Oral immunization with recombinant *Lactobacillus acidophilus* expressing the adhesin hp0410 of *Helicobacter pylori* induces mucosal and systemic immune responses

Running title: An oral vaccine for Helicobacter pylori

Fan Hongying*, Wu Xianbo*, Yu Fanga, Bai Yangb, Long Beiguo§

The School of Public Health and Tropical Medicine and The Southern Hospital of Southern Medical University, Avenue North No. 1838 Street, Guangdong, Guangzhou 510515, People’s Republic of China

*These authors contributed equally to this work

§Corresponding author. Tel.: +86 2062789123

E-mail address: gzfhy@smu.edu.cn
Abstract Helicobacter pylori infection is relatively common worldwide and is closely related to gastric mucosa-associated lymphoid tissue (MALT) lymphoma as well as chronic gastritis and stomach ulcers. A safe and effective mean to prevent H. pylori infection is therefore urgently needed. Given that developing an effective vaccine against H. pylori is one of the best alternatives, H. pylori adhesin Hp0410 was expressed in the food-grade bacterium Lactobacillus acidophilus. The recombinant live bacterial vaccine was then used to orally vaccinate mice and the immune protective effect of Hp0410-producing strains was investigated. H. pylori colonization in the stomach of mice immunized with the recombinant Lactobacillus acidophilus was significantly reduced compared with control groups. Furthermore, mucosal sIgA antibodies were elicited in the mucosal tissue of mice immunized with the recombinant bacteria, and specific anti-Hp0410 IgG responses were also detected in mouse serum. At the same time, there was a significant increase in the level of protection against gastric Helicobacter infection following attack by the H. pylori strain SS1. Our results collectively indicate that adhesin Hp0410 is a promising candidate vaccine antigen and recombinant Lactobacillus acidophilus expressing Hp0410 is likely to constitute an effective, low-cost live bacterial vaccine against H. pylori.
Key words: Helicobacter pylori vaccine; recombinant Lactobacillus acidophilus; Hz0410.
*Helicobacter pylori* is a Gram-negative bacterium found in different areas of the stomach, especially in the antrum. Approximately 50% of the world’s population are infected by the pathogen and up to 64% of China’s population (1, 2). *H. pylori* infections typically commence during childhood and last for life. The infection is transmitted within the family in childhood (3, 4), likely by fecal-oral or oral-oral transmission (5). Moreover, infection is strongly associated with the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer (6, 7). In 1994, the World Health Organization classified *Helicobacter pylori* as class I human carcinogen (8). Prevention of *H. pylori* infection is therefore highly topical and the subject of intense debate for many researchers.

At present, the treatment for *H. pylori* infection is a triple therapy consisting of two antibiotics and a proton pump inhibitor. *H. pylori* can be successfully eradicated in most situations. However, there are some problems with this treatment, such as patient compliance, antibiotic resistance and high cost, resulting in relapse after a short period in about 15% to 30% of patients (9-12). There is some evidence of *H. pylori* presence in the oral cavity that a recent meta-analysis related to gastric colonization and possible reinfection (13, 14). *H. pylori* presence in tonsils is controversial (15, 16, 17), if confirmed, it could help further understanding of *H. pylori* transmission and reinfection. Since antibiotic therapy is not ideal and *H. pylori* eradication therapy is designed for treatment after infection rather than prevention, vaccination against *H. pylori* would be the most effective approach.

A protective antigen is an essential part of vaccine construction. To date, many
protein molecules expressed by *H. pylori* have been identified as immunogenic, including urease (UreB), cytotoxin-associated antigen (CagA), neutrophil-activating protein A (NapA), *H. pylori* adhesin A (HpaA), vacuolating toxin A (VacA), catalase and outer membrane protein (Omp) (18, 19). Among these protein antigens, *H. pylori* adhesion (HpaA) is a flagellar sheath protein of *H. pylori*, and also is one of the bacterium main adherence factors (20). HpaA can bind to the many kinds of surface receptors of gastric epithelial cells, and then do further damage to the gastric mucosa (21). Previous research showed that HpaA gene is harbored in all *H. pylori* strains and considerably conserved for its nucleotide and amino acid sequences (22). Furthermore, there are genomic studies which show no significant sequence homologies of HpaA with other known proteins (23), and antibody against HpaA almost could be found in all *H. pylori*-infected patients’ sera (24). Taken together, adhesion HpaA is considered as a potential vaccine antigen. *H. pylori* adhesin 0410 (Hp0410) (accession number NC_000915.1) is a gene homologue of the *H. pylori* adhesion hpaA family, and is highly conserved, sharing 94% to 95% of its genome sequence with standard *H. pylori* strains, such as J99 and ATCC26695 (25). It would be a rational choice for a candidate antigen for an *H. pylori* vaccine (26, 27).

