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Use of Selected Lactic Acid Bacteria in the Eradication of *Helicobacter pylori* Infection

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Helicobacter pylori **is among the major pathogenic bacteria that cause chronic gastritis and peptic ulcer disease and is related to the development of gastric cancer. Several chemicals, including antibiotics, have been used to eradicate *H. pylori*; however, they do not always curb the infection. Ten representative type strains of lactic acid bacteria (LAB) were screened for antagonism toward *H. pylori* via inhibition of urease activity. Strains inhibiting the binding of *H. pylori* to human gastric cell line cells and suppressing *H. pylori*-induced interleukin-8 (IL-8) production were also screened. Of these, *Pediococcus pentosaseus* (SL4), which inhibited the adhesion of *H. pylori* to MKN-45 gastric cancer cells, *Bifidobacterium longum* (BG7), with urease inhibiting activity, and *Lactococcus lactis* (SL3), and *Enterococcus faecalis* (SL5), which suppressed *H. pylori*-induced IL-8 production within MKN-45 and AGS cells, were selected. In mouse model, these LAB stains in combination significantly suppressed IL-8 levels in serum. Gastric pH also recovered to normal values after the administration of these LAB. These stains effectively suppressed *H. pylori* viability, although not to the extent of antibiotic treatment. When used as probiotics, LAB may help decrease the occurrence of gastritis and reduce the risk of *H. pylori* infection without, inducing side effects.**

Keywords: lactic acid bacteria, *Helicobacter pylori*, urease activity, gastric pH, IL-8

Introduction

Helicobacter pylori is a microaerophilic gram-negative bacteria that causes chronic gastritis, peptic ulcer disease, and presumably gastric cancer. Accumulated evidence demonstrates that the eradication of these bacteria resolves *H. pylori*-associated diseases (Marshall, 1994). Multicenter studies have shown that triple therapy with a proton pump inhibitor (PPI), clarithromycin, and either amoxicillin or metronidazole (all taken twice daily) is among the most effective approaches to *H. pylori* eradication (Lind *et al.*, 1999). However, 5–10% of *H. pylori* strains are reportedly resistant to clarithromycin (Maeda *et al.*, 2000). In addition, one study noted a clarithromycin-resistant mutation in 63% of *H. pylori* strains from patients in whom treatment with a regimen including clarithromycin was unsuccessful (Maeda *et al.*, 1998). The treatment of *H. pylori* infection with antibiotics does not always eradicate the organism and is also often accompanied by deleterious side effects (Aiba *et al.*, 1998). Thus, although many experts believe that "untreatable" *H. pylori* is just ill-treated *H. pylori*, no clinical trial, to the best of our knowledge, has yielded a treatment that provides 100% eradication (Ierardi *et al.*, 2013).

Recently, probiotic lactic acid bacteria (LAB) have been reported to control *H. pylori*, and several studies have examined the efficacy of various probiotic preparations in *H. pylori* eradication with and without co-interventions (Sachdeva and Nagpal, 2009). Moreover, a number of clinical trials have been undertaken to test the hypothesis that probiotic bacteria inhibit *H. pylori* infection (Hsieh *et al.*, 2012). Probiotics inhibit enteric pathogens such as *Salmonella*, *Shigella*, and *Citrobacter rodentium* in both *in vitro* and animal models (Johnson-Henry *et al.*, 2005; Tsai *et al.*, 2005; Tien *et al.*, 2006), and their potential clinical benefits in preventing or resolving gastrointestinal diseases have been demonstrated (Fernandez *et al.*, 2003; Lionetti *et al.*, 2010). These microorganisms provide gut protection through several mechanisms, including decreasing luminal pH by producing lactic acid (Sartor, 2005; De Keersmaecker *et al.*, 2006) and competing with gut pathogens for host surface receptors (Mack *et al.*, 1999). Nonetheless, Coconnier *et al.* (1998) have shown that probiotics may inhibit *H. pylori* growth independent of pH and lactic acid levels. Another critical mechanism involving probiotics relates to changes in host immune responses to infection via reduced tumor necrosis factor alpha and interleukin-8 (IL-8) (Corr *et al.*, 2007; Matsumoto *et al.*, 2007). These changes result in anti-inflammatory effects in *H. pylori*-infected patients after brief contact between the flora of ingested probiotics and the gastric epithelium (Yang *et al.*, 2012).

