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Use of RAPD-PCR as a method to follow the progress of starter cultures in sauerkraut fermentation☆

V. Plengvidhya, F. Breidt Jr.*, H.P. Fleming

U.S. Department of Agriculture, Agricultural Research Service, NC State University, Raleigh, NC 27695-7624, USA
North Carolina Agricultural Research Service, Department of Food Science, NC State University, Raleigh, NC 27695-7624, USA

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Abstract

DNA fingerprinting methods were used to follow the progress of unmarked starter cultures in laboratory sauerkraut fermentations (1.2 and 13 l). Random prime PCR (RAPD-PCR) was used for strain-specific identification of *Leuconostoc mesenteroides* cultures. A comparative analysis of RAPD banding patterns for fermentation isolates and starter cultures was carried out using both genetically marked and unmarked cultures. While some variation in the RAPD patterns was observed, the results showed that the starter cultures dominated the fermentation during early heterofermentative stage for up to 5 days after the start of fermentation. Results from marked and unmarked starter cultures were confirmed by intergenic transcribed spacer (ITS)-PCR, and strain identify was confirmed by pulse field gel electrophoresis (PFGE) patterns. The results demonstrate the utility of RAPD to follow the progression of unmarked starter cultures of *L. mesenteroides* in sauerkraut fermentations.

Keywords: RAPD; Starter cultures; Sauerkraut; Fermentation; Lactic acid bacteria

1. Introduction

Commercial sauerkraut fermentation generally relies on the natural lactic acid bacteria (LAB) present on the cabbage for initiating fermentation. Microbiological methods have shown that four species are typically present in sauerkraut fermentation: *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Lactobacillus brevis*, and *Lactobacillus plantarum*. *L. mesenteroides* is the major species involved in the early stage of the sauerkraut fermentation and *L. plantarum* becomes predominant in the latter stage, beginning about 5 to 7 days after the start of fermentation. The correct sequence of organisms is essential in achieving a stable product with flavor and aroma typical of sauerkraut (Pederson and Albury, 1969).

Commercial sauerkraut production in the United States is usually carried out in bulk tanks ranging in capacity from 45 to 150 tons (Fleming et al., 1988). These tanks are used for both fermentation and bulk

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* Corresponding author. Tel.: +1-919-515-2979; fax: +1-919-856-4361.
E-mail address: breidt@ncsu.edu (F. Breidt).

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storage of the fermented cabbage. This can offer economic advantages, but can result in unpredictable concentrations of fermentation end-products. An extended holding period can result in excess acid formation and make the final product too acidic for consumption without dilution. However, dilution of the brine to reduce acidity may result in loss of flavor and nutritional value, and creates waste disposal problems (Fleming et al., 1995).

It may be possible to reduce salt waste by fermenting cabbage with less than the typical 2% salt concentration if a proper fermentation can be assured. Early predominance of heterofermentative LAB is considered to be essential in the production of good quality sauerkraut (Pederson and Albury, 1969). Therefore, based on current knowledge, the introduction of an *L. mesenteroides* starter culture to the fermentation could help ensure that a proper fermentation will occur (Fleming et al., 1995). To determine the ability of a starter culture to predominate over the naturally present microflora, a method for identifying individual strains of *L. mesenteroides* is needed. Because *L. mesenteroides* strains have very similar physiological properties and nutritional requirements, classification of individual strains is difficult. Although a variety of methods are now available for the differentiation of bacterial strains, these can be ineffective, time-consuming, costly, or technically difficult (Cocconcelli et al., 1995; Duffner and O'Connell, 1995; Johansson et al., 1995a; Bjorkroth and Korkeala, 1996; Drake et al., 1996; Farber, 1996; Villani et al., 1997).

Random amplified polymorphic DNA PCR (RAPD-PCR) is a powerful method for discriminating among bacterial strains. Several studies have reported success in using RAPD-PCR for differentiation of LAB strains (Cancilla et al., 1992; Cocconcelli et al., 1995; Du Plessis and Dicks, 1995; Johansson et al., 1995b; Drake et al., 1996; Nigatu et al., 1998). Although variability of RAPD fingerprints has been observed (Ellsworth et al., 1993; Meunier, 1993; Muralidharan and Wakeland, 1993), it is reported that reproducibility can be achieved under carefully controlled conditions (Farber and Addison, 1994; Neiderhauser et al., 1994). RAPD-PCR may have potential for use in following the progress of starter cultures in vegetable fermentations. The method does not require prior knowledge of target sequences, which makes it suitable for following the unmarked starter cultures in commercial fermentations where antibiotic-marked starter cultures are not applicable. In this study, we investigated the use of RAPD as a tool to follow the growth, survival, and predominance of a starter culture in sauerkraut fermentations.

