

# Microbial Community Structure of Korean Cabbage Kimchi and Ingredients with Denaturing Gradient Gel Electrophoresis

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Kimchi is a traditional Korean fermented vegetable food, the production of which involves brining of Korean cabbage, blending with various other ingredients (red pepper powder, garlic, ginger, salt-pickled seafood, etc.), and fermentation. Recently, kimchi has also become popular in the Western world because of its unique taste and beneficial properties such as antioxidant and antimutagenic activities, which are derived from the various raw materials and secondary metabolites of the fermentative microorganisms used during production. Despite these useful activities, analysis of the microbial community present in kimchi has received relatively little attention. The objective of this study was to evaluate the bacterial community structure from the raw materials, additives, and final kimchi product using the culture-independent method. Specifically, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to analyze the 16S rRNA partial sequences of the microflora. One primer set for bacteria, 341F<sup>GC</sup>-518R, reliably produced amplicons from kimchi and its raw materials, and these bands were clearly separated on a 35–65% denaturing gradient gel. Overall, 117 16S rRNA fragments were identified by PCR-DGGE analysis. *Pediococcus pentosaceus*, *Leuconostoc citreum*, *Leuconostoc gelidum*, and *Leuconostoc mesenteroides* were the dominant bacteria in kimchi. The other strains identified were *Tetragenococcus*, *Pseudomonas*, *Weissella*, and uncultured bacterium. Comprehensive analysis of these microorganisms could provide a more detailed understanding of the biologically active components of kimchi and help improve its quality. PCR-DGGE analysis can be successfully applied to a fermented food to detect unculturable or other species.

**Keywords:** Denaturing gradient gel electrophoresis, bacterial community, kimchi, kimchi ingredients

## Introduction

Kimchi is a well-known traditional fermented Korean dish made of salted vegetables such as Korean cabbage and radish, which are liberally seasoned with red chili pepper powder, garlic, green onion, ginger, radish, scallion, saeujeot (a salt-fermented shrimp sauce), and aekjeot (a fermented fish sauce), among other possible ingredients. Kimchi is rich in vitamin A, thiamine (B<sub>1</sub>), riboflavin (B<sub>2</sub>), calcium, and iron, and contains several types of lactic acid bacteria [3]. The popularity of kimchi has increased worldwide in recent years; indeed, *Health* magazine rated it as one of the top five “World’s Healthiest Foods” given that it is rich in

vitamins, aids digestion, and can possibly prevent cancer. Kimchi has also become popular in the Western world because of its unique taste and beneficial properties, including anticancer, antioxidant, and antimutagenic activities. These properties are derived from the various raw materials and secondary metabolites of fermentative microorganisms used in kimchi production [17].

Less than 1% of all bacterial cells in nature can be grown using culture-based approaches such as in culture media. Furthermore, these methods require a long incubation time, and the results may not accurately reflect the population dynamics of microbial communities because of variations in the environmental conditions such as temperature, pH,

and light [4]. However, molecular techniques (*i.e.*, culture-independent approaches) allow for more effective and accurate assessments of microbial community diversity. In particular, denaturing gradient gel electrophoresis (DGGE) analysis, based on direct recovery of rDNAs from samples, has facilitated the detection and characterization of low-abundance and unculturable microorganisms [6].

DGGE analysis is a molecular fingerprinting technique based on differences in the melting behavior of the double strands of polymerase chain reaction (PCR)-amplified 16S rDNA fragments in denaturing gradient gels (*i.e.*, gels containing chemical denaturants such as formamide and urea). DGGE can be used to differentiate between sequences of 16S or 18S rDNA from different microorganisms because these sequences denature at different concentrations of denaturants, resulting in a sequence-specific banding pattern [10, 11].

Recently, PCR-DGGE analysis has been widely used to study the bacterial diversity in fermented foods, including kefir grain [9], wine [1], salted duck [10], kimchi [2], fermented sausage [18], and cheese [5].

Although the final kimchi product is obtained from a complex community of microorganisms acting together during fermentation, little information is available concerning the correlation between the bacterial community of the final kimchi and that of its raw ingredients. Therefore, the objective of this study was to investigate the bacterial community structure in kimchi and its ingredients using a culture-independent method.

