Original paper

In Vitro and In Vivo Anti-Helicobacter pylori Activity of Probiotics Isolated from Mongolian Dairy Products

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Helicobacter (H.) pylori is known to be a bacterial risk factor for gastric cancer. In this study, 16 strains of lactic acid bacteria (LAB) isolated from Mongolian dairy products were screened for potential probiotic activity against H. pylori strain no. 130 in vitro. Lactobacillus (L.) paracasei 06TCa19 and L. plantarum 07MR044 were identified as LAB strains with anti-H. pylori activities. Compared to strain 07MR044, strain 06TCa19 was more potent in reducing H. pylori counts in co-culture. The ability of strain 06TCa19 to inhibit the growth of H. pylori was attributable to its rapid and excessive generation of lactic acid. Moreover, oral administration of strain 06TCa19 was found to significantly reduce the number of colonizing H. pylori in the stomach of H. pylori strain no. 130-infected mice. Thus, strain 06TCa19 is potentially effective against H. pylori infection.

Keywords: anti-Helicobacter pylori, probiotics, Lactobacillus paracasei, lactic acid, mongolian dairy products

Introduction

Helicobacter (H.) pylori, which inhabits more than 50% of the world’s population, is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the mucosa of the human gastric epithelium (de Vries et al., 2008). H. pylori infection is the major factor inducing chronic gastritis, peptic ulcer, and even gastric cancer in humans (Uemura et al., 2001). Although eradication therapies such as the administration of antibiotics are presently available, issues such as side effects of chemotherapy and increased antibiotic resistance of H. pylori have been reported (Cheng et al., 2012; Gerrits et al., 2006).

Therefore, prevention of H. pylori infection or eliminating its colonization in the stomach is critical. In addition, development of alternative medicinal products toward preventing the infection and proliferation of H. pylori, such as functional foods and natural compounds, is required. The anti-H. pylori activities of some natural products and foods have already been reported (Kudo et al., 2011; Lee et al., 2009; Pastene et al., 2010).

Lactic acid bacteria (LAB) have been used worldwide in the preparation of several kinds of food, especially dairy products. Some LAB, termed probiotics, are living microorganisms that
confer health benefits on the host when administered in adequate amounts (i). Several probiotic LAB have been shown to inhibit *H. pylori* growth in vitro (Hamilton-Miller, 2003). Some probiotics are reportedly effective in both suppressing *H. pylori* colonization and reducing gastric mucosal inflammation in humans (Boonyaritichaikij et al., 2009; Fujimura et al., 2006; Patel et al., 2014; Tamura et al., 2006). Thus, the identification of LAB strains possessing anti-*H. pylori* activity appears promising in developing effective foods, such as yogurt, capable of preventing *H. pylori* infection and proliferation.

We previously isolated LAB strains from traditional Mongolian dairy products (Takeda et al., 2011). Several of these strains exhibited tolerance to artificial gastric acids and adhered to human intestinal epithelial cells in vitro, hence these LAB strains were considered probiotic LAB candidates (Takeda et al., 2011). In the present study, we aimed to investigate the anti-*H. pylori* activities of these probiotic LAB candidates. To the best of our knowledge, this is the first work to investigate the anti-*H. pylori* activity of LAB from Mongolian dairy products, and we identified an LAB strain that exhibited high anti-*H. pylori* activity in vitro and in vivo.

### Materials and Methods

**Bacterial strains and culture conditions** The LAB strains used in this study were isolated from Mongolian dairy products, and their tolerance to gastric acid in vitro was confirmed previously (Takeda et al., 2011). The species and origins of the LAB strains are shown in Table 1. The LAB were cultured in de Man, Rogosa, and Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37°C for 18 h. *H. pylori* strain no. 130 (cag A+, vacuolating toxin+) used in this study was previously isolated from a gastric biopsy specimen (Kabir et al., 1997). *H. pylori* was cultured in brain heart infusion (BHI) medium (Difco Laboratories, MI, USA) containing 5% inactivated horse serum (HS) under microaerobic conditions, i.e., in an anaerobic jar with Anaero Pack MicroAero (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan), at 37°C for 48 h.