2. Materials and methods

2.1. Bacteria and culture methods

All bacterial strains were obtained from the culture collection maintained by the U.S. Food Fermentation Laboratory (FFL, USDA-ARS, Raleigh, North Carolina; Table 1). Cultures were maintained at −80 °C in MRS broth (Difco Laboratories, Detroit, MI) with 16% glycerol.

2.2. Cabbage fermentation

The cabbage used for all experiments was the Cecile cultivar from commercial sources. Fresh cabbage was trimmed of outer leaves and shredded to 1 mm thick with a food slicer. Cabbage fermentations were prepared by mixing sliced cabbage with granular food-grade salt to obtain a final concentration of approximately 2% (wt/wt) NaCl in duplicate sets of 1.2 l glass jars or 13 l fermentors, as described by Fleming et al. (1988). *L. mesenteroides* starter cultures

Table 1
L. mesenteroides strains used in this study

Strains	Sources	FFL no.[a]
C33	JRS[b]	LA 10
ATCC[c] 8293	ATCC	LA 81
ATCC 10830	NRRL[d]	LA 104
ATCC 27258	NRRL	LA 107
ATCC 13146	NRRL	LA 108
ATCC 10881	NRRL	LA 109
ATCC 10879	NRRL	LA 111
ATCC 10880	NRRL	LA 112
ATCC 10882	NRRL	LA 113
LA 113 M3	FFL	LA 195

[a] USDA-ARS Food Fermentation Laboratory (FFL) culture collection identification number.
[b] JRS=Dr. J. R. Stamer, Cornell University.
[c] ATCC=American Type Culture Collection (Rockville, MD).
[d] NRRL=USDA-ARS Northern Regional Research Laboratory (Peoria, IL).

were grown for 15 h at 30 °C in MRS broth, harvested by centrifugation, re-suspended in an equal volume of 0.85% NaCl, diluted, and sprayed onto the sliced, salted cabbage with a syringe prior to hand-packing the jars or fermentors. The cultures were diluted to give a starting concentration of approximately 10^6 CFU/g in the fermentations. Jars were capped with metal lids containing a rubber septum (serum stopper no. 7123-00, Rusch, Duluth, GA) to allow sampling the brine without opening the jars; brine samples were removed from the fermentors as described by Fleming et al. (1988). Alternatively, brine solution was added to the sliced cabbage prior to packing to allow equilibration at 0.5% NaCl. Cabbage fermentations were carried out at 18 °C for up to 14 days.

2.3. Cabbage juice preparation

Preparation of filter-sterilized cabbage juice broth (CJB) was carried out as described by Harris et al. (1992).

2.4. Collection of LAB isolates

Bacterial cell counts and LAB isolates from cabbage fermentations were obtained using a spiral plater (Spiral Systems model 4000, Spiral Systems, Cincinnati, OH) on MRS agar supplemented with 2% sodium azide (Fisher Scientific, Pittsburgh, PA; MMRS). The isolates were recovered from four separate laboratory fermentations (two inoculated with LA 195 starter culture and two uninoculated) and four pilot-scale fermentations (two inoculated with LA 10 and two with LA 81) and maintained at −80 °C in MRS broth with 16% glycerol. MRS broth cultures were incubated at 30 °C 12–16 h before performing DNA extraction. Gas production by heterolactic fermenting organisms was determined by using Durham tubes (6×50 mm, Kimble) with MRS broth. MRS agar supplemented with 5 μg/ml chloramphenicol and 5 μg/ml erythromycin was used to determine the antibiotic resistance phenotype.

2.5. DNA extraction and cell lysis

Genomic DNA was extracted using a Mini-Beadbeater (model 3110, Biospec Products, Bartlesville, OK) as described by Breidt and Fleming (1996). The DNA pellet was resuspended in 50 μl of deionized water, and the DNA solution was frozen at −20 °C until used for PCR reactions. Alternatively, total genomic DNA isolation from LAB cultures was carried out by using a Wizard™ Genomic DNA Purification kit (Promega, Madison, WI) in accordance with the manufacturer's instructions, with minor modifications. Twenty milliliters of stock 2.4 mg/ml mutanolysin (Sigma Laboratories, St. Louis, MO) was substituted for lysozyme.

