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ARTICLE

Anti-*Helicobacter pylori* Activity of Antimicrobial Substances Produced by Lactic Acid Bacteria Isolated from Baikkimchi

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Abstract The purpose of the present study was to determine the antagonistic activity of the 13 lactic acid bacteria strains isolated from Baikkimchi made with (*Brassica rapa*, subspecies *pekinensis* and *chinensis*) against *Helicobacter pylori* ATCC 43504 *in vitro*. Relatively good growth properties were found for *Lactobacillus brevis* BK11, *Lactobacillus acidophilus* BK13, and *Leuconostoc mesenteroides* BK26 strains with residual numbers of $>10^6$ CFU/mL after incubation for 2 h in artificial gastric juice. In co-culturing experiments, *Lactobacillus plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *Pediococcus pentosaceus* BK34, *Lactobacillus paracasei* BK57, *Enterococcus faecalis* BK61, and *Lactococcus lactis* BK65 showed significant antimicrobial ability against *H. pylori*. The cell-free culture supernatants (CFCSs) obtained from *L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. paracasei* BK57, and *L. lactis* BK65 strains producing very high levels of lactic acid dramatically decreased the viability of *H. pylori*. In addition, the bactericidal activity of *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *L. paracasei* BK57, and *E. faecalis* BK61 strains was significantly correlated with the bacteriocin production. The CFCS and bacteriocin solutions produced from the strains except for *E. faecalis* BK61 were effective in inhibiting the adhesion of *H. pylori* to human stomach adenocarcinoma cells and their urease activity.

Keywords adhesion · antimicrobial substance · *Helicobacter pylori* · lactic acid bacteria · urease

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Introduction

Helicobacter pylori, the pathogen allowing colonization of the harsh stomach environment, has recently been shown to be an important etiologic agent of chronic gastritis as well as peptic ulcer and gastric cancer (Hamilton-Miller, 2003). At present, the standard triple therapy consisting of two kinds of antibiotics (clarithromycin and amoxicillin) and a proton pump inhibitor is regarded as a good treatment to eradicate *H. pylori* infection (Malfertheiner et al., 2002). Although antibiotic-based therapies are efficient, the use of a large dose of antibiotics has caused a rapid emergence of antibiotic-resistant strains and several side effects such as diarrhea, vomiting, nausea, and metallic taste (Matsumoto et al., 1997). As a consequence, the need for novel therapeutic approaches, alternative or complementary to antibiotic therapy, has claimed the attention of many researchers (Canducci et al., 2002).

According to the previous study, one of the alternative anti-*H. pylori* treatments involves an application of probiotic strains, defined as living microorganisms that may confer a health benefit on the host (Patel et al., 2013). Probiotic organisms could be exploited as potential therapeutic agents to eradicate intestinal infections and as adjuncts to current therapy strategies, because lactic acid bacteria (LAB) may improve antibiotic therapy tolerability of the traditional eradication methods by reducing its side effects (De Bortoli et al., 2007).

Because LAB are acid-tolerant and able to persist in the stomach longer than other bacteria, some LAB preparations have been extensively studied for their ability to protect against pathogens such as *H. pylori* (Ryan et al., 2008; Tsai et al., 2004). *In vitro* and animal data indicate that probiotic LAB, *Lactobacillus* sp., and *Bifidobacterium* sp. can inhibit the growth of the pathogens and decrease urease activity necessary for *H. pylori* to remain in the acidic environment of the stomach (Aiba et al., 1998).

Several authors have previously indicated that probiotic

lactobacilli are able to inhibit potential pathogens growth owing to the production of antimicrobial substances (Midolo et al., 1995; Hamilton-Miller, 2003). Furthermore, distinct probiotic strains may facilitate the stabilization of gut mucosal barrier and the coaggregation with pathogens (Patel et al., 2013). Other mechanisms include the strengthening of gastric barrier function due to mucin production and the competition with *H. pylori* for adhesion sites on gut wall and nutrients (Kim et al., 2008). Animal studies suggested that probiotic bacteria could modify the humoral immune response of the host by interacting with epithelial cells and controlling the balance of proinflammatory and anti-inflammatory cytokines, which may result in reduction of gastric trouble symptoms (Murosaki et al., 2000). Probiotic LAB alter the composition of gastrointestinal flora and inhibit the growth of pathogens by producing the antimicrobial substances, such as short-chain fatty acids, diacetyl, hydrogen peroxide, as well as various bacteriocins (Midolo et al., 1995; Hamilton-Miller, 2003). Michetti et al. (1990) reported that a strain of *Lactobacillus acidophilus* produces an antimicrobial substance that reduced the viability of *H. pylori*. Tsai et al. (2004) indicated that the spent culture supernatant of *Enterococcus faecium* TM39 significantly inhibited the viability and the urease activity of *H. pylori*.

The purpose of the present study was to determine the antagonistic activity of the LAB strains isolated from Baikkimchi (Kimchi made without red pepper powder; *Brassica rapa*, subspecies *pekinensis* and *chinensis*) against *H. pylori* ATCC 43504 *in vitro*. Furthermore, the inhibitory effects of the adhesion of *H. pylori* cells to the epithelial cell lines and the urease activity of the adhered strains by the antimicrobial substances obtained from the isolated strains were evaluated.

Materials and Methods

LAB isolation, growth conditions, and identification. A total of 13 LAB strains were collected directly from 10 samples of Korean fermented vegetable food, Baikkimchi. The samples were serially diluted in sterile phosphate buffer solution (PBS, pH 7.0), spread directly onto the surface of Lactobacilli MRS agar (Difco, USA) supplemented with 1% $CaCO_3$, and incubated at 37°C for 48 h. Distinct colonies from each plate were randomly picked, purified by replating on MRS agar plates, and maintained on MRS agar slant for identification. Strains were maintained as frozen stock held at −80°C in MRS broth with 20% (v/v) glycerol. These isolates were first identified by comparing their morphological and biological characteristics. Carbohydrate fermentation pattern of the LAB isolates were also determined by using API 50 CHL identification system (bioMerieux, France). Furthermore, sequence analysis of 16S ribosomal DNA was used to classify and identify accurately the bacterial isolates. Genomic DNA was extracted from the isolates of LAB using a DNA extraction kit (Nippon Gene, Japan). Universal primers, 27F (5'-AGAGTTTGATCCTG GCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used to amplify 16S rDNA gene sequences of the isolates. 16S rDNA amplification was carried out by polymerase chain reaction (PCR, Bio-Rad Laboratories Ltd., Canada) using a PCR premix (Takara Bio, Japan). Thermal cycling was used as follows: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, for a total of 30 cycles. For sequence analysis of 16S rDNA, the PCR products were purified with QIA-quick PCR Purification System (Qiagen, USA). DNA sequencing was performed using an ABI 3730 DNA Analyzer (Applied Biosystems, USA). Sequence homologies were examined by comparing the sequences obtained from the National Center for Biotechnology Information (NCBI, USA) database.

