Characterization of Lactic Acid Bacteria Isolated from Sauce-type Kimchi

Suk Hee Jung¹, Joung Whan Park¹, Il Jae Cho¹, Nam Keun Lee¹, In-Cheol Yeo¹, Byung Yong Kim², Hye Kyung Kim³, and Young Tae Hahm†

¹Department of Biotechnology (BK21 Program), Chung-Ang University, Gyeonggi 456-756, Korea
²Department of Food Science and Biotechnology, Kyung Hee University, Gyeonggi 446-701, Korea
³Department of Food Science and Biotechnology, Hanseo University, Chungnam 356-706, Korea

Abstract

This study was carried out to investigate the isolation and characterization of lactic acid bacteria (LAB) from naturally fermented sauce-type kimchi. Sauce-type kimchi was prepared with fresh, chopped ingredients (Korean cabbage, radish, garlic, ginger, green onion, and red pepper). The two isolated bacteria from sauce-type kimchi were identified as Pediococcus pentosaceus and Lactobacillus brevis by 16S rDNA sequencing and tentatively named Pediococcus sp. IJ-K1 and Lactobacillus sp. IJ-K2, respectively. Pediococcus sp. IJ-K1 was isolated from the early and middle fermentation stages of sauce-type kimchi whereas Lactobacillus sp. IJ-K2 was isolated from the late fermentation stage. The resistance of Pediococcus sp. IJ-K1 and Lactobacillus sp. IJ-K2 to artificial gastric and bile acids led to bacterial survival rates that were 100% and 84.21%, respectively.

Key words: sauce-type kimchi, lactic acid bacteria, Pediococcus sp., Lactobacillus sp.

INTRODUCTION

Kimchis are fermented using vegetables mixed with spicy seasoning as traditional Korean fermented food and categorized by seasonings, areas, and ingredients (1). Cabbage kimchi, which is a fermented cabbage seasoned with radish, garlic, ginger, green onion, red pepper, and red pepper powder, is commonly prepared in Korean homes (2). The quality of kimchi produced in each household, however, differs because of fermenting microorganisms and various environmental conditions such as temperature, density of salt, pH, oxygen, species of cabbage, and side ingredients (3).

Kimchi has anti-cancer and anti-oxidative functional properties and is now prepared by industrial process in response to market factors that include nuclear family and women working outside (4-7). Recently, functional properties have contributed to the globalization of kimchi.

In Han’s study, multi-purpose sauce was developed by using kimchi (8). Sauce-type kimchi is different from mul-kimch, which is one type of kimchi enriched in seasoned water (9). In kimchi, selection of a proper starter is crucial because it should be fermentable under harsh conditions, such as high concentrations of seasoning. Many studies have been focused on the fermenting lactic acid bacteria (LAB) (4,10-14). Generally, Leuconostoc mesenteroides is found at an early fermentation stage and Lactobacillus plantarum generates over-ripeness and acidification after maturity of kimchi.

In this study, LAB was isolated from naturally fermented sauce-type kimchi. The isolated LAB were identified and analyzed for their physicochemical properties. Quality-controlled sauce-type kimchi was also produced with the isolated LAB as a starter and its properties were characterized.

MATERIALS AND METHODS

Preparation of sauce-type kimchi

To prepare sauce-type kimchi, fresh ingredients (Table 1) including Korean cabbage, radish, garlic, ginger, green

Table 1. Recipes of sauce-type kimchi

| Ingredients            | w/o starter culture | w/ starter culture |
|------------------------|---------------------|--------------------|
| Korean cabbage         | 20.0¹)              | 20.0               |
| Radish                 | 5.0                 | 5.0                |
| Garlic                 | 5.4                 | 5.4                |
| Ginger                 | 0.1                 | 0.1                |
| Green onion            | 1.5                 | 1.5                |
| Red pepper             | 1.2                 | 1.2                |
| Red pepper powder      | 2.3                 | 2.3                |
| Salt                   | 0.9                 | 0.9                |
| Sugar                  | 1.4                 | 1.4                |
| Starter                | 0.0                 | 20.0               |
| dH₂O                   | 82.2                | 62.2               |
| Total (%)              | 100.0               | 100.0              |

¹w/w %
onion, and red pepper, were purchased from a grocery store in An-seong, Korea, 2009. All ingredients were washed, chopped, mixed together, and fermented with and without a starter at room temperature with shaking (150 rpm) for 60 hr.