2.6. PCR amplification

PCR amplification was performed in a GTC-2 Genetic Thermal Cycler with a model LTM-2 refrigeration unit (Precision Scientific, Chicago, IL). For RAPD-PCR, a single arbitrary nucleotide sequence (5′ ACGCGCCCT3′) was used (Johansson et al., 1995b). The reaction mixture of 100 μl in a 0.6 ml microfuge tube (no. 3437, Continental Laboratory Products, San Diego, CA) consisted of 10 μl of 10× PCR reaction buffer (500 mM KCl, 100 mM Tris–HCl, pH 9.0, and 1.0% Triton® X-100, Promega), 16 μl of 25 mM $MgCl_2$ (Promega), 4 μl of 36.7 mM primer solution (Genosys Biotechnologies, The Woodlands, TX), 1 μl of dNTP mixture (25 mM each dNTP, No. 200415, Stratagene, La Jolla, CA), 2 μl of DNA preparation, 1.0 μl of *Taq* DNA polymerase (5.0 U/μl, Promega), and 66 μl of deionized water. The reaction was carried out with the initial heat denaturation step of 94 °C, 10 min; 36 °C, 3 min; and 72 °C, 2 min. The *Taq* polymerase was then added and followed by 94 °C, 45 s; 30 °C, 120 s; 72 °C, 60 s for four cycles, and 94 °C, 15 s; 36 °C, 30 s; 72 °C, 45 s for 36 cycles. The final extension step was performed at 75 °C for 10 min, followed by cooling to 4 °C.

The method of Breidt and Fleming (1996) was used for the intergenic transcribed spacer (ITS)-PCR. After PCR amplification, 20 μl of reaction mixtures containing DNA products was treated with 1 μl of *Rsa* I enzyme solution (16 U/μl, no. 500890, Stratagene) for 1 h at 37 °C in a water bath (model 18800, Lab Line Instruments, Melrose Park, IL). The restriction digest samples were stored at −20 °C prior to electrophoresis in 5% non-denaturing polyacrylamide gels using a vertical gel electrophoresis

box with a glass plate supplied by the manufacturer (BRL Model V16, Life Technologies, Gaithersburg, MD). Gels were run for approximately 6 h at 60 V in a Tris-borate buffer (TBE, Maniatis et al., 1982). Alternatively, PCR products were electrophoresed in 1.0% agarose (Sigma) or 2.0% Metaphor® agarose (FMC BioProducts, Rockland, ME) gels using a horizontal submerged gel apparatus (Bio-Rad, model 1704343, Bio-Rad Laboratories, Hercules, CA). A 100-bp DNA molecular weight marker (BRL) was used as a standard. After electrophoresis, the gels were stained with ethidium bromide (0.5 μg/ml, in water, Sigma) and photographed with UV illumination (UV Transilluminator, model 2040EV, Stratagene).

2.7. Analysis of RAPD banding patterns

Similarity analysis for comparison of RAPD patterns was accomplished with the Bio-Rad Molecular Analyst Software "PC Fingerprint Plus" (version 1.6). This software can produce similarity coefficients between two RAPD patterns using any of four similarity measures: fuzzy logic, dice, jaccard, and area. Each of the similarity measures yields values that range from 0 to 100% similarity. To determine which of these four similarity measures is most appropriate for RAPD comparison, we converted each of the percent similarity values to proportional similarity

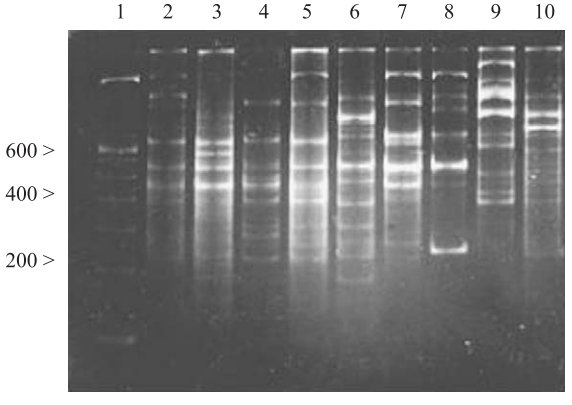

Fig. 1. RAPD patterns of different *L. mesenteroides* strains. Lane 1 contained the 100-bp DNA ladder size standards; lane 2, LA 10; lane 3, LA 81; lane 4, LA 104; lane 5, LA 105; lane 6, LA 106; lane 7, LA 109; lane 8, LA 111; lane 9, LA 112; lane 10, LA 113. DNA extraction was performed by shaking with glass beads.

