Antagonistic Activities of Lactobacilli against *Helicobacter pylori* Growth and Infection in Human Gastric Epithelial Cells

Xiaohua Chen,* Xiao-ming Liu,* Fengwei Tian, Qiuxiang Zhang, He-ping Zhang, Hao Zhang, and Wei Chen

Abstract: Lactobacilli have positive effects on bowel microflora and health in humans and animals. In this study, the antagonistic activities of *Lactobacillus gasseri* Chen, and *L. plantarum* 18 were assessed by agar plate diffusion assay and tests that determined the growth and urease activity of *Helicobacter pylori* cocultured with lactobacilli and the adherence of *H. pylori* to human gastric epithelial cells in the presence of lactobacilli. The results showed that the 2 *Lactobacillus* strains had significant anti-*H.pylori* activity, and this activity may be contributed by the cell-free supernatants (CFS) of lactobacilli and live *Lactobacillus* strains *in vitro*. The antagonistic activity of the CFS against *H. pylori* depended on the pH and the presence of metabolites, such as organic acids and proteases. Our results also indicated that 2 *Lactobacillus* strains could inhibit *H. pylori* adherence human gastric epithelial cells.

Keywords: gastric epithelial cells, *Helicobacter pylori*, lactobacilli

Practical Application: *Helicobacter pylori* causes chronic gastritis, peptic ulcer disease, and gastric cancer, and it infects about 50% of the world's population. Lactobacilli have been reported to have an inhibitory effect on *H. pylori* and can be used as probiotic to manufacture dairy products preventing *H. pylori* infection.

Introduction

Helicobacter pylori is a gram-negative microaerophilic human gastric pathogen, which infects more than half of the world's human population and causes chronic gastritis, peptic ulcer disease, and gastric cancer (Dunn and others 1997; Lehours and Yilmaz 2007). Treatment of *H. pylori* infections with antibiotics, such as proton pump inhibitors, amoxicillin, and clarithromycin may cause serious side effects; hence, natural food substances, such as apple peel polyphenols (Pastene and others 2010), green tea extract (Lee and others 2009), and probiotic foods, which can be used for adjuvant therapy, are being investigated.

Lactobacilli have positive effects on the intestinal microflora and the health of humans and animals. For using lactobacilli as probiotics, they must have certain properties, including adhesion, competitive exclusion capacity, and immunomodulation, to prevent pathogen infection of the gastrointestinal epithelium. Recently, some studies have reported that certain lactobacilli, including *Lactobacillus salivarius* (Kabir and others 1997), *L. casei* Shirota (Sgouras and others 2004), *L. johnsonii* La1(Bergonzelli and others 2006), *Lactobacillus* GG (Ouwehand and others 2000; Armuzzi and others 2001), *L. gasseri* (Sakamoto and others 2001), and *L. plantarum* have anti-*H. pylori* activities (Rokka and others 2006); however, the mechanisms of these activities are unclear and may be strain specific. Some studies reported that the antagonistic effect of the spent culture supernatants (SCS) of lactic acid bacteria (LAB) either is due to the production of organic acids or bacteriocins or is protein mediated (Aiba and others 1998; Barrett and others 2007; Deraz and others 2007). However, Ryan and others (2008) reported that the anti-*H. pylori* activity of *L. salivarius* UCC119 is neither due to acid production nor is mediated by a protein; it requires the presence of live cells. Further, Rokka and others (2006) found that the strains of *L. plantarum* have anti-*H. pylori* activity *in vitro*, and this activity is associated with the cell wall rather than with the SCS or intracellular fraction. Therefore, it is necessary to investigate the anti-*H. pylori* activities of lactobacilli.

A pilot study showed that *L. gasseri* Chen (LG Chen), and *L. plantarum* 18 (LP18) have potential anti-*H. pylori* activity (Chen and others 2010). In this study, we further assessed the activities of these *Lactobacillus* strains against the growth and infection of *H. pylori* in human gastric epithelial cells; the study was performed by using cell-free supernatants (CFS), fermentation broths (FB), live cells, and dead cells.