Tolerance of the LAB strain under conditions simulating the gastric juice. Tolerance of the LAB under conditions simulating gastric juice was evaluated by a modified method of Maragkoudakis et al. (2006). Briefly, the bacterial cells from overnight (18 h) incubation of lactobacilli cultures were harvested (7,000×g for 10 min at 4°C) and washed twice with PBS buffer, pH 7.2. Initial populations were adjusted to approximately 10^8 CFU/mL. One milliliter of the bacterial suspension was then transferred into the same volume of simulated gastric juice, PBS solution (pH 2) containing NaCl (125 mM), KCl (7 mM), $NaHCO_3$ (45 mM), and pepsin (1 mg/mL; Sigma, USA). For evaluating tolerance of the LAB isolates in gastric juice after incubation at 37°C for 0.5, 1, and 2 h. Viable cell counts were determined by pour plate method on MRS agar plates.

***H. pylori* cell growth.** *H. pylori* ATCC 43504 strain used in the present study was obtained from American Type Culture Collection (ATCC). *H. pylori* strain was cultured at 37°C for 48 h in Brucella broth (Difco) supplemented with 5% (v/v) fetal bovine serum (FBS, Gibco BRL, USA), 0.2% (w/v) 2,6-di--methyl--cyclodextrin (CD), and antibiotics (cefsulodin, vancomycin, trimethoprim, and amphotericin B) kept in microaerophilic conditions (10% CO_2, Anoxomat system, MART Co., Netherland). Stock culture was kept at −80°C in broth containing 20% (v/v) glycerol until further study.

Co-culture of *H. pylori* with the LAB strain. For co-culture experiment, the LAB strains cultured for 24 h were harvested and washed twice (7,000×g, 10 min, 4°C) with sterile PBS (pH 7.2), and finally resuspended in Brain Heart Infusion (BHI) broth (Difco) at 1.0×10^5 CFU/mL. *H. pylori* cells were grown in Brucella broth containing 5% (v/v) FBS, CD, and antibiotics at 37°C under microaerobic conditions. Cell pellets of two strains collected by centrifugation (7,000×g, 10 min, 4°C) were washed twice with sterile PBS (pH 7.0). Fresh *H. pylori* cells (1.0×10^5 CFU/mL) suspended in antibiotic-free Brucella broth (10 mL) containing the LAB cells were incubated under microaerophilic condition for 12 to 48 h at 37°C. Viability of *H. pylori* was evaluated based on the number of viable cells of *H. pylori* cultured under optimal condition.

Measurements of viable cell counts, pH, and titratable acidity. Overnight cultures of the LAB strains were inoculated in MRS broth and proliferated at 37°C for 24 h. After incubation, viable

cell counts were obtained by serial dilution with sterile PBS (pH 7.0) and measured by pour plate method. pH of the cultures incubated in MRS broth for 24 h at 37°C was directly measured using a pH meter (Hanna Instruments, Italy). In addition, titratable acidity was titrated with 0.1 N NaOH using 0.1% phenolphthalein as indicator and calculated as follows: [(0.1 N NaOH consumption ×0.1 N NaOH factor×0.009×dilution rate)/sample weight]×100.

Preparation and detection of antimicrobial substances. Lactic acid in the LAB strain was determined by high performance liquid chromatography (HPLC, Shimadzu, Japan) as described by Sgouras et al. (2004) with some modifications. The cultures of the tested strains were grown in MRS broth for 24 h at 37°C. Cell-free culture supernatant (CFCS) was prepared by centrifuging (7,000×*g*, 10 min, 4°C) the cultures, precipitated protein by the addition of 1 M $HClO_4$, and filtered through a 0.22-μm membrane filter (Millipore Corp., USA). Analysis was performed with HPLC equipped with an Aminex HPX-87H column (Bio-Rad, USA, 300 mm×7.8 mm) using 5 mM H_2SO_4 as a mobile phase at a flow rate of 0.5 mL/min. Injection volume of the sample was 50 μL. Lactic acid was determined by optical density (OD) measurements at 220 nm, and quantification of lactic acid was based on the external standard method.

Hydrogen peroxide production by the LAB strains was evaluated as described by Otero and Nader-Macias (2006). MRS agar supplemented with 1 mM 3,3'5,5'-tetramethylbenzidine (TMB, Sigma) and 2 U/mL type peroxidase (Sigma) were inoculated with LAB and incubated anaerobically for 48 h at 37°C. Plates were then exposed to air for 30 min. Colonies that produced hydrogen peroxide were discolored due to TMB oxidation; blue (+++), brown (++), light brown (+) or white (–).

The LAB cultures were grown in MRS broth at 37°C until the late logarithmic phase. They were then removed by centrifugation for 10 min at 7,000×*g*, and the supernatants were collected. One aliquot of the supernatants was adjusted to pH 6.5 with 1 N NaOH. Ammonium sulfate was gradually added to achieve 50% saturation. The samples were kept at 4°C with stirring for 12 h, and centrifuged for 30 min at 12,000×*g*, followed by filtration of the supernatant through a 0.45-μL pore size filter. For preparation of the bacteriocin solution (BS), the resulting pellet was solubilized in 20 mM sodium phosphate buffer (pH 6.5) and dialyzed for 12 h at 4°C using spectra-por dialysis membrane (molecular weight cut-off, 1,000; Spectrum Medical Industries, Inc., USA). Hydrogen peroxide was removed by the addition of catalase (1 mg/mL, Sigma) at 25°C for 30 min. Spot-on-lawn method as described by Martin et al. (2003) was used to detect the antimicrobial spectrum of the LAB-BS. *H. pylori*, *Listeria monocytogenes* KCTC 3569, and *Salmonella enteritidis* ATCC 13076 used as the indicator strains were propagated on selective medium. BS of the LAB strains were spotted (1 mm) onto the surface of the agar (0.8%, w/v) plates of indicator strains. After incubation for 48 h at 37°C, inhibition zone formed on plate was confirmed.

Antagonistic activity of CFCS and BS. Antimicrobial activity of CFCS obtained from the LAB strain was screened using agar-well diffusion method (Motta and Brandelli, 2002). In brief, BHI agar plates were overlaid with BHI top agar (0.8%, w/v) and inoculated with the culture of *H. pylori* (1.0×10^5 CFU/mL). After agar solidification, the well of 8-mm diameter was cut into agar plates, and 50 μL of CFCS were added to each well. Plates were then incubated for 48 h at 37°C and size of growth-free inhibition zones around the well was estimated.