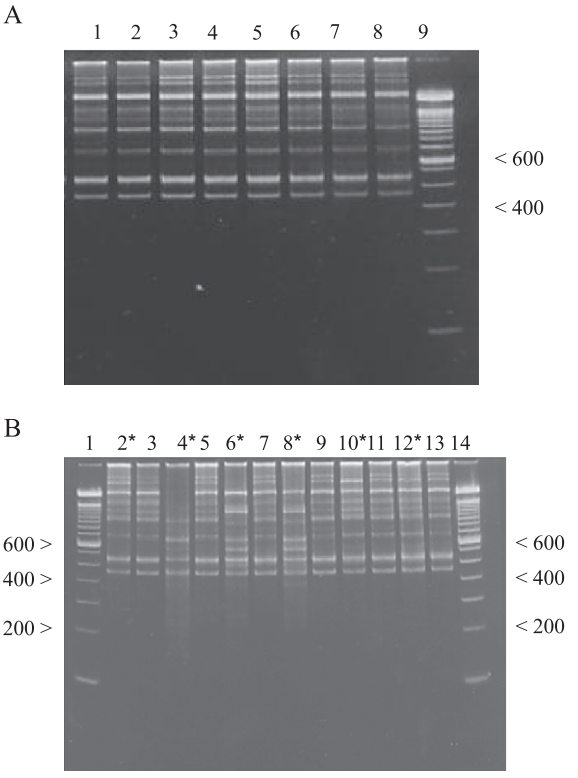

Fig. 2. Reproducibility of RAPD patterns. (A) Fingerprints generated by different cultures of *L. mesenteroides* LA 81. Lane 9 contained the 100-bp DNA ladder size standards; lanes 1 and 2, DNA template from 12 h culture; lanes 3 and 4, DNA template from 16 h culture; lanes 5 and 6, DNA template from 24-h culture; lanes 7 and 8, DNA template from 48-h culture. (B) RAPD patterns generated by freshly extracted DNA templates, and DNA templates extracted after frozen storage of the culture. Lanes 1 and 14, 100-bp standards; lanes 2–13, paired template preparations from individual colonies of LA 81 which were isolated from an inoculated sauerkraut fermentation; template prepared before (lanes denoted with an *, 2, 4, 6, 8, 10, 12) and the respective cultures after freezing (lanes 3, 5, 7, 9, 11, and 13).

by dividing by 100, and then we converted these numbers to distances by subtracting the similarity values from 1. We reasoned that an appropriate percentage similarity value could be converted to a distance measure that would work well for inferring the evolutionary relationship between the isolates. With the PHYLIP software package (Felsenstein, 1993), we applied the neighbor joining method (Saitou and Nei, 1987) to the four distance matrices that we derived from the four similarity matrices. For a

distance measure that fits the inferred phylogeny well, the distances between isolates along the inferred phylogeny will be very close to the corresponding entries in the distance matrix. In other words, a plot of the distance inferred along the tree versus the corresponding distance in the distance matrix should have a good fit to a line with a slope of 1 and a *y*-intercept of 0.

2.8. Pulse field gel electrophoresis (PFGE)

A modification of the procedure of Tanskanen et al. (1990) was used to prepare high molecular weight chromosomal DNA. Selected LAB were grown overnight at 30 °C in MRS broth and then diluted 1:100 into fresh media. When the absorbance of 590 nm reached 0.6 (approximately 6 h), chloramphenicol (Sigma) was added to a final concentration of 100 μg/ml, and incubation continued an additional hour. Cells were then harvested by centrifugation (13,000×*g* 5 min), washed and re-suspended in 1 ml of buffer: 1 M NaCl, 10 mM Tris–HCl (pH 7.6). Molten agar was added, 300 μl of a 2% solution of molten agarose NA (Pharmacia LKB, Upssala, Sweden), and the agar-cell suspension was added to fill the plug molds for the electrophoresis apparatus (Pharmacia). Once the agar had solidified, the cells were lysed in situ with EC buffer, which contained 6 mM Tris–Cl, pH 7.6, 1 M NaCl, 100 mM sodium EDTA, 1 mg of lysozyme per ml (all from Sigma), and 50 units of mutanolysin per ml (ICN Pharmaceuticals, Costa Mesa, CA). The agarose plugs were incubated for 12 to 14 h at 25 °C with 45 U of the restriction enzyme *Sma*I (Boehringer Mannheim, Indianapolis, IN) in 300 μl of a buffer solution recommended by the supplier. Electrophoresis of the restriction digests was performed through 1% (wt/vol) agarose NA (Phamarcia) gels in Tris–borate buffer in a Pulsaphor Plus electrophoresis system (model 2015, Phamarcia LKB Biotechnology) with a

