In vitro screening of lactobacilli with antagonistic activity against *Helicobacter pylori* from traditionally fermented foods

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ABSTRACT

Helicobacter pylori may cause stomach diseases such as chronic gastritis, peptic ulcer, and gastric cancer, and several studies reported that lactobacilli have inhibitory effects on *H. pylori*. In this study, 38 *Lactobacillus* strains were screened for anti-*H. pylori* activity using in vitro methods, including survivability under the simulated gastric conditions, agar plate diffusion, urease activity, coaggregation, autoaggregation, and hydrocarbon analysis. The results indicate that 2 *Lactobacillus* strains showed potential anti-*H. pylori* activity in vitro. *Lactobacillus plantarum* 18 had the largest zone of inhibition and markedly reduced the urease activity of *H. pylori*. *Lactobacillus gasseri* Chen had higher coaggregation rate (58.15%) and hydrophobicity (59.27%) compared with the other strains. Further research is needed to verify the activities of these strains against *H. pylori*.

Key words: *Lactobacillus*, *Helicobacter pylori*, antagonism, urease activity

INTRODUCTION

Helicobacter pylori is a spiral gram-negative microorganism that causes chronic gastritis, peptic ulcer disease, and gastric cancer. The mechanisms by which *H. pylori* infection leads to gastric mucosal damage include the direct effects of virulence factors produced by *H. pylori* such as *cagA*, *vacA*, or urease; propagation and perpetuation of inflammation; oxidative stress; and induction of apoptosis in infected gastric epithelial cells (Goodwin, 1997; Graham, 1997; Dhar et al., 2003; Lee et al., 2008). At present, triple therapies that use combinations of a proton pump inhibitor (omeprazole) plus

antibiotic (clarithromycin or amoxicillin) and metronidazole are typically used as the treatment for diseases introduced by *H. pylori*. However, problems associated with patient noncompliance and consequent relapse of *H. pylori* infections are common. Moreover, these drugs destroy the microenvironment in the stomach, leading to side effects. Therefore, it is desirable to search for a natural treatment against *H. pylori* infection.

Lactobacilli are natural inhabitants of the human intestinal tract and have a positive effect on bowel microecology and health. In previous studies, probiotics have been used as adjuvant therapy in the treatment of *H. pylori*; these probiotics include *Lactobacillus salivarius* (Kabir et al., 1997), *Lactobacillus casei* Shirota (Sgouras et al., 2004), *Lactobacillus johnsonii* La1 (Bergonzelli et al., 2006; Gotteland et al., 2008), *Lactobacillus* GG (Ouwehand et al., 2000; Armuzzi et al., 2001), *Lactobacillus gasseri* (Sakamoto et al., 2001), *Bacillus subtilis* (Pinchuk et al., 2001), and *Bifidobacteria* (Collado et al., 2005). *Lactobacillus gasseri* and *L. casei* strains have been found to decrease *H. pylori* density in the gastric mucosa (Sakamoto et al., 2001; Sgouras et al., 2004), and lactobacilli may decrease both gastric inflammation and side effects of therapy for *H. pylori* eradication (Lesbros-Pantoflickova et al., 2007). The mechanisms of anti-*H. pylori* activity of lactobacilli may be associated with decreases in urease activity and adhesive capability of *H. pylori*; secretion of lactic acid, bacteriocins, autolysins, and proteinaceous compounds; and suppression of *H. pylori*-associated IL-8 production (Lorca et al., 2001; Kim et al., 2003; Lesbros-Pantoflickova et al., 2007). The application of lactobacilli in foodstuffs could decrease colonization of *H. pylori* in infected asymptomatic subjects, increase eradication rates when administered together with antibacterial and other drugs in symptomatic subjects, or decrease the incidence and severity of possible adverse effects due to treatments (Boyanova et al., 2009). Traditional fermented foods are important resources for isolation and screening of probiotics with health benefits. Therefore, the aim of the present study was to screen lactobacilli from tra-

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ditional fermented foods in China for in vitro activity against *H. pylori*.

