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# Growth Inhibitory Effect of Fermented *Kimchi* on Food-borne Pathogens

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**Abstract** The effect of *kimchi*, traditional Korean fermented vegetables, on inactivating food-borne pathogens and the *kimchi* factors affecting the antimicrobial activity were investigated. More cells of *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella typhimurium* were inactivated in the *kimchi* that had low pH and high titratable acidity. Of the raw ingredients in *kimchi*, raw garlic showed the strongest antimicrobial activity against the pathogens. When *kimchi* was fermented at 0, 4, 10, or 20°C to pH 4.4, higher *kimchi* fermentation temperature resulted in higher titratable acidity. The greatest inactivation of *S. typhimurium* occurred in *kimchi* fermented at 20°C, while *L. monocytogenes* were inactivated in *kimchi* fermented at 0°C *in situ*. This study showed that appropriately fermented *kimchi* can inactivate various food-borne pathogens and that the fermentation temperature of the *kimchi* is an important factor in determining the ability of the *kimchi* to inactivate specific pathogens. Lactic acid bacteria (LAB) multiplication and organic acids produced according to LAB metabolism play a role in inactivating food-borne pathogens in *kimchi*.

**Keywords:** fermented *kimchi*, food-borne pathogen, fermentation temperature, lactic acid bacteria

## Introduction

Using a natural fermentation process has long been used as a food preservation method taking advantage of the low pH, organic acids, bacteriocins, $CO_2$, and hydrogen peroxide produced and the nutrient depletion caused by the dominant microorganisms (1).

*Kimchi*, a traditional Korean food consisting of naturally fermented vegetables, is produced from a mixture of cabbage, radish, pepper, garlic, ginger, spring onion, fish sauce, and salt. *Kimchi* is itself an ecosystem of diverse microflora, mainly lactic acid bacteria (LAB). The most well-known LAB identified in *kimchi* are members of the genera *Lactobacillus* and *Leuconostoc* (2). The taste becomes sour and palatable during *kimchi* fermentation due to LAB enzymatic activity and metabolic products (3). Moreover, some raw materials used in *kimchi*, such as garlic, are known for their anti-microbial properties (4-7). Although the nutrition and flavor characteristics of *kimchi* are well known (8,9), its properties as an antimicrobial agent have not been widely reported except for the characteristics of LAB isolated from *kimchi* (10-12). The extent to which properly fermented *kimchi* can inactivate food-borne pathogens in other foods when the *kimchi* is eaten together with the other foods has not been reported. There have been several reports demonstrating the potential of LAB to inactivate food-borne pathogens. In one of these studies, *Tempeh*, a fermented soybean Indonesian food containing 6 log CFU/g of *Lactobacillus plantarum* inactivated 6-7 log CFU/g of *Enterobacter aerogenes, Escherichia coli*, and *Salmonella infantis* (13). The authors suggested that this inactivation of pathogens during fermentation was partly due to competition between LAB and the food-borne pathogens for nutrition. In another study, the exponential growth phase of *Listeria monocytogenes* was halved and a 5-log reduction in its cell numbers occurred when this species was cultured with *Lactococcus lactis* (14). Recently, Inatsu *et al.* (15) showed that unfermented *kimchi* containing *E. coli* O157:H7, *Salmonella enteritidis*, *Staphylococcus aureus* and *L. monocytogenes* posed a potential risk to human health.

This study aimed to determine how well fermented *kimchi* can inactivate various food-borne pathogens at the temperature of the human body and investigate the microbiological and physicochemical factors of *kimchi* inhibiting food-borne pathogens such as microbial counts, pH, and titratable acidity. This is a preliminary study to address the hypothesis that the antimicrobial effect of *kimchi* can inactivate pathogens present in other foods in the human gastrointestinal tract. These results will be used as a basis for a further experiment modeling the conditions (pH, activity of digestion enzymes) in the human digestive tract and determining the response of food-borne pathogens to microorganisms in fermented food in that environment.

