caseinase / gelatinase named Vsp for vesicular serine protease, which is homologous to the VesA serine protease of *Vibrio cholerae*, was found to be specifically secreted through OMVs in which it is enclosed. Vsp was shown to participate in the virulence phenotype of LGP32 in oyster experimental infections. Finally, OMVs were highly protective against antimicrobial peptides, increasing the minimal inhibitory concentration of polymyxin B by 16-fold. Protection was conferred by OMV titration of polymyxin B but did not depend on the activity of Vsp or another OMV-associated protease. Altogether, our results show that OMVs contribute to the pathogenesis of LGP32, being able to deliver virulence factors to host immune cells and conferring protection against antimicrobial peptides.

Wednesday, 16:30-18:30

POSTERS

BACTERIA

Poster / Bacteria. Wednesday, 16:30. **BA-1**

A New Local Bio-Insecticide: Developing, Optimization, Toxicity and Determination of Activity

Kazım Sezen, Remziye Nalcacioglu, Ismail Demir, Hüseyin Tepe, Islam Yildiz, Ardahan Eski, Zihni Demirbag
Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Turkey
Address for Correspondence: remziye@ktu.edu.tr

The insects belonging to the order Coleoptera are one of the most harmful insect groups in our country and in all over the world. Members of coleopteran cause serious damages in the agricultural fields and the forested areas and the warehouses. So far, efforts to control coleopteran pests have mainly involved the use of chemical insecticides. These agents can have undesirable side-effects on humans, plant and other animal species, particularly predators and parasites of pests. In this study, we proposed to develop a biological preparation (bio-insecticide) against coleopteran pests using an insecticidal isolate of *Bacillus thuringiensis* subsp. *tenebrionis* (Mm2). Our results showed that the isolate has maximum growth at 30°C, at pH 7 in Tryptic Soy Broth containing 1% NaCl. Its sporulation was supported in synthetic medium and the bacterial cell suspension was produced in pilot fermenter. Powder bio-pesticide was produced using this cell suspension and necessary formulation materials in the spray dryer. The physical and biological properties like wettability, suspensibility, particle size, moisture content, and viable spores of the formulated powder were determined and noted as 24 s, 80%, 10 µm, 5% and $10x10^{12}$ (CFU/gdw), respectively. Insecticidal activity of the product against *Agelastica alni* and *Stophilus granarius* adults in laboratory conditions were investigated. Mortality results were identified as 37% against *S. granarius* and 100% against *Agelastica alni*.

Poster / Bacteria. Wednesday, 16:30. **BA-2**

`*Candidatus* Rickettsiella isopodorum´, a new lineage of intracellular bacteria infecting woodlice

Regina G. Kleespies[1]; Andreas Leclerque[1,2]
[1]Institute for Biological Control, Julius Kühn Institute (JKI), Germany; [2]Geisenheim University, Institute for Microbiology and Biochemistry, Geisenheim, Germany
Address for Correspondence: regina.kleespies@jki.bund.de

The taxonomic genus *Rickettsiella* (*Gammaproteobacteria; Legionellales*) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans. Ultrastructural together with genetic evidence is provided for a *Rickettsiella* bacterium occurring in Germany in the common rough woodlouse, *Porcellio scaber* (Isopoda, Porcellionidae). The new bacterium is found very closely related to a *Rickettsiella* strain from California that infects the pill bug, *Armadillidium vulgare* (Isopoda, Armadillidiidae). Both bacterial isolates display the ultrastructural features described previously for crustacean-associated bacteria of the genus *Rickettsiella*, including the absence of well-defined associated protein crystals; occurrence of the latter is a typical characteristic of infection by this type of bacteria in insects, but has not been reported in crustaceans. As demonstrated by a molecular systematic approach combining multilocus sequence analysis (MLSA) with likelihood-based significance testing, both bacteria - despite their distant geographic origins - form a tight sub-clade within the genus *Rickettsiella*. In the 16S rRNA gene trees, this sub-clade includes other bacterial sequences from woodlice. Moreover, the bacterial specimens from *P. scaber* and *A. vulgare* are found genetically or morphologically different from each of the four currently recognized *Rickettsiella* species. Therefore, the designation `*Candidatus* Rickettsiella isopodorum´ has been introduced for this new lineage of isopod-associated *Rickettsiella* bacteria.
Reference: Kleespies R.G., Federici B.A., Leclerque A. Ultrastructural characterization and multilocus sequence analysis (MLSA) of `*Candidatus* Rickettsiella isopodorum´, a new lineage of intracellular bacteria infecting woodlice (Crustacea: Isopoda). Systematic and Applied Microbiology, in press.

Poster / Bacteria. Wednesday, 16:30. **BA-3-STU**

Analysis and characterization of binary AB toxins in the honey bee pathogen *Paenibacillus larvae*

Julia Ebeling, Lena Poppinga, Anne Fünfhaus, Elke Genersch
Institute for Bee Research, Hohen Neuendorf, Brandenburg, Germany
Address for Correspondence: elke.genersch@rz.hu-berlin.de

The gram-positive spore-forming bacterium *Paenibacillus larvae* is responsible for American foulbrood in honeybees. Four *P. larvae* genotypes could be distinguished via genotyping with ERIC-primers, ERIC I – IV, with genotypes ERIC I and II being frequently isolated form outbreaks worldwide. The most important phenotypic difference between the genotypes are the differences in virulence. Recent studies show that binary AB toxins play an important role in the infection mechanism, presumably in breaching the larval midgut epithelium as crucial step in pathogenesis. AB toxins usually consist of two subunits which are encoded either by the same or different open reading frames (ORF). The A subunit is enzymatically active and modifies a cellular target, e.g. by mono-adenosine diphosphate (ADP)-ribosylation. Contrarily, the B subunit is responsible for cell surface receptor binding and the translocation of the A subunit into the cell. Recently, two binary AB toxins, Plx1 and Plx2, have been identified as virulence factors in *P. larvae* ERIC I. The study on further binary AB toxins in *P. larvae* will be

continued via exposure bioassays with knockout mutants. We aim at analyzing and characterizing the binary AB toxins in *P. larvae* in order to gain further insight into the pathogenic mechanisms of *P. larvae.*

