Quantitative Bio-Science 31(2), 151~159(2012)

# Isolation and Molecular Characterization of Lactic Acid Bacteria from Kimchi

Oh-Sik Kwon*, Seung-A Hwang Bo

Department of Microbiology, Keimyung University, Daegu 704-701, Korea
(Received October 15, 2012; Revised November 2, 2012; Accepted November 7, 2012)

## ABSTRACT

Five laboratory isolates from three kinds of kimchi (JA1-1, JA2-3 from Baechu kimchi, JB1-2, JB2-3 from Yeolmu kimchi and JC2-3 from Chonggak kimchi) were identified by a method of carbohydrate fermentation and a random amplified polymorphic DNA (RAPD)-PCR as a molecular method. In hexose tests all isolates could not ferment rhamnose at all but it was noticed that JB1-2 fermented all hexoses much better than other isolated lactic acid bacteria (LAB). With disaccharides, the isolated LAB were grouped into two different species. The JA2-3 and JB1-2 showed similar pattern in fermenting disaccharides but they also showed difference in melibiose like mannose. However, JB2-3 and JC2-3 were identical in fermenting all disaccharides. All isolated LAB revealed a dramatical pattern of fermenting trisaccharides. They couldn't ferment melezitose at all but fermented raffinose very well. From the results of carbohydrate derivatives, the five LAB were grouped into three kinds. This fact was clearly proved by the results of RAPD-PCR that revealed their genetic relationships at a molecular level. By application of primer LAB-01, the DNA band patterns of JA2-3 and JB1-2 were almost same although they were isolated from different sources of kimchi. The different kinds implied that they were three subspecies of *Leuc. mesenteroides*. This result was exactly as same as the results of the fermentation tests. Typical DNA bands of the five LAB were repeatedly produced by primer LAB-02. It again produced distinctive polymorphic DNA bands although the primer sequence was totally different from primer LAB-01. The LAB-02 also revealed indistinguishable DNA band patterns between JA2-3 and JB1-2. It implied that they were different only at a level of subspecies of *Leuc. mesenteroides*. This tendency was repeated in JB2-3 and JC2-3 indicating that five LAB have very similar genetic constitutions with the standard strains of *Leuc. mesenteroides*. Hence, RAPD-PCR appears to be a very efficient method to characterize the genetic relationships of the isolated *Leuconostoc* strains at the subspecies level.

**Key words :** Fermentation, Kimchi, LAB, *Leuconostoc*, RAPD-PCR

## Introduction

Lactic acid bacteria (LAB) are Gram positive bacteria showing a DNA G+C content of less than 50 mol% [1,2] and organisms of interest in the food industries because of their typical roles in inhibiting the growth of food spoilage bacteria [3]. Among the organisms, the genus *Leuconostoc* contains many commercially useful species for food industries [4,5]. Typically it consists of species of *plantarum, dextranicum, citreum* and *mesenteroides* isolated from kimchi that was determined by their physiological and biochemical characteristics. Since identification of the genus *Leuconostoc* remains uncertain and it consists of genetically heterogeneous strains which are not easy to identify by physiological and biochemical tests [6], characterization of the *Leuconostoc* genus is a difficult task. Thus, many LAB researchers recently oriented to determine its phylogenetic relationship at molecular levels including DNA-DNA hybridization with mol% G+C [7], RFLP [8] and mostly 16S rRNA sequence analysis [3,9,10]. From their results, it was con-

* Correspondence should be addressed to Dr. Oh-Sik Kwon, Department of Microbiology, College of Natural Science, Keimyung University, Daegu 704-701, Korea. Tel: +82-53-580-5539. Fax: +82-53-585-6949, E-mail: biv@kmu.ac.kr

cluded that the classical taxonomy for LAB was not close to its molecular classification.

However the molecular methods for identification of the genus *Leuconostoc*, RAPD-PCR is not a well understood method in the direct use of classifying the *Leuconostoc* species although it is known to be a PCR based DNA finger-printing method [11-13]. The method is now successfully used in classifying and determining genetic relationships of many organisms such as fungi [14], viruses [15] and some lactic acid bacteria species [6,16,17].

In this study, we attempted to isolate *Leuconostoc* species of LAB from various sources of kimchi, for examples, Baechu kimchi, Yeolmu kimchi and Chonggak kimchi known as Korean fermented food. For specific characterization of the isolated LAB, we applied two methods such as an essential biochemical tests and RAPD-PCR as a molecular method. And we expect that this kind of effort will give a better resolution for many LAB researchers to identify unknown species of LAB to be found in future.

## Materials and Methods

### 1. Bacterial strains

The standard strains of lactic acid bacteria (LAB) used in this study are listed in Table 1. Among them, *Leuconostoc mesenteroides* 2A9 and *Leuconostoc mesenteroides* 1B12 were isolated from our laboratory previously [18,19]. *Leuconostoc mesenteroides* subsp. *dextranicum* KCTC 3530 and *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722 were obtained from the Korean Collection for Type Cultures (KCTC), Genetic Resources Center, Daegeon, Korea. The bacterial strains were cultured in a lactobacilli MRS (Difco, USA) and stored in the storage solution containing 20% glycerol (Sigma) in a −80°C deep freezer until reactivation.

**Table 1.** Lactic acid bacterial strains used in this study

| Species & Strains | Sources |
|---|---|
| *Leuconostoc mesenteroides* 2A9 | Baechu kimchi |
| *Leuconostoc mesenteroides* 1B12 | Chonggak kimchi |
| *Leuconostoc mesenteroides* subsp. *dextranicum* KCTC 3530 | Type strain |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722 | Silage |
| Laboratory isolate LAB JA1-1 | Baechu kimchi |
| Laboratory isolate LAB JA2-3 | Baechu kimchi |
| Laboratory isolate LAB JB1-2 | Yeolmu kimchi |
| Laboratory isolate LAB JB2-3 | Yeolmu kimchi |
| Laboratory isolate LAB JC2-3 | Chonggak kimchi |

### 2. Isolation of LAB strains

Five lactic acid bacterial strains were isolated from various kimchi and tentatively named as JA1-1, JA2-3 (Baechu kimchi), JB1-2, JB2-3 (Yeolmu kimchi) and JC2-3 (Chonggak kimchi). For isolating new LAB strains, the kimchi was washed by 100 mL of sterile water in an Erlenmeyer flask and 10 mL of the kimchi solution was mixed with same volume of 6% saline solution to make 3% saline samples for final. After serial dilution ($10^{-3}$-$10^{-6}$), the solution was spreaded onto MRS plates containing 2% Bactoagar (Difco) for detecting numerable colonies. After successive pure culture, colonies were cultured in a refrigerated incubator (Sanyo, MIR-153) at 25°C for 3 days. In order to determine growth kinetics, isolated strains were subjected to incubate in the same incubator mentioned above and collected after 72 hr cultivation. The culture media were measured by a pH meter (Mettler, Model 225) after 20 minutes centrifugation at 3,000 rpm, 4°C.

### 3. Determining carbohydrate fermentation characteristics

In order to investigate fermentation characteristics of the isolates, MRS media without sugars was prepared. Each 22 kinds of 10% carbohydrate stock solution was mixed to the media at a ratio of 1 : 10. 20 μL of isolated bacterial solution was inoculated into the 5 mL of carbohydrate containing media in a 15 mL plastic tube (Corning, USA), After 48 hr incubation in the refrigerated incubator at 25°C, the acidity of the cultured media were measured by a pH meter (Mettler, Model 225) after 20 minutes centrifugation at 3,000 rpm, 4°C.

### 4. Random primers

Random primers were purchased from the Bioneer (Daegeon, Korea). Among them, oligonucleotide having GC contents of 50-80% were applicable for the purpose of *Leuconostoc* genomic DNA amplification. Nucleotide sequences of applied primers are AACGGGCAGC (LAB-01), CGCACCGCAC (LAB-02).

### 5. Enzymatic genomic DNA extraction

Sufficient quantities of genomic DNA of tested LAB strains was extracted by a method described by Kim et al. [20]. For improving recovery of the DNA, additional treatments were

applied. With modified methods, pure and intact genomic DNA was easily obtained for the purpose of RAPD-PCR after treatments of mutanolysin and RNase.

### 6. Random amplified polymorphic DNA (RAPD) PCR

The reaction conditions for the polymerase chain reaction (PCR) were performed with the following modifications for RAPD-PCR. Each 3 μL of purified genomic DNA was added to 17 μL of the PCR reaction mixture, which contained 10X *Taq* buffer, 40 mM dNTPs, oligonucleotide primers (200 pmol each), and 1 unit of *Taq* DNA polymerase (Applied Biosystems, USA). The 10X *Taq* buffer contained 500 mM Tris-HCl (pH 8.3), 25 mM $MgCl_2$ and 0.1% gelatin. In an automatic thermocycler (Applied Biosystems, Model GeneAmp 2700), the reaction tubes were subjected to the temperature cycles of Lac43 file. After 40 cycles of amplification, the samples were incubated for 7 minutes at 72°C.

### 7. Agarose gel electrophoresis and UV photography

Half of the PCR reaction (10 μL) was loaded onto a gel containing 2% SeaKem LE agarose (Cambrex) in Tris-borate buffer (8.9 mM Tris-HCl, 8.9 mM boric acid, pH 8.3) for the electrophoresis. For convenience, 0.5 μL of ethidium bromide solution (10 mg/mL) was directly added to each 10 mL agarose solution for DNA staining. The resulting gel was subjected to UV photography as described in Sambrook et al. [21] in order to verify the PCR results. Photos were taken with instant black & white film (FP-3000B, Fuji Film).

## Results and Discussion

### 1. Fermentation of pentoses and hexoses

From the results of pentose fermentation as shown in Fig. 1, all laboratory isolates were able to grow very well in media with L-arabinose as a sole source of sugar resulting in pH 4.6 ±0.1 but they were poorly grown with D-arabinose which gave final pH values of 5.5-5.8. This fermenting characteristic is unique in *Leuconostoc* species among LAB. JA2-3 (○) and JB1-2 (▼) showed same tendency in fermenting pentoses. Their abilities of fermenting pentose i. e., ribose and xylose were between pH 4.9-5.3. Interestingly, in case of xylose fermentation, JB2-3 (▽) and JC2-3 (■) gave better final pH values 4.9 and 5.1 respectively than JA1-1, JA2-3 and JB1-2. The difference indicates that JB2-3 (▽) and JB1-2 (▼) were not same strains although they were isolated from same source of kimchi (i. e., Yeolmu kimchi).

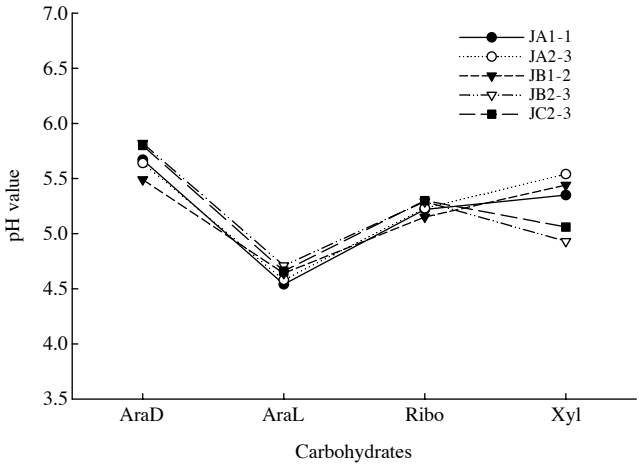


**Fig. 1.** Pentose fermentation characteristics of the isolated lactic acid bacteria from various kimchi and tentatively named as JA1-1, JA2-3 (Baechu kimchi), JB1-2, JB2-3 (Yeolmu kimchi) and JC2-3 (Chonggak kimchi). Abbreviations of the carbohydrates: AraD (D-arabinose), AraL (L-arabinose), Ribo (ribose), and Xyl (xylose).

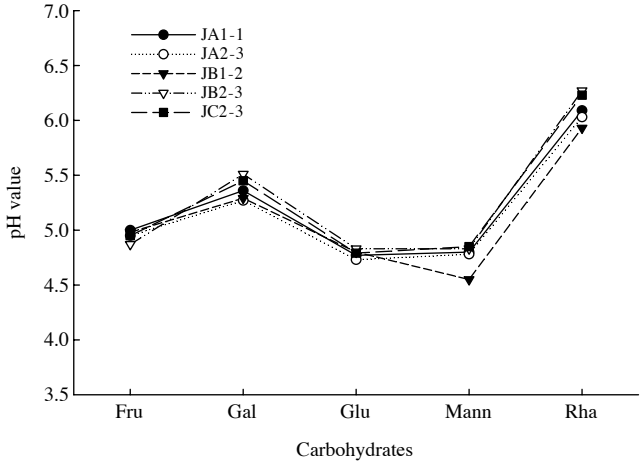


**Fig. 2.** Hexose fermentation characteristics of the isolated lactic acid bacteria from various kimchi and tentatively named as JA1-1, JA2-3 (Baechu kimchi), JB1-2, JB2-3 (Yeolmu kimchi) and JC2-3 (Chonggak kimchi). Abbreviations of the carbohydrates: Gal (galactose), Glu (glucose), Mann (mannose) and Rha (rhamnose).

This kind of difference was also found in hexose fermentation (Fig. 2). In case of mannose fermentation, JB1-2 (▼) from Yeolmu kimchi grew better than JB2-3 (▽) which final pH values were 4.5 and 4.8, respectively while they showed same fermentable characteristics with fructose, galactose and glucose. However, all the isolates could not ferment rhamnose at all that gave final pH values being more than 6.0. It was also noticed

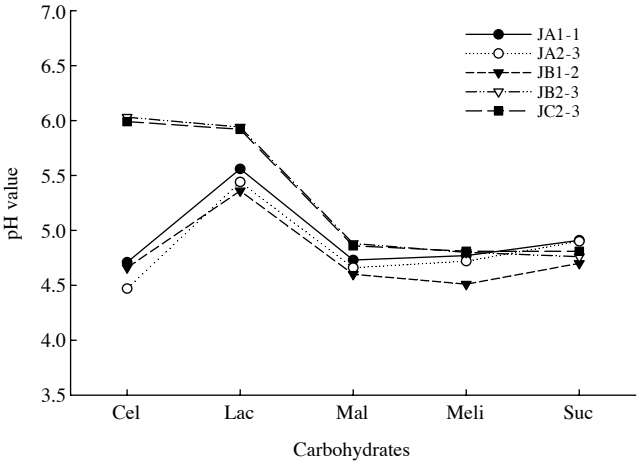


**Fig. 3.** Disaccharide fermentation characteristics of the isolated lactic acid bacteria from various kimchi and tentatively named as JA1-1, JA2-3 (Baechu kimchi), JB1-2, JB2-3 (Yeolmu kimchi) and JC2-3 (Chonggak kimchi). Abbreviation of the carbohydrates: Cel (cellobiose), Lac (lactose), Mal (maltose), Meli (melibiose) and Suc (sucrose).

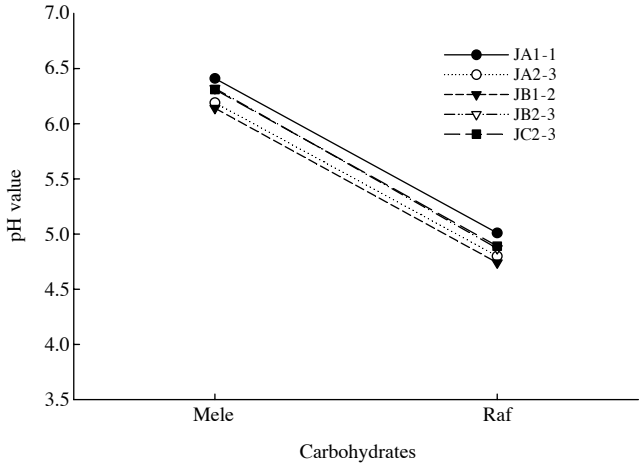


**Fig. 4.** Trisaccharide fermentation characteristics of the isolated lactic acid bacteria from various kimchi and tentatively named as JA1-1, JA2-3 (Baechu kimchi), JB1-2, JB2-3 (Yeolmu kimchi) and JC2-3 (Chonggak kimchi). Abbreviation fo the carbohydrates: Mele (melezitose) and Raf (raffinose).

that JB1-2 (▼) from Yeolmu kimchi fermented all hexoses much better than other isolated LAB.

## 2. Fermentation of disaccharides

Disaccharides are composed of two monosaccharides, for examples, sucrose (fructose and glucose), lactose (galactose and glucose) and maltose (two glucose molecules). In this study, five disaccharides were tested in order to identify fermentation ability of the isolated LAB (Fig. 3). Generally the isolated LAB fermented most disaccharides very well except lactose. This result implies that all the isolated LAB would be lactic acid bacteria originated from plants (i. e., Baechu, Yeolmu and Chonggakmu) rather than originated from animal. Especially JB2-3 (▽) and JC2-3 ( ■ ) could not ferment the lactose at all (final pH of the stains were both 5.9). The two strains showed that they also could not ferment cellobiose at all (final pH 6.0) although other stains such as JA1-1, JA2-3 and JB1-2 could ferment it well. Thus we can identify the isolated LAB being grouped at least into two different species. Although JA2-3 ( ○ ) and JB1-2 (▼) showed a similar pattern of carbohydrate fermentation, they showed some discrepancy in case of melibiose and sucrose as much as of mannose described in Fig. 2.

## 3. Fermentation of trisaccharides

Trisaccharides are also important fermentable sugars that used as critical indicators for characterization of LAB [19]. In this study melezitose (glucose+fructose+glucose) and raffinose (fructose+galactose+glucose) were tested as trisaccharides in order to enhance classifying the five strains of isolated LAB. As a result, all isolated LAB revealed a dramatical pattern of trisaccharide fermentation as showed in Fig. 4. All of them could not ferment melezitose at all but could ferment raffinose very well although both melezitose and raffinose were composed of same fermentable sugars such as glucose and fructose. This unique pattern of fermentation in trisaccharides was usually found in *Leuconostoc* species among LAB. From the results, we can conclude that all isolated LAB from different sources of kimchi will be grouped into *Leuconostoc* species. Again the strains of JA2-3 ( ○ ) and JB1-2 (▼) revealed closely related each other determined by production of acid after incubation and showed better growth (i. e., more acidic) than others.

## 4. Fermentation of carbohydrates derivatives

Another relationship between JA2-3 ( ○ ) and JB1-2 (▼) was found by a test of carbohydrate derivatives (Fig. 5). They showed difference only in fermenting mannitol that is an alcohol derivative of mannose (Fig. 5). In fermenting mannitol, JB1-2 fermented it better than JA2-3 (final pH 6.1 vs. 5.9, respectively). This indicates the two strains were not the same strains, that is, they may be classified into different subspecies within the same *Leuconostoc* species. It is also very valuable to notice that the JB2-3 (▽) and JC2-3 ( ■ ) showed same patterns of the carbohydrate derivatives. These similarities were also found in all other tested sugars described before. Furthermore the two

strains of LAB were totally different from others in an aspect of esculin fermentation. They could ferment esculin very well (pH 4.7) while others did not (pH 5.7). This discrepancy was very significant. It indicates that the JB2-3 (▽) and JC2-3 ( ■ ) can classify into different subspecies although they were grouped into species of *Leuconostoc mesenteroides*. Therefore, from the carbohydrate fermentation studies, we determine that the five isolated LAB were grouped into three kinds. The first group is composed of JA2-3 from Baechu kimchi and JB1-2. The second group is JB2-3 and JC2-3. Typically JA1-1 from Baechu kimch is the third group. Conclusively, same kind of LAB can obtained from different kimchi sources. Also the method of carbohydrate fermentation is very valuable in order to characterize unknown species of LAB isolated from kimchi.

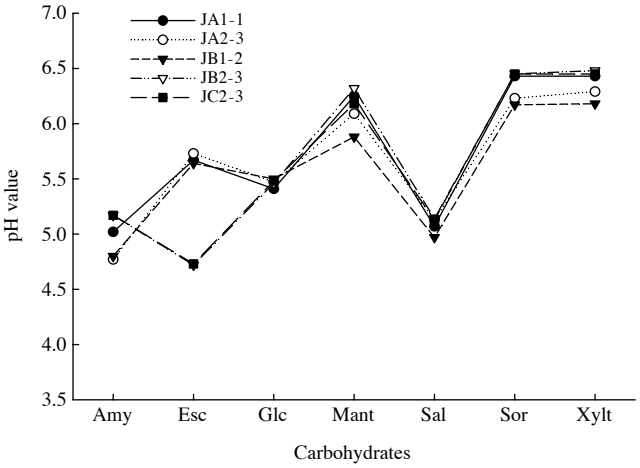


**Fig. 5.** Fermentation characteristics of carbohydrate derivatives in the isolated lactic acid bacteria from various kimchi and tentatively named as JA1-1, JA2-3 (Baechu kimchi), JB1-2, JB2-3 (Yeolmu kimchi) and JC2-3 (Chonggak kimchi). Abbreviations of the carbohydrates: Amy (amygdalin), Esc (esculin), Glc (gluconic acid), Mant (mannitol), Sal (salicin), Sor (sorbitol) and Xylt (xylitol).

## 5. Effect of enzymes for breaking LAB cell walls

Since lactic acid bacteria (LAB) are Gram positive bacteria, they have very thick cell walls composed of peptidoglycan comparing to Gram negative bacteria such as *E. coli*. The fact implies that obtaining chromosomal DNA from LAB is very difficult than Gram negative bacteria. In this study we tried to test enzymatic extraction method for the purpose of breaking cell wall of LAB. Initially we applied lysozyme (20 mg/mL) to the intact LAB and the result was shown in Fig. 6 (Left). As a result, it was found that bacterial cells of all strains were not effected by the lysozyme at all and the cells precipitated as intact condition. However, when mutanolysin (400 U/mL) was treated to cells, there was no precipitation of the bacterial cell, that is, the mutanolysin can break the cell wall of LAB very effectively (Fig. 6, Right). As a conclusion, cell wall of the LAB were degraded more susceptible by the mutanolysin treatment rather than lysozyme treatment.

## 6. Effect of freezing and thawing for cell wall breaking

In order to increase recovery of intact LAB DNA, we applied following methods and compared the results between two methods. First method was that mutanolysin was treated after a

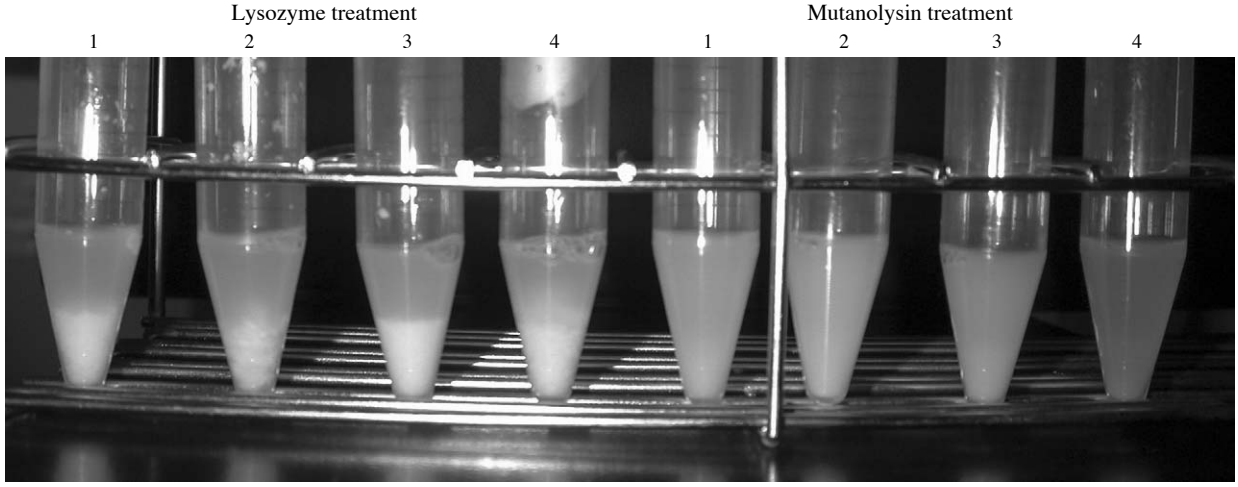


**Fig. 6.** Effect of cell wall breaking enzymes (lysozyme and mutanolysin) on tested LAB cells. 1, *Leuconostoc mesenteroides* 2A9; 2, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722; 3, JA1-1 (isolated strain); 4, JA2-3 (isolated strain). (Left) Lysozyme was treated by concentration of 20 mg/mL. (Right) Mutanolysin was treated by concentration of 400 U/mL.

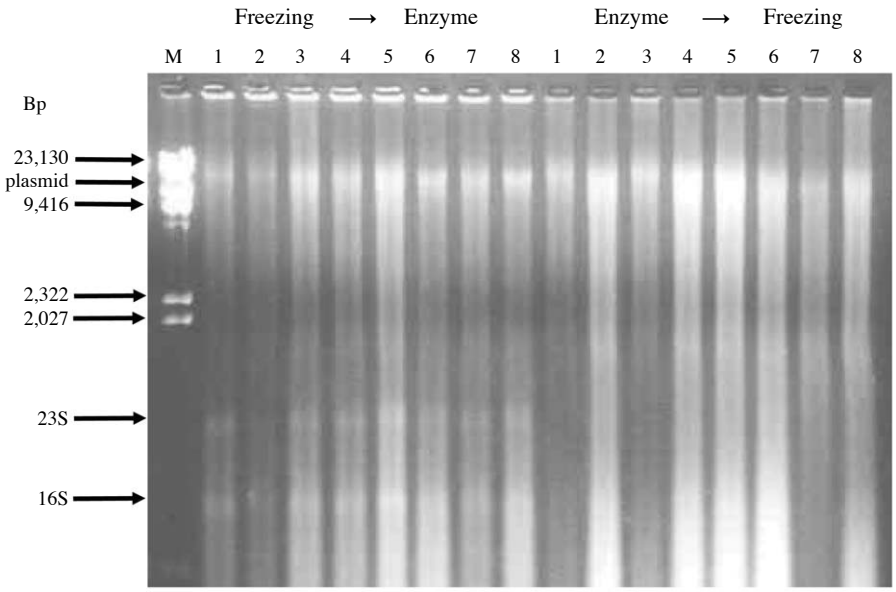


**Fig. 7.** Isolation of genomic DNA from tested LAB strains. (Left) Mutanolysin was treated after freezing-thawing. (Right) Mutanolysin was treated before freezing-thawing. Lane descriptions: M, marker DNA (Φλ *Hind*III digested, 1 μg); 1, *Leuconostoc mesenteroides* 2A9; 2, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722; 3, *Leuconostoc mesenteroides* 1B12; 4, JA1-1; 5, JA2-3; 6, JB1-2; 7, JB2-3; 8, JC2-3. Plasmids of the LAB were appeared between 23,130 bp and 9,416 bp. 23S rRNA (23S) and 16S rRNA (16S) were appeared distinctively in a Left method.

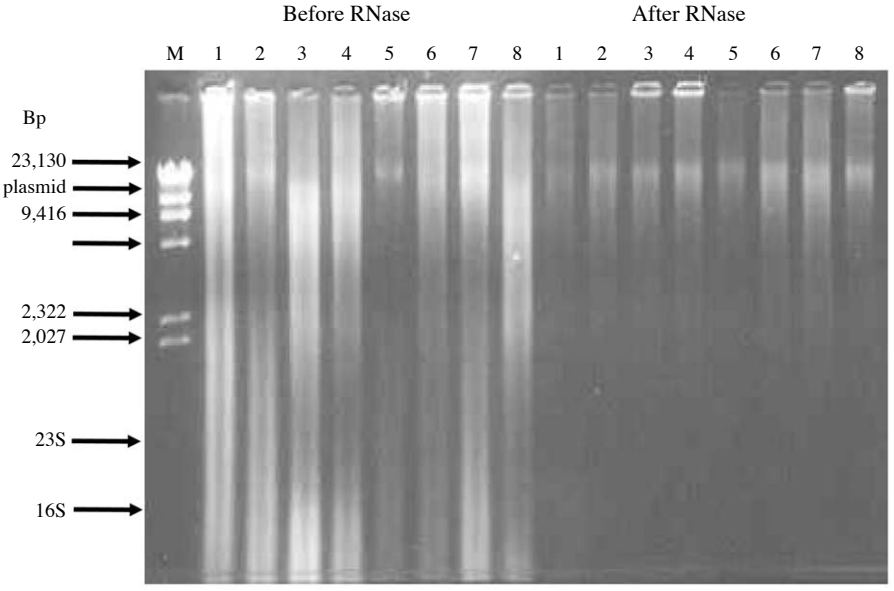


**Fig. 8.** Effects of mutanolysin and RNase treatments on the LAB genomic DNA. 200 U/mL of mutanolysin was treated (lane 1-4) and 400 U/mL of mutanolysin was treated (lane 5-8). Lane descriptions: M, marker DNA (Φλ *Hind*III digested, 1 μg); 1, *Leuconostoc mesenteroides* 2A9; 2, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722; 3, *Leuconostoc mesenteroides* 1B12; 4, JA1-1; 5, JA2-3; 6, JB1-2; 7, JB2-3; 8, JC2-3.

freezing-thawing method (Fig. 7, Left) and second method was that mutanolysin was treated before a freezing-thawing method (Fig. 7, Right). The first method gave less plasmid DNA (position between 23,130-9,416 bp) than the second method. However it is noticed that the first method revealed clearly 23S rRNA and 16S rRNA comparing to the second method. From the result, it was also determined that the size of LAB plasmid is between 23,130-9,416 bp and the LAB had high copy num-

ber of plasmid because the plasmid DNA revealed very thick DNA bands in variable DNA size.

### 7. Effect of RNase treatment for purifying LAB DNA

Since the DNA samples were heterogenous with celluar RNAs such as rRNA and mRNA after phenol/chloroform extraction, purification was done with treatment of RNase. The result showed in Fig. 8. The LAB DNA including plasmid was appeared very distinctively after RNase treatment. To verify effects of mutanolysin treatment, different concentration of mutanolysin (200 U/mL and 400 U/mL) was treated to cells before RNase treatment. Lanes 1-4 indicate treatment of 200 U/mL of mutanolysin and lanes 5-8 indicate treatment of 400 U/mL of mutanolysin. The figure showed clearly more chromosomal DNA and plasmid with the treatment of 400 U/mL (lanes 5-8). Thus it is concluded that treatment of higher concentration of mutanolysin gave more chromosomal DNA and plasmid.