Secondly, it is also important to choose an antigen delivery system. Recently, lactic acid bacteria (LAB) have been widely studied as mucosal surface vaccine delivery vehicles (28). This system has an advantage over traditional vaccines, in that LAB can colonize the respiratory tract, gastrointestinal tract, urinary tract and other mucosal epithelial cells and induce a strong mucosal immune response (29, 30). So far, several
bacterial and viral antigens have been produced in *Lactococcus lactis* (31-33), and immunization with these strains elicits immune responses specific to heterologous antigens (34-37). These reports indicate that recombinant *Lactobacillus acidophilus* used as oral vaccination strains can prevent gastric infection and allow direct contact between the antigen and the immune system. In addition, if *Lactobacillus acidophilus* is chosen as a vaccine vector, there is no need to culture pathogens and purify antigenic components (38). However, no effective and safe vaccine against *H. pylori* is currently available for humans.

In this study, we have successfully constructed a recombinant *Lactobacillus acidophilus* GIM 1.208/hp0410 strain, which expresses the foreign *H. pylori* protein, adhesin Hp0410. In order to explore a safe and convenient oral mucosal vaccine candidate against *H. pylori*, we measured and evaluated the effect on immunity in a mouse model by oral administration of the pathogenic *H. pylori* SS1 strains.

**Materials and methods**

**Bacterial strains, vector plasmid, and growth conditions**

*Escherichia coli-Lactobacillus* shuttle vector pMG36e plasmid was provided by Dr. J. Kok, University of Groningen, the Netherlands, University of Groningen, the Netherlands. *L. acidophilus* GIM 1.208 was grown in MRS medium supplemented with glucose at 30°C without agitation. The plasmid pMD19-T containing the Hp0410 gene of the *H. pylori*, was constructed by our laboratory. pMG36e-based plasmids were maintained in *L. acidophilus* on glucose-enriched MRS agar plates supplemented
with 1.25 µg/mL erythromycin. *Escherichia coli* was grown at 37°C in Luria-Bertani medium with 250 µg/mL erythromycin. The *H. pylori* strain was cultured on Columbia agar supplemented with 8% (v/v) defibrinated sheep blood, Dent selective supplement (Oxoid) in a microaerophilic atmosphere (85% N₂, 10% CO₂, 5% O₂) at 37°C for 3-5 days.

**Construction of recombinant plasmid pMG36e-hp0410**

To insert the hp0410 gene into the XbaI/HindIII site of the pMG36E plasmid, a PCR was performed using pMD19-T-S-hp0410 templates. The Hp0410 gene was amplified using the following primers (XbaI/HindIII sites are underlined): 5’-GAGAAAGCTTCTACTTTCTGTTTTTTCCATT-3’ and reverse primer; 5’-AGCGTCTAGAGATGAAAAAAGGTAGTTGTGCG-3’. The amplified Hp0410 (750 bp) was cloned into the XbaI/HindIII site of the pMG36e. The recombinant plasmid pMG36eH-hp0410, which was sequenced to ensure that the Hp0410 open reading frame was correct. Additionally, the recombinant plasmid pMG36e-hp0410 was digested with the restricted enzyme SalI/HindIII for verification (Figure 1). Finally, the plasmids pMG36e and pMG36e-hp0410 were transformed into *L. acidophilus* GIM 1.208 by electroporation as previously described (39).

**Cell lysate and Western blot analysis**

Bacterial cell lysates were prepared as described elsewhere (40). Briefly, the bacterial cells of *L. acidophilus* were suspended in phosphate-buffered saline (PBS), disrupted by sonication, and then incubated at 4°C for 30 min. After centrifugation at 16,000 g for 20 min, the supernatant and the pellet were obtained as the soluble
fraction and the inclusion body fraction of the cell lysates, respectively. Equivalent
amounts of cell extracts (15 μg) were electrophoresed on a 4-12% gradient sodium
dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and
electrotransferred to an Immobilon-P membrane (Millipore Corporation, Bedford,
MA). The membrane was probed with a primary antibody, and then with a
horseradish peroxidase-linked secondary antibody. Detection of each protein was
visualized using the ECL Western blotting detection system, according to the
manufacturer’s instructions.

