Growth of *Listeria monocytogenes* Scott A during Kimchi Fermentation and in the Presence of Kimchi Ingredients

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**ABSTRACT**

The fate of *Listeria monocytogenes* was determined during kimchi fermentation and in the presence of kimchi ingredients. The lag phase was increased in tryptic soy broth at 35°C in the presence of 3% garlic, ginger, and NaCl, but not in the presence of 5% red pepper. Low levels (10⁶ CFU/ml) of kimchi microflora did not inhibit the growth of *Listeria* in brain heart infusion (BHI) broth at 35°C. The growth of *L. monocytogenes* was inhibited in BHI broth containing a mixture of kimchi ingredients and 3% (wt/vol) NaCl at 21°C. During kimchi fermentation at 21 and 35°C, the numbers of viable cells of *L. monocytogenes* increased for the first 2 days of fermentation, but decreased during the next 10 days. The decrease at 35°C was more rapid. *L. monocytogenes* was largely inactivated by kimchi ingredients and low pH, but viable cells still remained after 10 days of fermentation. These results show that kimchi is a safe product with respect to low levels of *Listeria* contamination which can be achieved by using ingredients of good microbiological quality.

Key words: *Listeria monocytogenes*, kimchi, kimchi ingredients, growth inhibition

*Listeria monocytogenes* has achieved recognition as an important foodborne pathogen. Because of its ubiquitous presence in the environment, *L. monocytogenes* can contaminate fresh vegetables (9, 13). Vegetables contaminated on the farm or during prolonged cold storage in the raw state can be vehicles for transmission of listeriosis.

Kimchi, a spiced and fermented vegetable product, is an important part of the diet of the Korean people. Preparation of kimchi involves: slicing Chinese cabbage, which is then soaked in 10% (wt/vol) NaCl for 4 h, washed, and drained; mixing various ingredients with the cabbage, such as red pepper, garlic, ginger, and pickled seafood; placing the cabbage mixture in an appropriate vessel; and fermentation in a cellar (or buried container) for several days or weeks. After fermentation, the product is ready to consume. The proper combination of ingredients is important for organoleptically acceptable kimchi. Various studies have examined the presence and fate of *L. monocytogenes* in salads and fresh vegetables (2, 3, 12). However, there is little available information concerning the growth response of *L. monocytogenes* in fermented vegetable products destined for human consumption.

The present study determined the effect of kimchi ingredients on growth of *L. monocytogenes* in complex media and the fate of *L. monocytogenes* during kimchi fermentation.

**MATERIALS AND METHODS**

Cultures

*Listeria monocytogenes* Scott A was obtained from the culture collection of the Department of Food Science and Technology, University of Georgia, and was maintained on tryptic soy agar (TSA) (Difco) slants, stored at 4°C, and transferred monthly. Lactic acid bacteria, including *Lactic acid bacteria, Leucosnostoc mesenteroides* were maintained on deMan Rogosa Sharpe (MRS) agar slants, stored at 4°C, and transferred monthly. Intermediate cultures were prepared by transferring the stock culture into tryptic soy broth (TSB) or MRS broth and incubating at 35°C for 24 h. All culture media were obtained from Difco Laboratories (Detroit, MI).

Effect of kimchi ingredients and lactic acid bacteria on growth of *Listeria monocytogenes*

TSB was sterilized by autoclaving, after which raw kimchi ingredients were added under aseptic conditions. The active *Listeria* culture was inoculated into 100 ml of TSB (final level of about 1 x 10⁶ CFU/ml) containing 0, 3, and 5% (wt/vol) of macerated garlic, ginger, and red pepper and incubated at 35°C for 48 h respectively. To investigate the effect of ingredient mixtures and lactic acid bacteria on growth of *L. monocytogenes*, the active *Listeria* culture was inoculated into 100 ml of brain heart infusion (BHI) broth (final level of about 1 x 10⁶ CFU/ml containing lactic bacteria (1 x 10⁶ CFU/ml), 3 g of mixed macerated ingredients (equal parts of garlic, ginger, and red pepper), and 3 g of NaCl. Flasks were incubated at 21°C for 72 hr. Experiments were replicated three times.

Growth curve

One-milliliter samples were taken every 4 to 8 h throughout incubation and diluted in 0.1% peptone (Difco) solution. Appropriately dilutions of samples were surface plated on McBrade listeria agar (Difco). Plates were incubated at 35°C for 48 h before colony enumeration. Only colonies with the typical appearance of *L. monocytogenes* (using oblique transmitted light) were enumerated.
Preparation of kimchi
Washed Chinese cabbage was soaked in salt brine (10%, wt/vol) for 4 h and rinsed with fresh water. The cabbage was then submerged in water containing L. monocytogenes (10^5 CFU/ml) for 30 min and drained. A premix of macerated ingredients was added to the inoculated cabbage, and the mixture was fermented at 4, 21, or 35°C for 10 days. Chinese cabbage and kimchi ingredients were obtained at a local retail market of the Taegu area. During fermentation, a 1-ml sample of juice was taken every 2 days for enumeration of Listeria. The pH of the kimchi was measured by using a Corning pH meter (ion analyzer 150). Data are reported as the mean of three replications.

