

Effects of ingesting *Lactobacillus*- and *Bifidobacterium*-containing yogurt in subjects with colonized *Helicobacter pylori*¹⁻³

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ABSTRACT

Background: Evidence suggests that ingesting lactic acid bacteria exerts a suppressive effect on *Helicobacter pylori* infection in both animals and humans. Supplementing with *Lactobacillus*- and *Bifidobacterium*-containing yogurt (AB-yogurt) was shown to improve the rates of eradication of *H. pylori* in humans.

Objective: We administered AB-yogurt to subjects with asymptomatic *H. pylori* to test whether the yogurt could inhibit *H. pylori* growth.

Design: The in vitro inhibition of *H. pylori* growth was determined by inoculating *Lactobacillus acidophilus* La5 or *Bifidobacterium lactis* Bb12 on plates that were inoculated with *H. pylori*. Assessment of the viability of *H. pylori* was performed by the mixed culture method with La5 or Bb12. In an intervention study, 59 adult volunteers infected with *H. pylori* were given AB-yogurt (10⁷ colony-forming units of both La5 and Bb12/mL) twice daily after a meal for 6 wk. Eleven subjects positive for *H. pylori* infection were treated with milk placebo as control subjects. *H. pylori* bacterial loads were determined with use of the ¹³C-urea breath test, which was performed before and 4 and 8 wk after the start of AB-yogurt supplementation.

Results: Bb12 exerted an in vitro inhibitory effect against *H. pylori*, whereas La5 did not show an effect. Administration of AB-yogurt decreased the urease activity of *H. pylori* after 6 wk of therapy ($P < 0.0001$).

Conclusion: Regular intake of yogurt containing Bb12 and La5 effectively suppressed *H. pylori* infection in humans. *Am J Clin Nutr* 2004;80:737-41.

KEY WORDS Probiotics, *Bifidobacterium lactis* Bb12, *Lactobacillus acidophilus* La5, urea breath test, yogurt, *Helicobacter pylori*

INTRODUCTION

Helicobacter pylori is a spiral-shaped, gram-negative rod that can colonize epithelial cells lining the antrum of the stomach and survive in the acidic environment. *H. pylori* causes chronic gastritis, plays an etiologic role in the development of peptic ulcer disease, and is considered a risk factor in the development of gastric malignancies such as gastric mucosa-associated lymphoid tissue lymphomas and gastric adenocarcinoma (1-3). Despite the effective antibiotic-based therapies, we were concerned

about their possible induction of resistance to antibacterial drugs. Furthermore, the side effects of these kinds of therapies are a common cause of treatment discontinuation. Probiotics are live microbial food supplements that beneficially affect the host by improving its microbial balance (4). *Lactobacillus* and bifidobacteria are added to several fermented dairy products and are known to have an inhibitory growth effect on a wide range of intestinal pathogens in humans and animals. Midolo et al (5) demonstrated that inhibition of *H. pylori* growth occurred as a result of organic acid production by *Lactobacillus acidophilus* strains in vitro. Coconnier et al (6) showed that *L. acidophilus* supernatant decreased *H. pylori* viability in vitro and decreased urease activity and the histopathologic degree of gastric lesions in mice infected with *Helicobacter felis*. Conventional yogurt is fermented milk produced by adding *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to milk, and conventional yogurt bacteria have a poor resistance to acid and bile (7). However, *L. acidophilus* and bifidobacteria can tolerate pH 3 environments and 2-8% concentrations of bile acid (8). Administration of yogurt supplemented with *Bifidobacterium* spp. and *L. acidophilus* was shown to enhance mucosal and systemic immunoglobulin A responses to the cholera toxin immunogen (9). Sheu et al (10) discovered that supplement with yogurt containing *Lactobacillus* and *Bifidobacterium* could improve the intention-to-treat eradication rate of *H. pylori* and restore the depletion of *Bifidobacterium* in stools after therapy. On the basis of the increasing evidence that *L. acidophilus* and bifidobacteria have therapeutic properties, we investigated the effects of yogurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 on *H. pylori* infection in vitro and in humans.

