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

ABSTRACT BOOK OF THE SYMPOSIUM

TOWARDS UNDERSTANDING MICROBIAL INTERACTIONS

Friday, November 9TH, 2001

Louvain-la-Neuve

Under auspices of Académie royale des Sciences de Belgique,
Koninklijke Vlaamse Academie van België voor Wetenschappen,
FNRS,
D. Collen Research Foundation vzw

TOWARDS UNDERSTANDING MICROBIAL INTERACTIONS

Programme

9h00 Registration

9h15 Welcome address

9h25 **Taylor MJ.,** University of Liverpool, U.K.
"Interaction between **Wolbachia** ***and Nematodes"***

10h10 **Fonty G.,** Clermont-Ferrand, INRA, France
"Interactions between Micro-organisms in the Rumen ecosystem"

10h55 Coffee

Short communications of selected posters

11h15 **Karen Van der Meulen** Lab. Virology, Fac. Vet. Med., Ghent University
Absence of viral antigens and MHC-1 on the plasma membrane of EHV_1-infected PBMC in vitro: a mechanism of immune evasion?

11h30 ***Victor Ladero*****,** U.C.L. Unité de Génétique. Louvain-la-Neuve
Metabolic engineering of **Lactobacillus plantarum** ***for polyol production.***

11h45 Alan **Fauconnier,** *Nivelles Laboratoires ASBL, 24 rue de l'industrie, 1400 Nivelles*
Herpetamonas muscarum**, a trypanosomatid "bien de chez nous".**

12h00 General assembly of the Belgian Society of Microbiology

12h15 Lunch and poster session

13h30 Poster discussion groups A+D

14h15 Poster discussion groups B+C

coffee

15h00 ***Shapiro JA****.,* Department of Biochemistry and Molecular Biology,University of Chicago, Illinois, USA
"Thinking about bacterial populations as multicellular organisms"

15h45 ***Baranowski E.,*** Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain,
"Interaction between foot-and-mouth disease virus and its hosts"

16h30 General conclusion

16h45 End of symposium

Acknowledgment

This symposium is organised by the Belgian Society for Microbiology in collaboration with the National Committee for Microbiology

Financial support has been received from

Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique

D. Collen Research Foundation

Fonds National de Recherche Scientifique

Koninklijke Vlaamse Academie van België voor Wetenschappen en Kunsten

ABSTRACTS

ORAL COMMUNICATIONS

THE INTERACTION OF *WOLBACHIA* WITH FILARIAL NEMATODES

Mark Taylor
Cellular Immunology Laboratory, Division of Molecular Biology and Immunology, Liverpool School of Tropical Medicine, Liverpool, UK.

Filarial nematodes are parasitic worms, which cause some of the most devastating of all tropical diseases including elephantiasis and riverblindness. Or so we thought until recent research revealed that the real culprit is a symbiotic bacterium hidden inside of the nematode. Nematode *Wolbachia* are most closely related to the endosymbionts of arthropods, famous for their manipulation of host reproduction. In contrast to the facultative association of *Wolbachia* in arthropods, nematode endosymbionts appear to have evolved as essential symbionts of their hosts. Antibiotic treatment of filarial nematodes shows that the bacteria are essential for normal fertility and development of the worm and may even protect the parasites from the immune system. To date we know almost nothing of the molecular interaction between bacteria and nematode, although the genome sequencing of symbionts from two of the human filarial parasites will undoubtedly open the door to some of these secrets. In addition to uncovering a fascinating symbiotic relationship, this discovery means we can now consider using antibiotics as a new approach to the treatment of these debilitating diseases.

References

Taylor MJ and Hoerauf A (2001) A new approach to the treatment of filariasis. Current Opinion in Infectious Diseases. (in press)

Taylor MJ, Cross HF, Ford L, Makunde WH, Prasad GB, Bilo K. (2001). *Wolbachia* bacteria in filarial immunity and disease. Parasite Immunology 23(7), 401-9.

Taylor MJ. (2001). One in the eye for river blindness. Trends in Microbiology. 9(7), 310.

Taylor MJ. (2000). *Wolbachia* bacteria of filarial nematodes in the pathogenesis of disease and as a target for control. Transactions of the Royal Soceity for Tropical Medicine and Hygiene. 94(6), 596-8.

Taylor MJ, Bandi C, Hoerauf AM, Lazdins J. (2000). *Wolbachia* bacteria of filarial nematodes: a target for control? Parasitology Today. 16(5), 179-80.

Taylor MJ, Cross HF, Bilo K. (2000). Inflammatory responses induced by the filarial nematode *Brugia malayi* are mediated by lipopolysaccharide-like activity from endosymbiotic *Wolbachia* bacteria. Journal of Experimental Medicine. 191(8), 1429-36.

Taylor MJ and Hoerauf A. (1999). *Wolbachia* bacteria of filarial nematodes. Parasitology Today. 15(11), 437-42.

MICROBIAL INTERACTIONS IN THE RUMEN

Gérard FONTY
Unité de Microbiologie INRA Centre de Recherches de Clermont-Ferrand
Theix 63122 Saint-Genès-Champanelle - FRANCE

Ruminants and other herbivores occupy a unique ecological niche among mammals in the sense that they can use green plants and forages as their sole food source. Feed compounds, in particular plant structural polysaccharides (cellulose, hemicelluloses, pectins) are degraded and fermented in the rumen by a complex and very diverse microbial community into products (volatile fatty acids: acetate, propionate, butyrate), that the animal host can assimilate. This community is composed of numerous species of strictly anaerobic bacteria, fungi, protozoa and archaea organized in a trophic chain (fibrolytic, fermentative and hydrogenotrophic microorganisms).
Throughout evolution, a web of complex interactions has been established between the various microbial species forming this ecosystem. All the modes of life (synergism, mutualism, antagonism, amensalism, predation, etc.) are found in the rumen. The diversity of the microbial community and the diversity of the interrelationships between the microbes ensure both the stability of the ecosystem and the efficiency of the digestion. Microbial interactions play a major role in adaptations to different feed or changes in rations or other factors. Most of the interactions described up to now have been studied *in vitro* in cocultures and their mechanism explained [1,2]. Some of them have also been studied *in vivo*, in gnotobiotically-reared animals [3,4]. The presentation will detail some examples of positive and negative interactions which characterize the rumen ecosystem and which are of great importance for the animal host. The paper will particularly focuses on :

1. syngergism and competition among the fibrolytic microoganisms in cellulose breakdown
2. nutritional complementarity between fibrolytic microorganisms and fermentative bacteria
3. cross-feeding between cellulose-degrading bacteria and nitrogen- metabolising species
4. interactions in propionate formation
5. hydrogen transfer in methane production
6. predation by ciliate protozoa

Optimization of the rumen fermentations in the most favorable direction for both the animal host and the environment requires a better understanding of the microbial relationships existing in the rumen ecosystem. Utilization of the new molecular biology techniques will probably allow in the near futur the rapid improvement of our knowledges of the ecology of the rumen microorganisms.

1. Wolin MJ, Miller TL and Stewart CS. 1997. In « The Rumen Microbial Ecosystem » p. 466-489. Hobson PN and Stewart CS (Eds). Blackie Academic and Professional, London.
2. Dehority BA. 1993. In « Forage cell wall structure and digestibility ». ASA-CSSA-SSSA, Madison, USA
3. Hobson PN, and Fonty G. 1997. In « The Rumen Microbial Ecosystem » p. 661-684. Hobson PN and Stewart CS (Eds). Blackie Academic and Professional, London.
4. Fonty G, Williams AG, Bonnemoy F, Morvan B, Withers SE, and Gouet P. 1997. Anaerobe, 3, 383-389

THINKING ABOUT BACTERIAL POPULATIONS AS MULTICELLULAR ORGANISMS

James A. Shapiro,
University of Chicago, Department of Biochemistry and Molecular Biology, Cummings Life Sciences Center, IL 60637-4931, USA

Although most academic thinking has focussed on the autonomous single cell as the conceptual basis for understanding bacterial action, the truth is that bacteria operate almost always as multicellular populations. There are many examples where bacterial multicellularity provides important (often essential) benefits to the population and its component cells. Examination of even the most basic laboratory situation, colonies growing on an agar substrate, reveals extensive multicellular organization and differentiation. Using microscopy and time-lapse recording reveals the collective, interactive nature of of *E. coli* colony development. The use of intercellular signaling molecules in bacterial populations has been known for over 50 years. Molecular analysis shows that intercellular chemical signals are part of complex intra- and extra-cellular control circuits that govern a wide variety of bacterial functions that range from DNA uptake to virulence factor expression and sporulation. These circuits allow individual cells to evaluate their position in the larger population and integrate cellular functions to adaptive multicellular phenotypes.

INTERACTION BETWEEN FOOT-AND-MOUTH DISEASE VIRUS AND ITS HOSTS

E. Baranowski, C.M. Ruiz-Jarabo, N. Pariente, J.I. Núñez, F. Sobrino and E. Domingo.

Centro de Biología Molecular "Severo Ochoa", UAM, and CISA-INIA, Valdeolmos, Madrid.

The recent outbreak of foot-and-mouth disease virus (FMDV) in Europe has raised questions about the adequacy of measures implemented to cope with the emergence and re-emergence of human and animal diseases (Sobrino and Domingo, 2001). For successful protection against highly heterogeneous and fast evolving RNA viral populations, vaccines must match the antigenic properties of the circulating viruses, and include multiple virus-specific B-cell and T-cell epitopes in order to minimise selection of variant viruses (Domingo *et al*., 2001). Despite the success of whole-virus inactivated polyvalent vaccines classically employed for FMD control, the European Union adopted in 1991 a non-vaccination policy owing to evidence that a few outbreaks were associated with vaccine strains presumably due either to incomplete inactivation of infectivity or to escape of virus from vaccine factories (Sobrino *et al*., 2001). In the context of a global economy with open markets and frequent exchanges of goods over long distances, there is a need to understand antigenic variation of FMDV and other RNA viral pathogens, its molecular basis and its application to vaccine development and provision.
Work with FMDV and other picornaviruses has provided evidence that changes in antigenicity could be linked to modifications in receptor usage and cell tropism (Baranowski *et al*., 2001a). Although RGD-dependent integrins are probably the major class of receptor molecules employed by FMDV *in vivo* (Berinstein et al., 1995; Neff *et al.,* 1998; Jackson *et al*., 2000), viruses propagated in cell culture can render integrin-binding dispensable for infectivity and exploit alternative pathways to recognize and enter cells (Jackson et al., 1996; Sá-Carvalho *et al*., 1997; Fry *et al*., 1999; Baranowski *et al*., 2000). The capacity to develop and use multiple alternative receptors for entry even into the same cell type confers to FMDV the potential to modulate receptor usage in response to selective constraints (Baranowski *et al*., 2000), and has considerable implications for the evolution of virus antigenicity. Since the Arg-Gly-Asp (RGD) triplet located at the surface G-H loop of capsid protein VP1 is a key part of several epitopes recognized by neutralizing antibodies (Verdaguer *et al.*, 1995), dispensability of the RGD integrin-binding motif for cell entry greatly expanded the repertoire of antigenic variants of FMDV, and prompted the isolation of viable mutants with profoundly altered antigenicity that contained mutations at the RGD triplet (Martínez *et al.*, 1997; Ruiz-Jarabo *et al*., 1999). Accessibility of receptor-binding site to neutralizing antibodies suggests the possibility of a co-evolution of virus antigenicity and receptor usage (Baranowski *et al.*, 2001a).

The genomic changes that can endow FMDV with the capacity to use alternative mechanism of cell recognition are minimal (Baranowski *et al*., 2001a), and viruses with unusual receptor-binding specificities are likely to be present in the mutant spectrum of FMDV replicating in the animal host. Evidence came from experiments with cattle immunized with synthetic peptides representing B-cell and T-cell epitopes, including the RGD-containing G-H loop sequence (Taboga *et al.*, 1997). Peptides conferred only partial protection, and animals challenged with virulent virus developed lesions. Direct sequencing using viral genomes from lesions revealed unusual amino acid replacements affecting the RGD motif, or positions known to be critical for binding to some RGD-dependent integrins. The antigenic alterations produced by these replacements suggest that the emergence of these particular FMDV mutants *in vivo* is the result of selection of antigenic variants that escaped neutralization by anti-FMDV antibodies in peptide-vaccinated cattle (Taboga *et al*., 1997; Baranowski *et al*., 2001a).

Little is known of changes in cell tropism and antigenicity accompanying changes in host range in nature, but there is no reason to rule out that emerging and re-emerging FMDVs

could be endowed with such unusual biological properties. A recent study analysing the genetic changes selected during adaptation of FMDV to guinea pig, documented the progressive dominance of an unusual amino acid replacement affecting the antigenic structure of the G-H loop of capsid protein VP1 in the course of adaptation of FMDV to this new host (Núñez *et al.*, 2001). Construction of infectious cDNA clone of FMDV confirmed that this mutation was essential for virus interaction with integrin receptor molecules expressed in BHK-cells and various other cell lines commonly used to propagate FMDV. The isolation from two different animal species of FMDV mutants displaying altered cell tropism in association with antigenic changes illustrates the important adaptive potential of FMDV, and the capacity of this virus to explore new antigenic/receptor recognition structures upon replication in the host.

In view of the re-emergence of FMD in several Asian and European countries (Samuel and Knowles, 2001), the non-vaccination policy in the EU could be reconsidered. If new vaccines were needed, FMDV mutants defective in integrin recognition could be the basis of an inactivated vaccine. We have recently shown that dispensability of the RGD triplet in variant FMDVs can be extended to surrounding amino acid residues that are part of an important antigenic site of the virus termed antigenic site A in FMDV of serotype C (Baranowski *et al.*, 2001b). The possibility of producing viable viruses lacking antigenic site A may find application in the design of marker vaccines against FMD. If the virus were highly attenuated for the natural hosts of FMDV, dangers inherent to handling of live virus or its incomplete inactivation, would be greatly diminished, while preserving the capacity to distinguish infected from vaccinated animals, which is important for economic considerations. Evaluation of the infectivity of FMDVs lacking antigenic site A are in progress.

Baranowski, E., Ruiz-Jarabo, C. M., and Domingo, E. (2001a). Evolution of cell recognition by viruses. *Science* 292, 1102-5.

Baranowski, E., Ruiz-Jarabo, C. M., Lim, F., and Domingo, E. (2001b). Foot-and-mouth disease virus lacking the VP1 G-H loop: the mutant spectrum uncovers interactions among antigenic sites for fitness gain. *Virology*, in press.

Baranowski, E., Ruiz-Jarabo, C. M., Sevilla, N., Andreu, D., Beck, E., and Domingo, E. (2000). Cell recognition by foot-and-mouth disease virus that lacks the RGD integrin-binding motif: flexibility in aphthovirus receptor usage. *J. Virol.* 74, 1641-7.

Berinstein, A., Roivainen, M., Hovi, T., Mason, P. W., and Baxt, B. (1995). Antibodies to the vitronectin receptor (integrin $\square_V \square_3$) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. *J. Virol.* 69, 2664-2666.