## Materials and Methods

**Strains** The following strains were cultured at 37°C using Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise (the growth medium used for each strain is given in brackets): *L. monocytogenes* ATCC 19111, Brain heart

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infusion (BHI) broth (Difco, Becton Dickinson Co., Sparks, MD, USA), *S. aureus* ATCC 12600, nutrient broth (Difco), *E. coli* O157:H7 ATCC 43895, trypticase soy broth (Difco), and *S. typhimurium* ATCC 14028, nutrient broth. An overnight culture of each of these strains was inoculated into the appropriate growth medium (total volume 10 mL) and incubated at 37°C in a shaker incubator (200 rev/min) until $OD_{600\ nm}$ reached 0.5. The culture was then mixed using a vortex mixer and a 1 mL sample was taken and centrifuged at 8,000×g for 2 min in a microcentrifuge. The supernatant was removed and the pellet was washed with phosphate buffered saline (PBS: 7.650 g NaCl, 0.724 g $Na_2HPO_4$, 0.210 g $KH_2PO_4$ in 1 L distilled water at pH 7.4).

***Kimchi* manufacture** Whole Chinese cabbage was divided into quarters. Salt (1 quarter of the weight of the whole cabbage) and water (5 times the weight of the salt) were added and allowed to soak for 3 hr until the salt content of the cabbage became 2.5%. The water was removed from the salted cabbage and the cabbage was washed. The ratio of red pepper powder:garlic:ginger:green onion: cabbage:table salt was 23:15:4:31:1,000:5. The raw ingredients were mixed and 100 g portions of *kimchi* were vacuum-sealed. Otherwise, the *kimchi* was purchased in a supermarket in Gyeonggi-do for testing commercial *kimchi*.

***Kimchi* preparation** The manufactured *kimchi* was fermented in the laboratory at 0, 4, 10, and 20°C. One-hundred g sample of *kimchi* was prepared for blending for 90 sec using a sterile Waring blender (Model 31BL9; Waring Commercial®, New Hartford, CT, USA). The blended contents were filtered using 3 thicknesses of sterile gauze. The filtered solution was used for this study.

**Measurement of pH and titratable acidity** The pH of *kimchi* was measured using a pH meter. Determination of the titratable acidity of the *kimchi* during fermentation was conducted according to the method of Hong and Park (16). The *kimchi* was titrated to pH 8.30 by addition of 0.1 N NaOH solution and the titratable acidity was calculated as percentage lactic acid.

**Incubation and enumeration of food pathogens in *kimchi*** Based on the hypothesis of 2 hr gastric digestion followed by 2 hr duodenal digestion (17), a 1 mL sample of the washed cells in 1 mL PBS as mentioned above, was mixed with 9 mL of each *kimchi* solution and incubated for 0, 2, and 4 hr at 37°C. Each mixture of fermented *kimchi* with a single strain of pathogen was serially diluted in PBS and plated onto 2 selective agars: Baird-Parker agar (Merck, Darmstadt, Germany) for *S. aureus* and Oxford-Listeria-Selective agar (Merck) for *L. monocytogenes,* which were then incubated at 37C for 48 hr. Black colonies of *S. aureus* and white colonies of *L. monocytogenes* were enumerated. MacConkey sorbitol agar (Difco) for *E. coli* O157:H7 and Salmonella Shigella (SS) agar (Difco) for *S. typhimurium* were used for plating. The plates were incubated at 37°C for 24 hr and the colonies (white colonies for *E. coli* O157:H7 and black colonies for *S. typhimurium*) were enumerated. The resistance of the cells to *kimchi* was expressed as $N/N_0$ on a log scale where N is the number of viable cells in the *kimchi* at the incubation time *t* and $N_0$ is the original number of cells.

**LAB enumeration during *kimchi* fermentation** Enumeration of LAB in the *kimchi* was carried out by a modification of the method of Lee *et al.* (2). Members of the genus *Leuconostoc* were selected using phenyl ethyl alcohol sucrose agar medium (PES) [5 g tryptone (Difco), 0.5 g yeast extract (Difco), 20 g sucrose, 2 g $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$, 1 g $KH_2PO_4$, 15 g agar, 2.5 mL phenylethyl alcohol in 1 L distilled water] and were cultured at 20°C for 5 days by using an anaerobic jar. For selection of members of the genus *Streptococcus*, the samples were cultured in KF Streptococcus agar (Difco) at 37°C for 4 days. These were counted as red colonies. For *Lactobacillus* selection, Lactobacillus selective medium (LBS) with the addition of acetic acid and sodium acetate (modified LBS agar medium: 74.5 g Rogosa agar (Difco), 0.3 mL acetic acid, 15 g sodium acetate in 1 L distilled water) was used and these inoculated plates were incubated at 30°C for 3 days. Total cell numbers were determined using plate count agar (Difco) and inoculated plates were incubated at 30°C for 3 days.

