THURSDAY

CONTRIBUTED PAPERS — Thursday, 16:00 - 17:45
Painted Horse I-II

Bacteria III

Moderator: Juan Luis Jurat-Fuentes

Contributed Paper - Thursday, 16:00 — 172

Pathogenicity island in the Mexican *Serratia entomophila* mor4.1 active against *Phyllophaga blanchardi* larvae (Coleoptera).

Maria Eugenia Nuñez-Valdez - Facultad De Ciencias Agropecuarias, Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; ***Jean Chen*** - Depts. of Cell Biology and Neuroscience3, University of California, Riverside, Riverside, California, USA; ***Sarjeet Gill*** - Depts. of Cell Biology and Neuroscience, University of California, Riverside, Riverside, California, USA; ***Francisco Villalobos*** - Facultad De Ciencias Agropecuarias, Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; ***Zitlhally Rodriguez-Segura*** - Centro de Investigación en Biotecnología2, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico
Address for correspondence: eugenia@uaem.mx

The bacteria *S. entomophila* strain Mor4.1 (SeMor4.1; Enterobacteriaceae) was isolated in México from the haemocoel of a *Phyllophaga blanchardi* larvae. The bacteria is pathogenic to several species of the *Phyllophaga* genus (Coleoptera:Scarabaeidae; Nuñez- Valdez et al., 2008). These larvae feed on plant roots causing severe damage to some important crops world wide. The oral infection by *SeMor4.1* causes anti-feeding effect (AFE) and mortality. Insecticidal activity against *Anomala* sp and the lepidopteran *Manduca sexta* has been observed in bio-assays either by injecting the bacteria or by injecting cell free culture broths (Nuñez-Valdez et al., 2008 Appl. Environ. Microbiol. 74:802-10). We think that strain *Mor4.1* produce virulence factors with a wide spectrum of action, with toxic activities at the level of the insect gut and also at the level of the hemocoel. To identify the virulence factors, a genomic approach was followed by constructing an *S. entomophila Mor4.1* fosmid library in *E. coli*. The library clones expressing insecticidal activity by injection bio-assays were selected. We present the analysis of the DNA sequence of the 40 Kb of the clone named G8. We found that 19 genes were associated with virulence factors and some of them were located on a potential "Pathogenicity Island". Most ORFs (27) showed homology to *Serratia proteamaculans 568* proteins following almost the same gene order compared with *S. proteamaculans* genome, but with insertions of other genes in five positions. Two main virulence regions were identified in G8 i) a putative Lipopolysacharide (LPS) biosynthesis core (ORFs 29-40) and ii) a ppGpp gene operon (ORFs 9-13). There are evidences in other pathogenic bacteria, suggesting that the LPS (Kurz et al., 2003, EMBO J., 22:1451-1460) and the signaling molecule Guanosine Tetraphosphate ppGpp (Nakanishi et al., Molecular Microbiology 61(1), 194–205) might work as potential virulence factors in *SeMor4.1* to scarab larvae.

Contributed Paper - Thursday, 16:15 — 173

From insect to man: A functional genomic comparison of clinical and insect pathogenic strains of *Photorhabdus*.

Maria Sanchez-Contreras - University of Bath, Banes, UK; ***Andrea Dowling*** - University of Exeter, Penryn, Cornwall, UK; ***Richard ffrench-Constant*** - University of Exeter, Penryn, Cornwall, UK; *Nick Waterfield* - University of Bath, Banes, UK
Address for correspondence: bssnw@bath.ac.uk

Bioinformatics is becoming increasingly successful at the annotation of genomes and yet it still cannot detect novel virulence genes or prove functionality. With this in mind we developed a powerful technique named Rapid Virulence Annotation (RVA). RVA employs the parallel screening of large insert DNA libraries of a bacterial pathogen of choice, against multiple invertebrate hosts. The use of these "gain of toxicity" screens in otherwise harmless *E. coli* circumvents certain problems inherent in traditional gene knock-out screens. These include avoiding the issue of virulence gene redundancy, revealing less potent factors that may otherwise be masked by dominant toxins and the detection of the final virulence effectors rather than pleiotrophic regulators. We screen libraries against the nematode Caenorhabditis elegans, serving as an oral route model, the single-cell protozoa *Acanthamoeba polyphaga* used as a phagocytosis model, and two caterpillar models, the Tobacco hornworm *Manduca sexta* and the Waxmoth *Galleria mellonella,* both of which represent the more complex innate immune systems. Finally, we use the mouse BALB/c macrophage cell line J774-2 to represent the phagocytic component of the vertebrate immune system. The genus *Photorhabdus* can be split into 3 species based on multi-locus sequence typing. All three of these species exist in a symbiotic nematode-bacterial complex with an insect pathogenic nematode worm of the genus *Heterorhabditis.* Two of these species, *P. luminescens* and *P. temperata* are exclusive insect pathogens while the third, *P. asymbiotica,* is both an insect and human pathogen and may be considered an "emerging" human pathogen. We present a comparison of an RVA analysis of the two sequenced strains, P. luminescens TT01, an insect only pathogen, and the clinical isolate P. asymbiotica ATCC43949. We will discuss the communality of virulence factors and the implications for insect pathogens as a potential source of virulence factors and emerging infections in mammals.

