As genomics data are however inherently noisy we need comparative analysis between multiple sets of data to make reliable analyses and predictions. We have shown that, while the co-expression of genes in one species provides only a weak signal that their proteins functionally interact, when that co-expression is conserved between species, it does become a reliable signal (van Noort et al., 2003). Similarly, yeast-2-hybrid from either *S.cerevisiae* or *D.melanogaster* are notoriously noisy, and likely contain many protein-protein interactions that have no functional relevance. However the interactions that are observed in both species do appear to be "true": for the proteins among that set of which the functions are known it is also known that they interact (Huynen et al. 2004). One of the surprising observations of such "horizontal comparative genomics" between species, is the low level of conservation: less that 5% of genes that are co-expressed in *S.cerevisiae* are also co-expressed in *C.elegans*, and less than 25% of the yeast-2-hybrid interacting proteins from *S.cerevisiae* have been observed to interact in *D. melanogaster*. The question rises whether such low conservation reflects evolution and the changing relations between proteins or merely the noisy level of the datasets. When comparing yeast-2-hybrid data between species the level of conservation is only slightly lower than when comparing independently generated datasets from a single species, indicating that the indeed the low reproducibility of genomics data might be the main cause for the low level of measured conservation between species. To obtain a more direct answer to the question to what extent gene co-regulation is conserved in evolution we have constructed a set of reliably co-regulated genes in *S.cerevisiae* by combining co-expression data with transcription factor binding data from ChIP-on-chip experiments. For those gene-pairs for which we have multiple sources of evidence that they are indeed truly co-regulated in *S.cerevisiae*, the conservation of co-regulation in *C.elegans* is 78%. Co-regulation therefore appears well conserved in evolution (Snel et al., 2004).

In the last part of my presentation I will talk about reconstructing the proto-mitochondrial proteome and applying comparative genomics for protein function prediction. Using large-scale phylogenetic we have the reconstructed the proteome of the ancestor of the mitochondria, and therewith its metabolism (Gabaldon and Huynen 2003). The most surprising result is that the majority of the proto-mitochondrial proteins are not mitochondrial anymore, but presently function in other parts of the cell. The set contains a number of hypothetical proteins. Combining information from structural genomics data with yeast-2-hybrid data and the analysis of genome sequences we predict the function for one of its hypothetical proteins, BolA. We show that there is compelling "genomics" evidence that this widespread protein family is a reductase that functions in conjunction with a mono-thiol glutaredoxin.

References:

Gabaldon T, Huynen MA. (2003) Reconstruction of the proto-mitochondrial metabolism. Science 301:609

van Noort, V., Snel, B. and Huynen MA (2003) Predicting gene function by conserved co-expression Trends Genet. 19: 238-242.

Huynen MA, Snel B, van Noort V (2004) Comparative genomics for reliable protein-function prediction from genomic data. Trends Genet. 20: 340-344.

Snel B, van Noort V, Huynen MA. (2004) Gene co-regulation is highly conserved in the evolution of eukaryotes and prokaryotes. Nucleic Acids Res. 32: 4725-4731

Huynen MA, Spronk, C, Gabaldon T., and Snel B (2004) Combining data from genomes, Y2H and 3D structure indicates that BolA is a reductase interacting with a glutaredoxin. FEBS lett. *In press*

In silico study of transcriptional networks in micro-organisms

Kathleen Marchal[1,2], Pieter Monsieurs[1], Tijl Debie[1], Kristof Engelen[1], Gert Thijs[1], Sigrid De Keersmaecker[2], Bart De Moor[1], Jos Vanderleyden[2]

[1] ESAT-SCD, K.U.Leuven, Kasteelpark Arenberg 10, 3001 Leuven-Heverlee, Belgium
[2] Centre of Microbial and Plant Genetics, K.U.Leuven, Kasteelpark Arenberg 20, 3001 Leuven-Heverlee, Belgium

Important technological advances in molecular biology allow simultaneously measuring of data at large scale representative of different cellular processes e.g. transcriptomics, proteomics, and interactomics. Based on these "omics" data, the action of the regulatory network that underlies the organism's behavior can be observed. Having access to these rich data sources changes our view on biology. Genetic entities (genes, proteins, etc) can now be studied in their global context, as parts of the complex regulatory network that is active in the cell. The challenge of the 21st century is to convert this tremendous dataflow into biological relevant information.

The increasing number of "omics"-technologies thus urges the development of methods to extract low level information from the analysis of each of these datasets separately. This is a non-trivial task. High-throughput "omics" data are characterized by a low signal/noise ratio, a high dimensionality, and the presence of consistent sources of variation.

Besides this individual analysis, advanced methodologies to integrate and combine these data are required. Each of the "omics" data gives independent information on the same biological system. The combination of heterogeneous data sources therefore enhances the reliability in predicting regulatory networks. Moreover, because each type of "omics" data describes the system from a different point of view, integrating all data allows gaining a holistic insight into the network studied.

In this work we will focus on the analysis of cDNA microarray data, chromatin immunoprecipitation (ChIP) DNA microarray (ChIP-chip) data and motif data.

cDNA microarrays profile the mRNA expression of thousands of genes simultaneously. The chromatin (ChIP-chip) technology allows directly mapping of *in vivo* physical interactions between transcriptional regulators and their binding sites at a high-throughput level. A DNA-motif is a short conserved DNA-sequence located in the promoter region of a gene that serves as the recognition site of a transcriptional regulator. DNA-motifs thus reflect the direct transcriptional interactions between regulators and target genes.

We will discuss how sequence data can be converted into motif information (phylogenetic footprinting, Thijs et al., 2003, Marchal et al., 2004, Monsieurs et al., in press) and we will show how expression data, immunoprecipitation (ChIP) DNA microarray and sequence data can be analyzed concurrently to reconstruct transcriptional networks in micro-organisms (Debie et al., in press).

References

T. Debie, P. Monsieurs, K. Engelen, B. De Moor, N. Christianini, and K. Marchal. Discovering regulatory modules from heterogenous information sources. PSB2005, accepted.

K. Marchal, S. De Keersmaecker, P. Monsieurs, N. Van Boxel, K. Lemmens, G. Thijs, J. Vanderleyden, and B. De Moor. *In silico* identification and experimental validation of PmrAB targets in *Salmonella typhimurium* by regulatory motif detection. 2004. Genome Biology, 5(2):R9, Epub

P. Monsieurs, K. Marchal, S. De Keersmaecker, F. De Smet, B. De Moor, and J. Vanderleyden. Comparison of the PhoPQ regulon in *Escherichia coli* and *Salmonella typhimurium.* J. Mol. Evol. In press

G. Thijs, K. Marchal, M. Lescot, S. Rombauts, B. De Moor, P. Rouzé, and Y. Moreau. A Gibbs Sampling method to detect over-represented motifs in the upstream regions of co-expressed genes (2002). J. Comput. Biol., 9 (3):447-464.

Computational inference of alpha-proteobacterial genome evolution

Siv G.E. Andersson

Department of Evolution, Genomics and Systematics, Evolutionary Biology Center, Uppsala University, Sweden

Environmental sequencing of the Sargasso Sea has shown that the α-proteobacteria is one of the most highly abundant bacterial subdivisions in the analyzed water samples (1). The recent sequencing of a dozen alpha-proteobacterial genomes spanning the entire bacterial size range, including those of nitrogen-fixing soil-bacteria as well as our own completed genomes of *Rickettsia prowazekii* the typhus pathogen (2), *Bartonella quintana*, the agent of trench fever (3) and *Bartonella henselae*, the agent of cat-scratch disease (3), now enables bioinformatic studies of the processes that drive genome size alterations. Here, we discuss the use of computational approaches to study how alpha-proteobacterial genomes have evolved by genome reduction in intracellular lineages versus genome expansion in their nitrogen-fixing relatives and how these processes have influenced the structures, sizes and numbers of chromosomes (4). Our analysis suggests that the α-proteobacterial ancestor contained some 3,000 to 5,000 genes and was biochemically highly versatile, with a complete system for aerobic respiration and a broad biosynthetic capability (4). We follow the fate of these pathways in the various descendents and estimate the extent of gene loss, gene transfer and gene genesis that have contributed to shape the modern alpha-proteobacterial genomes. We suggest that the flux of genes within the alpha-proteobacteria reflects environmental changes and modified host-adaptation processes.

References

1) Venter C *et al* (2004) Environmental genome shotgun sequencing of the Sargasso sea. Science 304:66-74.
2) Andersson, S. G. E., Zomorodipour, A., Andersson, J. O., Sicheritz-Ponten, T., Alsmark, C. M. U., Podowski, R. M., Näslund, A. K., Eriksson, A.-C., Winkler, H. H. and C. G. Kurland. 1998. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* 396:133-140.
3) Alsmark, U. C. M., Frank, A. C., Karlberg, E. O., Legault, B., Canbäck, B., Ardell, D., Eriksson, A.-S., Näslund, A. K., Handley, S., Huvet, M. La Scola, B., Holmberg, M. and S. G. E. Andersson. 2004. The louse-borne human pathogen *Bartonella quintana* is a genomic derivative of the zoonotic agent *Bartonella henselae*. *Proc. Natl. Acad. Sci. USA* 101:9716-9721.
4) Boussau, B., Karlberg, O., Frank, C., Legault, B. and S. G. E. Andersson. 2004 Computational inference of scenarios for □-Proteobacterial genome evolution.
Proc. Natl. Acad. Sci. USA 101:9722-9726.

Computational Genomics: from *Aeropyrum* to *Yersinia*

Christos A. Ouzounis ,

European Bioinformatics Institute – Cambridge, UK

Exploring the RNA virus Universe

Alexander E. Gorbalenya

Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands (a.e.gorbalenya@lumc.nl)

Viruses comprise the most genetically diverse Kingdom of Life that includes six major and unevenly populated classes different in respect to the genome type and/or polymerase. Four classes are known to include viruses that employ double-stranded (ds) or single-stranded (ss) RNA as their genome and these classes encompass a majority of the identified viruses. The most dominant class includes viruses that use ssRNA of the positive polarity (ssRNA+) as their genome, which replication is mediated by virus-encoded RNA-dependent RNA polymerase (RdRp). Over the years, we have systematically applied comparative sequence analysis to the RNA virus Universe with aim at the reconstruction of the origin and evolution of viruses and the structure-function assignment of poorly characterized viral components. Our evolutionary-based models have served as input for experimental studies conducted in collaboration with many groups worldwide; success of these studies provides an ultimate proof for the original phylogenetic inferences.

Our recent results obtained in studies of three large groups of viruses illustrate the power of cooperation between theoretical and experimental approaches in the dissection of the remarkable diversity of RNA viruses. A common denominator of these studies is that they relied upon evolutionary models, which were derived from very remote relationships uncovered by in-depth bioinformatics analysis. In study of the ssRNA+ *Flaviviridae* family, a properly established relationship between three major genera of this family was instrumental in gaining a decisive insight into the mechanism of the NS2-3 cleavage, central to pathogenesis of Pestiviruses (1), and the structure of enigmatic replicative subunit NS5a of Hepaciviruses (2), both of which had resisted researchers' efforts for a long time. In analysis of the ssRNA+ *Nidovirales* order that is composed of three families and includes SARS coronavirus (3, 4), a significant advancement in the understanding of the composition of the viral giant replicase has recently been achieved. The obtained results indicate that the synthesis of diverse species of nidovirus RNA in cytoplasm may be mediated by the replicative machinery that is related to enzymes involved in two cellular splicing pathways (5, 6). Finally, an unprecedented kinship between RdRps of viruses of the dsRNA *Birnaviridae* family and the ssRNA+*Tetraviridae* family has recently been revealed (7). These RdRps share a non-canonical permuted organization of the ubiquitously conserved active site (7) that is compatible with replicative competence (8). Diverse implications of this major discovery will be outlined.

References:

1. Lackner, T. *et al*., (2004) J. Virol., **78** (19): 10765-10775.
2. Tellinghuisen, T.L. *et al*., (2004) J. Biol. Chem., **279**: *in press* (jbc.M407787200)
3. Gorbalenya, A.E. (2001) Adv. Exp. Med. Biol. **494**: 1-17.
4. Gorbalenya, A.E. *et al*., (2004) J. Virol., **78** (15): 7863-7866.
5. Snijder, E.J. *et al*., (2003) J. Mol. Biol., **331** (5): 991-1004.
6. Ivanov, K.A. *et al*., (2004) PNAS, **101** (34): 12694-12699.
7. Gorbalenya, A. E. *et al*., (2002). J.Mol.Biol. **324**, 47-62.
8. von Einem, U. I. *et al*., (2004) J. Gen. Virol. **85** (8): 2221-2229.

Bacterial physiology changes after pH stress

Sarah Baatout[1], Ruddy Wattiez[2], Larissa Hendrickx[1], Natalie Leys[1], Annik Dams[1], Max Mergeay[1].

[1]Laboratory of Radiobiology & Microbiolog, [2]Belgian Nuclear Research Center, SCK-CEN, Mol, Belgium.

Flow cytometry and cell intact mass spectrometry provide powerful means to measure a wide range of cell characteristics in microbiological research. In order to estimate physiological changes associated with pH stress, both technologies were employed to estimate the extent of damage on the maintenance of membrane integrity and potential, esterase activity, intracellular pH and production of superoxides in two bacterial strains (*Ralstonia metallidurans* and *Escherichia coli*). The physiology of the bacterial strains is being studied in order to understand their behaviour and resistance under extreme conditions. This knowledge is of importance in the light of the potential use of these strains for space biology and bioremediation.

Suspensions of *R. metallidurans* and *E. coli* were submitted to a 1-hour pH stress (2, 4, 5, 6, 7, 8, 9, 10 or 12). The laser desorption technologies could be used for the characterization of intact micro-organisms by generation of different ion spectra of molecules (especially proteins) desorbed from bacteria. Specific cell fingerprint of both bacteria strains were analysed in different pH stress conditions in linear MALDI configuration. For flow cytometry, fluorochromes, including propidium iodide, rhodamine-123, 3,3'-dihexyloxacarbocyanine iodide, fluorescein diacetate, carboxy-fluorescein diacetate or hydroethidine were chosen as analytical parameters for identifying the physiological state and the overall fitness of individual cells. An individual cell's physiologic state was assessed with a Coulter EPICS XL analyser based on the differential uptakes of these fluorescent stains.

The two bacterial strains exhibited varying staining intensities. For both bacterial strains, the physiological status was only slightly affected between pH 6 and 8 in comparison with pH 7 which represents the reference pH. Moderate physiological damage could be observed at pH 4 and 5 as well as pH 9. In both strains, membrane permeability and potential, esterase activity, intracellular pH and production of superoxide anion production were increased to high levels at pH 2, 10 and 12.

It is apparent that a range of significant physiological alterations occurs after pH stress. Fluorescent staining methods coupled with flow cytometry as well as cell intact mass spectrometry are useful and complementary for monitoring physiological changes induced not only by pH stress but also temperature, and oxidative stress, radiation, pressure, etc. These studies are currently being investigated in our laboratories.

This work is partly supported by ESA/ESTEC (contract number n°15680/01/NL/ND).

Isolation of interleukin-2 and expression/secretion in *Clostridium acetobutylicum*

S. Barbé, L. Van Mellaert and J. Anné

Laboratory of Bacteriology, Rega Institute, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

Although traditional anti-cancer therapies, such as surgical resection, radiotherapy and chemotherapy, are effective in the treatment of many patients, various alternative approaches for cancer treatment are being investigated. Gram-positive, strictly anaerobic and apathogenic bacteria such as certain *Clostridium* strains can be used as an alternative vector system for the treatment of solid tumors, which are characterised by the presence of hypoxic/necrotic areas. Following intravenous injection of *Clostridium* spores into tumor bearing organisms, these spores germinate only in the hypoxic/necrotic regions of the tumor into vegetative, metabolically active cells. Over the last few years, different apathogenic *Clostridium* strains were successfully transformed with the genes coding for the prodrug-converting enzymes cytosine deaminase and nitroreductase and the cytokine murine tumor necrosis factor alpha, resulting in local production and/or secretion of these therapeutic proteins in the tumor micro-environment.

To enlarge the number of therapeutic proteins and to increase the efficacy of this vector system, we have constructed a *C. acetobutylicum* DSM792 strain that could secrete biologically active rat interleukin-2 (rIL2). IL2 (T-cell growth factor) is a pluripotent cytokine that enhances both non-specific immune responses such as the activation of natural killer and lymphokine-activated killer cells resulting in neoplastic cell killing in a major histocompatibility complex (MHC)-unrestricted fashion, and MHC-restricted T-cell responses.

First, we amplified the rIL2 cDNA from a cDNA mixture derived from homogenised spleen cells of a WAG/Rij rat. Subsequently, the coding sequence of rIL2 was translationally fused to the endo-β-1,4-glucanase (*eglA*) signal sequence of *C. saccharobutylicum* P262 (previously named *C. acetobutylicum*) and placed under transcriptional control of the *eglA* promoter. Next, this expression cassette was cloned in the *E. coli-Clostridium* shuttle plasmid pIMP1. Finally, *C. acetobutylicum* DSM792 was transformed with the resulting plasmid pIMP1eglArIL2 by electroporation. The recombinant strain efficiently secreted up to 800 µg rIL2 per liter culture medium as measured by ELISA. A T-cell proliferation assay revealed that the produced rIL2 was biologically functional.

These results indicate that *C. acetobutylicum* DSM792 is able to secrete biologically active rIL2 in therapeutically sufficient amounts to reinforce the *Clostridium*-mediated transfer system of therapeutic proteins to solid tumors. The efficacy of this rIL2-producing *Clostridium* strain in tumor growth delay will be investigated in the near future.

Acknowledgements
S.B. is a research fellow of IWT-Flanders. The research is further supported by the E.U. (QLK3-CT2001-01737). We acknowledge financial support from Het Fonds voor Wetenschappelijk Onderzoek Vlaanderen (grant G.0375.02).

Intracellular models of infection to evaluate antibiotic activity

M. Barcia-Macay, S. Carryn, S. Lemaire, C. Seral, M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke

Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain. UCL7370 av. Mounier 73, 1200 Brussels, Belgium. vanbambeke@facm.ucl.ac.be

Introduction: Several bacteria are able to survive and multiply in eucaryotic cells causing recurrent, difficult-to-treat intracellular infections. The treatment of such infections is difficult to optimize since criteria like MIC or antibiotic cell accumulation may not apply to the intracellular milieu. We have developed a model of infected macrophages to better evaluate the activity of antibiotics against *Listeria monocytogenes* (cytosolic infection) or *Staphylococcus aureus* (phagolysosomal infection).
Methods: THP-1 human macrophages were exposed to *L. monocytogenes* (5 bact./cell) or opsonized *S. aureus* (4 bacteria/cell). Extracellular bacteria were eliminated by extensive washing (including a 1 h washing with 50 mg/L gentamicin for *S. aureus*-infected cells) and thereafter incubated over 24 h with the antibiotic under study or with gentamicin at its MIC (1 mg/L for *L.monocytogenes*. or 0.5 mg/L for *S. aureus*) to avoid extracellular contamination by bacteria escaping from the cells.
Results: the table compares the activity of selected antibiotics against the extracellular and intracellular forms of these infections, upon 24 h exposure to an extracellular concentration corresponding to the maximal concentration found in human serum, and relates activity to the cellular accumulation of these drugs.

antibiotic	Extracellular concentration (mg/L)	Cellular accumulation [a]	Activity against					
			L. monocytogenes			*S. aureus*		
				Change in log CFU over 24 h			Change in log CFU over 24 h	
			MIC	extra	intra	MIC	extra	intra
				+ 3.0	+ 3.2		+ 3.0	+ 0.9
azithromycin	0.4	38.0	0.5	+ 3.0	- 0.2	0.5	- 0.6	- 0.2
gentamicin	18	4.4	1.0	- 6.0	+ 1.6	0.5	- 6.0	- 0.7
vancomycin	50	6.3	1.0	nd	+ 0.3	1.0	- 4.6	- 1.3
ampicillin	50	1.0	0.5	- 0.6	- 1.8	0.06	- 4.9	- 1.5
moxifloxacin	4	7.6	0.5	- 4.2	- 3.5	0.06	- 4.6	- 2.5

[a] cellular to extracellular concentration ratio

L. monocytogenes grows at an almost similar rate in extracellular or intracellular milieus. In contrast, the multiplication of *S. aureus* is slower in cells than in the culture medium. Considering antibiotic activity, azithromycin, which accumulates to the highest levels, is the least active antibiotic in both models. Gentamicin and vancomycin are poorly active intracellularly, even though they are cidal extracellularly, probably because of their low accumulation. Moxifloxacin is the most active drug against extracellular and intracellular bacteria. However, it is less active intracellularly than extracellularly, despite its cellular accumulation. Quite surprisingly, ampicillin, which does not accumulate in cells, is nevertheless bactericidal intracellularly, and is even more active against intracellular than extracellular *L. monocytogenes*.
Conclusion: The data show that neither extracellular activity nor antibiotic accumulation are predictive *per se* of intracellular activity. Our models may help to understand which other factors may be critical in this context.

