Contributed paper. Wednesday, 15:45 **191**

How do vine mealybug, grapevine leafroll-associated virus and grapevine interact on a molecular level?

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Vine mealybug (VMB), *Planococcus ficus*, is one of the most damaging grapevine pests in the world, largely because it is a vector of grapevine leafroll-associated virus (GLRaV). Since interactions among VMB, GLRaV and grapevine are responsible for the extent of VMB damage and GLRaV spread, we investigated the relationships among the three organisms. The effect of GLRaV infection on VMB was determined using cDNA-AFLP analysis and validated with RT-qPCR. It was found that VMB responds to GLRaV by activating only a few genes, and possibly also by endosymbiont mediation. The effect of VMB feeding on grapevine was investigated with microarray analysis and validated with RT-qPCR. Grapevine was found to respond to VMB feeding by mounting a weak response within a narrow window of time. These results are useful for understanding the interaction among the organisms in the VMB system, and limiting the damage caused in vineyards.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

BACTERIA 4

Contributed paper. Wednesday, 14:00 **192**

Analysis of the bacterial community of the insect pest *Lymantria dispar* during its life cycle

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Gypsy moth (*Lymantria dispar,* Lepidoptera) outbreaks can cause great damage to forestry across Europe. In order to control this pest, there is need for new insecticidal bacterial strains for the development of more effective biopesticides. The insect gut microbiota represents all aspects of microbial relationships, ranging from pathogenic to obligate mutualistic interactions. Latest investigations suggest that there is competition between individual opportunistic pathogens and that they are able to upregulate the production of virulence factors according to their density within hosts, what renders them interesting for use in combination with biocontrol agents.
The objective of this work is to characterize the bacterial midgut community of *L. dispar* and to monitor 1) changes in diversity during its life cycle, 2) changes within larvae from spring to summer and 3) differences between individuals. Microorganisms were first analyzed using a culture dependent approach where midguts were extracted and plated on media. Growing bacteria were analyzed by colony characteristics - color, size, shape, opacity, margin, elevation and viscosity and then by 16S rRNA gene sequencing. In a second step bacterial midgut communities were analyzed by a culture independent method using PacBio technology to sequence full length 16S rRNA genes.
Results showed relatively simple composition of the gypsy moth midgut community. We observed differences between individual larva from the same time point and structural changes of diversity in bacterial communities over the season.

This project is conducted within the frame of the SCIEX program with ETH Zürich and the University of Daugavpils as partners

Contributed paper. Wednesday, 14:15 **193**

Contacting microbe induce grooming behaviour in *Drosophila*

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Insects remove and clean microbes from their surface by grooming behavior, which is considered as a behavioral defense against pathogen/parasite infection in some cases. It is well known that the insects like *Drosophila melanogaster*, which live in an environment littered with bacteria, fungi and other microorganisms developing on decaying material devote a lot of time to self-grooming which seems to contribute cleaning their cuticula from external particles. The mechanisms that trigger this behavior are still ambiguous, although grooming behavior was identified in many insects. In this work, we examined if *D. melanogaster* can sense microbe in their habitat and if they conduct any hygiene behavior like grooming after they have perceived microbe. To follow the behavioral reaction, we focus on a contact chemo-stimulus, which would activate taste neurons. Microbe, microbe-related compounds and standard chemicals were used as stimuli and influence of water and mechanical stimuli were removed by using Gal4-UAS system in control experiments. Grooming seems to be specifically triggered by the activation of taste neurons since flies showed strong cleaning behavior when contacted with taste stimuli.

Contributed paper. Wednesday, 14:30 **194**

Cultivable gut bacteria of scarabs inhibit *B. thuringiensis* multiplication

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The entomopathogen *Bacillus thuringiensis* is used to control various pest species of scarab beetle but is not particularly effective. Gut bacteria have diverse ecological and evolutionary effects on their hosts, but whether gut bacteria can protect scarabs from *B. thuringiensis* infection remains poorly understood. To investigate this we isolated 32 cultivable gut bacteria from *Holotrichia oblita*, *Holotrichia parallela* and *Anomala corpulenta*, and analyzed their effect on *B. thuringiensis* multiplication and Cry toxin stability. 16S rDNA analysis indicated that these gut bacteria belong to the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* phyla. A confrontation culture analyses of the 32 isolates against three scarab specific *B. thuringiensis* strains showed that the majority of the scarab gut bacteria had antibacterial activity against the *B. thuringiensis* strains. The Cry toxin stability analysis results showed that whilst several strains produced proteases capable of processing the scarab-specific toxin Cry8Ea, none were able to completely degrade it. These results suggest that gut bacteria can potentially affect the susceptibility of scarabs to *B. thuringiensis* and that this should be considered when considering future control measures.

