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

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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 KanΩ 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*II 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.

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