Dynamics and role of *Sphingomonas*/*Mycobacterium* populations during bioremdiation of weathered PAH-contaminated soils

Leen Bastiaens, Annemie Ryngaert, Nathalie Leys, Diane Van Houtven & Johan Gemoets (Flemish Institute for Technological Research-Vito, Mol, Belgium
Lode Goethals (ENVISAN, Aalst, Belgium)
Dirk Springael (Catholic University of Leuven-KUL, Leuven, Belgium)

Polycyclic Aromatic Hydrocarbons (PAHs) are major soil pollutants in many industrialized countries. During the last decennia, a diversity of PAH-degrading micro-organisms has been isolated, suggesting possibilities for bioremediation. However, biodegradation of PAHs in contaminated soils is not always successful. The low bio-availability of the PAHs is the major problem, especially in weathered soils. In these soils a tightly sorbed PAH-fraction is present which is in general hardly accessible for micro-organisms.

In order to bioremediate PAHs also in weathered soils, stimulation of bacteria which have special strategies to access sorbed organics may be a solution. *Sphingomonas* and *Mycobacterium* strains may represent such bacteria as (I) they are often isolated as PAH degraders, (II) they are ubiquitously present in PAH-contaminated soils, and (III) they display features which might promote bioavailability.

Lab- and pilot-scale experiments were set up in order (A) to study the dynamics of indigenous *Sphingomonas* and *Mycobacterium* populations during bioremediation, and (B) to evaluate their role in the biodegradation of the less bio-available PAH-fraction during treatment of an historic PAH polluted soil. The soil was treated under natural soil moisture conditions and slurry conditions. The experimental set-ups ranged from 2 g labscale test to pilot experiments in 1 ton biopiles and dry solid reactors (50 kg 70% dry matter soil). Different additives were evaluated for stimulation of the *Sphingomonas* and *Mycobacterium* population as a strategy to improve bioremediation of PAHs. The evolution of this microbial population was followed using culture–independent general and genus-specific PCR-based detection methods targeting the 16S rRNA genes of the eubacterial community, *Mycobacterium* or the *Sphingomonas* populations, respectively.

During the different bioremediation experiments that were conducted, the *Mycobacterium* population remained very stable, only minor changes were observed between the test conditions and in time. On the other hand, some additives and also time did lead to changes in the *Sphingomonas* population and the eubacterial population.

Under natural soil moisture conditions a reduction of the PAH concentration up to 70% of the initial values was obtained within 10 weeks. Addition of carbon-containing additives initially decreased the PAH-degradation rate explicable by preferential substrate use. This negative effect was not observed anymore lateron in the experiments. More sampling events are planned to evaluate the longer term effect of the additives. A reduction of the ‘residual PAH-fraction’ is targeted. Addition of nutrient had a negative affect on the degradation. Under soil slurry conditions similar trends were seen.

Prediction of CAH bioremediation potential at polluted sites using microcosms and molecular biological techniques

B. Borremans, R. Lookman, J. Gemoets, T. De Ceuster, A. Provoost, K. Hamonts, W. Dejonghe, L. Bastiaens, K. Vanbroekhoven, L. Diels

VITO, Boeretang 200, B-2400 Mol, Belgium
E-mail: brigitte.borremans@vito.be

In this work we present results of laboratory anaerobic degradation tests ('microcosms') conducted with groundwater, soil, aquifer and sediment material, sampled at 14 sites polluted with chlorinated aliphatic hydrocarbons (CAHs) and more specifically perchloroethylene (PCE), trichloroethylene (TCE), *cis*-dichloroethene (DCE), vinyl chloride (VC), 1,1,1-trichloroethane (TCA) and 1,1-dichloroethane (DCA). Different types of carbon source amendments, including lactate were screened for their potential to stimulate CAH degradation; they were all found to stimulate anaerobic biodegradation of chlorinated ethenes, although with a site-specific performance. PCE and TCE degradation occurred in almost all microcosms. However, for about 50% of the sites degradation stopped at *cis*-DCE, while the other 50% showed complete conversion to ethene (which is required for a successful bioremediation). All aquifer materials used in these microcosms were evaluated for the presence of *Dehalococcoides* sp. using PCR with specific 16S rRNA gene primers (Löffler et al., 2000 and Hendrickson et al., 2002) for this genus, of which the members are able to perform complete degradation of TCE. Furthermore, all aquifer samples were evaluated for the presence of the catabolic genes *tce*A and *pce*A encoding dehalogenases involved in dechlorination of TCE and PCE respectively by using specific primers (Regaerd et al., 2004).

For 10 out of 14 sites, we observed a good correlation between the degradation capacity in the microcosm and the PCR results obtained with the 16S rRNA gene primers. A clearly positive signal was detected only in the aquifers where a complete dechlorination was observed, whereas no signal was obtained for those aquifers which showed no or partial dechlorination in the microcosms. For three sites this correspondence was not observed: the presence of *Dehalococcoides* sp. was clearly detected though no or incomplete dechlorination occurred in these microcosms. These sites contained a mixed pollution were not only PCE/TCE but also TCA/DCA, DCM and/or BTEX was present, which might inhibit the chlorinated ethene dechlorination. For one site with only PCE/TCE contamination, we observed the presence of *Dehalococcoides* sp. but no complete degradation in the microcosm. A possible reason might be that, although *Dehalococcoides* sp. are present, certain environmental conditions (presence of high concentrations of alternative electron acceptors such as Fe(III) and sulphate) are preventing complete degradation at this specific site.

Using the specific primers for *tce*A and *pce*A positive signals were only observed for those samples which show a very fast degradation of TCE. In the other samples where complete degradation of TCE or PCE was observed, we could not detect *tce*A nor *pce*A, probably due to detection limitations or due to the presence of other catabolic genes not detected by the used PCR method.

References

Löffler et al; AEM 66:1369-1374 (2000)
Regaerd et al; J.of Microbiol. Meth. 56:107-118 (2004)
Hendrickson et al; AEM 68:485-495 (2002)

The *mntH* expression of *Salmonella enterica* strains quantified by real time RT- PCR

Botteldoorn N, Werbrouck H, Grijspeerdt K, Van Coillie E., Heyndrickx M. and Herman L.

Departement for Animal Product Quality and Transformation Technology, Brusselsesteenweg 370, 9090 Melle, Belgium. E-mail: N.Botteldoorn@clo.fgov.be

In bacteria the *mntH* gene (H^+-coupled manganese transport) was described as an orthologue of the eucaryotic *Nramp*1 (natural resistance-associated macrophage protein 1) gene. In the phagosome the bacterial and the eucaryotic NRAMP would compete for the transport of bivalent cations (Agranoff and Krishna, 1998). NRAMP1 mediates the transport of metal divalent cations. The cation influx provided Fe^{2+} and Mn^{2+} to the macrophage to supply the catalytic metal in superoxide dismutases (SOD's) peroxidases and catalases for the protection against reactive oxygen (ROS) and hydroxyl radicals generated in response to infection. At the same time, the bacteria will be depleted from the same divalent cations which are necessary for the synthesis of the similar protective enzymes in the bacterial cell and for intracellular survival. Competition between both transport systems would determine the intracellular survival of the pathogenic bacterium.
Three major transcription regulation sites are identified in the *mntH* promoter region: Fur (Fe^{2+}), MntR (Mn^{2+}) and OxyR (oxygen). The exact role of the *mntH* in the pathogenesis of *Salmonella enterica* is not clear. For *S*. Typhimurium only a minor virulence effect of a *mntH* mutant was noticed in BALB/c mice; however a strong induction of the expression after invasion of the macrophage was observed (Kehres et al. 2000). In our study, the expression of the *mntH* gene, quantified by real time RT-PCR, was compared for different *Salmonella enterica* strains.
In total 8 different serotypes were tested for the basal expression of the *mntH* gene. The majority of the tested strains were of the serotype Enteritidis (n=10) and Typhimurium (n=39). All the strains were grown in Brain Heart Infusion at 37°C to the beginning of the exponential phase ($\pm 10^7$ CFU/ml) or in the minimal medium with two concentration of iron (5µM and 1µM). The expression of the *mntH* gene was quantified before and after induction with hydrogen peroxide in the different growth conditions. After comparison of the basal expression (before induction) of the *mntH* gene relative to the control gene *16 rRNA*, no significant differences between the strains could be detected. After induction with hydrogen peroxide a 6 times higher expression of the *mntH* gene was determined and incubation in the minimal medium resulted in a 20 till 45 times higher expression.
Differences between serotypes and strains could not be made on the basis of the *mntH* expression tested on different growth conditions.

References:

Agranoff, D. D. and Krishna, S. 1998. Metal ion homeostasis and intracellular parasitism. Mol. Microbiol. 28: 403-412.
Kehres, D. G., Zaharik, M. L., Finlay, B. B. and Maguire, M. E. 2000. The NRAMP proteins of *Salmonella* Typhimurium and *Escherichia coli* are selective manganese transporters involved in the response to reactive oxygen. Mol. Microbiol. 36: 1085-1100.

Molecular diversity of freshwater cyanobacteria and detection of microcystin synthetase genes in Belgian surface waters

Boutte, C., Waleron, M., Grubisic, S., Schlösser, M., Wilmotte, A.

Center for Protein Engineering, Institute of Chemistry B6, University of Liège, 4000 Liège, Belgium

The BELSPO project B-BLOOMS (www.bblooms.ulg.ac.be) aims at characterising and determining the extent of cyanobacterial blooms, that are potentially toxic, in freshwater bodies in Belgium. For this study, two lakes (the 3 pre-dam reservoirs of the Eau d'Heure complex in Wallonia and the lake Blaarmeersen near Gent in Flanders) are studied by an intensive monitoring since April 2003. They are sampled weekly during the stratification period and bi-monthly during the other months. In addition to classical analyses (physico-chemical parameters, microscopy, pigments) made by the other partners, we study the molecular diversity of the cyanobacteria by construction of clone libraries based on 16S rRNA gene sequences. Moreover, the presence and expression of genes encoding microcystin synthetases, *mcy*E (Rantala et al., 2004) and *mcy*B (Nonneman & Zimba, 2002) are detected by PCR with specific primers. Additional samples are taken in other ponds and lakes when a bloom is observed, in order to document the cyanobacterial blooms in Belgium.

Results from three clone libraries support the dominance of potentially toxic cyanobacteria in the Eau d'Heure complex (*Anabaena/Aphanizomenon*), but not in lake Blaarmeersen where cyanobacteria are generally not abundant. Furthermore, molecular detection of the *mcy*E gene shows a positive signal for a majority of the samples from the Eau d'Heure complex, whereas this is less frequently observed in samples from lake Blaarmeersen. The presence of the *mcy* operon in the genome is not sufficient to know if the cyanotoxins are produced, and therefore, we have tested different protocols to study the expression of the *mcyE* and *mcyB* genes by RT-PCR.

Strain differentiation in a multimember linuron degrading bacterial consortium using FISH and reporter genes

Philip Breugelmans[1], Larissa Hendrickx[2], Stephan Bathe[3], Boris Joffe[3], Martina Hausner[3], Winnie Dejonghe[4], Dirk Springael[1]

[1] Laboratory of Soil and Water Management, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium
[2] Laboratory for Microbiology, SCK-CEN, Boeretang 200, B-2400 Mol, Belgium
[3] Lehrstuhl für Wassergute- und Abfallwirtschaft, Technische Universität München, Am Coulombwall, D-85748 Garching, Germany
[4] Environmental Technology, Vlaams Instituut voor Technologisch Onderzoek, Retieseweg, B-2440 Geel, Belgium

Phenyl urea herbicides are among the most widely used herbicides in agriculture, resulting in important contamination of both surface water and groundwater. Recently, several multimember microbial communities mineralizing phenyl urea herbicides such as linuron have been isolated from long-term treated agricultural soils indicating that bacteria adapted to the contamination.
The topsoil is considered as an important biological filter which determines the extent of pesticide degradation and hence, the extent of groundwater contamination. So far, little attention has been paid to the role of the solid surface on the structure, spatial organization, stability and activity of commensal pollutant degrading communities of natural origin. In order to study these parameters, we developed a method to differentiate between the four members of a linuron mineralizing bacterial consortium using FISH and reporter genes. *Variovorax* sp. WDL1 is the key member of the consortium as it degrades linuron to 3,4-dichloroaniline (3,4-DCA) and N,O-dimethylhydroxylamine. WDL1 is able to mineralize 3,4-DCA but at a low rate leading to leakage of 3,4-DCA. Residual 3,4-DCA is mineralized by *Comamonas testosteroni* WDL7. *Hyphomicrobium sulfonivorans* WDL6 degrades the intermediate N,O-dimethylhydroxylamine. The fourth member, i.e. *Pseudomonas* sp. WDL5, has no clear active role in linuron degradation but seems to stimulate mineralization.

A genus-specific 16S rRNA-targeting oligonucleotide probe was designed to identify *Variovorax* spp. *Comamonas testosteroni* WDL7 was labeled with a reporter gene encoding for the fluorescent protein Gfp, and a fluorescent nucleic acid stain (DAPI) was used to distinguish *Hyphomicrobium sulfonivorans* WDL6 from the specifically labeled strains.
The method showed to be effective in differentiating between *Variovorax* sp. WDL1, *Comamonas testosteroni* WDL7 and *Hyphomicrobium sulfonivorans* WDL6. Selection of appropriate fluorochromes will allow for the additional differentiation of *Pseudomonas* sp. WDL5 using probe GAM42a. The method will be applied in the study of the interactions between community members grown as mixed biofilms in flow chambers by means of CLSM.

Spa40 interacts with Spa32 and is required for the secretion of *Shigella* virulence factors via the type III secretion pathway.

Mustapha Chamekh[1], Anne Botteaux[1], Claude Parsot [2] and Abdelmounaaïm Allaoui[1]

[1] Laboratoire de Bactériologie Moléculaire, ULB, Faculté de Médecine
[2] Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur de Paris

Shigella, a Gram negative bacterium, is the cause of the bacillary dysentery, an invasive disease of the human colonic epithelium. Genes required for bacterial invasion are carried out by a 214-kb plasmid. A 31-kb of this plasmid is necessary for the entry of *Shigella* into epithelial cells and contains two sets of genes. The first one corresponds to the 21 *mxi-s*pa genes that encode mostly components of the Mxi-Spa type III secretion apparatus (TTSA). The second one corresponds to the 13 *ipa* and *ipgD* genes that encodes the IpaA-D, IpgB and IpgD secreted proteins, which are the major determinants of entry into cells. The TTSA is used by numerous Gram-negative bacteria that are pathogenic for humans, animals, or plants to inject virulence factors into eukaryotic cells. In *Shigella* this system is composed of a cytoplasmic bulb, a transmembrane domain and an external needle whose length is controlled by the Spa32 protein. In this study, we present a functional analysis of *spa40*, the last gene of the *spa* operon. Inactivation of the *spa40* gene resulted in the loss of a functional TTSA. Indeed, the null *spa40* mutant has lost its ability to secrete the Ipas invasine and consequently could not invade HeLa cells *in vitro*. The wild-type phenotype was restored by expressing, in the mutated strain, either Spa40 or a histag Spa40 version. Spa40 is predicted to be an integral membrane protein, which consists of four transmembranes domains and a large cytoplasmic one. Using the two hybrid system and co-immunoprecipitation assays, we identified interaction between the cytoplamic domains of Spa40 (ccSpa40) and Spa32. To define more precisely the amino-acid residus of Spa40 involved in such interaction, we performed directed mutagenesis on ccSpa40 and studied both secretion phenotype and interaction with Spa32. Our data suggest that the null *spa40* mutant either alone or complemented with some modified Spa40 are no longer able to assemble a correct TTSA. To test this hypothesis, we monitored the production/secretion of MxiH, the major component of the needle, in several *Shigella* strains. We found that all mutations that abolished the TTSA function are defective in MxiH secretion, indicating that at least their needles are not normally assembled. Finally, based on Spa32 homologous in other species, we present a model in which Spa40 could be the anchor point of Spa32 at the inner membrane and so, these two proteins could act in synergy for substrate specificity switching from the secretion of the needle component MxiH to the effector Ipa/IpgD molecules.

Immunomodulation of the inflammation in shigellosis using recombinant IL-10 delivered by the *Shigella* type III secretion system

Mustapha Chamekh[1], Armelle Phalipon[3], Isabelle Salmon[2], Philippe Sansonetti[3], and Abdelmounaaïm Allaoui[1]

Laboratoire de Bactériologie moléculaire[1], Laboratoire d'Histopathologie[2], Faculté de Médecine, Université Libre de Bruxelles, Route de Lennik, 808, 1070, Bruxelles, Belgium and Unité de Pathogénie Microbienne Moléculaire[3], Institut Pasteur, 75724 Paris Cedex 15, France

Shigellosis, caused by the invasion of intestinal epithelium by the pathogenic Gram-negative bacteria, *Shigella flexneri* is characterized by an acute inflammatory response that is responsible for the destruction of the colonic and rectal mucosa, leading to bloody diarrhea and dysentery. The virulence of these bacteria relies on different effectors secreted by a specialised molecular machinery called type III system that delivers a set of bacterial proteins to the cytoplasmic membrane or into the cytoplasm of the host cell, leading to a variety of signal transduction pathways. With the aim to monitor the inflammation process triggered during Shigellosis, we investigated the potential use of the type III secretion sytsem of *Shigella* to deliver a biologically active anti-inflammatory cytokine, IL-10. Different constructions based on the fusion of the IL-10 gene either with potential signal peptide contained within amino terminal of the *Shigella flexneri* Invasion Plasmid Antigen H (IpaH) or with the defined secretion domain of the *Yersinia* Outer protein E (YopE) were investigated. *In vitro* experiments showed that *Shigella flexneri* M90T allows a type III dependant secretion of recombinant IL-10 fused to 50 amino terminal of YopE or to 60 amino terminal of IpaH. Moreover, recombinant *Shigella* producing IL-10 hybrids fail to induce TNFα production by macrophages. In a murine pulmonary model, mice inoculated with IL-10 recombinant strains showed significant reduction in the mortality and displayed a drastic decrease of the inflammatory symptoms characterized by less tissue damage and reduced number of infiltrative polymorphonuclear and mononuclear cells within bronchi and alveoli. Moreover, we showed by real time RT-PCR experiments that the production of the major pro-inflammatory cytokines, TNFα, IL-1β and IL-6 was significantly decreased within infected lungs. These findings point out the efficiency of the type III secretion system induced upon contact with host cells for the delivery of biologically active cytokine and underline the anti-inflammatory potential of the IL-10 produced at the local site of infection in Shigellosis. This study opens new perspectives for the potential use of such system with highly attenuated bacteria for immunomodulation or gene therapy purposes.

A comparative genomics approach to the identification of non-coding RNAs in the *Burkholderia cenocepacia* J2315 genome

T. Coenye[1], D.W. Ussery[2] & P. Vandamme[1]

[1]Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium, and
[2]Center for Biological Sequence Analysis, Danish Technical University, Lyngby, Denmark
Corrresponding autho : Dr. Tom Coenye (Tom.Coenye@UGent.be)

Non-coding RNA (ncRNA) genes produce transcripts that function directly as structural or regulatory RNAs rather than expressing mRNAs that encode proteins. These untranslated RNA molecules are present in many different organisms, both pro- and eukaryotes. Although the exact function of many ncRNAs remains unknown, it is anticipated that they affect a large variety of cellular processes and play an important role in the regulation of gene expression. The goal of this study was to identify ncRNA genes in the genome of *Burkholderia cenocepacia* J2315, which consist of 3 chromosomes and has a total genome size of 7.9 Mbp.

We used the QRNA software package to identify putative ncRNA genes in the *B. cenocepacia* J2315 genome sequence. From the initial list generated using QRNA, we selected the ncRNA genes that were located in regions with marked secondary structure by comparing them to a Genome Atlas constructed for the *B. cenocepacia* J2315 genome. The ncRNA genes that were identified this way were further characterised using Kodon 2.0 and *mfold*.

