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ORIGINAL ARTICLE

Anti-*Helicobacter Pylori* Effect of Fermented Ginseng Extracts with *Lactobacillus plantarum* MG 208

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Abstract Water extract of ginseng was fermented using various lactic acid bacteria, and their anti-*Helicobacter pylori* activity was evaluated. The fermented ginseng extracts evidenced anti-*H. pylori* activity, including anti-bacterial, anti-adhesion, and urease inhibition effects. Among the four types of lactic acid bacteria, *Lactobacillus plantarum* MG 208 evidenced the most profound anti-*H. pylori* activity in fermented ginseng. Therefore, fermented ginseng extract containing *L.plantarum* MG 208 could prove useful as functional diet for the protection of the gastric environment against *H. pylori*.

Keywords fermentation · ginseng · *Helicobacter pylori* · *Lactobacillus plantarum*

Introduction

Helicobacter pylori, a microaerophilic bacterium, is generally regarded as the causative agent of chronic gastritis, duodenal and gastric ulcers, and gastric adenocarcinoma (Uemura et al., 2001; Kaji et al., 2002). As over 50% of Koreans are infected with *H. pylori* (Yim et al., 2007), the eradication of *H. pylori* is considered an essential step in the treatment of gastrointestinal diseases and the prevention of recurrence (Kim et al., 1997). The current eradication method for the treatment of *H. pylori* is 7–14 days of triple therapy involving the combined application of proton pump inhibitor (PPI), clarithromycin, and amoxicillin, with eradication power of 85% in cases with no resistance. However, some patients infected with the strain showed resistance or experienced deleterious side effects caused by ingestion of the drugs, resulting in drop of their compliance, and the eradication effect is markedly decreased due to antibiotic resistance. Therefore, in order both to reduce side effects and to enhance patient compliance with antibiotic medication regimes, there is a clear need for the development of new treatment methods that replace antibiotics.

Ginseng, the root of *Panax ginseng* C.A. Meyer, is broadly employed as a traditional medicine in Korea. The pharmacological effects of ginseng have been previously demonstrated in the central nervous system (CNS) and in the cardiovascular, endocrine, and immune systems (Cheng et al., 2006). Additionally, ginseng and its constituents have been reported to evidence anti-neoplastic, anti-stress, and antioxidant activities (Wang and Joseph, 1999; Yun et al., 2001; Wang et al., 2006), and several studies have reported that ginseng extract exerts a gastroprotective effect against *H. pylori*-associated damages (Lee et al., 2004; Park et al., 2005). The ginsenosides have been identified as the principal components of ginseng, and appear to be responsible for a number of important pharmacological effects (Lee et al., 2005; Tian et al., 2005; Park et al., 2006; Zhang and Wang 2006). According to previous studies on *in vitro* examination, several types of comparatively more deglycosylated ginsenosides exhibit anti-*H. pylori* activity (Bae et al., 2001; 2002).

Recently, ingestion of fermented food products was identified as a healthy part of a functional diet for longevity. In particular, lactic acid bacteria, including *Lactobacilli* and *Bifidobacterium*, exist as the predominant bacteria within the intestine, and generate lactic acid via the fermentation of saccharides; the lactic acid bacteria have also been identified as live bacterial activators that promote the growth of beneficial bacteria in the body. They have been shown to prevent a variety of diseases and to control physiology by improving gastrointestinal function, inhibiting cholesterol absorption, controlling immunity, enhancing absorption,

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improving utilization of nutrients, among others (Kim and Kim 2006; Shin et al., 2006; Gao et al., 2008). In the present study, the anti-*H. pylori* activity of fermented ginseng was evaluated using various lactic acid bacteria (LAB), and the potent anti-*H. pylori* effects of *L. plantarum* MG 208 fermented ginseng extracts are described.

Materials and Methods

Fermentation and preparation of samples. Dried ginseng water extracts (5%) were fermented using various lactic acid bacteria. including *L. plantarum* MG 208, *Lactobacillus casei* MG 311, *Lactobacillus acidophilus* MG 501, and *Lactobacillus fermentum* MG 590-A. The fermented samples were lyophilized after filtration through a membrane filter, followed by anti-*H. pylori* assay and chemical analysis.