Domingo, E., Biebricher, C., Eigen, M., and Holland, J. J., (2001). *Quasispecies and RNA virus evolution: principles and consequences*. Landes Bioscience, Austin, TX.

Fry, E. E., Lea, S. M., Jackson, T., Newman, J. W., Ellard, F. M., Blakemore, W. E., Abu-Ghazaleh, R., Samuel, A., King, A. M., and Stuart, D. I. (1999). The structure and function of a foot-and-mouth disease virus- oligosaccharide receptor complex. *EMBO J.* 18, 543-54.

Jackson, T., Ellard, F. M., Ghazaleh, R. A., Brookes, S. M., Blakemore, W. E., Corteyn, A. H., Stuart, D. I., Newman, J. W., and King, A. M. (1996). Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparan sulfate. *J. Virol.* 70, 5282-7.

Jackson, T., Sheppard, D., Denyer, M., Blakemore, W., and King, A. M. (2000). The epithelial integrin alphavbeta6 is a receptor for foot-and-mouth disease virus. *J. Virol.* 74, 4949-56.

Martínez, M. A., Verdaguer, N., Mateu, M. G., and Domingo, E. (1997). Evolution subverting essentiality: dispensability of the cell attachment Arg-Gly-Asp motif in multiply passaged foot-and-mouth disease virus. *Proc. Natl. Acad. Sci. U S A* 94, 6798-802.

Neff, S., Sá-Carvalho, D., Rieder, E., Mason, P. W., Blystone, S. D., Brown, E. J., and Baxt, B. (1998). Foot-and-mouth disease virus virulent for cattle utilizes the integrin $\square(v)\square 3$ as its receptor. *J. Virol.* 72, 3587-3594.

Núñez, J. I., Baranowski, E., Molina, N., Ruiz-Jarabo, C. M., Sanchez, C., Domingo, E., and Sobrino, F. (2001). A single amino acid substitution in nonstructural protein 3A can mediate adaptation of foot-and-mouth disease virus to the guinea pig. *J. Virol.* 75, 3977-83.

Ruiz-Jarabo, C. M., Sevilla, N., Dávila, M., Gomez-Mariano, G., Baranowski, E., and Domingo, E. (1999). Antigenic properties and population stability of a foot-and-mouth disease virus with an altered Arg-Gly-Asp receptor-recognition motif. *J. Gen. Virol.* 80, 1899-909.

Sá-Carvalho, D., Rieder, E., Baxt, B., Rodarte, R., Tanuri, A., and Mason, P. W. (1997). Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. *J. Virol.* 71, 5115-5123.

Samuel, A. R., and Knowles, N. J. (2001). Foot-and-mouth disease virus: cause of the recent crisis for the UK livestock industry. *Trends in Genetics* 17, 421-424.

Sobrino, F., and Domingo, E. (2001). Foot-and-mouth disease in Europe. *EMBO Reports* 2, 459-461.

Sobrino, F., Saíz, M., Jimenez-Clavero, M. A., Nunez, J. I., Rosas, M. F., Baranowski, E., and Ley, V. (2001). Foot-and-mouth disease virus: a long known virus, but a current threat. *Vet. Res.* 32, 1-30.

Taboga, O., Tami, C., Carrillo, E., Núñez, J. I., Rodriguez, A., Saíz, J. C., Blanco, E., Valero, M. L., Roig, X., Camarero, J. A., Andreu, D., Mateu, M. G., Giralt, E., Domingo, E., Sobrino, F., and Palma, E. L. (1997). A large-scale evaluation of peptide vaccines against foot-and-mouth disease: lack of solid protection in cattle and isolation of escape mutants. *J. Virol.* 71, 2606-14.

Verdaguer, N., Mateu, M. G., Andreu, D., Giralt, E., Domingo, E., and Fita, I. (1995). Structure of the major antigenic loop of foot-and-mouth disease virus complexed with a neutralizing antibody: direct involvement of the Arg-Gly-Asp motif in the interaction. *EMBO J.* 14, 1690-1696.

ABSTRACTS OF

POSTERS AND SHORT COMMUNICATIONS

- **List is ordered alphabetically**
- **Posters are grouped according to topics:**
  - **A. Environment/ Food**
  - **B. Detection, identification, typing, taxonomy**
  - **C. General microbiology, host-pathogen interaction - Bacteria**
  - **D. Host-pathogen interaction - Viruses**

- **Poster abstracts selected for oral communication**

a. Van der Meulen, K.M., Nauwynck, H.J. & Pensaert, M.B. Absence of viral antigens and MHC-1 on the plasma membrane of EHV_1-infected PBMC *in vitro*: a mechanism of immune evasion? (P25)

b. Ladero, V., Kleerebezem, M., Delcour, J. and Hols, P. Metabolic engineering of *Lactobacillus plantarum* for polyol production. (P9)

c. Dujardin, J.-C., Fauconnier, A., Pichon, B., Le Ray, D. and Couvreur, B. *Herpetamonas muscarum*, a trypanosomatid “bien de chez nous”. (P3)

1. **(B)** De Clerck, E., Vanhoutte, T., De Vos, P. Isolation and characterisation of *Bacillus* contaminants in gelatine samples which are rejected for consumption.
2. **(A)** E Doucet, D., Weyns, J. and Bragard, C. Diversity in *indian peanut clump* reference serotypes strains and soils isolates.
3. **(C)** Dujardin, J.-C., Fauconnier, A., Pichon, B., Le Ray, D. and Couvreur, B. *Herpetamonas muscarum*, a trypanosomatid "bien de chez nous".
4. **(B)** Dumon, I., Werbrouck, H., Wiszniewska, A.,§ D'Haese, E., Nelis, H., Fus, M. and Herman, L. Detection of viable *Mycobacterium avium subsp. paratuberculosis* cells in raw and pasteurized milk.
5. **(D)** Favoreel, H.W., Van Minnebruggen, G., Nauwynck, H.J., Enquist, L.W. & Pensaert M.B. A tyrosine-based motif in the PRV GB cytoplasmic tail is important for both cell-to-cell spread of the virus and antibody-induced internalization of viral glycoproteins.
6. **(C)** Galloy, C., Vanhooff, V., Delcour, J. and Hallet, B. Interaction between the catalytic and regulatory sites of TN*4430* TNPI/IRS site-specific recombination complex.
7. **(C)** Grossiord, B.P., Fontaine, L., Hols, P. and Delcour J. Analysis of the two component systems (TCS) identified by genome sequencing of the thermophilic lactic acid bacterium *Streptococcus thermophilus* LMG18311.
8. **(A)** Jaouani, A., Vanthournhout, M. and Penninckx, M. Screening of fungi for olive oil mill wastewater treatment**.**
9. **(A)** Ladero, V., Kleerebezem, M., Delcour, J. and Hols, P. Metabolic engineering of *Lactobacillus plantarum* for polyol production.
10. **(C)** Lebeau, I., Lammertyn, E., De Buck, E., Van Mellaert L. and Anné, J. Characterisation of *lpnR* 259/6, a gene that encodes a LuxR-homologue in *Legionella pneumophila*.
11. **(B)** Loens, K., Beck, T., Sillekens, P., Overdijk, M., Ursi, D., Ieven, M. and Goossens, H. Detection of *Legionella pneumophila* using real-time NASBA.
12. **(B)** Loens, K., Dirven, K., Ursi, D., Wouters, H., Ieven, M. and Goossens, H. Detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* by PCR in lower respiratory tract infections in outpatients.
13. **(B)** Loens, K., Dirven, K., Wouters, H., Ursi, D., Ieven, M. and Goossens, H. Detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* by PCR in respiratory specimens from patients hospitalised with a community acquired pneumonia.
14. **(A)** Mergeay, M., and Glansdorff, N. Nickel-resistant *Vibrionaceae* from deep-sea hydrothermal sources of the eastern pacific rise (N 12°45' W 103°59').
15. **(B)** Meunier, A., Schmit, J.F., Stas, A., Bragard, C. Multiplex RT-PCR for detection of Beet necrotic yellow vein virus, Beet soil-borne virus, Beet virus Q and their vector, P*olymyxa betae*.
16. **(C)** Michelet, N., Wauthier, N., Van Vooren, A., and Mahillon, J. Cereolysin O from *Bacillus cereus sensu lato*: distribution, diversity and potential role.

17. **(C)** Palumbo, E., Hols, P., Kleerebezem, M., Leer, R., Boonaert, C.J.P. and Delcour, J. Genetic study of the functional role of D-alanine substitutions of teichoic acids in *Lactobacillus plantarum*.

18. **(C)** Schaerlaekens, K., Schierova, M., Lammertyn, E., Geukens, N., Van Mellaert, L. and Anné, J. The twin-arginine translocation pathway in *Streptomyces lividans*.

19. **(B)** Schmit, J.F., Meunier, A., Bragard, C. Development of a quantitative competitive RT-PCR for the quantification of the virus of rhizomania in roots of sugar beet.

20. **(D)** Schynts, F., McVoy, M., Meurens, F., Epstein, A., Thiry, E. Low levels of tail-to-tail junctions are present in concatemeric DNA of bovine herpesvirus 1.

21. **(B)** Storms, V., Vaneechoutte, M., Willems, A., Haesebrouck, F., Verschraegen, G. and De Vos, P. Single base C-sequencing for the differentiation and identification of members of the *genera Corynebacterium* and *Arcanobacterium*.

22. **(A)** Toussaint, A., Springael, D., Merlin, C., Wyndham, C., Boucher, C. and Mergeay, M. The TN*4371* transposon from *R.eutropha*, a member of a new family of mobile elements?

23. **(A)** Vallaeys, T.,Wattiez, R., Noel-Georis, I., Toussaint, A., Wodak, S., Mergeay, M., Dunn, J., Taghavi, S., Van der Lierde, N., Boucher, C. Proteomic and genomic approaches to identify plasmid-borne genes for resistance to heavy metals in *Ralstonia metallidurans* CH24.

24. **(D)** Van de Walle, G.R., Favoreel, H.W., Nauwynk, H.J. & Pensaert, M.B. Antibody-induced internalization of viral glycoproteins interferes with complement-mediated lysis of pseudorabies virus-infected porcine monocytes.

25. **(D)** Van der Meulen, K.M., Nauwynck, H.J. & Pensaert, M.B. Absence of viral antigens and MHC-1 on the plasma membrane of EHV_1-infected PBMC *in vitro*: a mechanism of immune evasion?

26. **(D)** van Eyll, O. and Michiels, T. L* protein expression and persistence of Theiler's virus.

27. **(D)** Van Gucht, S., Van Reeth, K. and Pensaert, M. Porcine reproductive and respiratory syndrome virus infection sensitizes the lungs for massive production of pro-inflammatory cytokines upon LPS exposure.

28. **(A)** Van Landschoot, A., Vlaemynck, K., Vanderputten, D. Detection of contaminants in beer by impedance.

29. **(B)** Van Loock, M., Vanrompay, D., Herrmann, B., Vander Stappen, J., Volckaert, G., Goddeeris, B.M. and Everett, K.D.E. *C. psittaci* strain 84/2334: a missing link in the divergence of *C. abortus* from *C. psittaci*.

30. **(D)** Van Reeth K., Maddelein W., Riffault S., Charley B. and Pensaert M. Characterisation of the interferon-α producing cell in the lungs of swine influenza virus infected pigs.

31. **(A)** Van Trappen, S., Mergaert, J. and Swings, J. Diversity of heterotrophic bacteria in microbial mats from antarctic lakes**.**

32. **(C)** Verheust, C., Herman, M. and Mahillon, J. PGIL01, a linear molecule from *Bacillus thuringiensis* serovar *israelensis*.
33. **(C)** Vermis, K., Vandekerckhove, C., Nelis, H.J. and Vandamme, P. Antibiotic susceptibility analyses of *Burkholderia cepacia* complex bacteria**.**
34. **(B)** Willems, A., Schelkens, J., Gillis, M. Genospecies in *Bradyrhizobium* – in search of phenotypic differentiation.

35. **(B)** Lanoot, B., Vancanneyt, M., Cleenwerck, I., Wang, L., Li, W., Liu, Z., and Swings, J. Searching for synonymous species among *Streptomycetes* using a polyphasic approach.`

36. (**C)** Vancanneyt M., Krooneman J.,. Faber F., Alderkamp A.C., Oude Elferink S. J. H. W., Driehuis F., Cleenwerck I, Swings J. and Gottschal J.C.*Lactobacillus diolvorans* sp. nov., a 1,2-propanediol degrading bacterium isolated from aerobically stable maize silage

ISOLATION AND CHARACTERISATION OF BACILLUS CONTAMINANTS IN GELATINE SAMPLES WHICH ARE REJECTED FOR CONSUMPTION

E. De Clerck, T. Vanhoutte, P. De Vos
Laboratory of Microbiology, University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium. e-mail: elke.declerck@rug.ac.be

Gelatine is an animal protein mainly applied in food and pharmaceutical industries. Previous studies pointed out that *Bacillus* species might contaminate the gelatine production process and survive the extreme conditions during the production, resulting in a contaminated final product. This contamination most often affects the viscosity of gelatine and its derivatives. Furthermore, some of the contaminants may be pathogenic for men and animals. It is the aim of this study to develop a fast and sensitive genotypic identification and detection method in order to spot and identify these contaminants at different stages of the gelatine production process. Before such a method can be developed, the identity of the contaminants needs to be determined via isolation and polyphasic characterisation.

In a previous part of this study, contaminants from different stages of a gelatine production process were characterised. A considerable amount of the contaminants belonged to the genus *Bacillus* or related genera and amongst them *B. licheniformis*, *B. fumarioli* and *B. cereus* were found to be the most frequent ones. In this part of the study, samples were analysed from batches of gelatine that were rejected for consumption because of suspected *Bacillus* contamination as shown by the quality control of the company.

Out of 26 contaminated gelatine samples, 400 isolates were obtained and characterised polyphasically. After microscopic analysis, rep-PCR using the $(GTG)_5$ primer was performed to recognise closely related isolates. Based on these results, a selection was made for further identification by 16S rDNA sequencing and DNA-DNA hybridisations to confirm the exact species status. The isolates were also tested on their ability to liquify gelatine.

All the contaminants were found to belong to the genus *Bacillus*. This confirms the hypothesis that only spore forming bacteria are able to survive the extreme conditions of pH and temperature during the production process. Furthermore, all the isolates were gelatinase positive.

The majority of the isolates were identified as *B. licheniformis* (67 %), but also *B. fumarioli* (9 %) and *B. cereus* (5 %) were found frequently. A few isolates were identified as *B. pumilus* and two isolates were identified as *Brevibacillus agri*. A group of 67 isolates, arranged into 4 different rep-PCR fingerprinttypes, could not be identified. Probably they belong to one or more yet undescribed species.

More gelatine samples from different production plants are now being analysed. At this moment *B. licheniformis*, *B. cereus* and *B. fumarioli* seem important targets for the development of a fast and sensitive detection method.

