Analysis of the *Helicobacter mustelae* Surface Ring (*hsr*) Locus

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ABSTRACT

The DNA upstream of the gene encoding the *Helicobacter mustelae* surface ring (Hsr) protein of the ferret gastric pathogen *Helicobacter mustelae* was shown to contain several uncharacterised *hsr*-like repeat sequences in a total of 12 kb of *hsr*-related DNA, including the *hsr* gene (the *hsr* locus). The primary objective of this study was to analyse the *hsr* locus of *H. mustelae* strain 4298, in particular, to determine the extent and possible function of the *hsr*-related DNA repeat sequences.

H. mustelae was isolated from the stomachs of six New Zealand ferrets. This study represents the first successful isolation of *H. mustelae* from ferret stomachs from at least two geographically distinct locations in New Zealand. The Hsr proteins of the different *H. mustelae* strains exhibited variability in protein size and reactivity to anti-Hsr antisera. The DNA sequence of the strain 4298 15-kb *hsr* locus was completed and analysed for DNA repeats. There were 343 perfectly repeated sequences 12 – 741 bp in length, with up to 11 copies of each. Within the *hsr* gene of strain 4298, a 2.4 kb repeat region, the variable repeat region, was defined. The flanking *hsr*-related sequences were equally distributed and mostly (96%) inverted with respect to the *hsr* gene. DNA sequence alignments of nine different *H. mustelae* strains, showed a high level of sequence variation in the variable repeat region of the *hsr* gene, in contrast to the central and β domains. Alignment of sequenced DNA from the variable repeat region of different strains identified conserved-variable-conserved blocks (CVCs) of sequence, which may facilitate a recombination-based antigenic variation mechanism. Approximately 7 kb upstream and 3 kb downstream *hsr*-related flanking sequence may serve as a reservoir for sequence variation of the *hsr* gene. The searches for repeat elements have facilitated the identification of potential DNA regulatory elements involved in the abundant production of the Hsr protein.

The HSRL also contained an unrelated open reading frame, encoding Orf2, which had significant identity with LolA , a periplasmic lipoprotein carrier protein, but containing an N-terminal extension of 14 charged and polar amino acids. Insertional inactivation of *orf2* had no detectable effect on Hsr expression in the Hsr^+ strain 4298.

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RELATED PUBLICATIONS

Some of the material presented in this thesis has been published.

Forester, N.T., Parton, K., Lumsden, J.S., and O'Toole, P.W. (2000). Isolation of *Helicobacter mustelae* from ferrets in New Zealand. *New Zealand Veterinary Journal* **48**:65-69.

Forester, N., Lumsden, J.S., O'Croinin, T., and O'Toole, P.W. (2001). Sequence and antigenic variability of the *Helicobacter mustelae* surface ring protein Hsr. *Infection and Immunity* **69**(5):3447 – 3450.

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