International Journal of Food Microbiology 126 (2008) 159–166

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Analysis of yeast and archaeal population dynamics in kimchi using denaturing gradient gel electrophoresis

Ho-Won Chang [a], Kyoung-Ho Kim [a], Young-Do Nam [a,c], Seong Woon Roh [a,c], Min-Soo Kim [a], Che Ok Jeon [d], Hee-Mock Oh [a], Jin-Woo Bae [a,b,c,*]

[a] Biological Resource Center, KRIBB, Daejeon 305-806, South Korea
[b] Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, South Korea
[c] Korea University of Science & Technology, 52, Eoeun-dong, Yuseong-gu, Daejeon, 305-333, South Korea
[d] Department of Life Science, Chung-Ang University, Seoul 156-756, South Korea

ARTICLE INFO

Article history:
Received 10 April 2008
Received in revised form 14 May 2008
Accepted 14 May 2008

Keywords:
Kimchi
DGGE
Archaea
Yeast

ABSTRACT

Kimchi is a traditional Korean food that is fermented from vegetables such as Chinese cabbage and radish. Many bacteria are involved in kimchi fermentation and lactic acid bacteria are known to perform significant roles. Although kimchi fermentation presents a range of environmental conditions that could support many different archaea and yeasts, their molecular diversity within this process has not been studied. Here, we use PCR-denaturing gradient gel electrophoresis (DGGE) targeting the 16S and 26S rRNA genes, to characterize bacterial, archaeal and yeast dynamics during various types of kimchi fermentation. The DGGE analysis of archaea expressed a change of DGGE banding patterns during kimchi fermentation, however, no significant change was observed in the yeast DGGE banding patterns during kimchi fermentation. No significant difference was indicated in the archaeal DGGE profile among different types of kimchi. In the case of yeasts, the clusters linked to the manufacturing corporation. Haloarchaea such as *Halococcus* spp., *Natronococcus* spp., *Natrialba* spp. and *Haloterrigena* spp., were detected as the predominant archaea and *Lodderomyces* spp., *Trichosporon* spp., *Candida* spp., *Saccharomyces* spp., *Pichia* spp., *Sporisorium* spp. and *Kluyveromyces* spp. were the most common yeasts.

1. Introduction

Fermentation is a well-known and ancient technique that uses microorganisms to process and preserve food (Ross et al., 2002). Kimchi is a Korean food prepared as a result of fermentation and in recent years it has been recognized as a health-promoting functional food (Song, 2004). Since it is interesting to know what microbes are involved by kimchi fermentation, the fermentation process has been studied extensively by microbiologists with respect to its ecology, proteomics, genetics and physiology (Kim and Chun, 2005; Li et al., 2006; Nan et al., 2005). These studies have reported that kimchi is a healthy food, rich in β-carotene, chlorophylls, dietary fiber and various minerals (Park et al., 2003). It supports a wide range of microorganisms, including lactic acid bacteria (LAB), which perform significant roles during fermentation (Bae et al., 2005; Kim and Chun, 2005). Bacterial isolates from kimchi have been investigated with respect to production and characterization of beneficial enzymes such as dextransucrase and alcohol/acetaldehyde dehydrogenase (Eom et al., 2007; Koo et al., 2005), and for biodegradation of toxic compounds such as sodium nitrite and bisphenol (Oh et al., 2004; Yamanaka et al., 2007).

Several halophilic archaea have been isolated from jeotgal, traditional Korean fermented seafood used in kimchi as an ingredient (Roh et al., 2007a; Roh et al., 2007b). Since the average NaCl concentration of kimchi is 3% (Mheen and Kwon, 1984), we reasoned that it could support haloarchaea. Yeasts such as *Pichia* spp. have been isolated from kimchi at low pH (about pH 4) that have been fermented over a long period of time (Oh and Han, 2003). However, the overall molecular diversity of yeasts and archaea in kimchi has not yet been reported.

Molecular biology techniques are used frequently to explore the diversity and structure of microbial communities, and microorganisms are identified using certain molecular markers such as 16S or 26S rRNA. In particular, the study of microbial ecology has benefited greatly from the introduction of denaturing gradient gel electrophoresis (DGGE), which provided a molecular fingerprinting technique for studying community structure (Yeates et al., 1998). In DGGE analysis, PCR amplicons of the same size but different sequences can be separated (Muyzer and Smalla, 1998) and this technique has been applied widely for studying microbial dynamics in complex environments such as soil

* Corresponding author. Biological Resource Center, KRIBB, Daejeon 305-806, South Korea. Tel.: +82 42 860 4628; fax: +82 42 860 4677.
E-mail address: baejw@kribb.re.kr (J.-W. Bae).

0168-1605/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2008.05.013

Table 1
List of PCR primers used in this study

	Designation	16S or 26S rRNA target (positions)[a]	Sequence (5′→3′)	Reference
Bacteria-specific primers	GC338f	338–357	ACGGGGGGACTCCTACGGGAGGCAGCAG	Muyzer et al. (1993)
	518r	518–534	ATTACCGCGGCTGCTGG	Muyzer et al. (1993)
Archaea-specific primers	GCarch340f	340–366	GGCACGGGCCCTACGGGGYGCASCAG	DeLong (1992)
	arch915r	915–935	GTGCTCCCCCGCCAATTCCT	DeLong (1992)
Yeast-specific primers	GCNL-1	110–134	GCATATCAATAAGCGGAGGAAAAG	Mills et al. (2002)
	NL-4	690–709	GGTCCGTGTTTCAAGACGG	Mills et al. (2002)
	LS2	266–285	ATTAAACAACTCGACTC	Cocolin et al. (2000b)

A GC clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGC-3′) was added to primers 338f, arch340f and NL-1 for DGGE analysis.
[a] Numbering denotes positions in *E. coli* for bacteria and archaea and in *Saccharomyces cerevisiae* for yeast.

(Sharma et al., 2006), sea (Bowman et al., 2003), insects (Reeson et al., 2003), sludge (Xia et al., 2005) and permafrost-affected soils (Ganzert et al., 2007). This method has also been used to investigate yeast diversity in foods such as wine (Cocolin et al., 2000a), sausage (Rantsiou et al., 2005), sourdough (Meroth et al., 2003) and coffee (Masoud et al., 2004).