Poster / Bacteria. Wednesday, 16:30. **BA-4**

Interplay of Regulators Controlling Fit Insect Toxin Expression in the Biocontrol Bacterium *Pseudomonas protegens*

Nicola Imperiali [1], Flavia Büchler [1], Maria Péchy-Tarr [1], Peter Kupferschmied [1], Monika Maurhofer [2], and Christoph Keel[1]
[1] Department of Fundamental Microbiology, University of Lausanne, Switzerland, [2] Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland
Address for Correspondence: nicola.imperiali@unil.ch

The root-colonizing biocontrol agent *Pseudomonas protegens* CHA0 protect its plant hosts from fungal diseases by releasing toxic exoproducts into the rhizosphere. Remarkably, this microorganism might also function as a natural insecticide since it is capable of exhibiting potent oral and systemic insecticidal activity against various pest insect species. Recently, our group discovered an insecticidal protein toxin termed Fit, which makes essential contributions to insect killing. The Fit toxin gene *fitD* is located in a virulence cluster coding for a type I secretion system (FitA, FitB, FitC and FitE) essential for toxin transport and also for three regulators (FitF, FitG and FitH) of toxin expression. By using a Δ*fitF* Δ*fitG* Δ*fitH* triple mutant, in which each regulatory gene was individually reintroduced and expressed, we observed that the expression of the *fitABCDE* operon is positively regulated by the LysR-type regulator FitG and repressed by the response regulator FitH. We demonstrate that a phosphorylation of the conserved aspartate residue (D59) in the receiver domain of FitH is necessary to eliminate the repressive activity of the regulator, and that this residue is necessary for Fit toxin expression. Findings of an analysis of the heterologous expression of regulatory genes *fitG* and *fitH* in naturally Fit-locus deficient strains carrying a *gfp* reporter monitoring the *fitA* leader sequence activity strongly suggests that the LysR-type regulator FitG promotes Fit-toxin expression through specific binding to the promoter of the *fitABCDE* operon. These results allowed to improve the model explaining the regulation of Fit toxin expression in *P. protegens* CHA0.

Poster / Bacteria. Wednesday, 16:30. **BA-5-STU**

Identification and Characterization of *Bacillus thuringiensis* Strains with Nematicidal Activity

Luis A. Verduzco-Rosas and Jorge E. Ibarra
CINVESTAV IPN, Irapuato, Mexico
Address for Correspondence: lverduzco@ira.cinvestav.mx

Crystal proteins from the soil bacterium *Bacillus thuringiensis* (Bt) are globally used in agriculture as biological control agents against insect pest, but its use as a nematicidal control agent is still under development. In this work, a total of 310 Bt strains were screened for activity against the free-living nematode *Caenorhabditis elegans*. Strains LBIT-596 (serotype darmstadiensis) and LBIT-107 (serotype neoleonensis) showed significant toxicity levels. These strains were characterized by plasmid and RepPCR patterns, and flagellin gene sequencing. Preliminary bioassays of LBIT-596 and LBIT-107 spore-crystal complexes estimated LC_{50}s at 63.36 and 76.33 µg/ml, respectively, and 24.2 and 24.99 µg/ml, respectively, when pure crystals were tested. SDS-PAGE protein content analyses of LBIT-596 crystals showed two proteins (35 and 130 kDa) before activation, which turned into lower molecular-weight proteins (28 and 55 kDa) after activation. LBIT-107 also showed two major proteins of 28 and 70 kDa, before activation. Amplicons from the *cry*-gene conserved blocks and from *cyt1* gene group were cloned and sequenced. Sequence analyses indicated that LBIT-596 contains sequences identified within the *cry5B* and *cyt1A* gene families, while LBIT-107 contains sequences identified within the *cry14* and *cyt1A* gene families. Interestingly one of the amplicons from LBIT-107 showed only 88% identity with the *cry14A* gene. These results indicate a potential use of these toxins against economically important parasitic nematodes.

Poster /Bacteria. Wednesday, 16:30. **BA-6**

Evaluation of Culture media for maximal growth, Cry toxin production and insecticidal toxicity of *Bacillus thuringiensis*

M. Tripathi [1], A. Kumari[2], L. Saravanan [3], G.T. Gujar [4]
[1,4] Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, [2] TERI, India Habitat Centre, New Delhi, [3]Directorate of Medicinal and Aromatic Plants Research, Anand
Address for Correspondence: gtgujar@yahoo.com

Bacillus thuringiensis Berliner is a gram positive soil dwelling, aerobic bacterium which produces parasporal crystal (Cry) toxins that are highly specific and effective against insect species. During the course of isolation of native strains, *B. thuringiensis* AUG-05 was found the most effective with a wide range of activity against lepidopterans. Hence, studies were carried out on its fermentation in different media to evaluate the production of maximal Cry toxin as well as spore and colony forming unit (cfu) counts. Increase in concentration of the Luria Bertani [(LB), composed of casein, yeast extract and sodium chloride in 2:1:2 w/w)] medium in the fermentation broth from 1 to 2% enhanced cfu, spore and also Cry1Ac and Cry2Ab toxin content. Addition of 1% Wesson salt in 1% LB broth dramatically increased spore, cfu counts, and also that of Cry1Ac but not of Cry2Ab. Spore and cfu counts in media were positively correlated with Cry1Ac and Cry2Ab contents. Bt powders from each fermentation with varying ratios of Cry1Ac and Cry2A toxins were more toxic to the cotton bollworm, *Helicoverpa armigera* than the tobacco caterpillar, *Spodoptera litura* and. Of all media substituting LB with agroproducts, most did well in supporting *B. thuringiensis* culture except for medium VI and VII, suggesting need for balancing qualitative and quantitative nutrients in the medium for optimal growth of the bacterium. Medium consisting of 2% wheat flour, 2% soybean meal and 1% Wesson salt could be considered as an alternative to LB medium to achieve economy of production costs.