Until now, most studies on the control of *H. pylori* with LAB have focused on one or two probiotic effects. Therefore, the inhibitory effects on *H. pylori* may have been limited. To increase these inhibitory effects, we screened ten representative type strains of LAB, *Bifidobacterium lactis* (BL3), *Bifidobacterium bifidum* (BF3), *Bifidobacterium longum* (BG7), *Lactobacillus acidophilus* (LA1), *Lactobacillus casei* (LC5), *Lactobacillus plantarum* (LP3), *Lactobacillus rhamnosus* (LR5), *Lactococcus lactis* (SL3), *Pediococcus pentosaseus* (SL4), and *Enterococcus faecalis* (SL5) in three ways,

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and tested the selected LAB in combination. In a previous study, LAB were selected based on the adherence of *H. pylori* to the binding moiety in the stomach in an effort to find new LAB with activity against *H. pylori* (Nam *et al.*, 2002). Urease catalyzes the hydrolysis of urea to form CO_2 and NH_3 and reportedly functions in *H. pylori* infection to neutralize gastric acid by producing NH_3 (Kuwahara *et al.*, 2000). Therefore, we also screened LAB for antagonism of *H. pylori* via the inhibition of urease activity. Accumulating evidence indicates that IL-8, a potent neutrophil chemoattractant and activating agent, plays a major role in the mucosal inflammation induced by *H. pylori* (Innocenti *et al.*, 2002). In addition, lactobacilli administered after *H. pylori* implantation reduce both the degree of inflammation and the density of *H. pylori* in the stomach. Given these findings, we also selected LAB that suppressed *H. pylori*-induced IL-8 production in MKN-45 and AGS cell lines. *In vitro* assays were carried out to determine whether the combination of the four selected probiotic stains inhibits the growth of *H. pylori* and its adhesion to gastric cells and if the strains modulate chemokine and cytokine secretion by the host cells, thus impacting gastric acidity.

The current therapeutic regimen for *H. pylori* aims to eliminate bacterial growth with antibiotics and reduce the total acidity of gastric acid. In this study, we demonstrated in animal models that selected LAB strains in combination have beneficial effects similar to those of antibiotic therapy on *H. pylori*-infected gastric epithelium.

Materials and Methods

Probiotics and *H. pylori* preparation

The LAB strains used in this study were obtained from collections maintained at Cell Biotech Co. Ltd. (Korea). *Bifidobacterium lactis* (BL3) KCTC 11904BP, *Bifidobacterium bifidum* (BF3) KCTC 12199BP, *Bifidobacterium longum* (BG7) KCTC 12200BP, *Lactobacillus acidophilus* (LA1) KCTC 11906BP, *Lactobacillus casei* (LC5) KCTC 12398BP, *Lactobacillus plantarum* (LP3) KCTC 11782BP, *Lactobacillus rhamnosus* (LR5) KCTC 12202BP, *Lactococcus lactis* (SL3) KCTC 12396BP, *Pediococcus pentosaseus* (SL4) KCTC 10297BP, and *Enterococcus faecalis* (SL5) KCTC 10699BP were cultured at Cell Biotech Co. Ltd. The probiotic LAB strains were inoculated into deMan, Rogosa, and Sharpe broth (Difco, USA) and cultured at 37°C for 18–24 h. The cultures were then washed twice with sterile saline to remove any associated metabolic substances. *H. pylori* from the Korean Collection for Type Cultures (HpKTCC B0007; Korea) was used throughout this study. This strain was cultured on Brucella broth agar plates (BD Biosciences, USA) containing 10% fetal bovine serum (HyClone, USA) for 48 h at 37°C in an atmosphere of 10% CO_2 under aerobic conditions, harvested with a scraper, and sub-cultured once in the same type of fresh media.

Cell cultures

The human gastric adenocarcinoma cell lines AGS (KCLB 21739; Korea) and MKN-45 (KCLB 80103; Korea) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (HyClone) and penicillin/streptomycin 1% (Invitrogen, USA). The cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% air.