Using the comparative genomics approach described above, we identified 210 ncRNA genes in the *B. cenocepacia* J2315 genome (76 on chromosome 1, 115 on chromosome 2 and 19 on chromosome 3), varying in length between 53 and 1243 nucleotides (nt) (average±standard deviation : 235±179 nt). The ncRNA genes make up 0.62% of the complete genome (49543 nt) and are distributed randomly on the 3 chromosomes, with an average density of 26.4 ncRNA genes per million bp. The majority of the ncRNAs identified showed significant secondary structure, with free energies ranging from –27.9 to –665.4 kcal/mole (average±standard deviation : -122.4±94.4 kcal/mole). The presence of a large number of ncRNA genes in this organism may help to explain its complexity, phenotypic variability and ability to survive in a remarkably wide range of environments.

Identification of two genes responsible for the production of signal molecules in *Pseudomonas aeruginosa*

Pierre Cornelis[1], Séverine Aendekerk[1], Miguel Cámara[2], Steve Diggle[2], and Paul Williams[2]

1. *Laboratory of Microbial Interactions, Department of Molecular and Cellular Interactions, Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium*
2. *Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, U.K.*

We have shown previously that the PA4205-PA4208 genes form an operon coding for the components of an efflux pump (MexGHI-OpmD) contributing to the resistance of *P. aeruginosa* to vanadium (Aendekerk *et al*., 2002). Mutants in the *mexI* or *opmD* genes have a decreased fitness, and fail to produce the quorum sensing signal molecules 3-oxo-C12-homoserine lactone and C4-homoserine lactone, as well as the 2-heptyl-3-hydroxy-4(1*H*)-quinolone (PQS). Here, we show that the two genes, upstream of the operon, PA4203 and PA4204, are needed for the expression of the *mexGHI-opmD* genes as well as for the genes for the biosynthesis of PQS (as shown for *pqsA* and *phnA*). Conversely, mutants in *mexI* and *opmD* still express the PA4203 and PA4204 genes, indicating that these two genes are regulating the pump operon. PA4203 encodes a LysR regulator and PA4204 a putative periplasmic protein. Inactivation of PA4203 and PA4204 also results in the inability to produce the quorum sensing *N*-acyl homoserine lactones as well as PQS. Similarly to *mexI* and *opmD* mutants, both mutants in PA4203 and PA4204 show an important growth delay. Interestingly, inactivation of PA4204 also results in the loss of production of the siderophore pyoverdine. The same mutant was however still in state to utilize externally provided pyoverdine for its growth in the presence of the iron (III) chelator EDDHA. These two genes were therefore designated as *pspR* (PA4203) and *pspA* (PA4204), which stands for **P**seudomonas **s**ignal **p**roduction. These two genes were also inactivated in the PA14 strain, which is virulent in plants, and the resulting mutants were found to have lost their virulence. We conclude that PspR is a regulator for the *mexGHI-opmD* pump genes and that PspR, and especially PspA, are needed for the fitness of *P. aeruginosa*, including its capacity to sacvenge iron. Future research will focus on the function of these two genes by combining transcriptome and proteome analysis.

Reference:

Aendekerk, S., Ghysels, B., Cornelis, P., & Baysse, C. (2002). Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. *Microbiology* **14,** 2371-2381.

Fishing genes in lactic acid bacteria to become resistant to vancomycin

*Marie Deghorain**[1], *Laetitia Fontaine*[1], *Jean-Luc Mainardi*[2], *Bernard Hallet*[1], *Jean Delcour*[1] *and Pascal Hols*[1]

[1]Unité de Génétique, I.S.V., Université Catholique de Louvain, Belgique, [2]Laboratoire de Recherche Moléculaire sur les Antibiotiques, Université Paris VI, France

[1] 5/bte6 Place Croix du Sud, B-1348 Louvain-La-Neuve, Belgique. *Deghorain@gene.ucl.ca.be

Bacterial peptidoglycan is composed of glycan strands crosslinked by interpeptide bridges. *Lactobacillus plantarum* and *Lactococcus lactis* are two species of lactic acid bacteria wich are distinguished by their peptidoglycan composition. In *Lactococcus lactis*, peptidoglycan pentapeptides involved in crosslinks are ended by the usual D-alanyl-D-alanine dipeptide. The peptidoglycan of *L. plantarum* is characterised by the presence of D-lactate in the carboxy-terminal of its pentapeptide precursors. It was recently demonstrated in our laborary that D-lactate is essential for peptidoglycan synthesis in this species. Dramatical growth defects were observed in a mutant impaired in D-lactate production (P. Goffin *et al*., *in preparation*). D-lactate ended peptidoglycan confers resistance to vancomycin, which inhibits cell wall synthesis by binding the usually encountered terminus D-alanyl-D-alanine. Before their incorporation in peptidoglycan precursors, the two last residues are linked together by specific Ddl ligases wich are responsible for the formation of the dipeptide D-alanyl-D-alanine or the depsipetide D-alanyl-D-lactate. In vancomycin resistant enterococci, D-lactate ended precursors synthesis requires acquisition of the van-operon including two additional genes coding for a D-lactate dehydrogenase (VanH) and a D-alanyl-D-alanine dipeptidase (VanX), that is necessary to eliminate the pool of endogenous D-alanyl-D-alanine dipeptides.

In order to investigate the genetic determinants of the specificity of D-lactate incorporation in *L. plantarum* peptidoglycan, genes of *L. plantarum* were transferred and expressed in the vancomycin sensitive strain *Lactococcus lactis*. The phenotype of vancomycin resistance was examined and the composition of the peptidoglycan precursors was analysed.

The heterologous expression of the D-alanyl-D-alanine Ddl ligase from *L. lactis* in *L. plantarum* resulted in D-alanine ended precursors leading to vancomycin sensitivity. The reciprocal expression of the *L. plantarum* ligase associated with a D-lactate dehydrogenase D-Ldh in *L. lactis* was insufficient to confer vancomycin resistance. A *L. lactis ddl* mutant strain expressing solely the *L. plantarum* Ddl ligase was still sensitive. 40% of the precursors were ended by D-alanyl-D-alanine, demonstrating that the Ddl enzyme of *L. plantarum* is bispecific "*in vivo*". Interestingly, a *vanX*-like (*ddpXP*) gene with D-ala-D-ala dipeptidase activity was found in the genome of *L. plantarum*. Coexpression of this dipeptidase with the *L. plantarum* Ddl ligase in a *L. lactis ddl* mutant strain resulted in production of 100% of D-lactate-ended precursors, correlated with an increase of vancomycin resistance as observed with the enterococcal *vanX* gene. This result suggests involvement of the DdpXP dipeptidase in the selectivity of D-lactate incorporation in *L. plantarum*. Surprisingly, *ddpXP* inactivation did not modify the level of vancomycin resistance of *L. plantarum* and no D-alanyl-D-alanine-ending precursors were detected. However, the inactivation of DdpX increased the ratio of D-alanyl-D-alanine-ending precursors in a *L. plantarum* expressing the *L. lactis* D-alanyl-D-alanine ligase, indicating some level of activity *in vivo*. This indicates that the involvement of DdpXP in the control of the cell wall biosynthesis is more than likely. Our study suggests a possible evolutionary origin for the *van* operon from the recruitment of separate functions not primarily involved in vancomycin resistance.

In situ mesocosmos socks and molecular ecology techniques for monitoring on site colonization of carrier materials

W. Dejonghe, B. Hendrickx, W. Boënne, L. Houtmeyers, J. Geets, L. Bastiaens (Flemish Institute for Technological Research (Vito), Boeretang 200, 2400 Mol, Belgium), and D. Springael (Catholic University Leuven, Laboratory of Soil and Water Management, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium)

The colonization of different carrier materials (endemic aquifer material, filter sand and Bio-Sep® polymer beads) was monitored in a TEX contaminated groundwater plume under *in situ* conditions. This was accomplished by inserting *in situ* mesocosmos socks filled with these materials, into a monitoring well under the groundwater table and the application of PCR based molecular ecology techniques on DNA extracted from the incubated materials. Mesocosmos containing the materials were harvested from the well after 64 days and 203 days. Based on the 16S rRNA DGGE profiles, all carrier materials were colonized by different communities. There was only minor similarity between the microbial community present on the carriers and the community of the aquifer itself. On the other hand, the community structure was more similar to that of the groundwater. Although anaerobic conditions prevailed in the aquifer, genes involved in aerobic BTEX degradation were detected. *tmoA*-like BTEX mono-oxygenase genes were recovered from the aquifer and from all carrier materials. After 203 days, *todC1*- and *todE*-like genes could also be detected. The microbial communities on the carrier materials degraded TEX under aerobic and sulfate reducing conditions. The colonization of the materials by sulfate reducing bacteria (SRB) was confirmed by the detection of the β-subunit of the dissimilatory sulfite reductase (*dsrB*) gene of SRB. DGGE analysis of the *dsrB* amplicons showed that the inserted aquifer material and filter sand were colonized by similar SRB populations, while the Bio-Sep® polymer beads were colonized by a more diverse SRB population.

Comparison of porcine, human and murine sialoadhesins: conservation of the extracellular domains and divergence of the cytoplasmic tails

P.L. Delputte, N. Vanderheijden and H.J. Nauwynck

Laboratory of Virology, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke

peter.delputte@UGent.be

Mouse and human sialoadhesin (Sn) were previously characterized as sialic acid binding immunoglobulin-like lectins (Siglec), expressed only on specific subsets of tissue macrophages that are found mostly in spleen, lymph nodes, bone marrow, liver, colon and lungs. High Sn expression has also been detected on inflammatory macrophages in tissues from patients with reumatoid arthritis, and on infiltrating macrophages that make close contact with breast carcinoma cells. Sn is further characterized as a macrophage restricted lymphocyte adhesion molecule which selectively recognizes Neu5Acα2-3Galß1-3GalNAc and to a lesser extent Neu5Acα2-6Galß1-3GalNAc and Neu5Acα2-8Galß1-3GalNAc. Sn binds to neutrophils, bone marrow cells, blood leukocytes, lymphocytes and thymocytes, and all of these interactions are mediated via sialic acids present on the interacting cells.

Recently, we identified porcine Sn as a receptor for the porcine arterivirus. Upon cloning and sequencing of the porcine Sn cDNA, we performed amino acid sequence comparison with the human and mouse Sn. Porcine Sn showed an overal amino acid identity of 69% with mouse Sn and 78% with human Sn. A detailed analysis showed that the extracellular domain and the transmembrane domain are fairly well conserved in between species, and that important structural features, such as the N-glycosylation sites and the amino acids involved in the binding of sialic acids, are very well conserved. In contrast, major differences were found in the cytoplasmic tails of Sn, both in length and the amino acid sequences.

The porcine Sn was functionally characterized for its ability to bind sialic acids, since the amino acids that were shown to be crucial for mouse Sn to bind sialic acid are conserved. Porcine macrophages expressing Sn were incubated with sialic acid containing erythrocytes, and clear rosetting of the erythrocytes was observed on the macrophages. Incubating the macrophages with a porcine Sn specific monoclonal antibody, or removal of sialic acids from the erythrocytes with neuraminidase completely abolished erythrocyte rosetting on macrophages. From these results, it was concluded that porcine Sn, similar to mouse and human Sn, has the capacity to bind sialic acid.

Since major differences were found in between the cytoplasmic tails of porcine, mouse and human Sn, it was investigated if different amino acid motifs are present in the porcine Sn cytoplasmic tail, compared to the other Sn. The porcine Sn tail contained a putative internalization signal which is not present in mouse and human Sn. Therefore, Sn internalization was studied in porcine macrophages. Upon addition of a Sn specific monoclonal antibody to primary macrophages, Sn was internalized together with the monoclonal antibody. Using inhibitors of different internalization pathways and confocal analysis of double immunofluorescent stainings for Sn together with components of internalization pathways, internalization was shown to be clathri-dependent. In contrast to what was known for mouse and human Sn, we observed that porcine Sn mediates clathrin-dependent internalization. As a next step, the putative endocytosis motif in the cytoplasmic tail of porcine Sn will be modified by site-directed mutagenesis, and the effect on internalization will be analyzed.

Promoter engineering: a useful tool for fine tuning gene expression in *Escherichia coli*

Marjan De Mey[a, *], *Katja Van Nieuland*[a], *Jo Maertens*[b], *Wim Soetaert*[a] *and Erick J. Vandamme*[a]

[a] *Departement of Biochemical and Microbial Technology, Ghent University, Coupure links 653, 9000 Ghent, Belgium*
[b] *Departement of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure links 653, 9000 Ghent, Belgium*

The importance of fine tuning gene expression to perform metabolic optimization rather than massive overexpression or gene inactivation is growing. In this contibution, the construction of a library of synthetic promoters of *Escherichia coli* as a useful tool for fine tuning gene expression is discussed. A degenerated oligonucleotide sequence that encodes consensus sequences for *E. coli* promoters separated by spacers of random sequences has been designed and synthesized. This 57bp long sequence contains 24 conserved, 13 semi-conserved (W, R and D) and 20 random nucleotides. This mixture of DNA fragments was cloned into a promoter probing vector (pLT10LacZT3). The ligation mixtures were transformed into *E. coli* MC1061 □lac and the resulting clones were screened for β-galactosidase activity, via producing blue colonies on agarplates containing X-gal (5-bromo-4chloro-3-indolyl-β-D-galactoside); some clones gave dark blue colonies after 24 hours of incubation, others turned only blue after more than 1 week of incubation. The β-galactosidase activity of liquid cultures of these clones was compared; the clones cover a range of promoter activities from 0.00196 units/OD_{600} ml to 33,81767 units/OD_{600} ml.

[*] Corresponding author. Tel.: +32-9-2646028; Fax.: +32-9-2646231

E-mail address: Marjan.DeMey@UGent.be

Pseudorabies virus gD contains an endocoytosis motif that drives uptake of antibody-antigen complexes from the surface of infected cells

J. Ficinska[1,3], H.J. Nauwynck[1], G. Van Minnebruggen[1], K. Bienkowska-Szewczyk[3], and H.W. Favoreel[1,2]

[1]Laboratory of Virology and [2]Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, and [3]Laboratory of Molecular Virology, Faculty of Biotechnology, Gdansk University, Gdansk, Poland

Pseudorabies virus (PRV) is an alphaherpesvirus of swine, closely related to human herpes simplex and varicella-zoster viruses. Alphaherpesviruses have developed numerous strategies to avoid or delay elimination by the immune system. PRV especially excels at circumventing antibody-mediated elimination. This allows the virus to replicate and sometimes spread, via infected blood monocytes, in pigs that have been vaccinated with an inactivated vaccine. PRV-infected monocytes express viral antigens on their surface that can be bound by virus-specific antibodies. These antibody-antigen complexes should trigger antibody-mediated cell lysis. However, we found that PRV is able to induce rapid clathrin-mediated internalization of antibody-antigen complexes from the surface of the infected monocytes, which interferes with efficient antibody-mediated elimination of the monocyte. This internalization process depends on two viral proteins that are present on the cell surface: gB and gD.

It is known that clathrin-dependent internalization of membrane-integrated proteins is mediated by specific amino acid sequences in their cytoplasmic domain. The most common motifs are tyrosine-based (YXXΦ) and dileucine motifs (LL), which interact with the clathrin-associated AP-2 complex as a first step in the formation of clathrin-coated endocytosis vesicles.We found earlier that the viral gB protein contains a YQRL endocytosis motif that interacts with AP-2 and that is of crucial importance in driving efficient internalization of antibody-antigen complexes in infected monocytes. However, no endocytosis motif has, to date, been reported in the gD orthologue of any of the alphaherpesviruses.

In the current report, we found that gD also contains a functional endocytosis motif, YRLL. We introduced point mutations in this motif (Y384A; L386A; L387A; L386A/L387A; Y384A/L386A/L387A; K382stop), and cloned the mutated gD ORFs in a mammalian expression vector. PK15 cells were transfected with these constructs and assessed for endocytosis of gD. We found that wild type gD undergoes internalization from the plasma membrane in 51% of transfected cells. The Y384A mutation significantly reduced, but did not completely inhibit gD endocytosis (11%). In contrast, the L387A mutation almost completely abolished internalization (2%), which was similar as observed for the double and triple mutant, and the mutant with a truncation of almost the entire cytosolic domain (3%, 1%, and 1%, respectively). The single L386A mutation had no obvious effect on endocytosis efficiency (50%). We introduced a subset of these gD mutations in the PRV genome and assayed the resulting viruses for their capacity to induce internalization of antibody-antigen complexes from the surface of infected monocytes. We found that mutations that destroyed the endocytosis motif in gD (L387A and K382stop but not L386A) resulted in a reduction in internalization of antibody-antigen complexes that was comparable to the reduction observed when removing the entire gD ORF. In addition, we found that introducing the L387A gD mutation in a PRV strain that already harboured a mutation in the endocytosis motif in viral protein gB resulted in a reduction in antibody-antigen complex internalization that was more pronounced than observed when removing either the gB or gD ORF alone.

In conclusion, the YRLL motif in the PRV gD cytoplasmic domain is a functional endocytosis motif, and this motif acts in concert with an endocytosis motif in gB to drive internalization of antibody-antigen complexes from the surface of PRV-infected monocytes, a process that protects these cells from efficient antibody-mediated lysis.

A toxin-antitoxin Phd-Doc-like system in plasmid pGI1 from *Bacillus thuringiensis* H1.1

S. Fico and J. Mahillon

Laboratory of Food & Environmental Microbiology, Université catholique de Louvain, Croix du Sud 2/12, B-1348 Louvain-la-Neuve, Belgium. Email: fico@mbla.ucl.ac.be.

Plasmids are extrachromosomal elements carrying non-essential genes but conferring advantages (antibiotic resistance, virulence or metabolic pathways) to their hosts. During cell division, it is particularly important for low-copy plasmids to possess an active partitioning system to conserve these advantages trough the generations.

One of these systems of active partitioning is called “post-segregationnal killing system” [1]. Such systems are found in a large variety of prokaryotes. The plasmid to be maintained through the generations harbours a group of two genes, one coding for a stable toxin and the other coding for an unstable antitoxin. When the plasmid is present in the cell, both toxin and antitoxin are expressed. Since the antitoxin inhibits the toxic effect of the toxin, the host cell is not affected. However, if the plasmid is lost during cell division, the toxin and antitoxin proteins are present in the cytosol but cease to be expressed *de novo*. While the unstable antitoxin protein is rapidly degraded by proteases, the toxin acts on the cellular target, leading to cell death. Consequently, only cells harbouring the plasmid can survive.

Bacillus thuringiensis H1.1 is a Gram-positive bacterium belonging to the *Bacillus cereus* group. This bacterium occurs naturally in soil and on plants and is considered to be safe for human. It produces, during sporulation, delta-endotoxins active on insect larvae. It contains at least four large plasmids (> 30 kb) and three small plasmids: pGI1, pGI2, pGI3 [2]. The complete sequences of pGI2, pGI3 and recently pGI1 plasmids have been determined [2-4]. pGI1 is an 8.254 bp plasmid containing five ORFs with size greater than 100 residues. This includes a replication *rep*-gene, a mobilisation *mob*-gene and an ORF5 whose function remains cryptic [2]. Another ORF, downstream the *mob*-gene, is coding for a 133-aa protein and showed a high degree of similarity with the Doc toxin of bacteriophage P1. The last ORF, located downstream the putative toxin gene, could potentially code for an antitoxin counterpart.

Here, we describe the cloning and expression of the putative toxin-antitoxin system of pGI1 from *B. thuringiensis* strain H1.1 in the Gram-negative host *E. coli*. After cloning in the *E. coli* positive selection vector pCR4-TOPO, only clones possessing a mutation in the toxin gene survived. These results demonstrate that this group of two genes corresponds to an active toxin-antitoxin system. Further bioinformatic data also show the ubiquity of this system through the bacterial world.

References

1. **Jensen, R.B. and K. Gerdes** (1995) Programmed cell death in bacteria: proteic plasmid stabilization systems. *Molecular Microbiology*, **17** (2): 205-210.
2. **Andrup, L., et al.** (2003) The patchwork nature of rolling-circle plasmids: comparison of six plasmids from two distinct *Bacillus thuringiensis* serotypes. *Plasmid*, **49** (3): 205-232.
3. **Mahillon, J. and J. Seurinck** (1988) Complete nucleotide sequence of pGI2, a *Bacillus thuringiensis* plasmid containing Tn*4430*. *Nucleic Acid Res.*, **16** (24): 11827-11829.
4. **Hoflack, L., J. Seurinck, and J. Mahillon** (1997) Nucleotide sequence and characterization of the cryptic *Bacillus thuringiensis* plasmid pGI3 reveal a new family of rolling circle replicons. *J. Bacteriol.*, **179** (16): 5000-5008.