## Materials and Methods

### Preparation of Kimchi

Kimchi was prepared at a kimchi factory system, using the following ingredients obtained from a commercial farm in Gwangju, Korea: Korean cabbage (70%), radish (7.0%), garlic (1.4%), ginger (0.6%), red pepper powder (4.2%), Welsh onion (1.8%), salt-fermented shrimp (2.3%), salt-fermented anchovy (1.1%), fermented seafood sauce (2.0%), seaweed (0.3%), and glutinous rice paste (9.3%). Korean cabbage was steeped in 13% salt water for 17 h and then washed in tap water three times. Kimchi fermentation was conducted by refrigerated storage at 4°C for 2 weeks.

### Extraction of DNA

Kimchi and samples of the raw ingredients (approx. 500 g) were homogenized in a blender (Philips Electronics N.V., China) under the hood for 5 min. Homogenized samples (approximately 10 g each) were transferred into a sterile stomacher bag with 90 ml of a

sterile 0.85% NaCl solution and then mixed for 5 min with a stomacher (Interscience, Rockland, MA, USA). To directly extract the microbial DNA, 10 ml aliquots of the suspension of homogenized samples were centrifuged at 8,000 ×g for 15 min. The resulting pellets were washed with sterile phosphate-buffered saline (pH 7.0) and resuspended in 1 ml of TE buffer (10 mM Tris, 5 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0). The pellets were subjected to DNA extraction, with the addition of lysozyme (50 mg/ml; Sigma Chemical Co., St. Louis, MO, USA) for bacterial cell lysis. DNA was extracted from the pellets using a DNeasy tissue kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. The concentration and purity of the extracted DNA were determined using a biophotometer (Eppendorf, Hamburg, Germany).

### PCR

The 16S rRNA fragments were amplified using the universal PCR primers 341F forward (5'-CCTACGGGAGGCAGCAG-3') and 518R reverse (5'-ATTACCGGGCTGCTGG-3') [16], which are commonly used for amplifying DNA from microorganisms. For DGGE analysis of the PCR products, a 40 nucleotide GC-rich sequence (GC-clamp, 5'-CGCCCGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG-3') was appended to the 5' end of the primer [19]. PCR amplification was performed with a Mastercycler gradient (Eppendorf). The PCR mixture (20 µl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM MgCl<sub>2</sub>, 0.5 U Taq polymerase, 0.4 mM dNTPs, 20 pmol of each primer, 1 µl of template DNA (20 µg/ml), and sterile water. An initial denaturation step at 95°C for 5 min was followed by 35 cycles of amplification (denaturation at 94°C for 45 sec, annealing at 52°C for 45 sec, and extension at 72°C for 1 min), and a final extension at 72°C for 5 min. The PCR products were electrophoresed on 2% agarose gels with 1× TAE buffer (20 mM Tris, 10 mM sodium acetate, and 0.5 mM Na<sub>2</sub>EDTA, pH 8.0), stained with ethidium bromide (0.5 µg/ml), and then visualized with an ultraviolet transilluminator (Korea Bio-Tech Co., Seoul, Korea).

### DGGE Analysis

DGGE analysis was performed with a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). A 30–65% denaturing gradient was used (note that a 100% denaturing solution corresponds to 7.0 M urea (Sigma Chemical Co.) and 40% (v/v) formamide (Sigma Chemical Co.)) with 8% (w/v) polyacrylamide gels (Acryl/Bis 29:1, 40% (w/v) solution; Amresco, Solon, OH, USA). Electrophoresis was performed in 0.5× TAE buffer (20 mM Tris, 10 mM Na-acetate, and 0.5 mM Na<sub>2</sub>EDTA, pH 8.0), initiated at a voltage of 20 V for 30 min and then at a voltage of 60 V for 12 h at 60°C. After electrophoresis, the gels were stained in 1× TAE buffer containing a 1:10,000 dilution of SYBR safe DNA gel stain dye (Invitrogen, Eugene, OR, USA) for 20 min, and then visualized with a UV transilluminator (Bio-Rad Laboratories).

### Determination of Nucleotide Sequences

The 16S rRNA gene sequences from DGGE gels were sequenced using the PCR-based technique described above. The bands corresponding to the 16S rRNA were excised from the DGGE gel, and then the DNA was extracted by passive diffusion in 50  $\mu$ l of sterilized water overnight at 4°C. Subsequently, the DNA was used as the template for PCR with the 341F (without the GC clamp) and 518R primers. The PCR products were purified using a QIAquick PCR purification kit (Qiagen Inc.) and then sequenced. DNA sequencing was performed on an ABI 377 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Finally, the closest known relatives were determined in public data libraries (Ribosomal Database Project and GenBank) using the nucleotide-nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the DGGE bands.