Stability of pMG36e-hp0410 in recombinant L. acidophilus

Frozen aliquots of rL. acidophilus GIM 1.208 vaccine preparations were diluted
(1/100) in MRS medium and grown to an optical density at 600 nm of 0.8. A 1/100
dilution of this preliminary culture was grown a further 20 times under the same
conditions. Both cultures were used as inoculants at suitable dilutions for MRS agar
plates with or without erythromycin (25 μg/mL). The stability of pMG36e/hp0410 in
rL. acidophilus GIM 1.208 was evaluated by comparing the numbers of CFU
recovered on erythromycin-containing plates and non-erythromycin-containing plates.

Immunization

Eighty-four SPF, 6- to 8-week-old female C57BL/6 mice (weighing 18 to 22 g)
were obtained from the Southern Medical University Laboratory Animal Center
(Guangzhou, China) and housed in a pathogen-free environment. All experiments
involving animals were approved by the Institutional Animal Care and Use
Committee of the Southern Medical University. Animals were randomly divided into
three groups (n = 28 for each) and were orally immunized with approximately $1 \times 10^9$ CFU of recombinant strain *L. acidophilus* GIM 1.208/pMG36e-hp0410 or strain GIM 1.208/pMG36e or phosphate-buffered saline (PBS) as a negative control. All mice were immunized on days 1, 2, 3, 7, 10, 14 and 20 respectively, seven times in total. No boosting immunization was performed.

One week after the last immunization, 10 randomly selected mice from each group were collected blood from orbital vein and their sera were harvested for evaluation. At 10, 21 and 35 days after the last immunization, six mice from each group were killed by cervical dislocation and their gastrointestinal fluid was harvested. The intestinal tissue was then incised along the longitudinal axis of the intestinal tract and washed with PBS containing protease inhibitors. The resulting fluid was centrifuged at 3000g for 10 min and the supernatant was collected and stored at -20°C until use.

**Enzyme-linked immunosorbent assay (ELISA) for the detection of Hp0410-specific serum antibody**

ELISA plates were coated overnight at 4°C with 2 μg/mL recombinant purified Hp0410. Threefold serial dilutions of each serum sample starting from 1:100 were applied to the plates and incubated for 2 h at 37°C. Serum IgG and IgA were detected by peroxidase-labeled goat anti-mouse IgG (1:1000, 100μl) (Amersham Biosciences, American) and goat anti-mouse IgA (1:5000, 100μl) (Sigma, American). The reaction was stopped by the addition of 2 M H$_2$SO$_4$, and the absorbance was measured at 450 nm using an ELISA reader (Bio-TekEL 9800, USA).
ELISA for the detection of Hp0410-specific mucosal IgA

Hp0410-specific and total IgA in the gastric mucosa were quantified by ELISA. Portions of each plate were coated with an anti-mouse IgA monoclonal antibody (Sigma) and Hp0410, respectively. In addition to the diluted samples, a dilution series of purified IgA was applied to each plate to provide a standard curve. After incubation with peroxidase-labeled anti-mouse IgA (1:5000, 100 μl), the concentrations of Hp0410-specific and total IgA were determined using the standard curve. In order to address the possibility that increased IgA concentrations were the result of a polyclonal nonspecific response to mucosal stimulation, responses were expressed as the ratio of specific to total IgA.

Survival time of rLactobacillus acidophilus in mice

Twenty SPF-grade, six-week-old female C57BL/6 mice were divided into two groups (n=10 for each) and were orally immunized with 1 × 10^9 CFU of the recombinant strain L. acidophilus GIM 1.208/pMG36e-hp0410 or strain GIM 1.208/pMG36e as a negative control. Then, the two group mice feces were collected at the same time each day. The Hp0410 gene and pMG36e plasmid were amplified from fecal genomic DNA using a TIANamp Bacteria DNA Kit (Qiagen, Germany) to detect the presence of rL. acidophilus GIM 1.208/pMG36e-hp0410 or GIM 1.208/pMG36e in mice. The pMG36e plasmid primers used were 5’- pMG36e (5’-GAGTTATGAATTCTCTGA-3’) and 3’- pMG36e (5’-CGTTGGCGATTGAAAACC-3’), and the Hp 0410 gene primers according to the Construction of recombinant plasmid pMG36e-hp0410 section.
Immune protective effect of recombinant \textit{L.acidophilus} expressing adhesin Hp0410