Materials and Methods

Bacterial strains, culture medium, and growth conditions

LGG (ATCC 533103), LP18 (CGMCC nr 4286), and LG Chen were obtained from the Culture Collection of Food Microorganisms, Jiangnan Univ., China (CCFM-JU) and cultured in MRS broth (Hopebio Co., Qingdao, China) at 37 °C for 18 h and FB were obtained. CFS were prepared by centrifuging the

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cultures and filtering the supernatant with a 0.2 μM filter. Live cells were prepared by centrifuging the cultures and washing 3 times by phosphate-buffered saline (PBS; 25 mmol/L sodium phosphate and 0.9% NaCl, pH 7.2) and were adjusted to10^9 CFU/mL. The lactobacilli live cells were further diluted to a concentration of 10^8, 10^7, and 10^6 CFU/mL. Dead cells were prepared by live cells boiling at 100 °C for 20 min.

Helicobacter pylori strain SS1 was obtained from CCFM-JU and 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% CO_2 for 48 to 72 h.

Human gastric epithelial cells (SGC7901) were cultured at 37 °C in RPMI-1640 medium (Gibco, N.Y., U.S.A.) containing 10% Fetal Bovine Serum (Gibco) in a humidified atmosphere of 5% CO_2, and the medium was changed every 3 d. Before the experiment was initiated, the cells were plated at 10^4 cells/well in 96-well plates for 24 h in serum-free RPMI-1640 medium.

Lactobacillus-mediated inhibition of *H. pylori* growth

H. pylori was cultured on fresh, antibiotic-free, Columbia agar plates (10^8 CFU/plate); 6-mm dia oxford cups were placed in the plates, and 80-μL aliquots of fresh CFS of the *Lactobacillus* strains, CFS with varying values of pH, CFS with pretreatment by proteins (1 mg/mL trypsin, proteinaseK, pancreatin, or pepsin at 37 °C for 1 h), FB, live lactobacilli suspended in PBS, or dead lactobacilli suspended in PBS were added to the discs. The plates were incubated for 48 to 72 h under microaerophilic conditions at 37 °C, and subsequently, the diameters of inhibition zones around the discs were measured (Sgouras and others 2004; Ryan and others 2008). PBS and MRS medium were used as negative controls.

Growth and urease assay of *H. pylori* cocultured with live lactobacilli

The growth of *H. pylori* cocultured with lactobacilli was determined by the following method (Sgouras and others 2004): the fresh *H. pylori* SS1 cells (10^8 CFU/mL) suspended in antibiotic-free brain heart infusion broth (BHIB) containing 5% serum were incubated under microaerophilic conditions for 24 and 48 h at 37 °C in the presence of a 10% volume of live lactobacilli cells (10^8 CFU/mL). The viability of *H. pylori* was evaluated from the number of viable CFUs of *H. pylori* cultured as described above on *H. pylori*-selective plates.

Urease activity was determined by the phenol red method, with some modifications (Sgouras and others 2004). Briefly, 50 μL of bacterial coculture suspension was taken on a microtiter plate, and 150 μL of urease reaction buffer (20% [w/v] urea and 0.012% phenol red in phosphate buffer, pH 6.8) was added to it; the plate was incubated at 37 °C for 20 min. The absorbance at 550 nm was measured with Sunrise microtiter plate reader (MDC Spectramax; Molecular Devices Inc., Sunnyvale, Calif, U.S.A.).

Adherence of *H. pylori* to human gastric epithelial cells in the presence of lactobacilli

The adherence test was performed as described by Rokka, with some modifications (Rokka and others 2008). For infection studies, SGC7901 cells were grown on microtiter plates to form a confluent monolayer. *Lactobacillus* strains cultured in MRS broth at 37 °C for 18 h were harvested and washed twice with cold PBS, and then, the concentration of cultures was adjusted to 10^9 CFU/mL with serum-free RPMI-1640 medium. The lactobacilli samples were further diluted to a concentration of 10^7 CFU/mL.

