



COST 862 WG4 WORKSHOP

Bacterial Toxins for Insect Control. Safety, Ecology and New Strains

Programme and Abstracts

September 20 – 23, 2009

Business & Recreation Complex “Soplicowo” in Białowieża

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PROGRAMME

Sunday, September 20th, 2009

14.00 Departure from Warsaw Airport to Bialowieza.
20.00 Welcome at Business & Recreation Complex "Soplicowo".

Monday, September 21st, 2009

7.30 - 9.00 Breakfast

SESSION 1

Ecology of *Bacillus thuringiensis*

Chair: Bjarne Munk Hansen

9.00	<i>Bacillus thuringiensis</i> – an impotent pathogen?	Neil Crickmore
9.50	The presence of <i>Bacillus</i> genus bacteria in populations of significant forest pests	Julija Halimona
10.15	The evolutionary ecology of <i>Bacillus thuringiensis</i> toxin production in the field	Ben Raymond
10.40	Coffee Break	
11.15	Can temperature profiling and resistance to intestinal conditions be used to distinguish between pathogenic and non-pathogenic species of the <i>Bacillus cereus</i> group?	Andrea Wilcks
11.40	Natural isolates of <i>Bacillus thuringiensis</i> display genetic and psychrotrophic properties characteristic of <i>Bacillus weihenstephanensis</i>	Marek Bartoszewicz
13.00	Lunch	

Monday, September 21st, 2009

SESSION 2

***Bacillus thuringiensis* – safety and toxicity**

Chair: Neil Crickmore

14.00	Safety of biopesticides for non-target insects	Luca Ruiu
14.25	Impact of Bt maize cultivation on abundance of epigeic Collembola	Adriana Simanska
14.45	Alternative models based on invertebrates and Mammalian cell lines for risk assessment of microbial pest control agents	Bjarne M. Hansen
15.10	Coffee break	
15.45	Fate of <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> HD1 on vegetables in the field	Niels B. Hendriksen
16.10	Differential transfer dynamics of pAW63 plasmid among members of the <i>Bacillus cereus</i> group in food microcosms	Pauline Modrie
16.35	Distribution, diversity and potential mobility of extra-chromosomal elements related to the <i>Bacillus anthracis</i> pXO1 and pXO2 virulence plasmids	Xiaomin Hu
17.00	Collection of the travel reimbursement requests	Izabela Swiecicka
18.00	Dinner with regional food served during a bonfire in Bialowieza National Park. In case of rain dinner will be served in the hotel restaurant.	

Tuesday, September 22nd, 2009

7.00 - 8.00 Breakfast

Session 3

New *Bacillus thuringiensis* strains for insect control

Chair: Luca Ruiu

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|-------|---|------------------|
| 9.00 | New <i>Bacillus thuringiensis</i> isolates from sawflies (Hymenoptera: Symphyta) larvae and their insecticidal activity against Diprionidae | Alicja Sierpiska |
| 9.25 | <i>Bacillus thuringiensis</i> subsp <i>roskildensis</i> (H-45) – an emerging commercial strain for control of <i>Nasutitermis ehrhardti</i> ? | Bjarne M. Hansen |
| 9.40 | Coffee break | |
| 10.15 | Restriction of cry gene expression to insect wound site by using a wound-induced promoter (AoPR1) isolated from <i>Asparagus officinalis</i> | Selma Onarıcı |
| 10.40 | <i>Bacillus thuringiensis</i> serovar. <i>thompsoni</i> HD542 crystal proteins: research overview | Samir Naimov |
| 12.00 | Lunch | |
| 13.00 | Field trip to the Białowieża National Park. | |
| | In case of heavy rain we will visit The Museum of Białowieża National Park and a very specific orthodox church in Białowieża. | |
| 18.00 | Dinner | |

Wednesday, September 23rd, 2009

7.00 – 8.00 Breakfast

8.00 – 9.00 Departure to Warsaw Airport. Exact time of leaving will be given during the workshop.

