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Development of Species-Specific Primers for PCR Identification of *Lactobacillus hilgardii* and *Lactobacillus farciminis* in Kimchi

- Research Note -

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Abstract

The aim of this study was to develop species-specific primer sets for kimchi *Lactobacillus*. Known gene sequences of *Lactobacillus* 16S rRNA were collected from the NCBI Gene bank, and 69 primer sets were designed using the homologous gene sequence. Six species of kimchi Lactobacilli were used as reference strains: *Lactobacillus brevis* KCTC3102, *Lactobacillus farciminis* KCTC3681, *Lactobacillus fermentum* KCTC3112, *Lactobacillus hilgardii* KCTC3500, *Lactobacillus plantarum* KCTC3099, and *Lactobacillus sanfranciscensis* KCTC3205. PCR amplification and gel electrophoresis were performed to identify the accuracy and specificity of the developed primer set. The results show that the primer set of 5'-aagcctgcgaaggcaag-3' & 5'-aggccaccggctttg-3', 5'-acatactatgcaaatctaagagattagacg-3' & 5'-actgagaatggctttaagagattagcttac-3' resulted in a specific PCR band on *L. hilgardii*, and primer set of 5'-ctaataccgcataacaactactttcacat-3' & 5'-aacttaataaaccgcctacattctctttac-3' on *L. farciminis*. The results indicate that the developed primer sets can provide a useful tool for the identification and differentiation of *L. hilgardii* and *L. farciminis* from other *Lactobacillus* species of kimchi.

Key words: *Lactobacillus* species, *Lb. hilgardii*, *Lb. farciminis*, kimchi, PCR primer

INTRODUCTION

Lactic acid bacteria (LAB) are bacteria that predominantly ferment a sugar to lactic acid (1-3). LAB comprise a versatile group of microorganisms that are generally regarded as safe and have profound applications in food (4-6). Lately, a number of health benefits of LAB as probiotics have been claimed, including alleviation of symptoms of lactose intolerance, treatment of diarrhea, prevention of cancer, lowering cholesterol and blood pressure, and improvement immunity (7-9).

Lactobacillus, a major component of the LAB group, is a genus of Gram-positive facultative anaerobic or microaerophilic bacteria, named such because most of its species convert lactose and other sugars to lactic acid (10). Some *Lactobacillus* species are used industrially for the production of yogurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, and other fermented foods. *Lactobacilli*, especially *Lb. plantarum*, *Lb. kimchii*, *Lb. casei*, *Lb. mesenteroides*, *Lb. brevis*, etc., are some of the most common organisms involved in kimchi fermentation (11).

Kimchi is a typical traditional Korean food which is a group of vegetables fermented by LAB. The flavor of kimchi is dependent on the ingredients, fermentation conditions, and LAB involved in the fermentation process (11-13), including *Leuconostoc*, *Streptococcus*, *Pediococcus*, *Lactobacillus*, and *Weissella*. In the early stage of kimchi fermentation *Leu. mesentoroides* is the predominant species, but the levels decrease during the fermentation process, while *Streptococcus* and *Pediococcus* increase. The *Lactobacillus* genus, especially *Lb. plantarum*, proliferate prosperously with lactic acid production until later stages of fermentation (14,15).

The fermentation process of kimchi, along with various subsidiary ingredients, determines the unique taste of kimchi, especially umami. Organic acid and carbon dioxide in kimchi are the two main elements which determine the taste of kimchi. The amounts of organic acid and carbon dioxide vary according to the kind of microorganism in the mixture, the salt concentration and the temperature (16). Hence, specific detection and classification of LAB strains concerning certain characteristics, such as their fermentation profile and their functionality, is demanded for appropriately developing kimchi for the global market. With this need in mind, Lee et al. (17) developed LAB sets of kimchi in hetero- and homofermentum to enhance functionality and the savory flavor aspect of kimchi for globalization.

Identification of LAB is complicated by the species' richness and the closely related taxonomic groups within the heterogeneity. Notably, LAB isolated from kimchi

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have distinguishing characteristics, which are differentiated from LAB of dairy products. These characteristics include low digestibility of lactose and strong tolerance to acid and bile acid (18). Thus, it is difficult to use phenotypic method and traditional fermentation profiles to identify most of the lactic acid bacteria originating from kimchi. Lately, consumption of traditional fermented food has increased because of their potential therapeutic and prophylactic attributes, and because Lactobacilli also have been proposed as probiotics. However, little scientific research has been focused on quality control and standardized processes for industrialization (19).