DIVERSITY IN *INDIAN PE ANUT CLUMP* REFERENCE SEROTYPES STRAINS AND SOILS ISOLATES

D. Doucet, J. Weyns & C. Bragard
Unité de Phytopathologie Faculté d'ingénierie biologique, agronomique et environnementale,Université catholique de Louvain, Place Croix du Sud, 2 bte 3, B-1348 Louvain-la-Neuve

Indian peanut clump virus, member of the genus of *Pecluvirus*, is the agent of the peanut clump disease, an economically significant disease of groundnut in the Indian subcontinent. The virus can be transmitted by *Polymyxa graminis*, a soil *Plasmodiophoromycete*, and by seeds. There is no known plant resistance in groundnut. This disease affects also other dicotyledonous and several monocotyledonous species. To tackle this complex problem, research has been undertaken in order to study the localisation of the virus and its transmission by the vector *Polymyxa graminis*. The use of specific detection tools as sera and riboprobes to the virus serotypes infecting the plant tissues is therefore crucial.

Triticum aestivum (139 plants) and *Nicotiana benthamiana* (94 plants) and *Nicotiana tabacum* var Xanthi (14 plants) were grown on two Indian soils originating from Ludhiana state. Plant roots were checked by ELISA using three polyclonal anti-IPCV *sera* (serotype Ludhiana, Durgapura, Hyderabad) and one anti-PCV *sera.* The plants were also checked by RT-PCR using a primer pair targeting the 3' extremities of the viral RNA-1 and -2, for virus presence.

More than 22 viral strains including 15 reference serotype strains and 7 soils isolates were tested by RT-PCR using six primer pairs. These primer pairs amplify respectively the 3'non coding sequence of the RNA-2, the coat protein sequence on the RNA-2, the second protein sequence of the triple gene block, the third protein sequence of the triple gene block, the 5' non coding sequence of RNA-1 and -2, and a fragment of the RNA polymerase on the RNA-1.

Soil transmission was obtained in 29% of tobacco plants and 35% wheat plants. ELISA performed revealed in 16% of tobacco plants and 27% of wheat plants the presence of several serotypes present in two IPCV infested soils. The amplification results illustrate also the diversity observed by serology. The primer pairs amplifying the coat protein and the second and the third protein sequence of the triple gene block are more discriminating than the primer pairs amplifying the 3' and 5' extremities and the RNA polymerase sequences, the latter primers enabling amplifications in a large range of serotypes.

HERPETOMONAS MUSCARUM, A TRYPANOSOMATID "BIEN DE CHEZ NOUS"

Jean-Claude Dujardin[1], Alan Fauconnier[2†], Bruno Pichon[3], Dominique Le Ray[1] and Bernard Couvreur[2].

[1] Prins Leopold Instituut voor Tropische Geneeskunde, 155 Nationalestraat,B-2000 Antwerpen. [2] Nivelles Laboratoires ASBL, 24 rue de l'industrie, B-1400 Nivelles. [3] University College of Dublin, Belfield, Dublin 4, Ireland. [†] Corresponding author.

Trypanosomatids are primitive flagellated protozoa that comprise the agents of life-threatening leishmaniasis, sleeping sickness and chagas disease but also purely insect-dwelling species that are harmless to humans. We investigated the presence of endemic monogenic trypanosomatids in flies collected in the southern part of Belgium. During the first study, a number of fly species from the Namur area were screened for the presence of flagellates in the digestive tract. Whereas various monogenic trypanosomatids are described from a number of fly species, flagellates were only seen in the greenbottle *Lucilia caesar.* On the basis of its morphology, the flagellate was temptatively identified as *Herpetomonas muscarum*. Electron microscopy (EM) examination revealed structures reminiscent of endosymbiotic bacteria. Interestingly, no trypanosomatid could be observed in *Calliphora vomitaria* and *Sarcophaga carnaria*, which ecological niches are related to that of *L. caesar*. In a second study, seven *L. caesar* specimens were collected in the Rance area (Botte du Hainaut) and examined. All of them were found infected. From 4 dissected guts, three isolates were successfully grown in BHI medium without added hemin or blood extract, suggesting the presence of a bacterial endosymbiont. Molecular and EM characterisation of the new strains are now underway. Bacterial endosymbiosis have been described in Neotropical *Herpetomonas roitmani*, *Crithidia oncopelti* and *Blastocrithidia culicis*. However, only a few strains are actually available for experimental work. Endosymbiosis is currently the subject of an intense research effort as it may provide insights into the origin of organelles such as the mitochondria and the chloroplast.

DETECTION OF VIABLE *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* CELLS IN RAW AND PASTEURIZED MILK

***I. Dumon*[1]*, H. Werbrouck*[1]*, A. Wiszniewska*[2]*, E. D'Haese*[3]*, H. Nelis*[3]*, M. Fus*[2]*, and L. Herman*[1]**

[1]*Agricultural Research Center - Gent, Department Animal Product Quality and Transformation Technology, Brusselsesteenweg 370, B-9090 Melle, Belgium*
[2]*Warmia and Mazury University, Laboratory of Hygiene of Animal Food Products, Oczapowskiego Street, 10-730 Olsztyn, Poland*
[3]*Ghent University, Laboratory of Pharmaceutical Microbiology, Harelbekestraat 72, B-9000 Gent, Belgium*

Animals with Johne's disease shed *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in their milk and feces, which can consequently contaminate the raw milk. It is doubted whether MAP is totally killed by common industrial milk pasteurization. This way humans could be infected with MAP, which possibly plays a role in Crohn's disease.
To find viable MAP cells in milk, there is a conventional culturing method, for which several months are needed, as MAP is a very slow grower. To improve this method, it is necessary to test large milk volumes (150 ml), because only small numbers of MAP cells are expected in milk (up to 8 cfu/50 ml, Sweeney et al., 1992).
In this study, we optimized the recuperation method of viable MAP cells from the milk. The viable MAP cells are recuperated by chemical degradation of the milk to remove fats and proteins, followed by centrifugation. All components for the degradation were separately tested to check inactivation of *Mycobacterium smegmatis*, which was used as a test organism.
When we want to grow MAP from the degraded milk, a decontamination step is required to remove the background flora. Hexadecylpyridinium chloride (HPC) kills the milk bacteria, but is also harmful for MAP. Dundee L. et al. (2001) published an optimal incubation time of 5h. However, in combination with the chemical degradation of the milk, incubation with 0.75% HPC for 10 min. is sufficient. After 10 min. incubation with HPC, there is an inactivation level of 1 log of *M. smegmatis*, when started with 10^8 cells. After 1h, an inactivation level of 3-4 logs is reached.
From May 2000 on, a set of milk samples (38 raw milk, 42 tank milk, 40 pasteurized milk; samples of 150 ml) was analyzed for the presence of MAP. The samples were chemically degraded, followed by centrifugation and decontamination (0.75% HPC, 5h). The samples were inoculated on HEYM slopes (Herrold's Egg Yolk Medium) and on liquid Dubos. Growth from the slopes was checked with PCR, based on the presence of the *IS900* (X16293) fragment, which is specific for MAP. Primers p90/p91 (Moss M. T. et al., 1992) and p25/p26 (Caracappa S. et al., 1999) were used for this purpose. Two raw milk and one tank milk sample were found positive, but a second confirmation is needed. One sample of pasteurized milk is positive, confirmed by PCR and the sequencing of the PCR product. For the pasteurized milk sample, a phosphatase test ensured that the sample was well pasteurized.

Caracappa S. et al., 1999. Proceedings of the Sixth International Colloquium on Paratuberculosis 476-483.
Dundee L. et al., 2001. Lett. Appl. Microbiol.. 33: 173-177.
Sweeney R. W. et al., 1992. J. Clin. Microbiol.. 30: 166-171.

A TYROSINE-BASED MOTIF IN THE PRV GB CYTOPLASMIC TAIL IS IMPORTANT FOR BOTH CELL-TO-CELL SPREAD OF THE VIRUS AND ANTIBODY-INDUCED INTERNALIZATION OF VIRAL GLYCOPROTEINS

Favoreel, H.W., Van Minnebruggen, G., Nauwynck, H.J., Enquist, L.W.[1], & Pensaert, M.B.

Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium,
[1]Department of Molecular Biology, Princeton University, USA

Pseudorabies virus (PrV), is an alphaherpesvirus of the pig, and is known to be able to replicate in vaccination-immune pigs. Two mechanisms that may help PrV avoid recognition by the host immune system are i) direct cell-to-cell spread of the virus and ii) antibody-induced internalization of plasma membrane-anchored viral glycoproteins. The latter process occurs in PRV-infected monocytes and intereferes with complement-mediated cell lysis. Earlier studies have demonstrated that PRV glycoprotein gB is crucial during both processes.
In the current study, PRV Becker strains carrying point mutations in the two YXX□ motifs in the gB cytoplasmic tail were constructed using BAC technology. These mutants were tested for intra- and extracellular virus production, for expression levels of different viral glycoproteins on the plasma membrane, and for their capacity to induce cell-to-cell spread and antibody-induced internalization of viral glycoproteins. A point mutation replacing the tyrosine (Y902) in the YXX□ motif nearest the C-terminal end of the gB tail by an alanine (Y902A) resulted in i) a significant 34% reduction in cell-to-cell spread ($p<0.01$) and ii) a significant 44% reduction ($p<0.01$) in antibody-induced internalization of viral glycoproteins, which is similar to the 46% decrease observed when using a gB null virus. All other growth characteristics of the mutant were comparable to those of the wild type virus.
Thus, Y902 in the PRV gB cytoplasmic tail is important for efficient cell-to-cell spread of PRV and for efficienty antibody-induced internalization of viral glycoproteins.

INTERACTION BETWEEN THE CATALYTIC AND REGULATORY SITES OF TN*4430* TNPI/*IRS* SITE-SPECIFIC RECOMBINATION COMPLEX

Christine Galloy, Virginie Vanhooff, Jean Delcour, Bernard Hallet
Université Catholique de Louvain-La-Neuve. Institut des Sciences de la Vie. Unité de Génétique. 5, Place Croix du sud. B-1348 Louvain-La-Neuve. Belgium.
E-mail : hallet@gene.ucl.ac.be

The transposon Tn*4430* from *Bacillus thuringiensis* encodes a DNA site-specific recombination system (TnpI/*irs*) that resolves the cointegrate intermediates arising from replicative transposition. Recombination is mediated by the tyrosine recombinase TnpI at the level of the internal resolution site (*irs*) of Tn*4430*. The *irs* recombination site is composed of a core site (IR1-IR2) at level of which TnpI catalyses the DNA strand exchange reactions, as well as two additional TnpI binding sites (DR1 and DR2) that are repeated in direct orientation downstream of the core. The accessory sites DR1 and DR2 are required for the formation of a higher order nucleoprotein complex, the function of which is to prevent intermolecular recombination events by favoring recombination between sites that are present on a same DNA molecule (resolution selectivity mechanism).

The existence of an interaction between the core and accessory sites was examined by varying the distance between these two sites. If the spacing is increased by adding one or more complete DNA helix turns, resolution selectivity is conserved. In contrast, if the distance is increased by non integral helix turns, the recombination reaction is relaxed allowing inter- and intramolecular recombination events. These results demonstrate some flexibility in the recombination complex, allowing to alter the distance between the core and accessory sites, but not their relative positioning with respect to the DNA helix axis.

Flexibility within the recombination complex was further demonstrated by the finding that the *irs* accessory sites DR1-DR2 can be replaced by the accessory sequences of *E. coli* Xer recombination system, although in this case, interaction between the recombination sites is controlled by unrelated host proteins, PepA and ArcA. *In vitro* analysis of hybrid recombination sites containing the *irs* core site and Xer accessory sequences showed that Xer accessory proteins stimulate TnpI-mediated recombination. Topology analysis of the hybrid sites recombination products are consistent with a simple model describing the TnpI/irs recombination complex architecture.

ANALYSIS OF THE TWO COMPONENT SYSTEMS (TCS) IDENTIFIED BY GENOME SEQUENCING OF THE THERMOPHILIC LACTIC ACID BACTERIUM *STREPTOCOCCUS THERMOPHILUS* LMG18311

Benoît P. Grossiord, Laetitia FONTAINE, Pascal Hols, and Jean Delcour.
Université Catholique de Louvain (UCL), Unité de Génétique,
Croix du Sud 5, B-1348 Louvain-la-Neuve, Belgique,
Tel: 00 32 (0)10 47 34 86, Fax : 00 32 (0)10 47 31 09 E-mail : Grossiord@gene.ucl.ac.be

Streptococcus thermophilus is one of the main economically important lactic acid bacteria used in the dairy industry. Increasing knowledge of its metabolic properties and physiology is a prerequisite for the improvement of its industrial properties in order to engineer new starter cultures or to improve existing fermentation processes. Determination of the entire genome sequence (1.8 Mbp) of *S. thermophilus* was started in mid 1999 by the Biochemistry Unit (FYSA) and the Genetics Unit (GENE) of the Life Sciences Institute (ISV) at UCL. To date about 95% of the genome sequence is known. The primary sequence data have been used in this study to investigate the presence of two-component systems (TCSs) and their respective functions in *S. thermophilus* LMG18311, a strain originally isolated from yogurt.

TCSs are often used in bacteria to sense and respond to their environment. They play a key role in important physiological mechanisms such as virulence in group A streptococci (GAS), natural competence, bacteriocin production, stress responses or even in the viability of cells. They typically consist of a sensor or histidine protein kinase (HPK) and an effector or response regulator (RR); the genes encoding those proteins being located on the same locus.

We have been able to detect the presence of nine TCSs in the *S. thermophilus* genome, seven of them showing the gene order *rr*/*hk* (two with the gene order *hk*/*rr*). *S. thermophilus* TCSs were analysed and classified by comparison of their deduced amino-acid sequences with previously identified proteins. Two were homologous to previously described TCS1 & 2 from S. *thermophilus* and are also present in *S. pneumoniae*[2] and *Lactococcus lactis*[3] (TCS G & C respectively in *L. lactis*). Two others, TCS3 (TCS D *in L. lactis*) and CiaR/H (TCS F in *L. lactis*), are also found in *S. pneumoniae*, *S. pyogenes*[1] and *L. lactis*. Two extra TCSs appeared to be homologous to TCS11 and TCS13 (BlpR/H) of *S. pneumoniae* whereas another seemed to correspond to the CsrR/S pair of *S. pyogenes* involved in virulence (TCS A in *L. lactis*). A TCS related to bacteriocin production (homology to SpaR/K of *Bacillus subtilis*) was also detected whereas the last TCS identified is possibly related to the VirR/S system of *Clostridium perfringens*. The organisation and possible functions of those TCSs will be presented and discussed.

