Identification and characterisation of toxin-antitoxin systems (TA) in *Burkholderiapseudomallei*

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Abstract

The aim of this study was to identify and characterise type II toxin-antitoxin (TA) systems in *Burkholderiapseudomallei*, the causative agent of the human disease melioidosis.

8 putative TA systems were identified within the genome of *B. pseudomallei*K96243. 5 of these were located witihn genome islands. Of the candidate toxins, BPSL0175 (RelE1) or BPSS1060 (RelE2) caused growth to cease when expressed in *Escherichia coli*, whereas expression of BPSS0390 (HicA) or BPSS1584 (HipA) (in an *E. coli ΔhipBA* background) caused a reduction in the number of culturable bacteria. HicA also caused growth arrest in *B. pseudomallei*K96243*ΔhicAB.* These toxin induced phenotypes were enhanced by an <3kDa extracellular factor that accumulated in the spent medium during growth. Expression of the cognate antitoxins could restore growth and culturability of cells.

Expression of *hicA* in *E. coli* gave an increased number of persister cells in response to ciprofloxacin or ceftazidime. Site directed mutagenesis studies identified two key residues within the HicA toxin that were essential for both the reduced culturability and increased persistence phenotypes. Deletion of *hicAB* from *B. pseudomallei*K96243 did not affect persister cell or survival frequencies compared to the wild type following treatment with a variety of stress conditions.

Deletion of the Δ*hipBA*locus from *B. pseudomallei* K96243 also had no affect on bacterial persistence or survival under the conditions tested.

Contents

List of figures

Page number

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of tables

Chapter 1

Chapter 2

Chapter 3

Chapter 5

Chapter 6

Chapter 7