Antimicrobial activity of BS obtained from the LAB strain was screened using microtiter plate assay (Holo et al., 1991). Reaction mixtures in 24-well plate (Falcon, USA) were comprised of the appropriate growth medium for *H. pylori* (100 μL), 2-fold serially diluted BS (100 μL), and the pathogenic culture inoculums (100 μL), which were separated in BHI broth to achieve a final population of 10^5 CFU. After incubation for 24 h at 37°C, the extent of growth inhibition was determined using OD_{600} measurement. Bacteriocin activity was defined as the reciprocal of the highest dilution inhibiting the 50% growth of the indicator strain. The results for the bacteriocin activity were presented in arbitrary units per milliliter (AU/mL).

Preparation of AGS cell line culture. Human gastric adenocarcinoma epithelial cell line AGS (ATCC CRL 1739) was purchased from ATCC. AGS cells were routinely cultured to confluence in tissue culture flasks (Falcon) in RPMI 1640 medium (Gibco) including L-glutamine, $NaHCO_3$, kanamycin (60 μg/mL), and streptomycin (20 μg/mL) supplemented with 10% (v/v) FBS in a 5% CO_2 incubator at 37°C for 3 days. For replating, the monolayers were detached for 5 min using trypsin-EDTA and then washed twice with PBS (pH 7.0). The cells were seeded in flat-bottomed 24-well plates in complete culture medium approximately 5×10^4 cells/mL and grown at 37°C in the presence of 5% CO_2/95% air atmosphere.

Adhesion assay. When the cells became confluent, the monolayers on the tissue culture plates were washed three times with PBS (pH 7.0). Culture medium was replaced with fresh RPMI 1640 without antibiotics for 2 h before the inoculation of bacteria. Prior to the adhesion assay, the cells of *H. pylori* strains incubated in Brucella broth containing 10% (v/v) FBS were washed twice with PBS (pH 7.0) and collected by centrifugation for 5 min at 7,000×*g*. One hundred microliters of viable *H. pylori* at 1×10^8 CFU/mL was added to each well with non-supplemented RPMI 1640 and incubated for 2 h to allow *H. pylori* adhesion to AGS cells. After incubation, the cells in each well were washed three times with sterile PBS. One milliliter of fresh RPMI 1640 medium containing CFCS or BS obtained from the LAB strains was added to each well in triplicate and allowed to incubate for 2 h at 37°C in 5% CO_2 to exclude adherent *H. pylori* from AGS cells. Subsequently, each well was washed five times to remove nonadherent *H. pylori*. AGS cells were then lysed with 1 mL of 0.1% Triton X-100. Cell counts of *H. pylori* adhered to AGS cells were estimated by pour plate method using Brucella agar plates added with 10% FBS. Number of treated cells that adhered to AGS cells was expressed as a percentage compared to the number of untreated cells that adhered to AGS cells.

Urease assay. Effects of CFCS and BS on the urease activity of *H. pylori* adhering to AGS cells were determined by a modified phenol red method (Sgouras et al., 2004). Treatment conditions of CFCS and BS against *H. pylori* adhered to AGS cells were the same as those described previously for the exclusion assays. briefly, after washing the cells five times with PBS (pH 7.0), *H. pylori* cell suspension (10 μL) was added to 300 μL of urease reaction buffer [20% (w/v) urea and 0.012% phenol red in PBS, with the final pH adjusted to 6.5] on a microtiter plate. Subsequently, the plates were incubated for 1 h at 37°C, to allow the *H. pylori* to produce ammonia. Finally, OD value was measured at 550 nm with a microplate reader (Packard Instruments, USA).

Statistical analyses. All measurements were performed in triplicate. Results are expressed as means± standard deviation. The paired *t*-test was employed using SPSS software (ver. 12.0, SPSS Inc., USA), for comparisons of each variable across the study phases. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Identification of the LAB obtained from Baikkimchi. A total of 13 strains of the LAB were isolated from various home-made Baikkimchi samples and selected due to their distinct colony in MRS agar plates. In morphological and physiological characteristics, the LAB strains were found to belong to *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Enterococcus* spp., and *Lactococcus* spp. These strains were biochemically characterized using API 50 CHL systems in order to discriminate different LAB genera. Some isolates were identified mainly as *Lactobacillus brevis*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactococcus lactis*, and *Lactobacillus salivarius*, with high similarity (>99%) based on carbohydrate fermentation pattern. However, BK10, BK13, and BK24 strains showed relatively low confidence in sugar utilization. Furthermore, molecular identification of the isolates exhibited more than 98% identity with the related strains in GenBank. Based on database entries, these 13 strains were recognized as *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *Lactobacillus sakei* BK24, *Leuconostoc mesenteroides* BK26, *P. pentosaceus* BK34, *L. plantarum* subsp. *plantarum* BK42, *Lactobacillus curvatus* BK48, *L. pentosus* BK50, *Lactobacillus paracasei* BK57, *Enterococcus faecalis* BK61, *L. lactis* BK65, and *L. salivarius* BK73 (Table 1). LAB originated from kimchi play an important role in the taste and sensory qualities of products owing to the production of organic acids (lactic and acetic acids) and flavoring compounds (mannitol, amino acids) during fermentation (Jung et al., 2014). Over 100 species of microorganisms were identified in fermented kimchi. Diverse LAB including *L. mesenteroides*, *Leuconostoc citreum*, *Leuconostoc carnosum*, *Leuconostoc inhae*, *Leuconostoc gelidum*, *Lactobacillus kimchi*, *Lactobacillus miyukkimchii*, *L. sakei*, *Lactobacillus fermentum*, *L. plantarum*, *L. brevis*, *L. curvatus*, *Weisella koreensis*, *Weisella cibaria*, *Weisella kimchi*, *Weisella soli*, and *Weisella confuse* have been isolated from kimchi (Rhee et al., 2011; Jung et al., 2014). Many LAB strains associated with kimchi show health benefit and functions in addition to other useful properties, e.g., antimutagenic, immunostimulatory, antioxidant abilities, and antagonistic activity against various pathogens including *H. pylori*, as well as intestinal regulation (Lee et al., 2011).