**Antimicrobial activity of crude raw materials of *kimchi*** The raw materials of *kimchi*, including cabbage, spring onion, garlic, ginger, and pepper were washed. Garlic and ginger were peeled. The materials were blended for 90 sec by using the Waring blender described above. The blended *kimchi* contents were filtered through 3 thicknesses of sterile gauze. The filtered solution was centrifuged at 1,000 ×g for 10 min and filtered by using a 0.45-μm membrane filter. A 0.1 mL volume of $10^7$ cells/mL of the exponential phase pathogens was plated on their growth medium. After 30 min, 20 μL of each crude extract was loaded onto the sterile disc on the lawn of each pathogens and the plates were incubated at 37°C for 24 hr. The radius of each inhibition zone (clear zone) was measured.

**Statistical analysis** The mean±stardard deviations (SD) of the viable counts were transformed to the logarithm values. Statistical comparison of the scale of log reduction ($\log_{10}N/N_0$) was performed by the Student's *t*-test (18).

## Results and Discussion

**Reduction of the cell number of food-borne pathogens in commercial *kimchi*** All 4 brands of cabbage *kimchi* (S1, S2, S3, S4) on sale in Korea (Table 1) were tested in this study. This study found that *kimchi* S2 had the lowest titratable acidity of these 4 brands. *Kimchi* S3, which showed the highest titratable acidity, resulted in the greatest inhibitory effect on *S. typhimurium* ATCC 14028. *Kimchi* S3 inactivated *S. typhimurium* ATCC 14028 more than 6 log CFU/g after 2 hr of incubation (Fig. 1a). Generally, the rate at which the *kimchi* inhibited *S. typhimurium* ATCC 14028 was higher than the rate at which it inhibited *S. aureus* ATCC 12600, *L. monocytogenes* ATCC 19111, and *E. coli* O157:H7 ATCC 43895 (Fig. 1). However, the growth inhibitory effect of the food-borne pathogens was not observed after 4 hr incubation in the *kimchi* which were heated at 80°C for 10 min to kill the LAB and neutralized

**Table 1. The pH and titratable acidity of 4 brands of *kimchi* on sale in Korea[1)]**

| *Kimchi* | S1 | S2 | S3 | S4 |
|---|---|---|---|---|
| pH | 4.45±0.03 | 4.70±0.13 | 4.40±0.02 | 4.93±0.08 |
| Titratable acidity | 0.99±0.06 | 0.77±0.13 | 1.10±0.10 | 0.95±0.32 |

[1)]*Kimchi* S1, S2, S3, and S4 are purchased cabbage *kimchi* produced by different companies.