### 8. Molecular characterization by RAPD-PCR

After purification of LAB DNA, RAPD-PCR was carried out with random primers LAB-01 and LAB-02 which nucleotide sequences were described in Materials and Methods. PCR with the primers gave fine results which showed many distinctive polymorphic DNA bands as showed in Fig. 9 and Fig. 10. More distinctive polymorphic DNA bands were produced by primers LAB-01 and less polymorphic DNA bands were produced by primers LAB-02. All LAB DNA samples revealed their characteristic band patterns. In general, as showed in Fig. 9 of primers LAB-01, the isolated strains (5, JA1-1; 6, JA2-3; 7, JB1-2; 8, JC2-3.) produced more polymorphic DNA bands than standard strains (1, *Leuconostoc* mesenteroides 2A9; 2, *Leuconostoc* mesenteroides 1B12; 3, *Leuconostoc mesenteroides* subsp. *dextranicum* KCTC 3530; 4, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722). Dramatically, the DNA band patterns of the isolated strains such as JA2-3 (lane

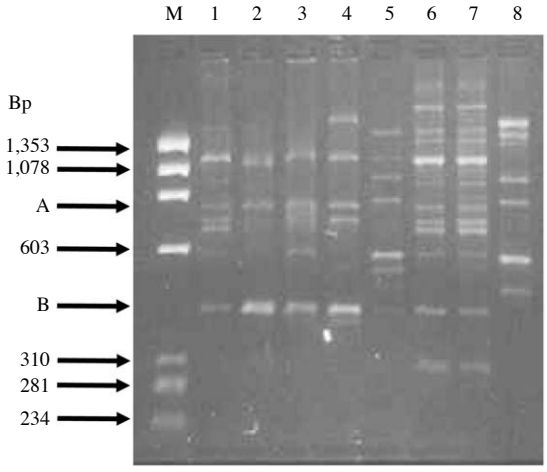


**Fig. 9.** RAPD-PCR results of tested LAB strains with primer LAB-01. It showed common RAPD markers (A, B) that can distinguish the LAB. Lane descriptions: M, marker DNA (ΦX174 *Hae*III digested, 1 μg); 1, *Leuconostoc mesenteroides* 2A9; 2, *Leuconostoc mesenteroides* 1B12; 3, *Leuconostoc mesenteroides* subsp. *dextranicum* KCTC 3530; 4, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722; 5, JA1-1; 6, JA2-3; 7, JB1-2; 8, JC2-3.

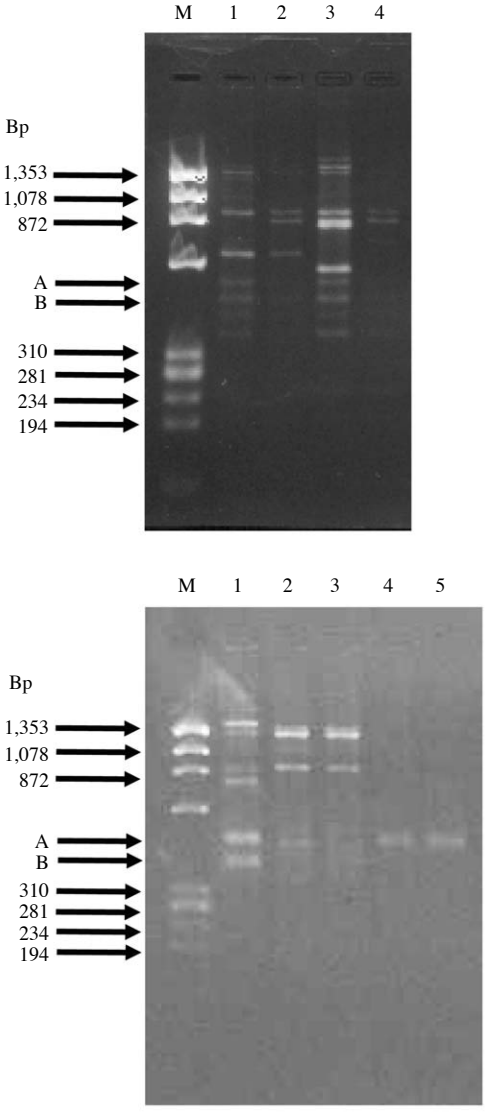


**Fig. 10.** RAPD-PCR results of tested LAB strains with primer LAB52. It showed common RAPD markers (A,B) that can distinguish the LAB. Lane descriptions: M, marker DNA (ΦX174 *Hae*III digested, 1 μg); (Top) 1, *Leuconostoc mesenteroides* 2A9; 2, *Leuconostoc mesenteroides* 1B12; 3, *Leuconostoc mesenteroides* subsp. *dextranicum* KCTC 3530; 4, *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3722. (Bottom) 1, JA1-1; 2, JA2-3; 3, JB1-2; 4, JB2-3; 5, JC2-3.

6) and JB1-2 (lane 7) were almost same although they were isolated from different sources of kimchi i. e., Baechu kimchi (JA2-3) and Yeolmu kimchi (JB1-2). This result was exactly as same as the results from the carbohydrates fermentation tests mentioned previously. Furthermore the band patterns appeared with the primer LAB-01 proved that the isolated strains were grouped into 3 different types of *Leuconostoc* species because lane 5 (JA1-1) was not the same as lane 6 & 7 (JA2-3 & JB1-2), and Lane 8 (JC2-3, from Chonggak kimchi). The RAPD-PCR results also revealed certain genetic relationship between isolated strains and standard strains that were indicating strains of *Leuconostoc mesenteroides*. Because all of them showed same common RAPD marker DNAs (A, B). Thus it is concluded that the five isolated strains can be classified as *Leuconostoc mesenteroides*.