**Isolation and identification of LAB**

To obtain LAB from the fermented sauce-type *kimchi*, the samples were independently fermented without a starter at room temperature for 20, 40, and 60 hr. The *kimchi* samples, diluted with 0.85% NaCl solution, were spread on MRS agar (Difco, Detroit, MI, USA) and incubated in a 2.5 L jar with an anaerobic pack at 37°C for 24 hr.

The isolated LAB was identified by analysis of the 16S rDNA sequence. The chromosomal DNA of LAB was extracted using a G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea) with Mutanolysin (Sigma-Aldrich, St. Louis, MO, USA) and used as PCR templates. PCR was carried out over 30 cycles (initial denaturation at 97°C for 5 min, denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and polymerization at 72°C for 1.5 min) with a final 4-min polymerization step at 72°C with forward primer 5'-AGA GTT TGA TCM TGG CTC AG-3' and reverse primer 5'-GG YTA CCT TGT TAC GAC TT-3' using PCR System 2700 (Applied Biosystems, Foster City, CA, USA) (15). PCR products were purified with a gel extraction kit (AtmanBio, Uiwang, Korea) and sequenced at Macrogen (Seoul, Korea). The sequences of the 16S rDNA gene were analyzed with the EzTaxon server (16). A phylogenetic tree was constructed by using the neighbor-joining method that produced a unique final tree under the principle of minimum evolution and the MEGA4 program (17,18).

**Physicochemical properties of the isolated LAB**

Isolated LAB was analyzed for morphological properties, gram staining, and catalase activity according to Bergey's Manual (19). The growth patterns and pH changes of isolated LAB were analyzed with MRS agar plate (Difco™, Franklin Lakes, NJ, USA) at 37°C for 48 hr and a pH meter (Orion 420A, Orion Research, Boston, MA, USA). The initial inocula of IJ-K1 and IJ-K2 were 5.6×10² and 9.4×10² CFU/mL, respectively. Salt resistances were analyzed with 2%, 4%, and 6% NaCl in MRS broth (20). Carbonate fermentation properties, fermentation efficacy of sugars, and enzyme activities were analyzed with an API 50 CH Kit, API 50 CHL System Kit, and API ZYM Kit, respectively, according to the procedures described by the manufacturer (BioMérieux, Lyon, France).

**Production of sauce-type *kimchi* concentrate using the isolated LAB**

The sauce-type *kimchi* concentrate was produced with the isolated LAB *Pediococcus* sp. IJ-K1 and *Lactobacillus* sp. IJ-K2 as starters. Starter (~1×10⁸ cells/mL) was inoculated with supplementary nutrients and fermented for 60 hr at room temperature. Various ratios of each starter strain were applied to the different batches of sauce-type *kimchi* concentrates, such as 5:0, 0:5, 2.5:2.5, 3.5:1.5, and 1.5:3.5 of *Pediococcus* sp. IJ-K1 to *Lactobacillus* sp. IJ-K2. Population of LAB, pH, and acidity of sauce-type *kimchi* concentrate were measured during fermentation (21,22).

**Sensory evaluation**

A panel of eight participants performed a sensory evaluation, assessing taste (bitterness, sourness, and savouriness), odor, and overall acceptability using the interval scale method (23). A score of 10 indicated that the feature assessed was very strong or very good, and a score of 1 indicated very weak or very poor.

**Statistical analysis**

All values of experimental data were obtained in triplicate and analyzed using the SPSS software package (SPSS, Chicago, IL, USA). Multiple comparisons were performed between all the data using Duncan’s multiple range test at p≤0.05.