ABSTRACTS

**Bacterial Toxins for Insect Control.
Safety, Ecology and New Strains**

***Bacillus thuringiensis* – an impotent pathogen?**

Neil Crickmore¹, Paul R. Johnston¹ and Ben Raymond².

¹ *Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK*

² *Mathematical Ecology Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK*

In 2006 Broderick et al (PNAS 103:15196-15199) published a thought-provoking paper in which they claimed that the toxicity of *Bacillus thuringiensis* towards *Lymantria dispar* was largely or wholly due to the effect of native bacteria within the gut of the insect larvae. In 2009 these findings were extended to a range of other lepidopteran insects (BMC Biology 7:11). Since a large body of literature exists which suggests that Bt is pathogenic without the need for synergists such as gut bacteria we decided to test this hypothesis on both *Plutella xylostella* and *Manduca sexta*. Should the Broderick hypothesis be correct we were also interested in whether differences in gut bacteria could result in differences in susceptibility to Bt. No such correlations could be found although we did replicate the findings of the above papers in that populations of insects whose gut bacteria had been removed through treatment with antibiotics were less susceptible to spore/crystal formulations of *Bt*. We then extended these studies to see whether the same results could be obtained using antibiotic-resistant strains of *Bt* and/or populations of insect whose gut bacteria had been removed through sterile techniques rather than through the use of antibiotics. Our findings do not support an obligate role of gut bacteria in the pathogenesis of Bt.

**Bacterial Toxins for Insect Control.
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**The presence of *Bacillus* genus bacteria in populations
of significant forest pests**

**Julija Halimona, Liga Jankevica, Zane Metla, Rita Seskena,
Valentina Petrova and Ivars Zarins**

Institute of Biology, University of Latvia, Miera iela 3, LV-2169 Salaspils, Latvia

Institute of Biology has been working on problems, which included development of microbiological methods for plant protection in Latvia for many years. Presence of bacteria from *Bacillus* genus has been studied.

Observations of natural epizootics of dendrophagous pests have been done regularly in different regions of Latvia. Collection of insect material was done in 4 sampling plots located in Kurzeme and 2 in Vidzeme region.

Dead, infected and living insects were collected from natural habitats applying standard methods. After collecting living insects were placed in sterile isolators for observations. Reasons of death were evaluated by applying microscopy (Olympus CX 41). Contents of dead and living insect's intestine were dissolved in sterile water and penetrated on artificial mediums. Then followed cultivation in incubator, colonies were counted on the 4th day.

Morphologically different bacteria were classified by carrying on necessary chemical reactions: Gram staining, Oxidase test, Indole test. *Bacillus* genus bacteria were classified applying Crystal GP identification system.

Bacillus circulans, *B. subtilis* and *B. sp.* were isolated from gypsy moth (*Lymantria dispar*). Two bacteria species from *Bacillus* genus were isolated from European pine sawfly (*Neodiprion sertifer*) imago and larvae. *B. thuringiensis* and *B. brevis* were isolated from pine looper (*Bupalus piniarius*). *Bacillus* sp. was found in populations of pine sawfly (*Gilpinia pallida*). During the study other insect toxins producing bacteria were isolated as well – *Serratia* sp., *Enterobacter* sp., etc. Pathogenicity of obtained isolates and produced endotoxins will be evaluated.

This work has been supported by the grant from the Foundation of Forest Development (08-S124) and Latvian Council of Science (09.1359).