1. Ferretti J.J., W.M. McShan, D. Ajdic, D.J. Savic, G. Savic, K. Lyon, C. Primeaux, S. Sezate, A.N. Suvorov, S. Kenton, H. Shing Lai, S. Ping Lin, Y. Qian, H. Gui Jia, F.Z. Najar, Q. Ren, H. Zhu, L. Song, J. White, X. Yuan, S.W. Clifton, B.A. Roe, and R. McLaughlin. 2001. Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. PNAS 98(8),4658-4663.
2. Lange R., C. Wagner, A. de Saizieu, N. Flint, J. Molnos, M. Stieger, P. Caspers, M. Kambler, W. Keck, and K.E. Amrein. 1999. Domain organization and molecular characterization of 13 two-component systems identified by genome sequencing of *Streptococcus pneumoniae*. Gene 237,223-234.
3. O'Connell-Motherway M., D. van Sinderen, F. Morel-Deville, G.F. Fitzgerald, S.D. Ehrlich, and P. Morel. 2000. Six putative two-component regulatory systems isolated from Lactococcus lactis subsp. cremoris MG1363. Microbiology 146, 935-947.

S. thermophilus page : http://www.biol.ucl.ac.be/gene/genome/

SCREENING OF FUNGI FOR OLIVE OIL MILL WASTEWATER TREATMENT

***Atef JAOUANI*[☀], *Maxime Vanthournhout*[✈] *and Michel PENNINCKX*[☀]**
[☀] *(UPEM) Laboratoire de Physiologie et d'Ecologie Microbiennes ; Université Libre de Bruxelles c/o Institut Pasteur, 642, Rue Engeland 1180 Bruxelles*
[✈] *Service de Chimie et de Biochimie Appliquées ; Faculté Polytechnique de Mons, Rue de l'Epargne, 7000 Mons*

The margines, liquid effluents from olive oil industry, constitute a critical problem for olive oil producing countries such as Tunisia. With a DCO and a DBO, which can reach, respectively, 200 and 100 g O_2/l, the treatment of these effluents by traditional ways seems to be impossible. A succession of various physicochemical and biological steps is thus necessary to detoxify and decolorize the effluent. In this work, we consider margines already pretreated by physicochemical means in order to attain a materiel with a DCO less than 100 g/l O_2 which is necessary before envisaging any further biological treatment; for example an aerobic processing with a fungus able to attack the recalcitrant polyphénolic compounds present in the margines.
57 fungi, described in the literature for their lignolytic activities, were screened. A double test on two different solid media was carried out:

1. A growth test on margines with 100 g/l DCO
2. A decolorization test of poly R-478 as model substrate added to malt extract medium

Thus, we defined 4 groups:

	Decolorization of model substrate (+)	No decolorization of model substrate (-)	Total growth or not on margines
Growth on margines (+)	Group A 12 fungi	Group D 15 fungi	27 fungi
No growth on margines (-)	Group B 16 fungi	Group C 14 fungi	30 fungi
Total decolorization or not	28 fungi	29 fungi	

It should be noted that the fungi of group A are all white rot fungi. Except *Agrocybe praecox*, all the fungi, which decolorize the model substrate, are white rot fungi. 16 out of 43 white rot fungi couldn't decolorize poly R-478.
Two other studies were conducted. The first aimed at defining the adequate physiological conditions for the decolorization of the model substrate as regard to carbon source, nitrogen concentration and Mn^{2+} addition. The malt extract medium was shown to be the best to test decolorization of the model substrate whereas the Mn^{2+} seems to have no effect on decolorization on solid media. Different groups of fungi were identified depending on their sensitivity to glucose and high nitrogen concentration.
In the second part, the limit of DCO, which allow fungal growth on the margines, was determined. A correlation between the decolorization of the model substrate and the decolorization of the diluted margines at 25 g/l O_2 of DCO was observed for all fungi of group A and some other fungi of group B.

METABOLIC ENGINEERING OF *LACTOBACILLUS PLANTARUM* FOR POLYOL PRODUCTION

[1]Victor Ladero *, [2]Michiel Kleerebezem, [1]Jean Delcour and [1]Pascal Hols.

[1] Université Catholique de Louvain (UCL). Unité de Génétique. Louvain-la-Neuve. Belgium.
[2] Wageningen Centre for Food Science (WCFS). Wageningen. The Netherlands

Body-weight control is a major concern in developed nations and obesity has an estimated cost of 4 % of total health expenses in these countries, so new food products containing low-calorie sugars are requested by consumers. Belonging to this low-calorie sugars family, Polyols have advantageous physiological properties. They are non metabolisable sugar alcohols that could replace sucrose, glucose or lactose in food products with equivalent sweetness and taste and they are slowly and incompletely absorbed from the small intestine.

In this context, Lactobacillus plantarum posseses some interesting characteristics: it is largely found as the dominant species in the last step of natural food fermentation, and, the development of a mutant defective in both lactate dehydrogenases, *L. plantarum* TF103, facilitates the metabolic engineering of this strain. The TF103 metabolism was investigated and it was found that mannitol is produced via a reaction that participates in the maintenance of the redox balance in the cell.

The main goal of the project will be to re-route the sugar flux from an intermediate of glycolysis (fructose-6P), in order to produce mannitol (mtl) and sorbitol (stl), by enhancing or activating the metabolism of these compounds.

The sequence of the operon involved in mtl and stl catabolism in *L. plantarum* NCIBM8826 were analysed. In all cases it was composed by the polyol dehydrogenase, a putative PTS system and regulatory genes. Different constructions containing sorbitol (stl1DH and stl2DH) dehydrogenase genes under the control of a strong constitutive promoter (P_{ldh}) were introduce into the *L. plantarum* TF103 mutant strain and were evaluated for their capacity to produce mtl or stl under different culture conditions. The results shown that this approach could be useful for the production of other products by re-routing the sugar fluxes from different intermediates of glycolysis instead of the classical metabolic engineering centred around the pyruvate dissipating enzymes.

CHARACTERISATION OF LPNR 259/6, A GENE THAT ENCODES A LUXR-HOMOLOGUE, IN *LEGIONELLA PNEUMOPHILA*

I. Lebeau, E. Lammertyn, E. De Buck, L. Van Mellaert and J. Anné
Laboratory of Bacteriology, Rega Instituut, Katholieke Universiteit Leuven, Minderbroeders-straat 10, 3000 Leuven, Belgium

Legionella pneumophila is the causative agent of Legionnaire's disease. It is an intracellular lung pathogen of humans that is amplified in the environment by intracellular multiplication within protozoa. Humans become infected following inhalation of contaminated aerosols in the lungs where alveolar macrophage phagosomes are the primary site for replication. The understanding of *L. pneumophila* pathogenesis is still rather limited although the number of genes important for infection and intracellular multiplication is constantly increasing. However, little is known about the regulation of these genes. The *L. pneumophila* genome contains a putative LuxR-homolog, designated LpnR 259/6. The LuxR-family is a member of the superfamily of transcriptional regulators. In other bacteria, LuxR-homologs play a central role in *quorum sensing*, a phenomenon in which bacteria are able to sense the cell density and to communicate using specific signal molecules. Detection of these signal molecules allows them to distinguish between low and high population density, and to control gene expression in response to changing cell numbers.

Characterisation of *lpnR 259/6* was initiated. Therefore, the gene was isolated from *L. pneumophila* serotype 1 and its presence in different *L. pneumophila* serotypes and different *Legionella* species was investigated. Through RNA slot blot hybridisation it was observed that LpnR 259/6 appears to be a negative regulator of *rpoS*, a gene that encodes a virulence factor of *L. pneumophila*. The effect of inactivation of *lpnR 259/6* was tested *in vitro* on the infection of and replication in amoebae and macrophages. Although a clear effect could be noticed for the intracellular growth in *Acanthamoeba castellanii,* the growth in macrophages was not affected.

DETECTION OF *LEGIONELLA PNEUMOPHILA* USING REAL-TIME NASBA

K. Loens*[1], T. Beck[1], P. Sillekens[2], M. Overdijk[2], D. Ursi[1], M. Ieven[1], and H. Goossens[1]

[1]Department of Medical Microbiology, Universitaire Instelling Antwerpen, Universiteitsplein 1 S3, B2610 Wilrijk, Belgium

[2]Organon Teknika, Boseind 15, 5281 RM Boxtel, The Netherlands

Introduction: *Legionella pneumophila*, the etiological agent of Legionnaire' s disease, has been recognised as an important potential cause of atypical pneumonia. Outbreaks in Belgium and The Netherlands confirm the need for a rapid and reliable test. However, conventional diagnostic methods such as culture, serology and detection of urinary antigen lack sensitivity and specificity.

Objectives: The aim of the study was to develop a real time NASBA assay for the detection of *L. pneumophila* in respiratory specimens based on NASBA amplification of a 16S rRNA target sequence using the NucliSens Basic Kit.

Methods: Oligonucleotide primers were derived from the *L. pneumophila* 16S rRNA. The assay was developed using the NucliSens Basic Kit® (Organon Teknika), including standardised reagents for nucleic acid extraction and subsequent RNA amplification and electrochemiluminescence (ECL) detection. For real-time detection, a molecular beacon was used. Specificity was established on a panel of bacterial strains. The analytical sensitivity of the assay was determined through testing of dilution panels of different *L. pneumophila* serotypes and *Legionella* species. Serial dilutions of *L. pneumophila* serotype 1 were added to pools of respiratory specimens and treated with protease. Finally, a limited number of *L. pneumophila* positive and negative specimens were analysed.

The results obtained with the real time *L. pneumophila* 16S rRNA assay were compared to the results obtained with ECL-detection.

Results: Specific detection of the 16S rRNA-derived amplicons was achieved: All *L. pneumophila* strains that were analysed tested positive whereas 26 other bacterial strains tested negative.

The primers enabled detection of as little as 0.1-1 CFU of serotypes 1 and 2 by the real-time NASBA assay and 10-100 CFU of the other serotypes and species were detected. The reaction was negative for *L. pneumophila* serotypes 4 and 5.

The sensitivity of NASBA for detection of *L. pneumophila* in spiked respiratory specimens was 1-10 CFU with both detection methods although the real time assay seems to be a little bit less sensitive than the ECL-detection. Finally, 11 PCR positive specimens were also positive using NASBA in combination with ECL-detection, 9 were positive with the real time assay.

Conclusions: The NucliSens Basic Kit® combined with real-time detection could become a fast, useful and userfriendly diagnostic tool for the development of a NASBA-based assay for the detection of *L. pneumophila.*

DETECTION OF *MYCOPLASMA PNEUMONIAE* AND *CHLAMYDIA PNEUMONIAE* BY PCR IN RESPIRATORY SPECIMENS FROM PATIENTS HOSPITALISED WITH A COMMUNITY ACQUIRED PNEUMONIA

K. Loens*, K. Dirven, H. Wouters, D. Ursi, M. Ieven, and H. Goossens
Department of Medical Microbiology, Universitaire Instelling Antwerpen, Universiteits-plein 1 S3, B2610 Wilrijk, Belgium

Introduction: Mycoplasma pneumoniae *and* Chlamydia pneumoniae *are common etiologic agents of respiratory tract infections in humans, each being responsible for 10-20% of community acquited pneumonia (CAP) and a wide range of extrapulmonary complications. Diagnosis of infection by these organisms is frequently based on serology because culture is slow and insensitive. We previously developed nucleic acid based amplification methods for the sensitive and rapid detection of* Mycoplasma pneumoniae *and* Chlamydia pneumoniae *and applied PCR to study their prevalence in hospitalised CAP patients.*
Methods: Between June 1, 2000 and March 31, 2001, 54 throat swabs, 67 sputa, 2 pleural fluids, 4 bronchoalveolar lavage specimens (BAL), 8 bronchus aspirates, and 6 other respiratory specimens were collected from 97 hospitalised patients with CAP in the Antwerp region. From the majority of patients two different respiratory specimens were obtained. DNA was extracted by the Qiagen method. The *M. pneumoniae* PCR was targeted at the P1 adhesin gene, in the *C. pneumoniae* PCR the Campbell primers were used. PCR products were detected by agar electrophoresis. Internal controls to detect inhibitors were used in both PCR assays.
Results: The results for 90 patients are as follows: 5/90 (5.6%) patients were *M. pneumoniae* positive; 1 in sputum, 1 in a throat swab, 3 in both sputum and throat swab. In four of them no other pathogenic organism was found. *C. pneumoniae* was not detected.
Conclusions: During the winter 2000-2001, *M. pneumoniae* was responsible for 5.6% of the CAP in hospitalised patients, while *C. pneumoniae* was not detected.

DETECTION OF *MYCOPLASMA PNEUMONIAE* AND *CHLAMYDIA PNEUMONIAE* BY PCR IN LOWER RESPIRATORY TRACT INFECTIONS IN OUTPATIENTS

K. Loens*, K. Dirven, D. Ursi, H. Wouters, M. Ieven, and H. Goossens

Department of Medical Microbiology, Universitaire Instelling Antwerpen, Universiteitsplein 1, B2610 Wilrijk, Belgium

Introduction: Mycoplasma pneumoniae and *Chlamydia pneumoniae* are common etiologic agents of respiratory tract infections in humans. Diagnosis of infection by these organisms is frequently based on serology because culture is slow and insensitive. We previously developed nucleic acid based amplification methods for the sensitive and rapid detection of Mycoplasma pneumoniae and *Chlamydia pneumoniae* and applied PCR to determine the prevalence of these organisms in an outpatient population with lower respiratory tract infections (LRTI).

Methods: We collected 187 throat swabs, 138 sputum specimens and 41 gargle specimens from 254 LRTI outpatients between October 15, 2000 and March 31, 2001. From the majority of these patients two different respiratory specimens were obtained. Four regions in Belgium (Antwerp, Hasselt, Leuven, Couillet) were surveyed. DNA was extracted by the Qiagen extraction method. The *M. pneumoniae* PCR was targeted at the P1 adhesin gene, the *C. pneumoniae* PCR used the Campbell primers. PCR products were detected by agarose gelelectrophoresis.

Results: The results of 212 patients could be analyzed: 23/212 (10.9%) patients were *M. pneumoniae* positive; 8 in sputum, 6 in the throat swab, 6 both in sputum specimen and throat swab, and 3 in the throat swab and gargle specimen. The majority of *M. pneumoniae* positive patients was found in the Antwerp region: 16.2%, versus 7.4% in Hasselt, 0% in Leuven and 6.9% in Couillet. *C. pneumoniae* was not detected.

Conclusions: *M. pneumoniae*, in contrast with *C. pneumoniae*, was an important cause of LRTI in our study population, especially in Antwerp, during the winter season 2000-2001.