MATERIALS AND METHODS

Bacterial Strains

Lactobacillus rhamnosus GG ATCC 533103 and 37 *Lactobacillus* strains isolated from traditional fermented milk and pickle (from Xinjiang, Sichuan, and Tibet provinces) were obtained from the Culture and Information Center of Industrial Microorganisms of China Universities, Jiangnan University (CICIM-CU; Wuxi, China), and cultured in de Man, Rogosa, and Sharpe (MRS) broth (Hopebio Co., Qingdao, China) at 37°C for 18 h. *Helicobacter pylori* type strains SS1, 11637, 26695, and 43504 and *H. pylori* clinical strain C0707 were obtained from CICIM-CU, and *H. pylori* was cultured on Columbia base agar (Oxoid, Basingstoke, UK) supplemented with 7% sheep blood and 0.4% *H. pylori* selective supplement (Oxoid) at 37°C and 5% CO₂.

Acid Tolerance Test

The acid tolerance test of *Lactobacillus* strains was carried out by the method of Liong and Shah with modifications (Liong and Shah, 2005; Ding and Shah, 2009). Briefly, MRS broth was adjusted to pH 3.0 with 5 M HCl. Approximately 10⁸ cfu/mL of each *Lactobacillus* strain was culture in the acidified MRS broth and incubated at 37°C for up to 18 h; absorbance was measured at 600 nm (A₆₀₀) before and after culture. The survival rates of the lactobacilli at pH 2.5 and 2.0 were determined by the method of Zhou et al. (2007). Briefly, 10⁸ cfu/mL of each *Lactobacillus* strain was inoculated into a medium simulating gastric conditions. The medium contained water, 5 g/L NaCl, and 3 g/L pepsin, and the pH was adjusted to 2.5 and 2.0 with HCl.

Growth Inhibition Assay

Cultures of *H. pylori* were plated on fresh Columbia agar plates without antibiotics (10⁸ cfu per plate), and 6-mm discs were put into the plates with *H. pylori*. Aliquots of 100 µL of fresh cultures of the *Lactobacillus* strains were added into the discs. Plates were incubated for 48 to 72 h under microaerophilic conditions at 37°C, and the diameters of the inhibition zones around the wells were measured (Sgouras et al., 2004; Ryan et al., 2008a). *Escherichia coli* DH5α culture and MRS medium were used as negative controls for in vitro anti-*H. pylori* activity, and metronidazole (0.0005–0.05 mg/mL) was used as a positive control.

Spectrophotometric Autoaggregation Assays

Autoaggregation abilities were determined and expressed as autoaggregation percentages, and were carried out by the method of Kos et al. (2003) with modifications. Briefly, cells from an 18-h culture of the *Lactobacillus* strains were prepared as described above. The A₆₀₀ was adjusted to 0.50 ± 0.05 to standardize the number of lactobacilli (10⁷–10⁸ cfu/mL). Then, the lactobacilli suspensions were incubated in aliquots at 37°C and were monitored for different durations (0, 2, 4, 16, and 24 h). The autoaggregation percentage was expressed as follows:

$$\text{Autoaggregation (\%)} = \left(1 - \frac{A_t}{A_0} \times 100 \right),$$

where A_t and A₀ represent A₆₀₀ of the upper suspensions at different times and total lactobacilli suspensions, respectively.

Coaggregation Assays of *H. pylori* with *Lactobacillus* Strains

The coaggregation test was performed as described by Kos et al. (2003) with modifications. Briefly, *Lactobacillus* strains and *H. pylori* suspensions were prepared as described in autoaggregation analysis, and equal volumes of cells (10⁷–10⁸ cfu/mL) of the different *Lactobacillus* strains and *H. pylori* were mixed and incubated at 37°C without agitation. Absorbance was determined for the mixture and for the bacterial suspensions alone. Coaggregation was calculated as follows:

$$\text{Coaggregation (\%)} = \frac{(A_x + A_y) - 2 \times A_{mix}}{A_x + A_y} \times 100,$$

where A_x and A_y represent A₆₀₀ of the mixed bacterial suspensions at time 0 h, and A_{mix} represents the A₆₀₀ of the mixed bacterial suspension at different times.

