Research Note

RAPD typing of *Lactobacillus brevis* isolated from various food products from Korea

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Abstract In the present study, the fingerprinting technique, random amplified polymorphic DNA-PCR was evaluated to characterize 13 strains of *Lactobacillus brevis*, isolated from different vegetable products of South Korea. Two primers i.e. 239 and KAY3 were used. The primer 239 produced bands ranged from 500-4,000 bp and KAY3 primer produced bands with sizes from 600-4,000 bp. Both primers produced thirteen different RAPD profiles. Phylogenetic dendrogram showed that all the isolates could be divided into six major clusters both the primers. However, a few strains of *L. brevis* had similar profiles and were not well differentiated by RAPD.

Keywords: *Lactobacillus brevis*, random amplified polymorphic DNA, primer

Introduction

The global demand for probiotics is expected to reach USD 96 billion in 2020 (1). Lactic acid bacteria (LAB) play an important role as probiotics, live microorganisms which provide health benefits to host when consumed. *Lactobacillus* is the most diverse genus of LAB. It belongs to the Lactobacillaceae family and contains around 152 species (2,3). *Lactobacillus* spp. are facultative anaerobic (aero-tolerant), Gram-positive, catalase-negative, non-spore forming, and have low GC content in their genome (4). They produce lactic acid as their major fermentation product. Important species of *Lactobacillus* include *L. brevis*, *L. delbrueckii* ssp. *bulgaricus*, *L. helveticus*, *L. acidophilus*, *L. gasseri*, *L. plantarum*, *L. pentosus*, *L. buchneri*, *L. curvatus*, *L. sakei*, *L. farciminis*, and *L. paracasei* (5). They have remarkable fermentation ability and also help by increasing nutrients and other health benefits in the products (6). Typically, LAB have been characterized using phenotypic methods, although these identification methods are not always reliable (7). Currently, several DNA-based molecular tools are used to identify novel strains and to assess diversity among strains.

Identification of LAB has significantly developed with the application of molecular biology techniques using 16S and 23S rRNA-targeted oligonucleotide probes (8), random amplified polymorphic DNA PCR (RAPD-PCR) (9-13), amplified fragment length polymorphism (AFLP) analysis (11), restriction fragment length polymorphism (RFLP) analysis (14), ribotyping (13,15), microarrays (16), and repetitive elements PCR (REP-PCR) (9,17). RAPD technique is conventionally

used to study genetic diversity and determine the overall similarity among different microorganisms or living organisms (18). It works on the principle that, a single, oligonucleotide primer of arbitrary nucleotide sequence, binds to different loci in the genome and amplify random segments of different sizes. This technique offers advantages such as no need of prior DNA sequence or DNA probe, requires very less amount of DNA and unit cost per assay is low (19). The present study focuses on the isolation, identification, and characterization of 13 strains of *L. brevis* isolated from various food products in Korea using RAPD typing.

Material and Methods

Bacterial strains and growth conditions Thirteen bacterial strains isolated from various food products (Table 1) were identified as *L. brevis* strains using 16S rRNA gene sequences. For further molecular biology analysis, LAB strains were cultured in MRS medium (BD, Franklin Lakes, NJ, USA) at 37°C.

DNA extraction Total chromosomal DNA from overnight broth cultures of the 13 *L. brevis* strains was extracted using AccuPrep Genomic DNA Extraction kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions.

PCR conditions and RAPD analysis Genetic fingerprinting was performed by RAPD-PCR typing using two RAPD primers, 239 (5-

Table 1. Source of *Lactobacillus brevis* strains isolated in this study

Strain no.	Area/locality	Source
200019	Seoul, Korea	Salted clam
B13	Seoul, Korea	Kimchi
B151	Seoul, Korea	Kimchi
B7	Seoul, Korea	Kimchi
200054	Seoul, Korea	Pickled vegetable
B2	Seoul, Korea	Kimchi
200086	Seoul, Korea	Mustard kimchi
B15	Seoul, Korea	Kimchi
G1	Seoul, Korea	Kimchi
KU15006	Seoul, Korea	Turnip kimchi
11348	Seongnam, Korea	Kimchi
11350	Seongnam, Korea	Kimchi
11351	Seongnam, Korea	Kimchi

CTGAAGCGGA-3) and KAY3 (5-CTGGCGACTG-3), at a concentration of 10 pmoles/ μ L. The premix kit (Bioneer; 10 mM Tris-HCl, pH 9.0, 40 mM KCl, 1.5 mM MgCl₂, 250 μ M dNTPs mix, and 1 U of *Taq* polymerase) was used for PCR amplification using 2 μ L of each primer for two separate reactions; 1 μ L of template DNA and 17 μ L of distilled water were added to make up a final volume of 20 μ L. Amplification conditions were as follows: initial denaturation at 94°C for 4 min, 2 cycles of denaturation at 94°C for 2 min, annealing at 39°C for 2 min, extension at 72°C for 2 min; 35 cycles of denaturation at 95°C for 15 s, annealing at 39°C for 15 s, and extension at 72°C for 20 s followed by final elongation at 72°C for 7 min. The amplified PCR products were checked by 1.5% (w/v) agarose gel electrophoresis with 100 bp and 1 kb DNA ladders (Takara, Shiga, Japan) and photographed using gel documentation system (Gel Doc XR⁺, Bio-Rad, Hercules, CA, USA). The band pattern was analyzed using Quantity One software (Bio-Rad) and phylogenetic trees were constructed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