Tolerance under conditions simulating the gastric juice. Effects of simulated gastric juices on the viability of the 13 LAB strains were evaluated. Viable counts of *L. plantarum* BK10, *L. sakei* BK24, *P. pentosaceus* BK34, *L. curvatus* BK48, *L. pentosus* BK50, *E. faecalis* BK61, *L. lactis* BK65, and *L. salivarius* BK73 dropped dramatically when the strains were exposed to simulated gastric juices for 2 h (Table 2). However, relatively good growth

Table 1 Identification of the LAB strains isolated from Baikkimchi according to API 50 CHL kit and 16S rDNA sequencing

Strain	API 50CHL		16S rDNA sequencing			Identification
	Species affiliation	Confidence (%)	Related strain in NCBI	Accession No.	Similarity (%)	
BK10	*Lactobacillus pentosus*	95.8	*Lactobacillus plantarum*	KJ026622	99.6	*Lactobacillus plantarum* BK10
BK11	*Lactobacillus brevis*	99.2	*Lactobacillus brevis*	EU147302	99.9	*Lactobacillus brevis* BK11
BK13	*Lactobacillus acidophilus*	95.0	*Lactobacillus acidophilus*	AB650590	98.0	*Lactobacillus acidophilus* BK13
BK24	*Lactobacillus curvatus*	92.5	*Lactobacillus sakei*	KJ558388	96.3	*Lactobacillus sakei* BK24
BK26	*Leuconostoc mesenteroides*	98.4	*Leuconostoc mesenteroides*	JQ658346	98.6	*Leuconostoc mesenteroides* BK26
BK34	*Pediococcus pentosaceus*	99.9	*Pediococcus pentosaceus*	JN036551	100	*Pediococcus pentosaceus* BK34
BK42	*Lactobacillus plantarum*	99.0	*Lactobacillus plantarum* subsp. *plantarum*	AB713898	98.9	*Lactobacillus plantarum* subsp. *plantarum* BK42
BK48	*Lactobacillus curvatus*	97.9	*Lactobacillus curvatus*	KC753455	100	*Lactobacillus curvatus* BK48
BK50	*Lactobacillus pentosus*	99.5	*Lactobacillus pentosus*	KF923751	99.5	*Lactobacillus pentosus* BK50
BK57	*Lactobacillus casei*	98.9	*Lactobacillus paracasei*	AB182642	99.2	*Lactobacillus paracasei* BK57
BK61	*Enterococcus faecium*	98.6	*Enterococcus faecalis*	HM776194	99.7	*Enterococcus faecalis* BK61
BK65	*Lactococcus lactis*	99.9	*Lactococcus lactis*	JX267125	99.9	*Lactococcus lactis* BK65
BK73	*Lactobacillus salivarius*	99.8	*Lactobacillus salivarius*	EF412986	99.0	*Lactobacillus salivarius* BK73

Table 2 Effect of simulated gastric juices on the viability of the selected LAB strains

Strain	Viable cell counts (CFU/mL)			
	Exposure time (h)			
	0	0.5	1	2
Lactobacillus plantarum BK10	$7.2\pm0.5\times10^{8}$	$9.2\pm2.5\times10^{7}$	$6.1\pm2.5\times10^{5}$	$4.8\pm2.6\times10^{3}$
Lactobacillus brevis BK11	$3.7\pm2.4\times10^{8}$	$4.0\pm2.6\times10^{7}$	$7.8\pm5.3\times10^{6}$	$5.5\pm3.2\times10^{6}$
Lactobacillus acidophilus BK13	$5.1\pm4.3\times10^{8}$	$2.5\pm2.1\times10^{7}$	$8.3\pm3.2\times10^{7}$	$3.0\pm2.3\times10^{7}$
Lactobacillus sakei BK24	$2.5\pm2.2\times10^{8}$	$7.9\pm1.5\times10^{6}$	$3.6\pm2.3\times10^{4}$	$1.8\pm0.7\times10^{3}$
Leuconostoc mesenteroides BK26	$3.4\pm0.3\times10^{8}$	$9.0\pm7.6\times10^{7}$	$6.5\pm1.4\times10^{6}$	$2.0\pm1.2\times10^{6}$
Pediococcus pentosaceus BK34	$5.0\pm2.8\times10^{8}$	$5.2\pm3.8\times10^{5}$	$5.2\pm0.9\times10^{4}$	$2.5\pm2.3\times10^{2}$
Lactobacillus plantarum subsp. *plantarum* BK42	$7.2\pm1.9\times10^{8}$	$1.7\pm1.0\times10^{6}$	$4.8\pm2.8\times10^{5}$	$6.1\pm4.4\times10^{4}$
Lactobacillus curvatus BK48	$6.4\pm2.5\times10^{8}$	$4.8\pm3.5\times10^{4}$	$2.6\pm3.2\times10^{3}$	$4.2\pm3.6\times10^{3}$
Lactobacillus pentosus BK50	$2.6\pm0.7\times10^{8}$	$5.5\pm4.3\times10^{3}$	$2.5\pm0.9\times10^{2}$	$<10^{2}$
Lactobacillus paracasei BK57	$4.2\pm3.6\times10^{8}$	$2.1\pm2.8\times10^{7}$	$5.0\pm3.6\times10^{6}$	$6.7\pm2.5\times10^{5}$
Enterococcus faecalis BK61	$5.3\pm2.0\times10^{8}$	$8.0\pm4.8\times10^{6}$	$6.3\pm2.9\times10^{4}$	$5.1\pm3.7\times10^{3}$
Lactococcus lactis BK65	$6.5\pm3.3\times10^{8}$	$7.0\pm2.7\times10^{4}$	$8.3\pm7.0\times10^{2}$	$<10^{2}$
Lactobacillus salivarius BK73	$4.3\pm1.5\times10^{8}$	$4.2\pm3.7\times10^{5}$	$9.5\pm6.3\times10^{3}$	$4.4\pm0.4\times10^{3}$

properties were found for *L. brevis* BK11, *L. acidophilus* BK13, *and L. mesenteroides* BK26 strains with residual numbers of $>10^{6}$ CFU/mL after incubation for 2 h in artificial gastric juice containing pepsin at pH 2.0. Therefore, these three strains had the ability to adapt to acidic environmental condition, which will thereby allow them to act as protective barriers against the pathogen in gastrointestinal tract.

Acid tolerance of these LAB strains may be an important factor in inhibition of the pathogen associated with gastritis, gastroduodenal ulcers, and gastric malignancies (Maragkoudakis et al., 2006). Tolerance mechanisms of LAB to acid originated from the presence of a constant gradient between extracellular and cytoplasmic pH by F_0F_1-ATPase proton pump and the alkalization of external environment with amino acid decarboxylation. On the other hand, the impairment of proton pumps and the inactivation of H^+-ATPase observed in sensitive strains to acid can cause disruption in the normal cellular function of the microorganisms and therefore lead to cell death (Lorca et al., 2001).

