J Appl Biol Chem (2015) 58(1), 89–95
http://dx.doi.org/10.3839/jabc.2015.015

Online ISSN 2234-7941
Print ISSN 1976-0442

Original Article: Food Science/Microbiology

Fermentation Characteristics and anti-*Helicobacter pylori* Activity of Aqueous Broccoli Fermented by *Lactobacillus plantarum* MG208

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Received: 8 December 2014 / Accepted: 2 February 2015 / Published Online: 31 March 2015

Abstract *Helicobacter pylori* infection causes gastrointestinal diseases such as chronic gastritis, peptic ulcers, and may lead to gastric cancer. Several studies have reported that lactobacilli present on broccoli show inhibitory activity against *H. pylori*. Here, we evaluated aqueous broccoli, fermented by *Lactobacillus plantarum* MG208, for its fermentation characteristics and anti-*H. pylori* activities including antibacterial activity, growth inhibition, anti-adhesion, and urease inhibition. The results indicated that the fermentation characteristics changed significantly depending on the amount of aqueous broccoli used for fermentation (p <0.05). There was no significant difference between the samples before fermentation (p >0.05). However, a significant concentration-dependent difference was noted in antibacterial activity and urease inhibition (p <0.05) following the addition of aqueous broccoli. Growth inhibition in the 10 mg/mL sample was significantly higher as compared to the negative control and similar to that with amoxicillin (positive control) (p <0.05). Anti-adhesion activity of aqueous broccoli was also significantly different (p <0.05) from the negative control. Therefore, aqueous broccoli fermented by *L. plantarum* MG208 could prove useful as a functional diet for protection of the gastric environment against *H. pylori* infection.

Keywords broccoli · fermentation *Helicobacter pylori* · *Lactobacillus plantarum* MG208

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Introduction

Helicobacter pylori is a spiral-shaped, gram-negative, acid-tolerant, microaerophilic bacterium found in the human stomach and duodenum (Montecucco and Rappuoli, 2001; Blaser and Atherton, 2004). It is regarded as the causative agent of chronic gastritis, duodenal and gastric ulcers, as well as gastric adeno-carcinoma (Uemura et al., 2001; Kaji et al., 2002). Since at any point in time, approximately 50% Koreans harbor an *H. pylori* infection (Yim et al., 2007), eradicating the bacterium is an essential step towards treatment of gastrointestinal disease and the prevention of recurrence (Kim et al., 1997).

The mechanisms by which *H. pylori* infection lead to gastric mucosal damage include direct effects of virulence factors produced by *H. pylori*, such as *cagA* (cytotoxin-associated gene A) and *vacA* (vacuolating cytotoxin A) 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).

The current eradication protocol for *H. pylori* infections includes 7–14 days of triple therapy consisting of a proton pump inhibitor (omeprazole), an antibiotic (clarithromycin or amoxicillin), and metronidazole, with an 85% eradication rate in the absence of resistance. However, some patients are either infected with a resistant strain, affecting the eradication rate or they experience toxic side effects caused by the drugs resulting in non-compliance, further compounding the drop in eradication success. Therefore, in order to both, reduce antibiotic side effects and improve patient compliance, there is a clear need for the development of new treatment approaches, replacing antibiotics. Recent reports show that natural phytochemicals in combination with lactic acid or lactate are promising alternatives to antibiotics (Pantoflickova et al., 2003; Miki et al., 2007; Apostolidis et al., 2011; Michael et al., 2011; Lin et al., 2011; Yang et al., 2012).

Lactic acid bacteria (LAB) including lactobacilli and bifidobacteria, are predominant within the intestine and produce lactic acid via fermentation of saccharides. LAB also function as live bacterial activators that promote the growth of beneficial bacteria in the

body, prevent a variety of diseases, and regulate physiological activity by improving gastrointestinal function, inhibiting cholesterol absorption, controlling immunity, enhancing absorption, improving utilization of nutrients, etc. Therefore, consumption of fermented food products are gaining acceptance as a healthy component of functional diets for longevity (Kim and Kim, 2006; Shin et al., 2006; Gao et al., 2008).

Vegetables from the Family *Cruciferae* such as broccoli (*Brassica oleracea italica*) contain less quantity of fat, are low in calories, and at the same time are rich in vitamins, inorganic matter, and fiber (Fahey et al., 1997; Jang, 2001). Broccoli contains high quantity of β-carotene, rutin, ascorbic acid, selenium, quercetin, and glutathione, which are potent antioxidants. Furthermore, broccoli is known to show detoxification effects and contains sulfur compounds associated with anti-cancer and mutation-inhibitory activity (Kurilich et al., 1999; Sok et al., 2003; Matusheski et al., 2004). Some *in vitro* experiments showed that sulforaphane (the primary isothiocyanate in broccoli) is effective in preventing cancers as well as in eradicating *H. pylori* (Fahey et al., 2002; Yanaka et al., 2009).

However, anti-*H. pylori* activity of LAB-fermented broccoli has not yet been studied. Therefore, the purpose of the present study was to examine the anti-*H. pylori* activity of broccoli fermented by *Lactobacillus plantarum* MG208.

