

and in particular an observed increase in parasite virulence and host resistance. Moreover, we found a potential for parasite local adaptation under coevolution.

Symposium. Thursday, 9:45 **230**

Means of fast virulence adaption: the plasmid and prophage equipment of selected *Bacillus thuringiensis* strains

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Strains of *Bacillus thuringiensis* (Bt) are used since decades as pest control in crop protection. A descriptive feature of the species is the existence of paracrystal bodies, which consist of δ -endotoxins, acting against specific classes of invertebrates. Over the years a solid amount of research has been achieved on the activity of δ -endotoxins on invertebrates as well as on the diversity of cry-toxin genes. In contrast surprisingly little is known on the genomic loci which encode this diversity of δ -endotoxins. Furthermore the knowledge on other invertebrate virulence factors encoded by Bt as well as on host adaptation factors is rather fragmentary. The observation of phenotypes that differ between strains indicates that they are encoded within the pan-genome of *Bacillus thuringiensis*. Since a pan-genome consists of the genes that are not shared by all members of species many of them are encoded on strain specific extra chromosomal elements. Here we present a comparative analysis of more than 40 extra chromosomal replicons such as plasmids and prophages of three nematocidal and two insecticidal Bt strains.

SYMPOSIUM 8 (Cross-Divisional) Thursday, 14:00-16:00

Host – Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing

Symposium. Thursday, 14:00. **231**

The *Bacillus thuringiensis* way of life: communicate to kill and survive in the insect host

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At the end of exponential growth, bacteria of the *Bacillus cereus* group (*ie. B. thuringiensis* and *B. cereus*) produce virulence factors allowing the bacteria to invade their host. In the insect gut, genes controlled by the PlcR quorum sensor allow the bacteria to damage the intestinal barrier and to gain access to the haemocoel. After the death of the insect, PlcR activates transcription of a gene encoding a second quorum sensor, NprR. NprR induces production of degradative enzymes and of a biosurfactant allowing the bacteria to survive in the insect cadaver and eventually to sporulate. The development of the sporulation process is controlled by the master regulator Spo0A, whose activity is regulated by Rap proteins. PlcR, NprR and Rap are quorum sensing regulators belonging to the RNPP family. Their activity depends on the

signalling peptides PapR, NprX and Phr, respectively. Altogether our results indicate that these three cell-cell communication systems, acting sequentially, coordinate virulence and adaptive properties with the general physiology of the bacterial cells. The PlcR-PapR complex induces the production of virulence factors allowing the bacteria to kill the insect. NprR-NprX activates transcription of genes allowing the bacteria to switch from a virulence state to that of survival in the host cadaver. Ultimately, the inhibition of the Rap proteins by the Phr signalling peptides triggers sporulation, thus allowing the bacteria to disseminate and to persist in the environment.

Symposium. Thursday, 14:30. **232**

The interplay of *Paenibacillus larvae* with honey larvae during infection

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Honey bees are attacked by numerous pathogens, some of them just causing covert infections others causing overt disease symptoms and even death of individuals and entire colonies. Among the latter group is the bacterium *Paenibacillus larvae*, the etiological agent of the epizootic American Foulbrood of honey bees (AFB). As the name suggests, AFB is a bacterial disease affecting only the larval stages of honey bees. *P. larvae* is an obligate killer because death of larvae and conversion of larval biomass into bacterial biomass are prerequisites for disease transmission within and between colonies. Hence, *P. larvae* must have evolved effective means to attack larvae, to circumvent the larval immune response and to finally kill and decompose larvae. We recently identified and characterized some of these virulence factors of *P. larvae*. We will present a model for molecular pathogenesis of *P. larvae* infections built upon these novel findings in order to further the understanding of the molecular basis of pathogen-host-interactions in American Foulbrood disease.

Symposium. Thursday, 15:00. **233**

Antimicrobial defense and persistent infection in insects revisited

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Antimicrobial peptides are mainly produced and used by multicellular organisms such as insects to interact with pathogenic and mutualistic micro-organisms. Antibiotics are mostly produced by single cell eukaryotes and bacteria. Here we provide a possible explanation for this dichotomy. Our hypothesis is based on the observation that antibiotics elevate bacterial mutation rates and we show that AMPs do not elevate bacterial mutation rates. Nevertheless we also found that bacterial resistance evolves readily against single AMPs in vitro, but the situation is already more complicated by the simultaneous action of two AMPs. I will contextualize these findings in the light of the immune responses of the beetle *Tenebrio molitor* and will use these findings to discuss some of the multiple roles AMPs have in host-microbe interactions: policing and killing.