***H. pylori* culture.** The *H. pylori* strain, ATCC 43504 (cag A+, vacA+), was cultured in a solid medium (a brain heart infusion (BHI) or brucella agar medium containing 7% sheep blood) and in a liquid medium (brucella medium containing 10% fetal calf serum) at 37°C. *H. pylori* was cultured in an anaerobic cultivator (BBL, Franklin Lakes, NJ) filled with a microaerophilic gas pack (MGC, Tokyo, Japan).

Anti-bacterial activity against *H. pylori*. The antimicrobial activities of the samples were evaluated via measurement of the formation of clear zones on 8-mm discs (Adventec, Toyo Roshi Kaisha, Japan). *H. pylori* was cultured for 5 days in a BHI agar medium containing 7% sheep blood, and recovered with a sterilized loop. After 3 min suspension, 200 μL (5×10^8 CFU/mL) was extracted, smeared, and placed on a sterilized disc. Each 50 μL (1 mg/mL) of sample was then added. After 72 h culturing at 37°C under microaerophilic conditions, antimicrobial activity was assessed by measuring clear zone formation around the disk. Each sample containing distilled water was compared with the Negative (distilled water) and positive (amoxicillin in distilled water, 5 μg/mL) control groups.

Anti-adhesion activity against *H. pylori*. The adhesion inhibitory effect was measured using the method described by Koo and Choe (2001). AGS gastric cells were divided into 96-well plates at 2×10^4 cells/well, and precultured for 48 h. Each 1 mg/mL samples were then added and the reaction was conducted for 30 min at 37°C. The cells were cultured in a solid-state medium for 5 days, and *H. pylori* bacteria were re-suspended in phosphate buffered saline (PBS) (1×10^8 CFU/mL, pH 7.4, Sigma, St. Louis, MO) for inoculation. The gastric cell samples were exposed for 90 min to *H. pylori* bacteria, and any *H. pylori* bacteria that did not adhere to gastric cells were removed by washing three times with PBS. After 30 min of incubation at 37°C with urease activity test solution (3 mM sodium phosphate, pH 6.8, containing 7 μg/mL phenol red and 110 mM urea), absorbance was measured at 540 nm.

Urease inhibitory effect. The urease inhibitory effect was measured via the alkalimetric method developed by Hamilton-Miller and Gargan (1979) and Mobley et al. (1988). *H. pylori* was activated by culturing in a Mueller-Hinton medium (BBL, Franklin Lakes, NJ) containing 10% fetal calf serum, washed in PBS, and recovered via centrifugation. In a 96-well plate, the bacterial suspension was mixed with the same amount of sample solution. Urea (0.33 μg/mL) and phenol red (7 μg/mL) were added to the mixture, and mixed thoroughly. After 60 min of incubation at 37°C, absorbance was measured at 540 nm at 10-min intervals.

Measuring total saponin content. To 2 g of lyophilized fermented-ginseng extracts, 100 mL of 80% menthanol (MeOH) was added and refluxed at 80°C. The supernatant was concentrated under reduced pressure and dissolved in 20 mL of distilled water. After washing in 20 mL of ethyl ether, the water layer was extracted three times using 20 mL of water-saturated *n*-butyl alcohol. The water-saturated *n*-butyl alcohol layer was concentrated under reduced pressure, and the saponin fraction residue was obtained. This residue was dissolved in MeOH and added to 300 μL of 8% vanillin solution [in ethanol (EtOH)]. In a cooling tank, 4 mL of 75% sulfuric acid solution was added and heated to 60°C for 10 min. After 20 min of cooling at room temperature, absorbance was measured at 545 nm. A standard curve was obtained using ginsenoside Re (WAKO Chem., Osaka, Japan).