\textit{H. pylori} Sydney strain 1 (SS1) \( (41) \) were recovered from storage at -80°C and resuspended in PBS to yield a concentration of \( 1 \times 10^9 \) CFU/mL. Twenty-six vaccinated mice (10 immunized with recombinant \textit{L.acidophilus} GIM 1.208/pMG36e-hp0410, 10 immunized with strain GIM 1.208/pMG36e, and six given PBS as a control) were administered (via the orogastric route, using an oral sonde) into the stomach with 0.2 mL \((2 \times 10^8)\) \textit{H. pylori} SS1. Animals were challenged with an equal dose of \textit{H. pylori} SS1 four times consecutively at 24-h intervals. Food and water were given 4 h after the challenge. Four weeks after receiving the challenge, mice were sacrificed by cervical dislocation. Their stomachs were washed twice in sterile 0.9% NaCl, and the gastric antrum from each stomach was assessed for \textit{H. pylori} colonization by the detection of urease activity, the catalase test, quantitative culture and histological analyses of bacterial cultures. Firstly, 50 \( \mu \)L of the succus gastricus was placed in 500 \( \mu \)L of urease reagent. Urease positivity was determined by an increase in pH and indicated by a color change from yellow to red within 24 h at 37°C. Strongly positive samples became red within 2 h and negative samples showed no change within 10 h. Secondly, for the catalase test, 3% \( \text{H}_2\text{O}_2 \) solution was dropped onto a plate containing gastric tissue washing fluid. The formation of bubbles indicated a positive result. For \textit{H. pylori} SS1 quantitative culturing, the stomach samples were weighed, homogenized in thioglycolate medium, serially diluted in PBS and plated on to Columbia agar plates with antibiotics. Bacterial counts were expressed as CFU per gram of gastric tissue. To histologically assess bacterial...
colonization, 5-µm-thick sections of the gastric antrum were cut and stained with hematoxylin and eosin (H&E). The degree of inflammation was analyzed on blinded slides by two pathologists, by assessing lymphocyte and monocyte density and grading the depth of the invasion of gastritis from 0 to 3 (0 denoting no infiltration, 1 mild, 2 moderate, and 3 marked). Scores were expressed according to the updated Sydney system (42).

Statistical analysis

Statistical analysis was carried out using SPSS 13.0 computer software, and data were expressed as the mean ± standard deviation (SD). Differences among three groups were determined by one-way analysis of variance using the Tukey post-hoc test. Differences in rates were analyzed by the chi-square test. P values of less than 0.05 were considered significant.

Results

Expression of adhesin Hp0410 by rLactobacillus acidophilus

To express the Hp0410 protein of H.pylori in the L.acidophilus delivery plasmid, the Hp0410 gene was inserted into the E.coli-Lactobacillus shuttle plasmid pMG36e, which was designated as pMG36e-Hp0410. The recombinant plasmid was constructed and confirmed by restriction enzyme digestion with XbaI/HindIII (Figure 1). Two plasmids (the recombinant plasmid pMG36e/hp0410 and the control plasmid pMG36e) were introduced into L.acidophilus GIM 1.208, resulting in strains GIM 1.208/pMG36e-hp0410 and GIM 1.208/pMG36e. Proteins were extracted and assayed by Western blot after SDS-PAGE (Fig 2a and 2b), using polyclonal antiserum to
Hp0410. The results indicate that rHp0410 was produced in the supernatant of GIM 1.208/pMG36e-hp0410, and was detected as a 34-kDa band on Western blot. In contrast, no band corresponding to the Hp0410 protein was detected in the supernatant of GIM 1.208 carrying an empty plasmid (Fig. 2b).

Stability of pMG36e-hp0410 in rLactobacillus acidophilus

The stability of pMG36e-hp0410 in recombinant L. acidophilus was 97% with erythromycin, and 90% without erythromycin (data not shown). We have therefore constructed a stable rL. acidophilus vaccine containing pMG36e expression vectors.