Materials and Methods

Materials. Powdered broccoli was obtained from Cannan Farmers School (Korea). *L. plantarum* MG208 was cultured in Mann, Rogosa, and Sharp (MRS) agar medium (Difco, USA), stored in a refrigerator at 4°C, and subcultured every 3 weeks at Mediogen Co., Ltd, (Korea) a contract research institute. In addition, for long-term storage, the strain was kept at −70°C (Operon-128c, Korea) in a medium containing 20% sterilized glycerol (Shinyo Chem., Japan).

Preparation of samples. The procedure for sample preparation is shown in Fig. 1. The LAB strain was cultured at 37°C in MRS liquid medium and LAB medium under stationary conditions. We also prepared media containing aqueous broccoli of different concentrations. Aqueous broccoli was obtained by adding reconstituted broccoli to distilled water at concentrations of 1, 5, and 10%. Culture medium containing 5% (1.0×10^6 CFU/mL) LAB was inoculated with the different concentrations of aqueous broccoli and incubated at 37°C for 28 h in an anaerobic chamber (BBL, USA). The cultures obtained were used as LAB-fermented broccoli samples for the evaluation of fermentation characteristics, i.e., cell count, pH, and titratable acidity (TA).

In order to evaluate anti-*H. pylori* activity, we prepared a control solution of 10% non-fermented aqueous broccoli. In addition, we prepared an *L. plantarum* MG 208 sample, containing 10% aqueous broccoli fermented by *L. plantarum* MG208. The samples were filtered using a 0.45-μm membrane filter (Whatman, USA) and lyophilized. The lyophilized powder was then suspended in distilled water at concentrations of 1, 5, and 10 mg/mL. MG208 refers to aqueous broccoli fermented by the LAB strain *L. plantarum* MG208; distilled water was used as another control.

***H. pylori* culture.** The *H. pylori* strain ATCC 43504 (*cag A*+, *vacA*+) was cultured in solid (brain-heart-infusion [BHI] or Brucella agar medium containing 7% sheep blood) and liquid medium (Brucella medium containing 10% fetal calf serum) at 37°C in an anaerobic incubator (BBL, USA) fitted with a microaerophilic gas pack (MGC, Japan).

Estimation of cell number, pH, and TA. To obtain the total bacterial count, we diluted 1 mL sample with 9 mL sterile physiological saline, mixed well, serially diluted and inoculated (1 mL) on bromocresol-purple plate-count agar with 1% glucose. The samples were evenly spread on the agar and plates were placed in an anaerobic box (BBL Gas Pak Anaerobic System) and incubated at 37°C for 48 h. The number of colonies was counted and the viable count per mL medium was calculated by multiplying the average colony count with the dilution factor.

The pH values of the sample were measured with a digital pH meter (Model 320, Thermo Orion, USA).

TA was determined by titrating each sample with 0.1 M NaOH to pH 8.3. The results are expressed as percentage of citric acid, determined according to standard procedures (AOAC, 1984).

Antibacterial activity against *H. pylori*. Antibacterial activity was evaluated using the modified method described by Gavidson and Parish (1989), where antimicrobial activity was determined by measuring the clear zones formed around the 8-mm discs (Advantec, Japan). *H. pylori* was cultured for 5 days on BHI agar containing 7% sheep blood, harvested using a sterilized loop and a suspension was prepared, of which, 200 μL (5×10^8 colony-forming units [cfu]/mL) was evenly spread on agar plates. Sterilized discs containing 50 μL of absorbed sample was placed on the agar. After incubation for 72 h at 37°C under microaerophilic conditions, the antimicrobial activity was assessed by measuring the clear zone that formed around each disc. Samples were compared with the negative and positive controls containing distilled water and antibiotic (5 μg/mL amoxicillin), respectively.

***H. pylori* growth inhibition.** Growth inhibition was measured using the modified method described by Gavidson and Parish (1989). *H. pylori* (1×10^8 cfu/mL) was spread evenly within each well of a 96-well plate and 40 μL sample was added to each well. The mixture was cultured with Brucella medium containing 10% fetal bovine serum for 24 h. Inhibition of *H. pylori* growth (i.e., changes in the multiplication rate of *H. pylori*) was analyzed by measuring the absorbance (optical density [OD]) at 450 nm.

Anti-adhesion activity against *H. pylori*. Inhibition of adhesion was measured using the method described by Koo et al. (2001). AGS gastric cells were seeded in 96-well plates at a density of 2×10^4 cells/well and pre-cultured for 48 h. Then, 1 mg/mL samples were added to the wells and the reaction was carried out for 30 min at 37°C. AGS gastric cells were cultured on solid medium for 5 days and *H. pylori* cells were re-suspended in phosphate-buffered saline (PBS) (1×10^8 cfu/mL) for inoculation. The gastric cell samples were exposed to *H. pylori* for 90 min and *H. pylori*

cells that did not adhere to gastric cells were removed by 3 washes with PBS. After 30 min of incubation at 37°C with the 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 inhibition. The inhibition of urease activity was measured by the alkalimetric method developed by Hamilton-Miller (1979) and Mobley et al. (1988). *H. pylori* was activated by incubation in Mueller-Hinton medium containing 10% fetal calf serum, washed in PBS, and recovered by centrifugation. In a 96-well plate, the bacterial suspension (10 μL) was mixed with the same amount of sample solution. Urea (330 μg/mL) and phenol red (7 μg/mL) were added to the mixture to obtain a final volume of 80 μL and mixed thoroughly. After 60 min of incubation at 37°C, absorbance was measured at 10-min intervals at 540 nm.

