

## Effect of Kimchi and Its Ingredients on the Growth of *Helicobacter pylori*

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### Abstract

Effects of kimchi and its ingredients, vitamin C and  $\beta$ -sitosterol on the growth of *Helicobacter pylori* were investigated. Three kimchi variations were studied: a standard recipe (kimchi I) and two functional variations for cancer prevention and treatment made with organically grown ingredients (kimchi II and III). Methanol extracts and juices from kimchi I and III did not inhibit the growth of *H. pylori*. However, 10 mm and 12 mm inhibition zones were formed by methanol extract and juice from kimchi II, which had higher concentrations of red pepper powder (RPP) than those of kimchi I and III. Among the major kimchi ingredients, methanol extracts of RPP, garlic and ginger substantially inhibited the growth of *H. pylori*. The maximal inhibition zone (30 mm) was attained with garlic treatment. Inhibitory effects of the RPP, garlic and the sub-ingredient mixture (prepared with radish, garlic, RPP, ginger, green onion, sugar and fermented anchovy juice) on *H. pylori* were decreased by lactic acid bacteria fermentation. Neither the fermented garlic nor the fermented sub-ingredient mixture inhibited the growth of *H. pylori*. But, the inhibition zone of fermented RPP was 12 mm, which was less than the 16 mm inhibition zone formed by the non-fermented RPP. Vitamin C and  $\beta$ -sitosterol which are known to be functional active compounds of kimchi also showed no inhibitory effect on the growth of *H. pylori* after 3 days of incubation. Further study is needed to determine why the inhibitory effect is removed or decreased by lactic acid fermentation, and to determine if fresh kimchi and lactic acid bacteria of kimchi can inhibit the growth of *H. pylori*.

**Key words:** *Helicobacter pylori*, kimchi, garlic, red pepper powder, ginger

### INTRODUCTION

Baechu (Korean cabbage) kimchi is the most popular lactic acid bacteria (LAB) fermented vegetable in Korea with baechu cabbage, red pepper powder (RPP), garlic, ginger, onion, salt, fermented anchovy juice among the major ingredients. The primary raw ingredients in kimchi are yellow-green vegetables and condiments, many of which contain large amounts of phytochemicals such as vitamin C,  $\beta$ -carotene, dietary fiber, chlorophylls, polyphenols, etc. The numbers of lactic acid bacteria are also greatly increased as the kimchi is fermented (1,2).

Recently, kimchi has been reported to reduce the risk of many types of cancers, including those of lung, esophagus, stomach, colon, liver, etc (3-7). RPP, garlic, ginger, green onion, baechu cabbage and radish, which are the main raw ingredients of baechu kimchi are known to contribute numerous health benefits (1). We previously reported the antimutagenic effects of both kimchi and its ingredients as revealed by Ames test, SOS chromotest and *Drosophila* wing spot assay system (1,3); and the anticancer effects have been confirmed in several cellular and animal

experimental trials (3-7). Kimchi inhibited the growth of human cancer cells and reduced the cytotoxicity and transformation induced by carcinogens in C3H/10T1/2 cells (6). In addition, kimchi extracts reduced tumor formation in Balb/c mouse and increased the phagocytic activity of macrophages, which was confirmed in an *in vivo* experimental system (7).

*H. pylori* infection is established as the major pathogenic factor in the etiology of chronic gastritis and peptic ulcer disease, and is a risk factor for the development of gastric carcinoma. Pathogenic *H. pylori* produce copious amounts of urease, which hydrolyzes urea to CO<sub>2</sub> and ammonia. This ammonia generated by *H. pylori* protects it from the environment of gastric acid in stomach, but directly damages gastric mucosal cells. Therefore, eradication of the bacteria is important for the treatment of patients with gastroduodenal diseases (8).

There have been several reports (9-13) that natural foods such as garlic, honey, and capsaicin can inhibit *H. pylori* *in vitro*, and each report has suggested that the natural ingredient can be used for treatment of the infection. Korea is a country where garlic and RPP are extensively used

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in the diet, and yet Korea has one of the highest rates of *H. pylori* infection as well as gastric cancer (14,15). Since baechu kimchi is prepared with red pepper powder and garlic, baechu kimchi has potential for use as a functional food with efficacy for the treatment or prevention of *H. pylori* infection. In this study, methanol extracts of kimchi, its ingredients, and active functional compounds such as vitamin C and  $\beta$ -sitosterol were investigated for their inhibitory effects against the growth of *H. pylori* *in vitro*.

