Isolation of CtpA, a copper transporting P-type ATPase which has significance for virulence of *L. monocytogenes*

by

Matthew S. Francis, B.Sc. (Hons.)

Department of Microbiology and Immunology,
The University of Adelaide.

A thesis submitted for the degree of Doctor of Philosophy
in
The University of Adelaide
(Faculty of Science)

December, 1996

CtpA is structurally similar to other reported bacterial P-type ATPases on the basis of aligned hydropathy profiles and prediction of transmembrane topology. Using these approaches, an N-terminal truncation was observed in CtpA in a domain normally attributed to initial cation binding. This truncation has only been described for one other P-type ATPase protein involved in copper transport in *Helicobacter pylori* (Ge *et al.*, 1995). Confirmation of this finding in future studies is required using N-terminal amino acid sequence analysis of purified CtpA. Nevertheless, conserved amino acid residues critical for protein function were identified in CtpA, located in putative functional domains of this protein. These domains were predicted to lie on the cytoplasmic side of the bacterial membrane, which is consistent with other membrane topology models reported for several ATPases.

To investigate the significance of CtpA for virulence, a mutant strain was constructed by insertion of an antibiotic resistance cartridge into the *ctpA* gene. A tissue culture internalisation assay, optimised in this study using the HeLa cell line, and mouse infection studies were used to compare *ctpA* insertion mutants and parental wild type strains. Mutants in CtpA, were unaltered for intracellular growth in J774 and HeLa cell lines. However, recovery of mutants from liver of infected mice was dramatically reduced compared with the wild type, and a significant impairment in terms of *in vivo* persistence in livers and spleens of mice following mixed-infection competition experiments was observed. These results demonstrated the significance of CtpA for establishment of an *in vivo* infection by *L. monocytogenes.* Given Cu^{2+} is an essential nutrient for growth of all lifeforms, and Cu^{2+} concentration is significantly reduced in mammalian cells upon infection, this suggested CtpA may be involved in scavenging free Cu^{2+} ions from the intracellular environment of an infected host.

Furthermore, DNA homologous to *ctpA* was not detected by Southern hybridisation analysis or PCR, in non-pathogenic *Listeria* spp. or in the animal pathogen *L. ivanovii.* However, the distribution of *ctpA* in *L. monocytogenes* was restricted to a population of environmental and clinical isolates predominantly associated with RLFP groups B and B1, which contain unique *Hin*dIII restriction fragment polymorphisms within the *hly* gene (Thomas, 1995).

Table of Contents

Chapter 2 Materials and Methods