Contributed Paper - Thursday, 16:30 — 174

The fate of toxin complexes in cultured cells.

Michelle Hares - University of Exeter, Penryn, Cornwall, U.K.; ***Stewart Hinchliffe*** - University of Exeter, Penryn, Cornwall, U.K.; ***Richard ffrench-Constant*** - University of Exeter, Penryn, Cornwall, U.K.
Address for correspondence: m.c.hares@exeter.ac.uk

The Toxin complex (Tc) genes were first identified in the insect pathogen *Photorhabdus luminescens* strain W14. These high molecular weight proteins (~1MDa) have been shown to be orally and injectably toxic to several orders of insects. They are encoded by four loci; tca, tcb, tcc and tcd, the genes within these loci labelled according to their order (tcdA, tcbB, tccC). Significant homology is observed between the loci and previous work has shown that three components are required for full toxicity, the tcdA-like [A], the tcbB-like [B] and tccC-like [C] genes. Interestingly these Tc's are seen in a variety of gram-negative pathogenic bacteria including Yersinia, suggesting an evolving function or targets for these proteins directed towards insect and /or mammalian hosts. Previous work has show that when the Tc's are applied to cultured cells membrane ruffling, vacuolation and multinucleation is induced. Transfection of A, B and C into these cells determined that A was responsible for multinucleation, B induced vacuolation, whereas C had no effect. Using GFP-tagged A, B and C proteins in combination with immunofluorescent markers for membrane trafficking compartments we present work to demonstrate the origin of these vacuoles and the fate of the Tc's.

21876

Contributed Paper - Thursday, 16:45 — 175

Bacillus thuringiensis biopesticide produced with different amounts of carbon and nitrogen.

Fernando Valicente - Embrapa Maize and Sorghum, Sete Lagoas, Minas

THURSDAY

Gerais, Brazil; ***Andre Horta*** - Agronomy Student, Jaboticabal, São Paulo, Brazil; ***Lucas Mendes*** - Agronomy Student, Patos de Minas, MG, Brazil; ***Amanda Lanza*** - Student, Sete Lagoas, MG, Brazil; ***Sandra Miranda*** - Student, Sete Lagoas, MG, Brazil
Address for correspondence: valicent@cnpms.embrapa.br

The amount of carbon and nitrogen used to produce *Bacillus thuringiensis* biopesticide may influence the quality of the final product. This research used different levels of carbon and nitrogen in 3 bioassays with 5 treatments each, and LB medium was the check treatment. The first bioassay used 5g/L of maize glucose for all treatments with yeast ranging from 5g/L to 60g/L. The second bioassay used 30g/L of yeast for all treatments with maize glucose ranging from 5g/L to 60g/L. The third bioassay used increasing amounts of nutrient ranging from 1g/L of maize glucose and 3g/L of yeast up to 20g/L of maize glucose and 60g/L of yeast. All media were enriched with salts (FeSO4, ZnSO4, MnSO4, MgSO4). The seed culture was produced using LB medium plus salts, at a stirrer speed of 200rpm, for 18 hours at 30°C. All media were sterilized and inoculated with Bt strain 344 (*B. thuringiensis tolworthi*) and maintained at 30°C for 72 hours at a stirrer speed of 250rpm. The H was measured at regular intervals, heat resistant spores were expressed as c.f.u/mL, cell mass produced in g/L-lyophilized, and spore counting per mL of medium. Results showed that pH followed the same pattern for all media tested, decreasing in the first 12-14 hours and increasing up to 8.7 (no pH control was made). The number of spores reached 4.9 x 109 spores/mL, and the lowest amount of 1.09 x 109 spores/mL. In the second bioassay the maximum number of spores was reached within 48h. Cumulative cell mass produced more than 30.0g/L in many treatments were the amount of nitrogen was higher. Mortality of 2-day-old *Spodoptera frugiperda* larvae was a 100% when treated with spores withdrawn at 24 hours from bioassay 3, and a 100% after 48 hours with spores withdrawn from bioassay 2 and 3.

Contributed Paper - Thursday, 17:00 176

Expression of aminopeptidases in *Ostrinia nubilalis* (Hübner).

