May 31st, 2006, Bialystok

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SCIENTIFIC REPORT OF THE COST ACTION 862 WORKSHOP "ECOLOGY OF BACTERIA USED FOR INSECT CONTROL"

To tend towards increasing interaction and cooperation of European microbiologists on entomopathogenic bacteria, we arranged the COST Action 862 workshop "Ecology of bacteria used for insect control" held in Goniadz (Poland) on 21–24th May, 2006. We apprise the COST Office that the workshop has been successfully completed.

In the workshop twelve microbiologists from five European countries (Belgium, Denmark, Italy, England, Poland) presented their newest study and discussed the problems concerning ecology of bacteria used for insect control. Special attention was given to *Bacillus thuringiensis*, the most commonly used microbial pesticide throughout the world. The three sessions we had organised, allowed us to debate on insecticidal activities of *B. thuringiensis* strains, their diversity, occurrence in environment, and their relationship with the vertebrate and invertebrate hosts.

The first session “Gene transfer among the *Bacillus cereus* group” chaired by **Dr. Alistar Bishop** (University of Greenwich, UK) was opened by **Prof. Jacques Mahillon** (Catholic University of Louvain, Louvain-la-Neuve, Belgium) lecture. Prof. Mahillon reviewed the newest issues on horizontal gene transfer, with most emphasis given to conjugation, among *B. thuringiensis* strains, as well as among the bacteria related to *B. thuringiensis*: *B. cereus*, *B. anthracis*, *and B. weihenstephanensis.* After a short introduction concerning the history of the main discoveries related to *B. cereus* and *B. thuringiensis* conjugation, Prof. Mahillon gave us descriptions and illustrations of the conjugation, mobilisation, conduction and donation phenomena. Moreover, the transfer of chromosomal genes, retrotransfer or interspecific transfers with other bacteria were also presented with details. Special attention Prof. Mahillon paid to the problems of horizontal gene occurrence in "real" environments.

In complementary to the above lecture, **Dr. Andrea Wilcks** (Danish Institute for Food and Veterinary Research, Denmark) presented her latest results of the study on *B. thuringiensis* behaviour in the gut of gnotobiotic rats. From her presentation it could be concluded that *B. thuringiensis* isolates, which in addition to production of insect-specific toxins, possess the genes coding enterotoxins that may cause diarrhoea in humans when they are ingested with food products. Moreover, it seems most likely that conjugation between *B. thuringiensis* and *B. cereus* strains occurs in gnotobiotic mammal guts. Concerning this subject **Sophie Timmery** (Catholic University of Louvain, Louvain-la-Neuve, Belgium) also observed plasmid exchange (conjugation and mobilisation) between relatives of *B. thuringiensis* in foodstuffs. Both processes, conjugation and mobilisation, were shown to occur at even significantly higher levels in dairy products, e.g. in milk, than in bacterial media. Thus, the potential for horizontal transfer among the various subspecies of the *B. cereus* group should lead to concerns regarding food safety and public health.

In the latest presentation of the first session **Magdalena Czajkowska** (University of Bialystok, Poland) presented her study on insecticidal properties of environmental *B. thuringiensis* strains with regard to the VIP toxin genes presence. *B. thuringiensis* activity is mainly attributed to parasporal crystal inclusions (δ-endotoxins) but also to vegetative insecticidal proteins (VIPs). However, in *B. thuringiensis* collection from NE Poland the *vip* genes exist in low proportion. Interestingly *B. thuringiensis* harboring the *vip3A* gene encoding toxins against Lepidoptera possess also *cry* genes encoding proteins specific to these insects.

In the second session of the workshop entitled "Ecology of *Bacillus thuringiensis*" chaired by **Prof. Jacques Mahillon**, six presentations concerning the environmental aspects of *B. thuringiensis* strains were given. **Dr. Alistar Bishop** presented his latest studies on ecology of *B. thuringiensis* on phylloplane, suggested for the first time that these bacilli, in opposite to their closely related *B. cereus* isolates, are present on the leaves as vegetative forms, able to colonize the leaves. Dr. Bishop presented us two media which selectively allow the recovery of *B. thuringiensis* vegetative cells from the phylloplane. repPCR and Multiple Locus Sequence Typing has been proposed by him for comparison of 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.

It is known that insecticidal treatments often cause reductions in beneficial insect populations. This issue was discussed by **Dr. Luca Ruiu** (University of Sassari, Sardinia, Italy) with regard to both a laboratory study on the housefly pupal parasitoid, *Muscidifurax raptor***,** and observations in cork-oak forests treated with *B. thuringiensis* formulations. Although some researches have evidenced a reduction in the number of non-target Lepidoptera species in cork-oak forests, *B. thuringiensis* treatments should be considered to have a main positive effect in the forest ecology.