The objectives of this study were to develop species-specific primer sets for the *Lactobacillus* species based on the 16S rRNA, and to provide a useful and rapid tool for identifying the naturally occurring *Lactobacillus* of LAB in Kimchi.

MATERIALS AND METHODS

Reference strains

The reference strains used in this study were obtained from the Korean Collection for Type Cultures (KCTC) were: *Lactobacillus brevis* KCTC3102, *Lactobacillus farciminis* KCTC3681, *Lactobacillus fermentum* KCTC-3112, *Lactobacillus hilgardii* KCTC3500, *Lactobacillus plantarum* KCTC3099, *Lactobacillus sanfranciscensis* KCTC3205. All strains were routinely cultured at 30°C for 2 days on MRS agar (Merck, Germany).

DNA extraction from lactic acid bacteria

The genomic DNA was isolated from the lactic acid bacteria with Genomic DNA Purification Kit (Promega Ltd., Korea). All strains were maintained frozen as stocks in 50 mM EDTA buffer solution at -20°C.

Development of identification primer

Known 16S rRNA gene sequence of *Lactobacillus* on the NCBI data were collected by Multalin (http://prodes.toulouse.Inra.fr/multaline/multalin.html). 16S rRNA gene sequence homology was compared based on NCBI gene sequence data and primer sequence of each gene was designed using the Primer3 program (http://www.broad.mit.edu/cgi-bin/primer3_www.cgi). The synthetic oligonucleotides primers used in the present study were manufactured by Bioneer (Daejeon, Korea).

PCR amplification

PCR amplification of 16S rRNA gene sequence was performed to identify the accuracy of the developed primer set. Template DNA was genomic DNA extraction of lactic acid bacteria reference strains. The amplification of the genomic DNA from reference strains was performed at least in duplicate for each sample and carried out by using an automated thermal cycler (PC808, ASTEC Inc., Fukuoka, Japan) in a total volume of 20 μL containing 10 μL PCR premix (1U thermostable DNA polymerase, 200 μM dNTP, 1.5 mM $MgCl_2$), 10 μL mixture of template DNA and each primer at a concentration of 1 μM. The thermocycle program was as follows: 1 cycle of pre-denaturation at 94°C for 1 min; 30 cycles consisting of denaturation at 94°C for 2 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min; final extension as 72°C for 7 min.

Electrophoresis analysis of PCR product

The amplification products were separated by electrophoresis in a 3% agarose gel stained with ethidium bromide (Et-Br) solution. After loading 8 μL dye solution, the gels were run for 40 min at 100 V in TAE electrophoration buffer and visualized by UV transilluminator. Appropriate DNA ladder (Bioneer Co., Daejeon, Korea) was used as molecular size marker.

RESULTS AND DISCUSSION

Primer design by 16S rRNA sequence

Known gene sequences on the NCBI data of LAB were collected, most of which were 16S rRNA sequences. In the genus *Lactobacillus*, 16S rRNA sequence data came from 47 species. We designed a total of 69 primer sets of various sizes－15 mer, 20 mer, 25 mer, and 30 mer－using the homologue of 16S rRNA sequence of *Lactobacillus* species, which were 2 sets from *Lb. acidophilus*, 5 sets from *Lb. brevis*, 1 set from *Lb. delbrueckii*, 2 sets from *Lb. farciminis*, 3 sets from *Lb. fermentum*, 9 sets from *Lb. hilgardii*, 2 sets from *Lb. maltaromicus*, 21 sets from *Lb. plantarum*, 11 sets from *Lb. reuteri*, 13 sets from *Lb*, *sanfranciscensis* (Table 1).

PCR analysis of *Lactobacillus* species with designed primers

69 primers were designed from 16S rRNA sequence of 10 *Lactobacillus* species include; *Lb. acidophilus*, *Lb. brevis*, *Lb. delbrueckii*, *Lb. farciminis*, *Lb. fermentum*, *Lb. hilgardii*, *Lb. plantarum*, *Lb. maltaromicus*, *Lb. reuteri*, *Lb. sanfranciscensis*. The primers designed for specific detection of *Lactobacillus* species were validated by performing PCR with DNA isolated from 6 standard *Lactobacillus* strains, which are *Lb. brevis*, *Lb. farciminis*, *Lb. fermentum*, *Lb. sanfranciscensis*, *Lb. hilgardii*, *Lb. plantarum* and the results are indicated in Fig. 1 and Table 2.