RESULTS AND DISCUSSION

Effects of individual ingredients and lactic acid bacteria
The growth of L. monocytogenes Scott A was inhibited in the presence of garlic (Fig. 1). Inhibition was primarily the result of an increased lag phase but the growth rate and maximum population, were also reduced. Inhibition increased as the concentration of garlic was increased.

![Figure 1. Growth of Listeria monocytogenes Scott A at 35°C in tryptic soy broth containing various concentrations of garlic.](image1)

The presence of ginger resulted in initial inactivation of L. monocytogenes to less than 1% of the inoculum level (Fig. 2). This could have been the result of sublethal injury. After 4 h of incubation, growth (or recovery from injury) was initiated, and after 32 h, the Listeria population in TSB with ginger was nearly equivalent to that in the control. An increase in the level of ginger caused greater initial inactivation (injury) of L. monocytogenes but did not affect the growth rate.

Aureli et al. (1) found that neither essential oils of ginger nor garlic inhibited the growth of L. monocytogenes. Lee and Kim (7) reported that growth of L. plantarum and L. mesenteroides, the principal microorganisms responsible for kimchi fermentation, were inhibited by garlic and ginger, and that ginger was more inhibitory to these organisms than garlic. The growth of L. monocytogenes Scott A was only slightly influenced by the presence of 3 and 5% red pepper (data not shown).

Addition of 3 or 5% salt to TSB increased the lag phase, but had a minimal effect on the overall growth rate of L. monocytogenes (Fig. 3). After 24 h of incubation, populations of L. monocytogenes in TSB with 1 or 3% salt were similar to those in the controls, and the population of L. monocytogenes in TSB with 5% salt was only slightly lower.

Various studies have reported on the salt tolerance of L. monocytogenes (4, 10, 11). Results of this study indicate that ginger, garlic, and salt, but not red pepper, have the potential to inhibit the growth of L. monocytogenes during kimchi fermentation.

![Figure 2. Growth of Listeria monocytogenes Scott A at 35°C in tryptic soy broth containing various concentrations of ginger.](image2)

Lactic acid bacteria associated with kimchi fermentation did not inhibit the growth of L. monocytogenes in BHI over a 48-h incubation (data not shown). The final pH values (5.7 to 5.8) were similar for treatments and controls. The inoculum of lactic acid bacteria used in this experiment was small (about 10^6 CFU/ml) in order to simulate a natural level. Kimchi fermentation, like other vegetable fermentations, is not initiated by the addition of a starter culture.

Combined effect of mixed ingredients and lactic acid bacteria
The growth of L. monocytogenes in BHI at 21°C was initially slowed by the presence of lactic acid bacteria, but this
effect was slight enough so that the overall effect of the lactic acid bacteria was minimal (Fig. 4). As in the previous experiment, a low inoculum level was used to simulate natural conditions. The addition of the ingredient mixture (garlic, ginger, and red pepper) to BHI produced substantial inhibition of growth of L. monocytogenes from both a lengthened lag phase and a reduced growth rate. The combination of mixed ingredients with 3% NaCl produced even better growth inhibition, with a lag phase of over 20 h.

Fate of L. monocytogenes during kimchi fermentation

Data on the survival of L. monocytogenes during kimchi fermentation at 4, 21, and 35°C is shown in Fig. 5. The high initial levels of Listeria in the kimchi indicate that the cabbage could have adsorbed bacterial cells when submerged in the inoculated water. Also, some growth of L. monocytogenes might have occurred between the time of inoculation and analysis. When incubation was at 21° and 35°C, viable L. monocytogenes increased for 2 days, and then declined in number. The decline was more rapid at 35°C than at 21°C.

The inactivation of L. monocytogenes was associated with a drop in pH to below 4.9 within 2 days. There was no substantial change in the level of L. monocytogenes during fermentation of kimchi at 4°C over the 10-day incubation period. The pH did not drop below 5.0 during this time.

The pH of good quality kimchi is about 4.3 (6, 8). L. monocytogenes can survive and even grow at pH values of less than 5.6 (11). Conner et al. (4) found a pH of < 4.8 in cabbage juice to be lethal for L. monocytogenes, a result very similar to that found for kimchi. Kimchi is potentially more inhibitory than acidified cabbage juice due to the presence of flavoring ingredients. Although the experiment reported here was terminated after 10 days, the earliest time after which kimchi might be consumed, storage of the product would likely lead to complete inactivation of the pathogen. The data reported here indicate that even though the growth of L. monocytogenes is inhibited by the kimchi ingredients and by the low pH of fermentation, if kimchi is prepared using Listeria-contaminated Chinese cabbage, there is a potential for the fresh product to contain the viable pathogen. Additional research on the occurrence and prevention of L. monocytogenes in Chinese cabbage and its removal by preprocessing treatments (cleaning, washing, etc.) is recommended. In addition, modifications in fermentation procedures or the use of natural antimicrobials during fermentation could lead to improved control of this hazard.

Figure 4. Growth of Listeria monocytogenes Scott A at 21°C in brain heart infusion broth containing lactic acid bacteria or a kimchi ingredient mixture (1% each of garlic, ginger, and red pepper); –O– control, –□– lactics, –●– ingredient mixture, –■– Ingredient mixture + 3% NaCl.

Figure 5. Survival of Listeria monocytogenes Scott A and pH changes during Kimchi fermentation at various temperatures.

REFERENCES