NICKEL-RESISTANT *VIBRIONACEAE* FROM DEEP-SEA HYDROTHERMAL SOURCES OF THE EASTERN PACIFIC RISE (N 12°45' W 103°59')

Max Mergeay (1) & Nicolas Glansdorff (2)
(1) Laboratory for Microbiology , SCK/CEN, B-2400-Mol, Belgium
(2) Institut de Recherches Microbiologiques Jean-Marie Wiame, 1 av E.Gryzon B-1070-Bruxelles, Belgium

The deep-sea hydrothermal sources and their specific animal communities shelter a variety of microorganisms that colonise the whole steep gradient of biologically compatible temperature from the mouths of the well-known "black smokers " to the Ocean floor (2°C) at the basis of the sources. The present report focuses on the mesophilic part of the involved microbial communities of the hydrothermal sources Genesis and Grandbonum (at a depth of 2600 m). Attention has been given to the phenotypes related to the dependence from or the tolerance to inorganic chemicals that are released from the black smokers: aerobic chemolithotrophy and tolerance to heavy metals. In this respect, the following enrichments in artificial sea water (ASW) were analysed; each of them proved to contain an overwhelmingly dominant population of one bacterium that was identified by 16s rDNA sequencing:
1°) strain EP71G isolated from diluted broth inoculated with scrapings from a tube of Riftia pachyptila annelid worm(Grandbonum black smoker site) was identified as *Alteromonas* macleodi
2°) a culture in autotrophic conditions (air + H_2 + O_2) in presence of a tiny fragment of an active chimney (Genesis black smoker site that lies at a distance of ~5 nautic miles from the Granbonum site) provided the strain AMO3-11 closely tightly related to the group *V.natriexigens/Listonella pelagia*
3°) direct platings from a crushed deep-frozen *Alvinella pompejana* annelid worm sampled on a black smoker from the Grandbonum site and an oligo-autotrophic enrichment from the same sample provided isolates that were 100% identical and tightly related to the Genesis bacteria (less than 0.5 % of different nucleotides): the representative strain was thus called *Vibrio/Listonella pelagia* AM013JA.
All these cultures and enrichments gave rise to rather homogenous platings where the cfu's were all resistant to Nickel chloride at a concentration of 1 mM on ASW medium provided with glutamate as a carbon source. Cfu's resistant to Cu were also found but to a much lesser extent. Cfu clearly resistant to Cd or Zn were not found. The observed response to Nickel looked intriguing especially because no Nickel selective pressure was exerted during the cultures or the enrichments. Indeed, A.macleodi EP71G and the Vibrio/Listonella pelagia AMO3-11 proved to be really resistant to Nickel with gluconate and succinate as carbon sources and to display a MIC (~6mM) similar to those observed in Nickel-resistant Klebsiella oxytoca (nre^+) and *Ralstonia metallidurans* (cnr^+). In contrast, strain AMO13JA is sensitive to Nickel when grown on gluconate or succinate (MIC:~0.4 mM).This strong difference in the Nickel-resistance phenotype might indicate the presence of a genetic determinant associated to a Mobile genetic element that could be transferred between the closely related strains AM03-11 and AM013JA.
These Vibrio's still reasonably grow on ASW media with no added carbon source. The growth is slightly enhanced in presence of carbonate but the strains do not appear to be hydrogenotrophs.
Further studies will examine how representative these Vibrio's and *Alteromonas* (both genera belong to the α-Proteobacteria) are from the microbial communities that are specific of the deep-sea hydrothermal sources (using PCR-based methods) and examine the genetic determinants involved in the tolerance to Nickel.

The present investigation was made possible by the participation to AMISTAD, the French microbiological expedition (IFREMER) in the Eastern Pacific Ocean (May-June 1999) organised by Dr Daniel Prieur and led by Dr Christian Jeanthon to whom we are deeply grateful as well as to the crews of "L'Atalante " and "Nautile".

MULTIPLEX RT-PCR FOR DETECTION OF BEET NECROTIC YELLOW VEIN VIRUS, BEET SOIL-BORNE VIRUS, BEET VIRUS Q AND THEIR VECTOR, P*OLYMYXA BETAE*.

Meunier, A., Schmit, J.F., Stas, A., Bragard, C.
Unité de phytopathologie – UCL, Croix du Sud, 2 bte 3, 1348 Louvain-la-Neuve
Tél. +32(0)10 47 37 47 Fax. +32(0)10 47 86 97

Rhizomania is a disease of sugar beet causing an important sugar yield reduction and roots proliferation. The agent responsible for the disease is the *Beet necrotic yellow vein virus* (BNYVV), a *Benyvirus* transmitted to the plant by a Plasmodiophoromycete called *Polymyxa betae* KESKIN. During the month of October 2000, sugar beets were collected in 69 fields in different sugar beet growing areas in Belgium. The BNYVV was detected by RT-PCR in 40% of those fields. More than 25 Belgian isolates of BNYVV were sequenced targeting the P25 protein of RNA-3, involved in the development of rhizomania symptoms. The P75 protein of RNA-2 of 6 of these aforementioned 25 isolates was also sequenced. Type B, determined by the presence of the corresponding restriction sites, is present in the western parts of Belgium while type A is mainly present in the eastern regions of the country. Two other viruses infecting sugar beet, the *Beet soil borne virus* (BSBV) and the *Beet virus Q* (BVQ), also transmitted by *Polymyxa betae* were also detected by RT-PCR in more than 80% of the fields collected. The pathogenicity of those two viruses on sugar beet is still matter of debate. However it is really of high interest to unravel the role of BSBV and BVQ in the development of rhizomania. Moreover sequencing data obtained for BSBV isolates revealed a high diversity among the capsid protein. Therefore the use of serological methods for the detection of BSBV does not seem reliable. In order to avoid the use of these serological methods and to gain time, a multiplex RT-PCR allowing the simultaneous detection of BNYVV, BSBV, BVQ and *Polymyxa betae* has been developed.

CEREOLYSIN O FROM *BACILLUS CEREUS SENSU LATO*: DISTRIBUTION, DIVERSITY AND POTENTIAL ROLE

N. Michelet[*], N. Wauthier, A. Van Vooren, and J. Mahillon
Laboratory of Microbial Genetics, Université catholique de Louvain, Place Croix du Sud, 2/12
B-1348 Louvain-la-Neuve, Belgium

Bacillus cereus sensu lato comprises six closely related microorganisms (2, 3) displaying a broad range of virulence spectrum (4): the food contaminant and opportunist *B. cereus*, the entomopathogenic *B. thuringiensis*, the human pathogen *B. anthracis*, the rhizoid commensal *B. mycoides* and *B. pseudomycoides*, and the psychrotolerant *B. weihenstephanensis*. *B. cereus sensu stricto* has been associated with nosocomial infections and, more frequently, with gastro-enteritis due to contaminated food (especially dairy products). Symptoms are divided into diarrheal and emetic syndromes, mostly associated with the HBL enterotoxin and cereulide, respectively. However, *B. cereus s.s.* possesses a large arsenal of others virulence factors (hemolysins, phospholipases, enterotoxins), whose role may be determinant in setting up and/or maintaining non-acute pathogenicity. Similarly, although the major virulence factors of *B. anthracis* and *B. thuringiensis* have been characterised, studies on the potential contribution of other minor virulence factors are still in their infancy. Other *B. cereus s.l.* strains producing bacteriocins or antibiotics used as probiotics have also been isolated, but again, little information is available on the contribution of secondary virulence factors to the biology of these strains. The aim of this research was to focus on the contribution of one of these virulence factors, the sulfhydryl-activated cytolysin cereolysin O (CLO) (1), to the pathogenic arsenal of *B. cereus.*

More than 100 strains originating both from reference collection and from natural sources (including form food poisoning cases) have been analysed for their hemolytic activities on Columbia media containing horse and sheep blood, and sheep erythrocytes. The same set of strains was also investigated for the presence and diversity of the *clo* gene using PCR and RFLP analyses. A series of *clo*-derived PCR products were also cloned and sequenced. A strategy of gene knockout, based a thermosensitive replicon, was then used to generate *clo*-minus mutants in the reference strain of *B. cereus.*

All the strains tested exhibited hemolytic activity, although the level varied considerably from strain to strain. Moreover, significant differences were observed among the medium used: the sheep erythrocytes turned out to be the most sensitive, followed by sheep blood, and by horse blood. In addition, no simple correlation could be observed between this phenotypic variation in hemolytic activity and bacterial subgroups, strain origin or strain pathogenicity. Using PCR, the *clo* gene was shown to be present in all strains, regardless of the bacterial species. The diversity of the *clo* genes, analyzed by RFLP, indicated a relatively good conservation among the different strains. However, the most striking observation was certainly the presence, in at least 20% of the strains, of more than one gene copy. Cloning and sequencing of a total of 13 *clo*-derived PCR products from 7 strains revealed a conservation varying between 90 to 99% among themselves, as compared to the 75% identity shared with the alveolysin gene of *Paenibacillus alvei*. The genes from the same strain also tended to be clustered. Knockout experiments are currently under way to determine the relative contribution of the CLO toxin to the hemolytic activity towards various target cells (human and animal erythrocytes, Vero cells or insect cells) or living animals (insect larvae and arthropods).

References

1. Alouf, J. E. 1999. Introduction to the family of the structurally related cholesterol-binding cytolysins ('sulfhydryl-activated' toxins), p. 443-456. *In* J. E. Alouf and J. H. Freer (ed.), The comprehensive sourcebook of bacterial protein toxins, 2nd ed. Academic Press, London.
2. Ash, C., J. A. Farrow, M. Dorsch, E. Stackebrandt, and M. D. Collins. 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. Int. J. System. Bacteriol. 141: 163-167.
3. Daffonchio, D., A. Cherif, and S. Borin. 2000. Homoduplex and heteroduplex polymorphisms of the amplified ribosomal 16S-23S internal transcribed spacers describe genetic relationships in the "*Bacillus cereus* group". Appl. Environ. Microbiol. 66:5460-5468.
4. Granum, P. E., and T. Lund. 1997. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. 157:223-228.

GENETIC STUDY OF THE FUNCTIONAL ROLE OF D-ALANINE SUBSTITUTIONS OF TEICHOIC ACIDS IN *LACTOBACILLUS PLANTARUM*

Palumbo Emmanuelle[1], Hols Pascal[1], Kleerebezem Michiel[3], Leer Rob[3], Boonaert Christophe J.P.[2], and Delcour Jean[1]

[1]Unité de génétique and Unité de chimie des interfaces[2], Université catholique de Louvain, Belgique; [3]Wageningen Center For Food Sciences, The Netherlands.
[1]U.C.L. 5, place Croix du Sud, 1348 Louvain-la-Neuve, Belgique
Tel : 003210 473486 ; Fax : 003210 473109, palumbo@gene.ucl.ac.be

Lipoteichoic acids (LTA) and cell wall teichoic acids (WTA) represent a major constituent of the Gram-positive cell wall. They consist of polyglycerolphosphate or polyribitolphosphate substituted with D-alanine residues whose amino groups partially compensate for the negative charges of the phosphates. As a first step towards elucidating the function of D-alanine substitutions, the *dlt* genes responsible for teichoic acid D-alanylation in *L. plantarum* were cloned. The 4 *dlt* genes (*dltA*, *dltB*, *dltC* and *dltD*) were found to be clustered, as already described in other Gram-positive species. In addition, a *pbpX* gene encoding a protein homologous to low molecular weight penicillin-binding proteins (PBP) was found to adjoin the *L. plantarum dlt* cluster. Northern blotting analyses using *dltA* and *pbpX* probes demonstrated that the 5-gene cluster was transcribed as a single polycistronic mRNA. A DltB minus strain unable to incorporate D-alanine in teichoic acids was constructed. It displayed an autolytic phenotype in stationary phase. This might be explained by an activation of autolysins as a consequence of the more negative character of teichoic acids due to the absence of D-alanine, as the cationic autolysins might bind more tightly to D-alanine deficient teichoic acids. Demonstration of an increased anionicity of the mutant cell wall was gained from the observation of an enhanced binding of the cationic protein cytochrome c. Further evidence for acidification of TA was sought by assaying the sensitivity of the Dlt^- mutant to the cationic antimicrobial compound nisin. The $DltB^-$ strain turned out to be 10 times more sensitive to nisin as compared to the control strain. We also characterized the cell surface by two physicochemical approaches. X-ray photoelectron spectroscopy (XPS) analysis showed a decrease of protonated nitrogen at the cell surface, in agreement with the loss of D-alanyl ester substituents. However, no significant modification of the global surface charge could be detected by microelectrophoresis. This can be explained by the small contribution of protonated amino groups to the overall charge of the cell surface relative to carbonyl and phosphate functions.

THE TWIN-ARGININE TRANSLOCATION PATHWAY IN *STREPTOMYCES LIVIDANS*

K. Schaerlaekens, M. Schierová, E. Lammertyn, N. Geukens, L. Van Mellaert & J. Anné
Laboratory of Bacteriology, Rega Instituut, Katholieke Universiteit Leuven,
Minderbroedersstraat 10, 3000 Leuven, Belgium

In bacteria, three different types of signal peptide-dependent pathways are described for the secretion of proteins: the Sec-dependent pathway, the signal recognition particle (SRP)-dependent pathway and the recently discovered twin-arginine-dependent pathway or Tat pathway, which is structurally and mechanistically related to the ΔpH-dependent pathway in chloroplast thylakoid membranes. A striking feature of this newly discovered translocation system is its ability to transport folded proteins eventually bound to a cofactor before export. The presence of one *tatC* and two *hcf106* homologues in the *S. lividans* genome[1] together with several precursor proteins with a twin-arginine motif in their signal peptide, suggested the presence of the twin-arginine translocation pathway in *S. lividans*.

To demonstrate its functionality, a *tatC* deletion mutant was constructed by gene replacement. The translocation of 3 different putative, Tat-dependent proteins was investigated in this Δ*tatC* mutant. It was shown that this deletion impaired the translocation of the chimeric construct pre-TorA-23K, known to be exclusively secreted via the Tat pathway in *Escherichia coli*. Also *S. antibioticus* tyrosinase, MelC2, that forms a complex with its transactivator protein MelC1 before export, and *S. lividans* xylanase C could not be translocated. Complemenation of the mutation partially restored the defect. This study therefore demonstrates that also in *Streptomyces* the Tat pathway is functional and can translocate folded proteins.

The existence of a functional Tat pathway in *Streptomyces* opens new perspectives for the secretion of heterologous proteins in this host.

[1] Schaerlaekens K, Schierova M, Lammertyn E, Geukens N, Anne J, Van Mellaert L. 2001. Twin-Arginine Translocation Pathway in *Streptomyces lividans.* J. Bacteriol. 183, 6727-6732.

DEVELOPMENT OF A QUANTITATIVE COMPETITIVE RT-PCR FOR THE QUANTIFICATION OF THE VIRUS OF RHIZOMANIA IN ROOTS OF SUGAR BEET.

Schmit, J.F., Meunier, A., Bragard, C.
Unité de phytopathologie – UCL, Croix du Sud, 2 bte 3, 1348 Louvain-la-Neuve
Tél. +32(0)10 47 37 47 Fax. +32(0)10 47 86 97

Beet necrotic yellow vein virus (BNYVV) is responsible for rhizomania, an economically important disease of sugar beet affecting sugar yield by reduction of sugar content. Control of the disease is hampered by the fact that rhizomania is able to survive in the soil for at least 10 years by means of the sporosori of its Plasmodiophoromycete vector, *Polymyxa betae*. The numerous movements of soil associated with sugar beet crop increase the extension of the disease which now touch every sugar beet growing areas of Belgium.
At present, the only control method used is to grow tolerant sugar beet cultivars. These tolerant cultivars limit the impact of the disease, but they still do allow replication.
In order to evaluate the potential risk of dissemination linked to the use of those tolerant cultivars and the possibilities of future breeding of the beet resistance to rhizomania, a tool allowing the quantification of the virus in the roots is necessary.