Viability of LAB was strain-dependent manner in low pH of gastric juice included intestinal enzymes. Maragkoudakis et al. (2006) demonstrated that a wide variation in survival was observed when the strains were subjected to the pepsin solution at pH 2. After 1 h, highest survival was observed with *L. casei* Shirota ACA-DC 6002, *L. casei* Imunitass ACA-DC 6003 (<1.0 log cycle reduction). Strains *L. paracasei* subsp. *paracasei* ACA-DC 130, *L. paracasei* subsp. *tolerancs* ACA-DC 196, *L. rhamnosus* ACA-DC 112, and *Lactobacillus* sp. ACA-DC displayed reductions ranging between 1.0 and 2.3 log cycles, whereas *L. paracasei* subsp. *paracasei* ACA-DC 117 and 118 strains displayed a loss of viability >2.5 log cycles. *Lactobacillus* strains were able to retain their viability when exposed to pH values of 2.5–4.0, but displayed loss of viability at lower pH values.

Inhibition of *H. pylori* growth by co-culture with LAB strain. To understand the viability of *H. pylori* co-cultured with or without the live LAB strains, a time-kill assay was performed. A 3-log decrease of *H. pylori* viability occurred after 24 h of contact with *E. faecalis* BK61, whereas the cells of *H. pylori* were similar with the control after treatment of *L. sakei* BK24 and *L. plantarum* subsp. *plantarum* BK42. Although the number of pathogen elevated in the negative control without co-culturing the LAB after 48 h incubation, *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. paracasei* BK57, *E. faecalis* BK61, and *L. lactis* BK65 strains showed significant antimicrobial ability against *H. pylori*. After 48 h contact of *L. plantarum* BK10 or *E. faecalis* BK61 with *H. pylori*, a dramatic decline in viable counts for the pathogen cells was observed (Table 3). These results showed that two LAB strains, *L. plantarum* BK10 and *E. faecalis* BK61, had better anti-*Helicobacter* effects than the other strains according to the time-kill assay.

Antibiotics treatment for *H. pylori* eradication has been reported to give rise to side-effects such as disturbance of normal oropharyngeal and intestinal microflora and emergence of resistant microbial strains (Adamsson et al., 2000). Recently, probiotics which might help to inhibit or reduce the growth of *H. pylori* were superior to placebo for the prevention of drug-related side-effects during this pathogen eradication therapy (Felley and Michetti, 2003). Because probiotic species such as lactobacilli and bifidobacteria, or their metabolic products, have been shown to be antagonistic to *H. pylori* both *in vitro* and *in vivo*, probiotics have attracted attention as the inhibitory and/or bactericidal factor against *H. pylori* (Bhatia et al., 1989). Therefore, probiotics play a role in the inhibition of its adherence to gastric epithelial cells as well as *H. pylori* growth, the stabilization of the gastric barrier function, and the decrease of mucosal inflammation (Gotteland et al., 2006).

H. pylori CCUG 17874 in mixed cultures with *L. acidophilus* CRL 639 showed a decrease of 2 log-cycle, and no viable count

Table 3 Live cell counts of *H. pylori* in the co-culture with the LAB strains

LAB co-cultured with *H. pylori*	Cell counts of *H. pylori* (CFU/mL)			
	Co-incubation time (h)			
	0	12	24	48
Control	$5.6\pm2.2\times10^5$	$9.0\pm1.5\times10^5$	$4.1\pm2.3\times10^6$	$6.5\pm2.4\times10^8$
Lactobacillus plantarum BK10	$4.6\pm0.8\times10^5$	$4.1\pm2.8\times10^5$	$5.2\pm3.5\times10^4$	$2.5\pm2.4\times10^3$
Lactobacillus brevis BK11	$5.0\pm2.8\times10^5$	$8.3\pm3.9\times10^4$	$1.0\pm2.4\times10^5$	$6.5\pm2.2\times10^4$
Lactobacillus acidophilus BK13	$3.8\pm2.2\times10^5$	$1.9\pm3.5\times10^5$	$4.2\pm1.0\times10^4$	$7.5\pm1.7\times10^4$
Lactobacillus sakei BK24	$6.1\pm1.4\times10^5$	$7.1\pm2.2\times10^5$	$5.3\pm5.0\times10^6$	$2.7\pm4.5\times10^7$
Leuconostoc mesenteroides BK26	$5.8\pm2.1\times10^5$	$6.2\pm5.2\times10^5$	$4.7\pm5.6\times10^5$	$5.3\pm4.7\times10^7$
Pediococcus pentosaceus BK34	$5.7\pm3.0\times10^5$	$4.0\pm2.1\times10^5$	$7.6\pm2.8\times10^4$	$4.2\pm3.5\times10^5$
Lactobacillus plantarum subsp. *plantarum* BK42	$4.1\pm1.0\times10^5$	$8.0\pm1.8\times10^5$	$7.2\pm3.3\times10^6$	$8.9\pm3.6\times10^7$
Lactobacillus curvatus BK48	$7.4\pm2.5\times10^5$	$5.1\pm2.4\times10^5$	$6.3\pm4.6\times10^5$	$4.9\pm5.5\times10^7$
Lactobacillus pentosus BK50	$4.3\pm1.4\times10^5$	$9.8\pm4.6\times10^4$	$5.3\pm1.5\times10^5$	$1.1\pm2.7\times10^7$
Lactobacillus paracasei BK57	$2.7\pm0.6\times10^5$	$5.0\pm4.3\times10^4$	$5.6\pm3.4\times10^4$	$5.9\pm6.1\times10^4$
Enterococcus faecalis BK61	$5.4\pm1.9\times10^5$	$3.1\pm4.4\times10^5$	$6.6\pm3.9\times10^3$	$8.2\pm4.5\times10^3$
Lactococcus lactis BK65	$7.1\pm3.8\times10^5$	$5.2\pm3.1\times10^4$	$8.6\pm2.8\times10^4$	$4.3\pm6.6\times10^5$
Lactobacillus salivarius BK73	$4.5\pm2.3\times10^5$	$1.4\pm3.6\times10^5$	$6.0\pm1.4\times10^5$	$6.2\pm4.8\times10^6$

Helicobacter pylori cultures maintained in Brucella medium without the LAB strain were used as a control.
Data were presented as mean ± SD from three independent experiments.

was detected after 48 h. Therefore, the bactericidal effect of the LAB strain is related to a proteinaceous compound released after cell lysis (Lorca et al., 2001). The inhibitory activity of LAB strains against *H. pylori* strains was strain-dependent. *L. acidophilus*, *Lactobacillus johnsonii*, *L. salivarius*, *L. plantarum*, *L. casei*, *L. GG*, *L. gasseri*, and *L. casei* subsp. *rhamnosus* inhibited *H. pylori* growth, whereas *Bifidobacterium bifidus*, *P. pentosaceus*, and *Lactobacillus bulgaricus* was not (Midolo et al., 1995). *In vivo*, *H. pylori* eradiation rate has been shown to improve in connection with administration of lactobacilli and *L. gasseri* OLL 2716 and *L. acidophilus* La1 decreased the ^{13}C urea breath test values and the gastric mucosal inflammation (Sullivan and Nord, 2002).