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**The evolutionary ecology of *Bacillus thuringiensis* toxin production
in the field**

Ben Raymond

*Mathematical Ecology Research Group, Department of Zoology, University of Oxford,
South Parks Road, Oxford, OX1 3PS, UK*

Bt toxin production is highly metabolically costly and the means by which this trait is maintained in *Bacillus* populations is still controversial. If we treat *Bt* toxins as cooperative traits some of this controversy may be resolved. *Bt* toxins behave according to the definition of a cooperative “public good”. Within hosts avirulent crystal null strains (or “cheats”) can exploit the toxins produced by virulent strains, and the growth rate of cheats within hosts is higher than that of virulent strains. However, toxin production is essential for pathogenicity and cheats are not expected to be able to persist in the absence of toxin producers. Kin selection and competition between patches with varying proportions of toxin-production can however, explain the maintenance of this trait. In a field experiment I competed near-isogenic virulent and avirulent cheats at varying densities and frequencies. Evidence for a kin-selection process was found in strong negative frequency dependence, in other words cheats rapidly invaded populations with high frequencies of toxin-producers, and toxin-producers rapidly invaded populations with high frequencies of cheats. Evidence of competition between cheats and toxin-producers was also found in the spatial distribution of these phenotypes across the experiment. Not only can *Bt* be used as a tool to test evolutionary theory but the evolutionary tension between cheats and toxin-producers can explain the phylogenetic distribution of toxin production as well as the rarity of *Bt* epizootics in the field.

**Bacterial Toxins for Insect Control.
Safety, Ecology and New Strains**

Can temperature profiling and resistance to intestinal conditions be used to distinguish between pathogenic and non-pathogenic species of the *Bacillus cereus* group?

Andrea Wilcks and Bodil Madsen

Division of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

Background: Surviving the gastric barrier and the small intestinal conditions is a prerequisite for causing disease in humans. These properties together with the ability to grow at high temperatures could be indicators determining the potential for a strain to be a human pathogen.

Materials & Methods: We have tested ten different strains of the *Bacillus cereus* group: four *B. cereus* clinical isolates, two non-pathogenic strains, and four commercial *B. thuringiensis* strains. The strains were tested for ability to grow at both low and high temperatures at aerobic and microaerophile conditions. Furthermore their resistance to gastric acid and bile acid were studied.

Results: The clinical strains had a tendency for better growth at low and high temperatures compared to the other strains studied. All strains were able to grow at pH5. At lower pH (2 and 3.5) two *B. thuringiensis* commercial strains were most sensitive, and the probiotic strain *B. cereus* var. *toyoi* was most resistant. The experiments with bile acid did not show any differences between the ten tested strains.

Conclusion: The clinical strains only showed a marked difference to the other strains in the temperature study. It is necessary to include more strains to be certain that a different temperature profile is a risk factor. If this is the case it could be interesting to identify the gene(s)/gene product(s) responsible for this difference in growth at especially elevated temperatures.

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Natural isolates of *Bacillus thuringiensis* display genetic and psychrotrophic properties characteristic of *Bacillus weihenstephanensis*

Marek Bartoszewicz, Magdalena A. Kroten, Maria Sztachelska and Izabela Swiecicka

*Department of Microbiology, Institute of Biology, University of Bialystok,
20B Swierkowa Street, PL15-950 Bialystok, Poland*

Bacillus thuringiensis, often found in the natural habitats and food products is known primarily for its entomopathogenicity. In present study we determined the potential of *B. thuringiensis* to be a psychrotolerant contaminant of cold-stored products. We assessed the genetic properties and diversity of cold-adapted isolates of *B. thuringiensis* based on (i) the presence of *cspA*, a genetic determinant that confers psychrotolerance in *Bacillus weihenstephanensis* (ii) 16S *rRNA* genes, and (iii) pulse-field gel electrophoretic (PFGE) genome profiles. We established the pathogenic potential of these isolates based on whether they harboured various combinations of known determinants (*nheA*, *hblA*, *cytK*). Of 36 non-clonal *B. thuringiensis* cultured from soil and milk, 21 harboured *cspA*, and of these, 16 (76%) were psychrotolerant and possessed genetic signatures typical of psychrotrophic *Bacillus* species. The majority of psychrotolerant isolates contained various combinations of *nheA*, *hblA*, and *cytK*. Our results show that natural isolates of psychrotolerant *B. thuringiensis* occur in soil and milk. Moreover, the presence of *cspA* in combination with *nheA*, *hblA*, and *cytK* could be of concern if commercial products are contaminated with strains that harbour these determinants.