Adhesion assay

The adhesion assay was carried out as described by Nam *et al.* (2002), with some modifications to the method. Briefly, each well of a 12-well tissue culture plate was seeded with MKN-45 cells. Then, 1,000 µl of RPMI 1640 (without serum) and penicillin/streptomycin 1% (Invitrogen) were added to each well and incubated at 37°C in an atmosphere of 5% CO_2 for 1 h. Probiotics and *H. pylori* were grown overnight. *H. pylori* and LAB cultures were diluted (1/10) with RPMI 1640 to obtain a bacterial concentration of 10^8 colony-forming units (CFU). Simultaneously, *H. pylori* and each probiotic LAB strain was added and incubated for 2 h. After incubation, all of the dishes were washed three times with phosphate-buffered saline (PBS) to release the unbound bacteria. The wells were stained with a Gram-staining kit (BD Biosciences) and observed using a microscope (1,000×).

Urease activity assay

Urease activity was determined using a modification of the phenol red method (Hazell *et al.*, 1987). To determine the urease activity of *H. pylori*, we suspended freshly prepared *H. pylori* or LAB at 1×10^8 CFU/ml in Brucella broth. The selected four LAB are mixed at the same amount (2.5×10^7 CFU). Then, 0.1 ml of this bacterial mixture was added to 0.9 ml of fresh Brucella broth, and *H. pylori* and each LAB strain were mixed in equal volumes. The bacterial mixtures were incubated for 4 h at 37°C in an atmosphere of 10% CO_2 under aerobic conditions. The supernatant was carefully transferred to a centrifuge tube. Only the supernatant was used in the assay. Next, 200 µl of a urease substrate solution was mixed with the supernatant and incubated at 37°C for 20 min. The reaction solution was analyzed using a urea assay kit (BioAssay Systems, USA), according to the manufacturer's instructions to measure NH_3 concentration. The amount of NH_3 generated by the urease contained in 1×10^7 CFU/ml of *H. pylori* was designated as mg/dl for the activity of urease.

Cytokine assay

The culture supernatants of AGS or MKN-45 cells from 24-well microtiter plates (BD Biosciences) seeded at a concentration of 1×10^5 cells/ml were collected after 24 h of treatment with *H. pylori* and probiotic strains at 10^8 CFU (Rokka *et al.*, 2008). The selected four LAB are mixed with the same amount (2.5×10^7 CFU). The amount of the secreted cytokine IL-8 was determined by performing enzyme-linked immunosorbent assay (R&D Systems, USA), according to the manufacturer's protocol. Absorbance values at 450 nm were measured using an i-Mark instrument (Bio-Rad Laboratories, USA).

Serum samples were collected from the hearts of the mice. The collected blood samples were left at room temperature for 2 h and then centrifuged (1,500×*g*) at 4°C for 15 min to separate the serum. The samples were kept at -80°C until

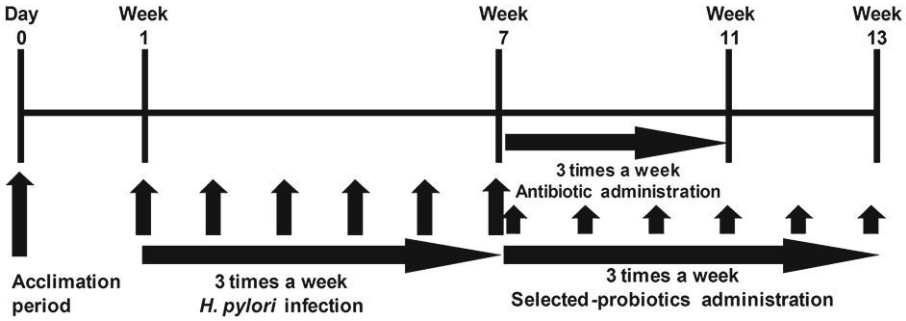

Fig. 1. Establishment of a *Helicobacter pylori*-induced mouse model in C57BL/6 mice and oral administration of selected probiotics. Incubated *H. pylori* was administered to mice 3 times over 6 weeks to induce infection. Oral administration of selected lactic acid bacteria (LAB) strains and antibiotics were begun at the same time at week 7. The experimental groups were as follows: 1×10^8 CFU, 1×10^9 CFU of selected LAB strains, and antibiotic, 500 mg/kg per day of clarithromycin and 30 mg/kg per day of amoxicillin, and distilled water (200 µl/kg per day). At weeks 11 (in the antibiotic treatment group) and 13 (in the probiotic treatment groups), the mice were killed, and samples were collected to measure treatment responses.

analysis. The serum was thawed for IL-8 analysis.

In vivo H. pylori inhibition experiments

Five-week-old male C57BL/6 mice were purchased from Orient Bio (Korea). The mice were placed in cages in groups of five. Breeding facilities is maintained at 24 ± 2°C and 40 ± 20% relative humidity with a 12-h lighting cycle.