Occurrence of bacterial chemotaxis towards Phenathrene in two polyclinic aromatic (PAH) contaminated soils

Line Fredslund[1, 2], Kirsten Bijdekerke[1], Carsten Suhr Jacobsen[2], Rene De Mot[3] and Dirk Springael[1]

[1] Laboratory for Soil and Water Management, Catholic University of Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium.
[2] Geological Survey of Denmark and Greenland, Øster Voldgade 10, DK-1370 Copenhagen, Denmark.
[3]. Centre for Microbial and Plant Genetics, Catholic University of Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium.

Biological removal of xenobiotics in soils is dependent on the presence of pollutant degrading bacteria. Recent studies indicate that direct contact between a degrading bacterium and the contaminant source, could be of major importance for utilization of the contaminants. This means that the bacteria must migrate to and seek contact with the substrate source as a first step in colonisation of that micro-environment. As such, migration or translocation of bacteria on a micro- and macro-scale towards the pollutant source could be an important feature for biodegradation of organic pollutants in soil, especially of hydrophobic low bioavailable pollutants such as polycyclic aromatics (PAHs). Recent years, chemotactic responses based on the detection of and active migration along a gradient towards pollutants like naphthalene, toluene, phenanthrene and different pesticides has been described for different bacterial isolates able to degrade these compounds. However, these data have been obtained with pure cultures. Not much is known about the actual occurrence of this feature in the microbial community of a polluted soil. Therefore, for two different PAH contaminated soils with different texture, the number of phenanthrene degrading bacteria were counted by means of MPN culturing in phenanthrene containing minimal medium. From relevant MPN cultures, phenanthrene degraders were isolated and their actual occurrence in the MPN cultures proven by DGGE analysis. The phenanthrene degraders were characterized by means of BOX-PCR, 16S rRNA gene sequencing and are currently being analyzed for their chemotactic response towards phenanthrene.

Pseudorabies virus US3 protein kinase blocks apoptosis induced by the virus, sorbitol, and staurosporine

K. Geenen[1], H.W. Favoreel[1, 2], G. Van Minnenbruggen[1] & H.J. Nauwynck[1]

[1]Laboratory of Virology and [2]Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

Most large DNA viruses, like herpesviruses, encode multiple anti-apoptotic genes. The US3 protein kinase (PK) of herpes simplex virus (HSV) participates in the inhibition of apoptosis induced by virus infection or other stress agents in a cell type-specific manner. In cells infected with the swine alphaherpesvirus pseudorabies virus (PRV), the US3 PK orthologue is expressed as a long (<5% of US3 protein) and short isoform. The long isoform differs from the short isoform in that it contains an N-terminally located functional mitochondrial localization signal. Whether the PRV US3 PK displays anti-apoptotic activity had not been carefully examined thus far.

Using swine testicle (ST) cells, wild type PRV, US3null PRV, and a US3 rescuant PRV strain, we found that PRV US3 has a strong capacity to protect cells from apoptosis induced by PRV, sorbitol or staurosporine (detected by active caspase-3 or TUNEL reactivity). To investigate whether US3 can restore the anti-apoptotic activity in *trans* in PRV US3null-infected cells, ST cells were transfected with plasmids encoding the short or long PRV US3 isoform and, 24h later, inoculated with US3null PRV. The US3 long isoform, which targets to mitochondria, blocked a significant part of apoptosis induced by US3null PRV, whereas no significant inhibition could be observed after transfection with the US3 short isoform.

Additionally, we investigated whether the anti-apoptotic activity of PRV US3 PK described here, accounts for the unusual high resistance of primary porcine trigeminal ganglion (TG) neurons to PRV-induced cell death that we observed before. These trigeminal ganglion neurons are the major site of establishment of latency (a quiescent form of infection, from which the virus can reactivate from time to time), not only for PRV, but also of other alphaherpesviruses like HSV-1 and bovine herpesvirus 1. Primary porcine TG cultures (consisting of neuronal and nonneuronal cells) were inoculated with US3null or wild type PRV and at 24 or 48hpi assessed for active caspase-3 or TUNEL reactivity. We found that, in nonneuronal TG cells, deletion of US3 resulted in a strong increase in apoptosis at 24 and 48hpi (13 to 43% and 37 % to 73%), whereas no such increase was observed in TG neurons at 24 and 48hpi (13 to 13% and 14 to 18%).

These results indicate that PRV US3 PK inhibits apoptosis induced by PRV infection or chemical reagents and suggest a role for the mitochondrial localization of US3 in this inhibition. Moreover, we found that porcine TG neurons, in contrast to other cells, do not critically depend on US3 to be protected against PRV-induced apoptosis.

Impact of long-term contamination with 2,4,6-trinitrotoluene (TNT) on soil microbial communities

George I., Eyers L., Stenuit B., Agathos S., El Fantroussi S.*

Bioengineering Unit (GEBI), Catholic University of Louvain, Place Croix du Sud 2/19, 1348 Louvain-la-neuve, Belgium (george@gebi.ucl.ac.be)*

The microbial diversity of soils contaminated with 2,4,6-trinitrotoluene (TNT) over two decades was characterized by 16S rRNA PCR-DGGE using bacterial universal primers. DGGE patterns were compared to those of reference non-polluted soils from the same TNT-destruction site. They showed a clear shift, from a complex fingerprint in the non-contaminated soils to a simpler pattern with fewer but more dominant bands in the polluted soils, indicating that a strong selection had occurred. Sequencing of the dominant bands in the polluted soils revealed a predominance of *Pseudomonas* sp. In parallel, soil samples were plated on poor solid media, eventually supplemented with (polluted or non-polluted) soil extract, and DGGE patterns of the culturable microflora were compared with those of the total microflora from the same soils. Finally, soils were screened in search of *Acidobacteria*, a very diverse and mostly uncultured new bacterial division usually recovered in large amounts from soils, but whose fate in connection with xenobiotic pollution hasn't been systematically explored so far. *Acidobacteria* were easily detected by PCR with specific primers in all non-contaminated soils. The low PCR signal recovered from TNT-contaminated soils indicates that TNT is probably toxic to most *Acidobacteria*, but that some members of this group can however survive in these heavily polluted environments (containing up to 26 g TNT/kg soil). We are presently in the process of characterizing these *Acidobacteria* populations.

Evolutionary and comparative genomics: gene and segmental duplication in bacterial genomes

Dirk Gevers[1,2], Olena Vinnyk[1], Klaas Vandepoele[1], Cedric Simillion[1], Siv Andersson[3] and Yves Van de Peer[1]

[1]Bioinformatics & Evolutionary Genomics, UGent/VIB, Belgium
[2]Laboratory of Microbiology, UGent, Belgium
[3]Dep. of Evolution, Genomics and Systematics, Uppsala University, Sweden

Completely sequenced genomes provide us with an opportunity to study the evolution of the genome at a comprehensive level. During the last decade, the increasing number of sequenced genomes has made it clear that bacterial genomes should not be regarded as static structures but rather as relatively variable and flexible structures. Evolutionary processes, including gene duplication, horizontal gene transfer, gene loss and chromosomal rearrangements act to optimize the genome organization allowing the best adaptive response of the cell within its natural environment. By comparing related genomes and inferring ancestral ones, it becomes possible to study the processes of genome evolution that shape bacterial genomes, and to reconstruct ancient evolutionary relationships.

Gene duplication is considered a mechanistic antecedent of gene innovation, and consequently of genetic novelty, that has facilitated adaptation to changing environments and exploitation of new niches. In the pre-genomic era it was suggested that bacterial genomes might have evolved from small to large genomes by several genome duplications. Previously we have analyzed the prevalence and genomic organization of duplicated genes (paralogs) in 106 bacterial genomes, showing that most of the paralogs in *Bacteria* seem to have been created by small gene duplication events. Nevertheless, paralogous genes comprise a significant fraction (up to 44%) of the bacterial genome coding capacity; a fraction that is found to be strongly correlated with genome size. Analysis of the functional classification of these paralogs reveals a preferential enrichment in functional classes that are involved in transcription, metabolism and defence mechanisms. Regarding the organization of paralogous genes within the genomes, it was found that 15% of the paranome (collection of paralogous genes) of the 106 bacterial genomes investigated consists of tandem duplicates, while 9,5% is located in duplicated segments. The majority of these segmental duplications resemble the typical bacterial operon size (3 - 4 genes), thereby indicating a putative mechanism for operon duplication and evolution. We will present an in dept analysis of segmental duplication that was performed for the α-*Proteobacteria*, a well-sampled taxonomic group of 11 completed genome sequences so far. It offers an excellent model system for comparative studies of bacteria that have descended from a common ancestor and yet have evolved many different types of interactions with their eukaryotic host cells. By implementing a visualisation tool we are able to examine the duplicated segments for divergence in gene content and organization (incl. gene loss, tandem duplications, gene fusion/fission, inversion and gene acquisition), in an attempt to obtain general patterns of segmental duplication and divergence. Additional detailed biological relevance to the described trends is determined by taking into account information on expression level (based on codon adaptation index and codon usage), essentiality and functional annotation of the genes in the segments. Placing the obtained intra-genomic results in a phylogenetic context (inter-genome comparisons) provides an extra evolutionary dimension on the conservation of the duplication event over larger evolutionary distances.

IpgB1 and IpgB2, two homologous proteins secreted by the type III secretion system, are synergistically required for both cell-to-cell spread and for the immunomodulation of the inflammation induced by *Shigella flexneri*

Abderrahman Hachani[1], Giacomo Rossi[2], Claude Parsot[3] , Ménard Robert[4], Maria Lina Bernardini[5] , and Abdelmounaaïm Allaoui[1]

[1]Laboratoire de Bactériologie Moléculaire, ULB, Faculté de Médecine, Route de Lennik, 808 1070 Bruxelles; [2]Facoltà di Medicina Veterinaria, Università di Camerino, Matelica, Italy; [3]Laboratoire de Pathogénie Microbienne, Institut Pasteur, 27-28 Rue du Dr Roux, 75725, Paris Cedex 15, France; [4]Institut Pasteur, 27-28 Rue du Dr Roux, 75725, Paris Cedex 15, France; [5]Dipartemento di Biologia Cellulare e dello Sviluppo, Università La Sapienza, Roma, Italy

Type III secretion systems are found in many pathogenic Gram-negative bacteria and are associated with the translocation of effector proteins into targeted host cells. In this study, we reported the first characterization of two *Shigella flexneri* homologous plasmidic genes, *ipgB1* and *ipgB2.* We found that IpgB2, like IpgB1, was secreted by the Mxi-Spa TTS apparatus. IpgB2 secretion, but not stability, required functional Spa15 chaperone. To investigate the role of these two genes in the virulence of *Shigella*, three strains were constructed by deleting the *ipgB1* and *ipgB2* genes either individually or together. *In vitro*, using the plaque assay, the *ipgB1* and *ipgB2* mutants were able to invade and to spread between cells similarly to the wild type strain. In contrast, a strain lacking both *ipgB1* and *ipgB2* genes were impaired in the dissemination stage. In vivo, using the sereny model of infection, *ipgB1* mutant exhibited a hypervirulent phenotype, the *ipgB2* mutant showed a moderate profile of virulence, while the double mutant *ipgB1ipgB2* was completely avirulent. Furthermore, using the murine pulmonary model of shigellosis, we evaluated the inflammation provoked by the three generated strains. Histopathological analysis of infected lungs revealed that the *ipgB1* mutant showed maximal lesions, the *ipgB2* one presented reduced lesions compared to the wild type strain, while the *ipgB1ipgB2* mutant exhibited a highly attenuated phenotype. Our findings indicate that IpgB1 and its homologue IpgB2 are new effectors involved in the pathogenicity of *Shigella flexneri* and are playing synergistic roles in the bacterial cell-to-cell spread and in the immuno-modulation of the bacterial inflammation.

Sediment biobarriers for chlorinated aliphatic hydrocarbons in groundwater reaching surface water

K. Hamonts, I. Clijsters, W. Dejonghe, R. Lookman, B. Borremans, J. Bronders, L. Diels (Vito, Boeretang 200, 2400 Mol, Belgium) and D. Springael (K.U.L., Laboratorium voor Bodem- en waterbeheer, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium)

Polluted groundwater in urban and industrial areas often represents a continuous source of diffuse contamination of surface waters. However, the fate of the infiltrating groundwater pollutants might be influenced by the sediment in eutrophic water bodies. Such sediments form an interface between groundwater and surface water and possess characteristic biological and physico-chemical degradation properties. Knowledge on natural attenuation of passing pollutants is however scarce or non-existent due to the lack of appropriate monitoring devices and tools to measure *in situ* mass balances of pollutants and other reactants/products. In this study, we wanted to explore the intrinsic capacity of eutrophic river sediment microbial communities to degrade Chlorinated Aliphatic Compounds (CAH) passing the sediment zone. Therefore, the structure and the catabolic potential of the microbial community present in the interface of the river Zenne (Belgium) at a place where groundwater contaminated with vinyl chloride (VC) and *cis*-1,2-dichloroethene (*cis*-DCE) was reaching the surface water, was studied.

Both batch degradation tests and molecular techniques (PCR or PCR-DGGE analysis of *dsr* (dissimilatory sulfite reductase) gene or 16S rRNA gene of Eubacteria and *Dehalococcoides* species) were performed on surface water and on aquifer material obtained from different positions in the interface. Batch degradation tests, performed with sediment material obtained from the interface, showed microbial degradation of both VC and *cis*-DCE under anaerobic conditions. *Cis*-DCE was reduced to VC, which was rapidly degraded to non-toxic ethene. This indicates that a high microbial degradation potential is present in the interface. To study the structure of the microbial community, undisturbed sediment/aquifer samples were taken with a macro-core sampler. The cores were frozen on dry ice immediately after sampling and divided into slices of approximately 0.5 cm in the laboratory. 16S rRNA gene-PCR-DGGE analyses were performed on each slice to study the microbial community as a function of its position in the interface. A complex DGGE pattern was obtained that was different from the pattern of the surface water. Certain bands appeared while others disappeared with increasing depth in the interface, which indicates that the structure of the microbial community changes with depth. PCR-DGGE analysis of the *dsr* gene revealed the presence of various sulfate-reducing bacteria in each position in the interface and showed a depth-related change in sulfate-reducing bacterial community structure in the eutrophic sediment. *Dehalococcoides* species, which are often involved in the degradation of CAHs, were also detected in the interface using PCR primers targeting the 16S rRNA gene of *Dehalococcoides* species. However, the *tceA* gene which codes for the trichloroethene (TCE) reductive dehalogenase of *Dehalococcoides ethenogenes* strain 195 that can transform TCE, DCE and VC to ethene, was not detected. Further research has to indicate whether this is due to the detection limit of the PCR or to the presence of more distantly related catabolic genes.

Our results indicate that the interface between groundwater and surface water may harbor a unique microbial community structure, capable of degrading groundwater contaminants before they reach the surface water. In this way, the interface may act as a natural biobarrier for groundwater pollutants passing through the interface, hereby reducing the risk of surface water contamination.

Culture-dependent study on the diversity of the denitrifying microbial community in activated sludge

Kim Heylen[1], Bram Vanparys[1], Nico Boon[2], W. Verstraete[2] & Paul De Vos[1]*

[1] Laboratory of Microbiology (WE10), Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; Tel: 09/264.51.01, Fax: 09/264.53.46, Email: Kim.Heylen@Ugent.be
[2] Laboratory of Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

Denitrification, the anaerobic respiration of nitrate and nitrite, is an important step in the nutrient removal of wastewater treatment plants (WWTP). The characteristic is widespread in the bacterial and archaeal domains and is therefore commonly studied through the use of *nir*-genes coding for nitrite reductases, a key enzyme of the pathway. However, these genes render no phylogenetic information and thus give no insight in the denitrifying community. It is generally accepted that new culturing efforts are required to link the functional information (*nir*-genes) with the diversity of the microbial population. We studied the composition of the denitrifying community of a municipal WWTP through isolation on different defined growth media and searched within the retrieved strains for *nirS* and *nirK* genes.

A dilution series from an activated sludge sample of the anaerobic compartment of a municipal WWTP was plated out on 16 growth media: 15 different defined growth media composed, specifically to enrich denitrifiers, and trypticase soy agar supplemented with sodium nitrate, the isolation medium most commonly used in similar studies. After a two-week period of anaerobic incubation, a total of 595 strains were isolated. Through phenotypic testing 54 isolates were considered to be capable of denitrification. Phylogenetically, they belonged to the *Alphaproteobacteria* (30), the *Betaproteobacteria* (16), the *Gammaproteobacteria* (5), and the *Firmicutes* (3). The presence of the *nirS* or *nirK* gene was investigated via a PCR with two sets of primers per gene: 41 strains contained either one gene or both. The amplicons were sequenced and compared with the *nir*-sequences of validly named microorganisms available in the EMBL database. This comparison led us to conclude that there are not yet enough *nir*-sequences available in the database to assess whether or not they can be used for phylogenetic analysis.

Novel *Bacillus* species from Drentse Grasslands

Jeroen Heyrman and Paul De Vos

Universiteit Gent, Faculteit Wetenschappen, Vakgroep BFM (WE10V), Laboratorium voor Microbiologie, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

Culture-independent studies on 16S rDNA directly extracted from soil in the Dutch Drentse grassland area revealed a dominant *Bacillus* group, named the BACREX-cluster, which accounted for at least 20 % of all soil bacteria present. Sequence analysis revealed that this cluster is subdivided into three lineages, clustering around *Bacillus niacini*, *Bacillus benzoevorans* and *Bacillus jeotgali*. To isolate members of the BACREX-cluster an extensive culture campaign was set up combined with a *Bacillus* specific multiplex PCR screening. The obtained isolates were characterized by a polyphasic approach including rep-PCR fingerprinting (using REP and GTG5 primers), partial 16S rDNA sequencing, gas chromatographic analysis of fatty acid methyl esters, DNA-DNA relatedness study and study of cultural and physiological characteristics. This approach revealed high genomic diversity among the isolates and several novel species were detected. In the neighborhood of *Bacillus niacini* five novel species were described: *Bacillus bataviensis*, *Bacillus drentensis*, *Bacillus novalis*, *Bacillus soli* and *Bacillus vireti*. The analysis of possible novel species in the neighborhood of *Bacillus benzoevorans* is in progress. The culturing campaign also yielded novel species outside the BACREX-cluster. Three novel *Bacillus* species were described: *Bacillus arvi*, *Bacillus arenosi* and *Bacillus humi*. In addition, several possible novel species belonging to *Paenibacillus* were isolated. From these data we can conclude that the Drentse grasslands accommodate a highly diverse *Bacillus* community including several novel species.

"CC"-COGENT: Two hundred completely sequenced and published genomes unified in one mySQL database

Paul J. Janssen[1], Anton J. Enright[2], Benjamin Audit[3], Ildefonso Cases[4], Leon Goldovsky[5], Nicola Harte[5], Dag Ahren[5], Victor Kunin[6], Christos A. Ouzounis[5]

[1]Belgian Nuclear Research Centre (SCK•CEN), Boeretang 200, 2400 Mol, Belgium; pjanssen@sckcen.be
[2]Sanger Institute, Genome Campus Hinxton, CB1 SD Cambridge, UK
[3]Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France
[4]National Centre for Biotechnology-CSIC, Campus UAM. Ctra Colmenar, 28049 Madrid, Spain
[5]European Bioinformatics Institute, Genome Campus Hinxton, CB1 SD Cambridge, UK
[6]Joint Genome Institute, Lawrence Berkeley National Laboratory, Walcreek, CA, USA

The constantly updated COmplete GENome Tracking (COGENT) database of fully sequenced and published genomes holds, as of 1 November 2004, the genomes of 162 Bacteria, 19 Archaea, and 19 Eukarya. The current total of 200 genomes represents a doubling of db size in about 2 years (up from 78, 15, and 7, respectively, on 24 October 2002) and a rate of 1 submisson each week. Taken together this represents a vast body of protein sequences (751,743) as compared to the nearly 2 milion entries in the non-redundant protein database (August 2004) and the 163,235 entries in the highly curated SwissProt database (release 45.0 of 25 October 2004). The core of the COGENTdatabase is composed of just two SQL tables that can be accessed via Perl DBI based scripts and modules allowing basic operations as well as complex querying. The genomes table contains genome related information such as the date of publication, source of data, proteome size, etc., while the proteins table holds the actual amino acid sequence data. Each genome is automatically assigned to a mnemonic species_code (e.g. HINF-KW2-01 for *Haemophilus influenza* strain KW2, version 01) and for each protein a unique identifier protein_id is produced, existing of the species_code followed by a dash and a number. This standard nomenclature contributes to a better consistency and reproducibility for large-scale computational analyses and improves the sharing of data and results. The core database is used as a front end in HTML [2] allowing selective downloads of protein sequence sets. The database is updated using DBI functionality but outdated genome information - including the older protein sequences - are retained in a separate table. Cogent can also be extended by a number of additional tables, for instance GeneQuiz derived annotations, cellular localizations, chromosomal positions, expression profiles, etc. The COGENT database is currently used in a number of projects [3,4,5]. For those that want to build a local SQL database a complete SQL dump (taking about 250 Mbytes of memory) of the core database is been made available.