Results and Discussion

Cell number, pH, and TA. Fermentation characteristics following addition of aqueous broccoli are shown in Table 1.

When medium containing aqueous broccoli was fermented with *L. plantarum* MG208, the viable counts of LAB increased, depending on the amount of aqueous broccoli added to the medium (p <0.05).

Before fermentation, the viable counts of LAB in media containing different amounts of aqueous broccoli were not significantly different (p >0.05)

Before fermentation, the pH of media to which aqueous broccoli was added, was between 5.93 and 6.04, whereas the pH decreased rapidly after fermentation of the aqueous broccoli by LAB, ranging between 3.15 and 2.64. The decrease in pH was dependent on the amount of aqueous broccoli added to the medium (p <0.05). Before fermentation, the pH of media containing different quantities of aqueous broccoli was not significantly different (p >0.05). We believe that the pH decreased on account of the production of various organic acids during fermentation by LAB in the presence of aqueous broccoli.

The TA value after fermentation by LAB increased significantly depending on the amount of aqueous broccoli added to the medium (p <0.05). Before fermentation, the TA values of media containing different quantities of aqueous broccoli were not significantly different (p >0.05).

With regard to the fermentation properties (cell count, pH, and TA), the results obtained in this study were similar to those previously obtained for vegetables, mulberry fruits, onion juice, and licorice extracts (Jung, 2007; Choi, 2009; Cheon, 2011; Kim, 2013).

Park et al. (2006), Lin et al. (2011), and Chen et al. (2010) reported fermentation properties (cell count, pH, and TA) which differed as per the type of LAB involved.

Antibacterial activity of aqueous broccoli against *H. pylori*. The effect of aqueous broccoli on the formation of clear zones around discs placed on *H. pylori*-seeded plates is shown in Table 2.

A significant difference was noted in all experimental samples (p <0.05). No clear zone was formed in the sample containing distilled water, which served as a negative control. The largest clear zone (diameter) was obtained with the 10-mg/mL sample of aqueous broccoli fermented with *L. plantarum* MG208, though not as large as that of the positive control (5 μg/mL amoxicillin). However, antibacterial activity significantly increased with the content of broccoli. This result suggests that aqueous broccoli fermented by *L. plantarum* MG208 showed antibacterial activity against *H. pylori*.

Antibacterial activity against *H. pylori* has been previously demonstrated by *Portulaca oleracea*, *Cistus laurifolius* leaves, thyme, tea extract, oregano, rosemary, mulberry leaves, mulberry

Table 1 Changes in viable counts of lactic acid bacteria, pH, titratable acidity (TA) by fermentation following addition of aqueous broccoli extracts

Sample	Concentration (%)	pH	Titratable acidity	Total viable counts
Before Fermentation	1	6.04±0.02^{a}	0.04±0.00^{d}	1.30×10^{8}±0.03^{e}
	5	5.93±0.02^{a}	0.04±0.00^{d}	1.30×10^{8}±0.03^{e}
	10	6.00±0.01^{a}	0.04±0.00^{d}	1.29×10^{8}±0.02^{e}
After Fermentation	1	3.15±0.02^{b}	0.20±0.00^{c}	7.15×10^{9}±1.57^{c}
	5	3.03±0.03^{c}	0.22±0.02^{b}	7.90×10^{9}±1.32^{b}
	10	2.64±0.01^{d}	0.28±0.00^{a}	9.51×10^{9}±1.71^{a}

$^{a-e}$Significant difference in sample concentrating to ANOVA followed by Duncan (p <0.05).

Table 2 Antibacterial activities of aqueous broccoli extracts fermented with *Lactobacillus plantarum* MG208

Samples	Concentration		Diameter of clear zone (mm)
Distilled water (negative control)	0	mg/mL	8.00±0.00^{e}
	1	mg/mL	11.17±0.58^{d}
	5	mg/mL	12.17±0.29^{c}
	10	mg/mL	13.33±0.29^{b}
Amoxicillin (positive control)	5	μg/mL	15.17±0.29^{a}

$^{a-e}$Significant difference in sample concentrating to ANOVA followed by Duncan (p <0.05).