In the present work, we studied population dynamics in the microbial community (bacteria, archaea and yeast) during fermentation of kimchi. We compared communities from various types of kimchi using culture-independent DGGE, which analyze 16S and 26S rRNA gene markers. For the best of our knowledge, this is the first report to reveal the diversity of archaea and yeast in kimchi analyzed by DGGE.

2. Materials and methods

2.1. Kimchi sampling

Kimchi was obtained from the distributors of commercially-available brands. Samples were taken immediately after production in the factory and then stored at 4 °C during the sampling period. Population dynamics were monitored during fermentation using a cabbage kimchi purchased from the 'C' corporation (designated P kimchi). P kimchi is the best-selling kimchi brand and the most representative kimchi in Korea. It is processed with a variety of ingredients such as cabbage, red pepper powder, garlic, ginger, onion, radishes and jeotgals. Samples (50 ml) of P kimchi soup were obtained periodically and after determining the pH, each sample was stored at −80 °C until extraction of bulk community DNA. In order to compare populations, seven types of kimchi and one spice were purchased from 3 different kimchi suppliers ('C' corporation, K1, K2 and K3; 'P' corporation, K4; and 'N' corporation, K5, K6 and K8). These varieties of kimchi were made using the following vegetables: Chinese cabbage, K1, K4 and K5; young radishes (yeolmoo in Korean), K2 and K6; and pony-tail radishes (chonggakmoo in Korean), K3. K7 is a spice made for K5 kimchi and mixture of a variety of ingredients: cabbage, red pepper powder, garlic, ginger, onion, radishes and jeotgals. K8 is a radish water kimchi (mulkimchi in Korean) that contains more water than ordinary kimchi. Samples were collected on day 11 after purchase, at which point fermentation was completed. They were then stored at −80 °C until DNA extraction.

2.2. DNA extraction and purification

Bulk community DNA was isolated from kimchi using the bead-beating method, as described previously (Yeates et al., 1998). All DNA samples were treated with RNase A (Sigma, St. Louis, MO) and then purified by ethanol precipitation. Extracted DNA was purified further using an UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA), with the following modifications. The bead-beating step and MD2 step were excluded. DNA solution was added to solution MD1 instead of the MicroBead solution. DNA concentrations were determined in triplicate using a spectrophotometer (Nanodrop Technologies, Rockland, DE).

2.3. PCR amplification

All primers used in this study are included in Table 1. For analysis of bacterial diversity, PCR amplification of the 16S rRNA gene was carried out using the bacteria-specific primer set GC338f-518r (Muyzer et al., 1993), as described previously (Henckel et al., 1999). For archaeal diversity analysis, PCR amplification of the 16S rRNA gene was performed using the arch340f-915r primer set (DeLong, 1992; Nakagawa and Fukui, 2003), as described previously (Ovreas et al., 1997). For analysis of yeast diversity, PCR amplifications of the 26S rRNA gene were performed as described previously (Prakitchaiwattana et al., 2004), with an initial amplification of the D1/D2 region using the eukaryotic universal primers NL-1 and NL-4 (Mills et al., 2002), followed by nested PCR using the primers GCNL-1 and LS2 (Cocolin et al., 2000b). DNA from each sample was subjected to DGGE following PCR amplification with each primer set (Table 1). All PCR amplifications were carried out in a final volume of 50 µl, containing 1 µl (50 ng/µl) template, 10 mM Tris HCl (pH 9.0), 40 mM KCl, 250 µM deoxynucleoside triphosphates (dNTPs), 2.5 U *Taq* polymerase, 1.5 mM $MgCl_2$ and 20 pmol of each primer. Reactions were performed in a PTC-220 DNA Engine Dyad MJ Research thermalcycler (PharmaTech, Seoul, Korea).

2.4. DGGE analysis

DGGE was performed using the D Code universal mutation system (Bio-Rad, Hercules, CA) for separation of PCR products. PCR products were applied directly onto 8% (wt/vol) polyacrylamide gels in a running buffer containing 1× TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA [pH8.3]) and a denaturing gradient of 30 to 60% urea and formamide for bacteria and yeast, or 35 to 55% urea and formamide for archaea (where 100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at 80 V for 14 h at a constant temperature of 60 °C. After electrophoresis, the gels were stained using SYBR Green I nucleic