References:

[1] Janssen *et al*. (2003). COmplete GENome Tracking (COGENT): a flexible data environment for computational genomics. Bioinformatics 19 (11): 1451–1452.
[2] http://maine.ebi.ac.uk:8000/services/cogent/
[3] Cases *et al.* (2003). Transcriptional regulation and environmental adaptation in bacteria. Trends in Microbiol. 11(6):248-253.
[4] Enright *et al.* (2003). Protein families and TRIBES in genome sequence space. Nucl. Acids Res. 31(15):4632-8.
[5] Kunin *et al.* (2004). Genomic perspective on phylogeny and comparison of species. Nucl. Acids Res. (In Press).

ACLAME: A Database for the Reticulate Classification of the Prokaryotic Mobilome

Raphaël Leplae[1], Gipsi Lima Méndez, Shoshana Wodak, Ariane Toussaint

Service de Conformation de Macromolécules Biologiques et de Bioinformatique, Université Libre de Bruxelles, Campus Plaine - CP 263, Boulevard du Triomphe, 1050 Bruxelles, Belgium
[1]raphael@scmbb.ulb.ac.be

The sequencing of the complete genomes of several strains of the same bacterial species brought a new dimension to our understanding of horizontal gene transfer (HGT) in prokaryotes, questioning the very meaning of strains and species (see for instance [1, 2]). Phages and plasmids are members of the Prokaryotic Mobile Genetic Elements (MGEs), which are central players in mobilizing and reorganizing genes, be it within a given genome (intra-cellular mobility) or between bacterial cells (inter-cellular mobility). Traditionally, MGEs have been classified as bacteriophages, plasmids or transposons. This classification becomes exceedingly obsolete as many chimerical elements are identified, as the many types of so called "genomic islands", which share genes or entire groups of genes. The modular nature of MGEs and the potential for reshuffling between modules has been long recognized [3, 4]. A systematic analysis of MGEs in terms of their modules might therefore be warranted. It is made difficult by the fact that these elements are not adequately annotated in the existing databases, out of which they cannot be easily retrieved. Complete genome annotations are not particularly helpful either. In the present gene ontologies, the vast majority of MGE gene functions are classified under the three very general "plasmid", "phage" or "transposon" related functional categories. Using as the basis the idea that MGE would best be described in terms of a hierarchy of functional modules, at both the protein and DNA levels, we undertook the development of the ACLAME database (http://aclame.ulb.ac.be). The ACLAME database is a collection and classification of prokaryotic mobile genetic elements (MGEs) from various sources, comprising all known phage genomes, plasmids and transposons. In addition to provide information on the full genomes and genetic entities, it aims at building a comprehensive classification of the functional modules of MGEs at the protein, gene, and higher levels. The current classification is produced automatically using TRIBE-MCL [5], a graph theory based Markov clustering algorithm that uses sequence measures as input, and then manually curated. The database is publicly accessible and open to expert volunteers willing to participate in its curation. Its web interface allows browsing as well querying the classification. The main objectives are to collect and organize in a rational way the complexity inherent to MGEs, to extend and improve the inadequate annotation currently associated with MGEs and to screen known genomes for validation and discovery of new MGEs.

References:

1. de la Cruz, F. and J. Davies, *Horizontal gene transfer and the origin of species: lessons from bacteria.* Trends Microbiol, 2000. **8**(3): p. 128-33.
2. Gogarten, J.P., W.F. Doolittle, and J.G. Lawrence, *Prokaryotic evolution in light of gene transfer.* Mol Biol Evol, 2002. **19**(12): p. 2226-38.
3. Casjens, S., G. Hatfull, and R. Hendrix, *Evolution of the dsDNA tailed-bacteriophage genomes.* Seminars in Virology, 1992. **3**: p. 383-397.
4. Couturier, M., et al., *Identification and classification of bacterial plasmids.* Microbiol. Rev., 1988. **52**: p. 375-95.
5. Enright, A.J., S. Van Dongen, and C.A. Ouzounis, *An efficient algorithm for large-scale detection of protein families.* Nucleic Acids Res, 2002. **30**(7): p. 1575-84.

Dynamic experiment design for identification of the lin-log kinetic parameters for the metabolic network of *Escherichia coli*

*J. Maertens[1], S. Van Den Broeck[1], A. Bhagwat[1], G. Lequeux[1], M, De Mey[2], P.A. Vanrolleghem[1],**

[1]BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure links 653, B-9000 Gent, Belgium
[2]Department of Biochemical and Microbial Technology, Ghent University, Coupure links 653, B-9000 Gent, Belgium

Dynamic metabolic network models are useful to predict and understand the effects of enzymatic kinetics on intracellular fluxes, metabolite levels and overall productivity.
However, both the selection of the model structure as the parameter identification, two steps of major importance in model building, are difficult undertakings.
Consequently, the objective of this research is dual. Primo, the potential of lin-log kinetics is investigated in view of a dynamic metabolic network of *Escherichia coli*. Secundo, starting from noisy raw data, collected during an in silico glucose pulse experiment, a statistically sound parameter identification procedure is applied, i.e. the effect of errors-in-variables is taken into account and the problem of correlated dependent variables is dealt with by means of, respectively, an error-in-variables model and principal component analysis and regression.
Finally, an optimal experiment is designed to obtain better intracellular concentration data that lead to more reliable parameter estimates.

***Correspondence** should be sent to: Peter Vanrolleghem, BIOMATH, Coupure Links 653, B-9000 Gent, Belgium; Telephone: +32(0) 92 645 939; Fax: +32(0) 92 646 220; E-mail: Peter.Vanrolleghem@UGent.be

Unusual Resistance Patterns Related to Alterations in the *Erm*(A) Attenuator in Macrolide-Resistant (MAC-R) *Streptococcus pyogenes* (SPY)

S. Malhotra-Kumar[1], A. Mazzariol[2], C. Lammens[1], J. Piessens[1], G. Cornaglia[2], H. Goossens[1].

[1]Univ. of Antwerp, Antwerp, Belgium, [2]Univ. of Verona, Verona, Italy.

Background

Erm(A)$^+$ MAC-R SPY express resistance to 14-membered (erythromycin, ERY) & 15-membered (azithromycin, AZI) macrolides, but not to lincosamides (clindamycin, CLD) that are non-inducers. We analyzed the genetic basis of 2 different phenotypes expressed by *erm*(A)$^+$ SPY recovered from skin and throat infections in Belgium and Italy.

Methods

Susceptibility tests were performed by double disk diffusion and MICs for ERY, AZI, and CLD according to NCCLS method. MAC-R genes {(*erm*(A), *erm*(B), *mef*(A)} were detected by PCR. Clonality was studied by *emm* typing. Double-strand sequencing of the entire *erm*(A), L4, L22, and portions of 23SrRNA genes was carried out.

Results

We isolated 7 SPY that were low-level ERY-R (MIC 4-8 µg/ml) and high-level AZI-R (MIC>256 µg/ml). Of these, 1 SPY showed constitutive CLD-R (MIC >256 µg/ml) and the 6 CLD-sensitive SPY (MIC 0.06-0.12 µg/ml) also became high-level CLD-R (MIC >256 µg/ml) upon induction with ERY. All 7 isolates carried *erm*(A). The 3 non-clonal (M1, M100-M104, and Not typable) Belgian isolates showed an A137C (Q15P) mutation while the constitutive CLD-R isolate carried an additional G98A (G2N) mutation in *erm*(A). All 4 clonal isolates (M77) from Italy showed a C140T (P16L) mutation. All changes were localized in the ORF of the 2nd leader peptide (LP2) in the *erm*(A) attenuator. Various point mutations were also noticed in 23SrRNA. No changes were found in L4 and L22.

Conclusions

Such *in-vivo* occurring alterations have not been documented in *erm*(A)$^+$ SPY. According to the translational attenuation model proposed for *erm*(A) regulation in *S. aureus*, single mutations localized in LP2 can present as varied phenotypes. However, contribution of the 23SrRNA mutations, if any, remains to be analyzed. Finally, our results reiterate the ease with which CLD-R can be selected in inducible SPY and warrant discouraging CLD therapy for infections caused by *erm*(A)$^+$ SPY.

Molecular epidemiology of macrolide- and telithromycin-resistant group A *Streptococcus* in Belgium, 1999-2003

Surbhi Malhotra-Kumar[1], Christine Lammens[1], Sabine Chapelle[1], Monique Wijdooghe[1], Jasper Piessens[1], Koen Van Herck[2], and Herman Goossens[1]

[1]Belgian Reference Center for Group A Streptococcus, [2] Department of Epidemiology and Community Medicine, Campus Drie Eiken, University of Antwerp, Antwerpen, Belgium.

Background

Increasing macrolide-resistance has prompted the introduction of the ketolide, telithromycin. During a national surveillance program from 1999 to 2003, we studied the temporal and molecular evolution of macrolide- and telithromycin-resistant group A streptococci (GAS) isolated from tonsillopharyngitis patients.

Methods

A total of 3866 GAS were screened for macrolide-resistance by the double-disk method and MICs. Macrolide-resistance genes (*erm*(A), *erm*(B), *mef*(A) were detected by PCR. Clonality was studied by *emm* typing and PFGE. The *erm*(B) gene (with promoter and control peptides) of 10 telithromycin-resistant GAS as well as L4, L22 ribosomal proteins and portions of 23SrRNA genes were sequenced.

Results

Overall, 506 (13%) GAS were macrolide-resistant and their yearly prevalence was 14%, 12%, 12%, 18%, and 9% in 1999, 2000, 2001, 2002, and 2003, respectively.

Telithromycin-resistance (MIC ≥ 4 µg/ml) was observed in 50/506 (10%) macrolide-resistant GAS harboring *erm*(B) or *erm*(B) and *mef*(A). cMLS (constitutively resistant, n=209) and M (resistant via efflux, n=279) phenotype GAS were divided into 20 and 51 PFGE types, respectively. Three clones (1, 1001, and 4), constituted 243 (48%) of the macrolide-resistant GAS. Clone 1/*erm*(B)$^{+}$/*emm*22 and clone 1001/*mef*(A)$^{+}$/*emm*1 caused outbreaks in 1999 and 2002, respectively. Sequencing of 5 telithromycin-resistant GAS showed a His118Arg substitution in *erm*(B).

Conclusions

Our results demonstrate the importance of both clonal spread and resistance gene transfer in response to selection pressure in the dissemination of macrolide-resistant GAS and emphasize caution while interpreting a ‘cause and consequence’ relation between macrolide use and resistance. Pre-existing telithromycin-resistance in GAS is of concern and needs monitoring

Multiplex PCR for Simultaneous Detection of Macrolide and Tetracycline Resistance Determinants in *S. pyogenes* (SPY) & *S. pneumoniae* (SPN)

S. Malhotra-Kumar[1], C. Lammens[1], H. Bruylants[1], J. Piessens[1], H. Goossens[1]

Univ. of Antwerp, Antwerp Belgium.

Background

A concomitant increase in resistance to macrolides (MAC) and tetracyclines (TET) is being observed among SPY & SPN. Resistance to both antibiotic groups tends to co-occur due to the well-known co-carriage of *erm*(B) and *tet*(M) on a single transposon as well as recent evidence suggesting linkage of *erm*(A) or *mef*(A) with *tet*(O). A multiplex PCR for detecting MAC and TET resistance determinants was developed to determine their simultaneous and specific presence in SPY & SPN.

Methods

The multiplex assay incorporated 8 primer pairs specific for MAC-R {*erm*(A), *erm*(B), *mef*(A/E)}, and TET-R {*tet*(M), *tet*(O), *tet*(K), *tet*(L)} genes and 16SrRNA as an internal control. Primers were designed using Primer3 software or obtained from published reports. The multiplex protocol was optimized on DNA pooled from reference cultures for all 7 resistance genes. Forty-one SPY & SPN, previously characterized for MAC and TET resistance based on erythromycin (ERY), clindamycin (CLD), and tetracycline (TET) MICs and on single-reaction PCRs using known primers for all 7 resistance genes were evaluated by the multiplex PCR protocol.

Results

The multiplex assay could specifically detect resistance determinants present in different combinations. *Erm*(B) alone or with *mef*(A) was detected in 28 isolates (MIC range ERY 64->512, CLD 0.03->512 µg/ml), m*ef*(A) alone in 11 isolates (MIC ERY 8, CLD 0.03 µg/ml), *erm*(A) in 2 (MICs ERY 1and 512 µg/ml, CLD 0.25 and 0.5 µg/ml), *tet*(M) in 10 (MIC range TET 0.03-64 µg/ml), and *t*et(O) in 9 isolates (MIC range TET 32-64 µg/ml).

Conclusions

Simultaneous and specific detection of multiple resistance genes is especially relevant in the current scenario where SPY & SPN carrying more than one MAC and /or TET resistance determinants are increasingly being noted. This single-step multiplex protocol will prove economical in terms of labor, time and cost for studies investigating large numbers of SPY & SPN for MAC- and TET-resistant determinants.

Analysis on the effects of spaceflight on the photosynthetic bacterium *Rhodospirillum rubrum* ATCC25903 used in Life Support Systems

Felice Mastroleo[1,2], Larissa Hendrickx[1], Sarah Baatout[1], Natalie Leys[1], Louis de Saint Georges[1], Ruddy Wattiez[2] and Max Mergeay[1].

[1]Belgian Nuclear Research Centre (SCK·CEN), Laboratory of Microbiology and Radiobiology, Mol, Belgium
[2]University of Mons-Hainaut (UMH), Department of Biological Chemistry, Mons, Belgium

The European Space Agency developed **MELiSSA** (**M**icro **E**cological **Li**fe **S**upport **S**ystem **A**lternative) required to provide food and water and to deal with gas and waste production on planetary bases or missions to Mars. It consists of a loop of interconnected bioreactors populated with bacteria and algae. In view of long haul space exploration missions, the organisms inhabiting the MELiSSA loop need to perform their tasks as optimally as possible. Proper functioning of the MELiSSA loop will be dependent on several physical, chemical and biological parameters including change in gravitational forces, ionizing radiation, oxidative stress and long time culturing.

Within this context, we focus on the mineralization compartment inhabited by the bacterium *Rhodospirillum rubrum* ATCC 25903. In this work, the first spaceflight experiment of *R. rubrum* ATCC25903 in the International Space Station (ISS) is presented.

Electron microscopic, flow cytometric, phenotypic and proteomic analysis were performed on the bacterial cultures, after spaceflight. Immediately after landing, electron microscopy and flow cytometry was performed on the 3 *R. rubrum* cultures that travelled into the ISS (MESSAGE 2 experiment). In a second phase, the phenotypes were characterized and allowed the identification of mutants, induced after space flight. At the proteomic level, analysis was performed using 2D-**PAGE** (**P**oly**A**crylamide **G**el **E**lectrophoresis) followed by **M**atrix-**A**ssisted **L**aser **D**esorption **I**onisation (**MALDI**)-**T**ime **o**f **F**light (**TOF**) mass spectrometry, giving a first glimpse on the bacterial response during spaceflight. This work presents the first results on the effects of spaceflight on the photosynthetic bacterium *R. rubrum* ATCC25903 used in life support systems.

Prophage Detection in Bacterial Genomes

Gipsi Lima Mendez[1], Raphael Leplae, Shoshana Wodak, Ariane Toussaint

Service de Conformation de Macromolécules Biologiques et de Bioinformatique, Université Libre de Bruxelles, Campus Plaine - CP 263, Boulevard du Triomphe, 1050 Bruxelles, Belgium
[1]gipsi@scmbb.ulb.ac.be

Sequencing of bacterial genomes confirmed the crucial role that bacteriophages play in bacterial evolution and the divergence between closely related bacterial strains and species. Temperate phages, while residing in their host as latent prophages, either integrated in the host genome or as a circular or linear plasmid, become a repository for the shuffling of genetic information by recombination with temporary resident phage, plasmid or other mobile element. ACLAME is a database dedicated to a reticulate classification of prokaryotic Mobile Genetic Elements including phages and prophages (Leplae, Hebrant et al. 2004). To populate the database with additional phage proteins, we developed a system to identify prophages in sequenced bacterial genomes. The procedure includes: - the detection of integrases in the bacterial genome based on sequence similarity with the integrases classified in the ACLAME database; indeed prophage integrase genes usually define one prophage end; - search for a direct repeat of at least 10bp between a 300 bp DNA fragment next to the integrase on one side and a 250 kb stretch on the other side of the integrase. This repeat, generated upon integration of the viral into the host genome, assigns the prophage boundaries . - similarity evaluation of the protein sequences in between the direct repeats with phage proteins stored in ACLAME allowing the definition of putative prophages. Applied on 155 bacterial genomes, the strategy identified ~1000 putative prophages. This initial dataset includes overlapping prophages, genomic islands and other non-prophage entities (based on visual inspection of the results). A second analysis step dedicated to filter them out is being finalised.

Reference:

Leplae, R., A. Hebrant, et al. (2004). "ACLAME: a CLAssification of Mobile genetic Elements." Nucleic Acids Res **32 Database issue**: D45-9.

Zinc tolerance and community structure of ammonia oxidizing bacteria in a long-term zinc contaminated soil

Jelle Mertens, Erik Smolders and Dirk Springael

Laboratory for Soil and Water Management, Kasteelpark Arenberg 20, 3001 Heverlee, BELGIUM

Nitrification is known to be reduced by heavy metals in laboratory contaminated soils. However, nitrification is generally unaffected by increasing heavy metal concentrations in long-term metal contaminated soils (Smolders *et al*., 2003). This paper attempts to explain this discrepancy in terms of bioavailability of the contaminant, ionic strength in soil pore water and metal tolerance of the microbial community.

Long-term zinc contaminated soils were sampled in grassland under and next to a galvanized electricity transmission tower (76-1643 mg Zn/kg). Potential Nitrification Rate ('PNR') in these soils did not significantly decrease with increasing Zn concentrations (25-32 mg NO_3-N/kg/d). However, addition of $ZnCl_2$ to the uncontaminated control soil significantly decreased PNR with increasing metal addition (32 to 0 mg NO_3-N/kg/d). Soil solution Zn concentrations in the field soils were lower than those in lab contaminated soils at equal total metal concentrations. A maximum of 80% reduction of PNR was expected in the field contaminated soils at soil solution Zn concentrations corrected to those in laboratory spiked soils (figure 1). However, no such inhibition was found, indicating zinc tolerance of the nitrifying community. Amplification of 16SrDNA of ammonia oxidizers with CTO-primers (Kowalchuk *et al*., 1997) and DGGE analysis showed a gradual change of the nitrifying community structure in the field contaminated soil samples with increasing Zn concentrations. This is an indication that the original nitrifying community changed during the long-term Zn contamination. Moreover, PNR after reinoculation of control soil in sterilized, lab contaminated soil was significantly higher than PNR after reinoculation of field-contaminated soil in the same lab contaminated soils and Zn concentrations at which PNR was reduced by 50 % ('EC50- values') differed significantly (figure 2). These were all indications that the original nitrifying community changed during the long-term Zn

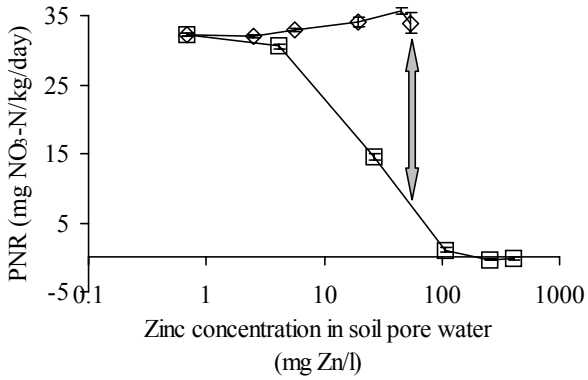

Figure 1: PNR in field contaminated soil samples (◊) and $ZnCl_2$ contaminated control soil (□) as a function of the zinc concentration in soil pore water.