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SUBJECTS AND METHODS

Preparation of the yogurt

The AB-yogurt (230 mL; President Enterprise Corporation, Tainan, Taiwan) used in this research was fermented milk containing sugar, high-fructose corn syrup, pectin, galactooligosaccharide, and an approximately equal mixture of *L. acidophilus* La5, *B. lactis* Bb12, *L. bulgaricus*, and *S. thermophilus* at a concentration of at least 10^7 bacteria/mL.

In vitro study

Disk diffusion test

H. pylori was isolated from the gastric biopsy specimens of 8 patients (2 gastric cancers, 2 gastric ulcers, 3 duodenal ulcers, and 1 case of gastritis). La5 and Bb12 were grown separately in medium consisting of 10% skim milk and 0.1% yeast extract incubated at 37 °C for 16 h. The suspension of La5 or Bb12 was inoculated into broth, and the concentration was adjusted according to the MacFarland standard [$\approx 6 \times 10^8$, 9×10^8 , 12×10^8 , 15×10^8 , and 21×10^8 colony-forming units (CFU)/mL, respectively] to reach the concentration of 9×10^9 CFU/mL. Diluted culture isolates (10, 30, and 50 μ L) were aspirated and incorporated into filter paper disks. The paper disks were placed onto previously inoculated *H. pylori* (concentration was adjusted to 3×10^8 CFU/mL) blood agar plates and then kept under microaerophilic conditions for 72 h. The inhibitory growth effect of *H. pylori* was interpreted by the inhibition clear halo zone, and the size of each inhibition zone was measured.

Mixed culture method

The concentration of 3 clinical isolates of *H. pylori* was adjusted to 3×10^8 CFU/mL. Both 7.5 g Bacto agar (Difco Laboratory, Sparks, MD) and 14 g *Brucella* Broth (Difco Laboratory) were dissolved in 500 mL distilled water. After autoclaving at 121 °C for 20 min, 50 mL 5% fetal calf serum was added at the temperature of ≈ 50 °C. Subsequently, broths of La5 and Bb12 (concentration was 10^8 CFU/mL), La5-containing yogurt, and AB-yogurt were poured into the culture dish when the temperature was ≈ 40 °C. When the medium became dry, the *H. pylori* isolates were administered for coculture. We counted the number of formed bacterial colonies under microaerophilic conditions after 72 h.

Subjects

Seventy volunteers infected with *H. pylori*, as identified by means of positive ^{13}C -urea breath test (UBT) and positive serology, were enrolled. Control samples were taken from individuals who were asymptomatic or who were dyspeptic but willing to undergo endoscopic examination. Volunteers with upper gastrointestinal tract lesions other than minimal antral erosions were excluded and treated with appropriate medical care. Other exclusion criteria included subjects who had taken antibiotics, bismuth salt, or proton pump inhibitors in the previous month; who had gastric or duodenal bleeding; and who had a history of gastric surgery or other life-threatening conditions. Informed consent was obtained, and the ethics committee of Kaohsiung Medical University Hospital approved the study.

Study protocol

Volunteers were assigned to consume a bottle of AB-yogurt (230 mL) or to consume an unfermented milk placebo twice a day after their morning and evening meals for the first 6 wk (AB-yogurt group: average age, 39 ± 10 y, range, 22–59 y; men: women = 22:37; $n = 59$. Placebo group: average age, 33 ± 9 y, range, 25–53 y; men: women, 5:6; $n = 11$). Subjects were instructed to maintain a regular dietary pattern and to avoid dairy products, Chinese herbal medicines, honey, spicy foods, cranberries, and products containing live lactic acid bacteria during the period of treatment.