High performance liquid chromatography (HPLC) analysis of ginsenoside contents. Five ginsenoside standard materials (ginsenoside Rb_1, Rb_2, Rd, Rf, Rh_1) and the saponin fraction residue were dissolved in MeOH and filtered through a 0.45-μm membrane filter, and 20 μL of filtered solution was injected into a Jasco HPLC system (Tokyo, Japan). Separation was conducted on a μBodapak C_{18} (Merck, Darmstadt, Germany) column maintained at 25°C. The acetonitrile/H_2O (3/7) solution was employed as the mobile phase at a flow rate of 1.0 mL/min. Detection was conducted at 203 nm.

Results

Anti-bacterial effect of fermented-ginseng extracts against *H. pylori*. The effect on formation of *H. pylori* clear zones is shown in Table 1. The fermented ginseng extract with *L. plantarum* MG 208 evidenced the largest clear zone diameter among four types of fermented ginseng extract at 1 mg/mL. These fermented-extracts exhibited similar anti-bacterial effects using 5 μg/mL amoxicillin as a positive control. Jee et al. (2008) reported that the white ginseng extract of 70% aqueous EtOH inhibit *H. pylori* growth at higher than 5 mg/mL.

Anti-adhesion effect of fermented-ginseng extracts. *H. pylori* infection of gastric epithelial cells resulted in gastric inflammatory damage. In an effort to determine whether fermented-ginseng extracts could inhibit the adhesion of *H. pylori* to gastric epithelial cells, AGS gastric cells was employed. AGS cells treated with *L. plantarum* MG 208 fermented-ginseng extracts evidenced relatively

Table 1 Anti-bacterial activities of fermented-ginseng extracts at 1 mg/mL

	Diameter of clear zone
CONTROL (Distilled Water)	10 mm
Lactobacillus plantarum MG 208	14 mm
Lactobacillus casei MG 311	10 mm
Lactobacillus acidophilus MG 501	11 mm
Lactobacillus fermentum MG 590-A	11 mm
Amoxicillin (5 μg/mL)	15 mm

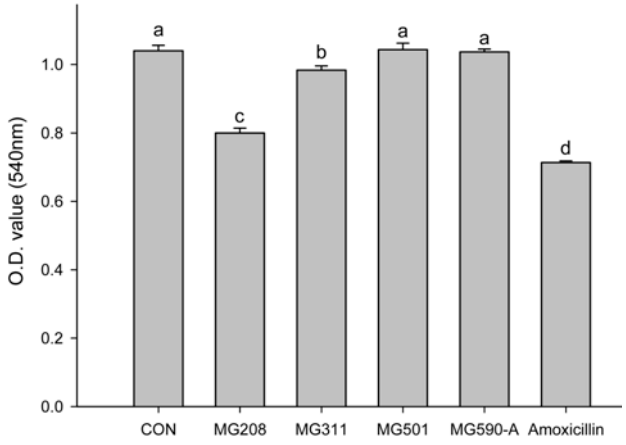

Fig. 1 Inhibition of fermented-ginseng extracts on adhesion of *H.pylori* to AGS human gastric epithelial cells at 1 mg/mL. [a-d]Mean values are significantly different as determined by Duncan's multiple range test (p <0.001). The concentration of amoxicillin is 5 μg/mL.

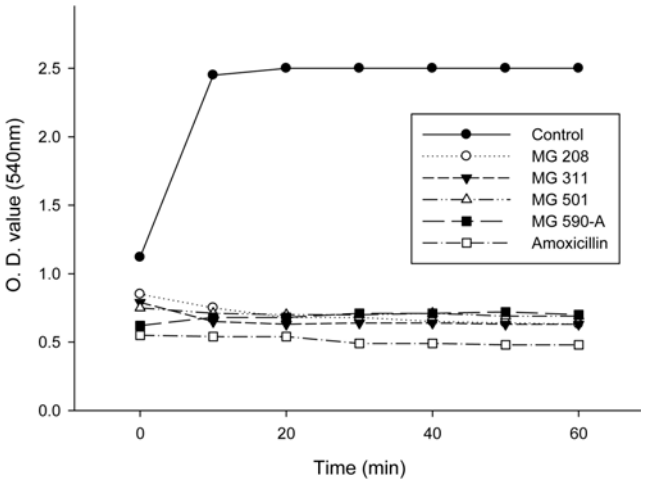

Fig. 2 Inhibition of fermented-ginseng extracts using different LAB on the urease activity of *H.pylori* at 1 mg/mL. The concentration of amoxicillin is 5 μg/mL.

