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The Genetic Characterisation of a Carbon Starvation
Gene of *Helicobacter pylori*

A thesis presented in partial fulfilment of
the requirements for the degree of
Master of Science in Microbiology
at Massey University, Palmerston North
New Zealand

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1998

Abstract

The carbon starvation gene (*csg*) of the gastric pathogen *Helicobacter pylori* was studied to elucidate the role of the gene product during the carbon starvation response. Studies were also conducted to investigate the relationship of *csg* to the glutamine permease operon (*glnP1*, *glnP2*, *glnQ*, and *glnH*), which is located directly upstream of *csg* on the *H. pylori* chromosome.

csg and *glnP1* were sequenced to determine the length of the genes, the base composition, and the location of putative promoters and regulator binding sites. The sequence information was then used to construct a *csg* mutant using a deletion:insertion strategy, whereby the promoter regions of *csg* and *glnP1* were removed and replaced with the Kan Ω antibiotic resistance marker. Phenotypic analysis was carried out on this mutant in complex, defined, and semi-defined media. The plasmid carrying the *csg* mutation was also transformed into *H. pylori* Sydney strain to allow colonisation studies in mice.

A *H. pylori* strain was constructed, which would allow the study of the expression of *csg* under various environmental conditions. The promoterless chloramphenicol acetyl transferase (CAT) cassette from pCM4 was used as the reporter gene and cloned into an engineered *Bgl*III site directly downstream of *csg* to disrupt the transcription terminator. The kanamycin resistance cassette from pILL600 was also cloned into this site in a three way ligation, to allow selection of transformants in *H. pylori*.

Sequence of the *csg* mutant showed putative σ^{70} responsive -10 and -35 sequences, and a putative cAMP receptor protein (CRP) binding sequence for *csg* in the 5' sequence of *glnP1*. The CRP site implied that *csg* may be regulated by cAMP. The location of the CRP binding site also suggested that *csg* and *glnP1* were linked, which was confirmed by PCR amplification of a region containing the *csg* and *glnP1* transcription start points and the intergenic region from ten *H. pylori* strains. The same PCR amplification failed for a *H. mustelae* strain, suggesting that this conservation of sequence is specific to *H. pylori* strains.

Phenotypic analysis of the *csg* mutant showed no difference compared to the wild type when grown in complex and semi-defined medium, both of which contained peptides. However, the *csg* mutant grew more slowly than the wild type in the defined medium, which contained glucose and amino acids as the sole carbon sources.

The role of *csg* could not be determined with certainty. The results suggested that Csg may scavenge for carbon sources to help the cell escape carbon starvation. This thesis work has shown *csg* to be non-essential *in vitro*. However, a non-functional *csg* gene *in vivo* may impair transmission, when carbon sources are lacking, but the mutation would be silent in the stomach where peptides are abundant.

Acknowledgements

I would firstly like to give a big thanks to my supervisor, Paul O'Toole, for letting me try and think for myself and then helping out when that didn't work! An especially big thanks for reading and correcting this tome – definitely no small feat!

I would also like to thank the members of the Department of Microbiology and Genetics, now part of the Institute of Molecular Biosciences, for their encouragement and support for us postgrads. Massey University also needs to be thanked, and I give thanks from the bottom of my heart for the scholarships which have helped over the past two years.

A big thank you goes to all members, past and present, of the Helipad. In particular, Tash for being a life saver on a number of occasions and also for being great as a sounding board for ideas; Steffen for technical support and the odd german lesson; Basil for being a fountain of knowledge on 80's music - we couldn't have done the Manic Medley without you; and Kirsty for being a great bench buddy. Thanks to everyone for making the last two years fun.

I must also thank my friends in the Department/Institute; Kirsty, Helen, Ross, BJ, James, Megan, Tash, and Anita for the smokos, lunches, and support.

An absolutely huge thank you to my family for their emotional and financial support over the last five years that I've been at varsity. Your encouragement has meant so much to me and I love you guys dearly.

Finally, to Ru. For your encouragement, for forcing me back to my room to study, for letting me vent about things that weren't working, for all the understanding (the non-scientific variety) and support, and for all of your love. You are my best friend and I love with all of my heart. Ta.

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