H. pylori was harvested from 1 to 2-d-old solid cultures and washed twice with cold PBS, and the bacterial concentration was then adjusted to 10^9 CFU/mL with serum-free RPMI-1640 medium. *H. pylori* samples were further diluted to concentrations of 10^7 CFU/mL. Before infection, the CFS of lactobacilli and 100 μL of live and dead *Lactobacillus* cells at 10^7 CFU/mL were pretreated with SGC7901 cells for 60 min, and the cells were washed 3 times with PBS. Thereafter, the cells were infected for 2 h with 100 μL of live *H. pylori* at 10^7 CFU/mL. Subsequently, each well was washed 5 times to remove nonadherent *H. pylori*. Urease test was performed by adding 200 μL of urease test solution into each well of the microtiter plate. After 3 h, the absorbance at 550 nm was measured with the Sunrise microtiter plate reader. The adherence of *H. pylori* was calculated as follows: Adherence = $([OD_{experimental} - OD_{negative}]/[OD_{positive} - OD_{negative}] \times 100)\%$. The negative control contained only SGC7901 cells; the positive control contained both epithelial cells and *H. pylori*, which were used to establish 100% adherence.

Organic acid analysis by high–performance liquid chromatography (HPLC)

The organic acid analysis method modified by Lin and others (2009) was used. Organic acids in LAB-CFS were determined by HPLC (Waters Corporation, Milford, Mass, U.S.A.). LAB-CFS was filtered through a 0.22-μm pore size filter. Filtered supernatants were diluted 10-fold and 5 μL were injected into an EC182546 C18 column (4.6 × 250 mm i.d., 5 μm particle size, Ecomsil). Elution was performed at 40 °C with methanol/water/phosphoric acid (5/95/0.05) at a flow rate of 0.8 mL/min. Organic acids was determined by optical density (OD) measurements at 210 nm. Standard solutions containing 10 mM of different organic acids, including lactic acid, acetic acid, oxalic acid, malic acid, and butyric acid were used as the standard. Quantification of organic acids in LAB-SCS was based on the external standard method.

Statistical analysis

All the analyses were conducted in triplicate, and the results were statistically analyzed by computing the means and standard deviations of the means. Differences between the means of the test and control groups were evaluated by Dunnett test, and $P < 0.05$ was considered statistically significant (1-way ANOVA and SPSS 13.0).

Results

Lactobacillus-mediated inhibition of *H. pylori* growth

A pilot study showed that LG Chen, and LP18 have potential anti-*H. pylori* activity (Chen and others 2010). However, the mechanism of these strains against *H. pylori* was still unclear. Therefore, in this study, *L. rhamnosus* GG (LGG) was used as a positive control against *H. pylori* and the antagonistic activities of the FB, CFS, live cells, and dead cells against *H. pylori* were respectively analyzed.

The inhibitory effect of CFS, FB, live, and dead of 3 lactobacilli against *H. pylori* was screened by measuring the diameters of inhibitory zones on plate. The results show that the fresh MRS medium used as a negative control also exhibited activity against

H. pylori, and it formed inhibition zones of approximately 10 mm. Comparing with MRS medium, 3 *Lactobacillus* strains had significant anti-*H. pylori* activities with inhibition zones of approximately 14 mm, which were present CFS and FB of 18-h cultures of lactobacilli (Figure 1). The FB had greater anti-*H. pylori* activities than the CFS, and the FB of LP18 had the greatest antibiotic activity. However, live or dead cells of *Lactobacillus* strains suspended in PBS did not inhibit the growth of *H. pylori*.

Table 1 shows the *H. pylori*-inhibitory activities of the CFS of the *Lactobacillus* strains, with or without adjusting the pH of the CFS at different values. The CFS with pH 4.0 and 6.5 of the *Lactobacillus* strains showed no inhibition ability comparing MRS, while the CFS at pH 3.5 showed inhibitory activity. The CFS whose pH was not adjusted also showed *H. pylori*-inhibitory activity. There was no difference between the inhibitory activities of CFS at pH 3.5 and primary CFS. Neutralized CFS lost its anti-*H. pylori* activity, and the residual activity appeared to be due to the MRS medium.

Characteristics of the antibacterial activity of the CFS were examined (Table 2). The untreated CFS was as a control. The inhibitory activities of the CFS of LGG and LP18 reduced after treatment with trypsin, pancreatin, and pepsin but were enhanced after treatment with proteinase K. However, the inhibitory activity of the CFS of LG Chen did not change after protease treatment.