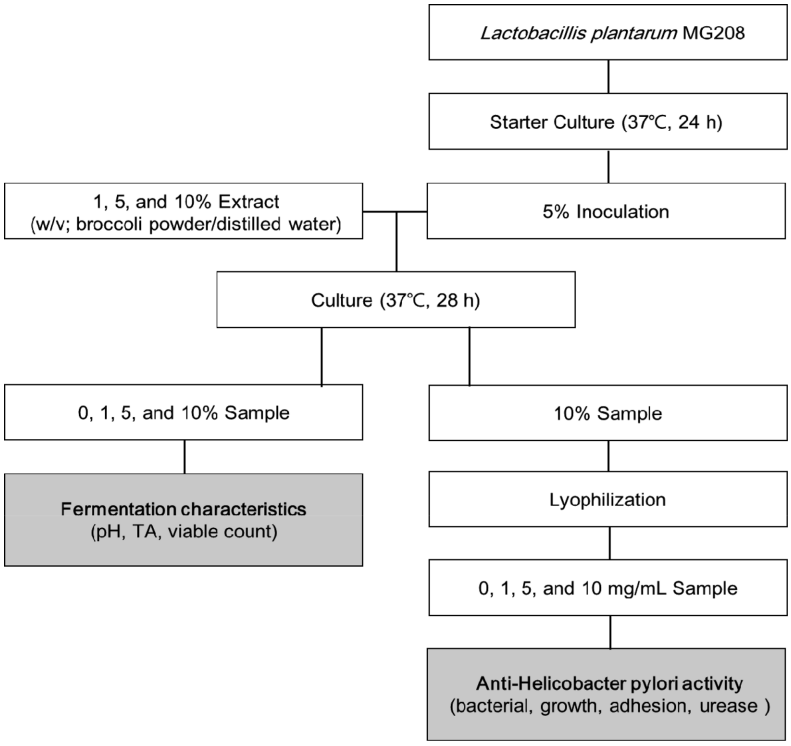

Fig. 1 Schematic diagram of the experimental design of the study.

fruit, and licorice (Diker and Hascelik, 1994; Tabak et al., 1996; Toshio et al., 2002; Cha et al., 2006; Cho et al., 2006; Kim et al., 2006; Osman et al., 2006; Cho et al., 2007; Jung, 2007; Son, 2007; Park, 2008; Lm et al., 2010; Kim and Cho, 2011). Chen et al. (2010) reported that among 16 LAB strains with anti-*H. pylori* activity, *L. plantarum* 18 strain showed the strongest antibacterial activity. Ko et al. (2011) reported that micro-encapsulated *L. casei* ATCC 393 also showed antibacterial activity against *H. pylori*.

Lin et al. (2011) used milk fermented by 10 different LAB strains and found that 3 LAB strains (LY1, LY5, and IF22) showed antibacterial activity against *H. pylori*. Apostolidis et al. (2011) reported that addition of 1% cranberry-chitosan oligosaccharide mixture to milk fermented by *L. plantarum* (NRRL, B4496) resulted in enhanced antibacterial activity against *H. pylori*.

Yang et al. (2012) reported that ginseng extract fermented by *L. plantarum* MG208 showed antibacterial activity against *H. pylori*.

***H. pylori* growth inhibition.** *H. pylori* growth was determined by a spectrophotometric method. The result is shown in Fig. 2.

All samples used in this experiment appeared effective in inhibiting the growth of *H. pylori*.

Compared to the negative control (amoxicillin), 1, 5, and 10 mg/mL samples showed strong growth inhibition, i.e., *H. pylori* growth was inhibited by 86.0, 75.8, 77.8, and 79.8%, respectively (p <0.05). This result shows that aqueous broccoli fermented by *L. plantarum* MG208 inhibits *H. pylori* growth, though the exact mechanism is unclear as there was no significant differences in the concentration of the samples.

The 5- and 10- mg/mL samples exhibited similar antibacterial activity as 5 μg/mL amoxicillin, which served as positive control. Mulberry fruits, *Portulaca oleracea*, and *Scutellaria baicalensis* have been previously shown to possess growth inhibitory activity against *H. pylori* (Cho, 2006; Park and Kim, 2006; Park, 2008). Cha et al. (2006) reported that 60% ethanolic oregano extract showed 72.2% inhibition of *H. pylori* growth.

Anti-adhesion activity against *H. pylori*. The attachment of *H. pylori* to the gastric epithelium is important for active inflammation of the mucosal layer. *H. pylori* shows a wide spectrum of different specificities regarding adhesion to host cells (Beil and Kilian, 2007). Several surface carbohydrates that mediate cell adhesion have been identified. In particular, the Lewis b blood group antigen, which is typically expressed on the surface of human gastric epithelial cells such as AGS cells, is known to mediate the adherence of *H. pylori* to human gastric mucosa (Lee et al., 2006). In this study, we used AGS cells in an effort to determine whether aqueous broccoli inhibited the adhesion of *H. pylori* to gastric epithelial cells. The result is shown in Fig. 3. All experimental samples were significantly different as compared to that of the control sample (p <0.05). However, the results of the 1 and 5 mg/mL samples were not significantly different from each other (p >0.05).

The adhesion of *H. pylori* to AGS cells was markedly reduced to 30, 32, and 41% in the 1, 5, and 10 mg/mL samples, respectively. Thus 10-mg/mL sample exhibited similar anti-adhesion activity (48%) to that observed with 5 μg/mL amoxicillin, which served as positive control.