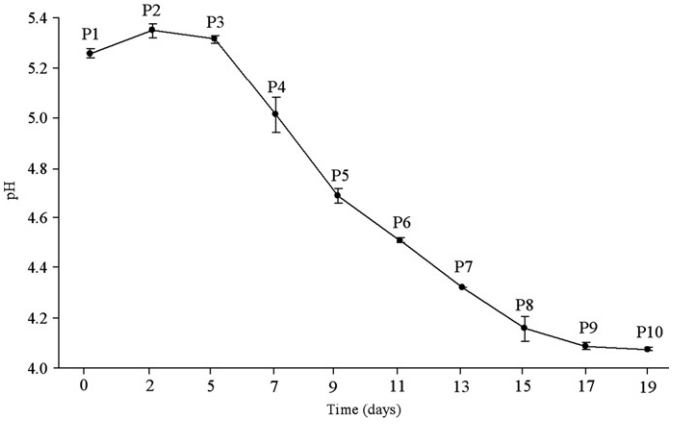


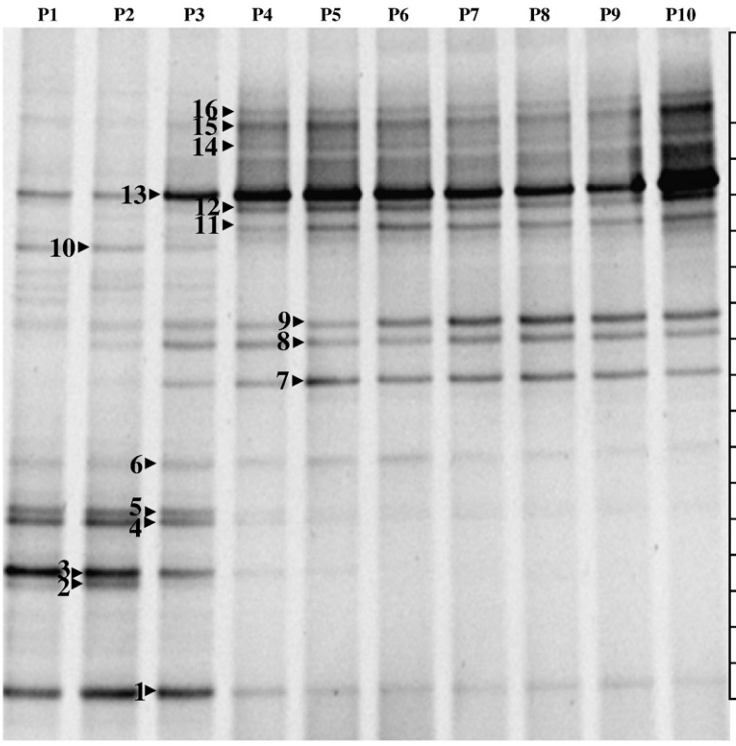
Fig. 1. pH change during P kimchi fermentation. The genomic DNA isolated from each sample was plotted against pH. Kimchi samples were named P1 to P10. Each error bar indicates the standard error.

acid stain (Bioneer) and photographed under UV transillumination. Sterile blades were used to excise bands from the gels, and these were then mixed with 20 µl of 0.1× TE buffer solution and incubated overnight at 4 °C. These solutions were then used for PCR amplification with the appropriate primer set.

2.5. Analysis of DGGE profiles

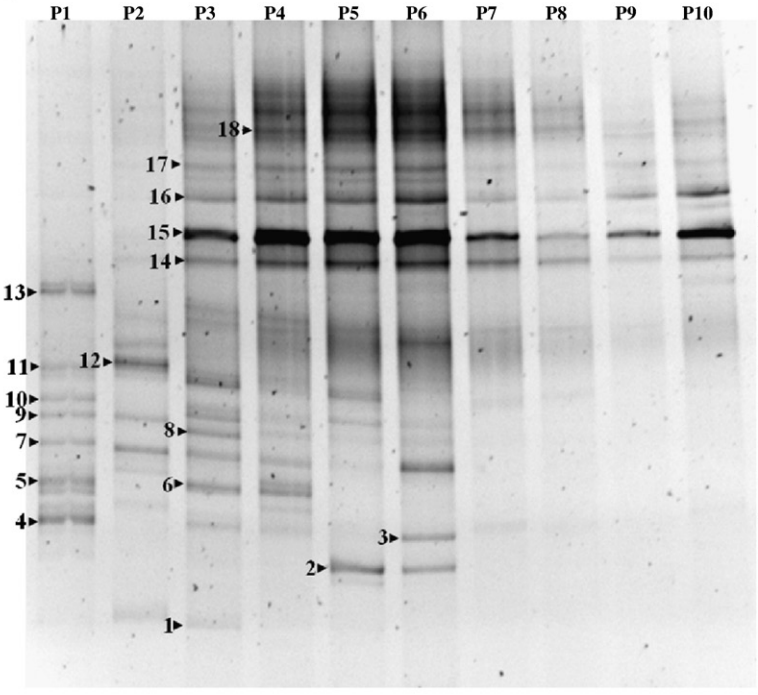
Cluster analyses of DGGE profiles were performed using BioNumerics software (BioSystematica, UK). For DGGE profile analysis, each lane was examined separately and common bands were selected as

A



DGGE Band No.	Closest relative	Sequence homology (%)
B1	*Atropa belladonna*	100
B2	*Arabidopsis thaliana*	99
B3	*Bacillus mycoides*	100
B4	*Bacillus pseudomycoides*	100
B5	*Zea mays chloroplast*	100
B6	*Weissella kandleri*	100
B7	*Leuconostoc gasicomitatum*	99
B8	*Weissella koreensis*	96
B9	*Lactobacillus sakei* subsp. sakei	98
B10	*Lactobacillus sakei* subsp. sakei	100
B11	*Weissella koreensis*	98
B12	*Weissella koreensis*	98
B13	*Weissella koreensis*	99
B14	*Weissella koreensis*	99
B15	*Weissella koreensis*	99
B16	*Weissella koreensis*	99