Biological and physicochemical characteristics of the LAB cultures. The cell counts, pH, and titratable acidity during LAB culturing, and the antagonistic activity of the antimicrobial substances obtained from these LAB cultures are shown in Table 4. After 24 h of incubation, the cell counts of all LAB strains increased to a level higher than 7-log units. Among these strains, the viable counts of strains *L. brevis* BK11, *P. pentosaceus* BK34, and *E. faecalis* BK61 showed higher growth ability compared with the other strains. Owing to the utilization of carbohydrates in fermentation, pH values of culture of all LAB strains after incubation for 24 h progressively decreased to pH 3.55–4.45, whereas titratable acidity increased continuously with incubation time. *L. plantarum* BK10, *L. brevis* BK11, *P. pentosaceus* BK34, and *L. paracasei* BK57 strains had lower pH values and higher titratable acidity than other tested strains. In particular, the cultures of *L. brevis* BK11 and *L. paracasei* BK57 contained higher concentration (more than 100 mM) of lactic acid than the other strains. The decline in pH value and the increase in titratable acidity were associated with the production of organic acids.

Furthermore, *L. plantarum* subsp. *plantarum* BK42, *E. faecalis* BK61, and *L. salivarius* BK73 strains produced hydrogen peroxide of high concentrations, but the CFCSs of these strains did not show antimicrobial effects. The CFCSs of *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. paracasei* BK57, and *L. lactis* BK65 strains after 24 h of incubation were found to possess the antagonistic activity to inhibit the growth of *H. pylori*. Mean diameters of the inhibition zones for these LAB strains ranged from 8.8 mm (*P. pentosaceus* BK34) to 13.0 mm (*L. acidophilus* BK13). Although the pH values of *L. sakei* BK24, *L. mesenterodies* BK26, *L. plantarum* subsp. *plantarum* BK42, *L. curvatus* BK48, *L. pentosus* BK50, *E. faecalis* BK61, and *L. salivarius* BK73 were relatively low, the CFCSs of these strains were ineffective for *H. pylori* inhibition.

Only 7 strains of the LAB strains (*L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. plantarum* subsp. *plantarum* BK42, *L. paracasei* BK57, *E. faecalis* BK61) were shown to produce the bacteriocin having the antagonistic activity against *L. monocytogenes*, *S. enteritidis*, and *H. pylori*. Among these strains, the BSs produced from *P. pentosaceus* BK34 and *L. plantarum* subsp. *plantarum* BK42 were active against *L. monocytogenes* and *S. enteritidis* but did not show the inhibitory action against *H. pylori*. However, the bacteriocin produced from *E. faecalis* BK61 exhibited the most efficient effects (1,024 AU/mL) on *H. pylori* inhibition. The bacteriocin activity of *L. brevis* BK11 (512 AU/mL) also exhibited higher inhibition capacity on *H. pylori* than *L. plantarum* BK10, *L. acidophilus* BK13, and *L. paracasei* BK57. Comparing the relationship between organic acid production and bactericidal activity, some strains which produced higher concentrations of lactic acid and titratable acidity showed strong inhibitory effect

Table 4 Biological and physicochemical characteristics of the LAB cultures and inhibitory effects of the antimicrobial substances obtained from the LAB strain against *H. pylori* ATCC 43504

Strain	Viable cell count (CFU/mL)	pH	Titratable acidity (%)	Activity of antimicrobial substance				
				Lactic acid (mM)	Hydrogen peroxide	Antimicrobial activity of CFCS (mm)[a]	BS	
							Spectrum[b]	Antimicrobial activity against *H. pylori* (AU/mL)
Lactobacillus plantarum BK10	$3.5±1.8×10^8$	3.88±0.15	1.25±0.09	62.5±16.1	+++	10.2	L/S/H	128
Lactobacillus brevis BK11	$6.1±3.5×10^9$	3.55±0.12	1.48±0.11	123.5±20.5	+	11.8	S/H	512
Lactobacillus acidophilus BK13	$2.4±0.2×10^8$	3.91±0.09	0.89±0.09	85.7±11.2	++	13.0	L/H	64
Lactobacillus sakei BK24	$4.9±2.6×10^7$	4.01±0.25	0.89±0.25	30.3±9.2	-	ND[c]	ND	ND
Leuconostoc mesenteroides BK26	$7.1±3.4×10^8$	4.20±0.21	0.74±0.21	47.6±7.0	-	ND	ND	ND
Pediococcus pentosaceus BK34	$8.6±0.9×10^9$	3.72±0.13	1.39±0.13	95.8±13.3	++	8.8	L/S	ND
Lactobacillus plantarum subsp. *plantarum* BK42	$5.6±3.7×10^8$	4.13±0.08	0.77±0.08	66.2±7.5	+++	ND	L	ND
Lactobacillus curvatus BK48	$6.7±3.6×10^7$	4.22±0.14	0.70±0.14	51.8±8.1	-	ND	ND	ND
Lactobacillus pentosus BK50	$7.0±3.4×10^8$	4.07±0.16	0.75±0.16	60.2±14.0	-	ND	ND	ND
Lactobacillus paracasei BK57	$8.7±5.5×10^8$	3.60±0.08	1.34±0.08	114.3±8.3	++	12.0	L/S/H	256
Enterococcus faecalis BK61	$1.2±0.4×10^9$	4.45±0.24	0.64±0.24	54.0±10.6	+++	ND	S/H	1,024
Lactococcus lactis BK65	$7.9±2.5×10^8$	3.76±0.17	1.22±0.17	73.2±11.4	+	10.8	ND	ND
Lactobacillus salivarius BK73	$3.6±5.2×10^8$	3.93±0.10	0.93±0.10	44.9±13.5	++	ND	ND	ND

Data were presented as mean ± SD from three independent experiments.
[a]Mean inhibitory zone diameters including well diameter (8 mm).
[b]Antimicrobial spectrum of the BS obtained from the LAB strains against *Listeria monocytogenes* KCTC 3569 (L), *Salmonella enteritidis* ATCC 13706 (S), and *H. pylori* ATCC 43504 (H).
[c]ND, not detected.

against the growth of *H. pylori*. Furthermore, the anti-*Helicobacter* activity of several strains was related to bacteriocin, but not due to presence of organic acids and hydrogen peroxide. Namely, the CFCSs obtained from *L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. paracasei* BK57, and *L. lactis* BK65 strains producing very high levels of lactic acid dramatically decreased the viability of *H. pylori*. In addition, the bactericidal activities of *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *L. paracasei* BK57, and *E. faecalis* BK61 strains were significantly correlated with the bacteriocin production.