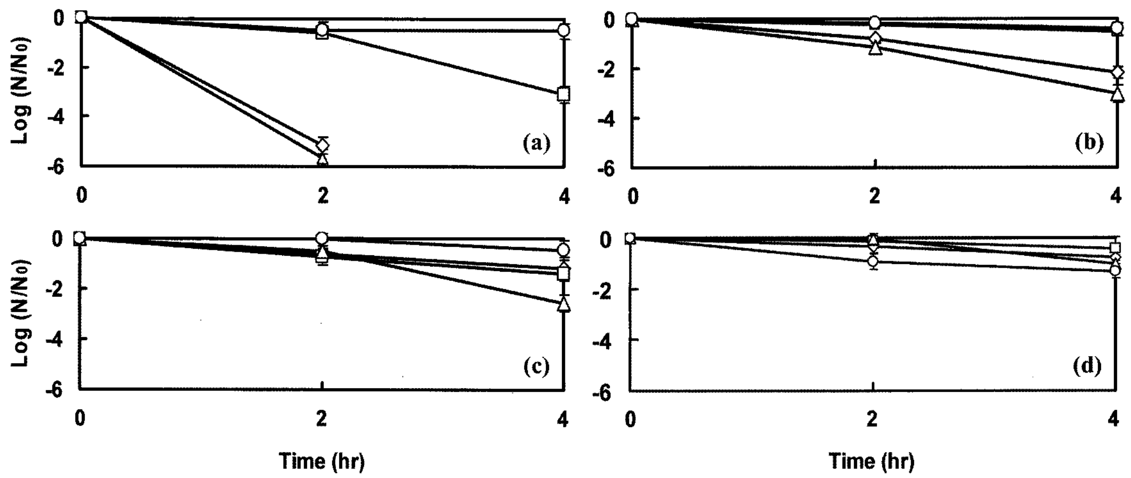


**Fig. 1. Comparison of the inhibitory effect of *kimchi* S1 (◇), S2 (□), S3 (△), and S4 (○) on *S. typhimurium* ATCC 14028 (a), *S. aureus* ATCC 12600 (b), *E. coli* O157:H7 ATCC 43895 (c), and *L. monocytogenes* ATCC 19111 (d).** Each pathogen was incubated at 37°C for 2 and 4 hr in blended and filtered *kimchi* solution. The resistance of the cells to *kimchi* is expressed as $N/N_0$ on a log scale where N is the viable cell number at incubation time *t* and $N_0$ is the original cell number.

from pH 4.4 to pH 7 (data not shown).

**Effect of fermentation temperature on the pH and the titratable acidity of *kimchi*** After confirming that fermented *kimchi* can inactivate food-borne pathogens, the characteristics of the *kimchi* that affected its antimicrobial activity were investigated. The antimicrobial activity of *kimchi* was compared at different fermentation temperatures. The pH of the *kimchi* was measured during fermentation to monitor the fermentation process. *Kimchi* fermentation took longer at low temperatures than at high temperatures, for example, *kimchi* fermentation at 0°C took up to 2 months while it took only 3 days to reach pH 4.4 when fermentation was conducted at 20°C. There was a long lag time before the pH began to decrease at 0 and 4°C (Fig. 2a). Titratable acidity in *kimchi* fermented at different temperatures was also measured to find out the relationship between fermentation temperature and titratable acidity (Fig. 2b). Interestingly, although the final pH of the *kimchi* (4.4) was the same regardless of fermentation temperature, the titratable acidity of the *kimchi* was dependent on fermentation temperature. *Kimchi* fermented at high temperatures (20°C) showed higher titratable acidity than *kimchi* fermented at low temperatures (0°C). The titratable acidity was in the range of 0.55-0.77 between fermentation temperatures of 0 and 20°C at pH 4.4 (Fig. 2). It was reported that the major organic acids produced during *kimchi* fermentation were lactic acid, malic acid, tartaric acid, citric acid, succinic acid, and acetic acid (19).

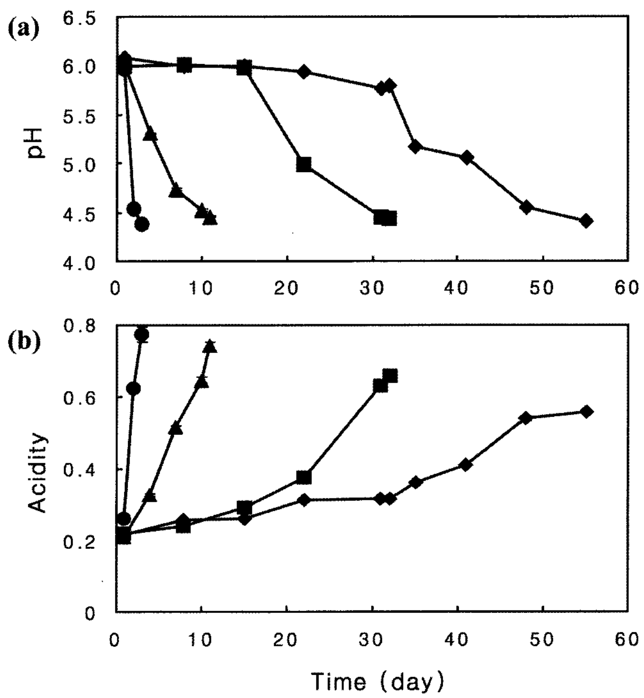


**Fig. 2. pH (a) and titratable acidity (b) of *kimchi* fermented at different temperatures.** Titratable acidity is expressed as lactic acid (%) in *kimchi* fermented at 0°C (◆), 4°C (■), 10°C (▲), and 20°C (●) until a pH of 4.4 was reached.