Poster / Bacteria. Wednesday, 16:30. **BA-7**

Gene organization of large plasmids of novel mosquitocidal *Bacillus thuringiensis* TK-E6

Mayu Noda, Naruhei Okamoto, Kimie Hayasaki, Yoshinao Azuma, and So Takebe
Faculty of Biology-Oriented Science and Technology, Kinki University, Wakayama 649-6493, Japan
Address for Correspondence: takebe@waka.kindai.ac.jp

A novel *Bt* strain, TK-E6, isolated from grove soil in Japan, produces a mosquitocidal inclusion body called crystal consisting of several Cry proteins during sporulation phase. We detected twelve genes belong to the *cry* family, by degenerate PCR from *Bt*. TK-E6. Nucleotide sequences of these genes were determined and deduced ORFs encoding 140 - 145 kDa Cry proteins were cloned into a *Bt* expression vector carrying *cyt1A* promoter and *cry4A* terminator. Each Cry protein was purified and used for mosquitocidal assay against *Ae*. *aegypti* larva, any protein, however, did not show the strong activity when used alone. These results suggested that there was a synergistic action with some proteins for mosquitocidal activity. Pulse-field gel electrophoresis analysis showed that *Bt*. TK-E6 had five

plasmids ranging 66 - 224 mDa. Southern hybridization experiments revealed that twelve genes we detected had been distributed on four of five large plasmids. Interestingly, insertion sequences and transposon structures are also found in the up- and downstream of all genes. It is very possible that some DNA rearrangement of gene amplification occurred in both intra- and inter-plasmids during the evolutional process of *Bt*. TK-E6. Elucidation of structure of *Bt.* TK-E6 large plasmids is very important to know evolution of *Bt*. Therefore, we are analyzing the gene organization of large plasmids by the next generation sequencer.

Poster / Bacteria. Wednesday, 16:30. **BA-8-STU**

Testing of Vip3 proteins for the control of caterpillar pests

Iñigo Ruiz de Escudero [1,2], Núria Banyuls [3], Yolanda Bel [3], Mireya Maeztu [1], Baltasar Escriche [3], Delia Muñoz [2], Primitivo Caballero [1,2], Juan Ferré [3]
[1]Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, 31192 Mutilva, Navarra, Spain. [2]Laboratorio de Entomología Agrícola y Patología de Insectos, Departamento de Producción Agraria, Universidad Pública de Navarra, 31006 Pamplona, Spain. [3]Departamento de Genética, Facultad de CC. Biológicas, Universitat de València, 46100 Valencia, Spain
Address for Correspondence: bafenu@uv.es

Vip3 insecticidal proteins are produced by *Bacillus thuringiensis* during the vegetative growth phase and most of them have activity against lepidopteran species. Five *B. thuringiensis* Vip3A proteins (Vip3Aa, Vip3Ab, Vip3Ad, Vip3Ae and Vip3Af) and their corresponding trypsin-activated toxins were tested for their toxicity against eight lepidopteran pests: *Agrotis ipsilon*, *Helicoverpa armigera*, *Mamestra brassicae*, *Spodoptera exigua*, *Spodoptera frugiperda*, *Spodoptera littoralis*, *Ostrinia nubilalis* and *Lobesia botrana.* Vip3Aa, Vip3Ae and Vip3Af were the most active proteins. Vip3Af was the protein active against most of the species tested. Contrarily, Vip3Ad was non-toxic to any species. *Agrotis ipsilon* was the species most susceptible to the four active proteins, whereas *O. nubilalis* was tolerant to all Vip3 proteins tested, with just some susceptibility to Vip3Af. The results obtained will help to design new combinations of insecticidal protein genes in transgenic crops or in recombinant bacteria for the control of insect pests.

Poster / Bacteria. Wednesday, 16:30. **BA-9**

Interactions between Cry and Vip proteins from *Bacillus thuringiensis* against different lepidopteran pests

Ana Rita Nunes Lemes[1], Camila Chiaradia Davolos[1], Paula Cristina Brunini Crialesi Legori[1], Odair Aparecido Fernandes[2], Juan Ferré[3], Manoel Victor Franco Lemos[1], Janete Apparecida Desiderio[1]
[1]Dpto de Biologia Aplicada à Agropecuária, UNESP/Campus de Jaboticabal, Brazil, [2] Dpto de Fitossanidade, UNESP/Campus de Jaboticabal, Brazil, [3] Dpto de Genética, Universidade de València, Spain
Address for Correspondence: mvictor@fcav.unesp.br

Second generation *Bt* crops (insect resistant crops carrying *Bacillus thuringiensis* genes) combine more than one gene coding for insecticidal proteins in the same plant to provide a better control of agricultural pests. Some of the new combinations involve co-expression of *cry* and *vip* genes. Since Cry and Vip proteins have different midgut targets and possibly different mechanisms of toxicity, it is important to evaluate possible synergistic or antagonistic interactions between these two classes of toxins. Three members of the Cry1 class and three from the Vip3A class were tested against *Heliothis virescens* for possible interactions. At the level of LC_{50}, Cry1Ac was the most active protein, whereas the rest of proteins were similarly active. However, at the level of LC_{90}, Cry1Aa and Cry1Ca were the least active proteins, and Cry1Ac and Vip3A proteins were not significantly different. In the experimental conditions used, we found an antagonistic effect of Cry1Ca with the three Vip3A proteins and a slight antagonism of Vip3Af with either Cry1Aa or Cry1Ac. The interaction between Cry1Ca and Vip3Aa was also tested on two other lepidopterans. Whereas antagonism was observed in *Spodoptera frugiperda*, synergism was found in *Diatraea saccharalis.* In all cases, the interaction between Vip3A and Cry1 proteins was more evident at the LC_{90} than at the LC_{50} level. The fact that the same combination of proteins may result in a synergistic or an antagonistic interaction may be an indication of different types of interaction with the host depending on the insect species tested.