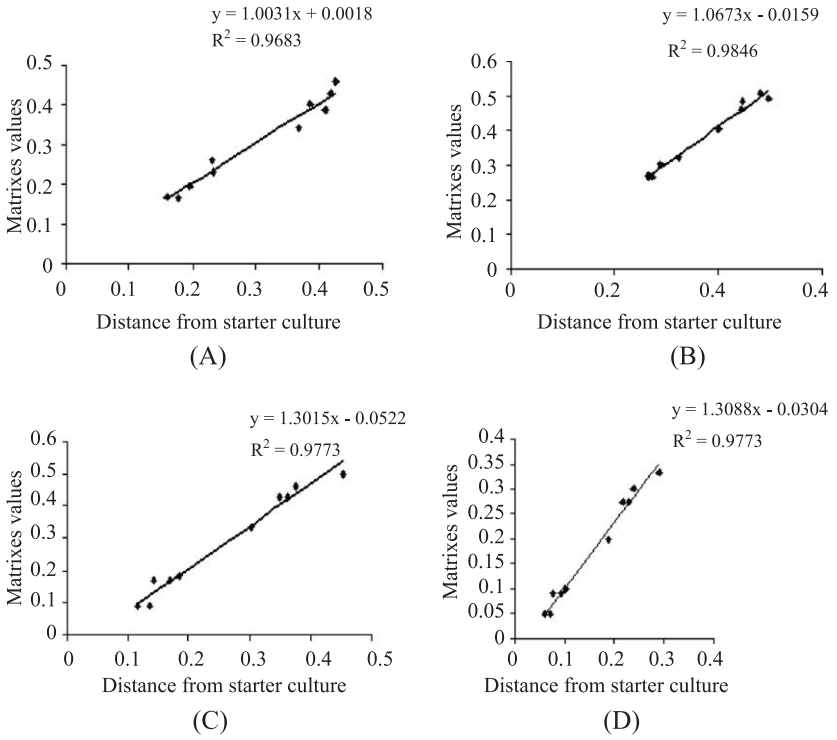

Fig. 3. Determination of relationship between matrix values and dendogram distances generated by different algorithms with the NJ clustering method. (A) Fuzzy logic; (B) Area; (C) Jaccard; (D) Dice.

model MgW K2R refrigeration unit (Lauda, Germany) for 22 h at 200 V at 15 °C. A low range PFGE marker (New England Biolabs, Bevery, MA) consisting of lambda DNA–*Hin*dIII fragments and lambda concatamers was used as the molecular size standard. After electrophoresis, the gels were stained and photographed as described above.

3. Results

3.1. Differentiation of L. mesenteroides strains using RAPD-PCR

To determine the potential of RAPD-PCR to differentiate LAB strains involved in sauerkraut fermen-