**Estimation of growth inhibition of *H. pylori* co-cultured with LAB** *H. pylori* and LAB strains were grown in BHI broth with 5% HS and in MRS broth, respectively. *H. pylori* and LAB cultures were centrifuged at 10,000 × g for 5 min and washed twice with sterilized phosphate-buffered saline (PBS). Then, the cells were suspended in the same volume of PBS as that in BHI broth with 5% HS or MRS broth. *H. pylori* and LAB suspensions were inoculated into fresh BHI broth with 5% HS (1% v/v). In screening for *H. pylori* growth inhibition by LAB, the initial cell density of each tested LAB strain in co-culture was 5.9–6.4 log₁₀ colony-forming units (CFU)/mL, as determined by colony counts using MRS agar. The initial concentration of *H. pylori* used in co-culture was adjusted to 6.0 log₁₀ CFU/mL in reference to a predetermined plot of CFU versus optical density at 660 nm of the *H. pylori* suspension prepared in PBS. Similarly, the cell densities of LAB were also adjusted in a similar manner, when necessary. The co-culture of LAB and *H. pylori* was performed at 37°C for 48 h under microaerobic conditions. As a control, PBS was used instead of LAB. After incubation, the co-culture was serially diluted with PBS, and the diluent was surface spread onto modified Skirrow’s agar for selective culturing of *H. pylori*. Modified Skirrow’s agar was prepared from BHI agar with 5% HS by supplementing the medium with 25 μg/mL tetrazolium violet (Sigma-Aldrich, MO, USA), 5 μg/mL trimethoprim (Wako Pure Chemical, Osaka, Japan), 2.5 U/mL polymyxin B sulfate (Sigma-Aldrich), 10 μg/mL vancomycin hydrochloride (Wako Pure Chemical), and 5 μg/mL bacitracin (Nacalai Tesque, Inc., Kyoto, Japan). Skirrow’s agar plates were incubated at 37°C for 4 d under microaerobic conditions. MRS agar plates were incubated at 37°C for 3 d, and subsequently LAB counts were determined.

**Preparation of LAB culture fractions and estimation of anti-*H. pylori* activity** The anti-*H. pylori* activity of LAB culture fractions was investigated by a modified well diffusion assay, as reported by Simova et al. (2009). Briefly, LAB were cultured in BHI broth with 5% HS, the broth was centrifuged at 10,000 × g for 5 min, and the LAB cells were collected separately from the supernatant. The harvested LAB cells were washed with PBS. Then, a portion of the cell suspension was heated at 100°C for 15 min. The pH of the separated supernatant was adjusted to 6.5 with 1 mol/L sodium hydroxide. A portion of this neutralized supernatant was treated with 10 mmol/L of phosphate buffer (pH 6.5) containing 1 mg/mL catalase (Sigma-Aldrich). The LAB cells, heat-treated LAB cells, and untreated and treated supernatants were separately lyophilized. Then, each lyophilizate was suspended in 10% of their original volume using sterilized distilled water and stored at −30°C until use. The supernatant was filtered through a 0.22-μm pore-size filter (Millipore, MA, USA) before use. *H. pylori* was inoculated into BHI broth with 5% HS containing 25 μg/mL tetrazolium violet and 0.7% (w/v) agar at a final concentration of 7.0 log₁₀ CFU/mL and then poured into Petri dishes. Wells (10 mm diameter) were made on the solidified agar and then filled with 100 μL of each sample. After incubation at 37°C for 48 h under microaerobic conditions, the diameter of *H. pylori* inhibition zones was measured.