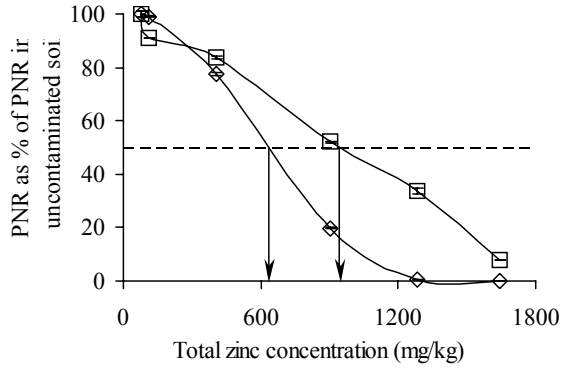

Figure 2: PNR after reinoculation of sterile soil with either uncontaminated control soil (◊) or field contaminated soil (□) as a function of the total zinc concentration. Arrows indicate the EC50-values for both inocula.

References

Kowalchuk, G.A., Stephen, J.R., De Boer, W., Prosser, J.I., Embley, T.M. and Woldendorp, J.W. (1997) Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class *Proteobacteria* in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Applied and Environmental Microbiology*, 63:1489-1497.

Smolders, E., McGrath, S.P., Lombi, E., Karman, C.C., Bernhard, R., Cools, D., Van den Brande, K., van Os, B. and Walrave, N. (2003) Comparison of toxicity of zinc for soil microbial processes between laboratory-contaminated and polluted field soils. *Environmental Toxicology and Chemistry*, 2:2592-2598.

Quantification of *mexAB-oprM* and *mexXY-oprM* gene expression level in *Pseudomonas aeruginosa* by QC-RT-PCR

N. Mesaros, N. Caceres, F. Van Bambeke, M.P. Mingeot-Leclercq and P.M. Tulkens
Dr. Narcisa Mesaros, MD

Université catholique de Louvain (Unité de pharmacologie cellulaire et moleculaire), Bruxelles, Belgique
UCL 7370, Avenue E. Mounier 73, B-1200 Brussels
narcisa.mesaros@facm.ucl.ac.be

Objectives
Survey of isolates for mechanism of resistance is a critical aspect in optimization of the therapeutic choices in a manner that will slow down the further development of resistance. This should now include the efflux systems, which are rarely identified as such in clinical microbiology setups because of absence of a rapid and versatile method. In this context, we developed and validated a new method, a QC-RT-PCR (Quantitative Competitive RT-PCR) to quantify the levels of expression of antibiotic resistance related genes *mexAB-oprM* and *mexXY-oprM* in *P. aeruginosa*. These two efflux systems are able to transport various antimicrobials.

Methods
P. aeruginosa PAO1 wild type strain, *P. aeruginosa* MexAB-OprM overproducer strain and *P.aeruginosa* MexXY-OprM overproducer strain were used in the study. Total RNA from all strains was isolated and used in RT-PCR reactions. The genes of interest were amplified by PCR from their respective cDNA in presence of primer -F and -R. For each gene an internal competitor DNA was generated by PCR [primer-40mer (primer-F+20 internal pb) and primer-R]. Triplicate PCR reactions were performed adding the internal competitor DNA at defined concentrations, identical aliquots of cDNA and primers -F and -R.

Results
With this method we detected that in the case of the MexAB-OprM overproducer strain the expression level is at least 5 fold higher than the level observed for the same gene in the wild-type strain. In MexXY-OprM overproducer bacteria compared to the wild-type strain, we observed a *mexXY-oprM* gene expression level of at least 10 fold higher.

Conclusions
The QC-RT-PCR is an advantageous method and allows for both a sensitive and rapid diagnostic procedure to quantify the expression level of efflux genes of interest.

The four replicons of *Ralstonia metallidurans* CH34 revealed by IS reassembly

Sébastien Monchy (1,2), Daniel van der Lelie (3), Sean McCorkle (3), Safyih Taghavi (3), John Dunn (3) and Max Mergeay (1)

(1) Laboratory for Radiobiology & Microbiology, Belgian Center for Nuclear Studies, SCK.CEN, Mol, Belgium, (2) Service de Conformation des Macromolécules Biologiques et de Bioinformatiques, Université Libre de Bruxelles, Belgium, (3) Brookhaven National Laboratories, Biology Department, Upton, NY11973-5000, USA

The species *Ralstonia metallidurans* includes various isolates of bacteria adapted to harsh industrial biotopes contaminated with heavy metals and / or organic pollutants. The genome of *R. metallidurans* CH34 is being sequenced by Joint Genome Institute and is presently assembled into 27 major contigs grouped in 13 scaffolds. This genome is rich in IS elements, which appear to be a handicap for the assembly. Nevertheless, IS elements can also be used as a tool to check the assembly and to propose contig closure.

IS elements are mobile genetic elements (from ~1kb to ~3kb) able to move by transposition within the genome. We identified 13 different IS elements, present in multiple copies, on the genome of *R. metallidurans* CH34, representing a total number of 48 IS elements. This relatively high number of IS elements leads to misassembly errors. By exploiting a general property of IS element, which is the presence of direct repeats on their extremities, we can confirm the assembly of fragments joined by IS elements (if direct repeats on both extremities are identical) and / or improve the assembly by closing contigs (a part of an IS element present on one contig extremity, the other part present on the extremity of another contig). To experimentally confirm the new assembly, PCR was performed with primers located on each IS element flanking region. Amplification, according to the prediction, was observed in most cases. To complete this analysis and to propose a scaffold for the *R. metallidurans* CH34 chromosome, we matched the new contigs to the biosynthetic marker map of the chromosome (Sadouk, Mergeay, 1993).

All the assembly improvements allowed us to build a new scaffolding for *R. metallidurans* CH34 genome and confirmed the presence of at least four replicons:

- One chromosome (~3.3 Mb) that is especially rich in house keeping genes and biosynthetic genes. This replicon was also observed in other Ralstonia genomes, and its proposed scaffold matched the biosynthetic map of the chromosome of strain CH34..
- One megaplasmid (~2.6Mb) that shares features with the (2.1Mb) megaplasmid of *R. solanacearum*.
- Two large plasmids: pMOL28 (171kb) that contains 19 heavy metal resistance genes located in three clusters and involved in nickel, cobalt (*cnr*), chromate (*chr*) and mercury (*mer* of Tn*4378*) resistance. pMOL30 (234kb) that contains around 45 genes for heavy metal resistance zinc, cobalt, cadmium (*czc*), lead (pbr), copper (*cop*) and mercury (*mer* of Tn*4380*), clustered opposite to the replication origin of the plasmid. The pMOL28 assembly was in close agreement with this plasmids restriction map as proposed by Taghavi et al (1997).

In the next future, sequencing the remnant gaps should allow us to finish the sequence of both replicons. Analysing *R. metallidurans* genome structure and it organisation into two mega replicons (characteristic for other *Ralstonia* species, such as *R. solanacearum*, *R. eutropha* and *R. taiwanensis*) will be of interest to understand their specific biotopes adaptation that seems to involve the megaplasmid and mobile genetic elements.

References:

Sadouk A., Mergeay M. (1993), Mol Gen Genet. 240(2):181-187.
Mergeay M, Monchy S, Vallaeys T, Auquier V, Benotmane MA, Bertin P, Taghavi S, Dunn J, van der Lelie D, Wattiez R (2003) FEMS Microbiol.Rev. 27:385-410
Taghavi S., Mergeay M., van der Lelie D. (1997), Plasmid 37: 22-34
Sequencing of the *R. metallidurans* genome is carried out at the Joint Genome Institute (JGI) under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program.

Comparison of the PhoPQ regulon in *Escherichia coli* and *Salmonella typhimurium*

Pieter Monsieurs[1], Kathleen Marchal[1], Sigrid Dekeersmaecker[2], Gert Thijs[1], Jos Vanderleyden[2], Bart De Moor[1]

[1] ESAT-SCD, K.U.Leuven, Kasteelpark Arenberg 10, 3001 Leuven-Heverlee, Belgium
[2] Centre of Microbial and Plant Genetics, K.U.Leuven, Kasteelpark Arenberg 20, 3001 Leuven-Heverlee, Belgium

The PhoPQ system is a relatively ubiquitous pleiotropic transcriptional regulator that responds to external Mg^{2+} in both *Escherichia coli* and *Salmonella typhimurium.* Mutations in the PhoPQ system in pathogenic species such as *Salmonella* result in an attenuated virulence phenotype showing an increased sensitivity to antimicrobial peptides and acid pH, a decreased resistance to bile salts, deficiency in epithelial cell invasion and the inability to survive within macrophages. As compared to the non-pathogenic strains such as *E. coli*, the PhoPQ regulon in *Salmonella* seemingly has obtained novel targets that allow bacterial survival in the intracellular environment of the host. From this observation, we hypothesized that the PhoPQ regulon in both related species, *E. coli* and *S. typhimurium* must have besides a core of common target genes, a set of genes that has been specifically acquired during evolution in each of the species. These specific gene sets would then contribute to the specificities of the phenotype in each of the organisms. To verify this hypothesis, the composition of the PhoPQ regulon was compared between *E. coli* and *S. typhimurium* using a combination of expression- and motif data.

To determine the overlap in the expression domain of the PhoPQ regulatory system of *Salmonella* and -*E. coli*, PhoPQ-dependent genes were isolated from microarray datasets obtained in the appropriate conditions from respectively *E. coli* (described by Minagawa *et al.* [3]) and *S. typhimurium* (kindly provided by Bader *et al.* [1]).
To distinguish direct from indirect targets, we searched for the presence of the regulatory motif (i.e. the binding site of a regulatory protein) in the promoter region of the identified PhoPQ-dependent genes. To this end a motif model corresponding to a small conserved DNA-sequence (T/G)GTTTA was used [2,3]. Based on this analysis that combines microarray- and sequence (motif) data, the direct PhoPQ-dependent regulon could be reconstructed in both species.
Subsequent comparison of the regulons pointed towards a limited overlap of PhoPQ-dependent genes between *S. typhimurium* and *E. coli* and suggests a specialized function of this two-component system in both species (e.g. pathogenesis in *S. typhimurium*).

References

1. M. W. Bader, W. W. Navarre, W. Shiau, H. Nikaido, J. G. Frye, M. McClelland, F. C. Fang, and S. I. Miller, Regulation of *Salmonella typhimurium* virulence gene expression by cationic antimicrobial peptides. *Mol.Microbiol.*, 50:219-230, 2003

2. S. Lejona, A. Aguirre, M. L. Cabeza, V. E. Garcia, and F. C. Soncini, Molecular characterization of the Mg^{2+}-responsive PhoP-PhoQ regulon in *Salmonella enterica*. *J. Bacteriol.,* 185:6287-6294, 2003

3. S. Minagawa, H. Ogasawara, A. Kato, K. Yamamoto, Y. Eguchi, T. Oshima, H. Mori, A. Ishihama, and R. Utsumi, Identification and molecular characterization of the Mg^{2+} stimulon of *Escherichia coli*. *J. Bacteriol.*, 185:3696-3702, 2003

Effects of *in situ* conditions on MTBE biodegradation kinetics and microbial community composition during long-term enrichment

David Moreels, Leen Bastiaens, Frans Ollevier, Roel Merckx, Dirk Springael, Ludo Diels

Vito, Flemish Institute for Technological Research, Environmental and Process Technology Centre, Mol, Belgium
KULeuven, Catholic University of Leuven, Laboratory for aquatic ecology, Leuven, Belgium
KULeuven, Catholic University of Leuven, Laboratory for soil and water management, Leuven, Belgium

Methyl tert-butyl ether (MTBE) is an anthropogenic chemical used as a gasoline additive with known toxicological effects and is being detected at increasing frequency in the environment. The effect of in situ important parameters (nutrients, oxygen, co-substrates e.g. benzene and propane) on the microbial population composition was studied under oligotrophic conditions using batch enrichment cultures of samples coming from a MTBE contaminated soil. The soil samples originated from 4 different depths from the vadose and saturated zone. Sustained MTBE degradation was observed using gaschromatographic methods for more than 800 days in all enrichment cultures of the 4 soil samples. The bacterial community composition was followed in function of enrichment condition and sample depth by denaturing gradient gel electrophoretic analysis of 16S rDNA genes amplified with general eubacterial primers. Initially, the microbial population in each of the 4 studied soil samples was different. During enrichment a clear shift in fingerprint pattern did occur, but no convergence in banding pattern was seen for the enrichment cultures of the 4 different soil samples. Not 2 enrichment cultures had identical fingerprint patterns, nonetheless some DNA bands common to multiple enrichment cultures could be detected. Sequencing of a 16 rDNA gene fragment amplified with specific primers indicated that a bacterium with 97 % homology to the known MTBE degrading bacterium *Rubrivivax gelatinosus* PM-1 is present. At this moment, cloning and sequencing of the most dominant bands observed in the fingerprint are in progress as well as isolation of the MTBE degrading bacteria.

Immune evasion properties are shared by leader proteins of neurovirulent and persistent Theiler's virus strains.

Sophie Paul, Sophie Delhaye, Vincent van Pesch and Thomas Michiels.

Christian de Duve Institute of Cellular Pathology, Université Catholique de Louvain, MIPA-VIRO 74-49, 74, avenue Hippocrate, B-1200, Brussels, Belgium.

Theiler's virus is a picornavirus responsible for infections of the central nervous system (CNS) of the mouse. The neurovirulent strains (GDVII and FA) of the virus induce an acute lethal necrotizing encephalitis. The persistent strains (DA and BeAn) provoke a chronic demyelinating disease considered as a model for multiple sclerosis. One aim of our work is to understand the striking ability of the latter strains to persist and multiply lifelong in the CNS, in the face of a strong specific immune response.

The leader (L) protein of Theiler's virus is crucial for persistence of DA1 virus *in vivo*[1].

On one hand, we showed that the L protein of persistent strains inhibits the transcription of type-I interferon genes in infected cells[1,2]. The leader protein appears to inhibit the transcription of other cytokine genes too, such as that encoding the RANTES chemokine. On the other hand, the L protein can trigger the subcellular redistribution of cellular proteins, probably by nuclear envelope permeabilization [3]. It can notably interfere with trafficking of interferon regulatory factor-3 (IRF-3), a cellular factor crucial for early transcriptional activation of interferon genes.

The link between IFN type-I inhibition and perturbation of nucleo-cytoplasmic trafficking by L protein is not yet established. However, perturbation of nucleo-cytoplasmic trafficking can be viewed as a strategy to escape immune responses since the expression of many immune effectors depends on the nuclear translocation of transcription factors.

Here, we tested whether L proteins from persistent and neurovirulent Theiler's virus strains are functionally exchangeable. Therefore, we constructed chimeric viruses by substituting the L coding region of neurovirulent Theiler's virus strain GDVII for that of the persistent DA1 strain. Both the parental DA1 virus and the recombinant virus expressing the GDVII leader protein could (i) inhibit transcription of the genes coding for IFN-α/β and RANTES, (ii) perturb nucleo-cytoplasmic trafficking of cellular proteins, and (iii) persist in the CNS of infected mice.

References:

1. van Pesch V., van Eyll O., and Michiels T. (2001),
The leader protein of Theiler's virus inhibits immediate-early alpha/beta interferon production.
J Virol **75:** 7811-7817.
2. van Pesch V and Michiels T. (2003),
Characterization of interferon-α 13, a novel constitutive murine interferon-α subtype.
J. Biochem **278**: 46321-46328.
3. Delhaye S., van Pesch V., and Michiels T. (2004),
The leader protein of Theiler's virus interferes with nucleocytoplasmic trafficking of cellular proteins. J Virol **78:** 43

Study of the cyanobacterial diversity and paleodiversity in microbial mats from Antarctic coastal lakes with molecular tools based on the rRNA gene sequences

Schmoker C., Taton A., Balthasart P., Grubisic S., Waleron K. and Wilmotte A

Center for Protein Engineering, Institute of Chemistry B6,University of Liège, Sart-Tilman, B-4000 Liège, Belgium.

Cyanobacteria are the dominant phototrophs in Antarctic biota, and they form conspicuous microbial mats at the bottom of lakes and ponds.

In the frame of the EC project MICROMAT (BIO4-CT98-040) and the OSTC project LAQUAN (EV/12/1B), a polyphasic approach was used to study the diversity of such communities and their geographic distribution. Molecular techniques based on 16S rRNA sequences were used to study 7 lakes of the Eastern Antarctic coast. The lakes were selected to cover a wide range of chemical environments.

Ten clone libraries were constructed from samples collected from the lakes Reid, Heart, Progress and Firelight (Larsemann Hills), Ace (Vestfold Hills), Fryxell (Dry Valleys), Rauer 2 and Rauer 8 (Rauer Islands). 546 clones were partially sequenced, and the sequences sharing more than 97.5 % similarity were grouped into 50 phylotypes. The cyanobacterial communities of the studied lakes appeared quite diverse, half of the phylotypes were unique to the lake where they were found. These variations in the genotypic cyanobacterial diversity probably reflect the heterogeneity of the ecological characteristics of the lakes (salinity, pH, depth, ice cover,…) in Antarctica.

The phylogenetic analysis has also revealed the existence of “Antarctic clusters” that might be endemic, sequences from putatively novel or yet unsequenced organisms.

The other aim of this project was to characterize the past diversity. Three lakes (lake Progress, lake Reid and lake Heart) were chosen to study the paleodiversity of cyanobacteria in fossil microbial mats that have accumulated since up to 50000 years B.P in these coastal Antarctic lakes.

In the case of cores from Progress and Reid lakes, it was observed that the ratio of cyanobacterial sequences in comparison to bacterial ones decreases with increasing age. Because the sediments in these lakes are cold but not frozen, it is probable that the spontaneous chemical decay of DNA can continue. In addition, many groups of bacteria seem to be living in the cores, and their DNA (of better quality) is isolated at the same time as fossil DNA.

In case of Lake Heart, the cyanobacterial sequences were dominant, perhaps due to salt preservation of the sediment core after seawater income to the lake 9000 years ago.

In the case of Lake Progress, we observed a stratification of microbial populations in dependence of the layer, chemical conditions and age.

Bacterial sequences from the analysed cores are similar to the sequences of bacteria occurring in soil environments in many different places around the World, and of bacteria which were observed in sediments from cold regions (e.g. Siberia).

Genetic relationships between caliciviruses isolated in bovine species in Belgium, other bovine caliciviruses and human noroviruses bases on polymerase and capsid partial sequences

Scipioni A.[1], Bourgot I.[1], Ziant D.[1], Czaplicki G.[2], De Mol P.[3], Lomba M.[4], Daube G.[5] and Thiry E.[1]

[1] Virology, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Belgium, [2] ARSIA (Association regionale de sante et d'identification animales de Loncin), Liege, Belgium, [3] Medical Microbiology and Virology, Faculty of Medicine, University of Liege, Belgium, [4] ARSIA de Ciney, Namur, Belgium, [5] Food Microbiology, Department of Food Sciences, Faculty of Veterinary Medicine, University of Liege, Belgium

Noroviruses (NV) from the family *Caliciviridae*, are responsible of a highly contagious mild human gastroenteritis which is transmissible by the fecal-oral way. They are involved in the majority of foodborne diseases. Humans were considered in past years as the sole host for these viruses but viruses closely related to NV were recently found in stool samples from cattle or pigs (1; 2; 3; 4). These results opened discussions about the potential for zoonotic transmission of these viruses. To investigate the situation in Belgium, stool samples from diseased cattle were assayed by RT-PCR using different sets of primers specific for the detection of NV from humans or bovines. To see the proximity between human and bovine viruses, at a molecular level, we work on RT-PCR product sequences from different part of genome.

Bovine and human stool specimens were collected from diagnostic laboratories. From February 2002 to December 2003, 317samples of all age group from cattle and 607 from human were collected.