Growth and urease assay of *H. pylori* cocultured with live lactobacilli and LAB-CFS

The count of *H. pylori* was measured by the plate count with or without coculturing live lactobacilli and LAB-CFS. With the time, the count of *H. pylori* decreased in the coculturing live lactobacilli and LAB-CFS. Figure 2 and 4 show the viable cell count of *H. pylori* cocultured with live lactobacilli and LAB-CFS. At 24 h, the count was reduced in cocultures with the 3 *Lactobacillus* strains and LAB-CFS. Further, at 48 h, this count was reduced only in cocultures with LG Chen and LP18 and their CFS; the cells of live LGG could not inhibit the growth of *H. pylori* at 48 h. Moreover, the cells of live LG Chen had a greater ability to inhibit the growth of *H. pylori* at 24 h than the other 2 strains. However, the CFS of LP18 had a greater ability to inhibit the growth of *H. pylori* than the other 2 strains. Figure 3 and 5 show the urease activity of *H. pylori* cocultured with lactobacilli and LAB-CFS. The 3 *Lactobacillus* strains inhibited the urease activity of *H. pylori* in the coculture. Further, compared to the other strains, the cell of live LG Chen had greater inhibitory ability at 24 h, and the cell of live

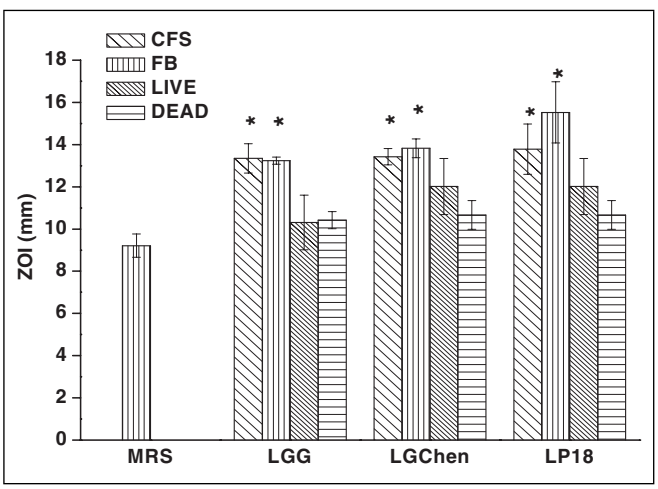

Figure 1–The antagonistic activities of lactobacilli against *H. pylori* SS1. Eighty microliter of the cell-free supernatants (CFS) of lactobacilli, fermentation broths (FB), live lactobacilli suspended into PBS (LIVE), and dead lactobacilli suspended into PBS (DEAD) were tested to inhibit *H. pylori* growth by the method of agar plate diffusion assay. MRS was as negative control. ZOI = zone of inhibition. *$P \leq 0.05$ compared with MRS.

Table 1 –The antagonistic abilities of fresh cell-free supernatants (CFS) of the *Lactobacillus* strains with different pH against *H. pylori* SS1.

	Average ZOI + SEM (mm)		
pH	**LGG**	**LG chen**	**LP18**
pH 6.5	11.65 ± 0.64^{a}	10.95 ± 0.49^{a}	10.60 ± 0.14^{a}
pH 4.0	12.00 ± 0.28^{a}	10.75 ± 0.07^{a}	10.65 ± 0.21^{a}
pH 3.5	13.84 ± 0.34^{b}	13.60 ± 1.13^{b}	12.05 ± 0.21^{c}
Primary CFS	13.40 ± 0.14^{b}	14.12 ± 0.85^{b}	11.37 ± 0.18^{b}

[abc] Column means containing different letters are significantly ($P < 0.05$) different. ZOI = zone of inhibition.

Table 2 –The antagonistic abilities of the antibacterial compounds produced by lactobacilli with protease treatment against *H. pylori* SS1.

	Average ZOI + SEM (mm)		
Enzyme	**LGG**	**LG chen**	**LP 18**
Protease K	14.02 ± 0.25^{c}	15.80 ± 4.95^{a}	12.61 ± 0.72^{b}
Pancreatin	12.80 ± 0.45^{b}	12.60 ± 3.25^{a}	11.93 ± 0.47^{ab}
Trypsin	11.00 ± 0.57^{a}	11.97 ± 2.22^{a}	11.45 ± 0.07^{a}
Pepsin	10.79 ± 0.58^{a}	11.02 ± 0.59^{a}	11.05 ± 0.21^{a}
Untreated CFS	13.40 ± 0.14^{bc}	14.12 ± 0.85^{a}	11.37 ± 0.18^{a}

[abc] Column means containing different letters are significantly ($P < 0.05$) different. ZOI = zone of inhibition. Untreated CFS was as a control.