**RESULTS AND DISCUSSION**

Isolation and identification of LAB from sauce-type *kimchi*

To isolate LAB from naturally fermented *kimchi*, sauce-type *kimchi* was produced without adding any starter culture. During fermentation, the pH values changed from 5.8 to 3.9, and total acidity reached 0.86% (Fig. 1). LAB were isolated in the initial, middle, and late stages of fermentation. Analysis of the 16S rDNA se-
Isolation of Lactic Acid Bacteria from Sauce-type Kimchi

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rDNA gene sequence. A, Pediococcus sp. IJ-K1; B, Lactobacillus sp. IJ-K2. Numbers of node are levels of bootstrap support (%) from 1000 resample dataset. Bar, 0.1, nt substitution per position.

The 16S rDNA gene sequences (≥1,400 bps) revealed that the LAB isolated from the initial and middle stages displayed 99.86% homology with Pediococcus pentosaceus ATCC 33316T. In LAB isolated from the late stage of fermentation, sequences were determined to share 99.93% homology with Lactobacillus brevis ATCC 14869T. Each strain was tentatively named Pediococcus sp. IJ-K1 and Lactobacillus sp. IJ-K2 (Fig. 2), which were predominant at each stage. The 16S rDNA gene sequences IJ-K1 and IJ-K2 were deposited in GenBank under accession number JX444059 and JX444060, respectively. Generally, Leuconostoc sp. is the dominant strain of traditional kimchi (24); however, this strain was not isolated from sauce-type kimchi. Besides Pediococcus sp. and Lactobacillus sp., Bacillus spp. were developed in the initial stage of fermentation. Microbial flora were influenced by various conditions such as composition of ingredients and culturing methods. In this study, Pediococcus IJ-K1 was pre-
dominant because of its halo-tolerance in the high salt condition of sauce-type kimchi (Fig. 4).

**Physicochemical properties of the isolated LAB**

Round-shaped *Pediococcus* sp. IJ-K1 and rod-shaped *Lactobacillus* sp. IJ-K2 cells were gram positive and catalase negative. In the growth patterns of the isolated LAB at 37°C, the duration of lag phase of *Pediococcus* sp. IJ-K1 and *Lactobacillus* sp. IJ-K2 was 6 hr and 12 hr and the stationary phase was reached after 18 hr and 36 hr of incubation, respectively. After incubation at 37°C for 30 hr, the pH values of bacteria culture of IJ-K1 and IJ-K2 were 4.03 and 4.95, respectively (Fig. 3). In addition, the halo-tolerance of *Pediococcus* sp. IJ-K1 was higher than that of *Lactobacillus* sp. IJ-K2 (Fig. 4). The stationary phase of *Pediococcus* sp. IJ-K1 was reached in 6% NaCl after 30 hr of incubation at 37°C. However, *Lactobacillus* sp. IJ-K2 was completely inhibited from the start of lag phase at the same culture condition. Utilization of carbohydrates with the API 50 CHL Kit is summarized in Table 2. 1-Arabinose, Ribose, d-Galactose...
Isolation of Lactic Acid Bacteria from Sauce-type Kimchi

Production of sauce-type kimchi concentrate using the isolated LAB

Sauce-type kimchi concentrate was produced with the isolated LAB as starter culture. The pH values of non-starter-treated sauce-type kimchi as control was 3.67 and starter-treated sauce-type kimchies were 3.42 to 3.77. Final acidities of starter-treated sauce-type kimchi samples were increased under the range from 0.79% to 1.0%, whereas final acidity in control was 0.65% (Table 4). Final pH was the lowest one (pH 3.42) and total acidity was the highest one (1.0%) at the inocula ratio of 0:5 (Pediococcus sp. IJ-K1 to Lactobacillus sp. IJ-K2). This sauce-type kimchi gave a high sourness taste during the sensory evaluation test (Fig. 5). Lee et al. (12) reported that L. brevis is very tolerant to acid and plays a role in the later stage of fermentation. These results suggested that the isolated Lactobacillus sp. IJ-K2 as starter was proper to produce sauce-type kimchi concentrate and its product gave better value concerning sourness taste, flavor, and overall evaluation.