Results and Discussion

RAPD-PCR has been successfully used for genetic fingerprinting in animals, plants, humans, and microorganisms such as *Salmonella*, *Lactobacillus*, *E. coli*, *Bacillus*, and yeast (20). In the present study, the PCR-based RAPD technique has been applied to assess the genetic diversity among the 13 *L. brevis* isolates, using two primers, 239 and KAY3. The band profiles of both primers on agarose gels were compared to each other. RAPD analysis generated 8 different RAPD profiles among the 13 *L. brevis* strains (Fig. 1 and 2). Maximum and minimum bands observed were 4,000 bp (very light) and 500 bp, respectively, with primer 239, and 4,000 bp (very light) and 600 bp, respectively, with primer KAY 3. Sample Strain B151 in lane 3 has a notably lighter RAPD profile compared to that of other samples with the primer 239. In RAPD profiling, 4 prominent bands 2,500, 1,250, 550, and 500 bp were observed with the primer 239, whereas 6

major bands 2,500, 2,000, 1,200, 900, 600, and 500 bp were observed with the primer KAY3. RAPD amplicons of strains 200019, 200054, G1, KU-15006, 11348, 11350, 11351 with the primer 239, and strains 200019, 200054, 200080, and G1 with the primer KAY3 have shown similarity in banding patterns. It implies that the high genetic similarity was probably because the RAPD primers used in this study yielded a low number of polymorphic bands in the selected bacterial species. The highest number of bands was observed for strain B-15 in lane 9 with both RAPD primers in this study. The only prominent band observed with the primer KAY3 consisted of a 900 bp band in two strains, B-7 and KU15006. RAPD and REP-PCR have been used for the identification of *L. hilgardii* and *L. brevis* (9). Similarly, RAPD with the primer 239 has also been used for identification and characterization of *Leuconostoc mesenteroides* (21).

RAPD analysis was performed to cluster the *L. brevis* isolates using the two primers, 239 and KAY3. Based on the analysis, the 13 *L. brevis* strains could be divided into six clades with the primer 239, in which the group 6 had the highest number of 7 strains in the group (200019, 200054, KU15006, G1, 11348, 11350, 11351) (Fig. 1). The 13 strains could be divided into two main groups with the primer KAY3, in which the group 6 consisted of the B-15, G1, KU15006, B-13 and B-151 strains (Fig. 2). The group 5 consisted of the B-15 and B-13 strains with the primer 239 and consisted of only one strain i.e. 200080 with the primer KAY3. For the remaining group 1, 2, 3, and 4, each were composed of the B-13, B7, 200080, and B-151 strains respectively, with the primer 239. The first five groups showed different banding pattern in gel images, as well (Fig. 1). In contrast to the primer KAY3, group 3 with primer 239 consisted of five strains i.e. B13-2, B151, B15, G1, and KU15006. The remaining group 1, 2, 4, and 5 with primer KAY3 consisted of the B-13, B7, 200019, and 200080 strains, respectively (Fig. 2).

In the absence of sufficient information available for the gene sequences in a particular bacterial species, the RAPD method can be significantly useful in determining important strain-specific information. However, reproducibility could be one of the main limitations associated with the fingerprints generated by the RAPD technique. These limitations associated with reproducibility could be overcome by precise optimization of the RAPD-PCR procedure (22). In conclusion, the results presented in this study indicate that the RAPD-PCR technique is useful for preliminary differentiation of *L. brevis* from different food products from Korea. Some of these *L. brevis* isolates are expected to be useful in starter cultures for various industrial applications. Further evaluation using additional molecular typing methods like REP-PCR and multilocus sequence typing is required to determine if isolated strains are of industrial value in improving the nutrition and controlling the growth of pathogen causing food spoilage in dairy industry.

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Fig. 1. (A) RAPD profiling of *Lactobacillus brevis* using primer 239. Lane M, 1 kb marker; Lane M, 100 bp marker; Lane 2, 200019; Lane 3, B13; Lane 4, B151; Lane 5, B7; Lane 6, 200054; Lane 7, B13-2; Lane 8, 200080; Lane 9, B15; Lane 10, G1; Lane 11, KU15006; Lane 12, 11348; Lane 13, 11350; Lane 14, 11351; Lane M, 100 bp marker; Lane M, 1 kb marker. (B) Dendrogram generated after cluster analysis of the *Lactobacillus brevis* strains isolated from various food products from South Korea after identification using RAPD typing with primer 239. The cluster analysis was done using UPGMA method by Quantity One software.

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Disclosure The authors declare no conflict of interest.

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Fig. 2. (A) RAPD profiling of *Lactobacillus brevis* using primer KAY3. Lane M, 1 kb marker; Lane M, 100 bp marker; Lane 2, 200019; Lane 3, B13; Lane 4, B151; Lane 5, B7; Lane 6, 200054; Lane 7, B13-2; Lane 8, 200080; Lane 9, B15; Lane 10, G1; Lane 11, KU15006; Lane 12, 11348; Lane 13, 11350; Lane 14, 11351; Lane M, 100 bp marker; Lane M, 1 kb marker. (B) Dendrogram generated after cluster analysis of the *Lactobacillus brevis* strains isolated from various food products from South Korea after identification using RAPD typing with primer KAY3. The cluster analysis was done using UPGMA method by Quantity One software.

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