Primer set No. 1 (5'-aagcctgcgaaggcaag-3' & 5'-aggccaccggctttg-3') was used for PCR amplification with 6 reference strains and the result indicated specificity to

Table 1. Designed primer sets for PCR detection of *Lactobacillus* species

Species	Primer No.	Primer sequence
Lactobacillus acidophilus	1	5'-aagcctgcgaaggcaag-3' & 5'-aggccaccggctttg-3'
	2	5'-tgcaactcgcctgcac-3' & 5'-aggccaccggctttg-3'
Lactobacillus brevis	3	5'-tggcggcatgcctaa-3' & 5'-gcctgcgctcgcttt-3'
	4	5'-tggcggcatgcctaa-3' & 5'-tccccaggcggagtg-3'
	5	5'-gcttcggctatcacttctgg-3' & 5'-taagccgaaggctttcacat-3'
	6	5'-aacacttggaaacaggtgctaatac-3' & 5'-aaggctttcacatcagacttaaaaa-3'
	7	5'-ttgcactgatttcaacaatgaag-3' & 5'-aaggctttcacatcagacttaaaaa-3'
Lactobacillus delbrueckii	8	5'-gctagcggcggatgg-3' & 5'-gcggcgttgctcca-3'
Lactobacillus farciminis	9	5'-gcgtattagctagttggtgaggtaa-3' & 5'-cttcaaacttaataaaccgcctaca-3'
	10	5'-ctaataccgcataacaactactttcacat-3' & 5'-aacttaataaaccgcctacattctctttac-3'
Lactobacillus fermentum	11	5'-ggcggacgggtgagt-3' & 5'-cgcggtgttgctcca-3'
	12	5'-cgtaggtaacctgcccagaa-3' & 5'-tatgcatcatcgccttggta-3'
	13	5'-acaacatttggaaacagatgctaat-3' & 5'-cgtcaacgtatgaacagttactctc-3'
Lactobacillus hilgardii	14	5'-cgccgcgtgagtgaa-3' & 5'-tccccaggcggagtg-3'
	15	5'-cgccgcgtgagtgaa-3' & 5'-gtggggtccccgtca-3'
	16	5'-gacccgcggcgtatt-3' & 5'-tccccaggcggagtg-3'
	17	5'-gacccgcggcgtatt-3' & 5'-gtggggtccccgtca-3'
	18	5'-gcggcgggtgctaat-3' & 5'-tccccaggcggagtg-3'
	19	5'-tggcttcggctaccactaat-3' & 5'-cggaaaccctccaacactta-3'
	20	5'-tggcttcggctaccactaat-3' & 5'-cctttgagtttcagccttgc-3'
	21	5'-gcgtattagctagttggtgaggtaa-3' & 5'-ggactaccagggtatctaatcctgt-3'
	22	5'-agcttgctctttaaccaagtgagt-3' & 5'-ggactaccagggtatctaatcctgt-3'
Lactobacillus maltaromicus	23	5'-ggcggacgggtgagt-3' & 5'-tccccaggcggagtg-3'
	24	5'-gcgtattagctagttggtgaggtaa-3' & 5'-gcagttactctcatccttgttcttc-3'
Lactobacillus plantarum	25	5'-gagcgcaggcggttt-3' & 5'-tccccaggcggaatg-3'
	26	5'-cgccgcgtgagtgaa-3' & 5'-tccccaggcggaatg-3'
	27	5'-gtcccgcggcgtatt-3' & 5'-tccccaggcggaatg-3'
	28	5'-gagcgcaggcggttt-3' & 5'-tgacgggcggtgtgt-3'
	29	5'-ggcggcgtgcctaat-3' & 5'-tccccaggcggaatg-3'
	30	5'-gtcccgcggcgtatt-3' & 5'-tgacgggcggtgtgt-3'
	31	5'-ggcggcgtgcctaat-3' & 5'-tgacgggcggtgtgt-3'
	32	5'-agcgttgtccggatttattg-3' & 5'-taaggttcttcgcgttgctt-3'
	33	5'-agccgacctgagagggtaat-3' & 5'-taaggttcttcgcgttgctt-3'
	34	5'-agcgttgtccggatttattg-3' & 5'-ttcatgtaggcgagttgcag-3'
	35	5'-agcgttgtccggatttattg-3' & 5'-tcatttgtcccaccttaggc-3'
	36	5'-agccgacctgagagggtaat-3' & 5'-ttcatgtaggcgagttgcag-3'
	37	5'-agccgacctgagagggtaat-3' & 5'-tcatttgtcccaccttaggc-3'
	38	5'-taggcggtttattaagtttgaagtg-3' & 5'-ggactaccagggtatctaatcctgt-3'
	39	5'-gcgtattagctagttggtgaggtaa-3' & 5'-cttcaaacttaataaaccgcctaca-3'
	40	5'-gcgtattagctagttggtgaggtaa-3' & 5'-ggactaccagggtatctaatcctgt-3'
	41	5'-taggcggtttattaagtttgaagtg-3' & 5'-actgagatcggttttaagtgatttg-3'
	42	5'-acatactatgcaaatctaagagattagacg-3' & 5'-aacttaatgctggcaactgataataagg-3'
	43	5'-acatactatgcaaatctaagagattagacg-3' & 5'-actgagaatggctttaagagattagcttac-3'
	44	5'-actctgttgttaaagaagaacatatctgag-3' & 5'-actgagaatggctttaagagattagcttac-3'
	45	5'-actctgttgttaaagaagaacatatctgag-3' & 5'-gttaccttgttacgacttcaccctaatc-3'
Lactobacillus reuteri	46	5'-ggcggacgggtgagt-3' & 5'-tccccaggcggagtg-3'
	47	5'-cggagcgggggataa-3' & 5'-gtgacgggcggtgtg-3'
	48	5'-ggctatcactctgggatgga-3' & 5'-tcagttgcagaccagacagc-3'
	49	5'-tgatgcatagccgagttgag-3' & 5'-cgagtttgcgactcgttgta-3'
	50	5'-ggctatcactctgggatgga-3' & 5'-cgagtttgcgactcgttgta-3'
	51	5'-tgcactgattgacgatggat-3' & 5'-cgagtttgcgactcgttgta-3'
	52	5'-gtaaagctctgttgttggagaagaa-3' & 5'-ggactaccagggtatctaatcctgt-3'
	53	5'-gcattagctagttggtaaggtaacg-3' & 5'-ggactaccagggtatctaatcctgt-3'
	54	5'-gtaaagctctgttgttggagaagaa-3' & 5'-gagaacggctttaagagattagctt-3'
	55	5'-gcattagctagttggtaaggtaacg-3' & 5'-gagaacggctttaagagattagctt-3'
	56	5'-acgggtgagtaacacgtaggtaac-3' & 5'-gagaacggctttaagagattagctt-3'

