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# Heterofermentative lactic acid bacteria as a starter culture to control kimchi fermentation

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ABSTRACT

Among 171 lactic acid bacteria (LAB) strains isolated from kimchi, the following three were selected: *Leuconostoc citreum* GR1, which has bacteriocin-enhancing activity, *L. citreum* C2, which produces mannitol but has no bacteriocin-enhancing activity, and *Pediococcus pentosaceus* MP1, which has the highest and broadest antibacterial activities. Selected LAB strains were independently introduced in the kimchi fermentation process to investigate the effects of the starter culture on kimchi fermentation. *L. citreum* GR1 and *P. pentosaceus* MP1 were predominant in kimchi during the fermentation process (60 days). Kimchi fermented using *L. citreum* GR1 had a high sensory quality and an extended shelf-life, whereas that fermented using *P. pentosaceus* MP1 had no significant improvement in the sensory quality. *L. citreum* C2 was predominant for 6 days only; thus the characteristics of kimchi fermented using *L. citreum* C2 were similar to those of kimchi fermented without starter culture. Controlling kimchi fermentation using heterofermentative LAB as a kimchi starter culture is difficult. However, this study revealed that controlling kimchi fermentation using heterofermentative LAB is possible. Therefore, the most important microbial characteristics of heterofermentative LAB as a starter culture was their bacteriocin-enhanced activities. Production of favorable metabolic products is required to obtain high sensory characteristics of kimchi.

## 1. Introduction

Kimchi, a traditional Korean food, is a popular lactic acid-fermented vegetable because of its nutritional and health-promoting properties (Cheigh & Park, 1994; Jo, Choi, Lee, & Chang, 2015; Park, Jeong, Lee, & Daily, 2014). In Korea, kimchi production has been traditionally performed on a small scale or at a household level for hundreds of years, whereas commercialization and mass production of kimchi on an industrial scale has rapidly increased over the recent years (Moon, Moon, & Chang, 2015). Industrial production of kimchi requires prolonged shelf-life and consistent and high sensory qualities within a regulated fermentation process. However, kimchi fermentation still relies on spontaneous fermentation, which often results in inconsistent production quality (Chang & Chang, 2010; Jung, Lee, & Jeon, 2014).

The application of a starter culture in kimchi fermentation for standardization of high sensory qualities has recently increased (Lee et al., 2015). However, it is difficult to control the fermentation process when introducing a starter culture for non-sterile, open fermentation of vegetables such as kimchi, sauerkraut, green olives, and Chinese sauerkraut production owing to the microbial succession of various lactic acid bacteria (LAB) species naturally present in the raw materials. Thus, the diversity of dominant LAB and periods of the starter cultures appear to depend on the characteristics of the starter fermentation culture. Specifically, when homofermentative LAB were used as a starter culture for vegetable fermentation, they easily predominated and maintained their concentrations throughout the fermentation process (Moon et al., 2015; Rao et al., 2013; Ruiz-Barba & Jiménez-Diaz, 2012; Wouters, Grosu-Tudor, Zamfir, & De Vuyst, 2013). However, heterofermentative LAB starter cultures were only predominant during the early stages of fermentation and could not maintain their dominance throughout the entire process. Thus, the dominance of a heterofermentative LAB starter culture is rapidly succeeded by homofermentative LAB (Choi et al., 2003; Kristek, Besio, Pavlovic, & Kristek, 2005; Wouters et al., 2013; Xiong, Li, Guan, Peng, & Xie, 2014).

In contrast, during kimchi fermentation, heterofermentative LAB produce $CO_2$, ethanol, and mannitol as metabolic products, while producing less acid than homofermentative LAB. These heterofermentative characteristics are responsible for the high sensory quality of kimchi along with its refreshing taste and pleasant flavor. Thus, heterofermentative LAB are generally considered to be better kimchi starter candidates than homofermentative LAB (Jeong & Lee, 2015; Jung et al., 2012). However, heterofermentative starter cultures can only control

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kimchi fermentation for a short time because of environmental factors that promote microbial succession (Choi et al., 2003; Jeong, Lee, Jung, Choi, & Jeon, 2013; Lee & Lee, 2010). Dominance of successive homofermentative LAB can result in the reduction of the sensory quality accompanied by excessive acidic taste, off flavor, and soft texture. The refrigerated shelf-life of kimchi in the market has been designated to be 28 days by a Korean kimchi manufacturer.

This study was aimed to determine the effects of a starter culture for controlling kimchi fermentation to improve quality and prolong shelf-life. To identify the properties that are important for controlling kimchi fermentation using a heterofermentative LAB starter culture, LAB isolates harboring strong antibacterial activities were selected to examine the culture characteristics. Furthermore, the selected LAB strains (homofermentative and heterofermentative) were introduced into kimchi as starter cultures to investigate their effects on kimchi fermentation.

## 2. Materials and methods

### 2.1. Bacterial cultures and media

LAB were propagated at 30 °C for 24 h in deMan, Rogosa and Sharpe broth (Difco Laboratories, Sparks, MD, USA). *Micrococcus luteus* was cultivated at 30 °C for 12 h in tryptic soy broth (Difco Laboratories), *Listeria monocytogenes* for 12 h in brain-heart infusion broth (Difco Laboratories), and the other bacteria at 37 °C for 12 h in Luria–Bertani broth (Difco Laboratories). For mannitol production, a LAB isolate was cultivated in simplified medium (Dols, Chraibi, Remaud-Simeon, Lindley, & Monsan, 1997) containing 3% fructose and 1.5% sucrose. The ATCC strain was purchased from the American Type Culture Collection (Manassas, VA, USA), the KCTC strain from the Biological Resource Center (Daejeon, Korea), and the KFRI strain from the Korea Food Research Institute (Bundang, Korea).