The quantitative competitive RT-PCR consists in the amplification of a target product along with a standard RNA sequence of known concentration, allowing the quantification of viral RNA within the target sample.
A 25 base pairs deletion was obtained by RT-PCR on the target sequence of 545 pb located on the readthrough protein of ARN-2 of BNYVV. This fragment of 520 pb was cloned into the plasmid pGEM-T (Promega, USA), before sequencing. Plasmid was then linearised by a single *Sac*I restriction site, before *in vitro* transcription using M-message-M-machine (Ambion technology, USA) and the T7 RNA polymerase without capping.
The concentration of the standard RNA produced was determined by spectrophotometry at 260 nm. Standard RNA was diluted from 5 X to 640 X, before mixture with the target viral RNA and RT-PCR amplification. The quantitative competitive RT-PCR was applied 10 different samples of infected sugar beet. collected in Belgium during the 2000 growing season.
The method will be used to compare 29 sugar beet cultivars for their resistance to BNYVV.

LOW LEVELS OF TAIL-TO-TAIL JUNCTIONS ARE PRESENT IN CONCATEMERIC DNA OF BOVINE HERPESVIRUS 1

Schynts F. *[1], McVoy M. [2], Meurens F. [1], Epstein A. [3], Thiry E. [1]*

[1]Department of Virology, Faculty of Veterinary Medicine, University of Liège, Belgium ; [2] Departments of Pediatrics and Microbiology & Immunology, Medical College of Virginia, Richmond VA USA ; [3] Centre de génétique Moléculaire et Cellulaire, CNRS, Lyon, France.

Bovine herpesvirus 1 (BHV-1) has a group D genome structure that is subdivided into a unique long segment (U_L) and a unique short segment (U_S). The latter is flanked by internal and terminal inverted repeats (IR_S & TR_S). U_S can invert relative to U_L to generate two genomic isomers. Genomes are cleaved from concatemeric DNA which consists of head-to-tail arrangements of tandemly repeated viral genomes. Here, the structure of BHV-1 concatemeric DNA was investigated. Concatemeric DNA from cells infected with BHV-1 ST strain was isolated and digested separately with *Pme*I and *Acl*I, which cut the genome only once within U_L (*Pme*I) or U_S (*Acl*I). The resulting fragments were separated by field-inversion gel electrophoresis and hybridized to detect specific restriction fragments. Consistent with other reports for viruses with group D genomes, our results showed that BHV-1 concatemeric DNA contains U_L segments that are inverted relative to each other, indicating that recombination occurs within or between replicative concatemers. More surprisingly, BHV-1 concatemeric DNA exhibited both U_L and U_S terminal fragments and low levels of tail-to-tail junctions (TR_S to TR_S). The latter were not detected in packaged virion DNA. The mechanism of how tail-to-tail junctions arise remains unknown. We conclude that BHV-1 replication intermediates share both common and distinct properties as compared with described herpesvirus concatemers and that their analysis should serve to better understand the mechanisms of herpesvirus DNA replication, cleavage, and packaging.

SINGLE BASE C-SEQUENCING FOR THE DIFFERENTIATION AND IDENTIFICATION OF MEMBERS OF THE GENERA CORYNEBACTERIUM AND ARCANOBACTERIUM

***Storms, V.**, Vaneechoutte, M., Willems, A., Haesebrouck, F., Verschraegen, G. and De Vos,*

P. RUG Laboratory of Microbiology, Ghent University, Ghent, Belgium.

The use of Single Base C-Sequencing of the first 500 bases of the 16S rRNA-gene (SBCS) was evaluated for the differentiation and identification of different species of the genera Corynebacterium and Arcanobacterium. For SBCS only dd-CTPs are used in the sequencing reactions instead of 4 dd NTPs and the generated fragments are separated by capillary electrophoresis (ABI310, Applied Biosystems http://www2.perkin-elmer.com/ab/index.htm). SBCS provides information on mutations including the appearance and disappearance of C-nucleotides, insertions and deletions. The electropherograms generated with SBCS consist of peak patterns with associated fragmentlength-tables. The tables can easily be exported to the software packet Bionumerics (Applied Maths, Kortrijk, B, version 2.0) for similarity calculations and clustering with reference strains of a constructed database. This renders SBCS a fast and cheap technique, making it attractive for use in routine clinical diagnostics. For the genus Corynebacterium, SBCS allowed us to differentiate between almost all species. *C. ulcerans* and *C. pseudotuberculosis* which are known to have a very high 16S rDNA similarity were indistinguishable. In the genus Arcanobacterium all species could be distinguished. Comparison between full sequencing of the first 500 bases of the 16S rRNA-gene and SCBS allowed the evaluation of SBCS for the differentiation of strains within the genera studied.

THE TN*4371* TRANSPOSON FROM *RALSTONIA EUTROPHA*, A MEMBER OF A NEW FAMILY OF MOBILE ELEMENTS ?

Ariane Toussaint (1), D.Springael (2), C.Merlin (3) C.Wyndham (4), C. Boucher (6) et M.Mergeay* (5,2).

(1) SCMBB, Université Libre de Bruxelles, Bruxelles, Belgique; (2)Vito, Mol, Belgïe; (3) Edinburgh University, Institute of Cell and Molecular Biology, UK (4) University of Toronto, Canada; (5) SCK-CEN, Laboratoire de Radiobiologie, Mol, Belgique; (6) Laboratoire de Biologie Moléculaire des Relations Plantes Microorganismes, CNRS – INRA, Castanet Tolosan, France

Tn*4371* is a 55 kb transposable element carrying a gene cluster (*bphSEGFA$_1$A$_2$A$_3$BCDA$_4$R*) involved in the metabolism of biphenyl and 4-chlorobiphenyl into benzoate/4-chlorobenzoate. The nucleotide sequence of this biphenyl catabolic transposon has been completed. It reveals a mosaic structure with genes coding for a tyrosine recombinase related to integrases from lambdoid phages, for biphenyl degradation enzymes very similar to those of *Pseudomonas* sp. KKS102 and for plasmid related genes. The latter constitute three blocks. Two contain genes coding for proteins involved in replication, partition (similar to those of the *M. loti* plasmid pMLb and an *E. stewartii* plasmid) and unknown functions (similar to those of plasmids of enterobacteria for some, of plasmids of Agrobacteria for others). The third block includes type IV secretion- conjugative transfer genes, similar to those found on IncP and Ti plasmids. These 3 blocks have also been found with a similar organization on the chromosome of *R. solanacearum* (α-Proteobacteria), a phytopathogenic bacterium, on chromosomal contigs of *R. metallidurans* chromosome(s) and on symbiotic islands of Rhizobia (α-Proteobacteria) where they are separated by groups of genes which are not found in Tn*4371*. None of these blocks were found on other available prokaryotic genomic sequence except for the integrase. These elements could thus be representatives of a new type of mobile element, particularly prone to invade soil bacteria.

Merlin,C., D.Springael, and A.Toussaint.1999. Tn*4371*: a modular structure encoding a phage-like integrase, a *Pseudomonas*-like catabolic pathway, and RP4/Ti-like transfer functions.Plasmid **41**:40-54

Merlin,C., D.Springael, M.Mergeay, and A.Toussaint.1997.Organisation of the *bph* gene cluster of transposon Tn4371, encoding enzymes for the degradation of biphenyl and 4-chlorobiphenyl compounds. Mol.Gen.Genet. **253**: 499-506

Mouz, S., C.Merlin, D.Springael, and A.Toussaint 1999.A GntR-like negative regulator of the biphenyl degradation genes of the transposon Tn*4371*.Mol.Gen.Genet.**262**:790-799

Springael,D., S.Kreps, and M.Mergeay.1993. Identification of a catabolic transposon, Tn*4371*, carrying biphenyl and 4-chlorobiphenyl genes in *Alcaligenes eutrophus* A5. J.Bacteriol. 175:1674-1681

PROTEOMIC AND GENOMIC APPROACHES TO IDENTIFY PLASMID-BORNE GENES FOR RESISTANCE TO HEAVY METALS IN *RALSTONIA METALLIDURANS* CH34

T. Vallaeys (1,4), R. Wattiez (3), I.Noel-Georis (3), A. Toussaint (5), S. Wodak (5), M. Mergeay (1,2)*, J. Dunn (6), S. Taghavi (2,6), N. Van der Lelie (2,6), C. Boucher (7)

(1) SCK/CEN, Mol, Belgium, (2) Vito, Mol, Belgium, (3) Université Mons-Hainaut, Belgium, (4) INRA, Jouy-en –Josas, France, (5) Université libre de Bruxelles, Belgium, (6) Brookhaven National Laboratories, USA, (7) CNRS – INRA, Castanet Tolosan, France

Metal–resistant species of the *Ralstonia* genus are peculiar for their colonisation of and adaptation to industrial biotopes as well as for their large plasmids containing a variety of operons and gene clusters involved in resistance to heavy metals, as it is the case in *Ralstonia metallidurans* CH34 plasmids pMOL28 (165 kb) and pMOL30 (250 kb). The draft sequence of the genome of *R. metallidurans* CH34 is available as well as the fully annotated sequence of *R.solanacearum*, a plant pathogen. It allows to make a first and preliminary catalogue of metal-resistance genes and to identify some chromosomal counterparts of plasmid-borne genes. Plasmids of *R.metallidurans* have equivalents of most metal resistance genes that have been identified in other bacteria and even in yeasts (with possible exceptions of metallothioneins and of resistance to arsenic oxyanions): involved metals are Cd, Co, Cr, Cu, Hg, Ni, Pb, Tl and Zn. They also contain some genes that were up to now neveridentified in other bacteria as the very peculiar regulation operon *cnr*YXH (resistance to cobalt and nickel)(pMOL28), as *copK* (copper), a gene of pMOL30 revealed via proteomics, or the *pbr*T, B, C and D in the pMOL30 *pbrTRpbrABCD* operon, which is involved in the resistance to lead. A first complete annotation of plasmid pMOL28 is now available as well as a new circular genetic map of pMOL30. This plasmid contains at least 55 genes and ORFs involved in of reminiscent of heavy metal resistances. Remarkably, it contains 5 loci involved in the synthesis of cation efflux P-ATPases . Plasmid-borne resistances to heavy metals in *R.metallidurans* CH34 include on pMOL30 (240kB) the *czc* cluster of genes: *czcNczcI czcCBA czcDRSE* genes (resistance to cobalt, zinc and cadmium). The CBA genes encode for a tricomponent cation/proton antiporter chemo-osmotic efflux, which has various counterparts. *cop* genes (resistance to copper ions) were found in both *Ralstonia* and phytopathogenic *Pseudomonas* genera as well in enterics: they were first identified in plasmids of *P.syringae* and *E.coli* (as a consequence of copper selection pressure) under the form *copABCDcopRS* and *pcoABCDpcoRSE* respectively). In *Ralstonia,* the arrangement *copSRcopABCD* (divergent operons) is observed on plasmid pMOL30 (where it is included in the large cluster of genes *copKcopSRcopABCDcopIcopGFcopH*, involved in responses to copper), and on the chromosomes of both *R.metallidurans* and *R.solanacearum*. Some metal resistance genes or ORFSs considered to belong to the chromosome of *R.metallidurans* CH34 (19 genes *czc , ars, pbr, chr* and *cop*) have also counterparts in *R.solanacearum* but these are all located on the 2.1 Mb megaplasmid of this phytopathogenic bacteria. The proteomic approach (2D PAGE) in *R. metallidurans* CH34 revealed new functions or genes (both on the chromosome and on the large plasmids) that are involved in tolerance to heavy metals, especially copper and zinc. It has revealed isoforms of the pMOL30 plasmid-encoded periplasmic protein CopC, which only appeared in cultures exposed to copper (0.8 mM) and differed by their isoelectric point. Isoforms are also observed for CopK and for CzcB (in this latter case, only in cultures exposed to zinc).This suggests a kind of post-traductional processing of these proteoins and looks to be a novel feature in the expression and regulation of metal resistance in *R.metallidurans.* Proteomic studies are now being complemented by a transcription approach via the use of RT-PCR. Natural genetic engineering is clearly at work in the various associations of modules (acting from the level of domains in a protein up to the clustering of operons in a megaplasmid) and their rearrangements for all the functions that are involved in the cell responses to heavy metals. These functions include the finely regulated homoeostasis of essential metals as well as the adaptation to high concentrations of heavy metals in harsh environmental situations.

RW is a research associate of the “Fonds national de la recherche Scientifique” (FNRS).

ANTIBODY-INDUCED INTERNALIZATION OF VIRAL GLYCOPROTEINS INTERFERES WITH COMPLEMENT-MEDIATED LYSIS OF PSEUDORABIES VIRUS-INFECTED PORCINE MONOCYTES

Van de Walle, G.R., Favoreel, H.W., Nauwynck, H.J., & Pensaert, M.B.
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium

Pseudorabies virus (PrV), an alphaherpesvirus of the pig, is capable of causing viremia and in some cases disease in well-vaccinated animals. It has been shown before that blood monocytes are essential to transport PrV throughout the body of vaccinated animals. These infected monocytes express viral proteins on their cell surface. Antibodies bound to these viral cell surface proteins should induce lysis of the infected cells via acitvation of the antibody-dependent component of the immune system (complement, phagocytes, NK cells).

Recently, we showed that the addition of PrV-specific polyclonal antibodies to PrV-infected porcine monocytes induces internalization of the viral cell surface proteins. This fast and efficient process leaves the infected cells with very few antibodies on their plasma membrane which may possibly interfere with efficient recognition of the cells by the antibody-dependent components of the immune system.

To examine whether this process indeed interferes with antibody-dependent lysis of the infected cells, a complement-mediated cell lysis assay was set up *in vitro*. Porcine monocytes were infected with PrV for 13h and incubated with porcine polyclonal FITC-labeled PrV-specific antibodies. Monocytes with internalized viral glycoproteins were enriched by magnetic cell sorting from 36.0% ± 6.2 to 74.3% ± 2.1. As a control, cells were incubated with the internalization inhibiting reagent genistein. Afterwards, cells were incubated with porcine complement for 1 h at 37°C. The percentage of dead cells was analyzed by flow cytometry. A significant decrease in the percentage of dead cells was observed from 46.0% ± 3.5 when internalization was inhibited by genistein to 21.0% ± 6.6 when internalization occurred ($p < 0.005$). This is the first report showing that antibody-induced internalization of viral cell surface proteins protects virus-infected cells from complement-mediated lysis *in vitro*.

ABSENCE OF VIRAL ANTIGENS AND MHC-I ON THE PLASMA MEMBRANE OF EHV_1-INFECTED PBMC *IN VITRO*: A MECHANISM OF IMMUNE EVASION?