Poster / Bacteria. Wednesday, 16:30. **BA-10**

Cry1Ac and Cry1F toxicity and binding sites study in two important soybean pests, *Anticarsia gemmatalis* and *Chrysodeixis (=Pseudoplusia) includens.*

Yolanda Bel [1], Ken Narva 2, Joel Sheets [2], Baltasar Escriche [1]
[1]Dept. Genetics, ERI BioTecMed, Universitat de València, Dr. Moliner, 50. 46100-Burjassot, Valencia, SPAIN; [2] Dept. Biochemistry/Mol. Biology. Dow AgroSciences LLC. 9330 Zionsville Rd. Indianapolis IN 46268, USA
Address for Correspondence: Baltasar.escriche@uv.es

Anticarsia gemmatalis (velvetbean caterpillar) and *Chrysodeixis (=Pseudoplusia) includens* (soybean looper) are two important defoliating insects of soybean that cause economic damage in soybean producing regions in the Americas. Both lepidopteran pests are currently controlled mainly with synthetic insecticides. Alternative control strategies such as biopesticides based on the *Bacillus thuringiensis* (Bt) toxins or transgenic plants expressing Bt toxins can be used and are increasingly being adopted. The studies on the insect susceptibility and mode of action of the different Bt toxins are crucial to determine management strategies to delay insect resistance. Also, these studies are necessary to help design pyramided transgenic plants involving more than one Bt toxin to ensure a crop long term protection.
In the present study the susceptibility of both soybean pests to Cry1Ac and Cry1F has been investigated. Bioassays performed in larvae show that both insects are susceptible to these two toxins. Competition-binding studies using brush border membrane vesicles indicate that Cry1F and Cry1Ac share some, but not all, binding sites in midguts of both insects. Incomplete shared binding indicates that there are resistance management benefits from combining the two proteins in Bt soybeans. Additional information on the receptors involved in binding and consequent cross-resistance potential are needed to more fully understand the long-term durability of combinations of Cry1Ac and Cry1F to control these two pests.

Poster / Bacteria. Wednesday, 16:30. **BA-11-STU**

***In vivo* and *in vitro* binding of Vip3Aa to *Spodoptera frugiperda* midgut and characterization of binding sites using ^{125}I-radiolabeling**

Maissa Chakroun and Juan Ferré
Department of Genetics, University of Valencia, 46100-Burjassot (Valencia), Spain
Address for correspondence: Chakrounmaissa7@gmail.com

Bacillus thuringiensis vegetative insecticidal proteins (Vip3A) have been recently introduced in important crops as a strategy to delay the emerging resistance to the existing Cry toxins. The mode of action of Vip3A proteins has been studied in *Spodoptera frugiperda* with the aim to characterize their binding to the insect midgut. Histological localization of Vip3Aa in the

midgut of intoxicated larvae using immunofluorescence showed that Vip3Aa bound to the brush border membrane along the entire apical surface. The presence of fluorescence in the cytoplasm of epithelial cells seems to suggest internalization of Vip3Aa or a fragment of it. Successful radiolabeling and optimization of the binding protocol for the ^{125}I-Vip3Aa to *S. frugiperda* BBMV allowed the determination of binding parameters of Vip3A proteins for the first time. Heterologous competition was performed using different protein competitors with the aim to determine if they share the same binding sites with Vip3Aa in *S. frugiperda* BBMV and thus select the appropriate candidates to be used in combination with the later in transgenic crops.

Poster / Bacteria. Wednesday, 16:30. **BA-12**

Comparative histopathology of two novel bacterial insecticidal proteins in *Tenebrio molitor* and *Diabrotica virgifera virgifera* larvae

Heba Abdelgaffar [1]; Cris Oppert [2], Jayme Williams [2], Deepa Balasubramanian [2], Juan Luis Jurat-Fuentes [1]
[1] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville (TN), USA; [2] Bayer CropScience, Morrisville (NC), USA
Address for Correspondence: jurat@utk.edu

Larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*) are the most devastating pest of corn in the US. Due to reports of field-evolved resistance, novel insecticidal proteins are needed as alternative candidates for expression in transgenic corn to control this insect pest. A novel insecticidal protein from a Gram negative bacterium (toxA) and a Cry-derived protein (toxB) have been identified and developed, respectively, as candidates for expression in transgenic corn targeting larvae of *D. v. virgifera*. In this work, we used *Tenebrio molitor* larval midgut as a model to characterize toxin binding and histopathology of toxA and toxB proteins in coleopteran larvae, and then compared to histopathology in *D. v.virgifera* larval midguts. While both toxins bound to the midgut brush border membrane, differences observed in H&E stained histological sections and TUNEL assays support differences in the mode of action of these toxins in coleopteran larvae.

