

Rapid Identification of *Lactobacillus plantarum* in Kimchi Using Polymerase Chain Reaction

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Abstract A polymerase chain reaction (PCR) was performed to rapidly identify *Lactobacillus plantarum* from type strains and kimchi samples. The PCR experiments were carried out using specific oligonucleotide primer sets based on the 16S rRNA gene sequences of *L. plantarum*. The expected DNA amplicate of 419 bp was obtained when either purified DNA or whole cells of *L. plantarum* strains reacted with LP primers, yet not with any of the other strains. The PCR product was confirmed by DNA sequencing. Accordingly, since the PCR method used is simple, specific, and rapid, it will be useful for monitoring and evaluating *L. plantarum* in the mixed microbial population found in kimchi.

Key words: *Lactobacillus plantarum*, polymerase chain reaction (PCR), kimchi

Lactic acid bacteria are used for the fermentation of a variety of dairy, vegetable, cereal, and meat products. Kimchi is a fermented vegetable and its characteristics depend on the lactic acid bacteria involved in the fermentation progress [5, 13, 18, 19, 22, 23]. Previous microbiological studies on the lactic acid bacteria in kimchi have been performed related to changes of microflora, identification, and classification. The common lactic acid bacteria in kimchi have been identified as *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Streptococcus faecalis*, and *Pediococcus pentosaceus* [10, 12]. *Lactobacillus plantarum* is present in large numbers following initial fermentation and produces the maximum acidity in later stages [14]. Therefore, since this bacterium can seriously affect the quality of the kimchi, its population needs to be monitored under various fermentation conditions. However, the detection and identification of this lactic acid bacteria by traditional biochemistry methods are labor intensive

and can take several days to complete [9, 16]. As many lactic acid bacteria have similar nutritional and growth requirements [7, 11, 17, 20], it is very difficult to identify them individually using conventional methods [25]. Accordingly, to study *L. plantarum* effectively, it is necessary to develop a rapid and accurate method for its specific detection and identification from the mixed lactic acid bacteria present in kimchi. Recently, the rRNA sequences have been generally accepted for the identification and phylogenetic analysis [3, 8, 15, 24]. The polymerase chain reaction (PCR) has been used for the identification of some *Lactobacillus* species using 16S rRNA gene [21] or 16S-23S rRNA intergenic space sequences [4, 26]. However, this identification technique has not yet been used to a large degree to identify to the species of lactic acid bacteria from fermented foods. Because of the above reasons, this study was undertaken for on a rapid and accurate technique for the identification of *L. plantarum* in kimchi as a vegetable fermented food using a PCR.

The type strains used in this study are listed in Table 1. All cultures were obtained from the Korean Collection for Type Cultures (Daejeon, Korea) and Korean Culture Center of Microorganisms (Seoul, Korea) and were cultured in an MRS broth (Difco, Detroit, U.S.A.) at 30°C. Unknown lactic acid bacteria were isolated from kimchi samples [6] at various phases of fermentation. The juices of kimchi were diluted to 10⁶-10⁸ with distilled water, spread onto MRS agar plates and incubated for 3 days at 30°C to allow colonies to develop. The Stock cultures were stored in a mixture of 20% glycerol and 80% MRS at -70°C. The PCR primers were selected from the specific 16S rRNA gene sequences of *L. plantarum*. The GenBank (National Center for Biotechnology Information, Bethesda, U.S.A.) program BLAST [1] was used to ensure that the designed primers (13LP and 18LP) were only complementary with the target species and not any other species. The primers were synthesized by Life Technologies, Inc. (Gaithersburg, U.S.A.) and the sequences of the 13LP primer and 18LP

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Table 1. Strains tested and results of PCR with designed primer sets.

Species	Strain	LP Primer
<i>Lactobacillus plantarum</i>	KCTC 3104	+
<i>Lactobacillus plantarum</i>	KCTC 3108	+
<i>Lactobacillus plantarum</i>	KCCM 11322	+
<i>Lactobacillus brevis</i>	KCTC 3498	-
<i>Lactobacillus brevis</i>	KCCM 11509	-
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	KCTC 3505	-
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	KCTC 3100	-
<i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i>	KCTC 3530	-
<i>Leuconostoc mesenteroides</i> subsp. <i>dextranicum</i>	KCCM 35046	-
<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	KCTC 3529	-
<i>Leuconostoc paramesenteroides</i>	KCTC 3531	-
<i>Leuconostoc citreum</i>	KCTC 3524	-
<i>Leuconostoc lactis</i>	KCTC 3528	-
<i>Lactococcus lactis</i>	KCCM 32406	-
<i>Pediococcus pentosaceus</i>	KCTC 3507	-

