Contributed paper. Wednesday, 9:15 **139**

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, a transcriptome analysis was initiated for *L. giganteum*, using various Next Generation Sequencing technologies. Homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins and revealed a full complement of plant pathogenic oomycete effector orthologs. The characterization of full-length transcripts corresponding to Cellulose Binding Elicitor Lectin (CBEL), Crinkler, and elicitin proteins demonstrated that *L. giganteum* is the first described animal pathogenic oomycete to secrete canonical Crinkler and CBEL effectors. In addition, phylogenetic analyses identified a Glycoside Hydrolase 5 (subfamily 27; GH5_27) as a putative virulence factor. Genome mining indicated that GH5_27 orthologs are shared by entomopathogenic oomycetes and fungi, but virtually absent in all other oomycetes and fungi. Using PCR, GH5_27 fragments were amplified and sequenced from additional entomopathogens, suggesting that oomycete and fungi underwent convergent evolution and that GH5_27 proteins may play a crucial role in insect/microbe pathosystems. Detailing the molecular basis of entomopathogenicity may allow for the use of oomycetes and fungi as control agents against insect pests, reducing the use of insecticides that can have negative impacts on the environment and human health.

SYMPOSIUM 6 (Bacteria) Wednesday, 10:30–12:30

Structure and Function of Novel Insecticidal Toxins

Symposium. Wednesday, 10:30 **140**

Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1

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Bacillus thuringiensis strains are well-known for the production of insecticidal proteins upon sporulation and these proteins are deposited in parasporal crystalline inclusions. The majority of these insect-specific toxins exhibit three domains in the mature toxin sequence. However, other Cry toxins are structurally and evolutionarily unrelated to this three-domain family and little is known of their three dimensional structures, limiting our understanding of their mechanisms of action and our ability to engineer the proteins to enhance their function. Amongst the non-three domain Cry toxins, the Cry34Ab1 and Cry35Ab1 proteins are required to act together to produce toxicity to the western corn rootworm (WCR) *Diabrotica virgifera virgifera* Le Conte via a pore forming mechanism of action. Cry34Ab1 is a protein of ~14 kDa with features of the Aegerolysin family (Pfam06355) of proteins that have known membrane activity, while Cry35Ab1 is a ~ 44 kDa member of the Toxin_10 family (Pfam05431) that includes other insecticidal proteins such as the binary toxin BinA/BinB. The Cry34Ab1/Cry35Ab1 proteins are important solutions for control of WCR having been developed as insect resistance traits in commercialized corn hybrids for control of WCR. The structures of Cry34Ab1 and Cry35Ab1 have been elucidated to a resolution of 2.15 Å and 1.80 Å, respectively. The solution structures of the toxins were further studied by small angle X-ray scattering (SAXS) and native electrospray ion mobility mass spectrometry. We present here the first published structures from the Aegerolysin and Toxin_10 protein domain families.

Symposium. Wednesday, 10:50 **141**

Structure/function studies of Cry5B via alanine-scanning mutagenesis

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Cry5B is a three-domain crystal protein that attacks nematodes. In collaboration with the laboratory of Partho Ghosh, the three dimensional structure of Cry5B has been solved. Cry5B shows significant similarities with three-domain insecticidal crystal proteins in domains I and III but significant differences with insecticidal proteins in domain II. To better understand structure function relationships in Cry5B, we performed alanine-scanning mutagenesis of the entire toxin domain in which each point variant was tested in bioactivity assays with the free-living nematode *Caenorhabditis elegans*. Alanine point variants were classified into three classes—those with reduced/no bioactivity, those with relatively normal bioactivity, and those with increased bioactivity against *C. elegans*. More than 400 point variants were successfully tested. Some of those in the latter class (increased bioactivity) have been selected for further study, including fully quantitative analyses and testing their spectrum of increased action against other nematodes. Our results, as well as their implications for crystal protein – nematode interactions, will be presented..

Symposium. Wednesday, 11:10 **142**

Insights into the structures of non-3-domain toxins through structural modeling

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A knowledge of the structure of insecticidal toxins is a major benefit in elucidating mode of action and is, therefore, fundamental for targeted mutagenesis to test mechanistic hypotheses, to alter target range and increase toxicity. Crystal structures of activated 3-domain toxins, Cyt toxins, Vip2, Mtx1 and anthrolysin are available and can be used to model structures for related proteins. There remains a significant

number of potent invertebrate-active toxins that do not fall within these classes. Crystallography is labour-intensive, requiring large quantities of pure, mono-disperse protein and often proves difficult. Recent developments in the field of *in silico, ab initio* structural modelling allow the generation of models in the absence of related sequences in the protein structure database. This may allow us to predict protein structures and use these predictions to develop testable hypotheses for the modes of action of the toxins. This procedure has been applied to several non-3-domain toxins and toxin-associated proteins. For one such protein, a structure is proposed, consistent with a pore forming mechanism of action. Analysis of secondary structure content is consistent with this model and evidence of pore formation has been produced. Mutagenesis of a region known to be important in structurally-related toxins was shown to eliminate toxicity. While further study is clearly required, modelling, thus, allows us to predict and test hypotheses related to the mode of action of toxins for which experimental structures are, as yet, unavailable.