**Comparison of LAB increase according to *kimchi* fermentation temperature** At first, the total number of microorganisms in the *kimchi* was counted during the fermentation process. In order to investigate the different titratable acidity even at the same pH 4.4 in *kimchi*, the changes of microflora producing organic acids in *kimchi*

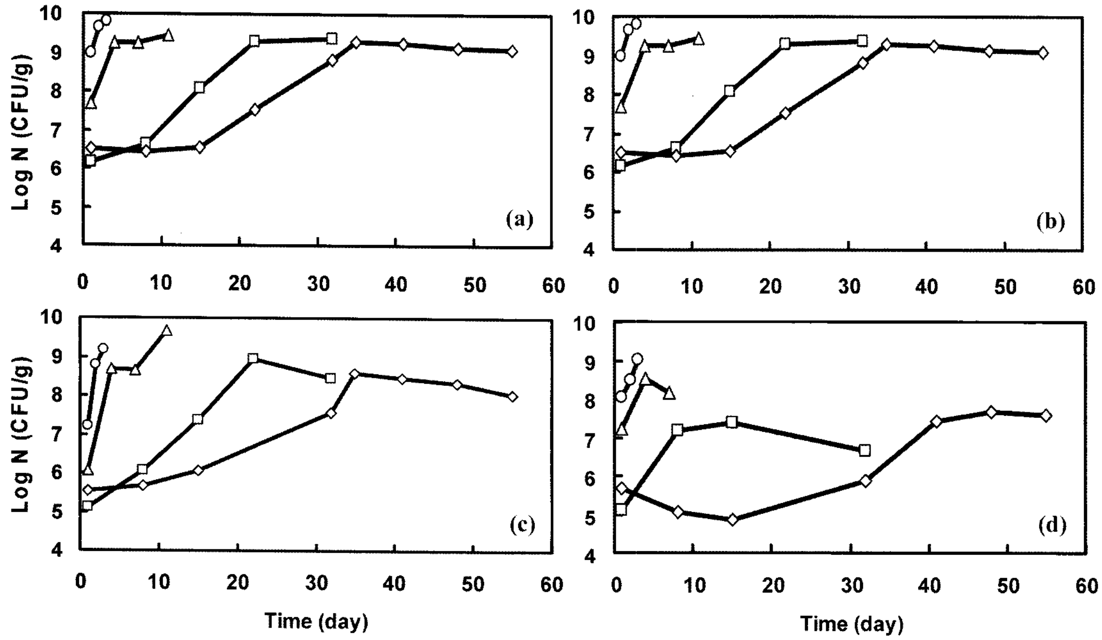


**Fig. 3. Total plate counts (a), *Lactobacillus* spp. (b), *Leuconostoc* spp. (c), and *Streptococcus* spp. (d) in *kimchi* fermented at 0°C (◇), 4°C (□), 10°C (△), and 20°C (○).** The total plate count was determined using plate count agar in *kimchi* fermented until a pH of 4.4 was reached. The standard deviation bars were removed for clarity.

were monitored. The cell numbers and growth response of total cells in *kimchi* was similar to that seen in the genus *Lactobacillus*. This shows that the dominant microflora in *kimchi* were members of the genus *Lactobacillus* (Fig. 3a, b). The cell numbers and growth response of *Leuconostoc* strains in *kimchi* (Fig 3c) was different from that of the lactobacilli (Fig. 3b). This reflects an effect of fermentation temperature since cell numbers of *Leuconostoc* strains started to decrease before pH reached 4.4 when fermentation was conducted at 4 and 0°C, while *Leuconostoc* and *Lactobacillus* strains both increased during fermentation at 20°C. The cell numbers and growth response in *Streptococcus* strains were also different in *kimchi* fermented at different temperatures since *Streptococcus* strains did not grow as much as the other strains at lower temperature. The different titratable acidity of *kimchi* fermented at the different fermentation temperature might be affected by the composition of the different lactic acid bacterial number at different fermentation temperatures.