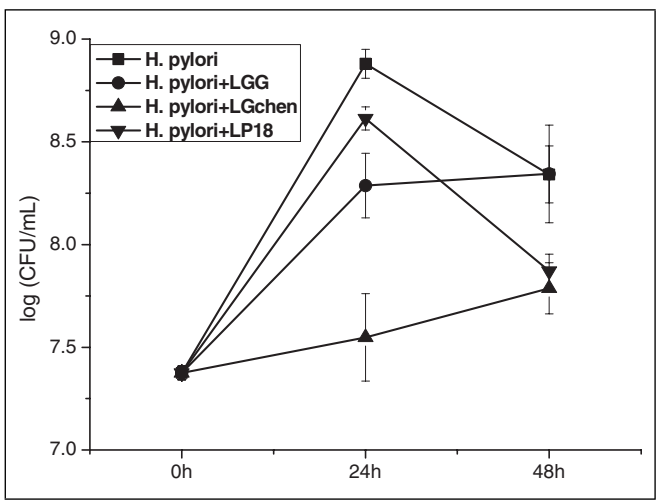

Figure 2–The live cell count of *H. pylori* SS1 in the coculture with lactobacilli. The fresh *H. pylori* SS1 cells (10^8 CFU/mL) suspended in antibiotic-free brain heart infusion broth (BHIB) containing 5% serum were incubated under microaerophilic conditions for 24 and 48 h at 37 °C in the presence of a 10% volume of live lactobacilli cells (10^8 CFU/mL). The viability of *H. pylori* was evaluated from the number of viable CFUs of *H. pylori* cultured on *H. pylori*-selective plates.

LGG had greater inhibitory ability at 48 h. However, the CFS of LP18 had greater inhibitory ability at 24 and 48 h.

Organic acids in LAB-CFS by HPLC

The organic acid was measured after 18-h culture of the lactobacilli cells by HPLC. The main organic acid present in the CFS of there lactobacilli were lactic acid with the range of content from 114 to 150 mM and acetic acid with the range of content from 30 to 65 mM. Moreover, oxalic acid and citric acid were also found and the range of contents from 4 to 8 mM and 10 to 24 mM, respectively (Table 3).

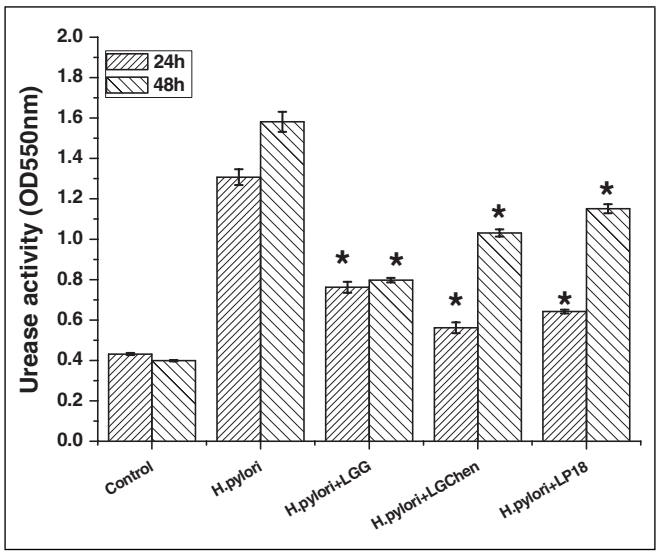

Figure 3–The urease activity of *H. pylori* SS1 in the coculture with lactobacilli. The fresh *H. pylori* SS1 cells (10^8 CFU/mL) suspended in antibiotic-free brain heart infusion broth (BHIB) containing 5% serum were incubated under microaerophilic conditions for 24 and 48 h at 37 °C in the presence of a 10% volume of live lactobacilli cells (10^8 CFU/mL). Urease activity was determined by the phenol red method. $^*P \leq 0.05$ compared with *H. pylori*.