Table 1. Continued

Species	Primer No.	Primer sequence
Lactobacillus sanfranciscensis	57	5'-gagcgcaggcggttt-3' & 5'-tccccaggcggaatg-3'
	58	5'-cgccgcgtgagtgaa-3' & 5'-tccccaggcggaatg-3'
	59	5'-gacccgcggcgtatt-3' & 5'-tccccaggcggaatg-3'
	60	5'-gagcgcaggcggttt-3' & 5'-tgacgggcggtgtgt-3'
	61	5'-ggcggcgtgcctaat-3' & 5'-tccccaggcggaatg-3'
	62	5'-gacccgcggcgtatt-3' & 5'-tgacgggcggtgtgt-3'
	63	5'-ggcggcgtgcctaat-3' & 5'-tgacgggcggtgtgt-3'
	64	5'-gcatgggtagcaaacaggat-3' & 5'-cagactgcaatccgaactga-3'
	65	5'-ggcctttgtgctagtgcttc-3' & 5'-cggaaaccttccaacaccta-3'
	66	5'-gcccagaagaaggggataac-3' & 5'-cggaaaccttccaacaccta-3'
	67	5'-ggcctttgtgctagtgcttc-3' & 5'-caccttcctccggtttatca-3'
	68	5'-ggcctttgtgctagtgcttc-3' & 5'-cagactgcaatccgaactga-3'
	69	5'-gcccagaagaaggggataac-3' & 5'-cagactgcaatccgaactga-3'

Table 2. Reactions of designed primers for *Lactobacillus* species amplification by PCR