**Effect of *kimchi* fermentation temperature on the reduction of food-borne pathogen in the fermented *kimchi* at pH 4.4** In the manufactured *kimchi* fermented in the laboratory at 0, 4, 10, and 20°C to pH 4.4, *S. typhimurium* ATCC 14028 was the most sensitive to *kimchi* of the pathogens tested and *S. aureus* ATCC 12600 was the most resistant to *kimchi*. More *S. typhimurium* ATCC 14028 cells were inactivated in *kimchi* fermented at high temperature (20°C) at pH 4.4 than at lower temperatures (Fig. 4a), while *L. monocytogenes* ATCC 19111 cells were inactivated more effectively in *kimchi* fermented at low temperature (0°C) (Fig. 4b). This shows that fermentation temperature affects the antimicrobial activity of *kimchi* against *S. typhimurium* and *L. monocytogenes*.

**Antimicrobial activity of raw ingredient of *kimchi* against food-borne pathogens** Finally, the effect of the raw ingredients of *kimchi* on food-borne pathogens was investigated. Extracts of the raw ingredients were applied to a lawn of *L. monocytogenes* ATCC 19111, *S. typhimurium* ATCC 14028, *S. aureus* ATCC 12600, and *E. coli* O157:H7 ATCC 43895. Of the raw ingredients in *kimchi*, including cabbage, spring onion, garlic, ginger, and pepper, the raw ingredient with the greatest antimicrobial activity was garlic, followed by spring onion. However, most of the raw ingredients of *kimchi* did not show any antimicrobial activity against the food-borne pathogens (Table 2). These ingredients could play a role in inactivating food-borne pathogens if there was a significant amount of them present in the *kimchi* at the beginning of fermentation.

Kang *et al.* (20) found that various food-borne pathogens including *L. monocytogenes*, *S. typhimurium*, *S. aureus*, *V. parahaemolyticus*, and *E. coli* O157:H7 were not detected in *kimchi* when the pH value was 3.7. A pH of 3.7 is very low for *kimchi* as this creates a very sour taste. Generally, pH 4.4 to 4.8 is regarded as the best pH for eating *kimchi*. *S. typhimurium* ATCC 14028 and *L. monocytogenes* ATCC 19111were reduced by 3 log CFU/mL by 4 hr incubation at pH 4.4 *kimchi* while the strains were reduced by nearly 1 log CFU/mL at pH 4.8 *kimchi* (data not shown).

This is the first study demonstrating how effectively food-borne pathogens can be inactivated if the pathogens meet appropriately fermented *kimchi* at the temperature of the human body before confirming the effect of preventing food poisoning by *kimchi* in the real pH and digestion enzymes of human digestion system. *S. typhimurium* was quite sensitive to fermented *kimchi* compared to *S. aureus*,