Three sets of primer, which had been developed for the detection of NV in stool specimens from humans or bovines, were used: the primer pair Jv12/Jv13 amplifies a segment of 326 bp in the polymerase gene region of NV (5), primer pair CBECU-F/CBECU-R amplifies a segment of 532 bp in the polymerase gene region of bovine NV (6) and primer pair CCV3/CCV4 amplifies a segment of 407 bp in the capsid protein gene of bovine NV (7). This allowed detection of 20 human positive samples and 14 bovine positive samples. From the 14 bovine samples, 21 sequences were obtained: 3 with primers JV12/JV13, 8 with CBECUF/CBECUR and 10 with CCV3/CCV4. These 14 samples were all confirmed NV positive by sequencing RT-PCR products of expected size with Megabase autosequencer in both directions with PCR primers. The sequences obtained were used for molecular analyses and compared with sequences from Genbank database. Multiple alignments were generated using ClustalW program and trees were drawn using TreeTop program.

The results provide further evidence that NV are present in cattle and not only restricted to humans. These findings do not support the hypothesis of zoonotic transmission, based on the data set we have.

References:

1. Dastjerdi et al. The bovine Newbury agent-2 is genetically more closely related to human SRSVs than to animal caliciviruses. Virology 254, 1-5, 1999

2. Liu et al. Molecular characterization of a bovine enteric calicivirus: relationship to the Norwalk-like viruses. J. Virol. 73, 819-825, 1999

3. Sugieda et al. Detection of Norwalk-like virus genes in the caecum contents of pigs. Arch. Virol. 143, 1215-1221, 1998

4. Van der Poel et al. Norwalk-like calicivirus genes in farm animals. Emerg.Infect.Dis. 6, 36-41, 2000

5. Vinjé et al. Molecular detection and epidemiology of Small Round-Structured Viruses (SRSV) in outbreaks of gastroenteritis in the Netherlands. J.Infect.Dis. 174, 610-615, 1996

6. Smiley et al. Reverse transcription-PCR assays for detection of bovine enteric caliciviruses (BEC) and analysis of the genetic relationships among BEC and human caliciviruses. J. Clin. Microbiol. 41, 3089-3099, 2003

7. Van der Poel et al. Epidemiology of Norwalk virus infections in cattle in The Netherlands. Vet. Microb. 92, 297-309, 2003

Metabolic Modelling and Engineering of Micro-Organisms (M^2EMO)

W. Soetaert[1], P. Vanrolleghem[2], M. De Mey[1], J. Maertens[2] & E. Vandamme[1]

[1] Laboratory of Industrial Microbiology and Biocatalysis (LIMAB), Faculty of Bioscience Engineering, Ghent University

[2] Department of Applied Mathematics, Biometrics and Process Control (BIOMATH), Faculty of Bioscience Engineering, Ghent University

M^2EMO is strategic research collaboration between the Department of Applied Mathematics, Biometrics and Process Control (BIOMATH) and the Laboratory of Industrial Microbiology and Biocatalysis (LIMAB) of Ghent University. The aim of the research collaboration is to develop efficient technology for the metabolic engineering of micro-organisms. This includes the development of a mathematical model of microbial metabolism and the tools for genetic modification of micro-organisms. Our model organism is *E. coli* but the technologies developed are applicable to other micro-organisms, particularly bacteria.

The developed model is a dynamic metabolic network model, an appropriate mathematical representation of the metabolic capacities of the cells. Contrary to steady-state models, dynamic metabolic models take into account the regulatory mechanisms and dynamic behaviour of microbial metabolism, leading to superior models with a significantly better predictive power. The model can be used to predict the effects of genetic modification of the production strain, before actually performing the genetic modification in practice. Thus, valuable time and effort is saved for developing highly efficient production strains. The model can also predict and optimize the effect of environmental conditions on the yield of fermentation processes, without performing laboratory experiments.

The long-term objective of this research project is to develop fermentation technology for the biocatalytic synthesis of chemical substances from renewable resources such as wheat, corn or sugar beets. The type case we investigate is the synthesis of succinate from renewable resources, an important base chemical that can serve as an intermediate for plastics, paints, solvents, detergents, etc.

The better knowledge and model of microbial metabolism will also allow to improve the production of more complex molecules such as recombinant proteins for therapeutic or diagnostic purposes. The type example we investigate is the expression of the enzyme β-galactosidase.

In another research topic, we are investigating whether oscillating environmental conditions can lead to better fermentations with higher product yields and productivity. Oscillating environmental conditions can significantly modify the metabolic flow in micro-organisms, thus leading to improved product formation.

Characterization of the murine beta interferon N-glycosylation

Caroline Sommereyns and Thomas Michiels

Christian de Duve Institute of Cellular Pathology, Université Catholique de Louvain, MIPA-VIRO 74-49, 74, avenue Hippocrate, B-1200 Brussels, Belgium

Type I interferons (IFNs-α/β) form a large family of multifunctional cytokines which play an important role in the host antiviral response. Human and mouse genomes contain about 20 genes encoding Type I IFNs, including 13 or 14 IFN-α subtypes, IFN-β, IFN-ε/τ IFN-κ IFN-ω and limitine (6). In spite of important sequence differences, all of these IFNs appear to share a similar three-dimensional structure and to bind the same receptor.

Interestingly, some of these IFNs are N-glycosylated and some are not (5, 6).
IFN-β appears to be N-glycosylated throughout the mammalian class, the number of predicted N-glycosylation sites ranging between one (human) (4) and five (dog) (2). Murine IFN-β has three predicted N-glycosylated sites (1). Interestingly, one of these sites (Asn29) aligns with a Cys residue of IFN-α involved in disulfide bridge formation and occurs in a region reported to be involved in receptor binding (3).

Thus we examined whether the three predicted N-glycosylation sites of murine IFN-β were indeed glycosylated.

We used site-directed mutagenesis to eliminate N-glycosylation sites in IFN-β sequence. Mutants were then compared to the parental form to check their glycosylation status and to compare their activities *in vitro*.

Our data show that the 3 N-glycosylation sites predicted by the sequence of IFN-β, and in particular Asn 29, were indeed glycosylated, but to different extents. Mutation of glycosylation sites or enzymatic deglycosylation of IFN-β resulted in an important loss of activity.
The presence of an N-glycosylation site in one region described to be important for receptor binding is curious. This might indicate that sugar chains are involved in receptor binding or that IFN-β differs from IFN-α in the way it contacts the receptor.

References:

(1) Higashi Y.et al., J.Biol.Chem., 1983, 258(15) : 9522-29. **(2)** Iwata A et al., J.Interferon cytokine Res., 1996, 16(10) : 765-70. **(3)** Piehler J et al., J.Biol.Chem., 2000 Dec.22; 275(51) : 40425-40433. **(4)** Taniguchi T.et al., revue, 1980, 285 (5766) : 547-9. **(5)** van Pesch V. and Michiels T., J.Biol.Chem., 2003, 278 : 46321-46328.
(6) van Pesch V.et al, J.Virol., 2004, 78(15) : 8219-28.

Evaluation of tDNA-PCR for the identification of *Mollicutes*

Tim Stakenborg[*1], *Jo Vicca*[2], *Rita Verhelst*[3], *Patrick Butaye*[1], *Dominiek Maes*[2], *Anne Naessens*[4], *Jos Bogaert*[5], *Geert Claeys*[3], *Catharine De Ganck*[3], *Freddy Haesebrouck*[2], *and Mario Vaneechoutte*[3]

[1] *Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Brussels, Belgium*
[2] *Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[3] *Microbiology & Immunology, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium*
[4] *Department of Microbiology, University of Brussels (VUB) Hospital, Laarbeeklaan 101, 1090 Brussels, Belgium*
[5] *Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium*

A total of 109 strains and DNA-extracts of 33 different species belonging to the genera *Acholeplasma*, *Mycoplasma* and *Ureaplasma* were studied by means of tDNA-PCR and capillary electrophoresis on an ABI310 (Applied Biosystems, Foster City, Ca.). For twelve of these, i.e. one *M. genitalium*, the three *M. gallisepticum* isolates and eight of the 24 *U. urealyticum* cultures, no amplification could be obtained. For all other strains, reproducible peak profiles were generated. Clustering revealed numerous discrepancies compared to the identifications that had been obtained by means of biochemical and serological tests. Final identification was obtained by 16S rRNA gene amplification followed by sequence analysis and/or restriction digestion (ARDRA). This confirmed in all cases the identification obtained by tDNA-PCR. Seven samples yielded an unexpected tDNA-PCR profile. Sequence analysis confirmed these findings, since these samples were mixed or had a very unique 16S sequence that did not match with any of the published sequences. In conclusion, we found tDNA-PCR to be a rapid and discriminative method to correctly identify a large collection of different species of the class of *Mollicutes*.

Characterization of *in vivo* acquired resistance of *Mycoplasma hyopneumoniae* to macrolides and lincosamides

Tim Stakenborg[*1], *Jo Vicca*[2], *Patrick Butaye*[1], *Dominiek Maes*[2], *Aart de Kruif*[2], *Johan Peeters*[1], *Freddy Haesebrouck*[2]

[1] *Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Brussels, Belgium*
[2] *Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*

Macrolides and related antibiotics are used to control mycoplasma infections in the pig industry worldwide. To characterize the mechanism of acquired resistance to macrolides and lincosamides, the phenotype and genotype of a resistant *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) field strain was compared to five susceptible isolates. The minimal inhibitory concentrations (MICs) were significantly higher for the resistant strain for all antibiotics tested. The MICs for the 16-membered macrolide tylosin ranged from 8 to 16 μg for the resistant strain and from 0.03 to 0.125 μg/ml for the five susceptible strains. The MICs for the 15-membered macrolides and lincosamides were higher than 64 μg/ml for the resistant strain while they were only 0.06 to 0.5 μg/ml for the susceptible strains. *M. hyopneumoniae* strains are intrinsically resistant to the 14-membered macrolides due to a G2057A transition (*E. coli* numbering) in their 23S rDNA. Therefore, high MICs were observed for all strains, although the MICs for the resistant strain were clearly increased. An additional, acquired A2058G point mutation was found in the 23S RNA gene of the resistant strain. No differences linked to resistance were found in the ribosomal proteins L4 and L22. The present study showed that 23S rRNA mutations resulting in resistance to macrolides and lincosamides as described in other *Mycoplasma* spp. also occur under field conditions in *M. hyopneumoniae.*

Virus subtype dependent clearance or persistence of Theiler's murine encephalomyelitis in RAW264.7 macrophages

Steurbaut S., Rombaut B. and R. Vrijsen.

Department of Pharmaceutical Biotechnology and Molecular Biology, Vrije Universiteit Brussel, B-1090 Brussels, Belgium, email: ssteurb@vub.ac.be

Within Theiler's murine encephalomyelitis virus (TMEV), a mouse picornavirus, a distinction is made between two subtypes, which are very similar on the basis of nucleotide and amino acid sequences. However, these subtypes cause a very different pathology. The neurovirulent subtype (e.g. strain GDVII) induces an acute and mostly fatal encephalitis. In the few mice that survive, the virus is cleared by the immune system. In contrast, the demyelinating subtype (e.g. strain DA) persists during the animal's life time and induces neuronal demyelination, very similar to multiple sclerosis (MS).

Since macrophages/microglia have been reported to be the viral reservoir for the demyelinating subtype, we are studying the replication of both strains GDVII and DA in the murine macrophage cell line RAW264.7. After initial replication of both strains, as measured by plaque titration, GDVII infectivity rapidly decreased and infectious virus could not be demonstrated anymore after 4 days. The DA strain however, replicated to higher titers and infectious virus was still present after 4 days. In fact, we were able to maintain the DA-infected macrophages at least for 6 months during which the cells were continuously producing virions. The outcome of the in vitro infection thus seems to mimic the in vivo situation. Our data suggest a role for the innate immune system, in particular an interferon type I response, to be responsible for the clearance of the GDVII strain. By inhibiting or limiting this antiviral response, the DA strain could have elaborated a strategy to survive and install a persistent infection which is a prerequisite to induce the inflammatory MS-like lesions in the mouse. Currently we are investigating the interferon as well as the cell death pathway which should yield new insights in the mechanisms of viral clearance and persistence.

Safety issues of recombination between glycoprotein E deleted marker vaccine and wildtype strains of infectious bovine rhinotracheitis virus

Thiry E., Schynts F., Muylkens B., Meurens F. **

Department of Infectious and Parasitic Diseases, Laboratories of Veterinary Virology, University of Liège, Belgium

Control of infectious bovine rhinotracheitis (IBR), a disease caused by bovine herpesvirus 1 (BoHV-1) is performed by intensive vaccination programs in countries which exhibit a high seroprevalence of the infection. In several European countries, glycoprotein gE negative live attenuated marker vaccines are used. They can be administered intranasally at the natural portal of entry of wild-type BoHV-1 virus. It is especially the case in young animals, where the risk of --natural infection is high and where it is appropriate to overcome the interference by colostral antibodies. It can be therefore postulated that situations of double nasal infections can be encountered in natural conditions. This can lead to the production of recombinant viruses. We have therefore examined three issues regarding the potential rise of such recombinant BoHV-1 viruses. The rise of recombinant viruses was studied by *in vitro* and *in vivo* experiments. In cell cultures and in primary calf infections, coinfection with two distinguishable BoHV-1 led to the rise of high amount of recombinant BoHV-1, including recombinants with a gE negative phenotype. However, after reactivation from latency, no gE negative viruses, either parental or recombinant, could be reisolated from calves. However, a situation of coinfection must be rare in natural conditions. We therefore examined the effect of superinfection on recombination. A time interval of 2 to 8 h between two successive infections allows the establishment of a barrier, which reduces or prevents any successful superinfection needed to generate recombinant viruses. The virulence of BoHV-1 obtained after recombination between a gE negative virus and a highly virulent wild type strain was studied *in vitro*. Some of the obtained gE negative recombinants still exhibited a significant virulence by three *in vitro* tests. In conclusion, based on the weak likelihood of coinfections in natural conditions, and on the results obtained in our studies, recombination and its potential adverse consequences must be rare events. However, based on the virulence studies, a single recombinant keeping virulence and acquiring the gE negative genotype is enough to severely impair IBR control programs based on vaccination. Measures should be therefore taken to reduce the risk of recombination in the field.

**FNRS research fellows*

Stimulation of poliovirus synthesis in a HeLa cell-free in vitro translation-RNA replication system by viral protein $3CD^{PRO}$

Bert Thys[1], David Franco[1,2], Harsh Pathak[3], Craig Cameron[3], Eckard Wimmer[2], Aniko V. Paul[2], and Bart Rombaut[1]

[1] Department of Pharmaceutical Biotechnology and Molecular Biology, Vrije Universiteit Brussel, B-1090 Brussels, Belgium. [2] Department of Molecular Genetics and Microbiology, School of Medicine, Stony Brook University, N.N. 11790. [3] Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania 16802. Email: brombaut@vub.ac.be

The positive stranded RNA genome of poliovirus is important in at least three major processes: it acts as mRNA to direct the synthesis of viral proteins; it serves as a template for genome replication; and it is packaged along with structural proteins during viral assembly. An alternative way to investigate those steps in the life cycle of poliovirus is offered by the HeLa cell-free translation-RNA replication system where these processes can be faithfully reproduced to yield viable poliovirus. An unbalance, however, is observed between the different replication steps resulting in an inefficient production of infectious virus compared to the synthesis in infected cells. After programming, the viral RNA is exclusively used as a template for viral translation. It takes hours before there is a switch from protein synthesis to RNA replication. A better balance of protein synthesis and RNA synthesis can be obtained when a mRNA, encoding the viral protein $3CD^{PRO}$, or the purified $3CD^{PRO}$ is added to the translation-RNA replication reactions of poliovirus RNA resulting in an increased virus yield of at least 2 $\log_{10}$. Studies have shown that $3CD^{PRO}$ stimulates virus production at two stages in the in vitro system: (1) there is a 2-3 fold stimulation of RNA synthesis; (2) the formation of 160 S viral particles (mature virions) is enhanced about 50 fold. No effect has been observed on the translation of the polyprotein. Optimal stimulation is observed only when $3CD^{PRO}$ is present early in the incubation period. Two domains of the protein have been identified by mutational studies which are required for its activity to stimulate virus production: an RNA binding site located in the $3C^{PRO}$ domain of $3CD^{PRO}$ and a site at interface I in the $3D^{POL}$ domain of $3CD^{PRO}$. Surprisingly, virus synthesis is strongly inhibited by the addition of both $3C^{PRO}$ and 3CD at the beginning of incubation. The effect of other viral proteins and cellular proteins on virus synthesis was also examined in the in vitro system. No enhancement of virus synthesis was observed with viral proteins 3BC, 3ABC, 3BCD, $3D^{POL}$, or $3C^{PRO}$ or with cellular protein PCBP2.

Distribution of the *Mycobacterium* community among different size fractions of a polycyclic aromatic hydrocarbon contaminated soil

Maarten Uyttebroek[1], Philip Breugelmans[1], Mieke Janssen[1], Boris Joffe[2], Ulrich Karlson[3], Jose-Julio Ortega-Calvo[4], Leen Bastiaens[5], Annemie Ryngaert[5] and Dirk Springael[1]

[1] Laboratory for Soil and Water Management, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium.
[2] Lehrstuhl für Wassergute- und Abfallwirtschaft, Technische Universität München, Am Coulombwall, D-85748 Garching, Germany.
[3] Environmental Chemistry and Microbiology, National Environmental Research Institute, Frederiksborgvej 399, DK-4000 Roskilde, Denmark.
[4] Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, Avenida Reina Mercedes 10, Apartado 1052, E-41080 Seville, Spain.
[5] Environmental Technology, Flemish Institute for Technological Research, Boeretang 200, B-2400 Mol, Belgium.

Fast-growing *Mycobacterium* spp. are often isolated from polycyclic aromatic hydrocarbon (PAH) contaminated soils as degraders of PAHs. Interestingly, *Mycobacterium* spp. are preferentially selected over other PAH degrading bacteria when the PAH is provided in the enrichments sorbed to hydrophobic carriers. Recent research using model systems indicates that close proximity of the *Mycobacterium* cells to the source of contamination might play an important role in efficient degradation of the PAH molecule. This could indicate that in nature, mycobacteria are closely connected to soil particles containing PAHs. However, little is known about the ecological niche of PAH degrading *Mycobacterium* spp. in PAH contaminated soil.

Therefore, a PAH contaminated soil was fractionated by sonication (200 J g^{-1} soil) into 4 particle size fractions (coarse sand, fine sand, loam and clay). *Mycobacterium* specific molecular biological techniques (PCR, DGGE, FISH) were used to examine the distribution of the *Mycobacterium* community among these soil fractions. The PAH degrading capacity of the indigenous microbial community in the soil fractions was examined by mineralization experiments with ^{14}C-phenanthrene and ^{14}C-pyrene. This was related to the physicochemical properties of the soil fractions including the distribution of the PAHs over the soil fractions.

We observed an accumulation of nutrients (PAHs, total C and total N) in the clay fraction (2-0.1 µm) of the examined PAH contaminated soil. An MPN-nested PCR approach for enumeration of *Mycobacterium* in soil showed an accumulation of *Mycobacterium* in this clay fraction. By MPN enumeration of PAH degraders in soil using the respiration indicator WST-1, based on growth on PAHs, we found an accumulation of phenanthrene and pyrene degraders also in this clay fraction. By FISH, we identified an important amount of these degraders as *Mycobacterium*. The indigenous microbial community of this clay fraction showed an immediate phenanthrene and pyrene degrading activity. In conclusion, we propose that the clay fraction of the examined PAH contaminated soil can be seen as ecological niche for propagation of PAH degrading *Mycobacterium* spp.

Microbial ecology of an acidic polycyclic aromatic hydrocarbon contaminated soil from a former gaswork manufacturing plant

Maarten Uyttebroek, Steven Vermeir and Dirk Springael

Laboratory for Soil and Water Management, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium.

Former gaswork manufacturing plants often contain locations contaminated with high concentrations of polycyclic aromatic hydrocarbons (PAHs) and with acidic pH. Two PAH contaminated soils, one with pH 7 and one with pH 2, sampled from a former gaswork plant were analyzed regarding microbial ecology and the presence of PAH utilizing bacteria. The two soils demonstrated different eubacterial communities based on DGGE 16S rDNA fingerprinting. On media with pH 7, heterotrophic CFU obtained for the low pH soil were 2 to 3 log lower than the corresponding CFU for the neutral pH soil. For both soils, CFU on media with lower pH (pH 5 and 3) decreased dramatically with CFU below the detection limit at pH 3. Both from the neutral pH and low pH soil, enrichment cultures were obtained utilizing PAH (pyrene and phenanthrene) as sole source of carbon and energy and this at different pH. From the neutral pH soil, pyrene/phenanthrene utilizing cultures were obtained at pH 5 and 7 while from the low pH soil, phenanthrene/pyrene cultures were obtained growing at pH 7, 5, 3 and 2. No pure strains could be recovered except from the phenanthrene utilizing cultures growing at pH 7 enriched from the neutral pH soil. Utilization of pyrene by the cultures enriched on pyrene was confirmed by mineralization of ^{14}C-pyrene. DGGE analysis of cultures utilizing pyrene and phenanthrene enriched from the low pH soil indicated that they consisted of one bacterial strain and this for all pHs. Interestingly, whereas the pyrene and phenanthrene utilizing cultures contained clearly a different strain, the pyrene utilizing cultures showed the same DGGE pattern for cultures enriched on pH 3, 5 and 7. Phenanthrene utilizing cultures enriched on pH 3 and 5 showed the same pattern but a different pattern for the culture enriched on pH 7.