Bacterial Adhesion to Hydrocarbons

The bacterial adhesion to hydrocarbons (BATH) test was performed according to the method of Collado et al. (2008) with some modifications. Cells were washed once with PBS and resuspended in the same buffer to an A₆₀₀ of approximately 0.50 ± 0.05 to standardize the number of lactobacilli (10⁷–10⁸ cfu/mL), and an equal volume of xylene was added. The 2-phase system was thoroughly mixed by vortexing for 5 min. The aqueous phase was removed after 1 h of incubation at room tem-

Table 1. Survival rate of selected lactobacilli under the simulated gastric conditions at pH 2.0 for 1 h and percentage affinity to hydrocarbons

Strain	Survival rate (%)	Affinity to hydrocarbons (%)
<i>Lactobacillus rhamnosus</i> GG	39.58 ± 1.43*	45.50 ± 5.38*
<i>Lactobacillus reuteri</i> C234	5.06 ± 1.38*	8.02 ± 0.66
<i>Lactobacillus casei</i> ssp. <i>casei</i>	0.06 ± 0.03	39.12 ± 10.03*
<i>L. casei</i> Zhang	0.08 ± 0.06	47.58 ± 7.75*
<i>L. casei</i> BD-II	0.00 ± 0.00	-1.23 ± 0.70
<i>L. casei</i> 2W	0.00 ± 0.00	18.72 ± 10.32
<i>Lactobacillus plantarum</i> S3	0.81 ± 0.16	6.24 ± 4.94
<i>L. plantarum</i> CZ211-2	0.04 ± 0.01	2.74 ± 0.66
<i>L. plantarum</i> DJ201	0.00 ± 0.00	3.29 ± 0.52
<i>L. plantarum</i> DJ102-1	0.00 ± 0.00	-4.62 ± 1.38
<i>L. plantarum</i> ST-III	4.93 ± 1.53*	7.64 ± 3.38
<i>L. plantarum</i> ZS2058	9.30 ± 4.05*	18.58 ± 14.92
<i>L. plantarum</i> 18	0.01 ± 0.00	10.12 ± 12.52
<i>Lactobacillus bulgaricus</i> L24	0.00 ± 0.00	7.19 ± 1.45
<i>L. bulgaricus</i> 2-3	0.00 ± 0.00	8.31 ± 11.79
<i>Lactobacillus gasseri</i> Chen	2.25 ± 1.62*	59.27 ± 5.91*

*Significant differences ($P < 0.05$) among all the bacterial strains tested.

perature and its A_{600} was measured. Affinity to hydrocarbons (hydrophobicity) was reported as the adhesion percentage according to the following formula:

$$\text{Affinity to hydrocarbons (\%)} = \frac{A_0 - A}{A_0} \times 100,$$

where A_0 and A are the absorbencies before and after extraction with organic solvents, respectively. Hydrophobicity was calculated as the percentage decrease in the optical density of the original bacterial suspension due to the cells partitioning into a hydrocarbon layer.

Urease Activity Assays

Urease activity was determined by a modified phenol red method (Sgouras et al., 2004). Briefly, 10 μ L of fresh lactobacilli or *Lactobacillus* supernatant was added to 40 μ L of *H. pylori* cell suspension on a microtiter plate and incubated for 48 h at 37°C. Then, 150 μ L of urease reaction buffer (20%, wt/vol, urea and 0.012% phenol red in phosphate buffer with the final pH adjusted to 6.8) was added to the microtiter plate, and the plate was incubated at 37°C for 20 min. The absorbance at 550 nm was measured with a Sunrise microtiter plate reader (MDC Spectramax, Molecular Devices Inc., Sunnyvale, CA).