^{13}C -UBT was performed on subjects who had fasted overnight on 3 occasions: weeks 0 (before the study), 4, and 8 (2 wk after end of treatment), respectively. A baseline sample of expiratory air was obtained just before the ingestion of 75 mg ^{13}C urea diluted in 75 mL water (Proto Pylorikit; Altachem Pharma Ltd for Isodiagnostika, Edmonton, Canada). Excess $\delta^{13}\text{C}\text{O}_2$ was measured by isotope mass spectrometry. A value of > 3.5 /mil was considered a positive result.

Anti-*H. pylori* immunoglobulin G antibodies were measured by using a specific enzyme immunoassay (Meridian Diagnostics Inc, Cincinnati, OH) according to the supplier's instructions. The test, with use of spectrophotometric single wavelength, was considered positive when the OD_{450} was more than 0.120. Specimens of gastric antrum and body (2 biopsies each with a total of 4 biopsies) were obtained at the initial time by gastroendoscopy and repeated at 4 wk after the end of treatment from 14 randomly selected subjects among the AB-yogurt group.

Histology

Biopsies were fixed in 10% formalin and stained with hematoxylin and eosin. Gastritis was graded according to the modified Sydney system (11, 12): the density of *H. pylori* was graded from 0 to 5, and the activity of gastritis (the density of infiltration with neutrophils) and gastric inflammation (the density of infiltration with mononuclear cells) was graded from 0 to 4.

Statistical analysis

Pretreatment and posttreatment values were compared by repeated measurements of two-factor analysis of variance. $P < 0.05$ was considered statistically significant. To identify the specific groups that were significantly different, we performed Scheffé multiple comparison procedures for comparisons of pairs of means. To express the variability of the results, SE was used. The correlation between pretreatment UBT data and its interval change among the AB-yogurt group was analyzed with use of Pearson correlation coefficient. The paired t test was used for comparison of pretreatment and posttreatment values of biopsies. All calculations in this study were determined by SAS version 8.0 (SAS Institute Inc, Cary, NC) and SPSS version 8.0 (SPSS Inc, Chicago) programs.

RESULTS

In vitro study

The concentration of inoculum of *H. pylori* was $\approx 3 \times 10^8$ CFU/mL. Bb12 had an inhibitory effect on *H. pylori* in samples from 8 patients, whereas La5 did not have any obvious inhibition effect. The minimal concentration of Bb12 to be inhibitory to *H.*



TABLE 1

Presence of *Helicobacter pylori* colonization after administration of various probiotic preparations in different volumes by mixed culture method

Type of probiotic preparation	Volume of probiotic preparation (mL) ¹				
	0.06	0.08	0.10	0.125	0.25
La5	3	3	3	3	3 ²
Bb12	3	3	3	2 ³	0
La5-yogurt	3	3	3	3	3
La5-plus Bb12-yogurt	3	3	3	1 ^{3,4}	0

¹ The concentration of various types of probiotic preparations was 10⁸ colony-forming units/mL.

² Cases with significant presence of colonies of *H. pylori*, n = 3.

³ One case lacked *H. pylori* colonies.

⁴ One case with nearly complete absence of *H. pylori* colonies.

pylori was 9 × 10⁸ CFU/mL. No inhibitory effect of the supernatant of La5 or Bb12 was observed. In samples of *H. pylori* from another 3 patients, cocultures of Bb12, La5, La5-containing yogurt, and both Bb12- and La5-containing yogurt with *H. pylori* were performed, respectively. As the volume of the preparations of Bb12 and La5 plus Bb12 yogurt increased, colonies of *H. pylori* first appeared to be absent in both conditions at 0.125 mL. *H. pylori* became totally absent at the volume of 0.25 mL, whereas La5 and La5 yogurt did not show any suppressive effect at the different concentrations. This finding demonstrated that only Bb12 possesses a suppressive effect (Table 1).