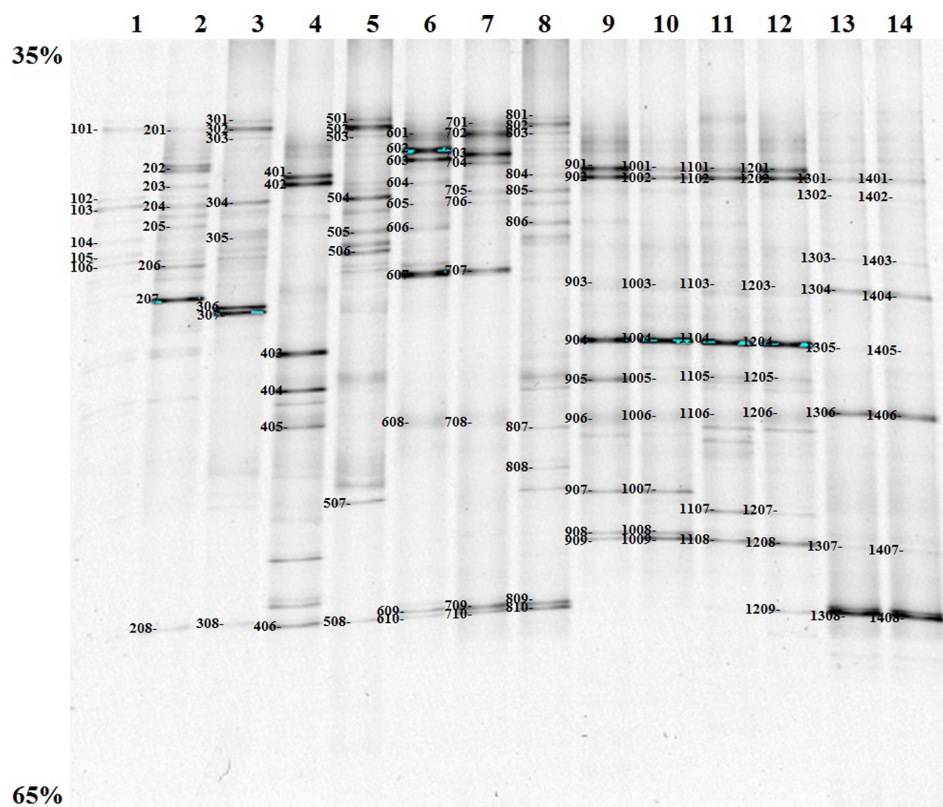
## Results

### Bacterial Diversity by DGGE Analysis

The results of the DGGE analysis of PCR-amplified 16S rRNA fragments directly extracted from kimchi and its

ingredients were used to determine the potential correlation of the bacterial community structures between the final kimchi product and its ingredients. Fig. 1 shows the distinct bands that appeared on the DGGE gel. For the salt-fermented anchovy, fermented seafood sauce, radish, ginger, garlic, and Welsh onion samples, the dominant microbial populations were characterized by one distinct band (207), two distinct bands (306 and 307), four distinct bands (401, 402, 403, and 404), two distinct bands (502 and 504), three distinct bands (602, 603, and 607), and two distinct bands (703 and 707), respectively. Furthermore, the banding patterns of DGGE gels were similar between raw Korean cabbage (outside and inside) and brined Korean cabbage (outside and inside).

In the samples of raw Korean cabbage (outside and inside), brined Korean cabbage (outside and inside), kimchi of 7-day fermentation, and kimchi of 14-day fermentation, the dominant populations were characterized by one distinct band (904), one distinct band (1004), one distinct band (1104), one distinct band (1204), two distinct bands



**Fig. 1.** DGGE analysis of PCR amplified bacterial 16S rDNA fragments from kimchi and its raw materials.

Lanes 1, Salt-fermented shrimps; 2, salt-fermented anchovy; 3, fermented seafood sauce; 4, radish; 5, ginger; 6, garlic; 7, Welsh onion; 8, seaweed (*Codium fragile*); 9, raw baechu cabbage (outside); 10, raw baechu cabbage (inside); 11, brined baechu cabbage (outside); 12, brined baechu cabbage (inside); 13, kimchi (1 week); and 14, kimchi (2 week). The PCR cycle number for the 341F<sup>GC</sup> and 518R primers was 30 cycles.