**Measurement of pH and organic acids in co-cultures** The pH of co-cultures was measured using a pH meter (F-12; Horiba Ltd., Kyoto, Japan). Co-cultures were centrifuged at 10,000 × g for 5 min. Then, 0.5 mL of the supernatant was ultrafiltered at 15,000 × g for 20 min to obtain the fraction with a MW <10kDa (Nanosep 10K OMEGA; Pall Corp., NY, USA). The obtained fraction was diluted two-fold with distilled water and then subjected to an analysis of organic acids using the HPLC Agilent SERIES 1100 System (Agilent Technologies Inc., CA, USA). For the analysis of organic acids, the diluted solution was injected into a reversed-phase column (Inertsil ODS-3; GL Sciences Inc., Tokyo, Japan). Elution was performed at 30°C with 50 mmol/L phosphate buffer (pH 2.8) at a flow rate of 0.7 mL/min. Organic acids were detected using absorbance at 210 nm. A solution containing 5.13 mmol/L...
lactic acid and 24.84 mmol/L acetic acid was used as the standard solution. Quantification of organic acids was based on the external standard method.

**Growth inhibition of *H. pylori* by lactic acid**  
L- and dl-lactic acid (Wako Pure Chemical), and dl-laetic acid (Wako Pure Chemical), and o-laetic acid (Bachem AG, Bubendorf, Switzerland) were filtered through a filter (0.22 μm pore size) and used for the test. *H. pylori* was cultured in BHI broth with 5% HS at an initial cell density of 6.0 log₁₀ CFU/mL at 37°C under microaerobic conditions. To investigate *H. pylori* growth inhibition by lactic acid during the early culture stage, each lactic acid solution was added to the cultures at 12 h incubation and cultures were further incubated at 37°C under microaerobic conditions. The *H. pylori* counts in cultures were estimated by using modified Skirrow’s agar, as described above. In addition, to investigate the anti-*H. pylori* activity of L-, DL-, and O-laetic acid, strain no. 130, which was pre-cultured in BHI broth with 5% HS, was inoculated into fresh culture medium containing different levels of lactic acid and incubated at 37°C microaerobically. After incubation, *H. pylori* counts were estimated using modified Skirrow’s agar, as described above. The inhibitory lactic acid concentration for a 50% reduction in *H. pylori* (IC₅₀) was also determined from a curve depicting the concentrations of lactic acid and the CFU obtained after incubation with these lactic acid concentrations.

**H. pylori infection in mice**  
*H. pylori* infection was induced in mice as described by Aiba et al. (1998). Briefly, 4-week-old male, germ-free BALB/c mice were obtained from CLEA Japan, Inc. (Tokyo, Japan). The mice were orally inoculated with *H. pylori* strain no. 130 at a concentration of 9.0 log₁₀ CFU for 3 consecutive days. For LAB administration, *H. pylori*-infected mice were orally administered LAB at 9.0 log₁₀ CFU once a day for 3 consecutive days at the age of 8 weeks and thereafter administered once at 9, 10, and 11 weeks. These doses were in reference to a previous report (Aiba et al., 1998). The mice were sacrificed at 12 weeks of examination. Their gastric organs were removed and homogenized in PBS on ice. The homogenate was diluted serially with PBS (10⁻¹ – 10⁻⁷) and cultured in modified Skirrow’s agar for *H. pylori* and in MRS agar for LAB. The experimental protocol was approved by the Animal Experiment Committee of Tokai University, Japan (Permission no. 133005). The animal experimentation guidelines of the university were followed in the animal studies.

**Statistical analysis**  
The ratio of *H. pylori* growth in screening, the inhibition zones of *H. pylori* by LAB culture fractions, and the bacterial counts in *vivo* were analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s test. The bacterial counts and pH of cultures inoculated with LAB or lactic acid were analyzed by two-way ANOVA, followed by Bonferroni’s test. The organic acid levels in the co-cultures of *H. pylori* and LAB were also analyzed by two-way ANOVA, followed by Bonferroni’s test. *p* < 0.05 was considered as statistically significant.

## Results and Discussion

**Effects of LAB on *H. pylori* growth in vitro**  
In screening the tested LAB for anti-*H. pylori* activity, LAB were co-cultured with *H. pylori* for 2 days, and *H. pylori* counts were subsequently determined. The growth ratio of *H. pylori* in the co-cultures is shown in Table 1. All of the tested LAB strains demonstrated a decrease in the growth ratio of *H. pylori*. In particular, significant differences were observed in the growth ratio of *H. pylori* co-cultured with *Lactobacillus (L.) plantarum* strains 05AM23 (37.3%) and 07MR044 (35.4%) compared to PBS (control) (*p* < 0.05, one-way ANOVA, followed by Tukey’s test). For *L. paracasei*, strain 06TCa19 showed the lowest *H. pylori* growth ratio (46.2%), but a significant difference was not observed.