Symposium. Thursday, 15:30. **234**

Vibrio and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes

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Vibrio tasmaniensis LGP32 is a *V. splendidus*-related strain pathogenic for *Crassostrea gigas* oysters. We recently showed that LGP32 invades the oyster immune cells, the hemocytes, through phagocytosis. Oyster hemocytes are professional phagocytes harboring microbicidal activities including a potent oxidative response. Interestingly, the phagocytosed LGP32 survives inside the oyster hemocytes, evading the host defense by preventing acidic vacuole formation and limiting reactive oxygen species production. When hemocytes were invaded by numerous LGP32, we observed cytotoxic effects such as membrane disruptions and cytoplasmic disorders. Cytotoxicity was shown to entirely depend on LGP32 entry into hemocytes, as cytochalasin D was sufficient to inhibit hemocyte death. By developing a transcriptomic approach based on RNA sequencing, we identified a series of *Vibrio* antioxidant genes whose expression is strongly induced within oyster hemocytes. We also observed an overexpression of genes involved in cation efflux. Overexpression of these molecular functions in the intraphagosomal stage was confirmed by RT-PCR. To determine how far those LGP32 genes are involved in resistance to intracellular killing and subsequent virulence, we constructed isogenic deletion mutants for two overexpressed antioxidants and two overexpressed cation transporters. Those mutants were phenotyped for intracellular multiplication, cytotoxicity and virulence in oyster experimental infections. Our data show that resistance to reactive oxygen species and efflux of cations are two important functions required for LGP32 intracellular survival, cytotoxic effects and virulence.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

MICROBIAL CONTROL 4

Contributed paper. Thursday, 14:00. **235**

Establishing the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)

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The fungal entomopathogen *Beauveria bassiana* (Balsamo)

Vuillemin (Ascomycota: Hypocreales) is known to survive as an endophyte in a wide range of plants and offer protection against an increasing number of insect pests. Although recent discoveries suggest that the fungus can also protect plants against plant pathogens, no studies are currently available on the efficacy of endophytic *B. bassiana* against plant viruses. We conducted experiments to determine whether endophytic *B. bassiana* could provide protection against Zucchini Yellow Mosaic Virus (ZYMV), one of the most economically important diseases of cucurbits worldwide. Four selected *B. bassiana* strains were able to successfully colonize squash plants following the foliar inoculation of plants with the conidial suspension of each respective strain. Disease incidence and severity, sampled weekly following the challenge inoculation of plants with ZYMV, were significantly lower in *B. bassiana*-inoculated plants as compared to control plants; irrespective of the *B. bassiana* strain being inoculated. Our study demonstrates, for the first time, that endophytic *B. bassiana* has the biocontrol potential for managing plant viruses. Further studies should be conducted to determine whether such endophytic *B. bassiana*-mediated protection against ZYMV in squash extends to other cucurbits.

Contributed paper. Thursday, 14:15. **236**

Bean plant *Phaseolus vulgaris* endophytically colonized by *Beauveria bassiana* and *Hypocrea lixii* acquires protection against *Liriomyza huidobrensis* (Diptera: Agromyzidae) in the field

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Field trials were carried out for two cropping seasons in two sites (Sagana and Narumoro, Central province of Kenya) to evaluate the prospects of endophyte isolates of *Beauveria bassiana* and *Hypocrea lixii* for the control of leafminer *Liriomyza huidobrensis* in *Phaseolus vulgaris*. Autodissemination device treated with conidia of *Metarhizium anisopliae* was also added as a treatment. The effects of endophytes on leafminer infestation (punctures and mines), number of pupae and parasitoids, and yield were evaluated. Both isolates successfully colonized different parts of *P. vulgaris* plants; however, colonization was greater with *H. lixii* than *B. bassiana* in both sites. Leafminer infestation was not significantly different during the first season while it was higher in the controls than in endophyte treatments at both sites during the second season. The number of pupae varied between 150-250 and 320-400 in endophyte and control treatments, respectively, during the first season; and from 100-200 and 350-500, respectively, in endophyte and control treatments during the second season. The number of parasitoids that emerged from pupae did not differ significantly among the treatments. Higher yield was obtained in endophyte than in control treatments. With exception to yield during season two, the inclusion of autodissemination device treatment did not have significant effect on all the parameters evaluated. There were no significant differences between the fungal isolates. Results of the present study suggest that both endophyte fungal isolates hold potential and could be considered for the control of leafminer. There is the need however to confirm these results on large-scale trials.