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Safety of biopesticides for non-target insects

Luca Ruiu

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

The registration of a new biopesticide requires studies on the risks for humans, animals and other non-target organisms. This involves testing on mammals, birds, aquatic organisms and non-target arthropods. Among the latter, insect pollinators (honeybee), predators (lacewing, ladybird) and parasitoids (wasps).

Focusing on the safety assessment for new entomopathogenic bacteria, an example concerning a new *Brevibacillus laterosporus* strain showing toxicity against the house fly (*Musca domestica* L.) is reported. A study on the potential hazard effects of this entomopathogen on beneficials, such as the honeybee (*Apis mellifera* L.) and the house fly pupal parasitoid, *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) are reported.

None of the bacterial fractions (spores, vegetative cells, culture supernatant) assayed either on bees or on wasps was significantly toxic. However spores of this bacterial strain are known to be highly effective against the house fly. Investigations on parasitoids were conducted also at sub-lethal level, studying various insect performance parameters (e.g. development time, fecundity, emergence rate, etc.) and the tritrophic interaction (house fly-bacteria-parasitoid).

Based on our results and on the fact that dosages and concentrations that bees or wasps may come into contact in the field are obviously lower than those assayed in our laboratory study, the use of the formulations based on *B. laterosporus* against insect pests, at the present state of the art, is to be considered safe.

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Impact of Bt maize cultivation on abundance of epigeic Collembola

Adriana Simanska, Ludovit Cagan

Department of Plant Protection, Slovak Agricultural University in Nitra, Slovakia

The aim of the study was to evaluate the effects of maize expressing the *Bacillus thuringiensis* Cry1Ab protein (Bt maize) on population structure of epigeic Collembola in commercial fields.

Collembolan populations were studied at six localities of Slovakia. From those, three localities were situated in the western part of Slovakia and three localities in the eastern part of Slovakia. At each locality there were used pitfall traps to collect the insects under the Bt-maize plants and their transgenic Bt near-isolines grown side-by-side. Numbers of Collembolan species were significantly influenced by the date of collection and locality. No significant effect of the Cry1Ab protein was found on different Collembolan species.

This work was supported by Slovak grant agencies projects APVV COST-0043-06 and VEGA 1/0358/08.

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**Alternative models based on invertebrates and mammalian cell lines
for risk assessment of microbial pest control agents**

**Bjarne Munk Hansen¹, Line Thorsen², Christina Nielsen-LeRoux³, Andrea Wilcks⁴
and Niels Bohse Hendriksen¹**

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²*Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Denmark*

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Background: Microbial Pest Control Agents (MPCA) are used as alternatives to chemical pesticides for plant protection. The guidelines for risk assessment are however more or less copied from the guidelines for chemical pesticides. Several of these guidelines are not optimal for living microbials. Studies with higher animals like rodents are included in the assessment, although studies show that these animals are not appropriate for evaluation of potential risk to humans of the MPCA. Our goal is to develop alternative models for the assessment of human safety of product containing living MPCA. However, one demand to a reliable model is at least one bacterial strain and an experimental model giving some kind of pathogenic response. Otherwise, a negative pathogenic response could be a false response. So we need to search among isolates originating from diseased humans to find a true pathogenic isolate.

Purpose: To identify and test new and more appropriate models for risk assessment of microbial pest control agents. The bacterium *Bacillus thuringiensis* are used as model organism. As *B. thuringiensis* is highly similar to *B. cereus*, we will search for positive control organisms among strains suspected to have caused disease in humans. The models are based on two different invertebrates and mammalian cell lines. The models will be compared with existing models for risk assessment. Implementation of these new models will reduce the number of test-animals and the costs used in the health evaluation of microbial pest control agents.