Van der Meulen, K.M., Nauwynck, H.J., & Pensaert, M.B.
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium

Equine herpesvirus type 1 (EHV_1) can cause abortion in immune horses. EHV_1-infected leukocytes are essential for abortion to occur. If there is expression of viral antigens and MHC-I-viral peptide-complexes at the plasma membrane of EHV_1-infected leukocytes, this should render these cells recognizable for the cellular immunity. Since this recognition appears to be inefficient, expression of EHV_1 antigens and MHC-I on the cell surface of blood mononuclear cells (PBMC) was examined in the current study. PBMC were inoculated with EHV_1 and at 6, 9, 12 and 24 h post inoculation (hpi) triple stainings were performed. Viral proteins on the cell surface were stained with FITC-labeled horse anti-EHV_1 IgG. Mouse monoclonal IgG against specific T-, B-, monocyte membrane proteins or MHC-I, followed by goat anti-mouse-TR were used to stain different cell types or MHC-I. Intracellular viral proteins were stained upon permeabilization of the cells and using biotinylated anti-EHV1 IgG followed by streptavidine-Alexa-350. Sixty to 100% of the infected cells had no expression of viral proteins on the cell surface in all cell types at all time points. The percentage of infected cells with MHC-I expression was reduced at 6 and 9 hpi (2- and 1.5-fold resp.). Absence of both MHC-I and EHV_1 antigens was observed in 64%, 52%, 13% and 11% of the infected PBMC at 6, 9, 12 and 24 hpi respectively. In conclusion, the majority of EHV_1-infected PBMC lack viral antigens on the cell surface, independently of cell type. Further, MHC-I expression on PBMC is reduced during early stages of infection. Absence of viral antigens and MHC-I on the cell surface may be a strong immune evasion tool of EHV_1.

L* PROTEIN EXPRESSION AND PERSISTENCE OF THEILER'S VIRUS

***van Eyll O*. and Michiels T**. *(vaneyll@mipa.ucl.ac.be)*
Christian de Duve Institute of Cellular Pathology, UCL, Belgium.

Theiler's virus (TMEV) is a picornavirus that infects the central nervous system of the mouse. The genome (a single strand RNA of positive polarity) of TMEV contains a large open reading frame (ORF) translated as a precursor polypeptide that is processed to yield all the protein necessary to the viral life cycle. In persistent but not in neurovirulent strains of Theiler's virus, a second AUG initiates the translation of an overlapping ORF coding a 18 KDa protein called L*.

To analyse the influence of the L* protein expression on the phenotype of the DA-1 (persistent) and GDVII (neurovirulent) strains, we mutated the L* ORF either by replacing the AUG initiation codon by an ACG codon or by introducing a stop codon in the L* ORF without affecting translation of the overlapping ORF encoding the polyprotein.

Analysis of the mutants confirmed the involvement of the L* protein in the infection of macrophages cell lines. Surprisingly, viruses carrying this stop codon mutation (and thus expressing a truncated L* protein) had a dramatically impaired ability to persist in the central nervous system of the mouse while mutants bearing the AUG to ACG mutation persisted almost as well as the wild-type virus.

Taken together, our data show that the L* protein is an important determinant of viral persistence and suggest that IRES (*Internal Ribosome Entry Site*)-mediated translation of the L* ORF could be initiated at the level of an ACG codon. This would indicate that neurovirulent strains of TMEV also express some levels of the L* protein and explain why these strains conserved the entire L* ORF during evolution in spite of the fact that the ORF lacked an AUG codon.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION SENSITIZES THE LUNGS FOR MASSIVE PRODUCTION OF PROINFLAMMATORY CYTOKINES UPON LPS EXPOSURE

Steven Van Gucht, Kristien Van Reeth and Maurice Pensaert
Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium

Porcine reproductive and respiratory syndrome virus (PRRSV) plays an important role in multifactorial respiratory disease. However, the secondary agents needed to induce disease and the disease mechanisms involved remain unknown. Lipopolysaccharides (LPS) are bacterial endotoxins which are present in high concentrations in organic dust in swine confinement units. It was the purpose of this study to examine whether exposure of PRRSV-infected pigs to LPS can potentiate respiratory disease and, if so, whether the proinflammatory cytokines tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) are involved.
Caesarian-derived and colostrum-deprived pigs were inoculated intratracheally with PRRSV followed by LPS at 1-, 3-, 5-, 7-, 10- or 14-day intervals, and euthanatized 6 hours after LPS. Controls were inoculated with PRRSV or LPS only or left uninoculated. Clinical signs, virus titres and (histo)pathological changes in the lungs, percentage of neutrophils and bioactive TNF-α, IL-1 and IL-6 levels in bronchoalveolar lavage fluids were examined.
Separate virus or LPS inoculations failed to induce respiratory disease or high and sustained cytokine levels. All PRRSV-LPS inoculated pigs, in contrast, developed severe respiratory disease. Prior PRRSV infection significantly enhanced cytokine production in response to LPS. Mean TNF-α, IL-1 and IL-6 titres were respectively 6.4, 7.6 and 3.8 times higher in PRRSV-LPS than in singly LPS-inoculated pigs. Virus titres were similar in the lungs of PRRSV-LPS and singly PRRSV-inoculated pigs. Neutrophil infiltration and pathological changes in the lungs of PRRSV-LPS inoculated pigs resembled the combined effects of the PRRSV and LPS inoculations and there was no synergy between both agents. We found a clear association between disease and cytokine levels, but not between disease and neutrophil infiltration or lung pathology.
These data demonstrate that viruses can synergize with LPS in the induction of proinflammatory cytokines, and that cytokines may be responsible for the observed respiratory disease. Our (histo)pathological observations suggest that disease may result from functional lung disorders, such as bronchial hyperreactivity, rather than structural lung changes. PRRSV and LPS may be important contributors to multifactorial respiratory disease in the field.

DETECTION OF CONTAMINANTS IN BEER BY IMPEDANCE

Anita Van Landschoot[1], Karen Vlaemynck[1], Dana Vanderputten[1], Joris Mergaert[2], Jean Swings[2,3] and Luc De Vuyst[4]

[1] *Hogeschool Gent, Department Industrial Sciences, Voskenslaan 270, B-9000 Gent, Belgium*
[2] *Universiteit Gent, Laboratory of Microbiology, Ledeganckstraat 35, B-9000 Gent, Belgium*
[3] *BCCM/LMG Culture Collection, Ledeganckstraat 35, B-9000 Gent, Belgium*
[4] *Vrije Universiteit Brussel, Research Group of Industrial Microbiology, Fermentation Technology and Downstream Processing, Pleinlaan 2, B-1050 Brussel, Belgium*

Impedimetric techniques are based on the observation that microbial growth can be measured in a culture medium over time by monitoring the electric changes in the medium. Electrically neutral or slightly ionised molecules of the medium (proteins, carbohydrates, etc.) are transformed by micro-organisms into smaller molecules with higher mobility and electrical charge (amino acids, lactate, etc.). The ionic changes (conductivity, impedance or capacity) are measured with minimum one pair of electrodes in the culture medium. The main advantage of the impedimetric technology is a time reduction of days in detecting spoilage bacteria when compared to standard microbiological techniques.

Methods

Three commercially available impedimetric instruments (BacTrac 4100 SY-LAB Foss, Bactometer Biomérieux and Rabit STAG) have been evaluated for brewery purposes for quality control of beer and for monitoring microbiological quality in the brewery. The four beer spoilage isolates used in the study were a *Pectinatus* strain, two strains of *Lactobacillus* and a *Pediococcus* strain. More than one hundred beers and brewing samples from ten medium-sized breweries, partners in the research project, have been analysed with the impedance technique and with standard microbiological methods.

Eleven media were tested as impedance media: BiMedia630A Base, WLN, WLD, LM, MRS, MRSD, TMA, WIBS, NBB-B, NBB-C and NBB-P. The influence of the test protocol, a direct or indirect method for measurement, temperature, addition of cycloheximide and paraffin and the use of membrane filters was investigated.

Results and discussion

The results of the impedance measurements were expressed as detection times and curves of % signal change plotted against time.

The Bactometer and Rabit gave better results than the BacTrac instrument. The three instruments are very sensitive to small temperature changes. Beer and other ionic compounds influenced the electrical signals. Cycloheximide, used to prevent yeast growth, and paraffin, applied to obtain anaerobic conditions, had no effect on the results. Some of the media were very suitable for impedance measurements for the detection of beer spoilage bacteria and for hygiene quality control in the brewery. Several types of membrane filters in the sample cell did not disturb the impedance measurements. The contaminants are faster detected at 28°C than at 25°C. The larger the number of bacteria in the sample cell the shorter the detection time was.

The study proves that with two of the three tested instruments the impedance technique/method is a fast methodology, which effectively addresses one of the major problems of conventional microbiology - the time required from analysis to results in breweries. It turned out that the detection times, and the course and intensity of the impedance signals give the necessary information about the impact and nature of the contamination of a beer sample, for instance an atypical beer spoiler (problem of hygiene) versus a typical beer spoiler.

The results of our study make the impedance method thus an easy and rapid alternative to conventional plating, which is normally used for quality control and detection of contamination traces in breweries.

Acknowledgements: This work was possible thanks to the HOBU project 980087 of IWT.

C. PSITTACI STRAIN 84/2334: A MISSING LINK IN THE DIVERGENCE OF *C. ABORTUS* FROM *C. PSITTACI*.

M. Van Loock[1], D. Vanrompay[2*], B. Herrmann[3], J. Vander Stappen[1], G. Volckaert[1], B.M. Goddeeris[1] and K.D.E. Everett[4].

[1]Department of Animal Sciences, Katholieke Universiteit Leuven, Kasteelpark Arenberg 21 (Laboratory of Gene technology) and 30 (Laboratory of Physiology and Immunology of Domestic Animals), 3001 Leuven, Belgium, [2]Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium, [3]Department of Clinical Microbiology, University Hospital, S-751 85 Uppsala, Sweden, [4]Department of Medical Microbiology and Parasitology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA.

Chlamydophila psittaci is a lethal intracellular bacterial species that causes avian chlamydiosis, epizootic outbreaks in mammals, and respiratory psittacosis in humans. *Chlamydophila abortus* causes causes abortion and fetal death in mammals, including humans. Genetic studies have suggested that these pathogens are closely related. With the recent availability of diverse *C. psittaci* strains and comparative data for several genes, we have set out to explore these relationships. We found that parrot strain 84/2334 had DNA sequences that were identical to extrachromosomal plasmid in duck *C. psittaci* strain N352, to ribosomal Rnase P RNA (*rnpB*) gene in strain R54 from a brown skua, and to the intergenic spacer of the rRNA operon (*rrn*) in parakeet strain Daruma (from Germany, England, Antarctica, and Japan). Further analysis of outer membrane protein A (*ompA*) and *rrn* spacer revealed progressive diversification of the strains, with 84/2334 resembling what might have been a recent ancestor of *C. abortus*. Another *C. psittaci* strain (VS225) showed evidence of having undergone convergent evolution toward the *C. abortus*-like genotype, whereas strain R54 diverged independently. We have designated R54 a *C. psittaci* strain and recommend that characterization of *C. psittaci* strains utilize more than a single method and more than a single gene.

CHARACTERIZATION OF THE INTERFERON-α PRODUCING CELL IN THE LUNGS OF SWINE INFLUENZA VIRUS-INFECTED PIGS

K. Van Reeth[1], W. Maddelein[1], S. Riffault[2], B. Charley[2], M. Pensaert[1]
[1] *Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Belgium*
[2] *Unité de Virologie et Immunologie Moléculaires, INRA, Jouy-en-Josas, France*

Massive amounts of interferon-alpha (IFN-α) are found in lung lavage fluids of pigs and in nasal lavage fluids of humans during acute influenza virus infection. Peak IFN-α production coincides with and probably mediates the development of typical illness and bronchopneumonia. However, the nature of the IFN-α (IPC) remains unknown. This study aimed at localizing and characterizing IPCs in vivo in the swine influenza virus-infected lung.
To this purpose, gnotobiotic pigs were inoculated intratracheally with the A/Sw/Belgium/1/83 (H1N1) influenza virus and euthanized at 12, 18, 24, 30, 48 or 72 hours post inoculation (h PI). Lung homogenates and bronchoalveolar lavage (BAL) fluids were used for titration of influenza virus and bioactive IFN-α respectively. Cryosections of the lungs were used in immunohistochemical (IHC) stainings with porcine IFN-α monoclonal antibody (Mab). To determine whether there is colocalization of virus-infected and IFN-α producing cells, double stainings were performed with Mabs against IFN-α and polyclonal swine influenza virus antibodies. To determine the nature of the IPCs, double stainings were performed with IFN-α Mab and porcine cell markers.
Intracellular expression of IFN-α protein was detected exclusively at 18 and 24 h PI, at the peak of virus replication (8.7 – 9.4 log10 EID50/g lung) and secretion of bioactive IFN-α (27800 – 337400 U/ml). IPCs were scarce (max 16 cells/ tissue section) and became undetectable from 30 h PI on, while titres of virus and bioactive IFN-α declined significantly from 48 h PI on. Most IPCs were present in between or underneath the bronchiolar epithelial cells and morphologically resembled small lymphocytes. Double stainings for IFN-α and influenza virus showed that IPCs were localized in the vicinity of influenza virus-infected epithelial cells. Confocal microscopy will be performed to see whether there are true double positive cells. Current stainings for IFN-α and cell markers will reveal the nature of the IPCs.
This is the first demonstration of IPCs in virus-infected lung tissue. The kinetics, frequency and morphology of IPCs in the lungs of pigs infected with swine influenza virus resemble those of IPCs in the intestine of pigs infected with transmissible gastroenteritis virus. The latter cells were recently characterized as non-macrophage MHC class II-positive leukocytes. In vitro, influenza viruses have been shown to induce IFN-α in a variety of cell types. However, the mechanisms of IFN-α induction by the virus are unknown and they are likely to differ in different cell types. We intend to study IFN-α induction mechanisms in cultures of the cells producing IFN-α *in vivo*.

DIVERSITY OF HETEROTROPHIC BACTERIA IN MICROBIAL MATS FROM ANTARCTIC LAKES

S. Van Trappen [1]***, J. Mergaert*** [1] ***and J. Swings*** [1,2]

Laboratorium voor Microbiologie, Vakgroep Biochemie, Fysiologie en Microbiologie [1]*,*
BCCM/LMG Bacteria Collection [2]*, Universiteit Gent, Belgium*

The microbial mats in Antarctic lakes are unique and very diverse habitats that are constituted of complex microbial communities. In these mats microorganisms are confronted with extreme life conditions, such as low temperatures, freezing-thawing cycli, UV-irradiation, dessication, and varying light conditions, salinities and nutrient concentrations. As such they have been under a high selection pressure and are potentially belonging to endogenous, as yet undescribed taxa.

Almost 800 heterotrophic bacterial strains, isolated from mat samples from lakes in the Dry Valleys (lakes Hoare and Fryxell), the Vestfold Hills (lakes Ace, Pendant, Druzhby, Grace, Highway, Organic and Watts), and the Larsemann Hills (lake Reid) were assigned to 41 major clusters by numerical analysis of their fatty acid profiles (Mergaert *et al.*, in preparation). 16S rDNA sequences were analysed from representative strains from these clusters. The strains belonged to the α-, β- and γ-subclasses of the *Proteobacteria*, the high and low percent G+C Gram positives and to the *Cytophaga-Flavobacterium-Bacteroides* branch. Many sequences showed pairwise sequence similarities of less than 97% to their nearest validly named neighbours, indicating that the clusters which they represent may form as yet unnamed new taxa.