It was demonstrated that the inhibitory effects of LAB on *H. pylori* growth showed LAB species inter- and intra-variability (Hütt et al., 2006; Rokka et al., 2006). In this study, *L. paracasei* strain

### Table 1. Tested LAB and their effects on the growth of *H. pylori*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Growth ratio of <em>H. pylori</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PBS</strong></td>
<td></td>
<td>100.0 ± 8.1</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em></td>
<td>06TCa19</td>
<td>46.2 ± 11.2</td>
</tr>
<tr>
<td></td>
<td>06TCa22</td>
<td>56.8 ± 19.0</td>
</tr>
<tr>
<td></td>
<td>06TCa43</td>
<td>49.3 ± 11.8</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei tolerans</em></td>
<td>06TCa39</td>
<td>53.5 ± 15.0</td>
</tr>
<tr>
<td></td>
<td>07LC080</td>
<td>57.1 ± 22.7</td>
</tr>
<tr>
<td></td>
<td>08LY034</td>
<td>59.5 ± 18.0</td>
</tr>
<tr>
<td></td>
<td>08LY065c</td>
<td>86.1 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>08LY075b</td>
<td>66.2 ± 5.8</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em></td>
<td>05AM23</td>
<td>37.3 ± 2.9*</td>
</tr>
<tr>
<td></td>
<td>06CC2a</td>
<td>48.6 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>06CC9c</td>
<td>41.4 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>06TCa8b</td>
<td>41.6 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>06TCa40b</td>
<td>42.6 ± 10.0</td>
</tr>
<tr>
<td></td>
<td>07MR044b</td>
<td>35.4 ± 7.3*</td>
</tr>
<tr>
<td></td>
<td>08MR029b</td>
<td>61.7 ± 2.7</td>
</tr>
<tr>
<td><em>Lactobacillus delbrueckii lactis</em></td>
<td>06TC3b</td>
<td>61.3 ± 11.9</td>
</tr>
</tbody>
</table>

Data represent mean ± standard deviation (SD).  
The experiment was independently carried out three times.  
The asterisks indicate significantly differences from the control (PBS) by one-way ANOVA, followed by Tukey’s test (*p* < 0.05). *Growth inhibition ratios were expressed as a percentage of the control. *H. pylori* strain no. 130 counts in co-culture with each LAB were determined on modified Skirrow’s agar.  
1LAB strains were isolated from *tarag*, a traditional Mongolian yogurt.  
2LAB strains were isolated from *airag*, an alcoholic, fermented horse milk.  
3LAB strains were isolated from *aaruul*, a traditional Mongolian cheese.  
4LAB strain was isolated from *urum*, a clotted cream made from milk.  
The tested LAB strains were isolated from ninety-five tarags, thirty-one aaruls, nine aaruuls and two urums.
Effects of LAB in co-culture with H. pylori. (A) The count of H. pylori in co-cultures with the strain 06TCa19 (●), 07MR044 (▲), and PBS (○). (B) The count of LAB in the co-cultures of H. pylori strain no. 130 with the strain 06TCa19 (●) and 07MR044 (▲). (C) pH in the co-cultures of H. pylori strain no. 130 with the 06TCa19 strain (●), 07MR044 strain (▲), and PBS (○). The experiments were independently carried out three times. Vertical bars represent SD. Values with different small letters indicate a significant difference at each incubation time by two-way ANOVA, followed by Tukey’s test. The inhibitory zones from the fractions of 06TCa19 untreated supernatant (●), heat-killed 06TCa19 and 07MR044 cells (▲), PBS (○), and 06TCa19 and 07MR044 cells were also significantly smaller than those of the culture solutions and the untreated supernatants (<0.05, one-way ANOVA, followed by Tukey’s test). In addition, heat-killed 06TCa19 and 07MR044 cells did not produce large inhibitory zones against H. pylori. Therefore, the inhibition of H. pylori growth may be attributed to the release of products by strain 06TCa19 growth or the increase in cell counts of strain 06TCa19.