Ko et al. (2011) reported that *L. casei* ATCC 393 showed anti-

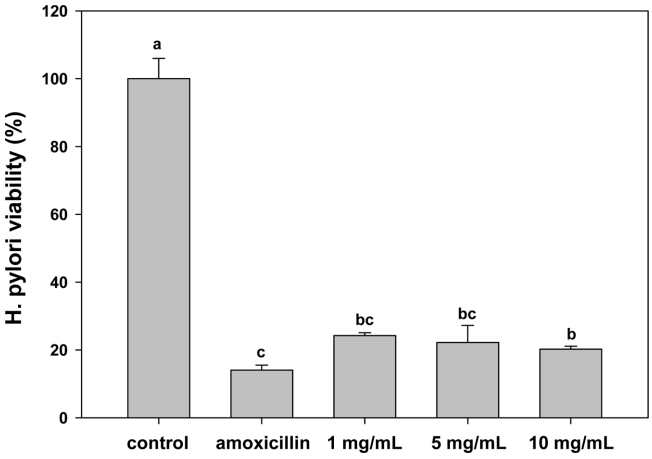

Fig. 2 Inhibitory effect of aqueous broccoli fermented by *Lactobacillus plantarum* MG208 on growth of *Helicobacter pylori*. [a-c]Mean values are significantly different as determined by Duncan's multiple range test (p <0.05). The concentration of amoxicillin used was 5 μg/mL.

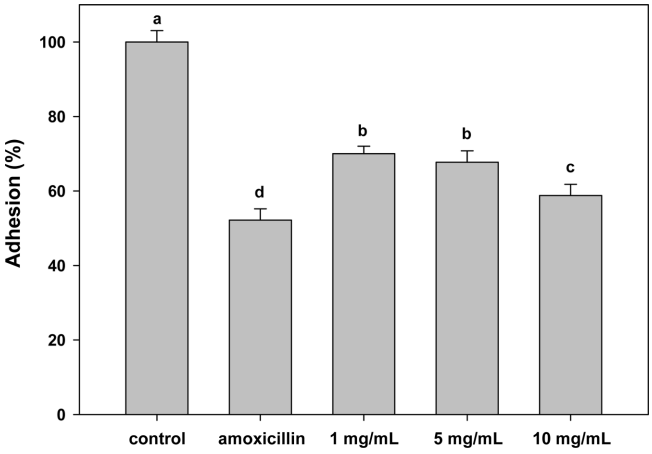

Fig. 3 Inhibitory effect of aqueous broccoli fermented by *Lactobacillus plantarum* MG208 on the adhesion of *Helicobacter pylori* to AGS human gastric epithelial cells. [a-d]Mean values are significantly different as determined by Duncan's multiple range test (p <0.05). The concentration of amoxicillin used was 5 μg/mL.

adhesion activity against *H. pylori*.

Lin et al. (2011) reported that milk fermented by LAB (fermented LY5-SCS and artificial LY5-SCS) showed significantly increased anti-adhesion activity against *H. pylori*.

Yang et al. (2012) reported that ginseng extract fermented with *L. plantarum* MG208 showed anti-adhesion activity against *H. pylori*.

Urease inhibition. Despite the acidic conditions of the gastric environment, *H. pylori* survives due to its acid-resistance, attributed to urease activity.

To evaluate the inhibitory activity of aqueous broccoli on the urease activity of *H. pylori*, aqueous broccoli was mixed with a suspension of *H. pylori* and urease activity was measured for 30 min. The result is shown in Fig. 4. Urease activity of *H. pylori*

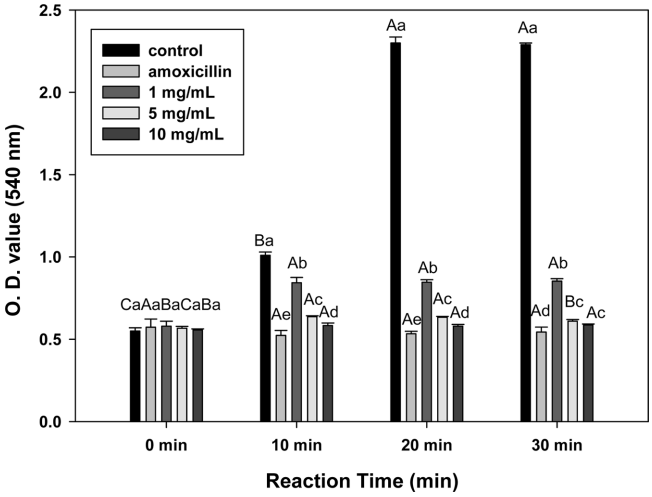

Fig. 4 Inhibitory effect of aqueous broccoli fermented by *Lactobacillus plantarum* MG208 on the urease activity of *Helicobacter pylori*. [a-e]Significant difference in treatment as per ANOVA followed by Duncan (p <0.05); [A-C]Significant difference in reaction time as per ANOVA followed by Duncan (p <0.05).

negative control began to appear within 10 min from the start of the reaction and increased significantly (p <0.05), reaching a plateau at 20 min.