To investigate the inhibitory effects of the selected LAB strains on the pathogenic bacterial proliferation, we prepared five experimental groups with nine mice per group (Fig. 1). For the *H. pylori* infection group, 6-week-old mice were orally inoculated three times per week with 1×10^9 CFU of freshly prepared *H. pylori* for 6 weeks. Each of the selected four LAB are mixed at the same amount. LAB was used per each 2.5×10^7 or 2.5×10^8 CFU. For the probiotic administration groups, the mice infected with *H. pylori* were inoculated orally three times per week for 6 weeks with 1×10^8 CFU or 1×10^9 CFU of the selected LAB strains. Mice infected with *H. pylori* were also administered with clarithromycin (500 mg/kg; Sigma-Aldrich, USA) and amoxicillin (30 mg/kg; Sigma-Aldrich) 3 times per week for 4 weeks. This treatment was administered for 4 or 6 weeks, and the mice were killed at 11 or 13 weeks, respectively, for examination.

Measurement of gastric pH

After 24 h of starvation, the mice were killed under ether anesthesia, and their stomachs were rapidly removed. To measure gastric pH, we prepared the mouse gastrointestinal tracts by first removing adherent fat and blood from the serosal surfaces. Stomach segments were divided longitudinally on paper towels, immediately before the pH measurement. The pH in the corpus area of the stomach was measured using a pH test paper (Sigma-Aldrich) (Ohana *et al.*, 2003).

Statistical analysis

The data were processed using Graphpad Prism™ 4.0, and the statistical parameters, mean values, and standard deviations were calculated and compared among the groups. Significance was determined via the analysis of variance ($P < 0.05$).

Results

Inhibition of adhesion and growth of *H. pylori* in gastric epithelial cells in the presence of LAB

The capability of the selected LAB strains to interfere with the adhesion of *H. pylori* to gastric epithelial cells was compared in the human gastric cancer cell line MKN-45. *H. pylori* and 10 species of probiotic LAB at 10^8 CFU were incubated in gastric cancer cell cultures simultaneously. The LAB strain SL4 out of the 10 species showed the highest degree of adhesion interference (Fig. 2A).

The urease activity test is among the most important indicators of *H. pylori* inhibition. Urease catalyzes the hydrolysis of urea to produce NH_3 and CO_2 and plays the crucial role of protecting bacteria in the acidic environment of the stomach. Two main types of substances have been implicated in the inhibition of *H. pylori* by lactic acid bacteria: short chain fatty acids (SCFAs) and bacteriocins. Such antimicrobial activity could be due not only to a direct effect on *H. pylori* but also to the inhibition of its urease activity, as shown with the high lactic acid producers *L. salivarius* and *L. casei* Shirota (Gotteland *et al.*, 2006). Ten representative type strains of LAB were screened for antagonism toward *H. pylori* via inhibition of urease activity. Most of the LAB strains showed approximately 30 mg/dl of urease activity, whereas the BG7 strain had the strongest inhibition (58.6%) of *H. pylori* urease with an activity of 20.6 mg/dl (Fig. 2B).

Cytokine secretion by AGS and MKN-45 cells exposed to *H. pylori* in the presence of LAB

The LAB strains suppressing *H. pylori*-induced interleukin-8 (IL-8) production in AGS and MKN-45 gastric cancer cell lines were also screened. AGS and MKN-45 cell cultures were exposed to LAB strains in the presence or absence of *H. pylori* cells, and the culture supernatants were assayed for secreted IL-8. The addition of *H. pylori* alone altered the cytokine levels to varying extents compared with the levels after the addition of PBS and in untreated AGS and MKN-45 cells. Similar inhibition patterns of IL-8 secretion by LAB strains were observed in the AGS and MKN-45 cell lines (Figs. 2C and 2D). The IL-8 level in AGS cell cultures with *H. pylori* alone was 337.2 ± 4.5 pg/ml, whereas exposure to LAB strains SL3 and SL5 reduced these levels to 76.1 ± 0.4