Poster / Bacteria. Wednesday, 16:30. **BA-13-STU**

Role of ABC-C2 in the interactions of *Heliothis virescens* with its host plants and Bt toxins

Anne Karpinski, Yannick Pauchet, Heiko Vogel and David Heckel, Department of Entomology, Max Planck Institute for Chemical Ecology, Jena Germany
Address for Correspondence: akarpinski@ice.mpg.de

Bacillus thuringiensis (Bt) Cry toxins are widely used biopesticides for reduction of crop losses caused by larvae from species such as *Heliothis virescens*. Until recently, Cadherin was identified as the major receptor for Bt toxins, albeit Bt resistance was shown to be genetically linked to an inactivating mutation in an ABC transporter. ABC (ATP-binding cassette) transporters are transmembrane proteins that hydrolyze ATP in order to conduct transport and other cellular processes. To date, we have no insights into the physiological role of this specific ABC transporter as well as into its role in the Bt toxin mode of action. We aim to investigate whether ABC-C2, the specific ABC transporter implicated in Bt resistance, acts as a receptor to Cry toxins. Furthermore, we want to find out whether an inactivated (mutated) ABC-C2 could cause a trade-off between Cry toxins and host plant secondary metabolites in Bt resistant insects. To address these two hypotheses, we first heterologously express *H. virescens* Cadherin and ABC-C2 in Sf9 cells. In addition, feeding assays with two *H. virescens* populations, JEN2 (wild type) and YEE (ABC-C2 mutant), are performed with different host plants as well as host plant secondary metabolites incorporated into artificial diet. The genes of interest were expressed successfully, generating the basis for our ongoing *in vitro* trials. Subsequently, the effect of different Cry toxins on transfected cells will be investigated. Our first feeding assays with homozygous Bt susceptible and homozygous Bt resistant insects revealed a trade-off between Cry toxins and host plant secondary metabolites

Poster / Bacteria. Wednesday, 16:30. **BA-14-STU**

AminomemtidaseN in *Popillia japonica* Newman larvae is putative *Bacillus thuringiensis* Cry8Da toxin receptor

Yuu Taniguchi, Takuya Yamaguchi, Hisanori Bando, Shin-ichiro Asano
Graduate School of Agriculture, Hokkaido University, Sapporo, Japan
Address for correspondence: sangaku@abs.agr.hokudai.ac.jp

Cry8Da from *Bacillus thuringiensis galleriae* SDS-502 has insecticidal activity against both the larvae and adult of Japanese beetle (*Popillia japonica* Newman). The receptor determines the specificity of the insecticidal activity of Cry proteins and hence, in order to reveal the mode of action of Cry toxin, receptor identification is a necessary step. However, a receptor for Cry8-type toxin has not been identified in the Scarabaeidae family of insects. Therefore, we aimed to identify the receptor of Cry8Da toxin in larvae *P. japonica* BBMV. A ligand blot showed the Cry8Da toxin bound to 110 kDa and 40 kDa protein in the BBMV of larvae *P. japonica*. The 110 kDa protein had higher binding affinity than the 40 kDa protein. In order to identify the Cry8Da toxin binding protein in the BBMV of larvae *P. japonica*, it was purified by column chromatography. The result of mass spectrometry indicated that the Cry8Da toxin binding protein in the BBMV of larvae was aminopeptidaseN which is commonly reported as receptors for Cry toxins in Lepidopteran and Dipteran insects. The 106 kDa APN homologous genes in larvae *P. japonica* could be amplified by PCR using degenerate oligonucleotide primers designed from a conserved sequence of Coleopteran APN. The 106 kDa APN is truncated into two peptides and tested to confirm the ability of binding with Cry8Da toxin. This experiment indicated the APN in larvae *P. japonica* is the receptor for Cry8Da toxin.

Poster / Bacteria. Wednesday, 16:30. **BA-15**

A Whole Genome Approach to Determine Cadherins associated with Bt toxicity in the Diamondback Moth, *Plutella xylostella*

Youngjin Park and Yonggyun Kim
Department of Bioresource Sciences, Andong National University, Andong 760-749, South Korea
Address for Correspondence: happy2pyj@gmaill.com

Diamondback moth, *Plutella xylostella*, is a main pest of Brassicaceae throughout worldwide and was first reported to evolve resistance to Bt toxins in field population. Cadherin has been known to be one of receptors of *Bacillus thuringiensis* Cry proteins and synergizes Cry toxicity against Lepidopteran, Dipteran, and Coleopteran insects by elevating a toxin oligomerization. Full genome analyses of several model insects suggest various number of cadherin genes in an organism and raise a fundamental question on which cadherin(s) is the Bt receptor. In a whole genome sequence of *P. xylostella*, 52 open reading frames were annotated to be cadherins, in which putative Bt receptors were chosen on the basis of three receptor motifs: a signal peptide, cadherin repeat, and transmembrane domains. Compared to other cadherins of *P. xylostella*

(PxCads), *PxCad1* has the highest homology with other lepidopteran insect cadherins previously associated to the Bt mode of action. *PxCad1* was expressed in all developmental stages especially in gut tissue. Expression of *PxCad1* was suppressed by feeding its specific double-stranded RNA (dsPxCad1) in the third instar. The suppression of *PxCad1* expression did not significantly influence on pupal and adult developments of *P. xylostella*. However, the larvae treated with dsPxCad1 (150 ng/larva) significantly reduced susceptibility to *B. thuringiensis* Cry1Ac toxin. In contrast, the dsPxCad1-treated larvae did not show any change in susceptibility to *B. thuringiensis* Cry1Ca toxin. Only one cadherin, PxCad1, out of 52 candidate cadherins is the Bt receptor and is responsible for the specificity to Bt toxin, Cry1Ac.

Poster / Bacteria. Wednesday, 16:30. **BA-16**

RNA Interference of Integrin subunit β1 Impairs Development and Immune Responses of the Oriental tobacco budworm, *Helicoverpa assulta* against Bacteria

Youngjin Park and Yonggyun Kim
Department of Bioresource Sciences, Andong National University, Andong 760-749, South Korea
Address for Correspondence: happy2pyj@gmaill.com

Integrin is a cell surface protein that is composed of α and β heterodimer and mediates cell interaction with extracellular matrix or other cells including microbial pathogens. A full length cDNA sequence (2,517 bp) of a integrin subunit β1 (*HaITGβ1*) was cloned from the oriental tobacco budworm, *Helicoverpa assulta*. Phylogenetic analysis showed that *HaITGβ1* was clustered with other insect β integrin subunits with the highest amino acid sequence identity (61%) to β1 of other Noctuidae such as *Spodoptera exigua* and *S. litura*. Structural analysis of the *HaITGβ1* possessed all functional domains known in other insect β1 integrins. RT-PCR analysis showed that *HaITGβ1* was expressed in all developmental stages and all tested tissues of *H. assulta*. Injection of double-stranded *HaITGβ1* RNA (dsHaITGβ1) into third instar of *H. assulta* suppressed *HaITGβ1* expression and resulted in significant delay from last larval stage to pupal stage. The dsHaITGβ1 injection significantly impaired nodule formation of *H. assulta* in response to bacterial challenge and hemocyte adherence. These results suggest that *HaITGβ1* plays crucial roles in cellular immune responses as well as development in *H. assulta*.