Origin 16S rRNA strain of specific primer	Primer No.	Test strain					
		Lb. brevis	*Lb. farciminis*	*Lb. fermentum*	*Lb. sanfranciscensis*	*Lb. hilgardii*	*Lb. plantarum*
Lb. acidophilus	1	−	−	−	−	+	−
	2	+	+	−	+	+	+
Lb. brevis	3	+	−	+	+	+	+
	4	+	+	+	+	+	+
	5	−	−	+	+	+	−
	6	+	+	+	+	+	+
	7	−	+	+	+	+	+
Lb. delbrueckii	8	+	+	+	−	+	+
Lb. farciminis	9	+	+	−	+	+	+
	10	−	+	−	−	−	−
Lb. fermentum	11	+	+	+	+	+	+
	12	−	−	+	+	+	+
	13	−	+	+	−	+	−
Lb. hilgardii	14	+	+	+	+	+	+
	15	+	+	+	+	+	+
	16	+	+	+	+	+	+
	17	+	+	+	+	+	+
	18	+	+	+	−	+	−
	19	−	+	+	−	+	−
	20	−	+	−	−	+	−
	21	+	+	+	+	+	+
	22	+	−	+	+	+	+
Lb. maltaromicus	23	+	+	+	+	+	+
	24	+	+	+	+	+	+
Lb. plantarum	25	+	−	+	+	+	+
	26	+	+	+	+	+	+
	27	+	+	+	+	+	+
	28	+	+	+	+	+	+
	29	+	+	+	+	+	+
	30	+	+	+	+	+	+
	31	+	+	+	+	+	+
	32	+	+	+	+	+	+
	33	+	+	+	+	+	+
	34	+	+	+	+	+	+
	35	+	+	+	+	+	+
	36	+	+	+	+	+	+
	37	+	+	+	+	+	+

Table 2. Continued

Origin 16S rRNA strain of specific primer	Primer No.	Test strain					
		Lb. brevis	*Lb. farciminis*	*Lb. fermentum*	*Lb. sanfranciscensis*	*Lb. hilgardii*	*Lb. plantarum*
Lb. plantarum	38	+	+	+	+	+	+
	39	−	+	−	+	+	+
	40	+	+	+	+	+	+
	41	−	+	+	+	+	−
	42	−	−	−	−	−	−
	43	−	−	−	−	+	−
	44	−	−	+	−	+	−
	45	−	+	+	+	+	+
Lb. reuteri	46	+	+	+	+	+	+
	47	+	+	+	+	+	+
	48	+	+	+	+	+	+
	49	+	+	+	+	+	+
	50	+	+	+	+	+	−
	51	+	−	+	+	+	−
	52	+	+	+	+	+	+
	53	+	+	+	+	+	+
	54	−	+	+	+	+	+
	55	+	+	+	+	+	+
	56	+	+	+	+	+	+
Lb. sanfranciscensis	57	+	−	+	−	+	+
	58	+	+	+	+	+	+
	59	+	+	+	+	+	+
	60	+	+	+	+	+	+
	61	+	+	+	+	+	+
	62	+	+	+	+	+	+
	63	+	+	+	+	+	+
	64	+	+	+	+	+	+
	65	+	+	+	+	−	+
	66	+	+	+	+	+	+
	67	−	+	+	−	−	−
	68	−	+	+	+	−	+
	69	+	−	+	+	+	+

Lb. hilgardii which express a band, but other strains did not. Primer No. 2 amplified all reference strains but *Lb. fermentum*, thus did not show species specificity. Designed based on 16S rRNA of *Lb. farciminis*, primer set No. 10 (5'-ctaataccgcataacaactactttcacat-3' & 5'-aacttaataaaccgcctacattctctttac-3') could only amplify *Lb. farciminis*, so it is expected to be useful for species level identification of *Lb. farciminis*. No. 43 primer set sequence of 5'-acatactatgcaaatctaagagattagacg-3' & 5'-actgagaatggctttaagagattagcttac-3', showed specific band on gel electrophoresis analysis with *Lb. hilgardii* and so could likely be used for species detection.

Lb. farciminis grows under 45°C, especially in the presence of high percentage of NaCl (10% to 12% optimally), and is generally isolated from meat products like raw sausages and also from sour dough (10). On average, kimchi contains 2～3% of NaCl which is preferable growth condition for *Lb. farciminis* as halophile. Ait-Belgnaoui et al. reported that *Lb. farciminis* treatment prevents stress-induced hypersensitivity, increases colonic paracellular permeability, and increases colonocyte MLC phosphorylation (20). *Lb. kimchii*, a single isolate derived from the kimchi, was found by taxonomic study using phenotypic characterization and phylogenetic and genetic methods to be a new species. Plus, it showed closest phylogenetic relatives with *Lb. alimentarius* (KCTC 3593) and *Lb. farciminis* (LMG 9200), with levels of 16S rDNA similarity of 98.4% and 98.2%, respectively (21).