Identification of the active anti-H. pylori component(s) in LAB culture To identify the substance(s) that contributes to the anti-H. pylori activity of strains 06TCa19 and 07MR044, six fractions from the respective BHI broth with 5% HS cultures were prepared and subjected to the well diffusion assay. The culture solutions of strains 06TCa19 and 07MR044 and their supernatants exhibited large inhibitory zones against H. pylori (Table 2). However, the fractions of pH-neutralized supernatant and pH-neutralized supernatant treated with catalase showed significantly smaller inhibitory zones than those of the culture solutions and the untreated supernatants (<0.05, one-way ANOVA, followed by Tukey’s test). The inhibitory zones from the fractions of 06TCa19 and 07MR044 cells were also significantly smaller than those of the fractions of the culture solutions or the untreated supernatants (<0.05, one-way ANOVA, followed by Tukey’s test). In addition, heat-killed 06TCa19 and 07MR044 cells did not produce inhibitory zones, respectively.

Some H. pylori growth-inhibiting activities by LAB are reportedly attributable to the production of organic acids (Aiba et al., 1998; Lin et al., 2009; Midolo et al., 1995). On the other hand, H. pylori growth inhibition by LAB has been reported to depend on the cell wall fraction and bacteriocin (Rokka et al., 2006; Simova et al., 2009). In this study, because the supernatant fractions of strains 06TCa19 and 07MR044 exhibited high inhibitory activity against H. pylori, the main inhibitory substances were thought to be contained in the supernatants (Table 2). In addition, neutralization of pH and catalase treatment were conducted to exclude the effects of organic acids and to eliminate the effects of hydrogen peroxide on anti-H. pylori activity, respectively; and
these fractions of strains 06TCa19 and 07MR044 showed remarkably small inhibitory zones against H. pylori. Therefore, the inhibitory activities of strains 06TCa19 and 07MR044 against H. pylori were thought to be caused by the production of organic acids. Also, the level of organic acids, including lactic acid, produced by the LAB strains that did not inhibit H. pylori growth (Table 1) may be lower than that produced by strains 06TCa19 and 07MR044.

Analysis of organic acids in co-cultured H. pylori and LAB. The organic acid levels in the supernatants of co-cultured H. pylori and LAB were analyzed. The lactic acid concentrations in the co-cultures of H. pylori and LAB increased in a time-dependent manner, but no lactic acid was detected in the supernatants of PBS (control) (Table 3). The concentration of lactic acid in the co-culture with strain 06TCa19 reached 25.0 mmol/L at 12 h incubation, and the lactic acid concentration was significantly higher than that in the co-culture with strain 07MR044 at 12 h (p < 0.05, two-way ANOVA, followed by Bonferroni’s test). On the other hand, no significant differences were observed in the concentrations of acetic acid in the control and co-cultures inoculated with the LAB strains.

As shown in Table 3, the increase in lactic acid concentrations in the co-culture of H. pylori and LAB was due to the growth of LAB, as no lactic acid was detected in the control. In addition, the lactic acid concentration of the co-culture inoculated with strain 06TCa19 was significantly higher than that with strain 07MR044 at 12 h after LAB inoculation (p < 0.05). Thus, strain 06TCa19 produced a larger amount of lactic acid compared to strain 07MR044 during early-stage co-culture with H. pylori. This ability of 06TCa19 may contribute to the anti-H. pylori activity. On the other hand, Lin et al. (2009) stated that the anti-H. pylori activity was due to the acetic acid as well as the lactic acid produced in the culture medium. Acetic acid was detected in the H. pylori culture inoculated with PBS, and no significant differences were observed in the concentrations of acetic acid in this study (Table 3). Therefore, it is suggested that acetic acid was not produced by strains 06TCa19 and 07MR044 and did not contribute to the inhibition of H. pylori in this study.