In vivo study

Seventy adult subjects were confirmed to have *H. pylori* infections. Serial UBT was performed on all 70 subjects. There was a significant interaction of time and treatment; administration of AB-yogurt lowered the ¹³C-UBT values after 6 wk of therapy (Figure 1). Among the AB-yogurt group, there were significant differences in the results of ¹³C-UBT values between week 0 and week 4, and between week 0 and week 8 (36.2 ± 19.4 compared

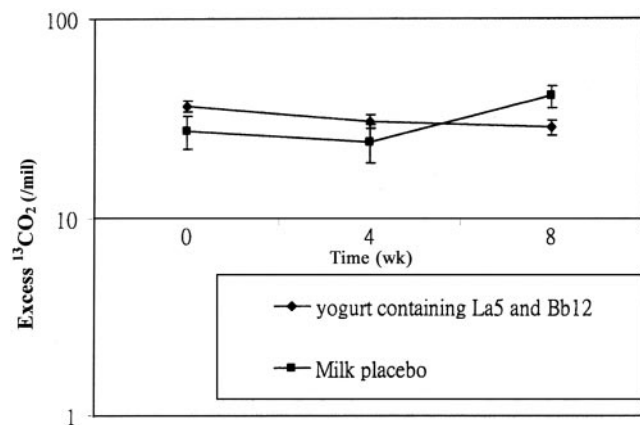


FIGURE 1. Mean (± SD) ¹³C-urea breath test (UBT) values before and after ingestion of AB-yogurt (n = 59 in yogurt containing La5 and Bb12 group) and milk placebos (n = 11 in milk placebo group) in subjects infected with *Helicobacter pylori*. ¹³C-UBT values are expressed as excess δ¹³CO₂. The y-axis is a log scale. ¹³C-UBT value continues to decrease with time in the La5 and Bb12 group, whereas it increases in the milk placebo group. P < 0.0001 for the interaction term by using repeated-measures two-factor ANOVA.

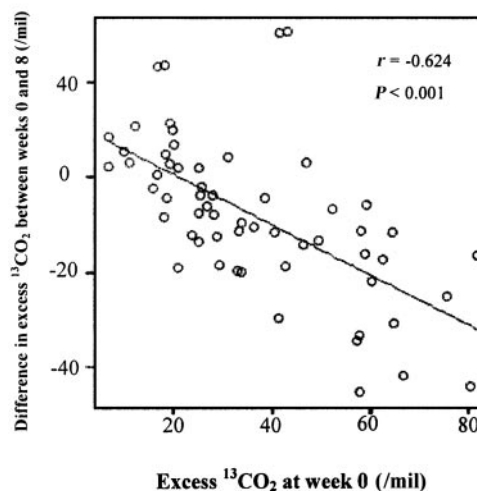
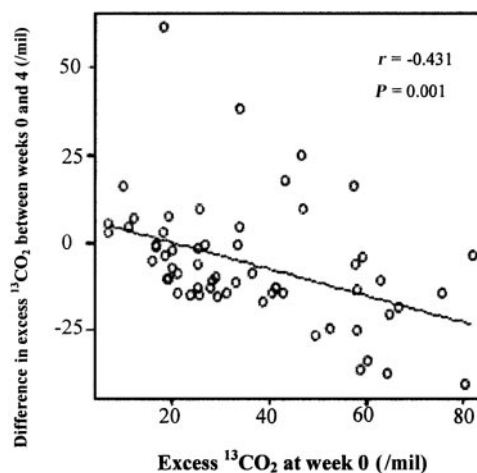


FIGURE 2. Correlations between urea breath test values at week 0 and the difference from weeks 4 and 8, respectively, among the AB-yogurt group. n = 59. r = correlation coefficient. P < 0.05 was considered statistically significant (Pearson’s correlation coefficient).

with 30.1 ± 19.6 and 36.2 ± 19.4 compared with 28.2 ± 15.8, P < 0.05 by using Scheffé multiple comparison method). Also, the pretreatment amounts of excess δ¹³CO₂ showed a significant negative correlation with the difference of excess δ¹³CO₂ amounts between weeks 0 and 4 and between weeks 0 and 8 (Figure 2) in the subjects in AB-yogurt group. For subjects whose ¹³C-UBT values declined at week 8, we had a further follow-up at weeks 12 and 16. One of the patients whose excess δ¹³CO₂ amount was negative at week 8 returned to a positive status at weeks 12 and 16, suggesting that *H. pylori* was not eradicated completely. Examination of antral biopsies showed reduced *H. pylori* density and gastritis activity (P = 0.006 and P = 0.015, respectively) from 14 subjects. No significant change was observed in the gastric body (Table 2).