Specific goals:

1. To identify *B. cereus* group isolates to be used as positive and negative controls
2. To analyse pathogenicity of *B. thuringiensis* in models based on the nematode *Caenorhabditis elegans*, insect larvae of *Galleria mellonella* and functional mammalian cell models

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3. The three models will be compared and evaluated. The evaluation will focus on the ability of the models to respond to known pathogenic and non-pathogenic bacterial isolates.
4. Expression of pathogenic traits in the bacteria will be analysed in the models by RT quantitative PCR

Methods: Our primary selection for a positive control organisms are, besides from their history, based on the ability to grow actively at high temperatures permitting active proliferation in the human body. Further, the bacteria are typed using Multi Locus Sequence Typing (MLST), a method which at detail level seems to cluster pathogenic isolates apart from non pathogenic. Also the ability of the strains to survive simulated gastric-gut conditions will be evaluated. The potential pathogenic isolates will next be tested in the three models. Only the *G. mellonella* larvae have earlier been used to test virulence of *B. cereus* group bacteria. Both functionally mammalian cells and the nematode *C. elegans* have successfully been used as models to investigate the virulence of other pathogenic microorganisms. When appropriate methods and positive control have been developed/found, selected *B. thuringiensis* strains will be analysed in the models.

Results from our attempts to develop the models for assessment of *B. thuringiensis* will be presented.

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**Fate of *Bacillus thuringiensis* subsp. *kurstaki* HD1 on vegetables
in the field**

Niels Bohse Hendriksen and Bjarne Munk Hansen

¹*Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, Aarhus University, Frederiksborgvej 399, DK-4000 Roskilde, Denmark*

Microbial pest control products based on the activity of *Bacillus thuringiensis* is in common use for the control of lepidopteran larvae. Several of these products contain the strain *B. thuringiensis kurstaki* HD1. Despite its wide use the survival and activity in the environment is scarcely known.

On the basis of experimental field releases with *B. thuringiensis kurstaki* HD1 on cabbage and curly kale, observations on horticultural uses of the pesticide Dipel, containing *B. thuringiensis kurstaki* HD1 and information from the open literature, a simple explanation model for the fate of *B. thuringiensis kurstaki* HD1 has been established.

The model will be presented and discussed in relation to the obtained results and current knowledge. Further, the implications for risk assessment of microbial pesticides based on *B. thuringiensis kurstaki* HD1, residues on vegetables and the exposure of humans will be discussed.

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**Differential transfer dynamics of pAW63 plasmid among members
of the *Bacillus cereus* group in food microcosms**

Pauline Modrie, Elise Beuls and Jacques Mahillon

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

The potential for plasmid transfer among members of the *Bacillus cereus* group, which includes the biopesticide *Bacillus thuringiensis*, the human opportunistic *Bacillus cereus sensu stricto* and the pathogen *Bacillus anthracis*, is of high economic and health interest. Indeed, virulence features of these Gram-positive bacteria are generally conferred by plasmids that encode virulence factors and often possess self-transfer capabilities.

In this work, dynamics of plasmid transfer between *B. thuringiensis* and *B. cereus* was assessed in various food microcosms using the *B. thuringiensis* pAW63 and *Staphylococcus aureus* pUB110 plasmids as models. The conjugative behaviour pAW63, which resembles the *B. anthracis* virulence plasmid pXO2, and the mobilization of pUB110 were investigated using kinetics studies performed in reference LB medium, full-cream and skimmed milks, soya milk and rice milk. Transfers of pAW63 and pUB110 were found to occur in the five tested media, with higher frequencies observed in food matrices, most notably in full-cream milk, skimmed milk and soya milk, where the mean transfer frequencies reached 10^{-3} transconjugants per recipient cell. The most notable observations were that the higher transfer frequencies obtained in foodstuffs compared to those observed in LB were due to an earlier onset of conjugation in combination with a higher transfer rate and/or a longer mating period. This new approach to study plasmid transfer provides insights for a better understanding of conjugation in food microcosms from both animal and vegetable origins among members of the *B. cereus* group.