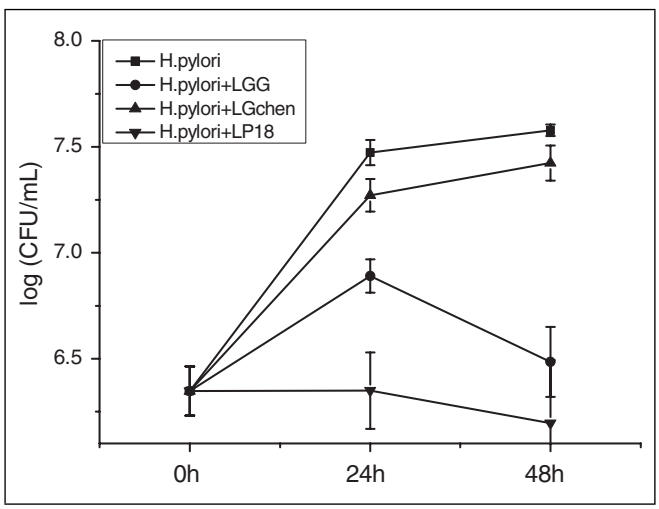

Figure 4–The live cell count of *H. pylori* SS1 in the coculture with the cell-free supernatant (CFS) of lactobacilli. The fresh *H. pylori* SS1 cells (10^7 CFU/mL) suspended in antibiotic-free brain heart infusion broth (BHIB) containing 5% serum were incubated under microaerophilic conditions for 24 and 48 h at 37 °C in the presence of a 10% volume of the CFS of lactobacilli. The viability of *H. pylori* was evaluated from the number of viable CFUs of *H. pylori* cultured on *H. pylori*-selective plates.

Adherence of *H. pylori* to human gastric epithelial cells in the presence of lactobacilli

After treatment with the CFS of lactobacilli and live and dead lactobacilli, the urease activity of *H. pylori* adhering to SGC7901 cells was examined. As shown in Figure 6, after 2-h incubation of *H. pylori* with the CFS and live and dead lactobacilli, the urease activity of *H. pylori* was significantly reduced. Considering that the adherence rate of *H. pylori* without treatment was 100%, this rate dropped to approximately 50% after treatment with the CFS and live and dead lactobacilli.

Discussion

Lactobacilli, such as *L. salivarius*, *L. casei* Shirota, *L. johnsonii* La1, LGG, *L. gasseri*, and *L. plantarum* are reported to have anti-*H. pylori* activities *in vitro* and *in vivo*. Agar plate diffusion assays performed in this study showed that LGG, LG Chen, and LP18 also inhibit the growth of *H. pylori*. The CFS and FB of these 3 *Lactobacillus* strains showed significant anti-*H. pylori* activities (Figure 1). The activities of CFS were lost after the pH of the CFS was adjusted to 4.0 and 6.5 (Table 1). This indicated that the antimicrobial activity was acidic or required an acidic environment to optimally develop its activity (Coconnier and others 1997). The inhibitory activities of the CFS of LGG and LP18 reduced after treatment with trypsin, pancreatin, pepsin, and trypsin, but were enhanced after treatment with proteinase K. However, the inhibitory activity of the CFS of LG Chen did not change after protease treatment (Table 2). The main organic acid present in the CFS of the there lactobacilli were lactic acid with the range of content from 114 to 150 mM and acetic acid with the range of content from 30 to 65 mM (Table 3). Therefore, it was indicated that the anti-*H. pylori* activities of these 3 *Lactobacillus* strains may depend on both the pH of and the metabolic compounds (such as organic acid and proteinaceous substances) present in the CFS of lactobacilli. The main metabolic end products of lactic acid fermentation, organic acids (lactic and acetic acids) are capable on interfering with the growth of pathogens (Vandenbergh 1993). Helander and others (1997) reported that organic acids inhibit *H. pylori* in a