To investigate in more detail the genomic diversity of strains belonging to two major fatty acid clusters, repetitive extragenic palindromic DNA (rep)-PCR fingerprinting (Rademaker & de Bruijn, 1997) was performed. For 46 strains from cluster AM, related to *Sulfitobacter* within the α-*Proteobacteria* (≤93 % 16S rDNA sequence similarity), REP1R-I and REP2-I primers were used (Versalovic *et al.*, 1991). More than 20 different fingerprints were obtained that correlated well with the geographical origin of the strains. For 74 strains from cluster AV, related to *Flavobacterium* (≤97 %), the GTG5-primer was applied, and at least 30 different fingerprints were detected. In contrast to cluster AM, strains showing the same fingerprint were often isolated from different lakes and even from different Antarctic regions. The rep-PCR fingerprinting results illustrate that the genomic diversity of heterotrophic bacteria in Antarctic microbial mats is extremely high. To investigate the genomic relatedness between the different rep-PCR groups within cluster AV, DNA-DNA hybridisations between representative strains were performed. The results indicated that they belong to at least 8 different species. 16S rDNA sequencing showed that these species are related to *Flavobacterium frigidarium, F. gillisiae* and *F. xanthum*, all recently isolated from Antarctica.

* Rademaker J.L.W., de Bruijn F.J. 1997. In DNA Markers: Protocols, Applications and Overviews, pp.151-171 (G. Caetano-Anollés, P. M. Gresshoff (eds.), John Wiley, New York).
* Versalovic J., Koeuth T., Lupski J.R. 1991. Nucleic Acids Research **19**, 6823-6831.

PGILO1, A LINEAR MOLECULE FROM *BACILLUS THURINGIENSIS* SEROVAR *ISRAELENSIS*

Céline Verheust, Murielle Herman and Jacques Mahillon.
Laboratory of Microbial Genetics, UCL, Place Croix de Sud 2/12, B-1348 Louvain-La-Neuve, Belgium

Linear DNA molecules, in comparison with their circular counterparts, have been discovered quite recently and are only present in a limited number of bacterial species. So far, both extrachromosomal linear plasmids and linear prophages have been characterised. Most of these elements can be classified in two different types: the first group includes molecules which have proteins bound to their 5' extremities, and the second group includes molecules with an "hairpin structure" at their covalently closed ends (2, 3, 5). Recently, a new linear element was found in *Bacillus thuringiensis* serovar *israelensis*, a bio-insecticide used to control black flies and mosquitoes, both important vectors of human and animal diseases. This bacteria belongs to the *B. cereus sensu lato* group which also includes *B. cereus sensu stricto*, *B. mycoides*, *B. pseudomycoides, B. weihenstephanensis*, and the human pathogen *B. anthracis*.

B. thuringiensis serovar *israelensis* contains at least seven extrachromosomal circular elements, in addition to a linear molecule (1). The present study aims at a detailed structuro-functional characterisation of this novel linear molecule, named pGIL01, in order to get new insights into the biology of *Bacillus* plasmids. Strain AND508, a *B. thuringiensis* serovar *israelensis* derivative cured of 3 small cryptic plasmids, was used in this work. The size of pGIL01 has been estimated to be ca 15 kb.

First, the distribution of pGIL01-related molecules among the *B. cereus sensu lato* group (100 strains were tested) was investigated by dot blotting and southern analysis : about 10% of the strains examined showed a positive reaction. Furthermore, hybridization done with the two linear plasmids previously observed in the reference strain ATCC14579 of *B. cereus sensu stricto* (A.-B. Kolstø, pers. comm.) and in *Paenibacillus polymyxa* (4) revealed that those molecules are not closely related to pGIL01.

In order to determine the structure of pGIL01 termini, experiments using exonucleases as well as proteases were undertaken: pGIL01 is sensitive to exonuclease III but resistant to lambda exonuclease. A lack of electrophoretic mobility of pGIL01 was also observed in agarose gels when proteinase K was omitted from the plasmid isolation. Those results strongly suggested that the 5' extremities of pGIL01 are tightly attached to a terminal protein, presumably via a covalent bond.

Interestingly, the current analysis of the nucleotide sequence of pGIL01 (11 kb have already been assembled) have revealed striking homologies to genes implicated in several phage systems. These features will be presented and discussed.

References

1. Gonzàlez, J. M. and B. C. Carlton. 1984. A large transmissible plasmid is required for crystal production in *Bacillus thuringiensis* variety *israelensis.* Plasmid. 11: 28-38.
2. Hinnebusch, J. and K. Tilly. 1998. Linear plasmids and chromosomes in bacteria. Mol. Microbiol. 10: 917-922.
3. Meijer, W. J. J., J. A. Horcajadas and M. Salas. 2001. ϕ29 family of phages. Microbiol. Mol. Biol. Rev. 65: 261-287.
4. Rosado, A and L. Seldin. 1993. Isolation and partial characterization of a new linear plasmid isolated from *Bacillus polymyxa* SCE2. J. Gen. Microbiol. 139: 1277-1282.
5. Rybchin, V. N. and A. N. Svarchevsky. 1999. The plasmid prophage N15: a linear DNA with covalently closed ends. Mol. Microbiol. 33: 895-903.

ANTIBIOTIC SUSCEPTIBILITY ANALYSES OF *BURKHOLDERIA CEPACIA* COMPLEX BACTERIA

***K. Vermis*[1]*, C. Vandekerckhove*[1]*, H.J. Nelis*[1] and *P. Vandamme*[2]**

Laboratorium voor Farmaceutische Microbiologie, Faculteit Farmaceutische Wetenschappen[1]*;*
Laboratorium voor Microbiologie, Faculteit Wetenschappen[2]*, Universiteit Gent, Ghent.*

Treatment of *Burkholderia cepacia* infections is challenging due to their high intrinsic resistance to antibiotics. To date, *B. cepacia* is actually a complex comprising at least nine phenotypically similar but genotypically different species: *B. cepacia* (genomovar I), *B. multivorans* (genomovar II), *B. cepacia* genomovar III, *B. stabilis* (genomovar IV), *B. vietnamiensis* (genomovar V), *B. cepacia* genomovar VI, *B. ambifaria* (genomovar VII), *B. anthina* (genomovar VIII) and *B. pyrrocinia* (genomovar IX). Strains of each genomovar can colonize the respiratory tract of cystic fibrosis (CF) patients. However, more than 80 percent of the infections in CF patients are caused by *B. multivorans* and *B. cepacia* genomovar III isolates. In the present study, we examined the in vitro activities of amikacin, cefotetan, cefoxitin, chloramphenicol, co-trimoxazole, imipenem, ofloxacin, polymyxin B sulfate, rifampin, ticarcillin and tetracyclin for a total of 144 *B. cepacia* isolates, representing all nine genomovars and originating from a range of clinical and environmental sources. The minimum inhibitory concentrations (MICs) and sensitivity were determined by the agar dilution and the agar disk diffusion method according to the NCCLS. The results indicated a good correlation between both methods ($p < 0,05$). Significant differences in MICs for amikacin, chloramphenicol, co-trimoxazole, cefoxitin, ofloxacin, ticarcillin and tetracyclin were demonstrated between environmental and clinical isolates. The data revealed susceptibility differences between the different genomovars. *B. vietnamiensis* is the most susceptible genomovar to amikacin, imipenem, tetracyclin and ticarcillin. *B. cepacia* genomovar VI is the most resistant species to chloramphenicol (MIC_{90}> 64 µg/ml compared to ± 32 µg/ml for the other genomovars). The overall high resistance against polymyxin B sulfate was confirmed. A concentration of 512 µg/ml (the highest concentration tested) inhibited less than five percent (strains of *B. stabilis*, *B. multivorans*, *B. ambifaria* and *B. pyrrocinia*) of all strains examined. In general, we conclude that *B. cepacia* genomovar III, *B. cepacia* genomovar VI and *B. ambifaria* are the most resistant members of the *B. cepacia* complex and *B. vietnamiensis* the most sensitive. Among the antibacterial agents tested, co-trimoxazole is the most active against *B. cepacia* complex bacteria.

GENOSPECIES IN *BRADYRHIZOBIUM* – IN SEARCH OF PHENOTYPIC DIFFERENTIATION

***Anne Willems**, Jeroen Schelkens, Monique Gillis*

Lab. of Microbiology, Dept. Biochemistry, Physiology & Microbiology, University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.

Bradyrhizobium contains three named species (*B. japonicum*, *B. elkanii*, *B. liaoningense*) and a number of unnamed groups from various host plants. In a study of *Bradyrhizobium* strains from *Faidherbia albida*, *Aeschynomene* species, and several small legumes from Senegal, as well as of *Bradyrhizobium* reference strains, we have previously described the presence of at least 11 genospecies (I to XI) in this genus (Willems *et al.*, 2001a&b), as revealed by DNA:DNA hybridizations. These genospecies form four subgeneric groups of genospecies inside the genus *Bradyrhizobium*. Genospecies inside each subgeneric groups were more closely related to each other (>40% DNA hybridization) than to other genospecies (<40% DNA hybridization). They consisted of (1) genospecies I (*Bradyrhizobium japonicum*), III (*Bradyrhizobium liaoningense*), IV and V; (2) genospecies VI and VIII; (3) genospecies VII and IX; and (4) genospecies II (*Bradyrhizobium elkanii*), X and XI. Photosynthetic *Aeschynomene* isolates were found to belong to at least 2 distinct genospecies in one subgeneric group. Genospecies I to VII were confirmed by sequence analysis of the internal transcribed spacer (ITS) between the 16S and 23S ribosomal RNA genes (Willems *et al.*, 2001a).

We have extended the ITS sequence analysis to include representatives of all 11 genospecies and these results confirm that this technique provides a good indicator of close genetic relationship. It can be used to predict the cases in which DNA:DNA hybridizations will be useful and can limit the number of DNA:DNA hybridizations needed for identification. A large benefit is that these sequence data permit easy comparison between laboratories.

Three of the 11 genospecies represent the currently recognized named species. The other eight genospecies can only be named if sufficient phenotypic differentiation is available. We used the following techniques on a subset of representative strains to attempt to find differentiating features: API ZYM and API 50CH tests, antibiotic sensitivity tests and fatty acid analysis. From our results it is evident that clear-cut phenotypic data for the differentiation of the different genospecies are difficult to obtain. For most of the techniques standard protocols had to be modified to allow for the slow growth of bradyrhizobia. Some groups, such as the photosynthetic genospecies (IV & VIII), may be recognized using fatty acid composition and antibiotic susceptibilities, but more strains from the different groups need to be included. The API 50CH system may provide further differentiation, but also here, more strains need to be studied.

References:

Willems, A., R. Coopman, and M. Gillis. 2001a. Comparison of sequence analysis of 16S – 23S rDNA spacer regions, AFLP analysis, and DNA-DNA hybridizations in *Bradyrhizobium*. Int. J. Syst. Evol. Microbiol. **51:**623-632.

Willems, A., F. Doignon-Bourcier, J. Goris, R. Coopman, P. de Lajudie, P. De Vos, and M. Gillis. 2001b. DNA-DNA hybridization study of *Bradyrhizobium* isolates. Int. J. Syst. Evol. Microbiol., **51:**1315-1322.

Searching for synonymous species among *Streptomycetes* using a polyphasic approach`

***Lanoot, B.*, Vancanneyt, M., Cleenwerck, I., Wang, L., Li, W., Liu, Z., and Swings, J.**
BCCM / LMG Bacteria Collection, K.L. Ledeganckstraat 35, Gent, Belgium.
CCCM / Institute of Microbiology, Chinese Academy of Sciences, Beijing, P.R. China

A collection of 93 *Streptomyces* reference strains, belonging to different phenotypically defined clusters, were investigated using SDS-PAGE of whole-cell proteins. Computer assisted numerical analysis revealed 21 clusters encompassing strains with very similar protein profiles. Five of them grouped several type strains with visually identical patterns. DNA-DNA hybridizations revealed homology values higher than 70 % among these type strains. ARDRA analysis confirmed high similarities among these strains. Following the current species concept we propose that *Streptomyces varsoviensis* LMG 19360^T is considered as a subjective synonym of *Streptomyces violatus* LMG 19397^T; *Streptomyces albosporeus* subsp. *albosporeus* LMG 19403^T as a subjective synonym of *Streptomyces aurantiacus* LMG 19358^T, *Streptomyces aminophilus* LMG 19319^T as a subjective synonym of *Streptomyces cacaoi* subsp. *cacaoi* LMG 19320^T; *Streptomyces niveus* LMG 19395^T and *Streptomyces spheroides* LMG 19392^T as subjective synonyms of *Streptomyces caeruleus* LMG 19399^T.

Lactobacillus diolvorans sp. nov., a 1,2-propanediol degrading bacterium isolated from aerobically stable maize silage

M. Vancanneyt[1*], J. Krooneman[2], F. Faber[2], A.C. Alderkamp[2], S. J. H. W. Oude Elferink[3], F. Driehuis[3], I. Cleenwerck[1], J. Swings[1], and J. C. Gottschal[2]

[1]BCCM/LMG Bacteria Collection, Laboratory of Microbiology, University of Ghent, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium; [2]University of Groningen, Department of Microbiology, P.O. Box 14, 9750 AA Haren, The Netherlands; [3]ID TNO Animal Nutrition, P.O. Box 65, 8200 AB Lelystad, The Netherlands

Fermentative treatment of forage crops with lactic acid bacteria is a common procedure to preserve cattle feed of high nutritional value in many countries all over the world. This ensiling procedure is based on lactic acid fermentation of water-soluble carbohydrates by lactic acid bacteria, which are common members of the natural epiphytic microflora of freshly harvested crops.

Inoculation of maize silage with *Lactobacillus buchneri* ($5{\cdot}10^5$ cfu g^{-1} of maize silage), prior to ensiling, resulted in the formation of aerobically stable silage. After 9 months, lactic acid bacteria counts were approximately 10^{10} cfu g^{-1} in these treated silages. An important subpopulation ($5.9{\cdot}10^7$ cfu g^{-1}) was able to degrade 1,2-propanediol, a fermentation product of *L. buchneri*, under anoxic conditions to 1-propanol and propionic acid. From this group of 1,2-propanediol-fermenting facultative anaerobic, heterofermentative lactobacilli, two rod-shaped isolates were purified and characterized. Comparative 16S rDNA sequence analysis revealed that the newly isolated bacteria have identical 16S rDNA sequences and phylogenetically belong to the *L. buchneri* group. DNA-DNA hybridizations, whole-cell protein fingerprinting and examination of phenotypic properties (including the pathway of anaerobic degradation of 1,2-propanediol) indicated that these two isolates represent a new species for which the name *Lactobacillus diolvorans* sp. nov. is proposed. Based on the ability of *L. diolvorans* to ferment 1,2-propanediol to 1-propanol and propionic acid at conditions which prevail in silage, *L. diolvorans* may play an important role in stabilizing maize silages.