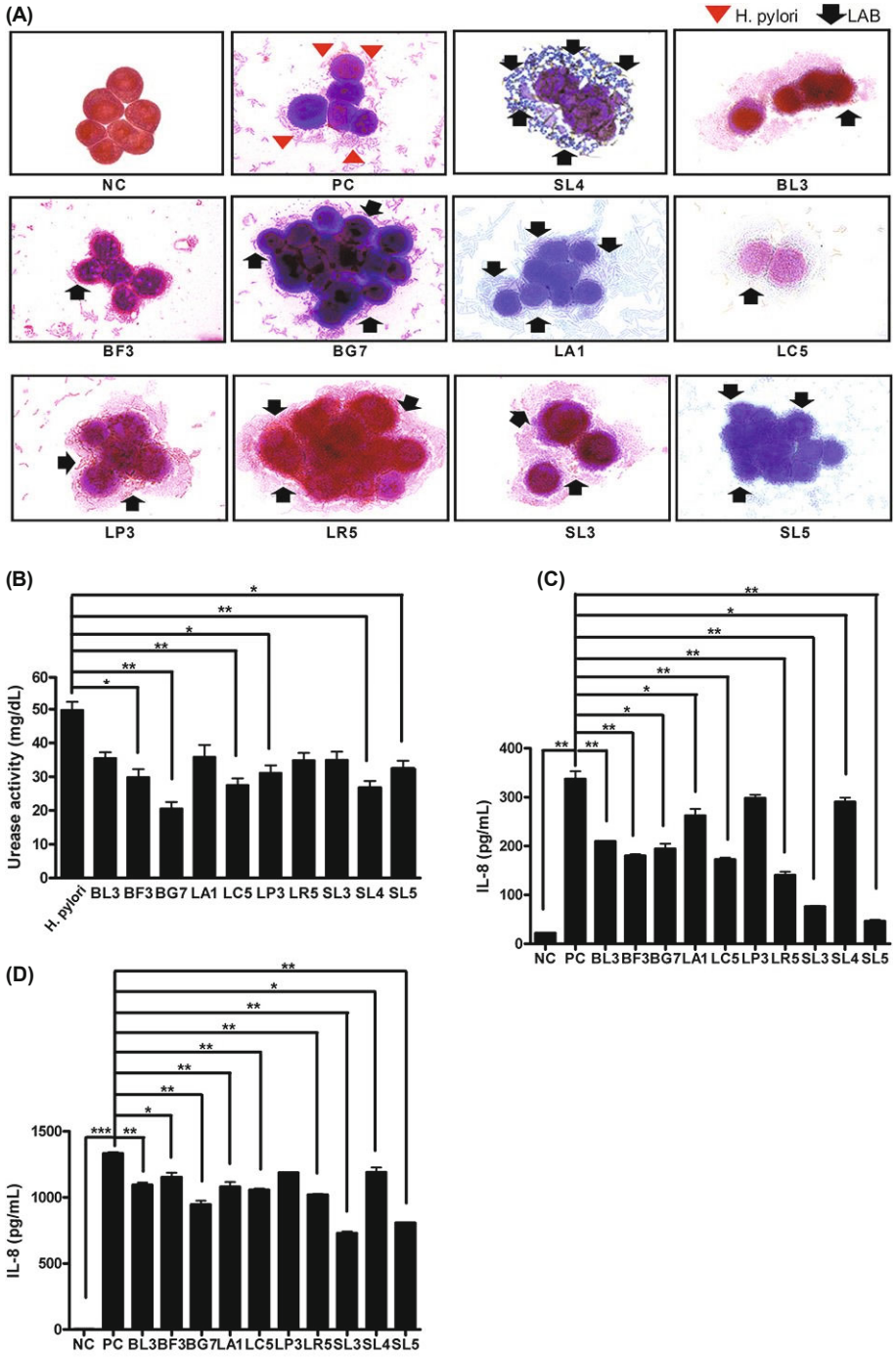

Fig. 2. *In vitro* screening of probiotics in *H. pylori* inhibition in human gastric cancer cell lines. (A) Effect of adhesion assay with *H. pylori* and several LAB strains in the MKN-45 cell line. The cells were examined in the absence, in the negative control (NC) group, in the presence of *H. pylori* alone, in the positive control (PC) group, and after treatment with SL4 probiotic cultured for 1 h. Pink and purple colours indicate *H. pylori* and SL4, respectively. (B) The *in vitro* effect of each probiotic LAB on *H. pylori* urease activity. *H. pylori* suspended in Brucella broth without antibiotics was incubated under microaerophilic conditions at 37°C. Samples were collected after 4 h and assayed for urease activity. Points represent the mean and standard error of the mean for three independent experiments. (C and D) AGS and MKN-45 cells treated with LAB strains were incubated in the presence of *H. pylori* for 24 h. Interleukin-8 (IL-8) was measured in the medium with enzyme-linked immunosorbent assay (ELISA). IL-8 levels in AGS and MKN-45 cells cultured in the presence of *H. pylori* (PC) were compared to those in the cells cultured in the presence of various LAB stains. Data shown represent the mean e standard deviation (SD). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the PC group (analysis of variance).