+, Positive reaction; -, negative reaction.

primer were 5'-TGATTGGTGCTTGCATCATG-3' and 5'-TGAACAGTTACTCTCAGATA-3', respectively. The positions of the 13LP primer and 18LP primer were nucleotides 82 to 101 and 481 to 500, respectively, in *L. plantarum* (GenBank M58827). Those colonies on the MRS plates that were at least 1.0 mm (diameter) were selected using sterile toothpicks, diluted with 30 μ l of distilled water in an Eppendorf tube, and mixed by shaking the tube for 30 sec. The bacterial solutions were heated at 95°C for 6 min, immediately cooled in ice water, and tested directly by a PCR without any isolation of the DNA. The genomic DNA was isolated from the lactic acid bacteria using the phenol-chloroform method [2]. After centrifugation, the cells were resuspended in 567 μ l of a TE buffer (10mM Tris-HCl pH 8.0; 1 mM EDTA), treated with 30 μ l of 10% (w/v) SDS and 3 μ l of 20 mg/ml protease K (Sigma, U.S.A.), and incubated for 1 h at 37°C. The samples were then treated with 100 μ l of 5 M NaCl and 80 μ l of a CTAB/NaCl solution. The mixture was incubated for 10 min at 65°C, and then extracted with phenol-chloroform. The DNA was then precipitated in isopropanol, washed with 70% ethanol, and finally resuspended in 100 μ l of a TE buffer (10 mM Tris-HCl pH 8.0; 1 mM EDTA). The PCR tests were performed using genomic DNA or a whole cell without isolating the DNA. The reaction mixture for the PCR consisted of 1 \times a reaction buffer (20 mM Tris-HCl, pH 8.4, 1.5 mM MgCl₂, 50 mM KCl), 200 μ M of each deoxynucleoside triphosphate, 0.4 μ M primer set, 5 μ l of the bacterial suspension (10⁶-10⁷ CFU) or 100 ng of purified DNA, and 1 unit of *Taq* polymerase (Gibco BRL, U.S.A.). The PCR was conducted in a PT-100 Programmable Thermal Controller (MJ Research, Massachusetts, U.S.A.). The amplification was at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. This was repeated for 40 cycles. The

program also included a preincubation at 94°C for 5 min before the first cycle and a further incubation at 72°C for 7 min after last cycle. The amplification products were separated by electrophoresis in 1.5% agarose gels containing ethidium bromide (0.2 μ g/ml). The gels were run for 40 min at 50 V in a TAE electrophoresis buffer (40 mM Tris acetate, 2 mM EDTA) without cooling and analyzed using Image Tool (Bioneer, Daejeon, Korea). The DNA molecular weight marker, *Hae*III- Φ X174 DNA marker (Gibco BRL, U.S.A.), was used as the standard. The nucleotide sequence of the amplified 16S rRNA fragment was determined using an ABI prism automatic sequencer (Perkin-Elmer, U.S.A.). Thus, the PCR fragment was excised from the gel and purified with a QIAquick gel extraction kit (Qiagen, U.S.A.). The nucleotide sequence of the 16S rRNA of *L. plantarum* was obtained from the GenBank (M58827) and homology searches were performed using the Gene Doc program (Multiple Sequence Alignment Editing and Shading Utility version 2.5).

The specificity of the primers was tested by performing the PCR with various type strains of lactic acid bacteria, as listed in Table 1. The DNA templates for the primers were obtained from the colonies using a simple lysis procedure, as described above. The PCR amplification from the purified DNA and whole cells using the LP primers consistently produced a fragment of 419 bp as expected. This lysis procedure was simple and convenient, and showed the same result as the experiments that used DNA isolated from the strains (Fig. 1). As the control for the PCR, type strains of *L. plantarum* were all reacted with the LP primer set whereas the other strains were not. The PCR products from the reference bacteria confirmed the specificity (Table 1). Based on the colonies isolated from the *kimchi* on the MRS plate, PCR with the specific

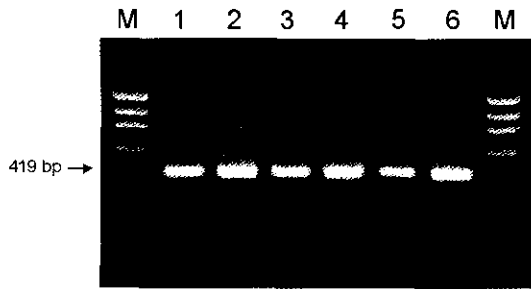


Fig. 1. PCR products obtained from amplification of whole cells (lanes 1, 3, 5) and purified DNA (lanes 2, 4, 6) with LP primers. Lanes: M, *Hae*III- Φ X174 DNA marker (Gibco BRL, U.S.A.); 1-2, *L. plantarum* KCTC 3104; 3-4, *L. plantarum* KCTC 3108; 5-6, *L. plantarum* KCCM 11322.

