The identification and characterisation of PPIases from *Burkholderia pseudomallei* and *Burkholderia thailandensis*

Submitted by Isobel Harriet Norville, to the University of Exeter as a thesis for the degree of Doctor of Philosophy

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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 been previously submitted and approved for the award of a degree by this or any other University.

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Abstract

The aim of this study was to identify and characterise peptidyl-prolyl *cis-trans* isomerases (PPIases) from the bacterium *Burkholderia pseudomallei,* the causative agent of the disease melioidosis. The longer term goal was to assess their potential as vaccine candidates or antimicrobial targets.

Using bioinformatic approaches, six putative FK506-binding proteins (FKBPs) proteins and three putative parvulin proteins were identified in *B. pseudomallei*. Of these, six were expressed and purified as recombinant proteins. The purified proteins were used to immunise BALB/c mice, with some providing protection against a subsequent *B. pseudomallei* infection. These proteins could therefore be proposed as potential vaccine candidates.

Homologues of Mip or SurA, which are associated with virulence in other bacterial species, were identified in *B. pseudomallei* and closely related *B. thailandensis.* Recombinant Mip or SurA homologues from *B. pseudomallei* were shown to have characteristic PPIase enzyme activity. To evaluate the role of the Mip homologue from *B. pseudomallei* in virulence, an unmarked deletion mutant was constructed. The mutant had reduced intracellular survival; defects in putative virulence mechanisms and attenuated virulence in mice. To assess the role of a SurA homologue, closely related *B. thailandensis* was used as a model organism, with deletion of the gene resulting in defects in intracellular infection, outer membrane integrity and virulence. This indicates that PPIases from *B. pseudomallei* and *B. thailandensis* represent novel virulence determinants and potential antimicrobial targets for therapeutics against melioidosis.

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