Statistical Analysis

All analyses were conducted in triplicate and the results were statistically analyzed by computing means and standard deviations of the mean. Differences between means of the test and control groups were

evaluated by the Dunnett's test, and $P < 0.05$ was considered significantly different by one-way ANOVA and SPSS 13.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Acid Tolerance Tests

The pH in the human stomach ranges from 1 to 4 (Chou and Weimer, 1999). Thirty-eight *Lactobacillus* strains were tested for tolerance to HCl at pH 3.0 for 24 h. Twenty-five (66%) of the 38 *Lactobacillus* strains could grow in MRS medium at pH 3.0 (data not shown). Because the essential culture conditions of the human stomach consist of a pH (2–3) and the presence of pepsin and NaCl, the 25 *Lactobacillus* strains were inoculated under simulated gastric conditions with pH 2.5 and 2.0 values. The results showed that 16 of 25 lactobacilli could survive under simulated gastric conditions at pH 2.5 for 3 h (data not shown), and 10 of 16 lactobacilli could survive under the simulated gastric conditions at pH 2.0 for 1 h (Table 1). The survival rates of 5 *Lactobacillus* strains were >1%, and *L. rhamnosus* GG exhibited the highest survival rate, approximately 39.58%. The survival rates from the highest to the lowest were in the following order: *L. rhamnosus* GG, *L. plantarum* ZS2058, *L. reuteri* C234, *L. plantarum* ST-III, and *L. gasseri* Chen.

Lactobacilli screened for activity against *H. pylori* should survive under simulated gastric conditions. If lactobacilli are capable of surviving for a significant length of time in the stomach, they might be useful in the treatment of gastric disorders (Ryan et al., 2008b). In this study, 10 out of 16 lactobacilli could survive

Table 2. Ability of 16 *Lactobacillus* strains to inhibit growth of *Helicobacter pylori*: the absorbance (optical density, OD, at 600 nm) and pH of the culture medium for each strain after 18 h of growth and zone of inhibition (ZOI; mm, mean $\pm$ SEM) and urease activity (optical density, OD, at 550 nm) of *H. pylori* coculture with lactobacilli supernatants

Strain	OD ₆₀₀	pH	ZOI (mm)	Urease activity (OD ₅₅₀ /per min)
<i>Escherichia coli</i> DH5 α	—	—	—	0.087 $\pm$ 0.002*
Metronidazole (0.005 mg/mL)	—	—	11.05 $\pm$ 0.79	—
Metronidazole (0.05 mg/mL)	—	—	20.92 $\pm$ 1.02**	—
de Man, Rogosa, and Sharpe (MRS) (pH 6.2)	—	6.2	13.97 $\pm$ 0.37	—
MRS (pH 4.0)	—	4.0	13.47 $\pm$ 0.65	—
<i>Helicobacter pylori</i> SS1	—	—	—	0.063 $\pm$ 0.002
<i>Lactobacillus rhamnosus</i> GG	1.911	3.71	15.42 $\pm$ 1.00	0.053 $\pm$ 0.006
<i>Lactobacillus reuteri</i> C234	1.813	4.23	9.25 $\pm$ 0.02	0.057 $\pm$ 0.010
<i>Lactobacillus casei</i> ssp. <i>casei</i>	1.608	3.81	11.31 $\pm$ 0.17	0.054 $\pm$ 0.002
<i>L. casei</i> Zhang	1.773	3.79	12.51 $\pm$ 1.92	0.053 $\pm$ 0.001
<i>L. casei</i> BD-II	1.764	4.23	12.68 $\pm$ 0.92	0.055 $\pm$ 0.002
<i>L. casei</i> 2W	1.067	4.31	11.06 $\pm$ 1.96	0.050 $\pm$ 0.001
<i>Lactobacillus plantarum</i> S3	2.154	3.69	16.30 $\pm$ 1.32	0.054 $\pm$ 0.002
<i>L. plantarum</i> CZ211-2	2.147	3.69	17.48 $\pm$ 2.14	0.047 $\pm$ 0.001
<i>L. plantarum</i> DJ201	2.113	3.71	15.54 $\pm$ 1.44	0.073 $\pm$ 0.006*
<i>L. plantarum</i> DJ102-1	1.952	3.76	15.84 $\pm$ 1.41	0.032 $\pm$ 0.012*
<i>L. plantarum</i> ST-III	1.773	4.25	13.18 $\pm$ 1.21	0.054 $\pm$ 0.003
<i>L. plantarum</i> ZS2058	1.770	4.22	11.96 $\pm$ 0.32	0.054 $\pm$ 0.004
<i>L. plantarum</i> 18	2.024	3.67	20.32 $\pm$ 0.36**	0.045 $\pm$ 0.001*
<i>Lactobacillus bulgaricus</i> L24	1.922	3.71	14.73 $\pm$ 0.62	0.050 $\pm$ 0.005
<i>L. bulgaricus</i> 2-3	1.789	3.94	15.84 $\pm$ 1.24	0.038 $\pm$ 0.006*
<i>Lactobacillus gasseri</i> Chen	1.305	4.01	15.53 $\pm$ 1.32	0.044 $\pm$ 0.002*