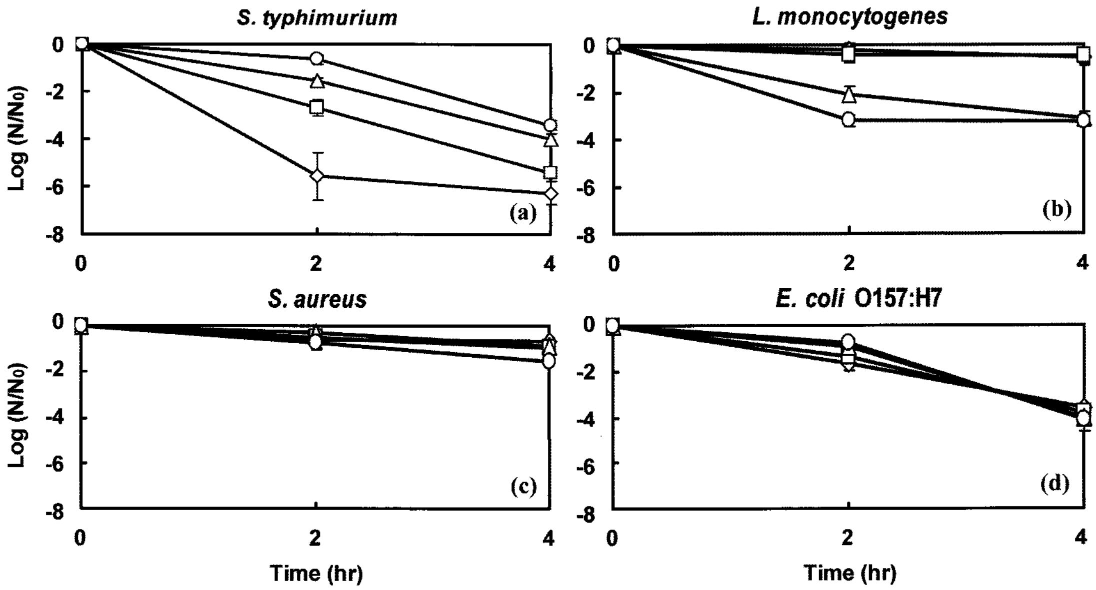


**Fig. 4. Antimicrobial effect of *kimchi* (pH 4.4) fermented at 0°C (◇), 4°C (□), 10°C (△), and 20°C (○) on *S. typhimurium* ATCC 14028 (a), *L. monocytogenes* ATCC 19111 (b), *S. aureus* ATCC 12600 (c), and *Escherichia coli* O157:H7 ATCC 43895 (d).** Each pathogen was incubated at 37°C for 2 and 4 hr in blended and filtered *kimchi* solution. The resistance of the cells to *kimchi* is expressed as $N/N_0$ on a log scale where N is the number of viable cells at incubation time *t* and $N_0$ is the original number of the cells.

**Table 2. Radius (mm) of inhibition (clear) zone of food-borne pathogens formed by addition of crushed *kimchi* raw ingredients**

| Strains | Garlic | Spring onion | Ginger | Cabbage | Red pepper | Radish |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* ATCC 12600 | 6 | 1.0±0.7 | ND[1)] | ND | ND | ND |
| *Salmonella typhimurium* ATCC 14028 | 6 | ND | ND | ND | ND | ND |
| *Listeria monocytogenes* ATCC 19111 | 6 | ND | ND | ND | ND | ND |
| *Escherichia coli* O157:H7 ATCC 43895 | 4 | ND | ND | ND | ND | ND |

[1)]Not detected.

*L. monocytogenes*, and *E. coli* O157:H7. Earlier Kim (22) emphasized the potent probiotic use of LAB obtained from *kimchi* by the studies that the isolated strains were resistant to artificial gastric fluid and the LAB showed antimicrobial effect against various food-borne pathogens. The work reported here has also shown that fermentation temperature affects *kimchi* fermentation time, titratable acidity, and the composition of its microflora. The difference in titratable acidity, represents the different composition of organic acids in the *kimchi* when it is fermented at different temperatures, even though the final pH is the same. Inactivation level of *S. typhimurium* was the most affected by *kimchi* fermentation temperature among the pathogens tested in this study. Interestingly, the more inactivation of *S. typhimurium* cells occurred in *kimchi* fermented at high temperature (20°C) while the more inactivation of *L. monocytogenes* ATCC 19111 cells occurred in *kimchi* fermented at low temperature (0°C), suggesting that the fermentation temperature of *kimchi* is important for the inactivation of specific food-borne pathogens. The fermentation temperature can affect the antimicrobial activity of garlic and spring onion, which might affect the antimicrobial activity of *kimchi*.

It has been reported that the kinds of organic acid produced during *kimchi* fermentation are dependent on the fermentation temperature (19,21,23). Park *et al.* (21) showed that more malic acid was produced in *kimchi* fermented at 20°C while as more lactic acid and succinic acid were produced in *kimchi* fermented at 10 or 4°C. They also showed the organic acid contents (mg/mL) of acetic acid, lactic acid, malic acid, and succinic acid was higher in *kimchi* fermented at 20°C (7.13 mg/mL) than at 0°C (5.27 mg/mL) to pH 4.3. The *kimchi* fermented at 20°C inactivated *S. typhimurium* more effectively than at 0°C, presumably because of the organic acids produced at high temperature.

Each component of fermented *kimchi* needs to be addressed to understand the effect of *kimchi* on inactivating food-borne pathogens. The combination of each characteristic of the *kimchi* could have a strong effect on the pathogens.

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