Table 2 Total saponin contents of fermented-ginseng extracts (dry-weight basis, %)

	Total saponin (%)
Lactobacillus plantarum MG 208	4.4473
Lactobacillus casei MG 311	4.4473
Lactobacillus acidophilus MG 501	4.8947
Lactobacillus fermentum MG 590-A	4.6578

low rates of *H. pylori* adhesion. However, the other LAB fermented-ginseng extracts evidenced no significant anti-adhesion activity (Fig. 1).

Urease inhibitory effect of fermented-ginseng extracts. Despite the acidic conditions of the gastric environment, *H. pylori* can survive due to its acid-resistant property, attributable to urease release. In order to evaluate the effects of fermented-ginseng extracts on the urease-release activity of *H. pylori*, fermented-ginseng extracts were mixed with *H. pylori* bacterial suspensions, and urease activity was measured for 60 min. Fermented-ginseng extracts potently inhibited the urease activity of *H. pylori* in all four species of the lactic strain (Fig. 2).

Contents of total saponin and various ginsenosides. Total saponin contents did not differ substantially among the fermented-ginseng extracts (Table 2). However, the *L. plantarum* MG 208 fermented-ginseng extract evidenced a different ginsenoside pattern than those observed with other fermented-ginseng extracts in HPLC-based quantitative analysis; the contents of ginsenosides Rd and Rh_1 in the *L. plantarum* MG 208 fermented-ginseng extracts were higher than the other fermented-ginseng extracts, and ginsenosides Rb_1, Rb_2 and Rf contents in this extract were significantly low (Table 3).

Discussion

Several studies have demonstrated that ginsenoside glycosides are poorly absorbed throughout the human intestinal tract (Odani et al., 1983; Han and Fang, 2006). The pharmacological activities of ginsenosides have been theorized to involve the manner in which glycosylated ginsenosides are deglycosylated by intestinal bacteria following oral ingestion, and the generated aglycone metabolites

Table 3 Comparison of seven ginsenoside contents in fermented-ginseng extracts (dry-weight basis, %)

	Rb_1	Rb_2	Rd	Rf	Rh_1
Lactobacillus plantarum MG 208	-	-	0.017	-	0.003
Lactobacillus casei MG 311	0.048	0.023	-	0.026	0.002
Lactobacillus acidophilus MG 501	0.0047	0.019	-	0.016	0.003
Lactobacillus fermentum MG 590-A	0.049	0.022	-	0.018	0.001

exhibit a broad range of pharmacological activities (Hasegawa et al., 1996; Tawab et al., 2003; Hasegawa, 2004).

In the metabolic pathway, ginsenosides Rb_1 and Rb_2 can be converted into metabolite Rd, and ginsenoside Rf can be converted into metabolite Rh_1 (Cheng et al., 2008; Ruan et al., 2009). The Rb_1, Rb_2 and Rf contents in the *L. plantarum* MG 208 containing fermented-ginseng extract were lower than in the other fermented-ginseng extracts, whereas the Rd and Rh_1 contents were higher. These results showed that *L. plantarum* MG 208 is easily fermentable in ginseng extracts, in which potent anti-*H.pylori* activities, including anti-bacterial, anti-adhension, and urease inhibition effects were evidenced. These results indicate that the *L. plantarum* MG 208 fermented-ginseng extract assessed herein may prove useful as a nutraceutical material for the prevention of *H. pylori*-induced gastric damage. Evaluation of the relevant clinical and dietary values of this extract using experimental animal models and clinical experiments remains for future studies.

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