pg/ml and 46.1 ± 0.8 pg/ml– inhibition rates of 77.4% and 86.3%, respectively (see Fig. 2C). In MKN-45 gastric cancer cells, the IL-8 level in cultures with *H. pylori* alone was 1,333.6 ± 2.7 pg/ml, whereas exposure to strains SL3 and SL5 reduced these levels to 808.6 ± 3.8 pg/ml and 729 ± 0.1 pg/ml–inhibition rates of 45.4% and 39.4%, respectively (see Fig. 2D). From the above results, we selected SL3 and SL5, which suppressed *H. pylori*-induced IL-8 production in MKN-45 and AGS cells, for the further study. We also selected SL4, which inhibited the adhesion of *H. pylori* on MKN-45 gastric cancer cells, BG7, which had urease inhibiting activity, and performed several experiments in mice to clarify whether a combination of these selected LAB strains was effective *in vivo*.

Analysis of cytokine IL-8 in serum

The IL-8 level in the serum of *H. pylori*-infected mice was measured to examine whether a combination of the selected LAB strains suppressed IL-8 production (Fig. 3). IL-8 con-

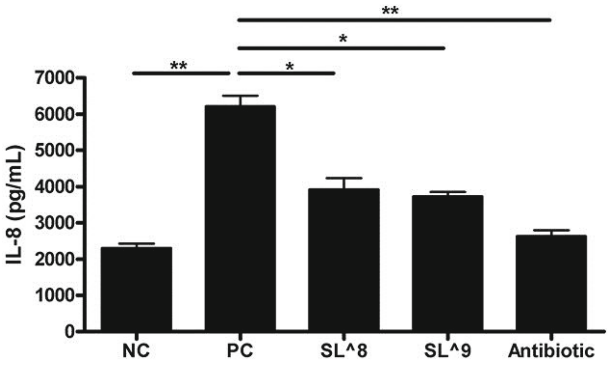

Fig. 3. Serum IL-8 levels in *H. pylori*–infected C57BL/6 mice. Blood was obtained from C57BL/6 mice treated with antibiotics or selected LAB strains (SL^8 at 10^8 CFU and SL^9 at 10^9 CFU) at week 11 or 13, respectively, and the serum levels of the indicated cytokines were determined with ELISA. Antibiotic, clarithromycin 500 mg/kg and amoxicillin 30 mg/kg. Data shown represent the mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$ compared with the PC group (analysis of variance). NC, negative control ; PC, positive control.

centration in the mouse serum of the negative control (NC) group was 2,306 ± 39 pg/ml, whereas that in the *H. pylori*-infected mouse (PC) group was increased by approximately 2.7-fold (6,215 ± 42 pg/ml). The mice were orally administered probiotic cultures of selected LAB strains, and the effect on IL-8 secretion in serum was determined. The selected LAB treatments (1×10^8 and 1×10^9 CFU) significantly decreased the concentration of IL-8 (3,924 ± 17 and 3,730 ± 27 pg/ml, respectively) in the serum at suppression rates of 40% and 36.9%, respectively. In the antibiotic group, the level (2,631 ± 18 pg/ml) of IL-8 decreased to that of the selected LAB treatments. These findings implied that the selected LAB strains in combination eradicated *H. pylori* in mice but not to the same extent as the antibiotics.

Gastric acid regulation with a combination of selected LAB

Normal stomach pH is approximately 2–3, but gastric pH during *H. pylori* infection ranges between 6.5 and 8 (Ohana *et al.*, 2003; Joseph and Kirschner, 2004). At the end of the experiment, the pH of the stomachs in the PC group was noticeably higher than that of the negative control (NC) (Fig. 4). The pH in the NC group was 2.4 ± 0.4 on average, whereas that of the PC group was significantly higher (5.1 ± 0.6). The oral administration of the selected LAB strains decreased the pH in the SL^8 (1×10^8 CFU) and SL^9 (1×10^9 CFU) groups to 3.8 ± 0.4 and 3.6 ± 0.4, respectively. With respect to pH reduction, antibiotic treatment decreased the pH to a near normal value (2.8 ± 0.3). These results indicate that the combination of the selected LAB strains has the potential to regulate gastric acidity.