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Distribution, diversity and potential mobility of extra-chromosomal elements related to the *Bacillus anthracis* pXO1 and pXO2 virulence plasmids

**Xiaomin Hu¹, Géraldine Van der Auwera¹, Sophie Timmery¹, Lei Zhu²
and Jacques Mahillon¹**

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Large plasmids of *Bacillus cereus* group species are important genetic carrier for their major phenotypical features and respective virulence spectra. In *Bacillus anthracis*, the anthrax toxin and capsule genes responsible for anthrax disease are located on the 182-kb pXO1 and 95-kb pXO2 plasmids, respectively. The presence of plasmids with extensive sequence similarity and broad synteny to these plasmids (named pXO1-like and pXO2-like plasmids) have been reported recently in clinical isolates of *B. cereus* (*sensu stricto*) and in strains of the biopesticide *Bacillus thuringiensis*. Furthermore, the pXO2-like plasmids pBT9727 and pAW63 have been shown to be conjugative and capable of mobilizing small plasmids.

In order to understand the ecological significance of pXO1- and pXO2-like plasmids on public health and safety issues, the distribution, diversity and potential mobility of extra-chromosomal elements related to the pXO1 and pXO2 virulence plasmids were evaluated. 1,000 *B. cereus* group isolates, originating from a wide range of geographical and ecological sources (soil, water, food, animals, plants and clinical samples), were analysed for the presence of pXO1- and pXO2-like replicons and for pXO2-related conjugative modules. The occurrence frequency of pXO1- and pXO2-like replicons in random environmental samples was ca. 6.6% and 7.7%, respectively. None tested positive for the anthrax virulence factors, but variations in size suggested that many of these plasmids carry some kind of genetic “payload” in addition to the conserved backbone. The highest prevalence of isolates carrying pXO1- and pXO2-like plasmids was found in soil, which may constitute a natural reservoir for these plasmids. Further screening revealed that 1.54% of the strains were positive for pXO2-like transfer module genes and only the strains harbouring a pXO2-like replicon also contained the corresponding transfer genes. Furthermore, 17 out of 22 pXO2-like plasmids containing the transfer modules displayed the capability of self-transfer and of mobilising small plasmids.

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Among the strains possessing the putative pXO2-like conjugative apparatus, variations on the presence of the group II introns B.th.I.1 and B.th.I.2 were observed, suggesting an important flexibility of the conjugation modules and their regulation. Interestingly, there was no consistent correlation between pXO2-like *repA* dendrogram and presence/absence of *tra* region, nor between *virB4* dendrogram and transfer capabilities. Incongruence between pXO2-like *repA* and *virB4* dendrograms was also observed, indicating that the evolution of pXO2 is an active process. The comparison based on plasmidic *versus* chromosomal genetic backgrounds of 80 sympatric soil-borne *B. cereus* group isolates, which were collected from two neighbouring sites in Belgium, identified probable instances of horizontal transmission of the pXO1- and pXO2-like plasmids across lineages within the *B. cereus* group.

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New *Bacillus thuringiensis* isolates from sawflies (Hymenoptera: Symphyta) larvae and their insecticidal activity against Diprionidae

Alicja Sierpinska

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The main purpose of the studies was exploration for new *B. thuringiensis* isolates from sawflies larvae and evaluation of their insecticidal activity against Diprionidae larvae, which belongs to the most dangerous forest defoliators in Poland. There were 2 projects concerning the above subject: the first was conducted in 1996-2000 and the second – in 2005-2008.

During the studies around 30 new *B. thuringiensis* isolates were found in sick and dead larvae of 4 sawflies species: *Diprion pini* L., *Gilpinia pallida* Kl., *Acantholyda posticalis* Mats. and *Cefalcia falleni* Dalm. In 1996-2000 the insecticidal activity of 12 isolates from *D. pini* was checked. In 1998 the most promising of them caused 71 % mortality of test larvae on 7th day of experiment. In 1999 the mortality of the same species test larvae estimated on 7th day was 34 % (mortality corrected with an Abbott formula). In the above experiments the origin of test larvae was the only difference concerning the test conditions. In the second project the insecticidal activity of 10 isolates (among them – the most promising one in the former project) was estimated. The isolate with the highest insecticidal activity caused 47 % mortality of test larvae on the 7th day of experiment. No feeding inhibition of *B. thuringiensis* treated larvae was observed during all experiments. A kind of synergistic reaction was observed, when larvae were feeding pine needles dipped in a mixed suspensions of one *B. thuringiensis* isolate and Dimilin (inhibitor of chitin synthesis). Implications of the results will be discussed.