Lb. hilgardii, is mostly isolated from wine and its optimal initial pH for growth and carbohydrate fermentation reactions is in the range of 4.5～5.5. This species highly related to the type strain of *Lb. brevis* and *Lb. reuteri*, especially *Lb. brevis*, one of the kimchi lactic acid bacteria (10). Lately, GABA, which is produced by decarboxylation of glutamic acid, has received interest from many researchers. GABA influences the development of neural progenitor cells via brain-derived neurotrophic factor expression (22) by regulating the proliferation of neural progenitor cells, the growth of embryonic and neural stem cells (23,24). Shinji (25) developed Pharma GABA made from glutamic acid fermen-

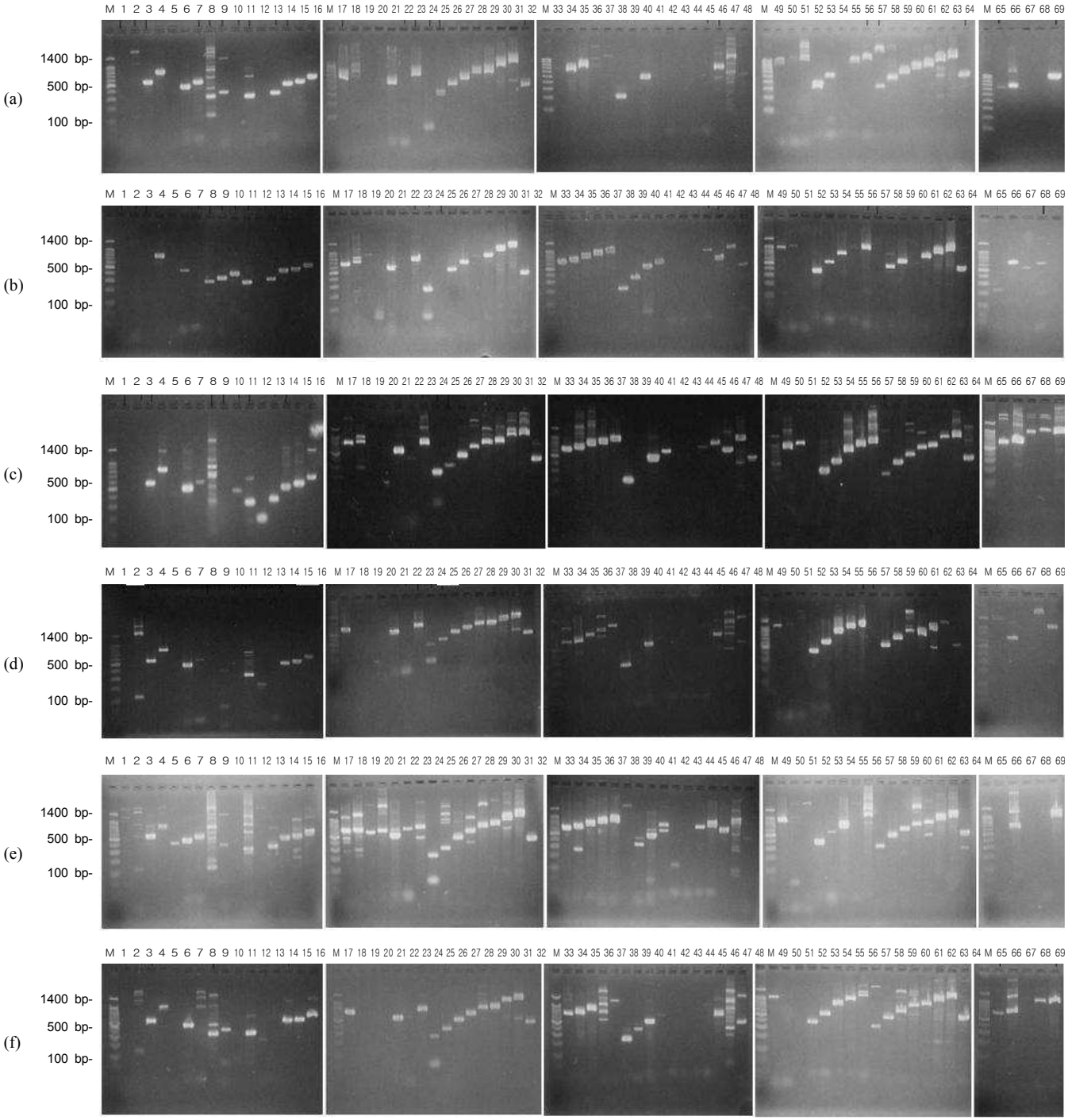

Fig. 1. Pattern of PCR products on general gel of reference strains (a) *Lb. brevis*, (b) *Lb. farciminis*, (c) *Lb. fermentum*, (d) *Lb. sanfranciscensis*, (e) *Lb. hilgardii*, (f) *Lb. plantarum*. Each lane means 69 designed primer sets based on 16S rRNA sequence homology of kimchi *Lactobacillus*. M: Ladder marker (100 bp), Lane 1～2 (*Lb. acidophilus*), 3～7 (*Lb. brevis*), 8 (*Lb. delbrueckii*), 9～10 (*Lb. farciminis*), 11～13 (*Lb. fermentum*), 14～22 (*Lb. hilgardii*), 23～24 (*Lb. maltaromicus*), 25～45 (*Lb. plantarum*), 46～56 (*Lb. reuteri*), 57～69 (*Lb. sanfranciscensis*).

tation by *Lb. hilgardii* which is isolated from kimchi.