Poster / Bacteria. Wednesday, 16:30. **BA-17**

A natural hybrid of a *B. thuringiensis* Cry2A toxin implicates domain I in specificity determination.

Guihua Chen [1,3], Changlong Shu[1], Jacob Evans [2], Fuping Song [1], Guoxun Li [3], Neil Crickmore [2], Jie Zhang [1]
[1]State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [2]School of Life Sciences, University of Sussex, Falmer, Brighton, UK; [3]College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao 266109, China
Address for Correspondence: jzhang@ippcaas.cn

A PCR-RFLP method was used to identify *cry2A* toxin genes in a collection of 300 strains of *Bacillus thuringiensis* were confirmed with *cry2* gene. Of the 81 genes identified the vast majority appeared to be *cry2Aa* (32) and *cry2Ab* (46) on the basis of their RFLP pattern. Three genes showed a different pattern and were subsequently cloned and sequenced. The gene cloned from strain HD395 was named *cry2Ba2*. The proteins encoded by the genes cloned from LS5115-3 and DS415 shared enough similarity with existing toxins that their genes were named *cry2Aa17* and *cry2Ab29* respectively by the toxin nomenclature committee. Despite this overall similarity these two toxins resembled natural hybrids with Cry2Ab29 resembling Cry2Ab for the majority of the protein but then showing identity to Cry2Aa for the last 60 amino acids. For Cry2Aa17, domains II and III resembled Cry2Aa whilst domain I resembled Cry2Ab. The toxicity of the recombinant toxins against three insects was tested, and it was found that the toxicity of Cry2Aa17 more closely matched the toxicity profile of Cry2Ab than that of Cry2Aa, thus implicating domain I in specificity determination. Analysis of all publically available Cry2Aa sequences identified other examples of natural hybrids.

Poster / Bacteria. Wednesday, 16:30. **BA-18**

***Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris quatuordecimpunctata* to *Beauveria bassiana* infection**

Yulin Gao, Zhongren Lei, Xuenong Xu
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, PR. China.
Address for Correspondence: ylgao@ippcaas.cn

The spotted asparagus beetle, *Crioceris quatuordecimpunctata* (Coleoptera: Chrysomelidae), is one of the most devastating pests of asparagus in China. Sprayed synthetic pesticides have been used to control *C. quatuordecimpunctata* damage, but they pose problems because of residues and harm to natural enemies. Neither the microbial coleopteran-specific toxin from *Bacillus thuringiensis tenebrionis*, Cry3Aa, nor the fungal pathogen Beauveria bassiana have sufficient activity to effectively control *C. quatuordecimpunctata* damage to asparagus. However, second instar C. quatuordecimpunctata larvae exposed to a sublethal dose of Cry3Aa toxin demonstrated significantly higher larval mortality when exposed to B. bassiana. Our results suggest that a combination of Cry3Aa and *B. bassiana* may be effective in reducing damage by *C. quatuordecimpunctata* larvae to asparagus.

Poster / Bacteria. Wednesday, 16:30. **BA-19**

InterVening Sequence (IVS) elements as genetic markers for the differential diagnosis of arthropod-associated *Rickettsiella* bacteria

Christina Schuster[1]; Katharina Saar[1]; Regina G. Kleespies[1]; Andreas Leclerque[1,2]
[1]Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; [2]Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany
Address for Correspondence: andreas.leclerque@hs-gm.de; leclerque@hotmail.com

Genomic analysis has revealed the presence of insertion sequences within 23S ribosomal RNA encoding genes of arthropod-associated *Rickettsiella* bacteria (*Gammaproteobacteria*). Secondary structure modelling shows that these insertions fulfill the structural criteria for RNase III processed bacterial intervening sequence (IVS) elements.
IVS elements have previously been identified within the rRNA operons of several *Alphaproteobacteria* and occur comparatively frequently within *Enterobacteriaceae*, but not in *Escherichia coli*. In these bacteria, IVS insertion sites have been shown to be conserved with respect to deduced rRNA secondary structures. 23S rRNA gene insertions in *Rickettsiella* occur at one of these conserved loci, more exactly within rRNA helix 25, and at a previously unidentified insertion site within helix 72.
Expression of the *Rickettsiella* 23S rRNA genes in the surrogate host *E. coli* by a plasmid replacement approach leads to rRNA fragmentation and thereby confirms that *Rickettsiella* insertion sequences at both sites can function as IVS elements. Given the

lack of sequence similarity with current GenBank database entries, IVS25 and IVS72 give rise to two unprecedented IVS element superfamilies. Whereas the IVS72 element is highly conserved across the full range of investigated *Rickettsiella* species and *Rickettsiella*-like bacteria, the sequence of element IVS25 strongly varies among different *Rickettsiella* strains. Using the sequence information available for both IVS elements, a PCR-based approach for the genus-specific identification and infra-generic characterization of *Rickettsiella* bacteria has been developed.