B. thuringiensis and related bacteria from the *B. cereus* group are regarded as soil microorganisms from which they may colonise different niches. **Marek Bartoszewicz** (University of Bialystok, Poland) hypothesized that bacilli of this group may contaminate milk and its products from soil. From his study it could be concluded that the most important milk contaminant is *B. cereus.* A number of these bacteria varies during seasons and reaches the minimum in winter and maximum in spring. The toxicity of *B. cereus* and *B. thuringiensis*, assessed by presence of enterotoxin genes, is similar, while the few *B. mycoides* strains isolated in this study possessed almost no genes of tested toxins. Majority of the strains isolated from milk, both *B. thuringiensis* and *B. cereus*, were psychro-tolerance. Furthermore **Dr. Niels Hendriksen** (National Environmental Research Institute, Denmark) noted that psychro-tolerant *B. thuringiensis* strains exist in nature, but their prevalence is related to climatic conditions. It is possible that psychro-tolerant and mesophilic strains might even occur within the same serotype.

The last two presentations were focused on the symbiotic relations between *B. thuringiensis* strains and their vertebrate and invertebrate hosts. It is widely accepted that members of the *B. cereus* group are opportunistic human pathogens. **Dr. Bjarne Munk Hansen** (National Environmental Research Institute, Denmark) 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. Further, the possibilities for assessment of human pathogenicity and the possibility for establishment of models was discussed. The hypothesis of close *B.* cereus-invertebrate interactions was further documented by **Dr. Izabela Swiecicka's** (University of Bialystok, Poland), who demonstrated that bacteria from the *B. cereus* group are symbionts of arthropods. In the experiment she presented, *B. cereus sensu stricto*, *B. mycoides/B. pseudomycoides*, 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. Moreover, properties of the arthropod gut lumens reveal suitable conditions for the *B. cereus s.l.* growth in them. Nevertheless, although *B. cereus s.l.* are true symbionts (commensals) of the arthropod digestive tract, it is difficult to predict their contribution to the processess present in the intestine of invertebrates.

During the third session "Common projects and future collaboration" chaired by **Dr. Bjarne Munk Hansen** we summarized the workshop and discussed the subjects and possibilities for future collaboration on *B. thuringiensis* ecology and safety. The main subjects for future collaboration were summarised:

1. Model for *B. cereus sensu lato* human toxicity:
    - insect model;
    - nematode model;
    - chemostat model for pathogenicity triggers.
2. Pheromone involvement in *B. cereus/B. thuringiensis* pathogenesis:
    - invasive *B. cereus* and *B. thuringiensis* strains;
    - why are the enterotoxin genes so wide-spread in *B. cereus/B. thuringiensis*?
3. *B. cereus/B. thuringiensis* epizootics:
    - isolation of strains e.g. from Sardinian cork forests;
    - what factors promote *B. cereus/B. thuringiensis* epizootics?
    - *B. thuringiensis* strains with activity against new orders e.g. flies, cockroaches.
4. Invertebrate / *B. thuringiensis* interaction.
    - role of Cry toxin in attachment of spore to invertebrate gut wall;
    - does *B. thuringiensis* produce high levels of ICPs when it is replicating in nature non-pathogenically?
    - attachment of vegetative *B. cereus* (Arthromitus) forms to host gut wall.

5. Clonality of *B. thuringiensis* isolates:
    - *B. anthracis* is the extreme example;
    - MLST programmes;
    - relate clonal structure to actual occurrence of strains by locality or invertebrate association.
6. Plasmid transfer:
    - good indicator of activity, clonal structure;
    - host range of the strains may be important. Use the same strain as donor and recipient;
    - matrices/environments: food (sterile/ non-sterile; pasteurisation);
    - emetic plasmid.
7. Triggers for gene expression:
    - plasmid/ chromosomal genes;
    - communication between *Bacillus* spp. and within strains;
    - genomics, proteomics, secretomics of *Bacillus* spp;
8. Financial support/ grant applications:
    - connection to environment is an important theme;
    - model for emerging pathogens, bioterrorism, risk assessment;
    - are *B. thuringiensis / B. cereus* the most widespread/ numerous food poisoning agents in the environment?
    - nosocomial infection;
    - ready to eat/ minimal processing food;
    - the role of biofilms in *B. thuringiensis / B. cereus* ecology.

In conclusion, the workshop was a good opportunity to get together for the few people working with ecology of bacteria used for control of insects. Several of the participants had not met before and the organizers feel that the meeting has resulted in an improved potential for future co-operations. Further, besides the "old" scientists, also young scientists participated and contributed actively in the workshop. All in all, the organisers feel that the workshop was successful with synergistic benefits for all participants.

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and

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