Programme and Abstracts

ECOLOGY OF BACTERIA USED FOR INSECT CONTROL

THE COST ACTION 862 WORKSHOP

May 21–24th, 2006

The Conference Centrum “Bartlowizna” in Goniadz
32 Nadbiebrzańska Street, 19-110 Goniadz, Poland

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COST 862 Workshop, May 21-24th, 2006, Goniadz, Poland
ECOLOGY OF BACTERIA USED FOR INSECT CONTROL

PROGRAMME

Sunday, May 21, 2006
16.00 – 17.00   Pick up of the participants at Warsaw Airport.
21.00     Supper at The Conference Centrum “Bartlowizna”

Monday, May 22, 2006
8.00 – 9.00    Breakfast
9.00 – 9.15   Welcome address       B.M. Hansen, I. Swiecicka

SESSION 1

9.15 – 11.45   Gene transfer among the Bacillus cereus group
Chair: Alistair Bishop

9.15 – 10.00  Horizontal gene transfer among Bacillus cereus
group members: a review     J. Mahillon
10.00 - 10.25 Germination and conjugation of Bacillus thuringiensis
in the gut of gnotobiotic rats    A. Wilcks
10.25 – 10.55 Coffee Break
10.55 - 11.20 Plasmid exchanges among members of the
Bacillus cereus group in foodstuffs    S. Timmery
10.20 – 11.45 Screening of environmental Bacillus thuringiensis
strains containing vip genes    M. Czajkowska
12.00 – 14.00 Lunch (Restaurant at The Conference Centrum “Bartlowizna”)  

SESSION 2

14.00– 17.00 Ecology of Bacillus thuringiensis
Chair: Jacques Mahillon

14.00 – 14.25 The ecology of Bacillus thuringiensis on the
phyloplane     A. Bishop
14.25 – 14.50 Effects of entomopathogenic bacteria
on non-target insects    L. Ruiu
14.50 – 15.15 Bacillus cereus sensu lato in milk from
north-east Poland    M. Bartoszewicz
15.15 – 15.45 Coffee Break
ECOLOGY OF BACTERIA USED FOR INSECT CONTROL

15.45 – 16.10 Psychro-tolerance in *Bacillus thuringiensis* N.B. Hendriksen
16.10 – 16.35 The symbiotic- and the pathogenic life cycles of *B. cereus* group bacteria B.M. Hansen
16.35 – 17.00 Bacilli of the *Bacillus cereus sensu lato* group are symbiont of the common sow bug (Porcellio scaber, Isopoda) I. Swiecicka

**Tuesday, May 23, 2006**

8.00 – 9.00 Breakfast

9.00 – 17.00 Trip to the Biebrza National Park and visiting marshes with plants attacked by *Lepidoptera*. During this trip we will have lunch in one of the restaurant in the Biebrza National Park.

18.00 Supper (Restaurant at The Conference Centrum “Bartlowizna”)

**Wednesday, May 24, 2006**

8.00 – 9.00 Breakfast

**SESSION 3**

9.00 – 11.00 Common projects and future collaboration. Chair: Bjarne Munk Hansen

12.00 Lunch and Departure from Goniadz to Warsaw Airport.
ABSTRACTS
Horizontal gene transfer among *Bacillus cereus* group members: a review

*Florence Hoton, Géraldine Van der Auwera, Sophie Timmery, Daniel De Palmenaer and Jacques Mahillon*

*Laboratory of Food and Environmental Microbiology, UCL, Croix du Sud, 2/12 B-1348 Louvain-la-Neuve, Belgium*

Members of the *Bacillus cereus* group are all genetically closely related. Yet, they display distinct virulence, from the entomopathogenic properties of *Bacillus thuringiensis*, to the emetic or diarrhoeic syndromes of some *B. cereus* food contaminants. Moreover, the strain diversity observed for the different (sub-)species varies considerably. All the *Bacillus anthracis* isolates are close-to-clonal and most emetic strains of *B. cereus* belong to the same homogenous group. This contrasts with the broad strains diversity encountered in the *B. thuringiensis* or *B. cereus* clusters. In this context, understanding the type and frequency of gene exchanges among these bacteria is essential.