(1306 and 1308), and two distinct bands (1406 and 1408), respectively (Fig. 1).

### Identification of Bacteria

Analysis of the 16S rRNA sequences of the samples allowed for identification of the species represented by the obtained DGGE bands (Table 1). The 16S rRNA sequences of bands 101, 201, 302, 502, 701, and 802 (Fig. 1) were 98% similar to *Pseudomonas putida*. The sequences of bands 303, 503, 601, 702, and 803 were 97% similar to uncultured bacterium, and those of the 901, 1001, 1101, and 1201 fragments were 97% similar to *Lactobacillus* sp. The sequences of the 203, 402, 604, 902, 1002, 1102, 1202, 1301, and 1401 fragments were 97% similar to *Weissella confusa*, and those of the 102, 504, 705, 805, 1302, and 1402 fragments were 96% similar to *Weissella* sp. The sequences of the 103, 204, 304, 605, and 706 fragments were 97% similar to *Pseudomonas denitrificans*. The sequences of the 104, 305, 505, 606, and 806 fragments were 97% similar to *Pseudomonas koreensis*, and those of the 206, 1303, and 1403 fragments were 97% similar to *Tetragenococcus halophilus*. The sequences of the 607 and 707 fragments were 97%

similar to *Pediococcus acidilactici*, and those of the 207, 903, 1003, 1103, 1203, 1304, and 1404 fragments were 97% similar to *Pediococcus pentosaceus*. The sequences of the 306 and 307 fragments were 97% and 96% similar to *Pediococcus ethanolidurans* and *Pediococcus* sp., respectively. The sequences of the 403, 904, 1004, 1104, 1204, 1305, and 1405 fragments were 97% similar to *Leuconostoc* sp. The sequences of the 404, 905, 1005, 1105, and 1205 fragments were 97% similar to *Lactobacillus brevis*, and those of the 405, 608, 708, 906, 1006, 1106, 1206, 1306, and 1406 fragments were 97% similar to *Leuconostoc citreum*. The sequences of the 507, 907, and 1007 fragments were 97% similar to *Cyanobacterium* sp., and those of the 909, 1009, 1108, 1208, 1307, and 1407 fragments were 97% similar to *Leuconostoc gelidum*. In addition, the 208, 308, 609, 709, and 809 fragments were 97% similar to *Streptomyces globisporus* and those of the 406, 508, 610, 710, 810, 1209, 1308, and 1408 fragments were 97% similar to *Leuconostoc mesenteroides*.

### Discussion

We observed that the 16S rDNA fragments identified to

**Table 1.** Bacterial community structure in kimchi and its ingredients as determined by denaturing gradient gel electrophoresis analysis.

Band No. <sup>a</sup>	Closest relative	GenBank Accession No.	BLAST similarity (%)
101, 201, 302, 502, 701, 802	<i>Pseudomonas putida</i>	EF529517	98
303, 503, 601, 702, 803	Uncultured bacterium	GQ245709	97
901, 1001, 1101, 1201	<i>Lactobacillus</i> sp.	KT380051	97
203, 402, 604, 902, 1002, 1102, 1202, 1301, 1401	<i>Weissella confusa</i>	KU055492	97
102, 504, 705, 805, 1302, 1402	<i>Weissella</i> sp.	KP126905	96
103, 204, 304, 605, 706	<i>Pseudomonas denitrificans</i>	AY747590	97
104, 305, 505, 606, 806	<i>Pseudomonas koreensis</i>	GU078448	97
206, 1303, 1403	<i>Tetragenococcus halophilus</i>	EU689056	97
607, 707	<i>Pediococcus acidilactici</i>	AJ305322	97
207, 903, 1003, 1103, 1203, 1304, 1404	<i>Pediococcus pentosaceus</i>	FJ390108	97
306	<i>Pediococcus ethanolidurans</i>	DQ400914	97
307	<i>Pediococcus</i> sp.	AF349938	96
403, 904, 1004, 1104, 1204, 1305, 1405	<i>Leuconostoc</i> sp.	KF999722	97
404, 905, 1005, 1105, 1205	<i>Lactobacillus brevis</i>	AB919113	97
405, 608, 708, 906, 1006, 1106, 1206, 1306, 1406	<i>Leuconostoc citreum</i>	KT626384	98
507, 907, 1007	<i>Cyanobacterium</i> sp.	KP869000	98
909, 1009, 1108, 1208, 1307, 1407	<i>Leuconostoc gelidum</i>	AB485958	97
208, 308, 609, 709, 809	<i>Streptomyces globisporus</i>	NR112593	99
406, 508, 610, 710, 810, 1209, 1308, 1408	<i>Leuconostoc mesenteroides</i>	GU458344	99

<sup>a</sup>Each number corresponds to the bands indicated in Fig. 1.