Contributed paper. Wednesday, 14:45 **195**

Interactions between the Med fly *Ceratitis capitata* (Wied.)and a new *Bacillus cereus sensu lato* strain

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The Med fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is a polyphagous species affecting many species of fruits and vegetables worldwide. Due to its high economical impact on crops, the management of this multivoltine pest is always necessary and mostly based on the application of various synthetic insecticidal formulations as foliage baiting or cover spraying. Besides the use of chemicals, the potential of entomopathogenic microorganisms (i.e. bacteria, fungi) against this pest has been highlighted. The lethal and sub-lethal effects of sporulated cultures of a novel *B. cereus sensu lato* strain lacking detectable *cry* genes and identified by its morphological and genetic features, have been studied in a larval based bioassay model. Sporulated cultures of this strain significantly reduced immature stages survival and development time, and the size of emerging Med fly adults. The toxicity has been associated to a specific parasporal fraction characterized through a proteomic approach (SDS-PAGE, 2D PAGE, LC MS/MS). The results of these analyses highlighted the possible role of different protein families produced also by other microbial entomopathogens and that have already been specifically associated to an insecticidal action. These proteins include molecular *chaperones* (GroEL), metalloproteases, aldehyde dehydrogenases, peptidases and other enzymes.

Contributed paper. Wednesday, 15:00 **196**

Long-term effect of *Bacillus thuringiensis* subsp. *israelensis* application on *B. cereus* group populations in Swedish riparian wetland soils

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The *Bacillus cereus* group (Bcg) commonly occurs in soil and includes the pathogens *B. cereus*, *B. thuringiensis* and *B. anthracis*, differing in pathogenicity and disease spectrum. The insect pathogenic *B. thuringiensis* subsp. *israelensis* (Bti) is available in products for augmentation biological control and has been applied worldwide to control larvae of the order Diptera. However, knowledge is limited on how long-term Bti application affects the structure of indigenous Bcg communities as well as the overall abundance of Bti. Based on new primer pairs targeting internal spacers located on the bacterial chromosome, group-specific quantitative PCR assays for Bcg and Bti in environmental samples were developed. On six occasions during the vegetation season, soil samples were collected in forest swamps and wet meadows which have been treated with Bti during the last 11 years as well as in untreated forest swamps, wet meadows and well-drained forests. Preliminary results from two of the time points indicate a decline of Bti abundance over time after the last treatment in wet meadows and forest swamps. These preliminary data also indicate that abundance of Bti in the untreated sites were lower than in the treated, independently of the sampling occasion. This study is coming up with the first specific PCR-primers for Bcg and Bti that target chromosomal DNA. These new tools will be useful for investigating the abundance and diversity of Bcg members in various environments and thereby for assessing the resident insecticidal potential of this bacterial group.

Contributed paper. Wednesday, 15:15 **197**

Proteomics of *Brevibacillus laterosporus* and its insecticidal action against noxious Diptera

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Brevibacillus laterosporus is a pathogen of invertebrates and an antimicrobial species, morphologically characterized by a typical spore surrounded by a firmly attached canoe-shaped parasporal body (CSPB). The biocontrol potential in agriculture of this bacterial species, is not limited to invertebrate pests (insects in different orders, nematodes and mollusks) but includes also phytopathogenic bacteria and fungi. This broad-spectrum activity is associated to a wide variety of molecules, including proteins and antibiotics, it produces. Whilst there are significant differences among strains in terms of virulence, the results of the recent whole genome sequencing of strains LMG 15441 and GI-9 revealed a conserved potential of this species to produce several polyketides, nonribosomal peptides, and toxins. Among genes encoding for putative toxins some show similarities to *Lysinibacillus sphaericus* mosquitocidal toxins.
Employing a *B. laterosporus-Musca domestica* bioassay model, associated to a proteomic and gene expression study, we have analyzed the implication in the microbial action of specific proteins produced during different bacterial life stages. Based on these results, new insights into the pathogenicity against noxious Diptera will be discussed.

Contributed paper. Wednesday, 15:30 **198-STU**

Outer membrane vesicles are vehicles for the delivery of *Vibrio* virulence factors to oyster immune cells

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V. tasmaniensis LGP32, a facultative intracellular pathogen of oyster hemocytes, was shown here to release outer membrane vesicles (OMVs) both in the extracellular milieu and inside hemocytes. Intracellular release of OMVs occurred inside phagosomes of intact hemocytes having phagocytosed few vibrios as well as in damaged hemocytes containing large vacuoles heavily loaded with LGP32. The OMV proteome of LGP32 was shown to be rich in hydrolases (29.8 %) including potential virulence factors such as proteases, lipases, phospholipases, hemolysins and nucleases. One major

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

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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

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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*

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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