The OD value of the amoxicillin sample did not change significantly during the entire measurement period (p >0.05). The OD values of the 1, 5, and 10 mg/mL samples were not significantly different at 0 min (p >0.05), but they increased significantly for up to 10 min (p <0.05) and were significantly different at 10, 20, and 30 min (p <0.05).

Compared to the negative control (amoxicillin), 1, 5, and 10 mg/mL samples showed urease inhibition with time; urease activity decreased by 48.2, 16.5, 37.0, and 42.2% at 10 min, 76.8, 63.2, 72.5, and 74.8% at 20 min and finally 80.5, 73.0, 78.1, and 78.9% at 30 min.

The antibacterial activity of aqueous broccoli was similar to that of 5 μg/mL amoxicillin. *Reynoutria elliptica* Migo, rosemary, and thyme have been previously shown to possess antibacterial activity against *H. pylori* (Tabak et al., 1996; Lee et al., 2003; Son, 2007). Chen et al. (2010) reported that among 16 LAB strains with urease inhibitory activity, 4 LAB strains (*L. plantarum* DJ102-1, *L. plantarum* 18, *L. bulgaricus* 2-3, and *L. gasseri* Chen) showed urease inhibitory activity against *H. pylori*.

Lin et al. (2011) reported that milk fermented by LAB (fermented LY5-SCS and artificial LY5-SCS) showed significant urease inhibition against *H. pylori*. Yang et al. (2012) reported that ginseng extract fermented by *L. plantarum* MG208 also showed urease inhibitory activity against *H. pylori*.

Broccoli and LAB are known as functional foods that enhance immune activity and physiological function control, respectively (Coconnier et al., 1998; Michetti et al., 1999; Canducci et al., 2000; Pantoflickova et al., 2003; Galan et al., 2004; Miki et al., 2007; Yanaka et al., 2009; Vrese et al., 2011; Tomofuji et al.,

2012; Takala et al., 2013). Therefore, broccoli fermented by LAB is likely to have both beneficial effects. Therefore, in order to obtain scientific evidence for the efficacy of broccoli fermented by LAB to inhibit *H. pylori* infection in this study, we determined the acidity and pH, viable count, antimicrobial activity, inhibition of growth as well as adherence to gastric cells, and urease inhibitory activity of fermented broccoli against *H. pylori*.

Broccoli fermented by LAB strain *L. plantarum* MG208 promoted the growth of LAB in a concentration-dependent manner. It is believed that decrease in the pH was due to the increasing amounts of different organic acids that were produced during fermentation. In addition, the total acidity after fermentation with LAB increased considerably depending on the amount of aqueous broccoli that was added.

With regard to the antimicrobial activity of fermented broccoli against *H. pylori*, the antimicrobial activity of the samples was significantly higher as compared to the negative control ($p < 0.05$); however, it was not as high as the antimicrobial activity of 5 μg/mL amoxicillin. The growth inhibition of aqueous broccoli against *H. pylori* was similar to that of the positive control, 5 μg/mL amoxicillin. With regard to adhesion inhibition, the 10-mg/mL sample reduced the adhesion of *H. pylori* to AGS cells by around 41%. In addition, the 10-mg/mL sample showed highest urease inhibition (around 78.9%) at 30 min.

In this study, we demonstrated the antimicrobial activity of fermented broccoli, the ability to protect gastric epithelial cells against *H. pylori* infection, and attempted to explain the underlying mechanisms. Our results provide scientific evidence for the use of fermented broccoli in eradicating *H. pylori*. This could be the basis for the development of functional broccoli products. However, the clinical utility of broccoli fermented by LAB remains to be established.

References

AOAC (1984) In *Official methods of analysis*. (14th ed.), Association of official analytical chemists, USA.

Apostolidis E, Kwon YI, Shinde R, Ghaedianc R, and Shetty K (2011) Inhibition of Helicobacter pylori by fermented milk and soymilk using select lactic acid bacteria and link to enrichment of lactic acid and phenolic content. *Food Biotechnol* **25**, 58–76.

Beil W and Kilian P (2007) EPs 7630, an extract from Pelargonium sidoides roots inhibits adherence of *Helicobacter pylori* to gastric epithelial cells. *Phytomedicine* **14**, 5–8.

Blaser MJ and Atherton JC (2004) *Helicobacter pylori* persistence: biology and disease. *J Clin Invest* **113**, 321–33.

Canducci F, Armuzzi A, Cremonini F, Cammarota G, Bartolozzi F, Pola P et al. (2000) A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. *Aliment Pharmacol Ther* **14**, 1625–9.

Cha WS, Kim JH, Lee KH, Kwon HJ, Yoon SJ, Chun SS et al. (2006) Antioxidative and Inhibition Activities on *Helicobacter pylori* of Spice Extracts. *J Korean Soc Food Sci Nutr* **35**, 315–20.

Chen X, Tian F, Liu X, Zhao J, Zhang H, Zhang H et al. (2010) *In vitro* screening of lactobacilli with antagonistic activity against *Helicobacter pylori* from traditionally fermented foods. *J Dairy Sci* **93**, 5627–34.

Cheon KH (2011) Fermentation properties of vegetables using lactic acid bacteria starter. MS Thesis, Sunchon national university, Korea.

