Isolation and characterisation of novel non-ribosomal peptide synthetase genes from the entomopathogenic *Xenorhabdus bovienii* T228

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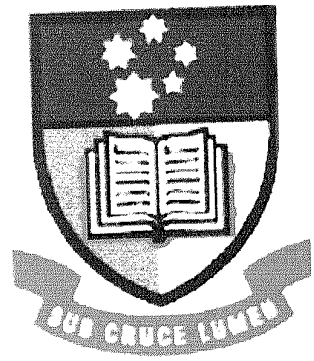

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Abstract

Xenorhabdus spp. are entomopathogenic bacteria grouped within the family Enterobacteriaceae. These bacteria exhibit a colony pleiomorphism, termed phase variation in which phase 1 and phase 2 forms differ in expression of a variety phenotypic characteristics. The main aim of this thesis was to identify and characterise genes involved in the expression of phase variant characteristics. A secondary aim was the construction of a RecA mutant of *X. bovienii* to facilitate genetic complementation analysis and the introduction of foreign DNA. This construct was also used to determine a possible role for *recA* in phase variation and virulence.

The *recA* gene of *Xenorhabdus bovienii* was cloned and sequenced. Phylogenetic analysis of *recA* sequence data confirmed the placement of *Xenorhabdus* within Enterobacteriaceae. A *recA* insertion mutant of *X. bovienii* was constructed using allelic exchange mutagenesis. The *recA* mutant displayed phase variation and did not show any differences in expression of the phase dependent characteristics phospholipase C, lipase, haemolysin, protease, antimicrobial activity or Congo Red binding. No differences in the virulence of *X. bovienii*, or the *recA* mutant, for *Galleria mellonella* were observed. These results suggest *recA* is unlikely to be involved in phase variation, or the expression of phase dependent characteristics.

Transposon mutagenesis was used to identify regions of the *X. bovienii* chromosome involved in expression of phase variant characteristics. Five transposon insertion mutants showing a disruption in the expression of phospholipase C, Congo Red, haemolytic and antimicrobial activity were further characterised. *X. bovienii* chromosomal DNA flanking each transposon insertion was mapped, cloned and sequenced. Three transposon insertion mutants; XB26(20), XB29(45) and XB34(45) inserted into a common 15,582 bp region of DNA showing significant homology at the amino acid level to non-ribosomal peptide synthetases (NRPSs). Two partial open reading frames (ORFs) (*xpsD* and *xpsC*) and two complete ORFs (*xpsA* and *xpsB*) were identified. ORF *xpsD* is 653 bp and shows significant homology at the amino acid level to ATP-binding cassette transporters required for the secretion of NRPSs pyoverdine and syringomycin. 788 bp downstream of *xpsD*, ORF *xpsA* has the potential to encode a protein of 1089 amino acids with a predicted *Mr* value of 122,980. 37 bp downstream of *xpsA*, ORF *xpsB* has the potential to encode a 3316 amino acid protein with a predicted *Mr* value of 368,263. The stop codon (ATGA) of *xpsB* overlaps the initiation codon (ATGA) of the 1177 bp ORF *xpsC*. ORFs *xpsA*, *xpsB* and *xpsC* are predicted to have an operon arrangement. BLASTX analysis of the *xpsABC* region shows

homology at the amino acid level to NRPSs such as the plant toxin syringomycin (*Pseudomonas syringae* pv. *syringae*) (Guenzi *et al.*, 1998), tumor suppressing cryptophycins (*Nostoc* sp. GSV224) (Subbaraju *et al.*, 1997) and the peptide antibiotic tyrocidine (Mootz & Marahiel, 1997b). The portion of *X. bovienii* NRPS identified to date is predicted to activate the amino acids serine, 6-*N*-hydroxylysine and glutamine. On the basis of this analysis, this NRPS is predicted to be a siderophore antibiotic.

To investigate regulation of the *X. bovienii* NRPS an *xpsA-lacZ* transcriptional fusion was constructed and introduced into the *X. bovienii* chromosome by transposon mutagenesis. Over a 96 hr incubation period the level of β-galactosidase activity increase 2 - 3 fold in *X. bovienii* broth cultures. This is in comparison to an *E. coli* based *xpsA-lacZ* construct, where levels of β-galactosidase activity remained constant over the 96 hr incubation period. These results suggest the expression of *X. bovienii* NRPS may be linked to a cell density dependent mechanism. The levels of β-galactosidase expression in *xpsA-lacZ* transcriptional fusion mutant cultures did not change when cultures were grown in 20% (v/v) conditioned culture medium. This result suggests a quorum sensing mechanism similar to that observed in *Serratia liquefaciens* (Lindum *et al.*, 1998) is not involved in expression of *X. bovienii* NRPS. Attempts to detect XpsA expression in *X. bovienii* whole cell lysates using an XpsA antiserum were unsuccessful, and this may reflect an extremely low level of XpsA expression.

To determine a function for *X. bovienii* NRPS, inframe deletion mutants in *xpsA*, *xpsB* and *xpsAB* were constructed by allelic exchange mutagenesis. Culture supernatants were tested for cytotoxic activity against cultured Schneider's cells. Also, whole bacterial cells were injected into *Galleria mellonella* to assess subsequent haemocyte damage by transmission electron microscopy. No significant difference in cytotoxic activity was observed between wild type and inframe deletion mutant strains.

The antimicrobial activity of phase 1 *X. bovienii* and in-frame deletion mutant supernatants against the indicator organism *M. luteus* was tested using a microtitre tray bioassay. Surprisingly, both the single and double in-frame deletion mutants showed a greater level of antimicrobial activity than the wild type *X. bovienii* supernatant. This result may be explained by the modular nature of NRPS. Deletion of one or more modules may result in production of a modified antimicrobial peptide. However, this observation can only be resolved by purification of the bioactive peptide and structural comparison of the wild type and mutant derived compounds in the absence of other background bacterial compounds.

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