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***Bacillus thuringiensis* subsp *roskildiensis* (H-45) – an emerging commercial strain for control of *Nasutitermis ehrhardti* ?**

Bjarne Munk Hansen and Niels Bohse Hendriksen

Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, Aarhus University, Frederiksborgvej 399, DK-4000 Roskilde, Denmark

Bacillus thuringiensis subsp *roskildiensis* was isolated from apple leaves collected in Copenhagen in August 1992. This special isolate is characterised by a rather amorphous crystal firmly attached to the spore. No known bio-control activity was associated with the isolate. Sato and Asano (2004) found that the strain encoded a putatively nematode-toxic crystal protein, Cry21Ba1. However, a paper from de Castilhos-Fortes in 2002 reported that *Bt roschildiensis* had activity against termites. The possibility for using this activity commercially is now being exploited. Available data on the isolate will be presented as introduction to a discussion of the possibilities for commercialisation of the strain.

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Restriction of cry gene expression to insect wound site by using a wound-induced promoter (AoPR1) isolated from *Asparagus officinalis*

**Selma Onarici¹, Mohsin Abbas Zaidi², Ibrahim Taga², Sebahattin Özcan³
and Illimar Altosaar⁴**

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²*Agricultural Biotechnology Laboratories, Department of Biochemistry Microbiology and Immunology, University of Ottawa, 451 Smyth Rd., Ottawa, ON, K1H 8M5, Canada*

³*Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110 Diskapi, Ankara, Turkey*

Recently, it was demonstrated that a wound-induced promoter (AoPR1) from *Asparagus officinalis* showed strong reporter *gus* gene expression in wound site and developing callus tissue, whereas it was virtually silent in non-wounded leaves, roots and tubers (the latter in the case of potato) tissue from the transgenic plants. The AoPR1 promoter was later fused to *cryIAc* gene and transferred to tobacco. The leaves of transgenic tobacco plants were evaluated for resistance against *Heliothis virescens* and *Manduca sexta* in insect bioassays in two different ways. The detached tobacco leaves were either fed directly to the insect larvae or they were first mechanically wounded followed by feeding 72 h post-wounding period. A complete protection of mechanically wounded leaves of transgenic plants was observed within 24 hours of the bioassay. The leaves of transgenic plants fed directly (without pre-wounding) to the larvae achieved the same level of protection from 24 to 72 h of the bioassay. An IRM strategy of limiting Bt expression to wound sites only by using the AoPR1 promoter might lower the selection pressure thus delaying the build-up of resistance in the target insect population. Moreover, studies based on the expression of the GUS reporter gene under the control of the AoPR1 promoter suggest that Bt toxin will not accumulate in pollen, unwounded plant organs, seed and crop residues, thus minimizing food and environmental concerns. Elimination of high levels of Bt toxin throughout the growth period of transgenic crops may also help improve the marketability of such Bt crops.

**Bacterial Toxins for Insect Control.
Safety, Ecology and New Strains**

***Bacillus thuringiensis* serovar. *thompsoni* HD542 crystal proteins:
research overview**

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Cry15Aa protein, produced by *Bacillus thuringiensis* serovar. *thompsoni* HD542 in a crystal together with a 40 kDa accompanying protein is one of a small group of non-typical, less well-studied members of the Cry family of insecticidal proteins, and may provide an alternative for the more commonly used Cry proteins in insect pest management. However, in many aspects the research data available is not complete or contradictory. Here we do present an overview of the Cry15Aa related publications and our own studies on insecticidal activity, crystal formation and the function of 40kDa protein.

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