BACTERIA 3

Contributed paper. Wednesday, 8:00-8:45

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 cqm1REC-1 and cqm1REC-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 transmission level of the both resistance alleles. Their frequency in the R2362 colony showed that the cqm1REC-1 predominated during a long period (>100 generations), however, it has been replaced by the cqm1REC-2 that became the most frequent allele. A colony established from the cross of homzygous individuals from each allele (1:1 ratio) showed that cqm1REC-1 assumed a higher frequency, compared to cqm1REC-2, during a period of 21 generations. An AS-PCR-screening detected the presence of cqm1REC-2 allele in larvae from field populations and its frequency and distribution was lower than that found for cqm1REC-1 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 PCL1925, 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 have not been discovered. 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. 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 encode potential virulence factors against insects. Histopathology to detect affected insect tissues and fluorescence microscopy to visualize the bacteria during the infection complete this study which reveals intriguing aspects on 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 the 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 A. aegypti larvae to L. sphaericus.