BACTERIA 3

Contributed paper. Wednesday, 8:00 **120**

Resistance alleles to *Lysinibacillus sphaericus* are co-select in a *Culex quinquefasciatus* colony and display distinct features

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Two alleles of the *cqm1* gene, containing mutations associated to resistance to the Binary (Bin) from *Lysinibacillus sphaericus,* were co-selected in a laboratory resistant colony of *Culex quinquefasciatus* (R2362). The goal of this study was to identify these alleles and to analyze the homozygous larvae for each one, through different approaches. The alleles named $cqm1_{REC\ and}$ $cqm1_{REC\text{-}2}$ are characterized by distinct mutations, however, they code for transcripts of truncated proteins that are not located in the midgut epithelium and cannot act as receptors for the Bin toxin. Homozygous larvae for each allele show high resistance to the Bin toxin, low specific binding of Bin toxin to midgut microvilli proteins and low transcription level of the both resistance alleles. Their frequency in the R2362 colony showed that the $cqm1_{REC}$ has predominated during a long period (> 100 generations), however, it has been replaced by the $cqm1_{REC\text{-}2}$ that became the most frequent allele. A colony established from the cross of homozygous individuals from each allele (1:1 ratio) showed that $cqm1_{REC}$ assumed a higher frequency, compared to $cqm1_{REC\text{-}2}$, during a period of 21 generations. An AS-PCR-screening detected the presence of $cqm1_{REC\text{-}2}$ allele in larvae from field populations and its frequency and distribution was lower than that found for $cqm1_{REC,}$ suggesting that this allele has a higher risk to be selected. The fitness cost of individuals homozygous is under study to evaluate the impact on the biological performance of individuals carrying these alleles.

Contributed paper. Wednesday, 8:15 **121-STU**

Untangling insect pathogenicity in plant-beneficial pseudomonads by a combination of comparative genomics, bioassays and histopathology

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The Pseudomonas fluorescens group harbors many root-associated plant-beneficial bacteria that suppress soil-borne fungal diseases and promote plant growth. Remarkably, two strains, Pseudomonas protegens CHA0 and Pseudomonas chlororaphis PCL1391, additionally display oral insecticidal activity towards lepidopteran larvae. This ability is associated with the Fit insect toxin and unknown GacA-regulated traits. However, the exact course of infection, the target organs and the virulence factors beyond Fit are yet undiscovered. To tackle these open questions we combined various methods. Fifteen strains of fluorescent pseudomonads, including four new isolates, were characterized for both their plant-beneficial traits and their insecticidal activity. Whereas the former were found throughout the entire *P. fluorescens* group, the latter was restricted to strains of *P. protegens* and *P. chlororaphis*. By next generation sequencing and subsequent comparative genomics we identified a small set of genes common to all insecticidal strains, but absent in non-insecticidal strains. These genes could therefore encode potential virulence factors against insects. Histopathology to detect affected insect tissues and fluorescence microscopy to localize the bacteria during the infection complete this study which reveals intriguing aspects on insect pathogenesis of plant-associated pseudomonads and identifies several strains with potent dual activity against root pathogens and insect pests.

Contributed paper. Wednesday, 8:30 **122**

Comparative analysis of the Cqm1 and Aam1 ortholog proteins from mosquitoes that have a differential capacity to bind to the Binary toxin from *Lysinibacillus sphaericus*

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The Cqm1 and Aam1 are ortholog proteins from the midgut of *Culex quinquefasciatus* and *Aedes aegypti* larvae, respectively. These related proteins, with 74% of identity, are expressed as membrane-bound alpha-glucosidases and, functionally, Cqm1 also acts as the receptor of the insecticidal Binary (Bin) toxin from *Lysinibacillus sphaericus*, while Aam1 does not. The major goal of this study was to analyze some features of these proteins produced in Sf9 cells. The recombinant proteins obtained in this expression system showed the same molecular weight and kept their differential capacity to bind to the Bin toxin, as the native proteins. The Cqm1 sequence presents three predicted N-glycosylation sites (PGS), however, the analysis of the recombinant protein suggested that it does not have glycans. On the other hand, Aam1 sequence has six PGS and analysis of the recombinant protein showed that four of them contain carbohydrates that can be removed by the glycosidase PNGase F. Site-directed mutagenesis of these PGS prevented the insertion of carbohydrates and these mutant proteins did not bind to the Bin toxin, similarly to the wild Aam1. In terms of their catalytic function, both recombinant proteins displayed alpha-glucosidase activity and Aam1 showed a two-fold increase compared to Cqm1. Analysis of protein sequences showed that one segment of the Cqm1, that is required for Bin toxin binding, is not conserved in the Aam1 and might be an important factor for their differential capacity to interact with the Bin toxin and, thus, for the refractoriness of *Ae. aegypti* larvae to *L. sphaericus*.