### 2.2. Antibacterial activity assay

Antibacterial activities against various Gram (+) or Gram (−) bacteria listed under Table 1 was assayed by the spot-on-the-lawn method (Hoover & Harlander, 1993). Briefly, plates were overlaid with each indicator organism at a concentration of 6.0 log CFU/mL, after which 10 μL of antibacterial sample was spotted onto lawn of indicator-seeded plates. Antibacterial activity expressed as arbitrary units (AU) per milliliter was defined as the reciprocal of the highest dilution at which bacterial growth was inhibited.

### 2.3. Preparation of crude antibacterial sample (CAS)

LAB samples cultivated at 30 °C for 24 h were centrifuged (9500 × *g*, 15 min) and then filtered (0.45 μm; Millipore, Beverly, MA, USA). The cell-free culture supernatant was freeze-dried (Lab Conco, Kansas, MO, USA), dissolved in 20 mmol/L Tris-HCl buffer (pH 7.6), and dialyzed against the same buffer at 4 °C for 6 h using a dialysis tube (MW < 1000; Spectrum, CA, USA). The dialyzed sample was then freeze-dried and dissolved in appropriate buffer solutions before use.

### 2.4. Characterization of CAS

The effects of pH, temperature, and different enzymes on CAS were investigated as previously described (Chang, Lee, & Chang, 2007). To determine the effects of pH, CAS was equilibrated to pH 3.0 (50 mmol/L glycine-HCl), pH 4.0–6.0 (50 mmol/L sodium citrate), and pH 7.0 (50 mmol/L Tris-HCl), for 6 h at 4 °C. To determine the effects of temperature, CAS was dissolved in 50 mmol/L sodium citrate buffer (pH 4.5) and treated at temperatures of 4, 30, 50, and 70 °C for 24 h, 100 °C for 30 min, and 121 °C for 15 min. Furthermore, CAS treated with the following proteolytic enzymes were analyzed: proteinase K (EC 3.4.32.64, Sigma, St. Louis, MO, USA) in 10 mmol/L Tris-HCl-50 mmol/L NaCl-5 mmol/L EDTA (pH 7.5), trypsin (EC 3.4.21.4, Sigma) and protease (type 1, Sigma) in 50 mmol/L Tris-HCl (pH 7.5). All enzyme reactions were performed at a final enzyme concentration of 2 mg/mL for 4 h at 25 °C.

### 2.5. Enhancement of antibacterial activity by LAB strain in the presence of *Lactobacillus spp.*

Enhancement of antibacterial activity by LAB strain was investigated in the presence of *Lactobacillus* strains such as *Lb. plantarum* KFRI 464, *Lb. delbruekii* KFRI 347, and *Lb. sakei* ATCC 15521, which are known to be predominant LAB species at the end of kimchi fermentation (Lee et al., 2015), as previously described (Chang et al., 2007). The three *Lactobacillus* strains were fractionated into intracellular, cell debris, and thermally inactivated (121 °C, 15 min) whole cell fractions.

**Table 1**
Antibacterial activities of lactic acid bacteria isolated from kimchi.

| Indicator microorganism | | | Activity (AU/mL) | | | | |
|---|---|---|---|---|---|---|---|
| | | | GR1 | C2 | PH1 | DM1 | MP1 |
| Gram (+) | LAB | *Lactobacillus plantarum* KFRI 464 | 400 | 400 | 0 | 0 | 1200 |
| | | *Lactobacillus plantarum* KFRI 236 | 0 | 0 | 0 | 0 | 1200 |
| | | *Lactobacillus delbruekii* KFRI 347 | 1200 | 1200 | 400 | 1200 | 2800 |
| | | *Leuconostoc mesenteroides* KFRI 218 | 1200 | 1200 | 400 | 1200 | 2800 |
| | | *Leuconostoc mesenteroides* KCTC 1628 | 2800 | 2800 | 1200 | 2800 | 6000 |
| | | *Lactobacillus sakei* ATCC 15521 | 2800 | 2800 | 1200 | 2800 | 6000 |
| | Other | *Bacillus subtilis* ATCC 6633 | 2800 | 2800 | 1200 | 2800 | 6000 |
| | | *Streptococcus mutans* ATCC 25175 | 400 | 400 | 0 | 400 | 2800 |
| | | *Listeria monocytogenes* ATCC 19111 | 0 | 0 | 0 | 0 | 400 |
| | | *Micrococcus luteus* ATCC 13513 | 0 | 1200 | 0 | 0 | 1200 |
| Gram (−) | | *Escherichia coli* ATCC 25922 | 400 | 400 | 400 | 400 | 2800 |
| | | *Escherichia coli* O157:H7 ATCC 43895 | 0 | 0 | 0 | 400 | 2800 |
| | | *Pseudomonas aeroginosa* ATCC 27853 | 2800 | 2800 | 1200 | 1200 | 6000 |
| | | *Salmonella enterica* serovar. Typhi ATCC 19430 | 0 | 0 | 0 | 0 | 400 |

GR1: *Leu. citreum* GR1.
C2: *Leu. citreum* C2.
PH1: *Leu. mesenteroides* PH1.
DM1: *Leu. mesenteroides* DM1.
MP1: *P. pentosaceus* MP1.