Effects of lactic acid produced by LAB during early-stage co-culture on H. pylori growth. To investigate the effect of lactic acid produced by strains 06TCa19 and 07MR044 during early-stage co-culture on H. pylori growth, 25 mmol/L L-lactic acid and 15 mmol/L Dl-lactic acid were added to the H. pylori culture at 12 h incubation, and H. pylori was enumerated. The concentrations and schedules for L- and Dl-lactic acid addition were based on the results given in Table 3, which showed the inoculation of strains

| Table 2. Effect of LAB culture fractions on H. pylori growth. |
|-----------------|-----------------|-----------------|
| Fraction of LAB culture | 06TCa19 | 07MR044 |
| Culture solution (containing cells) | ND | ND |
| Supernatant | 17.1 ± 2.3a | 18.0 ± 0.2a |
| Supernatant pH neutralized to 6.5 | 17.7 ± 1.8a | 18.7 ± 2.8a |
| Supernatant pH neutralized to 6.5 and treated with catalase | 3.9 ± 0.9b | 2.7 ± 0.7b |
| LAB cells | 2.8 ± 0.8b | 2.2 ± 0.1b |
| Heat-killed LAB cells | ND | ND |

Samples were prepared from three independent experiments and subjected to a well diffusion assay. Inhibition zones were expressed by subtracting the diameter of the well from that of each sample. Data represent the mean ± SD. ND, not detected. Values with different superscripts indicate a significant difference in each LAB group by one-way ANOVA, followed by Tukey’s test (p < 0.05).

| Table 3. Levels of organic acids in the co-cultures of LAB and H. pylori. |
|-----------------|-----------------|-----------------|
| Strain | 6 h | 12 h | 24 h | 48 h | 6 h | 12 h | 24 h | 48 h |
| PBS | ND | ND | ND | ND | 72.9 ± 2.3 | 68.2 ± 3.3 | 69.4 ± 2.9 | 69.8 ± 2.8 |
| 06TCa19 | 9.0 ± 1.0 | 25.0 ± 0.5 | 24.5 ± 0.6 | 23.3 ± 0.3 | 71.2 ± 2.6 | 73.4 ± 3.4 | 74.8 ± 4.9 | 79.3 ± 2.6 |
| 07MR044 | 8.7 ± 1.1 | 15.1 ± 2.2 | 22.6 ± 2.5 | 22.2 ± 1.5 | 69.6 ± 2.2 | 66.8 ± 2.5 | 71.0 ± 2.1 | 72.8 ± 5.3 |

Samples were collected from three independent experiments for analysis by HPLC. Data represent the mean ± SD. ND, not detected. Levels of lactic and acetic acids in the initial incubation were ND and 63.2 ± 2.0 mmol/L, respectively. Lower limits of detection were 2.5 mmol/L for lactic acid and 12.4 mmol/L for acetic acid. The asterisk indicates significantly different from the lactic acid level of strain 07MR044 at 12 h by two-way ANOVA, followed by Bonferroni’s test (p < 0.05).
06TCa19 and 07MR044 to *H. pylori* cultures, respectively. It was also confirmed that strains 06TCa19 and 07MR044 produced L-lactic acid and D-lactic acid, respectively (data not shown). As shown in Fig. 2, the addition of 25 mmol/L L-lactic acid at 12 h incubation significantly reduced *H. pylori* counts in the medium to lower than that observed with PBS or 15 mmol/L D.L-lactic acid addition at 24 h and 48 h incubation (*p* < 0.05, two-way ANOVA, followed by Bonferroni’s test). On the other hand, the addition of 15 mmol/L D.L-lactic acid did not produce a time-dependent reduction in *H. pylori* counts.

The anti-*H. pylori* activity mediated by lactic acid has been described in previous reports (Midolo et al., 1995; Zheng et al., 2014). The effect of lactic acid on *H. pylori* strain was also determined in this study. As shown in Fig. 2, the addition of 25 mmol/L L-lactic acid at 12 h incubation significantly reduced *H. pylori* counts in the stomach of mice, infected mice were orally administered the respective strains.