## MATERIALS AND METHODS

### Preparation of kimchi and experimental samples

**Kimchi** : To prepare standardized kimchi, Chungbang baechu, a kind of Korean cabbage (baechu) grown in Kimhae, Korea, was obtained. Garlic, ginger, RPP, radish and green onion were purchased from a local market in Busan, Korea. Two other variations of kimchi were also prepared, cancer preventive kimchi and anticancer kimchi. The cancer preventive and anticancer formulations were based on previous studies that demonstrated increased efficacy of kimchi made from organic ingredients, and that also demonstrated differential effects of various ingredients on cancer prevention and treatment (2-7) To prepare cancer preventive and anticancer kimchi, organically cultivated baechu cabbage, garlic, ginger, RPP, radish and green onion were obtained from Nongsuwon Co. (Gyeongju, Kuyongnam, Korea). Anchovy juice and Gueun salt (a heat processed salt, Sannaedle Co, Seoul, Korea) were purchased from a local market in Busan, Korea. The method and the proportion of ingredients for preparing kimchis were described in a previous report (4). The cabbage was brined in 10% salt solution for 10 hours, rinsed with fresh water, drained for 3 hrs, and mixed with the ingredients. The ratio of the ingredients in standardized kimchi (kimchi I) was 13.0 parts radish, 2.0 green onion, 3.5 RPP, 1.4 garlic, 0.6 ginger, 2.2 anchovy juice, 1.0 sugar and 2.5 final salt concentration in 100 parts salted baechu cabbage. The ratio of the ingredients in cancer preventive kimchi (kimchi II) was 11.0 parts radish, 2.0 green onion, 5.0 mustard leaf, 5.0 RPP, 2.8 garlic, 0.1 Chinese pepper powder, 0.6 ginger, 2.8 pear, 5.0 mushroom and sea tangle soup, 2.2 anchovy juice, 1.0 sugar and 2.5 final salt concentration in 100 parts salted organic baechu cabbage. The ratio of the ingredients in anticancer kimchi (kimchi III) was 11.0 parts radish, 2.0 green onion, 7.5 mustard leaf, 2.5 RPP, 2.8 garlic, 0.1 Chinese pepper powder, 0.6 ginger, 2.8 pear, 5.0 mushroom and sea tangle soup, 1.0 sugar and 2.2 final salt concentration in 100 parts salted organic baechu cabbage.

The prepared kimchis were packed in pint jars and

fermented for 4 days at 15°C (pH 4.3) and then used as test samples.

**Kimchi ingredients** : The sub-ingredient mixture was prepared with 13.0 parts radish, 2.0 green onion, 3.5 RPP, 1.4 garlic, 0.6 ginger, 2.2 anchovy juice, and 1.0 of sugar. To prepare fermented RPP, garlic and the sub-ingredient mixture, 2-fold of water and 2.5% salt were added to each of them and fermented to reach pH 4.3 at 15°C. Ten days were required days to reach pH 4.3 for RPP and garlic, and 8 days for the sub-ingredient mixture.

**Kimchi functional active compounds** : Vitamin C and  $\beta$ -sitosterol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Vitamin C was dissolved in distilled water and  $\beta$ -sitosterol was supplied as  $\beta$ -sitosterol-2-hydroxypropyl  $\beta$ -cyclodextrin complex. The molar ratio of  $\beta$ -sitosterol to cyclodextrin was maintained at 1:300.

### Extraction and preparation of kimchi juice

Freeze-dried and powdered kimchi and its raw ingredients were extracted with methanol (MeOH, 20-fold) three times by shaking for 8 hrs and then were taken as MeOH extracts. Each extract was dried in a rotary vacuum evaporator (Buchi 011 & 461, Switzerland) at 45°C and then dissolved in dimethyl sulfoxide (DMSO, Sigma Chemical Co., USA) for use in the experiment. To prepare kimchi juice, kimchi was crushed by a juicer (Angel juicer Co., Korea). The resulting juices were centrifuged at 9000  $\times$  g (4°C) for 30 min to obtain a clear supernatant, which was sterilized by filtration through a Milipore filter (0.45  $\mu$ m)

### *H. pylori* cultivation condition

*H. pylori* TK1054 was obtained from the college of medicine of Kosin University, Korea. *H. pylori* TK1054 was incubated microaerobically on brain heart infusion (BHI) agar (Difco, USA) supplemented with 5% human blood and 5% horse serum at 37°C for 3 days in GasPak jars (Mitsubishi Gas Kagaku, Tokyo, Japan) with AnaeroPack Campylo (Becton Dickinson and Company Sparks, MD USA).

### Growth inhibition test

Air-dried 8 mm paper discs were placed on BHI agar plates on which 0.1 mL of a suspension of *H. pylori* in saline phosphate buffer ( $10^8 \sim 10^9$  CFU/mL) had been spread. 50  $\mu$ L of sample solution (dissolved in distilled water) or 75  $\mu$ L of kimchi juice was loaded on discs in triplicates. Three sample-impregnated discs were placed on each of the lawned plates and a disc saturated in distilled water served as control. Two lawned plates, without discs, were also included as additional controls. All plates

were incubated under microaerophilic conditions for 72 hrs at 37°C, after which the maximum diameter of the inhibition zone appearing around each disc was recorded (16). All experiments were repeated three times.