primers were then performed to detect *L. plantarum* from the isolated lactic acid bacteria. A total of 27 single colonies of lactic acid bacteria were selected from *kimchi* sample on day 1 during *kimchi* fermentation at 20°C. Among them, 6 were identified as *L. plantarum*. Six each isolates as positive and negative of this PCR result are shown in Fig. 2. The nucleotide sequence of the 16S rRNA of *L. plantarum* KHF-2 isolated from the *kimchi* was determined and aligned with that of the type strain (*L. plantarum*, M58827). The sequence of *L. plantarum* KHF-2 isolated from the *kimchi* was identical with the reference sequence of *L. plantarum* (Fig. 3). The specific primer set designed and evaluated in this study allows for the identification of a small amount of lactic acid bacteria. This PCR method has two major advantages over the conventional identification techniques used for the identification of *L. plantarum* in *kimchi*. First, a reliable and rapid identification of *L. plantarum* in *kimchi* can be obtained within a few hours, one colony on an agar plate being sufficient. Second, the *L. plantarum* population can be monitored both during and after the *kimchi* fermentation process.

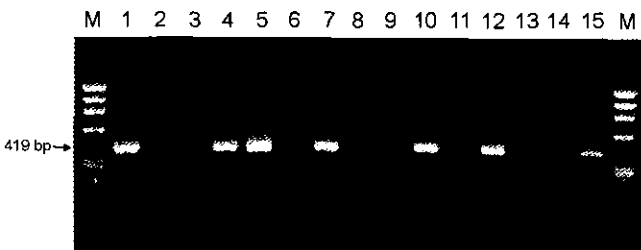


Fig. 2. PCR products obtained from amplification of type strains and lactic acid bacteria isolated from *kimchi* with LP primers. Lanes: M, *Hae*III- Φ X174 DNA marker (Gibco BRL, U.S.A.); 1, *L. plantarum* KCTC; 2, *Leu. mesenteroides* KCTC 3100; 3, *L. brevis* KCTC 3498; 4-15, isolates from *kimchi*. Lanes 4, 5, 7, 10, 12 and 15 detected for *L. plantarum*.

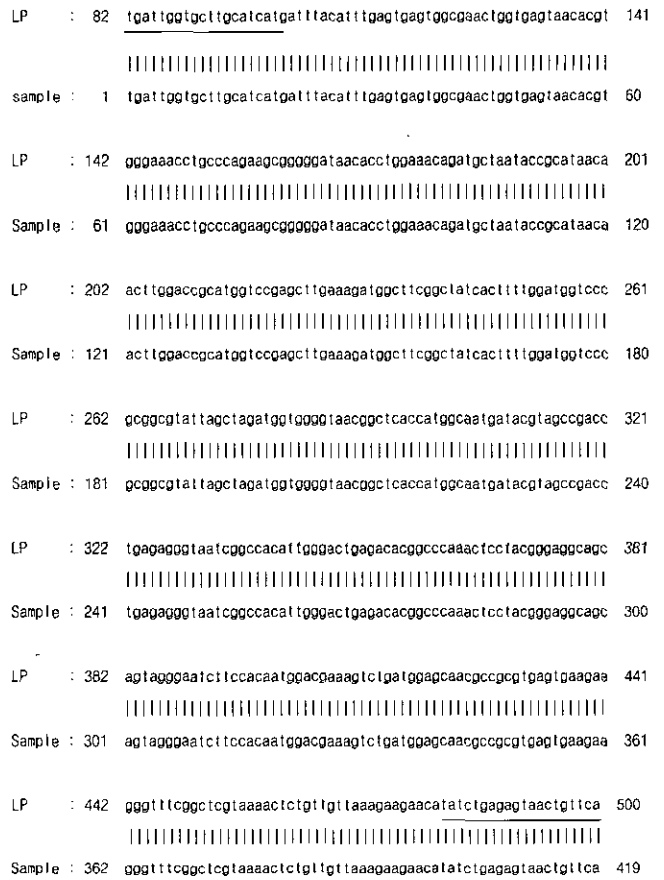


Fig. 3. Alignment of 16S rRNA sequence of type strain (*L. plantarum*) and *L. plantarum* KHF-2 isolated from *kimchi*. The underlined letters indicate the primers used for sequencing. The GenBank accession number of *L. plantarum* is M58827.

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