*Significant difference ($P < 0.05$) compared with urease activity of *H. pylori* SS1.

**Significant difference ($P < 0.05$) compared with ZOI of MRS medium inhibition growth of *H. pylori* SS1.

under simulated gastric conditions at pH 2.0 for 1 h, indicating that some lactobacilli could survive in the human stomach.

Growth Inhibition Assay

The activity of 16 lactobacilli against *H. pylori* SS1 was assessed by using the Oxford plate diffusion assay (Table 2). No inhibition of *H. pylori* was observed when *E. coli* DH5 α was used. The fresh MRS medium also exhibited activity against *H. pylori*, and it formed inhibition zones of approximately 14 mm. The inhibition zone diameters of the 16 lactobacilli varied from 9.25 to 20.32 mm. Because the diameters of the inhibition zones formed by 0.005 and 0.05 mg/mL metronidazole were approximately 12 and 20 mm, respectively, the activity of the 12 lactobacilli against *H. pylori* was equal to that of metronidazole at concentrations between 0.005 and 0.05 mg/mL. The range of pH in lactobacilli fermentation broths was from 3.6 to 4.3. Therefore, the antimicrobial activity of lactobacilli may be enhanced by metabolic products including acids. However, no difference in antimicrobial activity was observed between MRS media at pH 4.0 and pH 6.2, which indicated that the main inhibitory effect might not be due to the variation in pH of the medium.

Probiotics have a proven benefit in reducing the side effects of *H. pylori* therapy. However, the inhibitory ef-

fects of lactobacilli on *H. pylori* differ from strain to strain. Using the agar-well diffusion method, Ryan et al. (2008a) showed that *Lactobacillus salivarius* had high anti-*H. pylori* activity, and Boyanova et al. (2009) evaluated the activities of 6 strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* against 30 *H. pylori* strains and indicated that *L. delbrueckii* ssp. *bulgaricus* activity was strain-dependent and higher at low pH than at neutral pH against *H. pylori*. Similarly, in the current study, 9 *Lactobacillus* strains had zones of inhibition >14 mm, and 1 strain, *L. plantarum* 18, had significant anti-*H. pylori* activity.

Urease Activity Assays

Table 2 shows the urease activity of *H. pylori* coculture with 16 *Lactobacillus* supernatants. The urease activity of 5 strains differed significantly between the cocultures of *Lactobacillus* supernatants with *H. pylori* and individual *H. pylori* culture ($P < 0.05$). Four *Lactobacillus* strains showed significant inhibition of the urease activity of *H. pylori*; however, *L. plantarum* DJ201 could not inhibit the urease activity of *H. pylori*. Likewise, *E. coli* DH5 α could not inhibit the urease activity of *H. pylori*. *Lactobacillus* fermentation broths and viable cells showed good ability to inhibit the urease activity of *H. pylori*, whereas dead cells could not inhibit the activity (data not shown).

Helicobacter pylori can survive in the acidic environment of the stomach by producing urease, which catalyzes hydrolysis of urea to yield ammonia and elevates the pH of its environment (Celli et al., 2009). Although *H. pylori* can counteract stomach acid by producing urease, inhibition of urease by some lactobacilli could render *H. pylori* susceptible to lactic acid (Midolo et al., 1995; Boyanova et al., 2009). By increasing the outer membrane permeability of gram-negative bacteria, lactic acid could improve antibiotic activity (Boyanova et al., 2009). In the present study, the urease activity of *H. pylori* coculture with lactobacillus supernatants, fermentation broths, and viable cells decreased, whereas nonviable cells could not inhibit the activity. It was concluded that lactobacilli could act against *H. pylori* via inhibition of urease activity.