![Fig. 2. Effect of lactic acid on *H. pylori* counts.](image)

**Fig. 2.** Effect of lactic acid on *H. pylori* counts. *H. pylori* strain no. 130 counts in cultures inoculated with 25 mmol/L D.L-lactic acid (●), 15 mmol/L D.L-lactic acid (▲), and PBS (□) at 12 h incubation are shown. The experiment was independently carried out three times. Vertical bars represent SD. Values with different small letters indicate a significant difference by one-way ANOVA, followed by Bonferroni’s test (*p* < 0.05).

Effects of oral administration of LAB on bacterial counts in the stomach of *H. pylori*-infected mice

Strains 06TCa19 and 07MR044 on *H. pylori* colonization in the stomach of mice, infected mice were orally administered the respective strains. *H. pylori* counts in the stomach of infected mice administered strain 06TCa19 were significantly lower than in those administered PBS (control) or strain 07MR044 (*p* < 0.05, one-way ANOVA, followed by Tukey’s test) (Fig. 3A). Further, no reduction was noted in *H. pylori* counts in the stomach of infected mice administered strain 07MR044. On the other hand, LAB counts in the stomach of infected mice administered strain 06TCa19 were higher than in those administered strain 07MR044, although not significantly so (Fig. 3B).

Administration of several LAB strains has been demonstrated to reduce the number of *H. pylori* in the stomach of infected mice (Aiba et al., 1998; Kabir et al., 1997; Kimura et al., 2003; Ushiyama et al., 2003). In this study, oral administration of strain...
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06TCa19 to infected mice produced a significant reduction in *H. pylori* levels, and the oral administration of strain 06TCa19 showed a beneficial effect on reducing or eliminating *H. pylori* in the stomach in vivo (Fig. 3A). The beneficial effect of *L. salivarius* WB1004 administration on the eradication of *H. pylori* in the stomach of infected mice was due to its ability to adhere to gastric epithelial cells and produce high levels of lactic acid, in parallel with its rapid growth in the stomach (Aiba et al., 1998). In addition, *L. gasseri* OLL2716 showed strong anti-acid properties and tolerance to gastric juices in vitro, leading to the suppression of *H. pylori* infection (Fujimura et al., 2012; Kimura 2004). In our previous study, the tolerance of strain 06TCa19 to gastric acid was confirmed in vitro (Takeda et al., 2011), and the strain potentially adhered to the stomach of *H. pylori*-infected mice in this study (Fig. 3B). Furthermore, strain 06TCa19 was observed to produce l-lactic acid at a high rate and in large amounts in vitro (Table 3). Therefore, the administration of strain 06TCa19 is suggested to reduce the number of colonized *H. pylori* in the stomach of infected mice. Moreover, it is suggested that the l-lactic acid produced in the stomach by this strain can contribute to reductions in *H. pylori* levels, as shown by *L. salivarius* WB1004 and *L. gasseri* OLL2716. Further in vivo studies are required to clarify the mechanism of anti-*H. pylori* activity by strain 06TCa19. Notably, strain 06TCa19 can grow favorably in milk, and milk fermented using this strain has a good flavor and has been reported to improve human bowel habits (Takeda et al., 2013). Thus, strain 06TCa19 may be an effective LAB starter for the production of fermented milk with anti-*H. pylori* activity, and potentially aiding in intestinal regulation. Future investigations on whether oral administration of dairy products such as yogurt prepared with strain 06TCa19 can exert anti-*H. pylori* activity in humans are warranted.

In conclusion, *L. paracasei* strain 06TCa19 isolated from Mongolian dairy products was suggested to possess anti-*H. pylori* activity, in addition to potentially aiding human intestinal regulation. Strain 06TCa19 inhibited the growth of *H. pylori* strain no. 130 and produced significant amounts of l-lactic acid, likely contributing to the anti-*H. pylori* activity in vitro. Furthermore, strain 06TCa19 tended to adhere to gastric epithelial cells in *H. pylori*-infected mice in this study, leading to a reduction in *H. pylori* colonization.

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