The present review will focus on horizontal gene transfers characterised in the *B. cereus* group, with most emphasis given to conjugation. After a brief historical account of the main discoveries related to *B. cereus* and *B. thuringiensis* conjugation, a detailed description and illustration of the conjugation and mobilisation phenomena will be given, including the distinction between conduction and donation. This will also bring us to the contribution of transposable elements (IS231, IS232, Tn4430, Tn5401, or group II introns) to the horizontal gene flux. The most recent genomic data on conjugation will be given through the 70-kb conjugative plasmid pAW63.

Of particular importance for the scope of this meeting are the gene transfers in "real" environments. A review of experiments performed in various natural matrices will be presented. Finally, less documented observations related to transfer of chromosomal genes, retrotransfer or interspecific transfers with other bacteria will also be given.
Germination and conjugation of *Bacillus thuringiensis* in the gut of gnotobiotic rats


¹ Danish Institute for Food and Veterinary Research, Søborg, Denmark; ² National Institute of Occupational Health, Copenhagen, Denmark; ³ University of Copenhagen, Copenhagen, Denmark; ⁴ National Environmental Research Institute, Roskilde, Denmark

*Bacillus thuringiensis* (*Bt*) is an entomopathogenic Gram-positive spore forming bacterium used worldwide in the combat of insect pests. In addition to production of insect-specific toxins, *Bt* produces enterotoxins that may cause diarrhoea in humans. Since plant protection agents based on *Bt* are widely used on e.g. tomatoes and cucumbers there is a risk that humans ingesting those vegetables may also ingest *Bt* spores that could germinate in the gut and express enterotoxins causing disease.

We used germfree animals to study whether *Bt* spores are able to germinate, express enterotoxins and transfer plasmids in a mammalian gut. To study germination, the rats were fed spores of a *Bt* strain harbouring a plasmid encoding Green Fluorescent Protein, which enabled us to detect germination by flow cytometry. *In vivo* conjugation was studied by di-association of rats with a donor strain harbouring the conjugative plasmid pXO16 and an isogenic recipient strain. Both strains were given as spores and transfer was observed from the donor to the recipient strain. This is the first time that conjugation between *Bt* has been shown in a mammalian tract, and confirms that the strain was able to germinate *in vivo*, since conjugation can happen only between vegetative cells. However, *in vivo* enterotoxin production was detected only in one out of six animals.
Plasmid exchanges among members of the *Bacillus cereus* group in foodstuffs

**Sophie Timmery, Florence Hoton, Géraldine Van der Auwera and Jacques Mahillon**

_Laboratory of Food and Environmental Microbiology, UCL, Croix du Sud, 2/12 B-1348 Louvain-la-Neuve, Belgium_

The potential for horizontal transfer among the various subspecies of the *Bacillus cereus* group, which includes the human opportunist and pathogens *B. cereus sensu stricto* and *B. anthracis*, as well as the biopesticide *B. thuringiensis*, has led to growing concerns regarding food safety and public health. Horizontal transfers by conjugation and mobilisation within this bacterial group were studied in foodstuffs. The conjugative behaviour of *B. thuringiensis* strains was compared in LB medium, milk and rice pudding, using three plasmids: the conjugal plasmids pXO16 (350 kb) and pAW63 (72 kb), as well as the mobilisable element pC194 (2.9 kb). Both biparental and triparental matings were tested. Conjugation and mobilisation experiments were conducted at 30°C, during 4 hours without shaking, to mimic natural conditions.