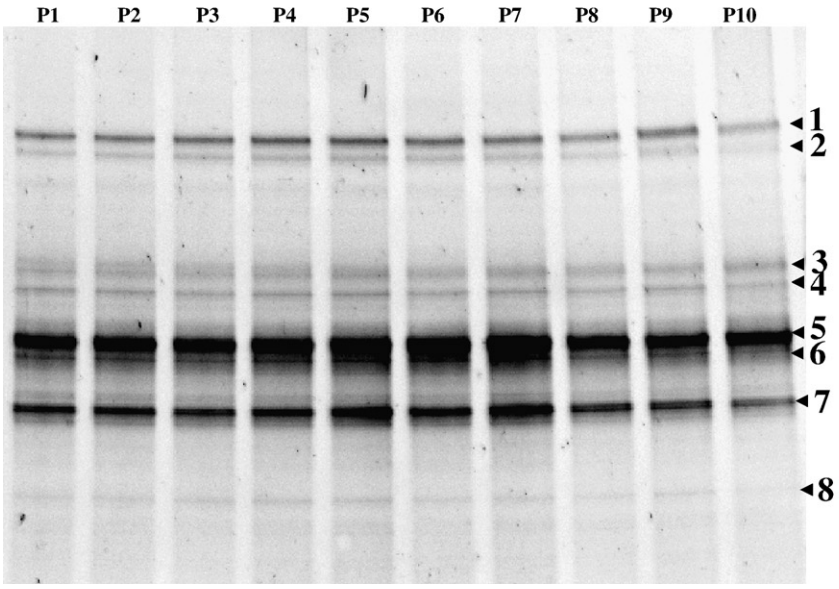
B



DGGE Band No.	Closest relative	Sequence homology (%)
A1	*Uncultured haloarchaeon* (AY396002)	97
A2	*Haloarchaeon* str. CSW2.24.4	91
A3	*Haloarchaeon* str. CSW2.24.4	92
A4	*Haloarchaeon* str. PW5.4	98
A5	*Halorubrum trapanicum*	98
A6	*Halococcus dombrowskii*	100
A7	*Halococcus dombrowskii*	99
A8	*Halococcus dombrowskii*	97
A9	*Halococcus dombrowskii*	99
A10	*Halococcus dombrowskii*	99
A11	*Haloterrigena thermotolerans*	90
A12	*Halococcus dombrowskii*	99
A13	*Halococcus dombrowskii*	96
A14	*Halococcus dombrowskii*	97
A15	*Halococcus dombrowskii*	98
A16	*Halococcus morrhuae*	97
A17	*Halococcus dombrowskii*	95
A18	*Halococcus dombrowskii*	96

Fig. 2. DGGE profiles of DNA extracted from kimchi samples (P1 to P10). (A) DGGE profiles of PCR-amplified DNA from the bacterial population in each sample. (B) DGGE profiles of PCR-amplified DNA from the archaeal population in each sample. (C) DGGE profiles of PCR-amplified DNA from the yeast population in each sample.

C



DGGE Band No.	Closest relative	Sequence homology (%)
Y1	*Lodderomyces elongisporus*	97
Y2	*Lodderomyces elongisporus*	95
Y3	*Trichosporon brassicae*	98
Y4	*Trichosporon middelhovenii*	98
Y5	*Candida sake*	98
Y6	*Trichosporon middelhovenii*	100
Y7	*Saccharomyces castellii*	99
Y8	*Kluyveromyces marxianus*	98

Fig. 2 (*continued*).

positions for normalization. The lanes were normalized to compensate for differences in migration distance due to gel heterogeneity. Dendrograms were calculated using the Pearson product moment correlation coefficient, with the unweighted pair group method and arithmetic averages clustering algorithm (UPGMA).

3. Results

3.1. Change in the microbial community during fermentation of kimchi

The pH change during fermentation of P kimchi is shown in Fig. 1. The DGGE banding patterns for bacteria, archaea and yeasts, were determined for each pH phase of kimchi fermentation (Fig. 2). pH decreased between P3 and P9, ranging between pH 5.3 and 4.1. The DGGE banding patterns of bacteria changed significantly between P3 and P4, but remained unchanged after P4 (Fig. 2A). Bands B1 to B5, which corresponded to two *Bacillus* and three eukaryotes, were not observed after P4, at which point the pH falls below 5.0. Bands B6 to B16 are amplification products from *Weissella kandleri*, *Weissella koreensis*, *Leuconostoc gasicomitatum* and *Lactobacillus sakei*. These bands could be observed in the initial lane (P1) and became prominent after P4, remaining until the end of fermentation. In addition, the sequences obtained from these bands showed great similarity to those bacteria isolated from kimchi in previous studies (Lee et al., 1997; Lee et al., 2002).

DGGE analysis of archaea indicated a different trend, in which the banding patterns of the initial points (P1, P2 and P3) were substantially different from each other. No significant differences had been observed between these three points in bacteria. The bands A14, A15, A16, A17 and A18 appeared at the third sampling point (P3), when pH started to decrease, and they were observed for the remainder of the fermentation (Fig. 2B). Bands A4, A5, A7, A9, A10, A11 and A13 were observed at P1 and corresponded to uncultured haloarchaea such as *Halococcus dombrowskii*, *H. thermotolerans*, and *Halorubrum trapanicum*. These bands were not detected after P2. Interestingly, no significant change was observed in the yeast DGGE banding patterns during kimchi fermentation. The bands Y1 to Y8 were found to be closely related to *Lodderomyces elongisporus*, *Trichosporon brassicae*, *Trichosporon middelhovenii*, *Candida sake*, *Saccharomyces castellii* and *Kluyveromyces marxianus*, and these bands were observed throughout fermentation (Fig. 2C).

3.2. Microbial diversity in various types of kimchi

Microbial diversity was investigated from seven types of kimchi and one spice. pH changes were monitored to determine appropriate sampling times and samples for DNA analysis were obtained on day 11, the first point at which pH stabilized (Fig. 3). DGGE profiles were used to compare the bacterial, archaeal and yeast communities in various types of kimchi (Fig. 4). The bacterial and archaeal DGGE profiles did not differ significantly between kimchi and sequencing of the dominant bands indicated the presence of well-known LAB such as *W. koreensis*, *Leuconostoc carnosum*, *Leuconostoc inhae*, *Lactobacillus nantensis* and *L.*

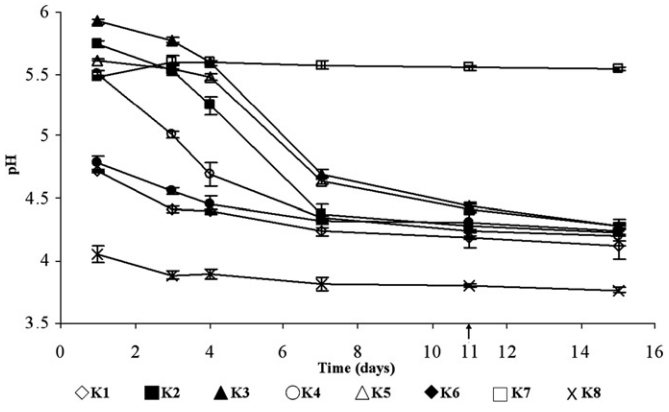


Fig. 3. pH change during fermentation of seven types of kimchi and one spice. Genomic DNA was extracted at day 11, toward the end of fermentation. K1, K4 and K5 are samples of Chinese cabbage kimchi from different suppliers. K2 and K6 are young radish kimchi (yeolmoo kimchi in Korean) from different suppliers. K3 is a pony-tail radish kimchi (chonggakmoo in Korean). K7 is a spice used in the preparation of K5 kimchi. K8 is radish water kimchi (mulkimchi in Korean), which is prepared from radishes and water. K1, K2 and K3 were all obtained from the same supplier (C corporation). K4 was from the P corporation and K5, K6 and K8 were from the N corporation. Each error bar indicates the standard error.

sakei (Fig. 4A), as well as halophilic archaea such as *Natronococcus jeotgali*, *Natronococcus zabuyensis*, *Natrialba aegyptiaca*, *Halosimplex carlsbadense* and *Halobiforma nitratireducens* (Fig. 4B). DGGE banding patterns for the yeast populations in various types of kimchi are shown in Fig. 4C. Their sequences corresponded to *Trichosporon domesticum*, *Trichosporon loubieri*, *T. brassicae*, *Trichosporon cutaneum*, *Sporisorium cutaneum*, *Saccharomyces unisporus* and *Pichia kluyveri*.

