The effect of mutations in lipopolysaccharide biosynthetic genes on the virulence of *Salmonella typhimurium* for the mouse

by:

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SUMMARY

In **Chapter 1** of the work, the literature pertaining to the pathogenesis of *Salmonella* in animals and humans is first reviewed. This section covers the genetic basis of bacterial virulence, as far as it is understood, and describes genetic modifications attenuating for the virulence of *Salmonella* strains. Some such strains have been used as live oral vaccines against salmonellosis of humans and animals. One mutation which is attenuating for the mouse-virulence of *Salmonella typhimurium* is *galE*. Strains with mutations in *galE* are affected in lipopolysaccharide (LPS) biosynthetic ability, among other features .The possibility that other mutations affecting LPS biosynthesis are also attenuating has not been explored for mutations in *rfc* or *pmi*. As this is the work of this thesis, Chapter 1 concludes with a review of the structure of LPS, and the enzymology and genetics of its biosynthesis.

Chapter 2 covers the Materials and Methods used in the work.

In **Chapter 3**, the *rfc* gene of *S. typhimurium* is cloned, by direct selection in an *rfc* mutant, and confirmed to complement the lesions in both an older point mutant in *rfc*, and a new IS*10* insertion mutant. In **Chapter 4**, the gene is sequenced and the sequence analysed. There is a high frequency of modulating codons in the presumptive gene, explaining why difficulties were experienced in visualisation of the protein product in a variety of systems.

In **Chapter 5**, the *pmi* gene of *S. typhimurium* is cloned and analysed, while **Chapter 6** details the sequence of the gene, which is very like that of *Escherichia coli* K-12. The protein product of this gene was readily visualised in minicells.

In **Chapter 7**, both of the wild-type *rfc* and *pmi* genes are interrupted *in vitro* by the insertion of a Km-resistance cassette, and these insertion mutations recombined (singly) into the genome of virulent *S. typhimurium* C5. Comparisons, in both LD_{50} tests, and in short-term colonisation experiments, of the wild-type C5, and the otherwise isogenic mutants, showed that either mutation greatly reduced the virulence of C5 for the mouse. Both mutants persisted well in the spleen following oral administration, unlike a *galE* mutant of C5. Both mutants were effective as live oral vaccines against C5 infection in the mouse.

In **Chapter 8**, the results of the work are briefly summarised . A conclusion is that an *S. typhimurium* strain which is capable of the biosynthesis of a full LPS core is able to colonise the spleens of animals infected orally with such a strain.

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