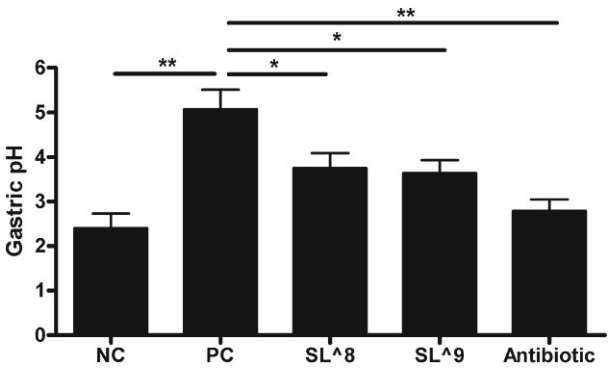

Fig. 4. Effects of *H. pylori* infection on gastric pH in C57BL/6 mice. At 11 and 13 weeks after antibiotic oral administration and treatment with selected LAB strains (either SL^8 at 10^8 CFU or SL^9 at 10^9 CFU), respectively. Antibiotic, clarithromycin 500 mg/kg and amoxicillin 30mg/kg. Data shown represent the mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$ compared with the PC group (analysis of variance). NC, negative control; PC, positive control.

Discussion

Chronic *H. pylori* infection weakens the natural defenses of the stomach lining, increasing susceptibility to stomach cancers (Wang *et al.*, 2014). *H. pylori* is difficult to eradicate from the stomach because it develops resistance to commonly used antibiotics (Maeda *et al.*, 2000; Chey *et al.*, 2007). Published guidelines in Europe and North America recommend a first-line treatment for *H. pylori* eradication that combines PPI with amoxicillin and clarithromycin (Malfertheiner *et al.*, 2007). Therefore, two or more antibiotics are usually given together with a PPI, bismuth-containing compounds, or both to eradicate the bacterium. These combinations of medications can be expected to cure 70–90% of infections (Chey *et al.*, 2007). However, a meta-analysis by Aydin *et al.* (2005) showed that the average *H. pylori* eradication rate with PPI-based triple regimens was 93.3% in 1996, and it decreased to 47.1% in 2004. Studies have also shown that resistance of *H. pylori* to clarithromycin is common among patients who have prior exposure to clarithromycin or other chemically similar macrolide antibiotics such as erythromycin. Therefore, the more antibiotics are used to cause the secondary side effects, or indiscriminate use of antibiotics is to cause it. Because antibiotic combinations can have side effects, and stomach cancers are infrequent in the United States, the risks of treatment for eradicating *H. pylori* in patients without symptoms or ulcers may not justify the unproven benefits of this treatment in preventing stomach cancer. Recent studies suggest that eradication rates achieved by first-line treatment with a PPI, clarithromycin, and amoxicillin have decreased to 70–85% due in part to increasing clarithromycin resistance (Chey *et al.*, 2007).

Antibiotic resistance is a major factor affecting the outcome of treatment. Given the increase in antibiotic resistance, alternative treatment approaches, including the use of probiotics, have been investigated for the treatment and eradication of *H. pylori* infection (Kamiji and de Oliveira, 2005). Studies investigating the use of probiotics for this purpose–most using *Lactobacillus* and *Bifidobacterium* species–have been conducted in both humans and animals (Kabir *et al.*, 1997; Aiba *et al.*, 1998; Sakamoto *et al.*, 2001; Sheu *et al.*, 2002; Ushiyama *et al.*, 2003; Johnson-Henry *et al.*, 2004; Sgouras *et al.*, 2004, 2005; Zhang *et al.*, 2008). Human clinical trials have shown that lactobacilli effectively increase the eradication rates of *H. pylori* and decrease *H. pylori* therapy-

related side effects when combined with antibiotic treatment (Kim *et al.*, 2008; Zou *et al.*, 2009). Furthermore, when used in combination with antibiotic therapy, probiotics reduce antibiotic-related side effects, and in some cases, increase the eradication rate of the infection (Kamiji and de Oliveira, 2005).