Serum anti-Hp0410 antibody responses after mucosal immunization

Groups of mice were immunized orally with rL. acidophilus GIM 1.208 expressing Hp0410 protein in the culture supernatant. Control mice were vaccinated in the same way with pMG36e strains of L. acidophilus GIM 1.208. One week after the final vaccination with GIM 1.208/pMG36e-hp0410, the level of Hp0410-specific IgG in the serum was significantly greater than in controls (P < 0.001), while Hp0410-specific IgA levels in the serum were lower and did not differ significantly from those observed with the vector control strain (pMG36e) or unvaccinated mice (Fig. 3).

Mucosal antibody responses

Significantly elevated Hp0410-specific IgA responses could be detected in fresh intestinal mucus from mice immunized orally with GIM 1.208/pMG36e-hp0410, but not in those mice given the control strain (Fig. 4). On day 21 after oral inoculation, the mean ratios of specific to total IgA detected in the group given pMG36e-hp0410
were about three times those in unvaccinated controls ($P < 0.01$). On days 10 and 35, the ratios in the pMG36e-hp0410 group were also significantly higher than in the control groups ($P < 0.05$). No significant differences could be detected between mice vaccinated with the pMG36e control strain and the unvaccinated control group at any of the time points.

**Survival time of r*Lactobacillus acidophilus* in mice**

As shown in Table 1, r*L. acidophilus* GIM 1.208/pMG36e-hp0410 were detected in all mice on the first and the second day after immunization through amplifying the pMG36e plasmid and the Hp0410 gene, GIM 1.208/pMG36e were detected pMG36e plasmid too. On the third day, the presence of recombinant bacteria could still be detected in the intestines of 70–80% of the mice; however, on the fourth day, no recombinant bacteria were detectable in any of the mice. These results indicate that r*L. acidophilus* can survive for at least 72 hours in mice, and suggest that *L. acidophilus* may be a suitable LAB-based delivery vehicle for human vaccines.

**Immune protective effect of recombinant Lactobacillus acidophilus**

Three weeks after immunization, mice were challenged with $2 \times 10^8$ *H. pylori* SS1. Four weeks later, all of the 10 stomach biopsies from the C57BL/6 mice immunized with live vaccine expressing Hp0410 were negative by urease assay and catalase test, but all were positive as assessed by colony counts. These 10 immunized mice had a range of *H. pylori* colonization loads from $10^4$ to $10^5$ bacteria per gram of stomach tissue. However, a significant difference in the level of protection was seen between the immunized mice and the control groups, which had higher bacterial loads...
Similarly, examination of histological sections of gastric tissue showed that (Fig. 5), in comparison with the control group, the histopathological changes in the *L. acidophilus* GIM 1.208/pMG36e-hp0410 group were relatively minor, being limited to the lamina propria without reaching the serosa, and leaving the glands largely intact. This difference was quantified and shown to be statistically significant, and the numbers of inflammatory cells and their exudates were also significantly reduced (Table 2). There was no significant difference in histology scores between the two LAB treated groups.

**Discussion**

*H. pylori* is a microorganism that has been linked to diseases such as gastritis and peptic ulcers. It has a high incidence of infection especially in developing countries, and antibiotic treatment is not only expensive but also likely to induce antibiotic resistance. Taking these issues into account, vaccination appears to be a promising intervention. To reduce the risk of *H. pylori* infection, it is important to induce a protective immune response in the mucosal surfaces.

In this study, our main objective was to develop an effective vaccine against *H. pylori* using Hp0410 as the antigen and *L. acidophilus* as the antigen delivery system. Our study showed that mucosal immune responses in the intestine could be elicited by oral vaccination with a *L. acidophilus* vaccine. We tested the effect of immunization with the recombinant strain *L. acidophilus* on protective immunity to *H. pylori* in C57BL/6 mice. Following oral immunization, although all animals were positive by culture (Table 2), the quantity of *H. pylori* in the mice immunized with...
pMG36e-hp0410 was significantly less than in control groups. Furthermore, all 10 of the mice in the experimental group were negative by the catalase and urease tests. Similar results have been obtained by immunization with whole bacterial sonicates and cholera toxin (43) or urease subunit B (44). Meanwhile, examination of histological sections of gastric tissue showed that, the *L.acidophilus* GIM 1.208/pMG36e-hp0410 group density of *H.pylori* in gastric mucosa was significantly lower than that of the control groups. These findings all suggest that *L.acidophilus* can significantly ameliorate the inflammatory response caused by *H. pylori* infection in immunized mice. However, no significant difference in gastric tissue lesions was found between the *L.acidophilus* GIM 1.208/pMG36e-hp0410 group and the GIM 1.208/pMG36e group. The reason is likely to be that *L.acidophilus* itself may also be able to improve the degree of inflammation caused by *H. pylori* infection. Possible mechanisms for this are as follows: (1) *L.acidophilus* may adhere to and colonize the gastric mucosa in advance, and thus partially inhibit adhesion and colonization by *H. pylori* (45); (2) *L.acidophilus* itself as a LAB may also stimulate the gastric mucosa to produce nonspecific sIgA antibodies, thereby enhancing the mucosal immune response (46-48).