Coconnier et al. (1998) noted that the supernatants of the human *L. acidophilus* strain LB dramatically decreased the viability of *H. pylori in vitro* and *in vivo*, depending on pH and lactic acid level. Recent studies have shown that probiotic LAB may inhibit the growth of *H. pylori* both *in vitro* and *in vivo* as well as undesirable pathogens causing diarrhea and other diseases in the human intestinal tract (Sgouras et al., 2004). These inhibitory substances (lactic acid, acetic acid, short-chain fatty acids, alcohol, hydrogen peroxide, bacteriocins, and other metabolites) produced from the probiotic bacteria may reduce not only the number of *H. pylori* cells but may also affect bacterial metabolism (Jack et al., 1995; Otero and Nader-Macias, 2006). There are many proposed mechanisms by which probiotics may protect the host against intestinal disease caused by *H. pylori*. Competitive inhibition for the adhesion sites of pathogens on intestinal epithelial surfaces and degradation of the bacterial receptor on the intestinal mucosa are another mechanisms of action for some probiotic strains. Competition for nutrients with pathogenic microorganisms has been proposed as antimicrobial mechanism of probiotics (Lesbros-Pantoflickova et al., 2007). In addition, the possible mechanisms included suggest that probiotics may stimulate the specific and non-specific immunity, inhibit *H. pylori* urease enzyme, and disrupt the bacterial cell membrane (Vitor and Vale, 2011).

Previous studies have indicated that the inhibitory role of the LAB strains against *H. pylori* was related to the acid production and low pH attained (Tsai et al., 2004; El-Adawi et al., 2013). Midolo et al. (1995) reported that lactic, acetic, and hydrochloric acids demonstrated inhibition of *H. pylori* growth in a concentration-dependent manner with the lactic acid demonstrating the greatest inhibition. Bhatia et al. (1989) found that lactic acid (about 100 mmol/L), the major by-product of the fermentation, was sufficient to inhibit the growth of *H. pylori* by decreasing pH. Aiba et al. (1998) demonstrated that as the amount of lactic acid increased, the number of *H. pylori* decreased in parallel with the increase in the number of colonizing *L. salivarius*. On the other hand, the inhibitory effect of *Lactobacillus* spp. against *H. pylori* strains was lost after neutralization of the supernatants *Lactobacillus* indicating pH-mediated effect (Medouakh et al., 2010).

H. pylori possess active catalase, which neutralize hydrogen peroxide (Medouakh et al., 2010). Andrzejewska and Szkaradkiewicz (2007) reported that the strains of *L. acidophilus* induce variable

Table 5 Effects of CFCS and BS obtained from the LAB strains on the adhesion ability and urease activity of *H. pylori* to human gastric epithelial AGS cells

Strain	Concentration of CFCS (μL/mL)				Concentration of BS (AU/mL)					
	100		200		256		512		1,024	
	Relative adhesion (%)	Urease activity	Relative adhesion (%)	Urease activity	Relative adhesion (%)	Urease activity	Relative adhesion (%)	Urease activity	Relative adhesion (%)	Urease activity
Control	100±8.1	0.32±0.02	100±9.0	0.30±0.02	100±11.5	0.31±0.02	100±5.8	0.30±0.03	100±7.1	0.32±0.03
Lactobacillus plantarum BK10	81.7±5.6	0.31±0.02	64.6±6.5*	0.26±0.01	69.4±6.9*	0.27±0.03	55.5±6.1*	0.26±0.03	31.6±4.2*	0.22±0.03*
Lactobacillus brevis BK11	50.6±8.3*	0.24±0.03*	28.1±4.3*	0.19±0.01*	ND	ND	ND	ND	77.4±8.7*	0.28±0.02
Lactobacillus acidophilus BK13	20.1±6.1*	0.16±0.02*	13.3±6.0*	0.14±0.03*	50.2±7.7*	0.25±0.01*	40.2±10.5*	0.22±0.01*	24.2±6.9*	0.16±0.02*
Pediococcus pentosaceus BK34	60.5±10.1*	0.26±0.03*	33.7±6.1*	0.20±0.02*	ND	ND	ND	ND	ND	ND
Lactobacillus paracasei BK57	46.1±7.0*	0.24±0.01*	28.5±8.2*	0.18±0.02*	ND	ND	95.6±10.0	0.29±0.03	46.4±7.4*	0.24±0.02*
Enterococcus faecalis BK61	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lactococcus lactis BK65	32.2±5.5*	0.20±0.01*	15.8±5.9*	0.13±0.01*	ND	ND	ND	ND	ND	ND

Data were presented as mean ± SD from three independent experiments.
*Statistical analysis performed with a Student *t*-test between control and treated *H. pylori* with the CFCS or BS showed a highly significant difference (p <0.05).
ND, not detected.

inhibition of growth in clinical isolates of *H. pylori*, but there is no significant differences between *H. pylori* growth inhibition induced by *L. acidophilus* strains, which produced or did not produce hydrogen peroxide (p >0.05). It is also unlikely that the production of H_2O_2 by *L. salivarius* UCC 119 was responsible for its anti-*H. pylori* activity, which was similar to the result of Ryan et al. (2008).

On the contrary, the growth inhibition of *H. pylori* was not due to lactic acid production by the lactobacilli, because there was no correlation between pH reduction achieved in the medium and the ability to inhibit growth of *H. pylori*. The bacteriocin obtained from probiotic LAB strains may be considered as a novel source for the control of microbial pathogens surviving in the human gastrointestinal tract, for instance towards *H. pylori* (De Vuyst and Leroy, 2007). A bacteriocin-like inhibitory substance with anti-*H. pylori* activity was identified in probiotic *L. johnsonii* strain LA1 and *L. acidophilus* strains LB (Coconnier et al., 1998). Lacticin A164 produced by *Lactococcus lactis* subsp. *lactis* A164 and lacticin BH5 produced by *L. lactis* BH5 inhibited the growth of *H. pylori in vitro* and may thus be used in the treatment of peptic ulcers (Piard et al., 1992). When assessed by the critical dilution micro-method, minimum inhibitory concentrations (MICs) of the lacticins against *H. pylori* ranged from 0.097 to 0.390 mg/L (DSM strains) or from 12.5 to 25 mg/L (ATCC 43504), supporting the strain-dependent sensitivity of the pathogen (Kim et al., 2003).