Typing and toxigenic profile of *Bacillus cereus* strains isolated from a fatal food intoxication in Belgium

Van Coillie, E.[*1], *Swiecicka, I.*[2], *Heyndrickx, M.*[1] , *Mahillon, J.*[3] , *Dierick K.*[4]

[1] *Min. of Flemish Comm., CLO–Ghent, DVK, Brusselsesteenweg 370, B-9090 Melle, Belgium.*
[2] *Dpt. of Microbiology, Univ. of Bialystok, 15 – 950 Bialystok, Swierkowa 20B, Poland*
[3] *MBLA, UCL, Croix Du Sud 2/12, B-1348 Louvain-la-Neuve, Belgium*
[4] *Inst. of Public Health - Food Section, J. Wijtsmanstraat 14, B-1050 Brussels, Belgium*

Introduction:

Bacillus cereus is a spore-forming bacterium that causes two types of food intoxications known as the emetic and the diarroeal types. For the emetic type, a heat-stabile emetic toxin named cereulide, that is preformed in the food, is responsible for the symptoms. Heat-labile enterotoxins, produced in the gut by vegetative cells, cause the diarrhoeal type.

In august 2003, a Belgian family had met with a serious food intoxication, by which several children fell ill and the youngest among them died after some hours. In the vomit of this patient and in various food and food-related products present in the kitchen of the family, *B. cereus* was detected. A total of 21 *B. cereus* strains were isolated from the different sources and subjected to further research in order to track the food product and the agent that had caused the intoxication.

Materials and methods:

- The genetic similarity among the strains was determined by the molecular typing method rep-PCR, using the (GTG)5-primer, and by pulsed-field gelelectrophoresis, using the restriction enzymes *Asc* I and *Not* I.
- To differentiate between *B. cereus*, *B. thuringiensis*, *B. mycoides* and *B. anthracis*, species-specific primers based on the *gyrase* B gene were used.
- Detection of *Bacillus cereus* emetic and enterotoxin production was performed by cell cytotoxicity assays, using Vero cells as toxin-sensitive cells and the WST-1 reagent (Roche Diagnostics) as the detection reagent for cell viability.

Results:

By Rep-PCR, four groups (1-4) and one subgroup (1a) were detected. With the exception of rep-type 2, all rep-types were confirmed as *B. cereus* with species-specific PCR tests within the *B. cereus* group. By PFGE using two different enzymes, four different patterns (A-D) were identified. All three strains isolated from the vomit of the patient showed the same pattern by Rep-PCR (rep-type 1) as well as by PFGE-analysis (PFGE-type C). One of the strains isolated from pasta salad belonged also to rep-type 1 and PFGE-type C. A selection of the strains was subjected to cytotoxicity assays to identify their potential to produce emetic and/or enterotoxins. It was shown that both strains of rep-type 1 (one from the vomit and one from the pasta salad) and the strains from rep-type 4 are capable to produce the emetic toxin. The only strain of rep-type 3 was demonstrated to be able to produce enterotoxins.

Discussion:

These results strongly indicate that at least the *B. cereus* strain isolated from the pasta salad was the causative agent of this severe food intoxication. The implication of the emetic toxin in this toxi-infection is also strongly suspected. Although only a limited number of severe food intoxications caused by the *Bacillus cereus* emetic toxin has been reported, this case illustrates the possible severity of the emetic syndrome. Because the emetic toxin is preformed in the food and can not be inactivated by heat-treatment, it is important to prevent the growth of *Bacillus cereus* and production of emetic toxin during storage.

Conjugative plasmid pAW63 from *Bacillus thuringiensis* and its relation to the *Bacillus cereus sensu lato* gene pool

Géraldine Van der Auwera, Jacques Mahillon

Laboratory of Food & Environmental Microbiology, Université Catholique de Louvain, 2/12 Croix du Sud, 1348 Louvain-la-Neuve, Belgium

The *Bacillus cereus sensu lato* family of Gram-positive bacteria contains six subspecies which are genetically very close but nonetheless have highly specialized lifestyles, especially as concerns their respective virulence spectra. Most notable are *B. cereus sensu stricto*, which is implicated in several human diseases, *B. anthracis,* the etiological agent of anthrax, and *B. thuringiensis* which unlike the first two does not affect mammals, but produces δ-endotoxin crystals in its spores that are toxic to insect larvae. These subspecies are thought to have emerged from a common ancestor following a series of genetic rearrangements mediated *inter alia* by mobile DNA elements (transposons, insertion sequences and phages), in synergy with various mechanisms of horizontal gene transfer (conjugation, transduction, transformation), leading to the acquisition of virulence genes. This is exemplified by the presence of large virulence plasmids in *B. anthracis* (pXO1 and pXO2) and *B. thuringiensis* that carry the genes responsible for the main phenotypic properties by which these bacteria can be distinguished.

While the inter- and intra-molecular movements of mobile elements can obviously have major consequences for the organization and composition of the host genome (insertion effects and ferrying of passenger genes), it is probably the mechanism of conjugation that best enables the dispersion of these elements throughout the gene pool. Several conjugation systems have been described in Gram-negative bacteria, with as leitmotiv the formation of a sex pilus to bring the participants in close contact, followed by the actual transfer of genetic material via a type IV secretion system. There is much less data available concerning conjugation among Gram-positive bacteria, but the present consensus distinguishes four main transfer strategies, the most common of which seems to be that of the so-called wide host spectrum plasmids.

The wide host-spectrum conjugative plasmid pAW63 was identified in *B. thuringiensis* serovar *kurstaki*, where it displays a highly efficient ability to conjugate in liquid medium, for both its own transmission as well as that of small mobilizable plasmids. Moreover, heterologous conjugation experiments have shown that it is also capable of transfer to its cousins *B. thuringiensis* serovar *israelensis* and *B. cereus*, as well as to less closely related species.

Sequencing has led to a functional map of the plasmid, yielding many insights into its conjugative apparatus, which includes T4SS-like components, as well as its resemblance to other large plasmids of Gram-positive bacteria. Of particular interest is the shockingly extensive homology shared between pAW63 and pXO2, the second virulence plasmid of *B. anthracis*. This bioinformatics-driven study, complemented by gene expression analysis and *in vivo* investigation of the conjugative transfer of pAW63 under various conditions, aims to shed new light on the dynamics of the *B. cereus sensu lato* gene pool as well as on the mechanism and regulation of Gram-positive conjugation.

References:

Wilcks, A., Jayaswal, N., Lereclus, D. and Andrup, L. 1998. Characterization of plasmid pAW63, a second self-transmissible plasmid in *Bacillus thuringiensis* subsp. *kurstaki* HD73. Microbiology **144:**1263-1270.

Wilcks, A., Smidt, L., Økstad, O.A., Kolstø, A.-B., Mahillon, J. and Andrup, L. 1999. Replication mechanism and sequence analysis of the replicon of pAW63, a conjugative plasmid from *Bacillus thuringiensis*. J. Bacteriol. **181:**3193-3200.

Multiplex PCR for the Detection of *asa1*, *gelE*, *cylA*, *esp* and *hyl* genes in European Hospital Isolates of *Enterococcus faecium*

V. Vankerckhoven[1], T. Van Autgaerden[1], C. Vael[1], C. Lammens[1], S. Chapelle[1], R. Rossi[2], D. Jabes[2], and H. Goossens[1]

[1]Univ. of Antwerp, Belgium; [2]Vicuron Pharmaceuticals, Gerenzano, Italy

Background

Several virulence factors have been described in *E. faecalis*, such as aggregation substance (*asa*1), gelatinase (*gel*E), cytolysin (*cyl*A), and enterococcal surface protein (*esp*). Recently *esp* and hyaluronidase (*hyl*) were described in *E. faecium*.

Methods:

A total of 271 [153 clinical (C) and 1118 fecal (F)] *E. faecium* isolates were included from at-risk wards of 13 hospitals in 8 European countries. 135 of these strains were vancomycin-resistant (VRE) whereas 136 vancomycin-susceptible (VSE). Five oligonucleotide primers were selected to amplify the genes of *asa1*, *gelE*, *cylA*, *esp* and *hyl*. MIC testing was performed according to NCCLS guidelines. Chi square and Fisher exact were used for statistical analysis. P<0.05 was considered statistically significant.

Results:

Multiplex PCR for the detection of five virulence factors was developed. *Asa1*, *gelE* and *cylA* were not detected. The prevalence of *esp* was significantly higher in C VRE (92 %) versus F VRE (73%) (P= 0.03), and *hyl* was found in 14 % F VRE versus 27 % C VRE isolates (P= 0.09). Prevalence of *esp* and *hyl* are reported in table.

	VRE + VSE (n=271)	VSE (n=136)	VRE (n=135)	Italy		UK		Others	
				VSE (n=19)	VRE (n=96)	VSE (n=14)	VRE (n=28)	VSE (n=103)	VRE (n=11)
% esp	65	53	77*[1]	68	91*[1]	71	50	48	18
% hyl	17	17	16	5	1	29	71*[1]	18	9

*[1] p<0.05, VRE versus VSE

PFGE showed a spread of two center-specific (*esp*-positive) VRE clones in Italy and one (*hyl*-positive) in the UK, all resistant to ampicillin, gentamicin and streptomycin.

Conclusions:

Italy and the UK show high *esp* and/or *hyl* prevalences, particularly among C isolates, of VRE, which is related to the emergence of 3 multi-resistant clones. A changing epidemiology of VRE in these European countries towards a more US pattern might be evolving.

Keywords: multiplex PCR, *E. faecium*, virulence

Contact: Vanessa Vankerckhoven, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium, T/F: +32 3 820 26 63, Email: vanessa.vankerckhoven

Inventory and identification of lactic acid bacteria used as probiotics

V. Vankerckhoven[1], T. Van Autgaerden[1], G. Huys[2], M. Vancanneyt[3], J. Swings[2,3] and H. Goossens[1]

[1]Univ. of Antwerp, [2]Laboratory of Microbiology, [3]BCCM/LMG Bacteria Collection, Ghent Univ., Belgium

Objectives

Within the framework of the European project PROSAFE – Biosafety evaluation of lactic acid bacteria used for human consumption – we made an inventory of commercial probiotic strains, verified their identification and collected relevant information.

Methods

Fifty-four companies involved in the production and/or distribution of probiotics were invited to submit their strains and to complete a questionnaire. Species identification of the strains was verified using Amplified Fragment Length Polymorphism (AFLP), repetitive DNA element (rep)-PCR fingerprinting, and protein profiling.

Results

Of the 54 companies contacted, 26 submitted their strains, 14 claimed not to manufacture probiotics and were therefore excluded from the survey, 2 did not wish to participate, and 12 (mostly US companies) did not respond. All 26 participating companies returned the questionnaire. In total 202 strains were submitted and received as *Lactobacillus* (54.0%), *Bifidobacterium* (26.7%), *Enterococcus* (5.9%), *Propionibacterium* (5.9%), *Lactococcus* (2.5%), *Pediococcus* (2.0%), *Streptococcus* (2.0%), *Bacillus* (0.5%) and *Oenococcus* (0.5%). The most frequently used identification techniques included biochemical characterisation (34.9%), DNA fingerprinting (21.8%) and 16S/23S rDNA sequencing (20.8%). Comparison with our current identification results for 174 strains, the identity of 17.2% of these strains did not correspond to the identification by the company. Out of 202 strains, 53.5% are of human origin, 44.5% of non-human origin, whereas for 2.0% the source of isolation is unknown. Two strains were submitted as genetically engineered. 46.5% of the strains are used for human consumption, 5.4% for animal use, 7.0% for both human and animal use, 7.4% were categorised as probiotic, 5.0% are industrial starters and 28.7% are still under investigation.

Conclusions

A large number of strains received from the contacted companies was correctly identified and the majority belonged to the genera *Lactobacillus* and *Bifidobacterium*. More than half of the strains are of human origin, and about the same number is used for human consumption.

Keywords: lactic acid bacteria, probiotics, identification

Contact: Vanessa Vankerckhoven, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium, T/F: +32 3 820 26 63, E: vanessa.vankerckhoven@ua.ac.be

Internalization of pseudorabies gB is mediated by an interaction between the YQRL motif in its cytoplasmic domain and the clathrin associated AP-2 adaptor complex

Geert Van Minnebruggen[1], Herman W. Favoreel[1, 2], and Hans J. Nauwynck[1]

[1]Laboratory of Virology and [2]Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium.

The gB envelope glycoprotein of the swine alphaherpesvirus pseudorabies virus (PRV) has been shown to undergo spontaneous endocytosis during the early phases of infection and to mediate internalization of antibody-antigen complexes from the surface of infected cells. The latter process protects PRV-infected cells from efficient antibody-dependent lysis and also depends on viral protein gD.

The cytoplasmic domain of PRV gB contains three putative internalization motifs: two tyrosine-based YXXϕ motifs and one dileucine (LL) motif. Previously, we have demonstrated that the tyrosine-based YQRL motif at position 902-905, but not the YMSI motif at position 864-867 and the LL doublet at position 887-888, is a functional endocytosis motif and is required for efficient gB-mediated internalization of antibody-antigen complexes from the surface of PRV-infected cells.

In the current study, we demonstrated that the YQRL motif is also crucial to allow spontaneous internalization of PRV gB during the early phases of infection, and thus that both types of PRV gB-mediated internalization occur through closely related mechanisms. Further, we found that, during internalization, PRV gB co-localizes with the cellular clathrin-associated AP-2 adaptor complex, and that this co-localization depends on the YQRL motif. In addition, by co-immunoprecipitation assays, we found that, during both spontaneous and antibody-mediated internalization, PRV gB physically interacts with AP-2, and that efficient interaction between gB and AP-2 is critically dependent on the integrity of the YQRL motif.

Collectively, these findings for the first time demonstrate that during internalization of an alphaherpesvirus envelope protein, i.e. PRV gB, a specific amino acid sequence in its cytoplasmic domain interacts with the clathrin-associated AP-2 complex, leading to the formation of AP-2-containing clathrin-coated internalization vesicles. Since similar motifs are known to drive internalization of many different alphaherpesvirus envelope proteins (PRV gD and gE, HSV gB and VZV gB, gE, gI and gH), a common AP-2-dependent mechanism may perhaps be used by different alphaherpesviruses to achieve internalization of envelope proteins from the cell suface of infected cells.

Characterization of the microbial ecology in the surroundings of an iron barrier

T. Van Nooten, B. Borremans, J. Dries, K. Vanbroekhoven and L. Bastiaens (Vito, Boeretang 200, 2400 Mol, Belgium) and D. Springael (KULeuven, Kasteelpark Arenberg 20, 3001 Heverlee Belgium)

The use of zero-valent iron in reactive barriers has been shown to be very effective for passive, long-term applications of groundwater remediation. Because the contaminants are removed by abiotic processes, little is known about the microbial activity and characteristics within and in the vicinity of the Fe^0-barrier matrix. Major uncertainties need to be resolved with respect to the adaptation of indigenous micro-organisms to the strongly reducing Fe^0 environment, changes in the microbial community composition, and their beneficial or detrimental effects on the longevity and long-term efficiency of the Fe^0 barriers. On the one hand, the accumulation of biomass, the production of gas bubbles, and the formation of mineral precipitates can have a negative impact on the reactivity of the barrier by blocking reactive sites. On the other hand, micro-organisms can positively affect the performance of the barrier by contributing to the degradation of contaminants, by consuming abiotically produced gas bubbles, and by contributing to mineral dissolution. The depletion of dissolved oxygen and the production of cathodic H_2 by Fe^0 corrosion provide a reducing environment favorable to a wide variety of hydrogen consuming anaerobic micro-organisms. These include sulfate- and metal-reducing bacteria, methanogens, and denitrifying bacteria within and downgradient of the barrier.

The objective of this study is to examine the microbial population in reactive iron barriers and to get a clearer view on the different groups of microorganisms that are present. Molecular methods including PCR and PCR-DGGE are being used for this purpose. These techniques have been applied on samples of lab-scale experiments. In the near future, samples from pilot-scale, or *in situ* reactive iron barriers will be examined.

Microbial diversity study of a nitrifying enrichment culture

Bram Vanparys[1], Kim Heylen[1], Nico Boon[2], W. Verstraete[2] & Paul De Vos[1]*

[1] Laboratory of Microbiology (WE10), Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium;
[2] Laboratory of Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
** Corresponding author: tel: 09/264.51.01, fax: 09/264.53.46, email: Bram.Vanparys@Ugent.be*

To optimize nitrification in aquacultures and aquaria, microbial inocula obtained after enrichment of nitrifiers may be used. In this study, the microbial community of one of these Nitrifying Enrichment Cultures or NEC's was studied using a combination of culture dependent and culture independent techniques. Since the studied NEC was developed by an enrichment of the autotrophic nitrifying community from natural surface water without addition of organic carbon, this study can give insights on the influence of low nutrient inflow on nitrifying communities and on which organisms can be favourable in obtaining a stable, highly active nitrifying consortium. On one hand a 16S rDNA clone library was generated, which showed *Nitrosomonas, Nitrobacter, Methylosinus, Hyphomicrobium, Rhodobacter*, and *Porphyrobacter* as most dominant genera. The total species richness was determined to be between 46 and 107 species. On the other hand a culture campaign using eight different media was set up, whereby members of more than 30 genera were retrieved. In addition, several isolates generated nearly complete 16S rDNA sequences with similarity values below 97 % versus known species, and probably represent new species. These isolates are studied in more detail at the moment.

Expression of virulence genes by *Listeria monocytogenes* strains of different origin and interactions with different human cell types

Werbrouck H.[1], Herman L.[1], Botteldoorn N.[1], Grijspeerdt K.[1], Rijpens N.[1], Van Damme J.[2] and Van Coillie E.[1]

[1]*Ministry of the Flemish Community, Department Animal Product Quality and Transformation Technology, Agricultural Research Centre-Ghent, Brusselsesteenweg 370, 9090 Melle, Belgium. E-mail: H.Werbrouck@clo.fgov.be*

[2]*Laboratory of Molecular Immunology, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium.*

Listeria monocytogenes has the capacity of causing severe infections in susceptible hosts. The pathogen is widely distributed in nature and generally transferred to humans by contaminated food. Despite the fact that many food products are contaminated with *L. monocytogenes*, the incidence of listeriosis is relatively low. Many studies suggest that beside the infective dose, the serotype and the host immunity, the virulence of the *L. monocytogenes* strain plays an important role in development of listeriosis.

The objective of this study was to track differences in virulence between *L. monocytogenes* isolates of different origin. Therefore, two groups of *L. monocytogenes* isolates, one including 27 clinical strains and the other including 27 food and environmental strains ("non-clinical" strains), were compared at different levels. On the one hand, the mRNA expression levels of specific virulence genes (e.g. *inlB* and *ami*, both important for invasion) were analysed by Real-Time RT-PCR. On the other hand, the *in vitro* invasion capacity for various human cell types (monocytic THP-1 cells and HepG2 liver cells) and the possibility to induce the production of the pro-inflammatory cytokine interleukin-8 (IL-8), were determined.

A statistical significant difference was observed in *inlB* expression ($p < 0.001$) between clinical and non-clinical *L. monocytogenes* strains, with the clinical strains showing a lower *inlB* expression level than the non-clinical strains. No significant difference was noticed for the *ami* expression. These observations were in accordance with *in vitro* invasion of HepG2 liver cells, where a higher invasion capacity of the "non-clinical" strains was detected compared to the clinical strains. Furthermore, it was shown that the "non-clinical" strains induce higher IL-8 levels in HepG2 cells than the clinical strains. In rather sensitive human monocytic THP-1 cells, no significant difference was observed in invasion capacity and IL-8 induction between clinical and "non-clinical" isolates.

The results of this study indicate that a differential expression of specific virulence genes such as *inlB* can play a role in the virulence capacity of *L. monocytogenes* strains.