Typical DNA band patterns of the five isolated LAB were repeatedly produced by RAPD-PCR with a random primer named as LAB-02 (Fig. 10). It also produced distinctive polymorphic DNA bands although the primer sequence was totally different from primers LAB-01. As showed in the Fig. 9 (Bottom) again the five isolated LAB divided into three distinctive types. That is, DNA band pattern of JA1-1 (lane 1) was not the same as others. Interesting enough JA2-3 (lane 2) and JB1-2 (lane 3) were very different from JB2-3 (lane 4) and JC2-3 (lane 5). Both of two sets showed exactly same polymorphic DNA band patterns. It revealed indistinguishable band patterns between JA2-3 (lane 2) and JB1-2 (lane 3). It implied that they were different only at the level of subspecies of *Leuconostoc mesenteroides* (see arrows A and B). This tendency was repeatedly presented in JB2-3 (lane 4) and JC2-3 (lane 5). The result indicates that all five LAB have very similar genetic constitutions comparing to the standard strains of *Leuconostoc mesenteroides* (Fig. 10. Top).

In conclusion, the five isolated strains of *Leuconostoc* as LAB from different sources of kimchi can be easily grouped into three types according to their genetical relationships generated by RAPD-PCR. And the results were fully supported from the results from carbohydrate fermentation characteristics (Fig. 1-5). Thus the choice of RAPD-PCR is very excellent as a molecular method for characterizing the isolated LAB strains, and it appeared to differentiate exactly LAB strains with ease at the level of subspecies.

## References

1. Pot B, Ludwig W, Kersters K, Schleifer KH. Taxonomy of lactic acid bacteria. In: De Vuyst L, Vandamme EJ, editors. Bacteriocins of lactic acid bacteria. Glasgow: Blackie & Professional; 1994. p. 13-90.
2. Vandamme P, Pot B, Gillis M, De Vos P, Kersters K, Swings J. Polyphasic taxonomy, a consensus approach to bacterial systematics. Micro. Reviews 1996;60:407-438.
3. Stackebrandt E, Teuber M. Molecular taxonomy and phylogenetic position of lactic acid bacteria. Biochimie 1988;70:317-324.
4. Baek YJ. Variety food using lactic acid bacteria. Micro. Industry 1993;19:46-48.
5. Gilmour A, Rowe MT. Microorganisms associated with milk. In: Robinson RK, editor. Dairy microbiology, Vol. 1. London and New York: Elsevier; 1990. p. 37-75.
6. Du Plessis EM, Dicks LMT. Evaluation of random amplified polymorphic DNA (RAPD)-PCR as a method to differentiate *Lactobacillus acidophilus, Lactobacillus crispatus, Lactobacillus amylovorus, Lactobacillus gallinarum, Lactobacillus gasseri*, and *Lactobacillus johnsonii*. Curr Microbiol 1995;31:114-118.
7. Dellaglio F, Torriani S. DNA:DNA homolgy, physiological characteristics and distribution of lactic acid bacteria isolated from maize silage. J Appl Bacteriol 1986;60:83-92.
8. Stahl M, Molin G, Persson A. Ahrne S, Stahl S. Restriction endonuclease patterns and multivariate analysis as a classification tool for *Lactobacillus* spp. Int J Syst Bacteriol 1990;40:189-193.
9. Chang YH, Kim JK, Kim HJ, Yoon JH, Kim WY, Choi YW, et al. Characteristics of *Lactobacillus reuterii* BSA-131 isolated from swine intestine. Kor J Appl Microbiol Biotechnol 1999;27: 23-27.
10. Collins MD, Rodrigues UM, Ash C, Aguirre M, Farrow JAE, Martinez-Murcia A, et al. Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. FEMS Microbiol Lett 1991;77:5-12.
11. Martin GB, Williams JGK, Tanksley SD. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 1991;88:2336-2340.
12. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 1990;18:7213-7219.
13. Williams JGK, Hanafey MK, Rafalski JA, Tingey SV. Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol 1993;218:704-740.
14. Min BR, Yang YJ, Choi YK. RAPD-PCR analysis in *Fusarium* species. Kor J Microbiol 1999;35:107-114.
15. Kwon OS, Lee JY. Fast genetic variation among coliphage quasispecies revealed by a random amplified polymorphic DNA (RAPD) analysis. J Microbiol 1996;34:166-171.
16. Torriani S, Zapparoli G, Dellaglio F. Use of PCR-based methods for rapid differentiation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. lactis. Appl Environ Microbiol 1999;65:4351-4356.

17. Son MJ, Jang EK, Kwon OS, Seo JH, Kim IJ, Lee IS, et al. Characterization of dextran produced from *Leuconostoc citreum* S5 strain isolated Korean fermented vegetable. Eur Food Res Technol 2008;226:697-706.
18. Kim SJ, Kwon OS. A study on the cell extract of *Leuconostoc mesenteroides* 1B12 inducing the death of carcinoma cell SNU-C4. Quantitative Bio-Science 2011;30(1):53-58.
19. Kwon OS, Lee SP. Isolation and characterization of high dextran producer *Leuconostoc mesenteroides* 2A9 from cabbage kimchi. Quantitative Bio-Science 2011;30(2):147-152.
20. Kim TW, Min SG, Choi DH, Jo JS, Kim HY. Rapid identification of *Lactobacillus plantarum* in kimchi using polymerase chain reaction. J Microbiol Biotechnol 2000;10:881-884.
21. Sambrook J, Fritsch EF, Manniatis T. Molecular cloning: A laboratory manual. 2nd ed. New York: CSHL Laboratory Press; 1989. p. 9.14-9.22.