The 16S rRNA fragments from the DGGE bands were aligned with GenBank reference sequences (<http://www.ncbi.nlm.nih.gov>) using the Basic Local Alignment Search Tool (BLAST) program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

the same species demonstrated a specific migration position on the DGGE gels, which validated the use of this technique for rapid species identification. Therefore, the DGGE profiles were used to provide details about the bacterial community, including the abundance of species among samples. The major goal of this study was to investigate the correlation between the bacterial community of the final kimchi and that of its raw ingredients using DGGE analysis. In this study, no significant change was observed in the DGGE banding patterns following kimchi fermentation. These results indicate that the bacterial community structures of kimchi samples likely originated mainly from radish and Korean cabbage. In particular, *W. confusa*, *P. pentosaceus*, *L. citreum*, and *L. gelidum* from kimchi and Korean cabbage corresponded to the highest intensity bands on the DGGE gel.

This result was similar to the findings of previous studies. Mheen and Kwon [12] reported that *L. mesenteroides*, *Leuconostoc pseudomesenteroides*, *Lactococcus lactis*, *L. brevis*, and *Lactobacillus plantarum* were identified as the predominant bacteria in kimchi; these bacteria were also shown to play an important role in the fermentation of many kimchi products and to influence the taste of kimchi. For example, the most common strains in kimchi are *Leuconostoc* species, including *L. mesenteroides*, *L. citreum*, *Leuconostoc carnosum*, *Leuconostoc gasicomitatum*, *Leuconostoc inhae*, *L. gelidum*, *Leuconostoc kimchii*, and *Leuconostoc miyukkimchii*, as well as *Lactobacillus* species, including *L. plantarum*, *L. brevis*, and *Lactobacillus sakei* [7, 10].

Lactic acid bacteria such as *Enterococcus*, *Pediococcus*, and *Streptococcus* were observed as the dominant microorganisms in the kimchi in recent studies [15]. Jung *et al.* [8] reported that genera *Leuconostoc*, *Lactobacillus*, and *Weissella* are likely to be mainly responsible for kimchi fermentation.

The most notable finding was the considerable difference in the bacterial diversity between kimchi and the ingredients used for kimchi fermentation. In particular, *Weissella*, *Pediococcus*, and *Leuconostoc* originating from Korean cabbage and radish were the dominant species in kimchi, and showed low abundance relative to other species in the other kimchi ingredients. Although there are various types of kimchi, differing mainly with respect to the primary ingredient used, the additional ingredients are usually the same.

This study also demonstrated a difference in the microbial relationships between kimchi and its ingredients. Bands of the 303, 503, 601, 702, and 803 fragments, and the 1107 and 1207 fragments were distantly located from the most closely related strain, uncultured bacterium and uncultured

*Lactobacillus* sp., respectively, suggesting that the species is an uncultured bacterium that may represent a new taxon at higher than the genus level. Therefore, phylogenetic analysis of 16S rRNA sequences is one of the most powerful methods for inferring the relationships between genera or between species belonging to a genus.

In summary, the bacterial community structures of the kimchi samples likely originated mainly from radish and Korean cabbage. In particular, *P. pentosaceus*, *L. citreum*, *L. gelidum*, and *L. mesenteroides* were the dominant bacteria in kimchi. The other strains identified were *Tetragenococcus*, *Pseudomonas*, *Weissella*, and uncultured bacterium. Therefore, a more comprehensive analysis of these microorganisms would provide a more detailed understanding of the biologically active components of kimchi and help improve its quality. Our results demonstrate that PCR-DGGE analysis can be successfully applied to kimchi to detect unculturable or more species than possible with a culture-dependent method. Thus, this technique is an effective and convenient culture-independent method for studying the microbial community in kimchi.

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