Compared with traditional vaccine vectors, LAB have several advantages, including being safer and non-toxic, being able to adhere to and colonize the respiratory tract, digestive tract and other mucosal epithelial cell layers, as well as functioning to express and secrete heterologous proteins, and to induce a strong mucosal immune response (49, 50). So far, several exogenous proteins have been
expressed in LAB. For example, the expression of ScFv, a fragment of the anti-streptococcal adhesion antibody single chain variable region in \textit{Lactobacillus} ATCC393, has been reported by Carina et al. (51). Gu Q et al. (44) showed that the urease UreB of \textit{H. pylori} was expressed in \textit{L. lactis} MG1363. Recombinant lactic acid bacteria, used to immunize mice through the oral and nasal routes, can induce humoral and cellular immune responses in the gastrointestinal mucosa, resulting in the secretion of sIgA antibodies (33,44, 52) which can protect the mice from a related infection. \textit{L. acidophilus}, used in this study as an oral vaccine antigen delivery vector, is safe, acid-resistant, bile-tolerant, and can also survive in the gastrointestinal tract for more than 72 hours. Compared with other lactic acid bacteria, a more prolonged gastrointestinal mucosal immune response can be stimulated when using \textit{L. acidophilus} (53), resulting in the production of specific sIgA antibodies, which are necessary to achieve the goal of preventing \textit{H. pylori} infection (44). Oral administration is also more convenient as a safe and effective vaccination pathway for some special groups, especially children.

Preliminary studies have used Bioinformatics Analysis and Phage Display to obtain the three B-cell epitopes of Hp0410, which all show a high degree of antigenicity (54). By using the purified Hp0410 to screen serum from gastric ulcer patients and normal healthy controls, they showed that the body produces high titers of anti-Hp0410 antibody with positive rates of 94.1% and 74.4%, respectively, consistent with the \textit{H. pylori} detection rates in gastric ulcer patients and normal healthy controls in vivo (55, 56). The Hp0410 monoclonal antibody specifically prevented the adhesion of \textit{H.
pylori to the stomach mucosa of gastric cancer patients as well as gastritis patients (25). Hp0410 does not participate in the inflammatory response, as its function is only to mediate adhesion (22).

In summary, recombinant *L. acidophilus* GIM 1.208 expressing the adhesin Hp0410 was successfully constructed, and the *L. acidophilus* GIM 1.208 induced the secretion of high levels of mucosal sIgA antibody. This vaccine was effective in giving some immune protection against gastric *H. pylori* infection in mice. However, no safe vaccine against *H. pylori* is currently available for humans, so further development of existing food-grade expression systems is necessary.

**Acknowledgments**

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Figure legends

Fig. 1 Identification of the plasmid digested with XbaI/HindIII.
1, The recombinant pMG36e-Hp0410 plasmid; 2, The recombinant pMG36e-Hp0410 plasmid digested with XbaI and HindIII, 750-bp Hp0410 and the bone of the plasmid; M, DNA Marker DL2k.

Fig. 2 The Hp0410 protein was detected by SDS-PAGE and western blot.
(a) SDS-PAGE of rL. acidophilus lysate and supernatant. M, marker; 1,4,7: rL. acidophilus GIM 1.208 lysate with pMG36e-hp0410; 2,3,6: rL. acidophilus GIM 1.208 lysate with pMG36e; 5,8: rL. acidophilus GIM 1.208 supernatant with pMG36e-hp0410.
(b) Western blot of Hp0410 protein expressed by rL. acidophilus, a 34-kDa band representing Hp0410 protein is visible in lane 2 but not in lane 1. M, marker; 1, rL. acidophilus GIM 1.208 containing the pMG36e; 2, rL. acidophilus GIM 1.208 containing the pMG36e-hp0410.