Cho YJ, Chun SS, Lee KH, Kim JH, Kwon HJ, An BJ et al. (2006) Screening of the antimicrobial activity against *Helicobacter pylori* and antioxidant by extracts from mulberry fruits. *J Korean Soc Food Sci Nutr* **35**, 15–20.

Cho YJ, Ju IS, Kim BO, Kim JH, Lee BG, An BJ et al. (2007) The antimicrobial activity against *Helicobacter pylori* and antioxidant effect from the extracts of mulberry leaves. *J Korean Soc Appl Biol Chem* **50**, 334–43.

Choi YJ (2009) Development of fermented onion juice using functional lactic acid bacteria. MS Thesis, Ewha Womans University, Korea.

Coconnier MH, Lievin V, Hemery E, and Servin AL (1998) Antagonistic activity against Helicobacter infection *in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. *Appl Environ Microbiol* **64**, 4573–80.

Dhar SK, Soni RK, Das BK, and Mukhopadhyay G (2003) Molecular mechanism of action of major *Helicobacter pylori* virulence factors. *Mol Cell Biochem* **253**, 207–15.

Diker KS and Hascelik G (1994) The bactericidal activity of tea against *Helicobacter pylori*. *Lett App Microbiol* **19**, 299–300.

Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK et al. (2002) Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. *Proc Natl Acad Sci* **99**, 7610–5.

Fahey JW, Zhang Y, and Talalay P (1997) Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci* **94**, 10367–72.

Galan MV, Kishan AA, and Silverman AL (2004) Oral Broccoli Sprouts for the Treatment of *Helicobacter pylori* Infection. Dig Dis Sci **49**, 1088–90.

Gao MT, Kaneko M, Hirata M, Toorisaka E, and Hano T (2008) Utilization of rice bran as nutrient source for fermentative. *Bioresour Technol* **99**, 3659–64.

Goodwin CS (1997) *Helicobacter pylori* gastritis, peptic ulcer, and gastric cancer: Clinical and molecular aspects. *Clin Infect Dis* **25**, 1017–9.

Graham DY (1997) *Helicobacter pylori* infection in the pathogenesis of duodenal ulcer and gastric cancer: a model. *Gastroenterology* 113, 1983–91.

Jang HG (2001) In *Food Material*, Sinkwang Press, Korea.

Jung SW (2007) Fermentation Characteristics of Yoghurt Using Lactic Acid Bacteria with High Exopolysaccharide Production Ability Isolated from Sourdough. MS Thesis, Dongguk University, Korea.

Kaji T, Ishihara S, Ashizawa N, Hamamoto N, Endo H, Fukuda R et al. (2002) Adherence of *Helicobacter pylori* to gastric epithelial cells and mucosal inflammation. *J Lab Clin* **139**, 244–50.

Kim BO and Cho YJ (2011) Evaluation of *In vivo* safety of Inhibitory Compounds from Cheongmoknosang Mulberry Leaves against *Helicobacter pylori*. *J Korean Soc Food Sci Nutr* **40**, 1404–10.

Kim HY, Cho BD, Chang WK, Kim DJ, Kim YB, Park CK et al. (1997) *Helicobacter pylori* infection and the risk of gastric cancer among the Korean population. *J Gastroenterol Hepatol* **12**, 100–3.

Kim JS (2013) Preparation of fermented mulberry beverage by lactic acid bacteria. MS Thesis, Catholic university of Daegu, Korea.

Kim MJ and Kim GR (2006) *In vitro* evaluation of cholesterol reduction by lactic acid bacteria extracted from Kimchi. *Korean J Culinary Res* **12**, 259–68.

Kim SJ, Shin JY, Park YM, Chung KM, Lee JH, and Kweon DH (2006) Investigation of Antimicrobial Activity and Stability of Ethanol Extracts of Licorice Root (Glycyrrhiza glavra). *Kor J Food Sci Technol* **38**, 241–8.

Ko JA, Lima HJ, and Park HJ (2011) Effect of microencapsulated precipitants of Lactobacillus casei ATCC 393 on *Helicobacter pylori* eradication. *Process Biochem* **46**, 631–5.

Koo JK and Choe TB (2001) Studies on Adherance Inhibition and Detachment of *Helicobacter pylori* Using Egg Yolk IgY and Additives. *Korean J Biotechnol Bioeng* **16**, 41–7.

Kurilich AC, Tsau GJ, Brown A, Howard L, Klein BP, Jeffery EH et al. (1999) Carotene, tocopherol, and ascorbate contents in subspecies of Brassicaoleracea. *J Agric Food Chem* **47**, 1576–81.