However, many substances have been reported to have anti-*H. pylori* activity, including lactic acid, bacteriocins, peroxides, proteinases, exopolysaccharides, and viable cells. Some studies have suggested that the production of relatively large amounts of lactate by lactobacilli is an inhibitory factor for *H. pylori* (Midolo et al., 1995; Aiba et al., 1998). Lactic acid, in addition to its antimicrobial effect resulting from lowering of pH, could inhibit *H. pylori* urease. However, other studies have demonstrated that lactobacilli exert inhibitory effects on *H. pylori* by lactic acid- and pH-independent mechanisms (Kim et al., 2003; Sgouras et al., 2004), and this inhibitory activity could be associated with the viable cells (Ryan et al., 2008a) and antimicrobial substances such as bacteriocins, peroxides, proteinases, exopolysaccharides, and cell wall components. Therefore, inhibition of lactobacilli against *H. pylori* could be attributed to inhibition of urease activity, the exposure of *H. pylori* to an acidic environment, or competitive growth with *H. pylori*.

Spectrophotometric Autoaggregation and Coaggregation Assays of *H. pylori* and Lactobacilli

The autoaggregation properties of lactobacilli were examined over a period of 24 h at 37°C by absorbance measurements at 600 nm; the results are shown in Table 3. In general, most lactobacilli exhibited a strong autoaggregating phenotype, and autoaggregation rates increased with incubation time. The highest autoaggregation rates were obtained with *L. bulgaricus* 2-3 and *L. gasserii* Chen. *Lactobacillus rhamnosus* GG, *L. casei* BD-II, and *L. plantarum* DJ201 also showed high autoaggregation rates (>45%) after 24 h of incubation.

Coaggregation abilities were tested using an indicator strain of *H. pylori* at different times, and the results are shown in Table 3. The coaggregation rates increased

Table 3. Rates of autoaggregation and coaggregation of lactobacilli with *Helicobacter pylori*