Maria Cristina Crava - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain; ***Yolanda Bel*** - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain; ***Barbara Manachini*** - Dep. Animal Biology. U. Palermo, Palermo, Italy; ***Juan Ferré*** - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain; ***Baltasar Escriche*** - Dep. Genética. Universitat de Valencia, Burjassot, Valencia, Spain
Address for correspondence: baltasar.escriche@uv.es

The aminopeptidases N (APNs) are a large family of enzymes with probable role in food digestion that have been detected in the midgut of several lepidopteran species. Aside of their insect physiological role, they have become relevant because of their function as membrane binding proteins involved in the mode of action of *Bacillus thuringiensis* (Bt) crystal protein biopesticidal toxins. In the present study, the expressions of 5 *apn* genes and *one puromicyne-sensitive aminopeptidase* (*psa*) gene have been characterized in *Ostrinia nubilalis* (Hübner), a key pest of Bt-corn The analysis by RT-PCR showed that all aminopeptidases were expressed along the whole larval development. The relative tissue expression analyses in 5th instar larvae by qRT-PCR showed that all aminopeptidase genes were transcribed in the midgut. Moreover, 2 *apns* (*Onapn4* and *Onapn8*) were also expressed in Malpighian tubules, and the *Onpsa* transcripts were found at similar levels in those tissues as well as in the fat body and carcass. The *Onapn8* was expressed in the Malpighian tubules and in the midgut tissue without statistically significant differences, whereas the *Onapn4* had a very low level of expression in the Malpighian tubules. The *in silico* structural putative aminoacidic sequence differences between APNs and PSA seems to be correlated with their expression patterns. The structural similarity and expression of the analyzed APNs suggest that more than a single class may be involved in the Bt toxin binding in the migut.

21877

Contributed Paper - Thursday, 17:15 177

Characterization, distribution and cloning *cry1* genes efficient against fall armyworm, *Spodoptera frugiperda*, in Brazil.

Fernando Valicente - Embrapa, Sete Lagoas, MG, Brazil; ***Edgard Picoli*** - Post Doc / Embrapa, Sete lagoas, MG, Brazil; ***Maria Vasconcelos*** - Embrapa, Sete Lagoas, MG, Brazil; ***Newton Carneiro*** - Embrapa, Sete Lagoas, MG, Brazil; ***Andrea Carneiro*** - Embrapa - Sete Lagoas, MG, Brazil; ***Cláudia T. Guimarães*** - Embrapa Sete Lagoas, MG, Brazil; ***Ubiraci G. P. Lana*** - Embrapa, Sete Lagoas, MG, Brazil
Address for correspondence: valicent@cnpms.embrapa.br

Brazil is located in South America and contains a rich and different biodiversity. A total of 4,459 Bt strains were isolated and evaluated regarding to *Spodoptera frugiperda* larval mortality, and 165 showed larval mortality above 75%. Molecular characterization was based on PCR electrophoresis profile using specific cry1 primers. Among these strains, 33 (20%) did not amplify the expected fragments; 103 (62,42%) amplified fragments corresponding to the presence of only one gene, while 25 (15,15%), 3 (1,8%) and 1 (0,6%) showed a profile of two, three and four different cry1 genes, respectively. SDS-page protein analyses were positive for the presence of cry1 genes. The most frequent (57.5%) was cry1D gene, whereas cry1Aa/cry1Ad and cry1C genes were the less frequent (1.2%). However, more than 60% of the evaluated strains presented cry1B and cry1E genes. Analysis of strains carrying cry1C, cry1B, cry1E, cry1F, cry1A, cry1G and cry1D genes showed that they were toxic to *S. frugiperda,* ranging from the most to the least toxic. The available sequences at http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ were used for designing primers to clone cry1C and cry1F genes. The amplified fragments with the expected size, approximately 2,046 bp, were purified, cloned and transformed into competent cells. The sequencing of 5' and 3' ends allowed the confirmation of the identity of the genes. Some strains that presented unspecific fragments, were also cloned, amplified, sequenced and showed sequences corresponding to cry1-type genes. Colonies holding clones of cry1F, cry1Ca and cry1Cb genes, were obtained only for two of the evaluated strains.

Contributed Paper - Thursday, 17:30 178

Plasmid capture system and its applications.

Jong Yul Roh - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Yong Wang*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Qin Liu*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Xueying Tao*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Jae Young Choi*** - Research Institute for Agriculture and Life Sciences, Seoul National University, Korea, Seoul, Seoul, Republic of Korea; ***Hee Jin Shim*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea; ***Hong Guang Xu*** - Department of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Korea, ***Soo Dong Woo*** - Department of