Contributed paper. Wednesday, 8:45 **123**

Resilience of the intestinal epithelium to the action of a bacterial pore-forming toxin and to xenobiotics in *Drosophila*

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The host defense against pathogens encompasses two complementary arms: i) resistance, attacking directly the pathogen, which is mediated by the immune system; ii) resilience, also referred to as tolerance, withstanding and repairing damages inflicted either by the pathogen or by the host's own immune system. We have discovered that the compensatory proliferation of *Drosophila* intestinal stem cells (ISCs) allows the intestinal epithelium to maintain its homeostasis during *Serratia marcescens* infection, and thus constitutes a *bona fide* resilience mechanism. Resilience is not limited to the control of ISC proliferation. Within three hours of ingestion of *S. marcescens*, the epithelium becomes very thin in the absence of cell death. Strikingly, epithelial cells are able to recover their shape and volume in the next 6-9 hours. Attack by *S. marcescens* hemolysin, a 2 nm-wide pore-forming toxin, leads to the controlled extrusion of the cytoplasm of epithelial cells. This may help in purging the cytoplasm from damaged organelles. We have initiated a molecular analysis using both a genetic and a transcriptomics approach and thus identified tens of genes required for the regeneration phase. One of them, a conserved cyclin of previously unknown function, plays a major role noncell-autonomously and is required for the expression of early response genes. Many of these genes are also induced by exposure to xenobiotics such as caffeine. We have found that the cyclin mutants are more susceptible to the ingestion of caffeine. Thus, we may have uncovered a novel stress response pathway that underlies a new resilience mechanism.

Contributed paper. Wednesday, 9:00 **124**

Cadherin mutations and Bt resistance: Field screening and fitness costs

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Insecticidal crystal toxins from *Bacillus thuringiensis* (Bt) of the Cry1A family bind to a 12-cadherin domain protein in the midgut of lepidoptera and eventually form pores in the midgut epithelium, leading to death of the insect. Mutations in this cadherin confer Cry1A resistance to several Lepidoptera. In the course of an F1 screen to estimate the frequency of such mutations in field populations of the tobacco budworm *Heliothis virescens*, a novel mutation was found. Like the first mutation found in this species, it is caused by insertion of a transposable element, but in a different location. Allele frequency changes were recorded over several generations of artificial selection for a homozygous mutant strain, showing a substantial fitness cost to knockout cadherin mutations, even under optimal conditions in the laboratory. Although this type of transposon-induced mutation may be moderately common in field populations, its high fitness cost makes it unlikely to threaten the sustainability of transgenic cottin expressing Cry1A toxins.

Contributed paper. Wednesday, 9:15 **125**

Down regulation and mutation of cadherin gene associated with Cry1Ac resistance in Asian corn borer

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Development of resistance in target insects is a major threat to long-term use of transgenic Bt crops. To delay the evolution of resistance in target insect through the implementation of the effective strategies, it is fundamental to understand the pests' resistance mechanisms. One of the most important mechanisms of insect resistance to Bt crops is the alteration of interaction between Bt toxin and its receptor in the insect midguts. Asian corn borer (ACB), *Ostrinia furnacalis*, is a key pest of maize to be targeted by Bt maize. A Cry1Ac resistant strain of ACB has been established in the laboratory. Compared to the membrane proximal extracellular region (MPR) of cDNA of *ofcad* that encodes a cadherin-like protein in ACB from the susceptible strain, there were three mutant alleles of *ofcad* (MPR-r1, MPR-r2, and MPR-r3) associated with resistance to Cry1Ac toxin. Each of those mutant alleles had 2-3 aa substitution in the putative-toxin binding region (TBR) of the cadherin, especially $Thr^{111} \rightarrow Ser^{111}$ was accurate. In addition, MPR-r2 had a deletion expected to eliminate 26 aa-residues in TBR, which resulted in decline in the binding of MPR to Cry1Ac in the resistant strain compared to the susceptible strain. Furthermore, down regulation of ofcad was associated with Cry1Ac resistance, response to the stress of low level Cry1Ac toxin in susceptible strain. These results suggest that Cry1Ac resistance in ACB is primarily associated with the down regulation of *ofcad*. Mutations in *ofcad* resulting in amino-acid substitutions and deletions might mediate higher level of resistance.

Contributed paper. Wednesday, 9:30 **126**

ABCC transporters mediate insect resistance to multiple Bt toxins revealed by BSA analysis

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Insect resistance to *Bacillus thuringiensis* (Bt) is one of the main threats for the long term use of Bt-based products, including Bt crops. Identification of genes conferring resistance to Bt will contribute to delaying the development of resistance as well as to provide additional information about the mode of action of these bacteria and its insecticidal toxins. By using linkage analysis based on high throughput sequencing, we have found a novel type of mutation in the ABCC2 transporter conferring resistance to Bt. In addition we have also found that different members of the ABCC transporters can act as receptors for not only Cry1A toxins but also for the Cry1C type toxins. The identified mutation in the ABCC2 transporter is

localized in a region that does not physically interact with the toxins but in the intracellular ATP-binding domain instead. Our toxin binding studies have revealed that such mutation correlates with a reduction in toxin insertion into the membrane (irreversible binding) and suggests that ABCC activity as transporter is necessary for the proper action of Bt toxins.

