Co-ordinated regulation of gene expression in H. pylori in response to low pH and iron limiting growth conditions

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Introduction

Establishing a successful infection within the gastric environment and its varied and changing niches requires adaptation achieved through regulation of bacterial gene expression. In the gastric environment in addition to the low pH, Helicobacter pylori is required to adapt to conditions of iron limitation. In this study, we used cDNA microarrays to identify genes whose expression was altered when the bacterium was grown under differing conditions of pH (5 versus 7) and iron limitation. cDNA microarray analyses following exposure to different growth conditions were carried out for three H. pylori strains 26695 (gastritis-associated), J99 (ulcer-associated) and AG-1 (atrophic gastritis-associated).

Design of experiments

Helicobacter pylori strains 26695 (gastritis-associated), J99 (ulcer-associated) and AG-1 (atrophic gastritis-associated) were grown at 37°C under microaerobic conditions on blood agar and used to prepare an inoculum for liquid cultures in Brucella Broth containing 10% horse blood serum, that subsequently were incubated microaerobically at 37°C. Bacteria from such liquid cultures (OD600 = 0.7) were harvested, re-suspended in medium whose pH was adjusted to 5.0 and pH 7.0, in the absence (iron replete) and presence of desferal (iron deplete). The incubation was continued for a further 1 h period before RNA extraction.

Results of Expression profiling

Figure 2. Differential regulation of gene expression in response to different conditions of stress

Differentially Expressed Genes

A. pH7- pH5 Shift
Transport and binding proteins
Transcription and Translation
Flagella and Motility
Energy metabolism
DNA replication, recombination, and repair
Cell division
Teichoic acids and Virulence
Cell envelope, surface structures
Signal transduction mechanisms
Amino acid and nitrogen metabolism

B. pH7 - No Fe
Transport and Binding
Transcription
Energy production
Metabolism
DNA replication, recombination, and repair
Cell division
Cell envelope and membrane associated
Amino acid and nitrogen metabolism

C. pH5 - No Fe
Transport and Binding
Transcription
Energy production
Metabolism
DNA replication, recombination, and repair
Cell division
Cell envelope and membrane associated
Amino acid and nitrogen metabolism

Figure 3. Ontological distribution of differentially expressed genes

Summary of Results

- Shift to low pH upregulated 68 genes including urease (ureA, ureB, ureD, UreF), amidase (aimE), flagellar synthesis (fliB, fimB, fliE), transport and genes encoding binding proteins (glnQ, glnH, exhB).
- At low pH 37 genes were down-regulated from different functional groups (e.g., outer membrane proteins and chaperones).
- At pH 7, iron limitation mainly altered the expression of motility-associated genes.
- Whereas, at pH5, iron depletion led to the increased expression of 21 genes including those coding for carbonic anhydrase.
- Genes encoding virulence factors (e.g., CagA and vacA) and proteins involved in motility showed altered expression in all 3 conditions.
- Our results emphasize the link between bacterial response to acidity, metal metabolism and virulence.
- Moreover, this response was similar in H. pylori strains associated with different pathologies.

Acknowledgements: