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Identification of Lactic Acid Bacteria in Kimchi Using SDS-PAGE Profiles of Whole Cell Proteins

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Abstract This study was conducted to evaluate the practical usefulness of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) fingerprinting of whole cell proteins for the identification of lactic acid bacteria in Kimchi. SDS-PAGE of whole cell proteins of the reference strains and lactic acid bacteria isolated from Kimchi yielded differential banding patterns that were highly specific fingerprints, thus making it possible to identify. Identification of the isolates from Kimchi was achieved by comparing the SDS-PAGE fingerprints of isolates to those of reference strains. In addition, the reliability of SDS-PAGE was examined by comparing the results with those of the API 50 CHL system assay and 16S rRNA gene sequence. SDS-PAGE assay showed a different identity to reference strains, while the API 50 CHL system and 16S rRNA gene sequence could not distinguish a few strains. Therefore, SDS-PAGE of the whole cell proteins is a specific and a reliable method that will be useful for the identification of lactic acid bacteria in Kimchi to the species level, and can be used as an alternative or complementary identification method.

Key words: Kimchi, lactic acid bacteria, identification, SDS-PAGE

The fermentation of Kimchi has been carried out naturally and it was found that Kimchi contained high levels of lactic acid bacteria when optimally ripened, resulting in an increase in acidity and formation of its unique flavor. Since these lactic acid bacteria greatly influence the quality of Kimchi, it is important to understand the ecosystem of the community in Kimchi. Many lactic acid bacteria have been isolated from Kimchi and identified as *Leuconostoc mesenteroides* subsp. *mesenteroides* [8, 10, 11, 14, 15, 17], *Leuc. mesenteroides* subsp. *dextranicum* [10, 17], *Leuc. paramesenteroides* [10, 15, 17], *Leuc. lactis* [17], *Leuc. gelidum* [5], *Leuc. citreum* [5], *Lactobacillus plantarum* [8, 10, 11, 14, 15], *Lb. brevis* [8, 11, 14, 15], *Lb. sake* [6, 8, 14], *Lb. fermentum* [14], *Lb. fructosus* [10], *Lb. maltaromicus* [10], and *Pediococcus pentosaceus* [8, 14, 15]. The identification of lactic acid bacteria isolated from Kimchi depends mainly on physiological and biochemical criteria.

Although physiological reactions can generally be used to determine the species of the lactic acid bacteria in Kimchi, inconsistencies in the test results make identification very difficult [17]. Therefore, a simple, rapid, and accurate identification method is one of the major concerns of the lactic acid bacteria research regarding Kimchi. A microbial cell expresses many different proteins that form a rich source of information for characterization, classification, and identification. Electrophoregrams of these protein are highly reproducible, it strains were cultivated under specific conditions, and standardized techniques were used. The comparison of whole cell protein patterns obtained by highly standardized SDS-PAGE allows fast screening of large numbers of strains and has been proved to be extremely reliable at the species level. Furthermore, the protein profiles can be stored in a database format and are routinely used for identification of unknown isolates [13]. For identification purpose, although SDS-PAGE requires an extensive database covering all known target species, it has an advantage of being fairly simple and convenient in performance. This technique has been used for taxonomic studies of lactic acid bacteria that were obtained from various environments. Recently, a technique has been described to used the unique patterns of cell proteins in identifying lactic acid bacteria, which made it possible to solve specific identification problems [1, 2, 4, 12].

However, the usefulness of this method for the identification of lactic acid bacteria isolated from Kimchi has rarely been assessed. In this study, the usefulness and reliability of SDS-PAGE of whole cell proteins as an alternative

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or complementary identification method were evaluated to identify lactic acid bacteria that were isolated from Kimchi.

MATERIALS AND METHODS

Isolation of Bacterial Strains

The reference strains used in this study were obtained from the Korean Collection for Type Cultures (Daejon, Korea), Korean Culture Center of Microorganisms (Seoul, Korea), American Type Culture Collection (Rockville, U.S.A.) and Korea Food Research Institute (Sungnam, Korea). They were cultured in a MRS (deMan, Rogosa, and Sharpe) broth (Difco, Detroit, U.S.A.) at 30°C. The reference strains are listed in Table 1. Lactic acid bacteria to be identify were isolated from Kimchi. The juice of Kimchi was diluted to 10^5–10^6 with distilled water, spread onto the surface of a MRS agar plate containing 0.2% $CaCO_3$ and 0.01% bromocresol purple. The juice was then incubated for 2–3 days at 30°C to allow for the colonies to develop. These selected colonies were recultured in a liquid MRS broth for 18 h at 30°C and respread on the MRS agar plate for purification and identification.

SDS-PAGE of Whole Cell Proteins

Strains were incubated overnight at 30°C in 5 ml of MRS broth and centrifuged at 12,000 ×g for 3 min at 4°C. The pellet of the sample was washed twice with deionized water and suspended in 50 µl of 50 mM Tris-HCl buffer (pH 8.0). Fifty mg of glass beads (diameter, 425 to 600 microns; Sigma, St. Louis, U.S.A.) was added to the tubes, and the bacteria were vortexed for 5 min. The pellet of the

Table 1. List of reference strains used.

Species	Strain	Lane No.[a]
Lactobacillus plantarum	KCTC 3104	1
Lactobacillus plantarum	KCCM 11322	2
Lactobacillus plantarum	KFRI 813	3
Lactobacillus casei	KCTC 3109	4
Lactobacillus casei	KFRI 709	5
Leuconostoc argentinum	KCCM 40710	6
Leuconostoc argentinum	ATCC 51355	7
Leuconostoc citreum	ATCC 13146	8
Leuconostoc citreum	ATCC 49370	9
Leuconostoc citreum	KCTC 3524	10
Lactobacillus sake	KCTC 3598	11
Lactobacillus fructosus	KCTC 3544	12
Lactobacillus amylophilus	KCTC 3160	13
Lactobacillus coryniformis	KCTC 3167	14
Lactobacillus hilgardii	KCTC 3500	15
Lactobacillus farciminis	KCTC 3681	16
Leuconostoc mesenteroides subsp. *mesenteroides*	KCTC 3505	17
Leuconostoc mesenteroides subsp. *mesenteroides*	KCTC 3100	18
Leuconostoc mesenteroides subsp. *dextranicum*	KCTC 3530	19
Leuconostoc mesenteroides subsp. *dextranicum*	KCCM 35046	20
Pediococcus pentosaceus	KCTC 3507	21
Lactobacillus lactis	KCCM 32406	22
Leuconostoc lactis	KCTC 3528	23
Leuconostoc fallax	KCTC 3537	24
Leuconostoc carnosum	KCTC 3525	25
Weissella kimchi	KCTC 3746	26
Lactobacillus brevis	KCCM 11509	27
Lactobacillus brevis	KFRI 805	28
Lactobacillus maltaromicus	KCTC 3602	29
Lactobacillus fermentum	KCTC 3112	30
Lactobacillus reuteri	KCTC 3677	31
Weissella minor	KCTC 3604	32
Weissella confusa	KCTC 3499	33
Weissella viridescens	KCTC 3504	34
Leuconostoc paramesenteroides	KCTC 3531	35
Leuconostoc gelidum	KCTC 3527	36

[a]Numbers correspond to the lane numbers in Fig. 2.

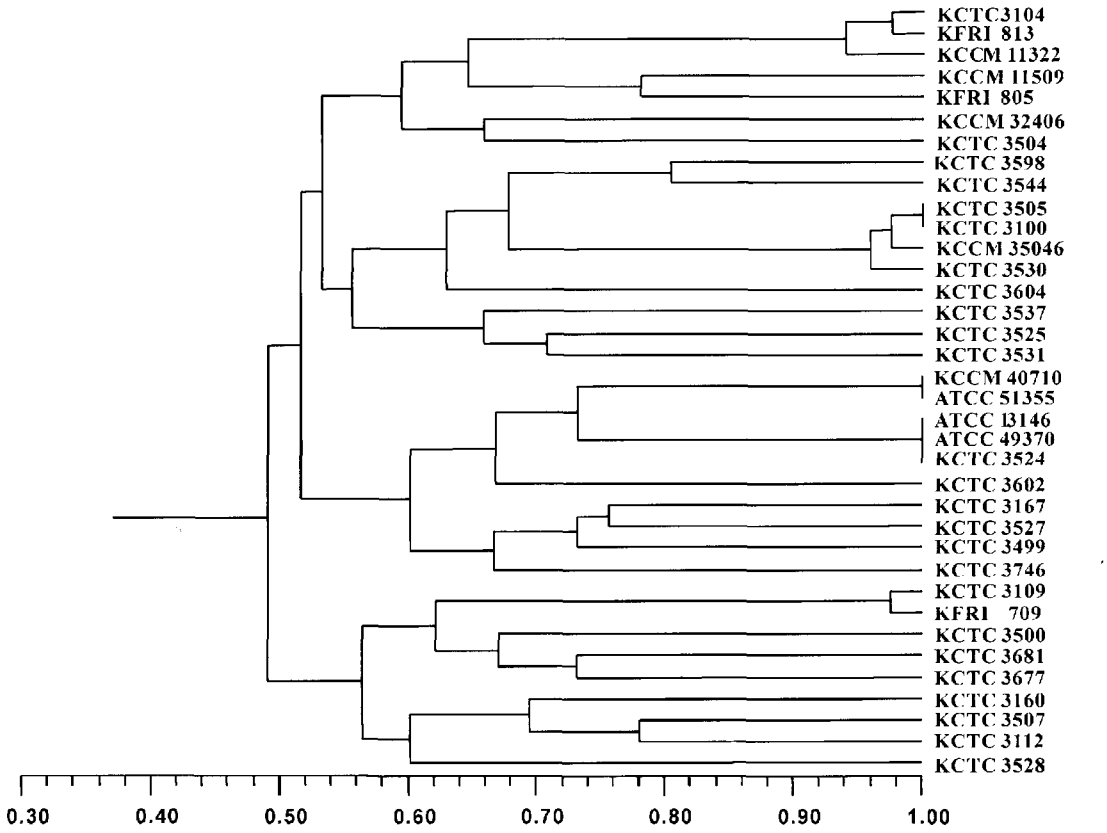

Fig. 1. Dendrogram of similarity based on SDS-PAGE of whole cell protein pattern analysis.
Genetic similarity was analyzed by UPGMA clustering. The right side of the figure is a strain number.

sample was resuspended by adding an equal volume of sample buffer [2× SDS sample buffer; 25 ml of 4× Tris-HCl/SDS (pH 6.8), 20 ml of glycerol, 4 g of sodium dodecyl sulfate, 2 ml of 2-mercaptoethanol, 1 mg of bromophenol blue, and H_2O added to 100 ml]. For protein denaturation, samples were heated for 5 min at 95°C. The cell debris was removed by centrifuge, and the supernatants were collected for analysis by gradient (8–16%) SDS-PAGE. SDS-PAGE was preformed on vertical slab gels. After electrophoresis, the gel was stained for 2 h with 0.05% Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, U.S.A.), and destained with 10% acetic acid and 30% methanol solution for 2 h. The destained gels were scanned for further analysis. Grouping of the patterns for whole cell proteins among reference strains was performed with the program NTSYS-pc (Numerical taxonomy system by using multivariate statistical programs, version 2.02j) and UPGMA (unweighted pair group method using average linkage) cluster analysis [16].

Identification of Strains by API 50 CHL System

Identification of strains by carbohydrates utilization was determined by using the API (Montalieu Inc., Vercieu, France) 50 CH strips and API CHL medium. Before testing, the strains were subcultured twice overnight in MRS broth at 30°C. The tests were carried out according to

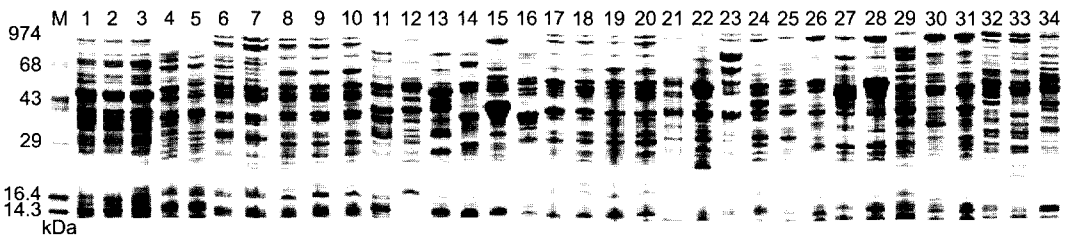

Fig. 2. SDS-PAGE profiles of whole cell proteins of reference strains tested.
Lanes: M, Protein molecular weight markers (kDa). Numbers correspond to the lane numbers indicated in Table 1.

Table 2. Identification of reference strains and the isolates from Kimchi according to API 50 CHL and SDS-PAGE of whole cell proteins results.

Species	Identification results by	
	API 50 CHL	SDS-PAGE
Leuc. mesenteroides KCTC 3100	*Leuc. mesenteroides*	*Leuc. mesenteroides*
Leuc. citreum ATCC 13146	*Lb. brevis*	*Leuc. citreum*
Leuc. argentinum ATCC 51355	*Leuc. mesenteroides*	*Leuc. argentinum*
Leuc. paramesenteroides KCTC 3531	*Leuc. mesenteroides*	*Leuc. paramesenteroides*
Leuc. lactis KCTC 3528	*Leuc. lactis*	*Leuc. lactis*
Leuc. gelidum KCTC 3527	*Leuc. mesenteroides*	*Leuc. gelidum*
Lb. sake KCTC 3598	*Lc. lactis* subsp. *lactis*	*Lb. sake*
Lb. plantarum KCTC 3104	*Lb. plantarum*	*Lb. plantarum*
Lb. fermentum KCTC 3112	*Ped. pentosaceus*	*Lb. fermentum*
Lb. casei KCTC 3109	*Lb. paracasei*	*Lb. casei*
Lb. brevis KCCM 11509	*Lb. brevis*	*Lb. brevis*
Ped. pentosaceus KCTC 3507	*Ped. pentosaceus*	*Ped. pentosaceus*
KHU-316	*Leuc. mesenteroides*	*Leuc. mesenteroides*
KHU-418	*Lb. brevis*	*Leuc. citreum*
KHU-82	*Lc. lactis* subsp. *lactis*	*Lb. sake*
KHU-22	*Lb. plantarum*	*Lb. plantarum*

the manufacturer's instructions and the results were read after the strains were incubated at 30°C for 2 days. The API LAB program was used to analyze the results.

RESULTS AND DISCUSSION

The reproducibility of the SDS-PAGE technique was investigated by electrophoretic runs of duplicate protein extracts of strains, which reached an average value of about 96±1%. The dendrogram obtained after numerical comparison of the protein patterns of reference strains is shown in Fig. 1, and the whole cell protein patterns are displayed in Fig. 2. Numerical analysis of the SDS-PAGE protein patterns clearly discriminated all species that were investigated. SDS-PAGE profiles of whole cell proteins were highly reproducible and showed similar patterns among the strains of the same species. Identification of the isolates by SDS-PAGE was performed with the comparison of the protein fingerprints that were derived from reference strains with almost known species of lactic acid bacteria in Kimchi (Table 2).

The API 50 CHL system is a widely employed method for identification of lactic acid bacteria isolated from Kimchi [3, 9, 15]. Twelve reference strains (*Leuc. mesenteroides* KCTC 3100, *Leuc. citreum* ATCC 13146, *Leuc. argentinum* ATCC 51355, *Leuc. paramesenteroides* KCTC 3531, *Leuc. lactis* KCTC 3528, *Leuc. gelidum* KCTC 3527, *Lb. sake* KCTC 3598, *Lb. plantarum* KCTC 3104, *Lb. fermentum* KCTC 3112, *Lb. casei* KCTC 3109, *Lb. brevis* KCCM 11509, and *Ped. pentosaceus* KCTC 3507) in addition to 4 isolates from Kimchi were tested by applying the API 50 CHL system. Their results were compared with those of SDS-PAGE of whole cell proteins patterns. The comparison of the phenotypic analysis by using API 50 CHL system and SDS-PAGE assay showed different results for identifying reference strains and the isolates from Kimchi (Table 2). Compared with the result of the SDS-PAGE assay, the API 50 CHL system assay was found to misidentify a few strains tested. SDS-PAGE of whole cell proteins of the reference strains and lactic acid bacteria isolated from Kimchi yielded differential banding

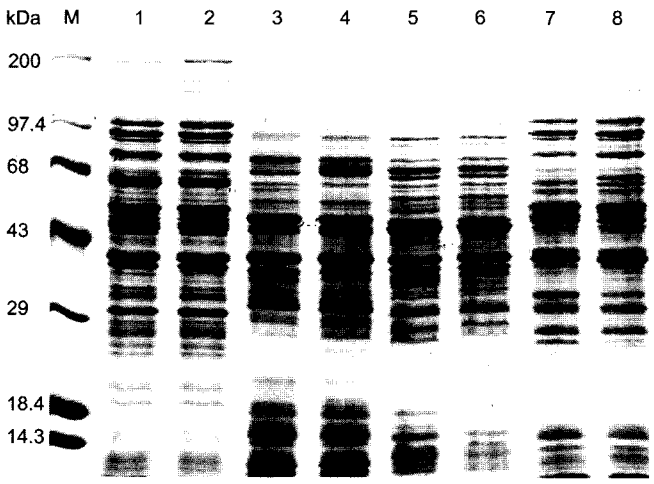

Fig. 3. SDS-PAGE profiles of whole cell proteins of reference strains and the isolates from Kimchi.
Lanes: M, Protein molecular weight markers (kDa). 1: *Leuc. citreum* ATCC 13146; 2: Isolate KHU-418; 3: *Lb. sake* KCTC 3598; 4: Isolate KHU-82; 5: *Lb. plantarum* KCTC 3104; 6: Isolate KHU-22; 7: *Leuc. mesenteroides* KCTC 3100; 8: Isolate KHU-316.

Table 3. The similarity of the 16S rRNA gene sequence among *Leuconostoc* spp. strains.

	1	2	3	4	5	6
1		99.2	93.9	99.6	97.4	83.7
2	99.2		94.2	98.9	97.4	84.0
3	93.9	94.2		94.1	94.2	81.0
4	99.6	98.9	94.1		97.6	83.9
5	97.4	97.4	94.2	97.6		85.1
6	83.7	84.0	81.0	83.9	85.1	

The number indicated as follows : 1, *Leuc. argentinum*; 2, *Leuc. citreum*; 3, *Leuc. gelidum*; 4, *Leuc. lactis*; 5, *Leuc. mesenteroides*; 6, *Leuc. paramesenteroides*.

patterns, which were specific for lactic acid bacteria isolated from Kimchi. Previously, KHU-82 was identified as *Lb. sake* by 16S rDNA sequencing [6] and the protein patterns of SDS-PAGE of whole cell proteins offered an accurate and specific result (Fig. 3). Although physiological reactions can generally be used to determine the species of the bacteria, inconsistencies in the test results can make the identification quite difficult. *Leuconostoc* spp. share several physiological characteristics, which sometimes makes it difficult to identify these organisms at the species level [2, 18]. Some *Leuconostoc* spp. strains tested in this study were misidentified by API 50 CHL system assay. In addition, the similarity of the 16S rRNA gene sequence was high among *Leuconostoc* spp. strains, therefore making PCR amplification of this region difficult to distinguish these strains (Table 3). However, the SDS-PAGE patterns of whole cell proteins of these *Leuconostoc* spp. strains showed significant differences, which were able to discriminate various *Leuconostoc* spp. strains (Fig. 4). Thus, the comparison of the soluble whole cell protein patterns of the unidentified bacterium and the patterns from the standard strains can be

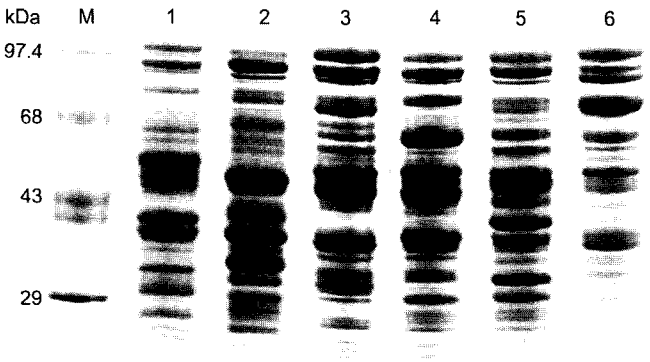

Fig. 4. SDS-PAGE profiles of whole cell proteins of *Leuconostoc* spp. strains.
Lanes: M, Protein molecular weight markers (kDa). 1: *Leuc. mesenteroides* KCTC 3100; 2: *Leuc. argentinum* ATCC 51355; 3: *Leuc. gelidum* KCTC 3527; 4: *Leuc. citreum* ATCC 13146; 5: *Leuc. lactics* KCTC 3528; 6: *Leuc. paramesenteroides* KCTC 3531.

used to clarify ambiguous physiological results. Tsakalidou *et al.* [19] previously reported that SDS-PAGE of whole cell proteins is beneficial in quickly identifying large numbers of strains to the species or subspecies level without performing any preidentification experiments. In fact, several authors actually applied this method to lactic acid bacteria to solve specific identification problems [7, 12].

In summary, SDS-PAGE of whole cell proteins is a reliable and specific method, and it can be used for identifying lactic acid bacteria in Kimchi to the species level as an alternative or complementary identification method.

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References

1. Descheemaeker, P., B. Pot, A. M. Ledeboer, T. Verrips, and K. Kersters. 1994. Comparison of the *Lactococcus lactis* differential medium (DCL) and SDS-PAGE of whole cell proteins for the identification of *Lactococci* to subspecies level. *System. Appl. Microbiol.* **17:** 459–466.
2. Elliott, J. A. and R. R. Facklam. 1993. Identification of *Leuconostoc* spp. by analysis of soluble whole-cell protein patterns. *J. Clin. Microbiol.* **31:** 1030–1033.
3. Hahn, Y. S. and J. Y. Oh. 1999. Dextransucrase activity of *Leuconostoc* sp. strains isolated from Kimchi. *Kor. J. Appl. Microbiol. Biotechnol.* **27:** 86–89.
4. Hebert, E. M., R. R. Raya, and G. S. de Giori. 2000. Use of SDS-PAGE of cell-wall proteins for rapid differentiation of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus helveticus*. *Biotech. Lett.* **22:** 1003–1006.
5. Kim, B. J., H. J. Lee, S. Y. Park, J. H. Kim, and H. U. Han. 2000. Identification and characterization of *Leuconostoc gelidum*, isolated from kimchi, a fermented cabbage product. *J. Microbiol.* **38:** 132–136.
6. Kim, T. W., J. Y. Lee, S. H. Jung, Y. M. Kim, J. S. Jo, D. K. Chung, H. J. Lee, and H. Y. Kim. 2002. Identification and distribution of predominant lactic acid bacteria in Kimchi, a Korean traditional fermented food. *J. Microbiol. Biotechnol.* **12:** 635–642.
7. Kunene, N. F., I. Geornaras, A. Holy, and J. W. Hastings. 2000. Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented weaning food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. *Appl. Environ. Microbiol.* **66:** 1084–1092.
8. Lee, C. W., C. Y. Ko, and D. M. Ha. 1992. Microfloral changes of the lactic acid bacteria during Kimchi fermentation and identification of the isolates. *Kor. J. Appl. Microbiol. Biotechnol.* **20:** 102–109.

9. Lee, K. S., Y. S. Shin, and C. H. Lee. 1998. Acid tolerance of *Lactobacillus brevis* isolated from Kimchi. *Kor. J. Food Sci. Technol.* **30:** 1399–1403.
10. Lim, C. R., H. K. Park, and H. U. Han. 1989. Reevaluation of isolation and identification of gram-positive bacteria in Kimchi. *Kor. J. Microbiol.* **27:** 404–414.
11. Mheen, T. I. and T. W. Kwon. 1984. Effect of temperature and salt concentration on Kimchi fermentation. *Kor. J. Food Sci. Technol.* **16:** 443–449.
12. Pot, B., C. Hertel, W. Ludwig, P. Descheemaeker, K. Kersters, and K. H. Schleifer. 1993. Identification and classification of *Lactobacillus acidophilus*, *L. gasseri* and *L. Johnsonii* strains by SDS-PAGE and rRNA-targeted oligonucleotide probe hybridization. *J. Gen. Microbiol.* **139:** 513–517.
13. Pot, B., P. Vandamme, and K. Kersters. 1994. Analysis of electrophoretic whole-organisms protein fingerprints, pp. 493–521. *In*: *Chemical Methods in Bacterial Systematics*, John Wiley and Sons, Chichester.
14. Shim, S. T., K. H. Kyung, and Y. J. Yang. 1990. Lactic acid bacteria isolated from fermenting Kimchi and their fermentation of Chinese cabbage juice. *Kor. J. Food Sci. Technol.* **22:** 373–379.
15. Shin, D. H., M. S. Kim, J. S. Han, D. K. Lim, and W. S. Bak. 1996. Changes of chemical composition and microflora in commercial Kimchi. *Kor. J. Food Sci. Technol.* **28:** 137–145.
16. Smith, M. L. and J. B. Anderson. 1989. Restriction fragment length polymorphism in mitochondrial DNAs of *Armillaria*. *Mycolog. Res.* **93:** 247–256.
17. So, M. H. and Y. B. Kim. 1995. Identification of psychrotrophic lactic acid bacteria isolated from Kimchi. *Kor. J. Food Sci. Technol.* **27:** 495–505.
18. Stiles, M. E. and W. H. Holzapfel. 1997. Lactic acid bacteria of food and their current taxonomy. *Int. J. Food Microbiol.* **36:** 1–29.
19. Tsakalidou, E., E. Manolopoulou, E. Kabaraki, E. Zoidou, B. Pot, K. Kersters, and G. Kalantzopoulos. 1994. The combined use of whole cell protein extracts for the identification (SDS-PAGE) and enzyme activity screening of lactic acid bacteria isolated from traditional Greek dairy products. *System. Appl. Microbiol.* **17:** 444–458.