CONTRIBUTED PAPERS Wednesday, 8:15-9:45

DIS. OF BENEFICIAL INVERTEBRATES 2

Contributed paper. Wednesday, 8:15 **128**

Nosema ceranae News: Update on Species Competition and Host-Pathogen Interaction Studies

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The apparent recent invasion of *Nosema ceranae* and its dominance over *Nosema apis* in honey bee populations in the USA and elsewhere have presented both an enigma and a treatment problem for apiculturists and scientists. Several studies, including those of our research group, have shown that *N. ceranae* produces more mature spores than *N. apis,* and we demonstrated that reproduction of *N. ceranae* recovers more quickly from fumagillin treatment than does *N. apis.* In addition, *N. ceranae* hyperproliferated in the presence of very low fumagillin concentrations in laboratory bioassays. Proteomic-level studies of fumagillin-*N. ceranae*-honey bee interactions continue and we are investigating the mechanisms of protein regulation in response to infection and fumagillin treatment. In studies of infectivity, we found that *N. ceranae* consistently has a higher IC_{50} than *N. apis.* The effect is most pronounced at 1 day post eclosion. We investigated the interaction of *N. ceranae* and *N. apis* in individual bees and found that *N. apis* produced more spores than *N. ceranae* in 62% of bees infected with equal dosages of both *Nosema* species. Mixed species infections negatively affected survival time (15-17 days) compared to single species infections (20 and 21 days for *N. ceranae* and *N. apis,* respectively) and uninfected bees (27 days). Midgut spore counts were higher for mixed species infections than for single species infections, but we did not find evidence that *N. ceranae* outcompetes *N. apis* in an individual host..

Contributed paper. Wednesday, 8:30. **129**

Influence of temperature on the development of _Nosema apis_ and _Nosema ceranae_

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Nosema apis and *Nosema ceranae* are two fungal pathogens infecting the European honey bee, *Apis mellifera*. These obligate intracellular pathogens, belonging to the phylum Microsporidia, infect epithelia cells of the midgut and elicit nosemosis. Recent studies suggested that *N. ceranae* is more virulent than *N. apis* and can lead to severe colony losses. These colony losses are so far only reported from the Southern parts of Europe. In the Northern parts (e.g., Denmark, Sweden, Finland, and Germany) *N. ceranae* could not be correlated to colony losses so far. While *N. ceranae* seems to have replaced *N. apis* in the bee population in South Europe, this is not the case for the Northern parts of Europe. Both findings suggest a climatic angle for spread, assertiveness, and virulence of *N. ceranae*. Exact whether parameters as temperature or humidity, which hinder or favor *N. ceranae* infections, are not determined so far. Spanish colleagues recently showed that *N. ceranae* has a better adaptation to complete its endogenous cycle at warmer temperatures. However, the results based on *in vivo*-infections only give a minor hint on different proliferation of both obligate intracellular pathogens exposed to different temperatures. We here present our results on the intracellular development of *N. apis* and *N. ceranae* exposed to different temperatures using our recently established cell culture model for *Nosema* spp.

Contributed paper. Wednesday, 8:45 **130-STU**

The involvement of bumblebee small interfering RNA pathway against two different bee viruses

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Wild pollinators such as bumblebees are in global decline. They share a pathogen network with other pollinators, consisting of multi-hosts pathogens and multi-pathogens hosts. Disturbance of these associations could lead toward the further host decline. Insects have developed certain immune pathways to combat viruses, of which the small interfering RNA pathway (siRNA) is important. By unveiling the interaction of the virus with the host defense pathway we can better understand the virulence of certain viruses in specific hosts. Here we use two viruses, israeli acute paralysis virus (IAPV) and slow bee paralysis virus (SBPV), representing two infection types after injection in *Bombus terrestris*, i.e. IAPV presents an overt acute infection resulting in mortality, while SBPV results in a covert persistent infection. First, to determine viral replication dynamics by following the negative and positive strands, we developed a new method in combining multiplex ligation-dependent probe amplification and qPCR. The results show both viruses experienced an exponential-plateau phase, and their replication strand were relatively low compared with genome (positive) stand. Second, both viruses increased the expression of Dicer-2 and SID, thereby activating siRNA. Finally we performed small RNAs sequencing to screen if differences in the siRNA production could explain different viral virulence.

Contributed paper. Wednesday, 9:00 **131**

Impact of Wolbachia endosymbionts on the evolution of sex determination in the isopod Armadillidium vulgare

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Terrestrial isopods are crustaceans that represent a major component of the litter ecosystem, as they mainly feed on dead plant material and participate in litter decomposition. In the isopod *Armadillidium vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). However, many *A. vulgare* populations harbor maternally-inherited *Wolbachia* bacterial endosymbionts. These bacteria are reproductive parasites that convert genetic males into phenotypic females, leading to populations with female-biased sex ratios. The W sex chromosome has been lost in lines infected by *Wolbachia* and all individuals are ZZ genetic males. The female sex is determined by the inheritance of *Wolbachia* by the *A. vulgare* individual. Surprisingly, some *A. vulgare* lines exhibit