Strain	Autoaggregation (%)				Coaggregation (%)			
	2 h	4 h	16 h	24 h	2 h	4 h	16 h	24 h
<i>Lactobacillus rhamnosus</i> GG	9.03 ± 1.99	10.60 ± 6.03	39.31 ± 1.44	46.80 ± 4.84	11.33 ± 0.23	17.60 ± 0.80	52.40 ± 0.92*	59.25 ± 0.69*
<i>Lactobacillus reuteri</i> C234	8.53 ± 6.70	13.88 ± 2.09	36.86 ± 1.73	44.84 ± 1.31	4.20 ± 1.36	10.32 ± 0.37	40.07 ± 3.34	45.41 ± 1.97
<i>Lactobacillus casei</i> ssp. <i>casei</i>	5.13 ± 0.81	11.39 ± 2.34	38.95 ± 0.65	44.65 ± 0.27	7.49 ± 0.11	15.79 ± 0.11	47.44 ± 0.69*	51.83 ± 0.46*
<i>L. casei</i> Zhang	6.70 ± 0.57	8.14 ± 1.04	31.32 ± 0.92	38.18 ± 0.34	14.16 ± 2.53	21.59 ± 1.26	44.88 ± 0.11	51.96 ± 0.69
<i>L. casei</i> BD-II	4.87 ± 2.09	7.80 ± 1.60	40.90 ± 3.89*	47.04 ± 2.63*	7.16 ± 0.23	15.16 ± 1.31	44.23 ± 0.24	49.54 ± 0.12
<i>L. casei</i> 2W	9.80 ± 0.23	12.81 ± 0.37	29.26 ± 0.22	34.85 ± 0.06	12.17 ± 0.55	19.70 ± 1.22	46.31 ± 1.55	49.76 ± 0.89
<i>Lactobacillus plantarum</i> S3	4.61 ± 0.58	8.75 ± 0.83	25.88 ± 3.38	32.12 ± 3.12	10.29 ± 1.47	19.88 ± 1.59	43.47 ± 0.24	49.01 ± 0.24
<i>L. plantarum</i> CZ211-2	5.38 ± 2.61	9.56 ± 1.94	35.04 ± 3.64	35.76 ± 0.92	13.13 ± 1.47*	22.28 ± 0.02*	44.30 ± 1.09	49.65 ± 1.58
<i>L. plantarum</i> DJ201	6.36 ± 0.85	8.52 ± 0.55	38.16 ± 2.82	46.41 ± 3.75	6.69 ± 1.63	12.72 ± 3.38	41.21 ± 1.63	46.33 ± 0.01
<i>L. plantarum</i> DJ102-1	5.58 ± 0.21	8.97 ± 2.46	31.62 ± 1.523	34.22 ± 1.72	10.95 ± 0.36	17.90 ± 0.66	40.66 ± 0.00	47.35 ± 4.44
<i>L. plantarum</i> ST-III	8.14 ± 0.55	11.96 ± 0.76	33.92 ± 0.31	40.39 ± 1.17	10.59 ± 0.36	19.07 ± 0.36	39.66 ± 2.16	44.75 ± 0.96
<i>L. plantarum</i> ZS2058	10.75 ± 1.01	12.82 ± 1.07	32.15 ± 0.22	40.12 ± 0.76	7.76 ± 1.02	15.28 ± 1.24	38.64 ± 1.69	43.12 ± 1.68
<i>L. plantarum</i> 18	4.57 ± 0.72	8.79 ± 1.15	28.75 ± 0.77	33.36 ± 0.83	5.66 ± 0.01	13.23 ± 0.12	39.85 ± 2.00	46.01 ± 2.00
<i>Lactobacillus bulgaricus</i> L24	4.94 ± 4.14	9.83 ± 1.01	32.99 ± 5.61	42.58 ± 0.83	6.13 ± 0.23	12.91 ± 0.01	43.71 ± 2.80	48.84 ± 0.23
<i>L. bulgaricus</i> 2-3	9.77 ± 1.77	14.44 ± 3.66*	52.00 ± 2.01*	66.36 ± 0.11*	19.91 ± 1.26*	26.03 ± 1.47*	43.33 ± 0.74	48.25 ± 0.10
<i>Lactobacillus gasserii</i> Chen	6.95 ± 0.22	10.68 ± 2.38	42.83 ± 1.53*	54.93 ± 1.88*	12.69 ± 0.73	18.01 ± 0.73	50.94 ± 0.73*	58.15 ± 0.72*

*Significant differences ($P < 0.05$) among all bacterial strains tested at the same incubation time.

Figure 1. Graphical representation of the principal component analysis of lactobacilli properties from the trained panel showing principal component (PC)1 versus PC2: (A) factor loadings; (B) factor scores. LP = *Lactobacillus plantarum*; LC = *Lactobacillus casei*; LB = *Lactobacillus bulgaricus*; LR = *Lactobacillus reuteri*; LG = *Lactobacillus gasseri*; and LGG = *Lactobacillus rhamnosus* GG.

with the incubation time, and the highest coaggregation percentages were obtained with *L. rhamnosus* GG (59.3%) and *L. gasseri* Chen (58.2%) after 24 h of incubation. However, no significant differences ($P > 0.05$) were found between the 2 strains.

Bacterial Adhesion to Hydrocarbons

The adhesion percentages of lactobacilli to xylene are shown in Table 1, and the percentage affinity to hydrocarbons (hydrophobicity) ranged between 0.0 and 59.3% for the test strains. The most hydrophobic strain was *L. gasseri* Chen (59.3%); *L. rhamnosus* GG and *L. casei* Zhang also had high hydrophobicities, which were 45.5 and 47.6%, respectively. In general, most lactobacilli tested showed high hydrophobicity but *L. casei* BD-II and *L. plantarum* DJ102-1 had low hydrophobicity (below zero).