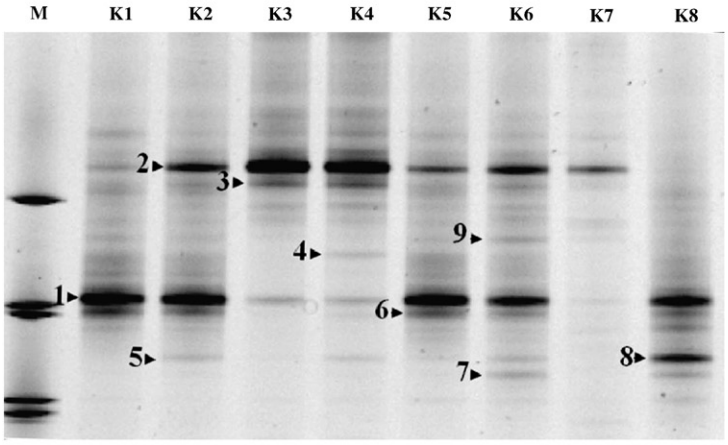
3.3. Cluster analysis of DGGE profiles

Cluster analysis of the bacterial DGGE profile indicated that the spice (K7) formed an out-group and that the two clusters (K1, K2, K5, K6 and K3, K4) showed >90% similarity with each other (Fig. 5A). These data confirm that the bacterial populations do not differ significantly between kimchi. Archaeal DGGE profiles divided into three clusters, group 1 (K1 and K2), group 2 (K3 and K4) and group 3 (K5 and K6), with members of each group showing about 85%, 98% and 78% similarity to each other, respectively. The cluster K1, K2, K5 and K6 exhibited about 72% similarity (Fig. 5B). In analysis of yeasts, the clusters linked to the manufacturing corporation, with different kimchi samples from the same supplier showing higher similarities to each other than to those from other suppliers; similarities between K5, K6 and K8 were >80% and between K1, K2 and K3 were >75% (Fig. 5C).

4. Discussion

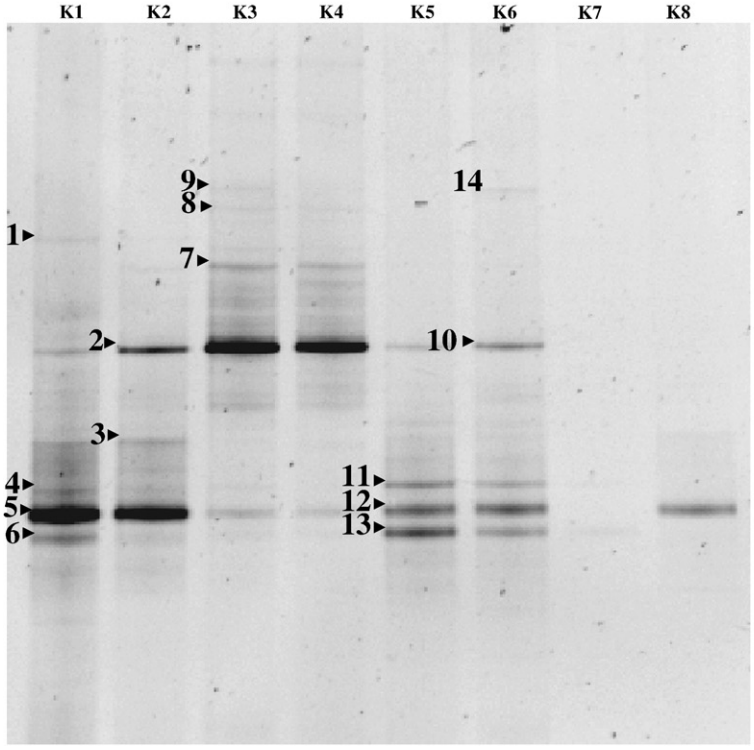
In general, the major ingredient of kimchi is Chinese cabbage and does not require the use of a starter culture. It is ripened by lactic fermentation and alcohol fermentation, a process performed primarily by LAB at low temperatures. Its main ingredients are vegetables such as cabbage or radish, and it can include additional ingredients such as onion, garlic, ginger and pepper. Currently, kimchi is in the health food spotlight because it is rich in nutrients and is recognized as a health-promoting functional food (Song, 2004). Although it has

A



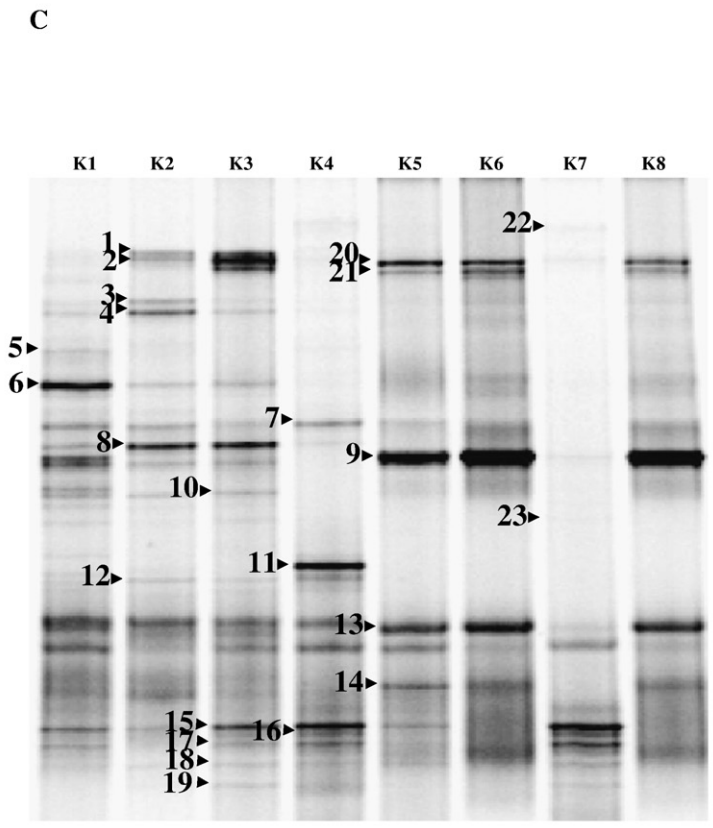
DGGE Band No.	Closest relative	Sequence homology (%)
DB1	*Lactobacillus sakei*	100
DB2	*Weissella koreensis*	100
DB3	*Weissella koreensis*	100
DB4	*Leuconostoc carnosum*	100
DB5	*Leuconostoc inhae*	100
DB6	*Lactobacillus sakei*	99
DB7	*Lactobacillus nantensis*	99
DB8	*Leuconostoc inhae*	100
DB9	*Lactobacillus sakei*	98

B



DGGE Band No.	Closest relative	Sequence homology (%)
DA1	*Natronococcus jeotgali*	97
DA2	*Natrialba aegyptiaca*	99
DA3	*Natrialba aegyptiaca*	99
DA4	*Natronococcus zabuyensis*	99
DA5	*Natronococcus jeotgali*	99
DA6	*Halosimplex carlsbadense*	99
DA7	*Natrialba aegyptiaca*	100
DA8	*Natrialba aegyptiaca*	99
DA9	*Natrialba aegyptiaca*	99
DA10	*Natrialba aegyptiaca*	97
DA11	*Natronococcus zabuyensis*	99
DA12	*Natronococcus jeotgali*	99
DA13	*Halosimplex carlsbadense*	99
DA14	*Halobiforma nitratireducens*	98

Fig. 4. DGGE profiles of PCR products amplified from various types of kimchi using specific primers for bacteria (A), archaea (B) and yeast (C).



DGGE Band No.	Closest relative	Sequence homology (%)
DY1	*Trichosporon domesticum*	98
DY2	*Trichosporon domesticum*	100
DY3	*Trichosporon domesticum*	100
DY4	*Trichosporon loubieri*	98
DY5	*Trichosporon brassicae*	98
DY6	*Trichosporon cutaneum*	98
DY7	*Trichosporon cutaneum*	97
DY8	*Trichosporon cutaneum*	98
DY9	*Saccharomyces unisporus*	99
DY10	*Trichosporon cutaneum*	94
DY11	*Sporisorium moniliferum*	98
DY12	*Trichosporon cutaneum*	97
DY13	*Saccharomyces unisporus*	100
DY14	*Saccharomyces unisporus*	100
DY15	*Pichia kluyveri*	99
DY16	*Pichia kluyveri*	98
DY17	*Pichia kluyveri*	99
DY18	*Pichia kluyveri*	99
DY19	*Pichia kluyveri*	99
DY20	*Saccharomyces unisporus*	100
DY21	*Saccharomyces unisporus*	100
DY22	*Pichia kluyveri*	100
DY23	*Pichia kluyveri*	98

Fig. 4 (*continued*).

been studied by food microbiologists, there has been no investigation of its yeast and archaeal diversity. Here, we examined the population dynamics of yeasts and archaea during fermentation of various types of kimchi using DGGE.

We determined that the pH of kimchi represented a significant factor in the diversity of bacteria and archaea. For example, the LAB detected by DGGE were found to proliferate during the initial period of ripening, producing lactic acid and hydrogen ions as byproducts of fermentation (Cheigh and Park, 1994). Decreasing pH suppressed growth of other bacteria and the population of bacteria did not change significantly after its decrease. *W. koreensis*, *L. sakei* and *L. inhae* were identified as the predominant bacteria in kimchi and they have also been found in various other fermented vegetable foods (Kim and Chun, 2005). Since it appears that these microorganisms perform essential roles in many food fermentations, they could also affect the taste and flavor of kimchi (Kim and Chun, 2005).

Some archaea disappeared as the pH decreased, while others were detected throughout the fermentation process. Even though most haloarchaea have been reported to grow well at near neutral pH, it would appear that some can survive at low pH (4–5) (Grant et al., 2001). Although the breakdown of proteins and other macromolecules occurs in high salinities, halophilic archaea inhabiting hypersaline environments are capable of living in high NaCl concentration (Kushner, 1985). In order for halophilic archaea to survive in halophilic environments, organic compounds such as amino acids and sugars accumulate in the cytoplasm, and osmotic adaptations can involve the selective influx of K^+ ions into the cytoplasm (Santos and da Costa, 2002). Most of the halophilic archaea studied, have been isolated from salt crystals, soda lakes and salty soil (Kanal et al., 1995; Vreeland et al., 2002; Xu et al., 2005) and these organisms require at least 9% NaCl for growth (Oren, 2000). However, some haloarchaea from coastal salt marshes have been shown to grow on 2.5% NaCl (Purdy et al., 2004) and kimchi also has a comparatively low concentration of NaCl (3%).

Most yeasts detected in the DGGE bands had been found previously in fermented foods such as cheese (*K. marxianus* and *S. unisporus*; Callon et al., 2006), cider and olives (*C. sake*; Coton et al., 2006), orange juice (*P. kluyveri*; Arias et al., 2002) and various fermented foods (*Pichia* spp., *Saccharomyces* spp. and *Kluyveromyces* spp.; Coton et al., 2006; Mills et al., 2002). However, *Trichosporon* spp. have rarely been identified from fermented foods and they were detected frequently in all types of kimchi. Furthermore, some of these were not closely related to commonly cultured strains (94–98%).