Lee IS, Im HG, and Lee SO (2003) Growth inhibition of Helicobacterpyloriby

Reynoutria elliptica Migo. *Korean J Food Sci Technol* **35**, 1182–7.
Lee JH, Shim JS, Lee JS, Kim JK, Yang IS, Chung MS et al. (2006) Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (Camellia sinensis). *J Agric Food Chem* **54**, 8717–23.
Lee SY, Shin YW, and Hahm KD (2008) Phytoceuticals: Mighty but ignored weapons against *Helicobacter pylori* infection. *J Dig Dis* **9**, 129–39.
Lin WH, Wu CR, Fang TJ, Guo JT, Huang SY, Lee MS et al. (2011) Anti-*Helicobacter pylori* activity of fermented milk with lactic acid bacteria. *J Sci Food Agric* **91**, 1424–31.
Matusheski NV, Juvik JA, and Jeffery EH (2004) Heating decreases epithiospecifier protein activity and increases sulforaphane formation in broccoli. *Phytochemistry* **65**, 1273–81.
Michetti P, Dorta G, Wiesel PH, Brassart D, Verdu E, Herranz M et al. (1999) Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (johnsonii) La1 on *Helicobacter pylori* infection in humans. *Digestion* **60**, 203–9.
Miki K, Urita Y, Ishikawa F, Iino T, Shibahara-Sone H, Akahoshi R et al. (2007) Effect of *Bifidobacterium bifidum* Fermented Milk on *Helicobacter pylori* and Serum Pepsinogen Levels in Humans. *J Dairy Sci* **90**, 2630–40.
Montecucco C and Rappuoli R (2001) Living dangerously: how *Helicobacter pylori* survives in the human stomach. *Nat Rev Mol Cell Biol* **2**, 457–66.
Osman U, Berrin O, Yakut A, Ufuk A, and Erdem Y (2006) Flavonoids with anti-*Helicobacter pylori* activity from Cistus laurifolius leaves. *J Ethnopharmacol* **108**, 457–61.
Pantoflickova D, Corthesy-theulaz I, Dorta G, Stolte M, Isler P, Rochat F et al. (2003) Favourable effect of regular intake of fermented milk containing *Lactobacillus johnsonii* on *Helicobacter pylori* associated gastritis. *Aliment Pharmacol Ther* **18**, 805–13.
Park SH (2008) The Effect of Portulac aoleracea extracts on antimicrobial activity against *Helicobacter pylori* and antioxidant activity. MS Thesis, Keimyung University, Korea
Park SJ, Kim DH, Paek NS, and Kim SS (2006) Preparation and quality characteristics of the fermentation product of ginseng by lactic acid bacteria (FGL). *J Ginseng Res* **30**, 88–94.
Park YS and Kim YH (2006) The Effect of Medicinal Herb Extract on Antimicrobial Activity against *Helicobacter pylori* and Antioxidant Activity. *J East Asian Soc Dietary Life* **16**, 199–206.
Shin KO, Jeon JR, Lee JS, Kim JY, Lee CH, Kim SD et al. (2006) Lactic acid fermentation of Chinese yam (Dioscorea batatas Decne) flour and its pharmacological effect on gastrointestinal function in rat model. *Biotechnol Bioprocess Eng* **11**, 240–4.
Sok DE, Kim JH, and Kim MR (2003) Isolation and identification of bioactive organosulfur phytochemicals from solvent extract of broccoli. *J Korean Soc Food Sci Nutr* **32**, 315–9.
Son EH (2007) A Inhibitory Effects of Rosmarinus officinalis L. on the growth of Helicobacter pylor. MS Thesis, Keimyung University, Korea.
Tabak M, Aromom R, Potasman I, and Neeman I (1996) In vitro inhibition of *Helicobacter pylori* by extracts of thyme. *J Appl Bacteriol* **80**, 667–72.
Takala PN, Salmieri S, Boumail A, Khan RA, Vu KD, Chauve G et al. (2013) Antimicrobial effect and physicochemical properties of bioactive trilayer polycaprolactone/methylcellulose-based films on the growth of foodborne pathogens and total microbiota in fresh broccoli. *J Food Eng* **116**, 648–55.
Tomofuji T, Ekuni D, Azuma T, Irie K, Endo Y, Yamamoto T et al. (2012) Supplementation of broccoli or Bifidobacterium longum-fermented broccoli suppresses serum lipid peroxidation and osteoclast differentiation on alveolar bone surface in rats fed a high-cholesterol diet. *Nutr Res* **32**, 301–7.
Toshio FMA, Kiyoshi K, Toshihisa K, Sumio T, and Taro N (2002) Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life Sci* **71**,1449–63.
Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M et al. (2001) *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* **11**, 784–9.
Vrese M, Kristen H, Rautenberg P, Laue C, and Schrezenmeir J (2011) Probiotic lactobacilli and bifidobacteria in a fermented milk product with added fruit preparation reduce antibiotic associated diarrhea and *Helicobacter pylori* activity. *J Dairy Res* **78**, 396–403.
Yanaka A, Fahey JW, Fukumoto A, Nakayama M, Inoue S, Zhang S et al. (2009) Dietary Sulforaphane-Rich Broccoli Sprouts Reduce Colonization and Attenuate Gastritis in *Helicobacter pylori*. Infected Mice and Humans. *Cancer Prev Res* **2**, 353–60.
Yang JW, Choi SY, Park SJ, Paek NS, and Kim SS (2012) Anti-*Helicobacter pylori* Effect of Fermented Ginseng Extracts with *Lactobacillus plantarum* MG 208. *J Korean Soc Appl Biol Chem* **55**, 53–6.