CONCLUSION

The objective of this study was to isolate and identify LAB from the naturally fermented sauce-type kimchi and to characterize their physicochemical properties. During

tose, d-Glucose, d-Fructose, d-Mannose, N-Acetylgluco-
samine, Amygdalin, Arbutin, Esculine, Salicin, Cel-
lobiose, Maltose, Lactose, Melibiose, Sucrose, d-Raffinose,
Gentiobiose, and d-Tagatose were utilized by Pediococcus sp. IJ-K1. Lactobacillus sp. IJ-K2 showed higher util-
ization rates with l-Arabinose, Ribose, d-Xylose, d-
Galactose, d-Glucose, d-Fructose, Mannitol, α-Methyl-d-
glucoside, N-Acetylglucosamine, Maltose, Gluconate, and 2-Ketogluconate. In contrast to Pediococcus sp. IJ-K1, Lactobacillus sp. IJ-K2 did not utilize lactose. Although Lactobacillus sp. IJ-K2 is one of many lactic acid bac-
teria, all LAB do not necessarily utilize lactose (25). Comparison with the API database (https://apiweb.biomerieux.com) (26) revealed 99.2% homology of IJ-
K1 with P. pentosaceus, and 99.9% homology of IJ-K2 with L. brevis. In the API ZYM enzyme assay kit, Pediococcus sp. IJ-K1 exhibited the enzymatic activities of esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, β-galactosidase, α-glucosidase, and N-acetyl-β-glucosamidase. In Lactobacillus sp. IJ-
K2, the enzymatic activities of leucine arylamidase, α-
galactosidase and β-galactosidase were confirmed (Ta-
ble 3). β-Glucuronidase is a carcinogenic enzyme (27), but its activity was not detected in either isolated strain.

**Table 3. Enzyme activities of Pediococcus sp. IJ-K1 and Lactobacillus sp. IJ-K2 as determined by API ZYM KIT**

| Enzyme                  | IJ-K1    | IJ-K2    |
|------------------------|----------|----------|
| Control                | ND<sup>1</sup> | ND<sup>1</sup> |
| Alkaline phosphatase   | ND       | ND       |
| Esterase (C<sub>1</sub>) | 1        | ND       |
| Esterase lipase (C<sub>4</sub>) | 1        | ND       |
| Lipase (C<sub>14</sub>) | ND       | ND       |
| Leucine arylamidase    | 5        | 2        |
| Valine arylamidase     | 2        | ND       |
| Crystine arylamidase   | ND       | ND       |
| Trypsin                | ND       | ND       |
| α-Chymotrypsin         | ND       | ND       |
| Acid phosphatase       | ND       | ND       |
| Naphthol-AS-BI-       | 5        | ND       |
| phosphohydrolase        |           |          |
| α-Galactosidase        | ND       | 2        |
| β-Galactosidase        | 4        | 2        |
| β-Glucuronidase        | ND       | ND       |
| α-Glucosidase          | 2        | ND       |
| β-Glucosidase          | ND       | ND       |
| N-Acetyl-β-glucosamidase | 5      | ND       |
| α-Mannosidase          | ND       | ND       |
| α-Fucosidase           | ND       | ND       |

<sup>1</sup> Enzyme activity was determined by using color-strength values: 0, negative reaction; 1, 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, > 40 nmol. ND, not detected.

<sup>2</sup> ND, not detected.

**Table 4. Sensory quality of sauce-type kimchi produced with the isolated lactic acid bacteria**

| Starter ratio (IJ-K1 : IJ-K2)<sup>1</sup> | Final acidity (%) | Final pH |
|------------------------------------------|-------------------|---------|
| Control<sup>2</sup>                     | 0.65              | 3.67    |
| 5:0                                      | 0.82              | 3.77    |
| 0:5                                      | 1.0               | 3.42    |
| 2.5:2.5                                  | 0.93              | 3.51    |
| 3.5:1.5                                  | 0.79              | 3.64    |
| 1.5:3.5                                  | 0.97              | 3.56    |