DISCUSSION

In the present study, we demonstrated that yogurt containing *B. lactis* Bb12 had a suppressive effect on *H. pylori* in vitro. Our results indicated that a 6-wk ingestion of yogurt containing La5 and Bb12 significantly decreased the values of UBT. In addition, endoscopic biopsies of gastric mucosa taken from the antrum

TABLE 2

Histologic aspects of gastric specimens of volunteers before and after treatment with yogurt containing La5 and Bb12¹

Aspects and biopsy site	Before treatment (week 0)	After treatment (week 12)	P ²
<i>Helicobacter pylori</i> density			
Antrum	6.00 ± 0.65	3.86 ± 0.54	0.006
Body	3.25 ± 0.82	2.00 ± 0.35	0.119
Activity of gastritis ³			
Antrum	3.43 ± 0.27	2.36 ± 0.17	0.015
Body	1.83 ± 0.32	1.67 ± 0.26	0.586
Gastric inflammation			
Antrum	4.57 ± 0.17	5.14 ± 0.29	0.071
Body	3.67 ± 0.38	3.33 ± 0.28	0.220

¹ All values are $\bar{x} \pm SE$. $n = 14$ specimens. Treatment was 6 wk long and the posttreatment period was defined as 12 wk.

² $P < 0.05$ was considered significant (paired t test)

³ Gastritis was graded according to the modified Sydney system (11, 12). Biopsies were performed twice at each site. Each value is the sum of the grades of the 2 biopsies.

showed significantly decreased gastritis activity and *H. pylori* density. Values of UBT provided a semiquantitative assessment of the density of *H. pylori* colonization of gastric mucosa, in addition to using histologic examination (13).

Michetti et al (14) used the cultured supernatant of *L. acidophilus* La1 to demonstrate a marked decrease in ¹³C-UBT values in humans. Felley et al (11) demonstrated that a 3-wk intake of acidified milk containing *Lactobacillus johnsonii* La1 decreased *H. pylori* density in humans. Furthermore, a decrease in antral inflammation and antral gastritis was also observed. The design of polymicrobial preparation in this study obtained results similar to previous experiments, whereas only live *B. lactis* Bb12 had a bacteriostatic effect on *H. pylori* in vitro. Because increased density of *H. pylori* on the gastric mucosa was related to more severe gastritis and increased incidence of peptic ulcers, we inferred that a reduction of the density here might help to decrease the risk of developing pathologies (15–17).

Current well-studied probiotics are usually lactobacilli but not bifidobacteria. Lactic acid bacteria in fermented milk need to be present in high concentrations and to survive the digestive process to exert the maximal influence on gastrointestinal functions and to act as probiotics. *Lactobacilli* are the predominant bacteria found in a normal fasting stomach, although their number is small, ranging from 0 to 10³/mL fluid (18). *L. acidophilus* is a strain of human origin that can tolerate very low acidic pH and can survive passage through the gastrointestinal tract (19). Being acid resistant, *L. acidophilus* persists in the stomach longer than other bacteria (Drasar BS, unpublished observations, 1987). Kabir et al (20) found that *L. acidophilus* can exhibit a significant inhibitory effect on the attachment of *H. pylori* to the gastric epithelial cell lines. Wendakoon et al (21) examined the efficacy of a specially designed fermented milk product containing selected lactic acid bacteria with anti-*H. pylori* properties (including *L. casei*, *L. acidophilus*, and *S. thermophilus*). After administration of the yogurt for 1 mo, only 1 subject of 27 was found to have had a complete eradication.