## RESULTS AND DISCUSSION

### Effect of kimchi on the growth of *H. pylori*

As we have already reported, different kinds of sub-ingredients can be added when kimchi is prepared. Thus, it is possible to design kimchi recipes with specific cancer preventive functionality (17-19). The cancer preventive kimchi and anticancer kimchi were prepared from organically cultivated baechu cabbage by adding more sub-ingredients such as garlic, mustard leaf, Chinese pepper, etc; and by manipulating the fermentation method (17-19). As shown in Table 1, methanol extracts and juices from the fermented (pH 4.3) standard kimchi (kimchi I) and anticancer kimchi (kimchi III) were unable to inhibit the growth of *H. pylori*. However, 10 mm and 12 mm inhibition zones (IZ) were formed by methanol extract and juice of the cancer preventive kimchi (kimchi II), respectively. The RPP level of the cancer preventive kimchi was higher than those of the standardized kimchi and the anticancer kimchi. RPP contains high levels of capsaicin, which is known to inhibit the growth of *H. pylori* (13). Therefore, the inhibitory effect of the cancer preventive kimchi was probably due to the higher content of RPP. Further study is needed to determine whether fresh kimchi or other kinds of kimchi may inhibit the growth of *H. pylori*.

### Effect of kimchi ingredients on the growth of *H. pylori*

In the standard method of processing baechu kimchi, the brined baechu cabbage is mixed with sliced radish, green onion, RPP, garlic, ginger, etc (20). Among the kimchi ingredients, methanol extracts from RPP (IZ: 28 mm), garlic (IZ: 30 mm) and ginger (IZ: 24 mm) inhibited the growth of *H. pylori* (Table 2). The maximal inhibition

**Table 2.** Antibacterial effects of methanol extracts from kimchi ingredients against *H. pylori*

Ingredients	Maximum diameter of inhibition zone <sup>1)</sup>
Baechu cabbage	8 mm (NI) <sup>2)</sup>
Red pepper powder	28 mm
Garlic	30 mm
Ginger	24 mm
Radish	8 mm (NI)
Green onion	8 mm (NI)

<sup>1)</sup>Diameter of the discs, each containing 50 µL (200 mg/mL) of the extract, was 8 mm.

<sup>2)</sup>No inhibition.

zone was attained with garlic, whereas methanol extracts of baechu cabbage, radish, and green onion showed no bactericidal effect on *H. pylori* after 3 days.

RPP contains high levels (25~80 mg %) of capsaicin (21). Jones et al. (13) demonstrated that capsaicin effectively exerted a time- and concentration-dependent inhibition of the growth of *H. pylori in vitro*. The effect of capsaicin was specific for *H. pylori*, as demonstrated by a lack of bactericidal activity against a nonpathogenic human commensal *E. coli* strain. Several studies (9-11) have reported that allicin (allyl 2-propene thiosulfinate) is the main active compound that is responsible for the anti-*H. pylori* activity provided by garlic. In the cooking process, allicin is converted to diallyl sulfide (DAS) and other garlic sulfides. Allicin has been shown to be more potent than diallyl sulfide (9). Therefore, the inhibitory effects of red pepper powder and garlic against *H. pylori* are probably due to capsaicin and allicin, respectively (9-11, 13,21).

### Effect of fermented kimchi ingredients on the growth of *H. pylori*

We were curious why ripened (fermented) kimchi (kimchi I and kimchi III) was unable to inhibit the growth of *H. pylori*. In order to further investigate the effect of fermentation of the sub-ingredients on the growth of *H. pylori*; RPP, garlic and the sub-ingredient mixture (prepared with radish, garlic, RPP, ginger, green onion, sugar and fermented anchovy juice) were fermented at 15°C until they reached pH 4.3. The inhibitory effects of RPP, garlic and the sub-ingredient mixture on *H. pylori* were all decreased by the fermentation (Table 3). Fermented garlic and the sub-ingredient mixture did not inhibit the growth of *H. pylori* at all. However, RPP retained part of its inhibitory capacity, with inhibition zones of 12 mm and 16 mm for fermented and non-fermented RPP, respectively. Cellini et al. (10) reported that heat treatment of garlic extracts reduced their inhibitory or bactericidal activity against *H. pylori*; the boiled garlic extract showed a loss of efficacy from two- to four-fold for minimum

**Table 1.** Antibacterial effects of methanol extracts and juices from kimchi samples against *H. pylori*

Kimchi	Maximum diameter of inhibition zone <sup>4)</sup>	
	Methanol extract	Juice
I (Standardized kimchi) <sup>1)</sup>	8 mm (NI) <sup>5)</sup>	8 mm (NI)
II (Cancer preventive kimchi) <sup>2)</sup>	10 mm	12 mm
III (Anticancer kimchi) <sup>3)</sup>	8 mm (NI)	8 mm (NI)

<sup>1-3)</sup>See the Materials and Methods.