Principal Component Analysis

Principal component analysis (PCA) was performed to investigate the correlation among the various properties of *Lactobacillus* (aggregation, coaggregation, and hydrocarbons) and the ability to inhibit *H. pylori* growth and urease activity and tolerance to acids. The results, as seen in Figure 1a, were defined by 4 principal components (PC): (1) the first (PC1), which explained 28.87% of the variance in the model, was characterized by inhibition zone; (2) the second (PC2) explained 24.17% of the variance in the model and was characterized by coaggregation and hydrophobicity; (3) the third (PC3) explained 19.40% of the variance in the model and was characterized by urease activity and aggregation; and (4) the fourth (PC4) explained 14.54% of the variance in the model and was characterized by the survival rate in acids.

Aggregation properties, together with the ability to coaggregate with the potential pathogen, could be used for preliminary selection of probiotic bacteria against the pathogen (Vlkova et al., 2008). Aggregation plays an important role in the formation of biofilms. Although coaggregation is not indicative of biofilm formation, it may play an important role in eliminating pathogens from the gastrointestinal tract (Todorov et al., 2008). Cell surface hydrophobicity is a nonspecific interaction between microbial cells and host cells. Bacterial cells with a high hydrophobicity usually form strong interactions with mucosal cells. Therefore, multiple properties of *Lactobacillus* may affect its inhibitory activity against *H. pylori*; however, the exact mechanism of inhibition against *H. pylori* should be further studied.

Table 4. The inhibition of the growth of *Helicobacter pylori* standard and clinical strains by *Lactobacillus plantarum* 18 and *Lactobacillus gasseri* Chen

Strain	Zone of inhibition (mm; mean $\pm$ SEM)			
	<i>H. pylori</i> 43504	<i>H. pylori</i> 11637	<i>H. pylori</i> 26695	<i>H. pylori</i> C0707
<i>L. plantarum</i> 18	14.79 $\pm$ 0.58*	13.16 $\pm$ 2.24	13.95 $\pm$ 1.36*	16.25 $\pm$ 1.10*
<i>L. gasseri</i> Chen	14.58 $\pm$ 0.60*	12.14 $\pm$ 1.78	12.61 $\pm$ 1.66	16.24 $\pm$ 1.89*
MRS	11.49 $\pm$ 0.93	10.95 $\pm$ 1.28	10.44 $\pm$ 0.56	12.73 $\pm$ 0.54

*Within-column significant difference ($P < 0.05$) compared with zone of inhibition of de Man, Rogosa, and Sharpe (MRS) medium.

As seen in Figure 1b, *L. plantarum* 18, *L. plantarum* DJ102-1, *L. plantarum* S3, and *L. plantarum* CZ211-2 had the highest contribution to PC1, whereas PC2 was more correlated with *L. rhamnosus* GG, *L. gasseri* Chen, and *L. casei* Zhang. The characteristics of *L. gasseri* Chen were the most similar to those of *L. rhamnosus* GG, which inhibited *H. pylori*, and these findings suggested that *L. gasseri* Chen could be used as a potential probiotic against *H. pylori*. *Lactobacillus plantarum* 18 had the largest zone of inhibition and markedly reduced the urease activity of *H. pylori*. Therefore, according to PCA and the ability of inhibition growth of *H. pylori*, *L. plantarum* 18 and *L. gasseri* Chen showed potential against *H. pylori*.

To further evaluate the activities of *L. plantarum* 18 and *L. gasseri* Chen against other strains of *H. pylori*, the inhibition abilities of these 2 lactobacilli on the growth of *H. pylori* were tested (Table 4). Although all 4 *H. pylori* strains were inhibited by these 2 lactobacillus strains, the degree of inhibition varied between *H. pylori* strains.

Therefore, *L. plantarum* 18 and *L. gasseri* Chen showed great potential for applications in prevention and treatment of *H. pylori* infection. Further research is required to investigate the mechanisms of the selected lactobacilli underlying the activity against *H. pylori* infection in vitro.

CONCLUSIONS

This study utilized a multi-step method to screen lactobacilli against *H. pylori*, and the results indicated that *L. plantarum* 18 and *L. gasseri* Chen had potential capacity to inhibit the growth and urease activity of *H. pylori*. Moreover, these 2 strains survived under the simulated gastric conditions. Therefore, *L. plantarum* 18 and *L. gasseri* Chen show potential anti-*Helicobacter pylori* activity in vitro, although further research is needed to verify the activities of these strains against *H. pylori*.

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