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Incorporating COST862 Action: Bacterial Toxins for Insect Control

PROGRAM and ABSTRACTS

3-7 August 2008

University of Warwick,

Coventry, UK

TUESDAY AM

Poster / Bacteria. Tuesday, 10:30. **B-29**

Characterization of environmental isolates of *Bacillus thuringiensis* from northeastern Poland harbouring *vip3A* gene homologues

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Various strains of *Bacillus thuringiensis* have been used effectively as biological insecticides due to their production of highly specific crystalline proteins, the so-called Cry or d-endotoxins. Recently, vegetative insecticidal proteins (VIPs) secreted during vegetative growth of certain *B. thuringiensis* strains have been described. As VIPs, particularly VIP3A, are known to be active against lepidopteran larvae, there is significant interest in identifying or developing strains with novel Cry and VIP combinations for applied use. To this end, the purpose of this study was to determine (i) the presence of *vip3A* homologues in *B. thuringiensis* collected in northeastern Poland; (ii) the correlation between the *vip3A* and *cry* genes contents, as well as the diversity in chromosomal DNA patterns; and in particular, (iii) the diversity of *vip3A*. Of 166 *B. thuringiensis* isolated from small wild mammals, soil, and milk products, 16 (~10%) harboured *vip3A* homologues with high levels of sequence conservation. These *vip3A*-positive isolates were shown to contain genes encoding known lepidopteran-active toxins, such as *cry1* (11 isolates), *cry2* (8 isolates), and *cry9* (2 isolates). Finally, PFGE analysis of DNA profiles demonstrated marked diversity among these isolate. As such, further studies are required to determine whether these isolates vary in toxicity against lepidopterans.

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Poster / Bacteria. Tuesday, 10:30. **B-30**

Characterization of a novel Cry9Bb δ-endotoxin from *Bacillus thuringiensis*

Jd. 31109

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The *Bacillus thuringiensis* serovar *japonensis* strain S725 produces spherical crystals harboring a major protein of about 130 kDa. This protein showed immunoafinity and high level of N-terminal sequence identity with Cry9 delta-endotoxins. A *cry9*-like gene from Bt S725 was cloned, sequenced and expressed in Bt. The cloned gene sequence contains a 3492 bp ORF, which encodes a polypeptide of 1163 amino acids, with a predicted molecular mass of 131.4 kDa. The deduced amino acid sequence was unique and showed 73% identity with Cry9Ba. The novel d-endotoxin was assigned to a new subclass, Cry9Bb, by the Bt Toxin Nomenclature Committee. The 130 kDa Cry9Bb protein formed crystals and produced two fragments around 69 and 58 kDa upon trypsin activation. It exhibited activity against the lepidopterans *Manduca sexta* and *Anticarsia gemmatalis*. The biological effect of an amino acid residue substitution, A84P, was investigated. The LC_{50} for the Cry9Bb crystals against *M. sexta* neonate larvae was 6.84 $\mu g/cm^2$, while the LC_{50} for Cry9BbA84P crystals was 0.78 $\mu g/cm^2$. PCR screening revealed that, in addition to *cry9Bb*, Bt strain S725 also contains *cry11* and *vip3* genes. Transcription analysis, using RT-PCR, showed that the *cry11* gene was transcribed at T_2 and T_5 stages of sporulation.

Poster / Bacteria. Tuesday, 10:30. **B-31 STU**

Identification and cloning of novel *cry* genes from *Bacillus thuringiensis* strain Y41

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Four novel *cry* genes were cloned by PCR-RFLP method from *Bacillus thuringiensis* isolate Y41, which was isolated from Hainan Province. The toxins accumulating within the cells consisted of major proteins of 66 and 140 kDa and forming spherically shaped crystals. Compared the sequences of these fragments with known holotype *cry* genes, the result indicated that three of them are similar with *cry40Aa1*, *cry30Aa1*, and *cry19Aa1* respectively, and one of them is not distinct similar with any reported *cry* genes. All toxins have typical characteristic of delta-endotoxin and containing five homology blocks (1-5) which present in most *B. thuringiensis* delta-endotoxins. These four novel *cry* genes were deposited in GenBank and named by the *B. thuringiensis* delta-endotoxin nomenclature committee as *cry40Ca1*, *cry30Da1*, *cry52Aa1* and *cry53Aa1* respectively.

Poster / Bacteria. Tuesday, 10:30. **B-32 STU**

The characterization of novel Bt toxins

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Despite the large numbers of Bt toxins already discovered there remains the potential for the discovery of new toxins or the creation of variants with improved activities through traditional or directed evolution techniques. Although many techniques now exist for the directed evolution of new proteins the screening of variant toxins for improved activities remains a labour-intensive and resource heavy activity. Using the toxins Cry1Ac, Cry1Ah and Cry1Ie we have investigated the possibility of using an in vivo method for the selection of improved recombinants. We will describe the basis of this selection procedure and present the results of initial feasibility studies.

Poster / Bacteria. Tuesday, 10:30. **B-33 STU**

Identification of new *cry* genes of *Bacillus thuringiensis* through the use of a system of universal primers

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Based on the known *cry* gene sequences of *B. thuringiensis*, three pairs of primers were designed from the 5 conserved blocks found in the delta-endotoxin coding region. Designed primer pairs amplify the regions between blocks 1 and 5, 2 and 5, and 1 and 4, respectively. *In silico* analyses indicated that up to 96% of the known sequences can be amplified by one or more of these pairs. Their ability to detect new *cry* genes was tested when DNA from *B. thuringiensis* strains showing atypical crystal morphology was used as template. Some 175 strains recorded as "atypical" in the CINVESTAV-IPN (LBIT-series) collection log were further selected by phase contras microscopy, SDS-PAGE, and SEM analyses. After a systematic amplification and sequencing of amplicons obtained from 27 strains, 5 putative *cry* genes showed