Poster / Bacteria. Wednesday, 16:30. **BA-20**

Type IV Secretion System (T4SS) substrates as potential virulence factors of arthropod-pathogenic *Rickettsiella* bacteria

Andreas Leclerque
Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany
Address for Correspondence: andreas.leclerque@hs-gm.de; leclerque@hotmail.com

Rickettsiella bacteria (*Gammaproteobacteria: Legionellales*) are intracellular pathogens of arthropods that multiply inside replicative vacuoles within host cells. Delivery of bacterial proteins across the vacuole membrane to the host cell's cytosol is believed to be of key importance for successful infection and pathogenesis.
Comparative genomic analysis of *Rickettsiella* and related bacteria has revealed the presence of a complete set of gene clusters presumably encoding a type IVB secretion system (T4SS) in two *Rickettsiella* strains of the pathotypes `*R. melolonthae´* and `*R. armadillidii´*, i.e. infecting, respectively, the European cockchafer and the pill bug. Hypothetical *Rickettsiella* T4SS key components show high similarity to orthologs in the Dot/Icm systems of the related vertebrate pathogens *Legionella pneumophila* and *Coxiella burnetii*, and T4SS gene cluster organization is very similar in these bacteria. In *Legionella* and *Coxiella*, involvement of Dot/Icm systems and several of their substrates into infection and pathogenesis has been demonstrated previously. In *Legionella*, transcriptional regulation of both T4SS structural and substrate genes is most likely mediated by several bacterial two-component systems, but only one of these, PmrAB, seems to be conserved in the genomes of both *Coxiella* and *Rickettsiella*. Expression studies in the surrogate host *Escherichia coli* that lacks an own T4SS, have demonstrated that `*R. melolonthae´* PmrAB drives expression from the promoter regions of the presumed homologous T4SS gene clusters. Comparative *in silico* analysis of PmrAB regulons reveals a very high degree of divergence in hypothetical T4SS substrates sets that is in line with expectations from the specific host-adaptation of these bacterial pathogens

Poster / Bacteria. Wednesday, 16:30. **BA-21**

Unbalanced Polyphosphate Levels Impair Insect Pathogenicity in Plant-Beneficial *Pseudomonas protegens*

Maria Péchy-Tarr[1], Nicolas Wenner[1], Peter Kupferschmied[1], Romane Keller[1], Monika Maurhofer[2], Christoph Keel[1];
[1]Department of Fundamental Microbiology, University of Lausanne, Switzerland; [2]Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland
Address for Correspondence: christoph.keel@unil.ch

Pseudomonas protegens is a plant-associated bacterium with lifestyles that potentially may be exploited for its use as a biological control agent in agricultural applications. The bacterium is a highly competitive root colonizer and produces antifungal compounds that ward off soil-borne plant pathogenic fungi and oomycetes. *P. protegens* is also capable of killing larvae of various pest insects following oral or systemic infection. We are exploring global regulatory mechanisms that control insect pathogenicity of the plant-beneficial bacterium. Here, we provide evidence that altering cellular levels of polyphosphate (PolyP) may strongly impair insect pathogenicity in *P. protegens.* The polymer is known for its involvement in regulation of diverse cellular and metabolic processes contributing to bacterial survival and virulence. *P. protegens* mutants with deletions in *ppk1*, encoding a PolyP kinase, or *ppx*, encoding an exopolyphosphatase, had a markedly reduced capacity to kill larvae of the Large White *Pieris brassicae* following oral infection. Oral toxicity could be restored by reintroducing the respective intact alleles into the mutant strains. Deletion of *ppk1* or *ppx* resulted in reduced in situ expression of a major virulence factor required for insect pathogenicity in *P. protegens*, i.e. the insecticidal toxin Fit, in insect larvae. We hypothesize that altering PolyP levels affects stress tolerance of *P. protegens* in the insect host thereby impacting virulence of the bacterium.

Poster / Bacteria. Wednesday, 16:30. **BA-22-STU**

***Paenibacillus larvae* and the virulence factor SplA- an ERIC II specific S-layer Protein**

Henriette Knispel, Lena Poppinga, Anne Fünfhaus, Elke Genersch
Institute for Bee Research, Hohen Neuendorf; Division of Molecular Microbiology and Bee Pathology, Friedrich-Engels-Str. 32, D-16540 Hohen Neuendorf, Germany
Address for Correspondence: elke.genersch@rz.hu-berlin.de

Paenibacillus larvae is the causative agent of the notifiable epizootic American Foulbrood of honey bees. Four genotypes, ERIC I - IV of this pathogen do exist, with only ERIC I and II being frequently isolated from outbreaks worldwide. Despite the importance of the disease, molecular and cellular details of pathogen-host interaction during pathogenesis of AFB in honey bee larvae are poorly understood. Recently, the surface layer protein SplA was identified and functionally characterized as the first virulence factor of the *P. larvae* genotype ERIC II. Through a gene-disruption strategy expression of the *spl*A-gene was successfully interrupted. In infection assays, SplA-deficient *P. larvae* and the parental wild-type bacteria were compared and it was demonstated that lack of SplA expression resulted in a significant decrease in total mortality. To futher investigate the role of SplA in virulence of P. larvae, SplA has been expressed in in the natural SplA-deficient genotype *P. larvae* ERIC I. We will present our most recent data on this SplA-expressing ERIC I-mutant in respect to growth characteristic in the lab and in larvae and, most importantly, to virulence parameters in exposure bioassays when compared to the parental wild type strain and to naturally SplA-expressing ERIC II.