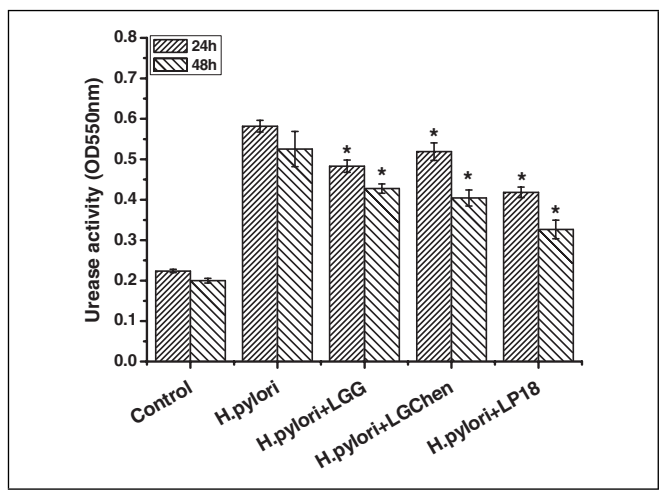

Figure 5–The urease activity of *H. pylori* SS1 in the coculture with the cell-free supernatant (CFS) of lactobacilliThe fresh *H. pylori* SS1 cells (10^7 CFU/mL) suspended in antibiotic-free brain heart infusion broth (BHIB) containing 5% serum were incubated under microaerophilic conditions for 24 and 48 h at 37 °C in the presence of a 10% volume of the CFS of lactobacilli. Urease activity was determined by the phenol red method. $^*P \leq 0.05$ compared with *H. pylori*.

concentration-dependent manner. Recently, several studies have reported that the LAB-SCS of LAB has anti-*H. pylori* activity (Michetti and others 1999; Lin and others 2009). Nam and others (2002) found that when *H. pylori* was treated with SCS, the helical form of the cells changed to the coccoid form and the cells became necrotic; these changes further led to the loss of infectivity of *H. pylori*.

The method of plate diffusion assay was analyzed antagonistic activities of components of lactobacilli against *H. pylori* and the antagonistic activities of lactobacilli were also further analyzed by the count and urease activity of *H. pylori* in the coculture lactobacilli with *H. pylori*. In the coculture conditions, the cells of LAB and the LAB-CFS both can reduce the number and the urease activity of *H. pylori*. *Helicobacter* urease, which is a surface protein component of *H. pylori*, produces ammonia from the host's urea and allows the survival of *H. pylori* by neutralizing the acidic environment. These results suggest that live cells may play an important role in the antibacterial action, and viable metabolically active cells are required for inhibition to take place; perhaps, the inhibitor is produced when the lactobacilli came in contact with *H. pylori*.

The ability to adhere to mucosal surfaces is important for bacterial maintenance in the human gastrointestinal tract. Bernet and others (1994) reported that adherent LAB may inhibit cell association and invasion by pathogens. Lin and others (2009) reported that LAB-SCS inhibits *H. pylori* infection and adhesion to AGS cells. Tsai and others (2004) found that both the SCS and the cells of *Enterococcus faecium* TM39 inhibit the binding of *H. pylori* to TSGH 9201 cells. Jankowska and others (2008) reported that *L. paracasei* inhibits the adhesion of pathogenic *Salmonella enterica* to Caco-2 cells; further, a coincubation experiment indicated that the inhibition mediated by the SCS of *Lactobacillus* was weaker than that mediated by the whole *L. paracasei* culture. Similar results were noted in this study. SGC7901 cells were used to evaluate the efficacy of the CFS and live and dead lactobacilli in the inhibition *H. pylori* adhesion to gastric cells. The results showed that the CFS and live and dead lactobacilli are efficacious in inhibiting *H. pylori* adhesion to SGC7901 cells.

The results of agar plate diffusion assay and tests determining the growth and urease activity of *H. pylori* cocultured with lactobacilli and the adherence of *H. pylori* to gastric cells in the presence of lactobacilli showed that lactobacilli have anti-*H. pylori* activities *in vitro*. However, it is necessary to investigate the *in vivo* activity of lactobacilli against *H. pylori* infection. Previous studies have shown that during chronic infection with the mouse-adapted *H. pylori* strain SS1, the organisms colonize the stomach of C57BL/6 mice and cause gastric inflammation and an increase in cytokine production (Sutton and others 2000; Garhart and others 2002). Johnson-Henry and others (2004) reported that probiotics could reduce *H. pylori* colonization and bacteria-induced mucosal inflammation in mice. Yi Cui and others (2010) reported that 2 *Lactobacillus* strains from the human stomach significantly decreased the density of *H. pylori* and relieve of mucosal inflammation in the gastric antrum and gastric body. We also observed that lactobacilli could reduce *H. pylori*-induced gastric mucosal inflammation in mice (Data not shown). The mechanisms of the activities of these 2 *Lactobacillus* strains against *H. pylori* infection *in vivo* require further investigation.