Different species of *Candida*, *Pichia* and *Trichosporon* were detected in kimchi and since these can grow in 2 to 4 M NaCl (Lages et al., 1999), many have been isolated previously from hypersaline environments (Butinar et al., 2005). A number of food spoilage yeasts were detected in kimchi including *Pichia* spp., *Saccharomyces* spp. and *Kluyveromyces* spp., all of which have been reported to grow well between pH 4.0 and 7.0 (Praphailong and Fleet, 1997). However, the diversity of yeast species did not change according to pH. This finding implies that growth of yeasts was suppressed during kimchi fermentation and that indigenous yeast might not be involved in or affect kimchi fermentation. However, it has been reported that foreign yeasts can affect the characteristics of kimchi, i.e. when yeast was used as starter, a high-

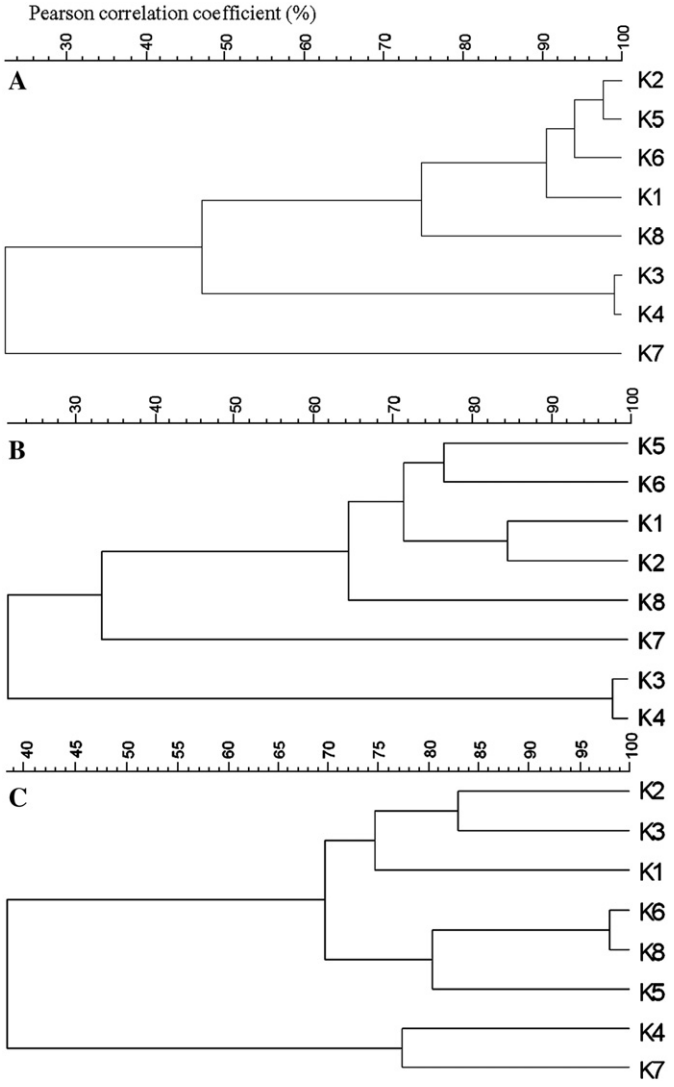


Fig. 5. Cluster analysis of the profiles obtained from DGGE banding patterns for bacteria, archaea and yeast. Dendrograms were calculated using the Pearson product moment correlation coefficient of similarity with the unweighted pair group method and the arithmetic averages clustering algorithm (UPGMA). Each kimchi sample was described in Fig. 3.

quality kimchi was produced that had less of a sour taste and smell than would be produced without starter culture (Kim et al., 1997).

This study also demonstrated that microbial diversity in kimchi is associated with supplier. Manufacturers made kimchi with mixtures of the main ingredients, as well as several additional ingredients. Although the different kinds of kimchi alter with respect to the main ingredient, the additional ingredients are usually the same. In the case of bacteria and archaea, it seems that the population differs a little between suppliers because of the generalized manufacturing process and ingredients. However, the yeast population was affected more by supplier than by type of kimchi.

The DGGE technique was effective and a convenient means for determining population structures. The combination of DNA-based molecular techniques allowed us to monitor changes in overall microbial composition during fermentation and show relationships between samples. From this study, we have identified members of the microbial community in kimchi and investigated the association between pH and population dynamics of bacteria, archaea and yeast during fermentation. Moreover, we detected novel archaea and yeast that could be important for inclusion in starter cultures and may have a capacity for production of beneficial compounds. Although bacteria such as LAB are isolated easily from kimchi, it is necessary to identify the other microorganisms in the community in order to establish their relationships and functional significance for the beneficial characteristics kimchi.

Acknowledgements

This work was supported by the KRIBB Research Initiative Program, the Environmental Biotechnology National Core Research Center Program (KOSEF: R15-2003-012-02002-0) and the Conservation Technology Research and Development project hosted by the National Research Institute of Cultural Heritage (of the Cultural Heritage Administration). The first author was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2007-512-C00016).

References

Arias, C.R., Burns, J.K., Friedrich, L.M., Goodrich, R.M., Parish, M.E., 2002. Yeast species associated with orange juice: evaluation of different identification methods. Applied and Environmental Microbiology 68, 1955–1961.

Bae, J.W., Rhee, S.K., Park, J.R., Chung, W.H., Nam, Y.D., Lee, I., Kim, H., Park, Y.H., 2005. Development and evaluation of genome-probing microarrays for monitoring lactic acid bacteria. Applied and Environmental Microbiology 71, 8825–8835.

Bowman, J.P., McCammon, S.A., Gibson, J.A., Robertson, L., Nichols, P.D., 2003. Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Applied and Environmental Microbiology 69, 2448–2462.

Butinar, L., Santos, S., Spencer-Martins, I., Oren, A., Gunde-Cimerman, N., 2005. Yeast diversity in hypersaline habitats. FEMS Microbiology Letters 244, 229–234.