Poster / Bacteria. Wednesday, 16:30. **BA-23**

Influence of (varying) population size on host-parasite coevolution: an experimental approach

Andrei Papkou, Rebecca Schalkowski, Mike-Christoph Barg, Ines Braker, Hinrich Schulenburg
Evolutionary Ecology Genetics, Zoological Institute,CAU Kiel
Address for Correspondence: apapkou@zoologie.uni.kiel.de

Host-parasite interaction is one of the most common and important type of interaction among species, which has a strong impact on species evolution. The signatures of this impact have been identified in genomes, natural communities and on a phylogenetic level. It is not surprising that many aspects of host-parasite relationships have received particular attention from evolutionary biologists. Paradoxically, one indispensible and basic property of host-parasite interaction, population size oscillations, has been overlooked as a factor in host-parasite

coevolution. Parasites, by reducing host fecundity and survival, strongly affect population size of the host, which very often is their only ecological niche. Already in the 1920s Lotka and Volterra showed that antagonistic interactions between species would lead to interdependent oscillations in their population size. However, most of the current models of host-parasite coevolution ignore population size changes or use a deterministic approach which cannot realistically imitate the finite nature of real populations. Similarly, in most experimental studies on host-parasite coevolution the population size is kept constant as a matter of good practice. To enhance a more realistic understanding of the coevolutionary dynamics, we performed laboratory-controlled evolution experiments with the model nematode host *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis* and specifically varied the factor population size. Here, we will show our results on temporal changes in host fitness and parasite virulence under different population size regimes.

Poster / Bacteria. Wednesday, 16:30. **BA-24**

An *in vivo* experimental evolution system for analyzing bacterial adaptation and evolution of *Bacillus cereus sensu lato* in an insect model

Rafael Patiño Navarrete[1,2], Isabelle Jéhanno[1,2], Christina Nielsen-Leroux[1,2] and Vincent Sanchis[1,2]
[1]INRA, UMR1319 Micalis, F-78350 Jouy-en-Josas, France
[2] AgroParisTech, UMR Micalis, F-78350 Jouy-en-Josas, France
Address for Correspondence: Rafael.patino@jouy.inra.fr

The continuous exposition of a pathogenic bacterium in a host during a serial passage experiment (SPE) may drive the fixation of mutations that favour its growth and multiplication in the host environment [1]. These changes that are usually associated with an increase in virulence, can be now traced during an SPE by whole genome sequencing of the evolved variants [2]. Here we describe the set up and initial results of a SPE using a *Bacillus thuringiensis* crystal minus strain (Bt407 Cry-) [3] using *Galleria mellonella* larvae. A new infection protocol has been established which permits bacterial multiplication inside the intestine following force-feeding with spores. The genomes of experimentally evolved bacteria that show significant changes in virulence or persistence will be sequenced and compared with the initial parental strain. Such a global genome based approach of pathogen evolution analysis should allow us to describe the history of the events which arose during the evolution of the *B. cereus* group in one of its natural hosts and explain phenotypic variations based on genotypic differences.

DISEASES OF BENEFICIAL INVERTEBRATES

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-1-STU**

Identification and Characterization of Immune Inhibitor A Metalloprotease of the Honey Bee Pathogen *Paenibacillus larvae*

Birte Arlt[1,2]; Gillian Hertlein[1]; Lena Poppinga[1]; Eva Garcia-Gonzalez[1]; Elke Genersch[1,3]
[1]Institute for Bee Research Hohen Neuendorf, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany; [2]Technische Universität Berlin, Institute of Biotechnology, 13355 Berlin, Germany; [3]Freie Universität Berlin, Institute of Microbiology and Epizootics, 14163 Berlin, Germany
Address for Correspondence: elke.genersch@rz.hu-berlin.de

Honey bees (*Apis mellifera*) are essential pollinators of various agricultural crops and fruit but also of many wild plants. Therefore, it is crucial to maintain honey bee health and prevent or cure diseases. The most contagious and fatal bacterial disease of honey bee brood is American Foulbrood (AFB) caused by *Paenibacillus larvae*, a Gram positive, spore-forming bacterium. Infection spreads among the whole hive, eventually leading to the loss of entire colonies resulting in considerable losses in apiculture. Despite the enormous impact of this disease and intensive research, molecular mechanisms involved in the pathogenesis are still not fully understood. Recently we have identified and characterized four genotypes of *P. larvae* (ERIC I-IV) which differ, among other factors, in virulence. Here we present our data on immune inhibitor A (InhA), a metalloprotease which is exclusively secreted by *P. larvae* ERIC II. In homologs of other pathogenic bacteria, InhA has been shown to have multiple functions such as degradation of anti-microbial peptides and cleavage of tight junctions. Here we functionally characterize InhA of *P. larvae* by combining transcriptomic, proteomic and histological studies as well as *in vivo* exposure bioassays with wild type and mutant P. larvae, the latter being deficient in InhA expression.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-2**

Awareness and Concept of Insects in a Korean Population

Sung Min Bae, Tae Young Shin, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo
Department of Agricultural Biology, Chungbuk National University, Chungju, Korea
Address for Correspondence: sdwoo@cbnu.ac.kr

To investigate the degree of individuals' concept and awareness of insects, a survey study was conducted with students and adults living in Korea. The misconception rate for insects was about 50% for both students and adults, but it was lower for students and people who had experienced insect-related events than for adults and those who had not. The highest misconception rate was obtained in answer to a question about the basic structure of an insect. Most people had a high preference of insects. Significant differences and correlations for the preference of insects were found between students and adults, men and women, people who had experienced insect-related events and those who had not. The experience of an insect-related event most influenced preference of insects. These results suggest that increasing people's interest in insects and utilizing insects in treatment situations may be beneficial for the field of mental healthcare.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-3**

Virus Epizootiology in Managed and Native Bee Populations

John P. Burand[1]; Matthew Boucher[2]; Anne Averill[3]
Departments of [1]Microbiology, [2]Biology and [3]Environmental Conservation, University of Massachusetts - Amherst, Amherst, MA 01003, USA
Address for Correspondence: jburand@microbio.umass.edu

The possible cross host-genus transmission of several honeybee viruses into native bee populations has recently been proposed. Given current pollination practices and the number as well as high levels of different viruses found in honeybees, the cross genus transmission of these viruses could have a dramatic impact on the health of native bees. In order to examine this possibility we initiated a study of the prevalence of the two honeybee viruses; deformed wing virus (DWV) and black queen cell virus (BQCV) in *Apis* and *Bombus sp.* where *Apis* was maintained under different conditions. These included sites