Probiotics have no observable side effects and also protect the stomach walls and stabilize the function that has already been reported. In addition, probiotic supplementation may resist this change or diminish the overgrowth of harmful bacteria or yeast induced by antibiotic treatment. Some previous studies have shown that probiotics have a positive impact on *H. pylori* eradication without therapy-related side effects (Cremonini *et al.*, 2002). Despite these advantages, probiotic treatment presents several challenges. First, probiotics must be consumed for a relatively long time. Daily consumption of probiotics provides robust preventive and therapeutic effects, but compared to the effects of drugs, these effects do not occur in a short period of time (Boonyaritichaikij *et al.*, 2009). Second, all probiotics do not have the same effects (Hamilton-Miller, 2003; Vitor and Vale, 2011).

Most studies of the control of *H. pylori* using LAB have focused on one or two probiotic effects. Therefore, the inhibitory effects on *H. pylori* may have been limited. In this study, LAB were screened with three assays, and selected LAB were tested in combination to increase their inhibitory effects. A total of 10 representative type strains of LAB were screened for antagonism toward *H. pylori* through the inhibition of urease activity. Strains inhibiting the binding of *H. pylori* to human gastric cell line cells and suppressing *H. pylori*-induced IL-8 production were also screened. From these results, SL4, which inhibited the adhesion of *H. pylori* on MKN-45 gastric cancer cells, BG7, which had urease-inhibiting activity, and SL3 and SL5, which suppressed *H. pylori*-induced IL-8 production in MKN-45 and AGS cells, were selected. We also confirmed the inhibition of urease activity with selected four LAB *in vitro*. Selected four LAB showed 18.4 mg/dl of urease activity (inhibition rate of 63.1%). As a result, the mixture of the selected LAB strains showed slightly higher inhibition rate than single strain. The IL-8 level in AGS and MKN-45 cell cultures by the LAB mixture was 46.8 ± 2.8 pg/ml and 562.5 ± 25.1 pg/ml-inhibition rates of 86.1% and 57.8%, respectively (data not shown). This is also similar or slightly higher than the levels by the single strain. The adhesion of *H. pylori* to gastric epithelial cells is a primary event in the development of inflammatory response. Such adherence is indispensable in the stimulation of IL-8 secretion (Sgouras *et al.*, 2005). Therefore, the inhibition of *H. pylori* adhesion on gastric epithelial cells by SL4 may also be effective in suppressing IL-8 production. In the mouse experiments in the present study, IL-8 secretion has been halved by treatment with selected LAB strains in combination. And *H. pylori* was blocked from the approach to the stomach epithelial cells by SL4, which is reducing the chance of reinfection of *H. pylori*. BG7 having urease-inhibiting activity in combination with SL4 could also contribute to inhibit *H. pylori* adhesion synergistically.

Most important, the pH value of the stomach decreased significantly with LAB administration. These results imply that a large number of *H. pylori* bacteria were eliminated. Even if a combination of selected LAB strains is less effective than antibiotics in suppressing cytokines or lowering pH, taking selected LAB strains in combination with conventional treatment may be a superior choice in the long-term treatment, because it is safe for the human body. In addition, When SL^8 and SL^9 of LAB were administrated, neither IL-8 level nor gastric pH in mouse was deemed significantly different. This result demonstrates that 10^8 CFU is sufficient to be effective.

Nothing unusual was observed in the gastric tissue (data not shown), which may be due to the short length of the experiment with C57BL/6 mice. Generally, observation of gastric tissue abnormalities requires approximately 8 months of persistent infection (Lee *et al.*, 1997). If the ingestion of the selected LAB strains both treated and prevented *H. pylori* infection, consistent intake of these strains would likely have an effect in eradicating peptic ulcer disease, and by extension, in preventing gastric cancer. The selected LAB strains are not only effective against *H. pylori* but also beneficial for intestinal health as probiotics.

H. pylori can be detected in the stomachs of approximately 50% of the world population. Therefore, with no vaccine available, the possibility of avoiding exposure to these bacteria seems very difficult or impossible. Even if the common symptoms of *H. pylori* infection are few, they are not insignificant. Therefore, LAB strains showing excellent efficiency in the eradication of *H. pylori* may be appropriate for prophylaxis. In summary, given the results of the *in vitro* and *in vivo* studies described herein, we propose that the effectiveness of probiotics to eliminate *H. pylori* may be due to nonspecific bacteriostatic activity on the gastric epithelial cell surface, competition in adherence to gastric epithelial cells, and suppression of IL-8 production.

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