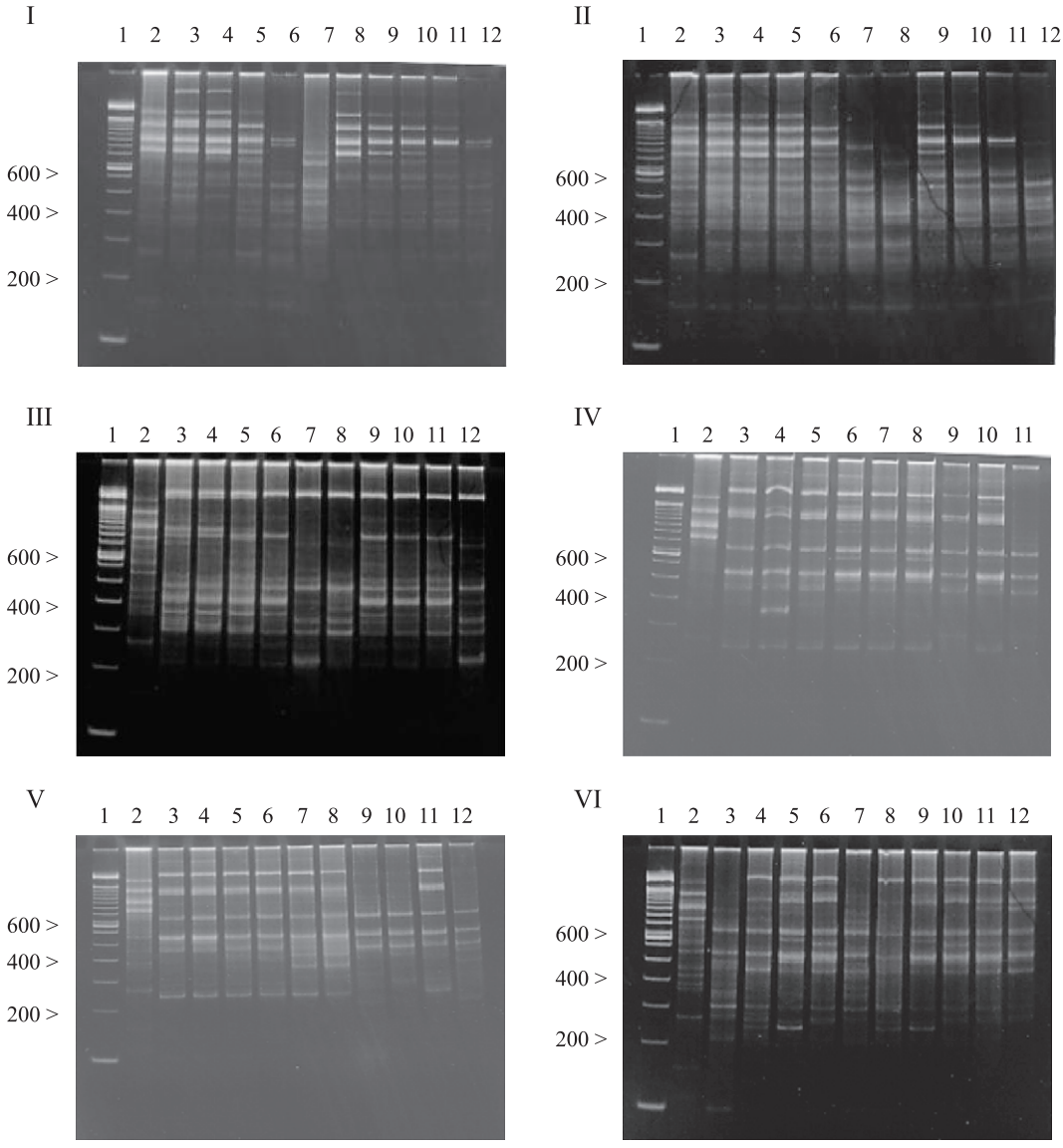

Fig. 4. Comparison of RAPD patterns for the starter culture LA 195. DNA template from inoculated (I–III) and uninoculated (IV–VI) fermentations. Isolates were taken at 2 (I and IV), 5 (II and V), and 14 (III and VI) days after the start of the fermentation. For each gel picture, lane 1 has 100-bp DNA ladder size standards; lane 2, the starter culture RAPD pattern; the remaining lanes show individual isolates.

tations, nine strains of *L. mesenteroides*, were tested. RAPD-PCR produced patterns with products ranging from approximately 200 bp to more than 1500 bp (Fig. 1). All nine strains of *L. mesenteroides* were distinguished from each other by their RAPD profiles.

3.2. RAPD-PCR reproducibility

CJB was chosen as a model system for evaluation of RAPD-PCR reproducibility because the medium was previously shown to be a satisfactory substitute for shredded cabbage (Stamer et al., 1971). RAPD patterns were consistent and reproducible when compared among DNA templates prepared from a culture sample of *L. mesenteroides* taken at different times during a growth curve (Fig. 2A). Surprisingly, variations were observed when comparing DNA templates from the same culture from an inoculated sauerkraut fermentation (LA 81, below) before and after frozen storage (Fig. 2B).

3.3. Laboratory fermentations

Sauerkraut fermentations were prepared in 1.2-l glass jars with and without LA 195, which contain plasmid-borne chloramphenicol and erythromycin resistance genes (Breidt et al., 1995). Isolates (120) were recovered from four separate laboratory fermentations (two inoculated with LA 195 starter culture and two uninoculated). Ten isolated colonies were taken from the spiral plates from each fermentation at 2, 5, and 14 days of the fermentation. Fig. 3 shows such plots for distances between the LA 195 starter culture and selected isolates taken from the fermentations. All four percentage similarity measures yielded data with good fits to a straight line, but the fuzzy logic coefficient also yielded best fitting lines with a slope estimate near 1 and a *y*-intercept estimate near 0. For this reason, we selected the fuzzy logic percentage similarity coefficient for our further analyses.

All 40 isolates from both inoculated and uninoculated fermentations at 2 and 5 days exhibited heterofermentative characteristics. Only the 20 isolates at 2 and 5 days from the fermentations inoculated with LA 195 (Cm^r Em^r) were able to grow on MRS agar containing chloramphenicol and erythromycin. As expected (due to microbial succession in the sauerkraut fermentation), none of the 20 isolates from 14 days of fermentation from both the uninoculated and inoculated exhibited the antibiotic resistance characteristic. Most of the 14-day isolates were homofermentative based on gas production in MRS broth. The results of the test for antibiotic resistance and gas production suggested that the starter culture predominated in the early part of fermentation and was typically replaced by homofermentative LAB at 14 days. The patterns of DNA fragments amplified by RAPD-PCR from isolates from each time-point (2, 5, and 14 days) were compared to RAPD-PCR products from the starter culture (Fig. 4) for both the

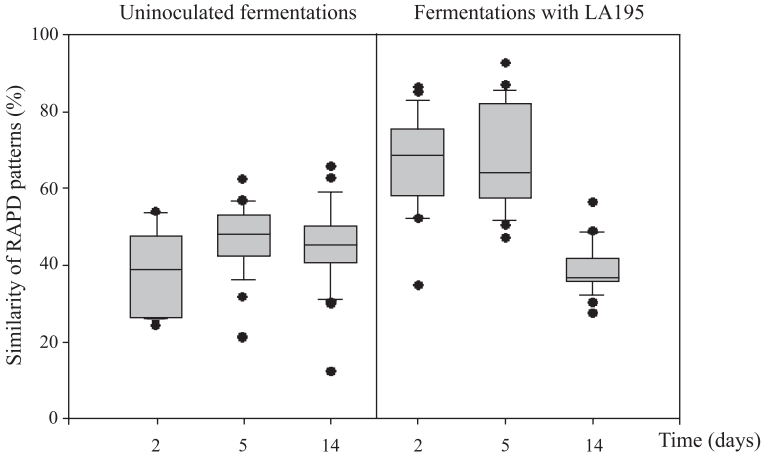

Fig. 5. Similarity of RAPD patterns between isolates and LA 195 starter cultures. The isolates were obtained from uninoculated and fermentations inoculated with LA 195 starter cultures.

Table 2
The mean similarity scores from RAPD patterns comparison for the starter cultures and isolates

	Day 2	Day 5	Day 14
Uninoculated fermentations	38.39	46.94	44.73
Fermentations with LA 195	66.99	67.82	39.05
Fermentations with LA 10	66.99	57.60	53.41
Fermentations with LA 81	69.09	71.15	68.84

inoculated and uninoculated fermentations. While none of the RAPD patterns from the isolates from the inoculated cultures were identical to the starter with scores of 35.0% to 92.4%, the mean similarity scores were greater than 66% for the inoculated fermentation RAPD patterns from days 2 and 5 (Fig. 5 and Table 2). Specific common bands were observed between the starter culture and all isolates, but only one isolate exhibited more than 90% similarity when analyzed by using fingerprinting comparison software. Some isolates from the inoculated fermentations with the antibiotic-resistant characteristic were found to have very low similarity with the starter culture. For example, lanes 6 and 7 in Fig. 4(I) had 52.5% and 35.0% similarity to the starter culture. The mean similarity scores, however, for both the uninoculated fermentation samples and the isolates from the inoculated fermentation at 14 days were less than 47%, as shown in Fig. 5 and Table 2. This indicated that the starter culture was the predominant organism in the fermentation at 2 and 5 days after the start of fermentation, but was then replaced by other (homofermentative) cultures at day 14.

3.4. Pilot-scale fermentations

To determine the predominance of starter cultures in pilot-scale fermentations (13 l), 117 isolates, including starter cultures, were tested to determine the patterns of the PCR products produced. The similarity of RAPD profiles among isolates and starter cultures was determined as described above using the Fuzzy logic coefficient and NJ clustering method. The mean similarity scores of the RAPD patterns were greater than 66% for the fermentations inoculated with LA 81, as well as LA 10 inoculated fermentations at 2 days, as shown in Fig. 6 and Table 2. From these data, we concluded that the starter cultures were the predominant organism during the early (2 and 5 days) part of these fermentations. We observed for LA 81, that this starter culture was still the predominant organism at 14 days.

ITS-PCR was used to confirm RAPD-PCR identification for differentiating starter cultures and isolates. The selected isolates had RAPD patterns with similarity scores ranging from 37.6% to 81.9% when compared to the starter cultures. The patterns observed from the isolates taken at 2, 5, and 14 days after the start of the inoculated fermentation with either LA 10 or LA 81 were identical to the starter

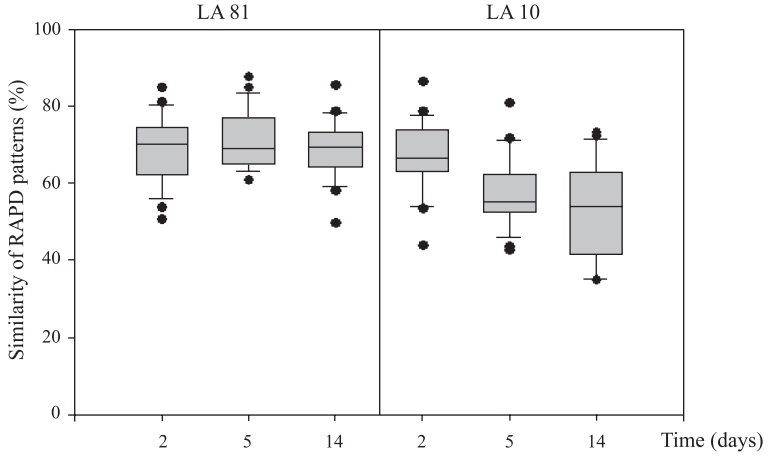

Fig. 6. Similarity of RAPD patterns between isolates and starter cultures. The isolates were obtained from sauerkraut fermentation with LA 81 and LA 10.