Conjugation and mobilisation of these plasmids were shown to occur at significant levels in both dairy products, reaching the highest transfer frequencies in milk, with an approximately ten-fold increase in conjugal transfer in this growth medium as compared to liquid LB. Furthermore, the ability of an emetic strain of *B. cereus* to function as either plasmid donor or recipient partner in heterologous biparental matings with *B. thuringiensis* was demonstrated in these food matrices.
Screening of environmental *Bacillus thuringiensis* strains containing the *vip* genes

**Magdalena Czajkowska, Edyta Czech, Diana Drozd, Izabela Swiecicka**

*Department of Microbiology, Institute of Biology, University of Białystok, 15-950 Białystok, Swierkowa 20B, Poland.*

The growing public concern about environment protection has led to the reduction of the use of chemical pesticides, and rather to utilise the activity of microorganisms as environmentally safe pest control agents. The Gram positive bacterium, *Bacillus thuringiensis*, has been used as a biological insecticide for several decades. Its insecticidal activity is mainly attributed to parasporal crystal inclusions (known as Cry proteins or δ-endotoxins), which are active on lepidopteran, dipteran and coleopteran insect larvae, and even some nematodes and protozoa. Recently, vegetative insecticidal proteins (VIPs) secreted in the culture supernatant during the vegetative growth of certain *B. thuringiensis* strains have been described as a novel source of entomotoxins. The purpose of the present study was to determine (i) the presence of the *vip* genes in the environmental *B. thuringiensis* collection from north-east Poland (ii) a correlation between the *vip* genes content, their *cry* genes presence, and the DNA patterns of the *vip*-positive strains.

Over 50 *B. thuringiensis* strains isolated from wild small mammals, soil, and a few from milk were screened by PCR to determine the occurrence of the *vip3A*, encoding toxins active against Lepidoptera larvae, as well as *vip1* and *vip2* encoding proteins toxic against Coleoptera. The most frequent was the *vip3A* gene, present in over 10% of isolates studies. The *vip1* and *vip2* genes were found in significantly lower proportion. Interestingly, no correlation was observed between the *vip* and *cry* gene content and DNA profiles investigated in pulsed field gel electrophoresis. Future work will focus on the *vip* genes diversity, their sequence and expression in *B. thuringiensis* and *Escherichia coli.*
The ecology of *Bacillus thuringiensis* on the phylloplane

*Alistair Bishop¹, Bizzarri, M.F¹. and Sylwia Andrzejczak¹,²*

¹ School of Science, University of Greenwich, Chatham Maritime, Kent, ME4 4TB, U.K.
² University of Wroclaw, Institute of Genetics and Microbiology, Wroclaw 51-148, 63 Przybyszewskiego Street, Poland

Ecological studies on *Bacillus thuringiensis* (Bt) to date have concentrated on recovering the spore form from a variety of environments. Here we report the development and application of two media which selectively allow the recovery of Bt in vegetative form from the phylloplane. The appearance of these ‘vegetative isolates’ on clover (*Trifolium hybridum*) over a growing season is presented in comparison with that of the spore form of Bt and its close relative, *B. cereus*. Inoculation of spores of Bt to sterile and non-sterile soil resulted in the colonisation of the emergent plants with these strains. The *cry* gene content of representative ‘vegetative isolates’ showed a large variation in the number of genes carried, as determined by PCR. These strains did not produce insecticidal crystals in abnormal quantities or with unusual potency to *Pieris* larvae. Currently repPCR and Multiple Locus Sequence Typing is being used to characterise the ‘vegetative isolates’ in terms of their phylogenetic relationship to each other, their fluctuating appearance over a growing season and in relation to strains already typed. Future work will include investigation of the factors that promote the presence of Bt on the phylloplane, competition with other leaf micro-organisms and interactions with invertebrates.
Effects of entomopathogenic bacteria on non-target insects

Luca Ruiu, Ignazio Floris, Alberto Satta, Andrea Lentini, Pietro Luciano

Department of Plant Protection, Entomology section, University of Sassari, Sardinia, Italy

During the last years the Entomology Section of the Department of Plant Protection, University of Sassari (Sardinia, Italy) has carried out studies on some side effects and ecological risks connected with the use of entomopathogenic bacteria, such as Bacillus thuringiensis, for the control of insect pests.

It is known that insecticidal treatments are often responsible of reduction in density of non target organisms which can have ecological significance in the environment and, in many cases, a decrease in the variety and abundance of insect species has been observed. Last but not least, treatments often cause reductions in beneficial insect populations.