Antibiotic therapy of *H. pylori* destroyed the microenvironment in the stomach leading to side effects and the rapid spread of resistant *H. pylori* strains. Considering the long history of use of lactobacilli in a variety of food applications all over the world and in the view that lactobacilli could be predominant probiotic, the study on the food containing LG Chen and LP18 with antagonistic activity against *H. pylori* could lay the foundation for the food application for preventing or adjuvant therapy gastric diseases caused by *H. pylori*.

植物乳杆菌LP18（CN2018）显著幽门螺杆菌的生长

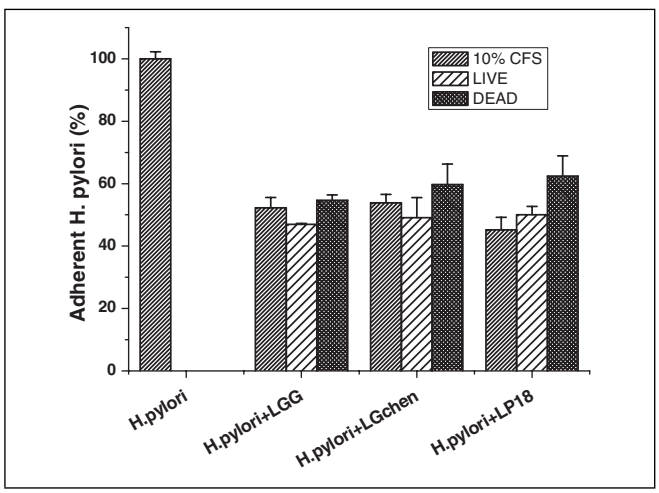

Figure 6–The adhesive rate of *H. pylori* to SGC7901 in the present of lactobacilli. The percentage of attached *H. pylori* was calculated as follows: Attached% = (ODexperimental − ODnegative)/(ODpositive − ODnegative) × 100.The negative control contained only SGC7901 cells, and the positive control contained the epithelial cells and *H. pylori*, which were used to establish 100% attachment.

Conclusion

In conclusion, the 2 *Lactobacillus* strains,namely LG Chen and LP18, showed significant anti-*H. pylori* activity, and these antibiotic activities may be contributed by the CFS and live *Lactobacillus* strains *in vitro*. The antagonistic activity of the CFS against *H. pylori* depended on the pH of the CFS and metabolites present in it, which may be organic acids and proteinaceous substances. These 2 *Lactobacillus* strains could also inhibit *H. pylori adherence* human gastric epithelial cells. It would be of great interest to further explore the role of such probiotic strains in the complex regulation of anti-*H. pylori* activities *in vivo*. Therefore, the current study on LG Chen and LP18 with antagonistic activity against *H. pylori* could lay the foundation for validation of these 2 strains as potential probiotics and the application of the strains for prevention or adjuvant therapy chronic gastritis and peptic ulcer disease caused by *H. pylori*.

植物乳杆菌LP18（CN2018）能够缓解由幽门螺杆菌造成的胃部损害

Table 3–Organic acids in the cell-free supernatants (CFS) after culturing LAB in MRS broth.

LAB strain	Oxalic acid (mM)	Lactic acid (mM)	Acetic acid (mM)	Citric acid (mM)	Malic acid (mM)
LGG	4.2 ± 0.3	114.9 ± 2.0	47.3 ± 3.7	14.1 ± 0.1	1.5 ± 0.3
LG Chen	7.7 ± 0.6	123.2 ± 3.5	65.5 ± 4.7	24.0 ± 1.3	NA
LP18	4.6 ± 0.4	150.4 ± 1.0	29.0 ± 1.7	9.6 ± 0.5	NA

ND = not detected.

Acknowledgments

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