Effects of CFCS and BS on the urease activity and the adhesion of *H. pylori* to AGS cells. When the CFCS and BS obtained from the strains having antimicrobial activity against *H. pylori* were evaluated with regard to their ability to inhibit the adhesion of *H. pylori* to human AGS gastric epithelial cells, a significant inhibition of *H. pylori* attachment by these antimicrobial substances was observed (Table 5). The adhesion of *H. pylori* to AGS cells without interruption by the LAB strain was assigned as 100%. *H. pylori* adhesion was reduce significantly by the CFCS or BS obtained from the LAB strains having the antimicrobial activity. Notably, the CFCS of *L. acidophilus* BK13 exhibited the most potent anti-adhesive effects against *H. pylori* at the concentration of 100 μL/mL. In addition, the significant inhibition of the bacterial attachment was observed with a reduction of above 50% by the CFCSs (100 μL/mL) of *L. lactis* BK65 and *L. paracasei* BK57 strains. Although the CFCS of *L. plantarum* BK10 significantly showed the antagonistic effect against *H. pylori*, this strain exhibited lower inhibition activity in adherence of the pathogen compared with the other strains. Meanwhile, the BSs (1,024 AU/mL) obtained from four LAB strains, *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, and *L. paracasei* BK57 significantly reduced the number of *H. pylori* adhering to the monolayers of cultured gastric epithelial AGS cells (p <0.05). Specially, the anti-adhesion ability of the BSs produced from *L. plantarum* BK10 and *L. acidophilus* BK13 against *H. pylori* was significantly higher than those of *L. brevis* BK11 and *L. paracasei* BK57. However, the BSs of *P. pentosaceus* BK34, *E. faecalis* BK61, and *L. lactis* BK65 strains did not show inhibition effects for *H. pylori* infection of AGS cells. The adhesion ability of *H. pylori* to AGS cells was significantly decreased by increased concentration of the CFCS and BS (p <0.05).

Furthermore, after treatment with the CFCS or BS, the urease activity of *H. pylori* adhering to AGS cells was examined. After 2 h incubation of *H. pylori* with the CFCS of several strains (*L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. paracasei* BK57, and *L. lactis* BK65), the urease activity of *H. pylori* adhered to AGS cells was significantly reduced (p <0.05). The BSs of *L. plantarum* BK10, *L. acidophilus* BK13, *L. paracasei* BK57 were predominant species which can express high inhibitory effects on the urease activity of *H. pylori* (0.16±0.02), whereas that of by BS (1,024 AU/mL) of *L. acidophilus* BK13 was

decreased by more than half of the control (0.32±0.03). The BSs of *P. pentosaceus* BK34, *E. faecalis* BK61, and *L. lactis* BK65 did not show any effect on the urease activity of *H. pylori*. These findings further imply that the CFCS and BS of the strains showed the antagonistic activity except for *E. faecalis* BK61 were effective in inhibiting the adhesion of *H. pylori* to AGS cells and their urease activity.

H. pylori possibly interacts with epithelial cells through secretory components, thus the adhesion of *H. pylori* to epithelial cells is important in determining the outcome in *H. pylori*-associated diseases (Coconnier et al., 1997). Some studies showed that *L. johnsonii*, *L. salivarius*, and *L. acidophilus* inhibit the attachment of *H. pylori* to intestinal HT-29 cells or to MKN 45 gastric cell lines (Lin et al., 2009; Jung et al., 2014). Although all six *H. pylori* isolates were inhibited by *L. salivarius* UCC 119, the degree of inhibition varied among strains. It is likely that the effectiveness of *L. salivarius* in the eradication of *H. pylori* is due to its high affinity for binding to gastric epithelial cells, thereby enabling to produce a sufficient amount of lactic acid to interfere with the infection of *H. pylori* (Ryan et al., 2008). There are several possible mechanisms by which LAB can inhibit the adhesion of *H. pylori*: the inhibition binding of *H. pylori* to specific glycolipid receptors, the switching of *Bab*A gene expression of *H. pylori*, which facilitates bacterial colonization and augments a nonspecific immune response, and the integrity of the host cells, which prevents the adhesion of *H. pylori* (Mukai et al., 2002; El-Adawi et al., 2013).

El-Adawi et al. (2013) noted that the pretreatment of *H. pylori* with the most potent antibacterial extracts of *L. plantarum*, *L. fermentum*, *L. bulgaricus* DSMZ 20080, and *S. lactis* prevent the remaining viable *H. pylori* from adhering to cell lines. Both *L. helveticus* CU631 and CFCS had a strong inhibitory activities in urease and cytotoxin- producing activities of *H. pylori* NCTC 11637 and CJH12 (Yoon and Won, 2002). Lin et al. (2011) suggested that in the AGS cell culture test, both *L. acidophilus* LY5-CFCS and artificial LY5-CFCS significantly reduced *H. pylori* infection and urease activity (p <0.05).

Urease produced by *Helicobacter* catalyzes the hydrolysis of urea into bicarbonate and ammonia, which allows the bacterial survival by neutralizing the acidic environment. These metabolites play an important role in colonization of the stomach by *Helicobacter* and may damage hepatocytes adjacent to the bacteria (Dunn et al., 1997). McGowan et al. (1996) reported that a dramatic decrease in *H. pylori* viability occurs *in vitro* at pH 2, and survival of *H. pylori* at this pH is markedly enhanced in the presence of urea resulting from the urease activity of *H. pylori*. Kang and Lee (2005) showed that the culture supernatant of *E. faecium* GM-1 significantly decreased the viability and urease activity of *H. pylori*, and this inhibitory activity remained after adjustment of pH of culture supernatant to neutral. Coconnier et al. (1998) found that *L. acidophilus* strain LB-SCS treatment inhibits the *H. pylori* urease activity *in vitro* and in *H. pylori* that remained was associated with the cultured human mucosecreting HT29-MTX cells.

In conclusion, *L. brevis* BK11 and *L. acidophilus* BK13 strains, which were tolerant to artificial gastric juice containing pepsin at pH 2.0 were better candidates than the other strains for further studies as a therapy adjunct due to its excellent anti-*H. pylori* activity *in vitro*. These two strains could remarkably inhibit the adhesion of *H. pylori* to AGS cell lines and the urease activity of the adhered pathogen due to their metabolic by-products (lactic acid and bacteirocin) exerting antimicrobial effects. Therefore, *L. brevis* BK11 and *L. acidophilus* BK13 could help to prevent the infection and colonization of *H. pylori* on the gastric mucosa and reduce the risk of gastrointestinal disease resulting from this pathogenic strain. For the future, the role and activity of their antimicrobial substances obtained from probiotic organisms in the eradication of *H. pylori in vivo* remains to be evaluated.

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