<sup>4)</sup>Diameter of the discs, each containing 50 µL (200 mg/mL) of the extract and 75 µL of juice, was 8 mm.

<sup>5)</sup>No inhibition.

**Table 3.** Antibacterial effects of non-fermented and fermented kimchi ingredients against *H. pylori*

Ingredients	Maximum diameter of inhibition zone <sup>2)</sup>	
	Raw (Non-fermented)	Fermented <sup>1)</sup>
Red pepper powder	16 mm	12 mm
Garlic	28 mm	8 mm (NI) <sup>3)</sup>
Subingredients mixture <sup>1)</sup>	15 mm	8 mm (NI)

<sup>1)</sup>See the Materials and Methods.

<sup>2)</sup>Diameter of the discs, each containing 50  $\mu$ L (200 mg/mL) of the extract, was 8 mm.

<sup>3)</sup>No inhibition.

inhibitory concentration and the minimum bactericidal concentration compared with fresh aqueous garlic extract. Thus, the elimination or reduction of the effect of fermented kimchi on the growth of *H. pylori* appears to be due to the destruction of active compounds during fermentation.

#### Effect of vitamin C and $\beta$ -sitosterol on the growth of *H. pylori*

Kimchi (*baechu* kimchi) contains high levels (30~70 mg%) of vitamin C (22). Vitamin C may play an important preventive role in the formation of gastric carcinoma. It has been demonstrated that the concentration of gastric vitamin C in people infected with *H. pylori* were several times lower than those of healthy individuals (23,24). Vitamin C (50~1000  $\mu$ g/mL which are almost the same levels of vitamin C found in kimchi) showed no bactericidal effect on *H. pylori* after 3 days of incubation in this experiment (Table 4). However, Chuang et al. (25) showed that *H. pylori* growth was inhibited by vitamin C at the concentrations of 2048, 512 and 128  $\mu$ g/mL at pH values of 7.4, 6.0 and 5.5 after more than 12 h exposure, respectively. These results indicate that the *H. pylori* inhibitory activity of vitamin C could be pH dependent. We previously identified  $\beta$ -sitosterol as an active compound that exhibits anticancer activity in kimchi (26). However, as shown in Table 4,  $\beta$ -sitosterol (50~1000  $\mu$ g) also did not inhibit the growth of *H. pylori*.

From these results, we can conclude that raw RPP, garlic and ginger, which are main kimchi sub-ingredients, inhibit the growth of *H. pylori*, but their fermentation products have decreased or no inhibitory activity. Fermented kimchi might inhibit the growth of *H. pylori* depending on the preparation condition. The levels of lactic acid bacteria including *Lactobacillus* sp. are greatly increased as the kimchi is fermented (17,19,20). Several intestinal *Lactobacillus* species are believed to play beneficial biological roles to hosts, and they are widely used as probiotics. It was recently reported that some *Lactobacillus* strains could inhibit the growth or attachment of *H. pylori* to human

**Table 4.** Antibacterial effect of vitamin C and  $\beta$ -sitosterol against *H. pylori*

Sample ( $\mu$ g/mL)	Maximum diameter of inhibition zone <sup>2)</sup>
Vitamin C 50	8 mm (NI) <sup>3)</sup>
100 <sup>1)</sup>	8 mm (NI)
200	8 mm (NI)
1000	8 mm (NI)
$\beta$ -sitosterol 50	8 mm (NI)
100	8 mm (NI)
200	8 mm (NI)
1000	8 mm (NI)

<sup>1)</sup>Vitamin C contents in 50  $\mu$ L (200 mg/mL) of methanol extract from kimchi.

<sup>2)</sup>Diameter of the discs was 8 mm.

<sup>3)</sup>No inhibition.

gastric epithelial cells *in vitro* and exhibit antagonistic activities against *H. pylori* *in vivo* (27-29). Therefore, lactic acid bacteria in kimchi have antithetical effects on *H. pylori*; while they inhibit *H. pylori* growth they also destroy the anti-bacterial agents in the raw ingredients of kimchi. Further studies are needed on the *H. pylori* inhibitory effects of kimchi LAB; fresh kimchi with high levels of raw RPP, garlic and ginger; and combinations of active compounds from kimchi and its ingredients at different pH levels.

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