Even though *Lb. hilgardii* and *Lb. farciminis* are not the dominant lactic acid bacteria, studies support the idea that these species normally take part in kimchi fermentation and, furthermore, that they have the possibility of acting as probiotics.

As explained above, primer set No. 1, 10, and 43 produced a specific PCR band with *Lb. hilgardii*, *Lb. farciminis*, *Lb. hilgardii*, respectively. But other than those 3 primer sets, many various PCR bands were observed over the *Lactobacillus* reference strains, which could be caused by the strong relationship among the species and

the similarity of the 16S rRNA gene sequence, so that primer attached to and amplified specific portion of all strains. These primers seem to be difficult to use as species-specific primers for *Lactobacillus*. However, the fact that the 16S rRNA primer sequence formed bands on all species would have potential application in development of a genus-specific primer set. While primer set No. 42 formed no amplification bands on any of the species, primer sets No. 20, 44, and 67 amplified 2 species, *Lb. farciminis* and *Lb. hilgardii*, *Lb. fermentum* and *Lb. hilgardii*, *Lb. farciminis* and *Lb. fermentum*, respectively. These primers needed to be analyzed under different PCR conditions, including denaturation temperature, annealing temperature, and reaction time, to determine the optimum condition for each strain however, this study performed PCR amplification under consistent conditions, which may not have been optimum for all species.

Most studies of microbial identification in kimchi fermentation are carried out using biochemical and morphological analysis methods. These traditional methods have several limitations, such as the use of a selective medium to identify specific strains, and difficulties in identifying differences between colonies, due to the biological similarities of LAB. To solve these problems, molecular biology techniques are being widely used for rapid classification and identification.

According to recent studies, reevaluation of the *Leuconostoc* and *Lactobacillus* strains by PCR during kimchi fermentation (26), and specific detection (27), analysis of lactic acid bacteria by denaturing gradient gel electrophoresis (28), such as the introduction of molecular biological methods have been tried. However, because of the continuous new discovery of bacteria involved in kimchi fermentation, there is a limitation of bacteria identification by existing primers. Thus, new varieties of primer sets are required for the analysis of lactic acid bacteria in the wide range of environment.

Consequently, this study developed PCR primer sets for species-specific identification based on 16S rRNA sequence of *Lactobacillus* species. This identification can be used as a foundation for rapid experimental detection of the microbial community in the ripening kimchi. The primers we developed in this study can be useful for species identification, but a more advanced primer set will be required for a commercialized PCR assay kit. The advancement of analytical technology for lactic acid bacteria identification provides an easier way to separate strains and develop them as probiotic functional foods and food additives. Furthermore, biochemical and physiological studies on characteristics of lactic acid bacteria are also important for the study of its probiotic applications.

CONCLUSION

In this study, one of the important kimchi lactic acid bacteria, *Lactobacillus* species' 16S rRNA sequence was investigated. Based on the results of this investigation, 69 species-specific primer sets were developed. To evaluate the accuracy of the designed primer sets, PCR was performed. As a result, primer sets No. 1, No. 10, and No. 43 produced specific amplification band for *Lb. hilgardii*, *Lb. farciminis*, *Lb. hilgardii*, respectively. Each of these primers could be available for the identification of the specific species of *Lactobacillus*.