<sup>1</sup>IJ-K1: Pediococcus sp. IJ-K1, IJ-K2: Lactobacillus sp. IJ-K2.
<sup>2</sup>Naturally fermented kimchi without adding any starter culture.
the 60 hr fermentation, naturally fermented sauce-type kimchi was investigated for pH, acidity and dominant microbial strains. The pH values of sauce-type kimchi were changed from 5.8 to 3.9 and the acidity reached 0.86%. The dominant LAB were isolated and identified as Pediococcus sp. and Lactobacillus sp. by the 16S rDNA sequencing and tentatively named Pediococcus sp. IJ-K1 and Lactobacillus sp. IJ-K2, respectively. The pH values of Pediococcus sp. IJ-K1 and Lactobacillus sp. IJ-K2 cultures after 24 hr incubation at 37°C were 4.03 and 4.95, respectively. Pediococcus sp. IJ-K1 was more resistant to NaCl than Lactobacillus sp. IJ-K2.

Sauce-type kimchi was produced with the isolated LAB and analyzed. An inoculum ratio of 0:5 (Pediococcus sp. IJ-K1 to kimchi to kimchi overall evaluation score, 6.5. The isolated LAB are

ACKNOWLEDGMENTS

This research was supported by the Chung-Ang University Research Scholarship Grants.

REFERENCES

1. Cheigh HS, Park KY. 1994. Biochemical, microbiological, and nutritional aspects of kimchi (Korean fermented vegetable products). Crit Rev Food Sci Nutr 34: 175-203.
2. Park WS, Lee IS, Han YS, Koo YJ. 1944. Kimchi preparation with combined Chinese cabbage and seasoning mixture stored separately. Korean J Soc Food Sci Technol 26: 231-238.
3. Lim CR, Park HK, Han HU. 1989. Re-evaluation of isolation and identification of gram-positive bacteria in kimchi. Korean J Microbiol Biotechnol 27: 404-414.
4. Park KY. 1995. The nutritional evaluation, and antimutagenic and anticancer effects of Kimchi. J Korean Soc Microbiol Nutr 24: 169-182.
5. Lee YO, Park KY, Cheigh HS. 1996. Antioxidative effect of Kimchi with various fermentation periods on the lipid oxidation of cooked ground meat. J Korean Soc Microbiol Nutr 25: 261-266.
6. Choi WY, Park KY. 1999. Anticancer effects of organic Chinese cabbage Kimchi. J Food Sci Nutr 4: 113-116.
7. Park JY, Rhee SH, Park KY. 2000. Enhancement of anticancer activities of Kimchi by manipulating ingredients. J Food Sci Nutr 5: 126-130.
8. Han GI, Shin DS, Cho YS, Lee SY. 2007. Development of a multi-purpose sauce using Kimchi. Korean J Food Cookery Sci 25: 281-287.
9. Lee SK, Ji GE, Park YH. 1999. The viability of bifidobacteria introduced into kimchi. Lett Appl Microbiol 28: 153-156.
10. So MH, Shin MY, Kim YB. 1996. Effects of psychrotrophic lactic acid bacterial starter on Kimchi fermentation. Korean J Soc Food Sci Technol 28: 806-813.
11. Mheen TI, Kwon TW. 1984. Effect of temperature and salt concentration on Kimchi fermentation. Korean J Food Technol 16: 443-450.
12. Lee CW, Ko CY, Ha DM. 1992. Microfloral changes of the lactic acid bacteria during Kimchi fermentation and identification of the isolates. Korean J Appl Microbiol Biotechnol 20: 102-109.
13. Lee HJ, Baek JH, Yang M, Han HU, Ko YD, Kim HJ. 1993. Characteristics of lactic acid bacterial community during kimchi fermentation by temperature downshift. Korean J Microbiol 31: 346-353.
14. Park JA, Heo GY, Lee JS, Oh YJ, Kim BY, Kim CK, Ahn JS. 2003. Change of microbial communities in Kimchi fermentation at low temperature. Korean J Microbiol 39: 45-50.
15. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173: 697-703.
16. Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW. 2007. EzTaxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequence. Int J Syst Evol Microbiol 57: 2259-2261.
17. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.
18. Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599.
19. Park JA, Heo GY, Lee JS, Oh YJ, Kim BY, Kim CK, Ahn JS. 2003. Change of microbial communities in Kimchi fermentation at low temperature. Korean J Microbiol 39: 45-50.