However, Olson and Maier (22) found that hydrogen is available in the gastric mucosa of mice and that its use greatly increased the stomach's colonization by *H. pylori*. Bifidobacteria


were shown to survive in the intestine and could play an important role in the maintenance of gastrointestinal health. Also, ingestion of yogurt could increase the numbers of stool bifidobacteria and suppress coliform bacteria (23). *Clostridium perfringens* and *Escherichia coli* are organisms that produce copious amounts of hydrogen gas.

It could be possible that the decreases in these organisms after a short-term treatment, which increases colonic bifidobacteria, will result in decreased colonic hydrogen gas production. Therefore, we speculated that yogurt containing Bb12 might change the production of hydrogen gas and thereby lessen the severity of *H. pylori* infection.

The activity of gastritis, used as a measure of response to therapy, decreased after ingestion of AB-yogurt. This decreased activity could be related particularly to the decreased density of *H. pylori*. No significant change in gastric inflammation represented by the density of mononuclear cells in the lamina propria was observed. Because *H. pylori* nearly always has a predominantly antral distribution (24), similar changes were not observed in the body of the stomach. However, the temporary inhibitory effect after stopping ingestion of AB-yogurt could be partially because of its stimulation of immunity. Administering fermented milk with lactic acid bacteria (*L. bulgaricus* and *S. thermophilus*, *L. acidophilus*, *L. casei*, and *Bifidobacterium* spp.) apparently enhanced the immune response in animal studies by activating macrophages and lymphocytes (25). In humans fed yogurt, results showed an increase of B and natural killer cells in the lymph nodes and increased interferon- γ production (26–28). The long-term anti-inflammatory effect and its immunomodulatory properties of AB-yogurt merits further investigation.

Pedrosa et al (29) demonstrated that neither *S. thermophilus* nor *L. bulgaricus* was recovered from the stomach or small intestine of subjects fed yogurt. Bb12 was considered to be responsible for the efficacy for suppressing *H. pylori* in our study. The mechanisms could be explained through reciprocal inhibition between different bacterial species. In our in vitro study, Bb12 exerted a significant inhibitory effect on *H. pylori* growth on the plate (mixed culture method), which might be related to its competition for nutrients. Bacterial disk diffusion method showed an annular radius of inhibition on the plate by the Bb12. Moreover, we found that subjects with higher intragastric bacterial load of *H. pylori* predicted by the ¹³C-UBT values had more significant rates of reduction after treatment. Because the ability to adhere to epithelial cells and thereby colonize the stomach is probably of crucial importance in its interference with *H. pylori*, this phenomenon could be explained by the barrier effect as a result of the attachment of La5 or Bb12 to gastric epithelial cells.

Because there is a high prevalence of lactose intolerance in our country, it is difficult to have a larger number of control subjects. The reason we administered milk placebos in our study was because of their considerable difference from fermented yogurt with regard to bacteriologic composition and lactase activity. When the ingestion of AB-yogurt was stopped, the UBT values returned to pretreatment levels at week 16 in some subjects (data not shown). Therefore, the necessity for continuous ingestion is apparent. It was also necessary to give doses regularly instead of assuming that a few doses would allow the organism to colonize the gut permanently (30). However, we need more research to standardize the dosages and timing of taking probiotics to reduce *H. pylori* infection in humans.

In summary, probiotic preparations of yogurt containing La5 and Bb12 are determined to be effective in suppressing *H. pylori* infection in humans for a 6-wk treatment. 

S-NL participated in the interpretation of the data and in writing the manuscript. C-SL participated in the interpretation of the data. T-NW participated in the design of the study, in the analysis, and in the interpretation of the data. WMW contributed to various stages of the study, including the design of the experiment, collection of the data, analysis of data, and writing of the manuscript. D-SP, Y-CS, D-CW, C-MJ, and C-HL participated in the data collection. No author had any financial or personal relations with the company or organization sponsoring the research. None of the authors had a conflict of interest.

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