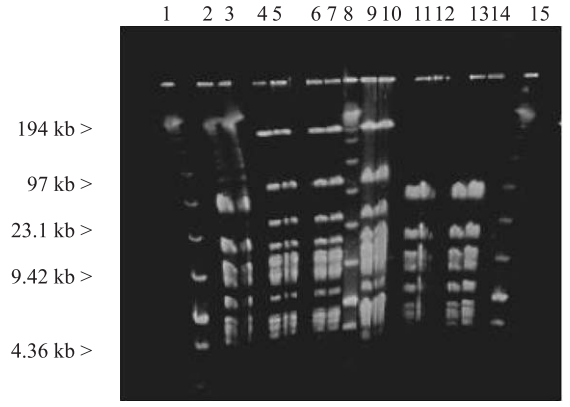

Fig. 7. PFGE patterns of *Sma*I digests of genomic DNA of *L. mesenteroides* LA 10, LA 81 starter cultures, and isolates from sauerkraut fermentations. Lane 1, λ DNA–*Hin*dIII and λ concatamers marker; lanes 2 and 3, LA 10; lanes 4 and 5, LA 81; lanes 6 and 7, isolates taken from fermentation inoculated with LA 81 at 5 days; lane 8, λ marker; lane 9 and 10, isolates taken from fermentation inoculated with LA 81 at 2 days; lanes 11 to 14, isolates taken from fermentation inoculated with LA 10 at day 14 (lanes 11 and 12) and day 2 (lanes 13 and 14); lane 15, λ marker.

cultures (data not shown) and were typical of *L. mesenteroides*, as described by Breidt and Fleming (1996).

To confirm the identity of strains presumed to be the same but showing dissimilar RAPD patterns, *L. mesenteroides* LA 10 and LA 81 starter cultures, as well as selected isolates, were examined by PFGE (Fig. 7). The selected isolates from days 2 through 14 had RAPD pattern similarity scores ranging from 53.3% to 76.8% when compared to the starter cultures. The patterns of LA 10 and LA 81 starter cultures were different for each other, but similar to their respective isolates. PFGE banding patterns, suggesting that the isolates taken from inoculated sauerkraut fermentations were the same strain as starter culture.

4. Discussion

The RAPD-PCR technique has been used to differentiate among *Lactococcus* and *Lactobacillus* at the subspecies level (Cancilla et al., 1992; Cocconcelli et al., 1995; Drake et al., 1996). In this study, various strains of *L. mesenteroides* exhibited different RAPD banding profiles in contrast to the similar banding patterns observed by PCR-ribotyping (Breidt and Fleming, 1996). However, comparison between RAPD profiles among starter cultures and isolates from laboratory sauerkraut fermentations using an antibiotic resistance gene-marked starter culture showed variations in RAPD profiles. The mean distribution of similarity scores for the data, however, clearly showed the predominance of starter cultures in the early stages of fermentation (Figs. 5 and 6). The mean similarity scores for LA 10 and LA 195 were lower for day 14 isolates when compared to the day 1 and 5 isolates, while the scores for LA 81 were consistently above 66% through the 14-day sampling. From these data, we conclude that LA 81 may be able to predominate longer in the fermentation than either LA 10 or LA 195. This information may be useful when evaluating or comparing the ability of an unmarked starter cultures to predominate in commercial scale fermentations, where genetically marked cultures are not used.

RFLP analysis of the rRNA-ITS region was also used to evaluate LAB isolates and starter cultures. Most isolates had RAPD patterns similar to, or slightly different from the starter cultures. The results only identified starter cultures and isolates at the species level, *L. mesenteroides*, and not the strain level, as described by Breidt and Fleming (1996). However, the selected isolates were confirmed as the added starter cultures when typed by PFGE.

There have been suggestions that RAPD are sensitive to variations and the patterns are not always reproducible (Ellsworth et al., 1993; Meunier, 1993; Muralidharan and Wakeland, 1993). These have been observed when we used the techniques for comparison between starter cultures and isolates from sauerkraut fermentation, which have been presumptively identified as starter cultures. Freshly extracted templates and templates from frozen cultures of the same isolates also exhibited variations in the RAPD fingerprints. However, DNA templates prepared from different cultures of the single LAB isolate produced consistent and reproducible RAPD patterns.

Our results indicate that the variations seen in RAPD are probably due to minor variations in template preparation. However, the techniques are relatively simple to perform and require no prior knowledge of specific sequences of starter cultures for characterizing and distinguishing LAB at the subspecies level. It is also possible to study a large number of isolates in a short time due to the rapid PCR

procedure. We used RAPD in a comparative manner only, basing our analysis on the similarity of starter culture RAPD fragment patterns and that of the isolates. We have shown that, by using the mean similarity values and the distribution of similarity scores, we can effectively use RAPD to follow unmarked cultures in mixed culture vegetable fermentations.

Acknowledgements

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