REFERENCES

1. Bottazzi V. 1988. An introduction to rod-shaped lactic-acid bacteria. *Biochimie* 70: 303-315.
2. Stiles ME, Holzapfel WH. 1997. Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol* 36: 1-29.
3. Axelsson L. 1998. Classification and physiology. In *Lactic Acid Bacteria: Microbiology and Functional Aspects*. Salminen S, Wright AV, eds. 2nd ed. Marcel Dekker, New York, USA. p 1-72.
4. Caplice E, Fitzgerald GF. 1999. Food fermentations: role of microorganisms in food production and preservation. *Int J Food Microbiol* 50: 131-149.
5. Leroy F, De Vuyst L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol* 15: 67-78.
6. Drider D, Fimland G, Hechard Y, Mcmullen LM, Prevost H. 2006. The continuing story of class IIa bacteriocins. *Microbiol Mol Biol Rev* 70: 564-582.
7. Ballongue J. 1998. Bifidobacteria and probiotic action. In *Lactic Acid Bacteria: Microbiology and Functional Aspects*. Salminen S, Wright AV, eds. 2nd ed. Marcel Dekker, New York, USA. p 519-587.
8. Shah N. 2000. Probiotic bacteria: Selective enumeration and survival in dairy foods. *J Dairy Sci* 83: 1-14.
9. Shah NP, Wu C. 1999. Aflatoxin B1 binding abilities of probiotic bacteria. *Biosci Microflora* 18: 43-48.
10. Kandler O, Weiss N. 1986. Genus *Lactobacillus* Beijerinck 1901. 212A-L. In *Bergey's Manual of Systematic Bacteriology*. Sneath PHA, Mair NC, Sharpe ME, Holt JG, eds. 2nd ed. Williams and Wilkins Co., Baltimore, USA. p 1209-1234.
11. Mheen TI, Kwon TW. 1984. Effect of temperature and salt concentration on kimchi fermentation. *Korean J Food Sci 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. *Kor J Microbiol Biotechnol* 20: 102-109.
13. Cheigh HS, Park KY. 1994. Biochemical, microbiological and nutritional aspect of kimchi. *Food Sci Nutr* 34: 175-203.
14. Han HU, Lim CR, Park HK. 1990. Determination of mi-

crobial community as an indicator of kimchi fermentation. *Korean J Food Sci Technol* 22: 26-32.
15. Cho JS. 1991. Changes of microflora in commercial kimchi. *Korean J Dietary Culture* 6: 479-501
16. Lee CJ, Park HW, Kim KY. 1998. *The book of Kimchi*. Korean Overseas Culture and Information Service, Seoul, Korea. p 93-95.
17. Lee MK, Jang DJ, Kim YJ, Kim DS, Kim SH, Yang HJ. 2009. Development of Kimchi microbes resources and local type for kimchi globalization. In *Studies for globalization of top 5 Korean traditional foods*. 1st ed. Annual Research Paper of Korean Food Research Institute. p 63-102.
18. So MH, Kim YB. 1995. Identification of psychrotrophic lactic acid bacteriaisolated from kimchi. *Korean J Food Sci Technol* 27: 495-505.
19. Jo JS, You HY, Kim YM. 1993. Recent trends and new developments of traditional fermented food industry. *Bioindustry News* 6: 11-15.
20. Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, Theodorou V. 2006. *Lactobacillus farciminis* treatment suppresses stress induced visceral hypersensitivity: a possible action through interaction with epithelial cell cytoskeleton contraction. *Gut* 55: 1090-1094.
21. Yoon JH, Kang SS, Mheen TI, Ahn JS, Lee HJ, Kim TK, Park CS, Kho YH, Kang KH, Park YH. 2002. *Lactobacillus kimchii* sp. nov., a new species from kimchi. *Int J Sys Evol Microbiol* 50: 1789-1795.
22. Obrietan K, Gao XB, van den Pol AN. 2002. Excitatory actions of GABA increase BDNF expression via a MAPK-CREB-dependent mechanism－a positive feedback circuit in developing neurons. *J Neurophysiol* 88: 1005-1015.
23. LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. 1995. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 15: 1287-1298.
24. Haydar TF, Wang F, Schwartz ML, Rakic P. 2000. Differential modulation of proliferation in the neocortical ventricular and subventricular zones. *J Neurosci* 20: 5764-5774.
25. Shinji H. 2002. Novel physiological functions of GABA and its application (Okinawa Prefecture. S., Japan Science and Technology Corporation. S). *Dai1kai Chiiki kara Hasshin suru Kagaku Gijutsu Shinpojiumu Yokoshu. Heisei 14 Nendo. Sangakukan no Renkei to Kenko Baio Bijinesu no Soshutsu* S: 37-42
26. Choi JY, Kim M, Lee JH. 2002. Reevaluation of the change of *Leuconostoc* species and *Lactobacillus plantarum* by PCR during Kimchi fermentation. *J Microbiol Biotechnol* 12: 166-171.
27. Kim TW, Min SG, Choi DH, Jo JS, Kim HY. 2000. Rapid identification of *Lactobacillus plantarum* in Kimchi using polymerase chain reaction. *J Microbiol Biotechnol* 10: 881-884.
28. Park JA, Heo GY, Lee JS, Oh YJ, Kim BY, Min TI, Kim CK, Ahn JS. 2003. Change of microbial communities in Kimchi fermentation at low temperature. *Kor J Microbiol* 39: 45-50.

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