

**Identification of an immunogenic 18 kDa protein  
of *Helicobacter pylori* using alkaline  
phosphatase gene fusions**

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## Abstract

Secreted or surface-associated proteins play an important role in the immunopathogenesis of *Helicobacter pylori* infection. The aim of this study was to identify, using a genetic approach, *H. pylori* exported proteins and assess their role in the host immune response to infection. As part of this work, an *H. pylori* expression library was constructed and screened with a monoclonal antibody raised to a component of outer membrane vesicles from *H. pylori*, identified and characterised in a separate study. The screening strategy identified a locus of the genome containing two genes encoding exported proteins. Subsequent expression studies identified the gene product detected by the antibody as Lpp20, which encodes a well characterised lipoprotein from *H. pylori*. In addition, the use of alkaline phosphatase (AP) gene fusion methodology enabled the identification of a large number of other *H. pylori* exported proteins. Immunoscreening of a selection of enzymatically active *H. pylori* AP fusion proteins was carried out by Western blot analysis with patient sera and lymphocyte proliferation assays using peripheral blood mononuclear cells from *H. pylori* infected individuals. These assays identified a novel *H. pylori* exported antigen which was recognised by antibody derived from *H. pylori* infected individuals. Southern blot analysis revealed that the gene encoding the protein was absent in other *Helicobacter* species tested and sequence analysis of the gene demonstrated that it is highly conserved among *H. pylori* isolates. In order to obtain pure recombinant protein, the gene encoding the protein was cloned and expressed as a Beta-galactosidase ( $\beta$ -gal) fusion in *Escherichia coli* and the protein purified by affinity chromatography. The size of the recombinant protein released (18 kDa) was consistent with the calculated molecular mass of the polypeptide deduced from the DNA sequence. In Western blot assays, the purified protein was recognised by 71% of sera taken from patients infected with *H. pylori*, but by only 16% of sera taken from patients with unrelated or with no gastrointestinal disease. These results indicated that the 18 kDa protein from *H. pylori* was immunogenic and expressed *in vivo*. In other experiments, it was found that oral administration of this antigen did not protect mice against *H. pylori* colonisation following challenge with *H. pylori*.

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