Symposium. Wednesday, 10:30 **143**

Novel MTX Toxins for Insect Control

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In addition to the conventional 3-domain Cry proteins, the Gram-positive bacteria Bacillus thuringiensis can also harbor other classes of insecticidal toxins with distinct structures, receptors, and modes of action. Among them are a group of proteins that share significant similarities to MTX2/3 toxins at the structural level, but are very divergent at the amino acid sequence level. In this presentation, we will discuss the general features of these MTX toxins, and agriculture applications for the control of insect pests.

Symposium. Wednesday, 11:50 **144**

Insecticidal toxins from *Photorhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins

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Photorhabdus luminescens and *asymbiotica* live in the gut of entomopathogenic nematodes, which invade insect larvae, where they release the bacteria. Here, the bacteria produce toxins, which kill the insects. We studied tripartite Tc toxins from *P. luminescens* and a novel toxin (PaTox) from *P. asymbiotica*.
Tc toxins consist of three components TcA, TcB and TcC, which occur in several isoforms. TcA is responsible for the binding and up-take of the toxin, B is a linker and C carries the biological activity. Recent crystal structure analysis revealed a novel type of syringe-like injection mechanism, which depends mainly on TcA but needs all components (1). We studied the biological activity of TccC3 and TccC5, which are isoforms of TcC. TccC3 ADP-ribosylates actin at threonine148, thereby actin polymerization is enhanced (2). TccC5 ADP-ribosylates Rho proteins at glutamine61, a modification which persistently activates of Rho GTPases. Both modifications of actin and Rho proteins induce clustering of the actin cytoskeleton (2).
The *P. asymbiotica* toxin PaTox glycosylates Rho proteins by attaching GlcNAc at tyrosine32/34 (3). The modification inhibits Rho signaling, because Rho activation and interaction with effectors are blocked. In addition, PaTox harbors a deamidation domain, which activates heterotrimeric G proteins, including Gq/11 and Gi family proteins. Functional consequences of the actions of *Photorhabdus toxins* on actin and GTP-binding proteins are discussed.
References
1. Meusch et al. (2014) Nature 508, 61-65.
2. Lang et al. (2010) Science 327, 1139-1142.
3. Jank et al. (2013) Nat. Struct. Mol. Biol. 20, 1273-1280..

Symposium. Wednesday, 12:10 **145**

Molecular basis of parasporin-2 action toward cancer cells

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Parasporin-2 (PS2) is a crystal toxin isolated from parasporal inclusions of *Bacillus thuringiensis* A1547. PS2 has a strong cytocidal activity in liver and colon cancer cells without showing a typical insecticide. Accumulated molecular, cellular and *in vivo* experimental observations on PS2 indicate that the protein form a pore in membrane with a mega size assembly. The crystal structure of active PS2 monomer reveals that the protein elongates like a short rod, comprising almost β-strands. The polypeptide folding is similar to a class of aerolysin-like β-pore-forming toxins while there is no homology to insecticidal Cry toxins. N-terminal domain of PS2 is rich in aromatic residues and forms a groove which could be capable to grapple the target molecule. Amino acid substitutions of PS2 in the region indicate that the residues could be involved in cell-binding. The C-terminal domain contains β-sandwiches and the surface of the protein has a unique extensive track of exposed side chains of serine and threonine where thought be related to PS2 oligomerization and membrane pore formation. Single-particle EM analysis reveals that PS2 oligomer shows a ring shape with the 24nm length, 8 nm diameter and a 4nm pore while a structure of pore-forming aerolysin is the ring-like mushroom structure with a central pore. We would like to introduce current observations on anti-cancer toxin PS2 in vitro and vivo in this symposium.

CONTRIBUTED PAPERS Wednesday, 10:30-12:30

MICROBIAL CONTROL 2

Contributed paper. Wednesday, 10:30 **146**

Evaluation of the non-target effects of *Bacillus thuringiensis* subspecies *israelensis* in standardized aquatic microcosms

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Malaria, one of the most deadly vector-borne diseases in the world, is transmitted by the bite of an infected female *Anopheles* mosquito. *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is a gram-positive, aerobic, spore-forming bacterium that produces crystalline inclusions that contain insecticidal proteins. *Bti* has been shown to be highly insecticidal to larvae of mosquitoes and blackflies, but is considered to have weak insecticidal activity against non-dipteran invertebrates in aquatic environments. Few studies have comprehensively studied the non-target effects of *Bti* under reproducible and standardized conditions. The objective of this study was to evaluate the effects of *Bti* on key non-target invertebrates in a highly reproducible synthetic multi-species system, the