Fig. 3 Serum anti-Hp0410 IgG and IgA levels elicited by recombinant L. acidophilus.
Groups of six mice were immunized orally with rL. acidophilus expressing Hp0410 (pMG36e-hp0410) or a control strain (pMG36e). On day 35 after initial treatment, blood samples were collected and Hp0410-specific serum IgG (dark gray) and IgA (white) titers were measured by ELISA as OD values at 450 nm. Significantly higher levels of Hp0410-specific IgG but not IgA were seen in the pMG36e-hp0410 group but not in control groups. Bars, mean ratios; error bars, SD.

*P < 0.001. OD: Optical density.
Fig. 4 Comparison of the ratio of specific to total IgA levels in the intestinal mucus of mice immunized orally with *L. acidophilus* expressing Hp0410 (pMG36e-hp0410) or the control strain (pMG36e). Intestinal mucus was collected from groups of six mice on days 10 (dark gray), 21 (white) and 35 (light gray) after initial treatment. The samples were tested by ELISA for Hp0410-specific and total IgA, and the results expressed as the ratio of OD values at 450 nm. The ratios were significantly higher in the pMG36e-hp0410 group than in the pMG36e group or unvaccinated controls at all time points but were greatest on day 21. Bars, mean ratios; error bars, SD.

* P < 0.05. OD: Optical density.

Fig. 5 Gastric histology in the mice post-challenge with *H. pylori*. (HE stain 100×)

A, Gastric tissue from control mice vaccinated with PBS showing severe inflammatory infiltrates in the mucosa and submucosa; B, A histologically gastric mucosa immunized with *rL. acidophilus* GIM 1.208/ pMG36e; C, A histologically gastric mucosa immunized with *rL. acidophilus* GIM 1.208/ pMG36e-hp0410; D, A histologically normal gastric mucosa.
Table 1 Survival time of *rL. acidophilus* GIM 1.208/pMG36e-hp0410 in the mouse gastrointestinal tract after immunization

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Origin primers</th>
<th>Days after immunization*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td><em>rL. acidophilus</em></td>
<td>Hp0410 gene</td>
<td>10/10</td>
</tr>
<tr>
<td>GIM 1.208/pMG36e-hp0410</td>
<td>pMG36e plasmid</td>
<td>10/10</td>
</tr>
<tr>
<td><em>rL. acidophilus</em></td>
<td>Hp0410 gene</td>
<td>0/10</td>
</tr>
<tr>
<td>GIM 1.208/pMG36e</td>
<td>pMG36e plasmid</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* Results are expressed as number of mice out of the total of 10 in which the Hp0410 gene and pMG36e plasmid could be detected in fecal DNA using a TIANamp Bacteria DNA Kit.
Table 2 Protection of mice from *Helicobacter pylori* SS1 infection following immunization with live r*L. acidophilus*

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Number of mice colonized by <em>H. pylori</em> (n = 10)</th>
<th>Hp counts of mouse gastric mucosa. (mean±SD)</th>
<th>Histology (Chronic gastric inflammation score) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None <em>b</em></td>
<td>10/10</td>
<td>6.02±0.01</td>
<td>2.22±0.21</td>
</tr>
<tr>
<td>pMG36e <em>c</em></td>
<td>10/10</td>
<td>6.28±0.60</td>
<td>1.49±0.64</td>
</tr>
<tr>
<td>pMG36e-hp0410 <em>d</em></td>
<td>0/10</td>
<td>4.55±0.17</td>
<td>1.21±0.17</td>
</tr>
</tbody>
</table>

*a* Histopathology of infected mice was assessed from hematoxylin and eosin-stained gastric mucosal sections. Gastric inflammation was graded on a scale of 0-3 for each group.

*b* Orally immunized with PBS.

*c* Orally immunized with r*Lactobacillus acidophilus* (pMG36e).

*d* Orally immunized with r*Lactobacillus acidophilus* (pMG36e-hp0410).

▲ *P* < 0.05 vs. Control group; * *P* < 0.05 vs. pMG36e group