P02 Microbiology, Molecular Genetics and Genomics

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DIVERSITY AND PHYLOGENY OF THE *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEIN ENCODING GENE HOMC

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The genetic diversity and evolution of the homC gene was evaluated in a panel of approximately 200 clinical and reference strains, isolated from patients from different geographical origins and presenting different gastric diseases. PCR, sequencing and bioinformatics analyses were used.
All the strains tested harboured a complete homC gene at a conserved locus. Phylogenetic reconstruction of homC showed a geographical segregation, with three predominant groups: Western, East Asian/Amerindian and African. A similarity plot analysis suggested a conserved profile of gene segmentation, where three segments were defined. In the first segment (5′ end extremity), sequences were separated according to the geographical origin of the strain. A higher level of diversity (<50%) was observed in the middle segment, while the third segment (3′ end extremity) was the most conserved (~90%). In the middle segment, eight allelic variants were identified, with geographic specificity regarding the most prevalent ones. The AI allele was predominant and exclusive of Western strains. The AII allele was predominant in African strains and was the only allele present in the three geographical groups. The AIV allele was predominant in East Asian/Amerindian strains and was not observed in Western strains. The Western group showed greater molecular distance while the sequences from the East Asian/Amerindian group were the closest.
Overall, the regular presence of homC and its allelic variability suggest that this gene is a good candidate to be part of the pool of *H. pylori* outer membrane proteins involved in bacterial persistence.

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HIGH WORLDWIDE CONSERVATION OF A *HELICOBACTER PYLORI* OUTER MEMBRANE PROTEIN GENE, HOMD

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The genetic diversity and evolution of homD, coding for *Helicobacter pylori* outer membrane protein (OMP) was investigated in a panel of approximately 200 clinical and reference strains, isolated from patients from different geographical origins and presenting different gastric diseases. PCR, sequencing and bioinformatics analyses were used.
The homD gene was present in all strains, at a conserved locus, and showed a low genomic diversity, displaying high similarity at both nucleotide and amino acid level. A similarity plot analysis also showed a high level of sequence conservation, although a small region (~30 nucleotides) differed between Western strains and the other strains (East Asian/Ameridian and African). This region was also found in some allelic variants of another hom family member, the homC gene, suggesting the existence of recombination events between these two OMP encoding genes.
Sequence analysis of the HomD predicted protein showed a N terminus region with a variable number of KP motif repeats (2 9 KP), with a correlation between the lowest number of KP motif repeats (≤4 KP) and peptic ulcer disease and the highest number of repeats (≤7 KP) and gastritis. In silico analysis of the HomD protein showed that the region of KP motif repeats exhibits a strong hydrophilicity and antigenicity and a high probability of being exposed to the bacterial surface, suggesting that HomD is immunogenic.
These results suggest that homD gene is an important *H. pylori* antigen and, because of its high global conservation, it is likely to constitute a new vaccine target.

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DIVERGENT MECHANISMS OF INTERACTION OF *HELICOBACTER PYLORI* AND *CAMPYLOBACTER JEJUNI* WITH MUCUS AND MUCINS

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Helicobacter pylori and *Campylobacter jejuni* are related organisms specifically adapted to colonise the mucus layers of the gastric mucosa and intestine, respectively. This study aimed to examine the interaction of the organisms with mucins from various animal species and how they colonise the adherent mucus layer of mucus secreting cells. Mucus secreting HT29-MTX-E12 (E12) cells, mucin secreting HT29-MTX cells and HT29 cells (non mucin/mucus secretors) were each infected with *H. pylori* and *C. jejuni* organisms. Binding of *H. pylori* and *C. jejuni* to mucins purified from E12 cells and various animal species was assessed. Both *C. jejuni* and *H. pylori* displayed a tropism for chicken or porcine mucin respectively compared to mucins from other natural sources. *H. pylori* colonised E12 and to a much lesser extent HT29-MTX cells but not HT29 cells indicating that the presence of an adherent mucus layer was essential for efficient infection. In contrast, *C. jejuni* infected all three cell lines. However, the presence of an adherent mucus layer in E12 cells enhanced colonisation by *C. jejuni*. *C. jejuni* bound to E12 mucin. However, *H. pylori* bound not to mucin but to Lewis^b containing non mucin fractions of E12 mucus. Although the presence of mucus was important for effective infection by both *H. pylori* and *C. jejuni* the mechanisms underpinning mucus colonisation by these two organisms differed. This study highlights the role of mucus in promoting bacterial infection and the importance of host glycans in mediating the interaction of bacteria with host tissue.

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USE OF MICROFLUIDIC DEVICES TO INVESTIGATE CHEMOTAXIS OF *HELICOBACTER PYLORI* IN RESPONSE TO GASTRIC CANCER CELLS

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Background: Host-pathogen interaction is of increasing interest in the study of infections. In the present study, we use custom-made microfluidic devices to examine chemotaxis of *Helicobacter pylori* to gastric epithelial cells.
Methods: Microfluidic devices were prepared using polydimethylsiloxane (PDMS) bound to a glass cover slip, forming three channels. The channels were then coated with Poly-D-Lysine. AGS cells and bacteria were seeded in two separate channels, while the third was filled with culture medium (nutrient). The setup therefore provides the pathogen the choice of interacting with AGS cells or nutrient. The devices were sacrificed by paraformaldehyde fixation at time intervals. The bacteria were first treated with an Anti-*H. pylori* antibody for four hours followed by a Cy3-tagged secondary antibody for another four hours. Cell nuclei9 were stained using DAPI. The devices were then analysed using Confocal Laser Scanning Microscopy (CLSM).
Results and Conclusions: The bacteria were stained red and appeared as bright red dots of high intensity at the interface between the collagen and the channel with the AGS cells. However, the intensity at the interface between the collagen and the culture medium channel was qualitatively low. It shows that *H. pylori* has a predilection for AGS cells than the nutrient. This preliminary study demonstrates that microfluidic device is a potential useful tool for evaluation of chemotaxis of pathogens. Experiments on cell-bacteria interaction and bacteria-bacteria communication are in progress.

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PRODUCTION OF MONOCLONAL ANTIBODY AGAINST ALKYL HYDROPEROXIDE REDUCTASE (AHPC) OF *HELICOBACTER PYLORI*

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Introduction: Stool-antigen detection kits for diagnosis of *H. pylori* infection have been widely used because of their full non-invasive nature. Because *H. pylori*

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