We report some results of our researches on the effects of entomopathogenic bacteria on non-target insects, including both a laboratory study on the housefly pupal parasitoid, Muscidifurax raptor Girault and Sanders (Hymenoptera: Pteromalidae) and observations in cork-oak forests treated with Bacillus thuringiensis formulations.

Muscidifurax raptor, like other pteromalid wasps, is a microhymenopteran parasitoids of synanthropic and other flies which play an important role as a natural biological control agent. In various part of the world it has been studied in connection with its possible use in fly control programmes in poultry and livestock facilities, especially against the housefly, Musca domestica L. and the stable fly, Stomoxys calcitrans (L.). Compatibility of entomopathogenic bacteria with these natural occurring biological control agents, is required to maximize pest control from natural mortality factors. We are investigating the susceptibility of M. raptor to B. thuringiensis and other entomopathogenic bacteria known to be toxic to the housefly.

Many researches have evidenced a reduction in the number of non-target Lepidoptera species in cork-oak forests, after treatments with B. thuringiensis against Lepidoptera defoliators, such as the gypsy moth Limantria dispar L. These negative and
unwanted side effects have also been confirmed by laboratory bioassays. However, many abiotic and biotic factors are involved in the population dynamic of these insects. For this reason, studying the effects of treatments on these species should take into account the role of any other factor involved. For example, in the case of cork-oak forests, we experienced a reduction in the abundance and variety of Lepidoptera species, due to a combination of factors, including the effects of defoliation by Lepidoptera pests, whereas *B. thuringiensis* was not really able to make any significant changes in the natural fluctuation of non-target Lepidoptera population density. In these conditions, *B. thuringiensis* treatments should be considered to have a main positive effect in the forest ecology.
Psychro-tolerance in *Bacillus thuringiensis*

*Niels Bohse Hendriksen and Bjarne Munk Hansen,*  
*National Environmental Research Institute, Roskilde, Denmark*

*B. cereus* has been divided into two species on the basis of psychro-tolerance. *B. cereus* is defined as a mesophilic species and *B. weihenstephanensis* as a psychro-tolerant species. The mesophilic species is characterised by its ability to grow at 42°C and not below 7°C, while the psychro-tolerant species is characterised by the opposite to this: growth below 7°C, no growth at 42°C. Two PCR-based methods have been developed to discriminate mesophilic isolates from psychro-tolerant. The first is based on the amplification of a segment of a cold-shock protein in psychro-tolerant strains. The second method takes advantage of specific sequence differences between psychro-tolerant and mesophilic strains in the 16S rDNA gene sequence. We have tried to differentiate *B. thuringiensis* strains on the basis of their temperature dependent growth and the two PCR-based methods into psychrotolerant and mesophilic isolates. We found that both groups exist, but that mesophilic strains dominate in the culture-collections. The results will be discussed in the context of the world-wide distribution of *B. thuringiensis* and its ecology.
**Bacillus cereus sensu lato in milk from north-east Poland**

*Marek Bartoszewicz, Emilia Klejbuk, Beata Osmolska and Izabela Swiecicka*

*Department of Microbiology, Institute of Biology, University of Białystok*

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Ecologically clean, not transformed environment and long agricultural traditions of north-east Poland allow our country to be one of the major milk producers in Europe. There are several dairies proceeding daily up to 1 mln liters of milk each. Thus the assessment of Polish milk quality is very important both to food industry and public health. The goal of the study was to determine the number of *Bacillus cereus sensu lato* spores in milk collected in different seasons. Samples of raw and heat-treated milk were collected during half a year study from one dairy farm and two large dairies from NE Poland. Rep-PCR fingerprinting allowed assessment of the relationship between strains, whereas pathogenic potential was assessed on the basis of enterotoxin genes presence. *Bacillus cereus sensu stricto* was found to be the most important contaminant, while *Bacillus thuringiensis* and *Bacillus mycoides* were isolated occasionally. Least of rods occurred in winter (40-70 cfu L⁻¹), whereas the highest number of spores was found in spring (200-420 cfu L⁻¹). Most of *B. cereus s.s.* and *B. thuringiensis* strains possess *nheA* enterotoxin gene. The *hblA* and the *cytK* genes were detected in almost 50% of *B. cereus s.s.* and *B. thuringiensis* isolates, while *B. mycoides* did not react with primers for those genes. Most of isolates show unique rep-PCR fingerprints, but strains with identical profile of chromosomal DNA were also found in milk from different sampling points.

We suppose that the greatest impact on the quality of heat-treated milk has the contamination during milking, probably due to soil and faeces contamination of teats and milking equipment. Post-pasteurization contamination (if occurs) does not lead to worsening of the final product quality. Number of spores in properly stored milk is too low to provoke food poisonings in people, even if enterotoxin genes are present in their genotype.
The symbiotic- and the pathogenic life cycles of \( B. \, cereus \) group bacteria

\textit{Bjarne Munk Hansen and Niels Bohse Hendriksen}

\textit{National Environmental Research Institute, Roskilde, Denmark}

Members of the \( B. \, cereus \) group are considered opportunistic human pathogens. It has been suggested that \( B. \, cereus \) group bacteria can have two life cycles, a symbiotic life cycle with invertebrate hosts, and only occasionally a pathogenic life cycle in invertebrates or vertebrates (Jensen et al., 2003).

The regulation of many genes supposed to be involved in the pathogenic life cycle, is mediated through the pleiotrophic regulator, PlcR, which is expected to control expression of around 50-100 pathogenesis related genes. On the other hand, these genes involved in pathogenesis could also be considered normal household genes. However, the knowledge of environmental factors controlling the pathogenic life cycle is almost absent. Preliminary data has shown a very low expression of PlcR regulated genes in the absence of PlcR. Further, variations of \textit{in vitro} culture conditions, probably causing variations in \( \text{CO}_2 \) and \( \text{O}_2 \) availability, has resulted in significant variations in Vero toxicity, a measure for virulence.

The general presence of genes encoding products considered pathogenic to humans in the \( B. \, cereus \) group causes concerns in the risk assessment of \( B. \, thuringiensis \). The possibilities, for studying the symbiotic and the pathogenic life cycles of \( B. \, cereus \) group bacteria will be discussed.
Bacilli of the Bacillus cereus sensu lato group are symbiont of the common sow bug (Porcellio scaber, Isopoda)

Izabela Swiecicka 1, Jacques Mahillon 2

1 Department of Microbiology, Institute of Biology, University of Bialystok, 15-950 Bialystok, Swierkowa 20B, Poland
2 Laboratory of Food and Environmental Microbiology, Université catholique de Louvain, Croix du Sud 2/12, B-1348 Louvain-la-Neuve, Belgium

Up to now, many B. cereus sensu lato isolates originating from hospital or food samples have been extensively studied, whereas less attention has been given to those isolated from soil or associated with animals, including invertebrates. Consequently, little is known about B. cereus s.l. ecology and diversity in soil, and even less about their relationship with other soil inhabitants e.g. invertebrates. In the work reported here, the commensal behaviour of B. cereus s.l. in the arthropod digestive tract was analysed using antibiotic resistant derivatives re-introduced into the sow bugs. The genotypic diversity of arthropod-borne B. cereus s.l. isolates was investigated using pulse-field gel electrophoresis (PFGE) following the whole genome DNA digestions with NotI and Ascl, and PCR amplification of virulence genes.

The majority of the sow bug Bacillus cereus sensu stricto isolates originating from the same but also from various sites displayed identical PFGE patterns, virulence gene content and enterotoxicty, indicating strong genetic and genomic relationships. The sow bug Bacillus mycoides/Bacillus pseudomycooides strains displayed a higher diversity. B. cereus sensu stricto, B. mycoides/B. pseudomycooides, and Bacillus thuringiensis apparently behaved as active residents of the digestive tract of arthropods since their antibiotic resistant derivatives. Both spores and vegetative cells of B. cereus s.l. were recovered from sow bugs over a 30-day period, strongly suggesting that these bacteria are natural residents of terrestrial isopods.
### List of the Workshop Participants

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