Callon, C., Delbes, C., Duthoit, F., Montel, M.C., 2006. Application of SSCP-PCR fingerprinting to profile the yeast community in raw milk Salers cheeses. Systematic and Applied Microbiology 29, 172–180.

Cheigh, H.S., Park, K.Y., 1994. Biochemical, microbiological, and nutritional aspects of kimchi (Korean fermented vegetable products). Critical Reviews in Food Science and Nutrition 34, 175–203.

Cocolin, L., Bisson, L.F., Mills, D.A., 2000a. Direct profiling of the yeast dynamics in wine fermentations. FEMS Microbiology Letters 189, 81–87.

Cocolin, L., Manzano, M., Cantoni, C., Comi, G., 2000b. Development of a rapid method for the identification of *Lactobacillus* spp. isolated from naturally fermented Italian sausages using a polymerase chain reaction-temperature gradient gel electrophoresis. Letters in Applied Microbiology 30, 126–129.

Coton, E., Coton, M., Levert, D., Casaregola, S., Sohier, D., 2006. Yeast ecology in French cider and black olive natural fermentations. International Journal of Food Microbiology 108, 130–135.

DeLong, E.F., 1992. Archaea in coastal marine environments. Proceedings of the National Academy of Sciences of the United States of America 89, 5685–5689.

Eom, H.J., Seo, D.M., Han, N.S., 2007. Selection of psychrotrophic *Leuconostoc* spp. producing highly active dextransucrase from lactate fermented vegetables. International Journal of Food Microbiology 117, 61–67.

Ganzert, L., Jurgens, G., Munster, U., Wagner, D., 2007. Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. FEMS Microbiology Ecology 59, 476–488.

Grant, W.D., Kamekura, M., McGenity, T.J., Ventosa, A., 2001. Class III. Halobacteria class. nov. In: Boone, D.R., Castenholz, R.W. (Eds.), Bergey's Manual of Systematic Bacteriology. Springer, New York, USA, pp. 294–334.

Henckel, T., Friedrich, M., Conrad, R., 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Applied and Environmental Microbiology 65, 1980–1990.

Kanal, H., Kobayashi, T., Aono, R., Kudo, T., 1995. *Natronococcus amylolyticus* sp. nov., a haloalkaliphilic archaeon. International Journal of Systematic Bacteriology 45, 762–766.

Kim, M., Chun, J., 2005. Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. International Journal of Food Microbiology 103, 91–96.

Kim, H.J., Kang, S.M., Yang, C.B., 1997. Effects of yeast addition as starter on fermentation of Kimchi. Korean Journal of Food Science Technology 29, 790–799.

Koo, O.K., Jeong, D.W., Lee, J.M., Kim, M.J., Lee, J.H., Chang, H.C., Kim, J.H., Lee, H.J., 2005. Cloning and characterization of the bifunctional alcohol/acetaldehyde dehydrogenase gene (adhE) in *Leuconostoc mesenteroides* isolated from kimchi. Biotechnology Letters 27, 505–510.

Kushner, D.J., 1985. The Halobacteriaceae. In: Woese, C.R., Wolfe, R.S. (Eds.), The bacteria, vol. 8. Academic Press, Inc., New York, pp. 171–214.

Lages, F., Silva-Graca, M., Lucas, C., 1999. Active glycerol uptake is a mechanism underlying halotolerance in yeasts: a study of 42 species. Microbiology 145, 2577–2585.

Lee, J.S., Chun, C.O., Hector, M., Kim, S.B., Kim, H.J., Park, B.K., Joo, Y.J., Lee, H.J., Park, C.S., Ahn, J.S., Park, Y.H., Mheen, T.I., 1997. Identification of *Leuconostoc* strains isolated from Kimchi using carbon-source utilization patterns. Journal of Microbiology 35, 10–14.

Lee, J.S., Lee, K.C., Ahn, J.S., Mheen, T.I., Pyun, Y.R., Park, Y.H., 2002. *Weissella koreensis* sp. nov., isolated from kimchi. International Journal of Systematic and Evolutionary Microbiology 52, 1257–1261.

Li, S.J., Paik, H.Y., Joung, H., 2006. Dietary patterns are associated with sexual maturation in Korean children. British Journal of Nutrition 95, 817–823.

Masoud, W., Cesar, L.B., Jespersen, L., Jakobsen, M., 2004. Yeast involved in fermentation of *Coffea arabica* in East Africa determined by genotyping and by direct denaturating gradient gel electrophoresis. Yeast 21, 549–556.

Meroth, C.B., Hammes, W.P., Hertel, C., 2003. Identification and population dynamics of yeasts in sourdough fermentation processes by PCR-denaturing gradient gel electrophoresis. Applied and Environmental Microbiology 69, 7453–7461.

Mheen, T.I., Kwon, T.W., 1984. Effect of temperature and salt concentration on kimchi fermentation. Korean Journal of Food Science Technology 16, 443–450.

Mills, D.A., Johannsen, E.A., Cocolin, L., 2002. Yeast diversity and persistence in botrytis-affected wine fermentations. Applied and Environmental Microbiology 68, 4884–4893.

Muyzer, G., Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Leeuwenhoek 73, 127–141.

Muyzer, G., De Wall, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695–700.

Nakagawa, T., Fukui, M., 2003. Molecular characterization of community structures and sulfur metabolism within microbial streamers in Japanese hot springs. Applied and Environmental Microbiology 69, 7044–7057.

Nan, H.M., Park, J.W., Song, Y.J., Yun, H.Y., Park, J.S., Hyun, T., Youn, S.J., Kim, Y.D., Kang, J.W., Kim, H., 2005. Kimchi and soybean pastes are risk factors of gastric cancer. World Journal of Gastroenterology 11, 3175–3181.

Oh, J.Y., Han, Y.S., 2003. Purification and characterization of L-galactono-γ-lactone oxidase in *Pichia* sp. isolated from kimchi. Korean Journal of Food Science Technology 35, 1135–1142.

Oh, C.K., Oh, M.C., Kim, S.H., 2004. The depletion of sodium nitrite by lactic acid bacteria isolated from kimchi. Journal of Medicinal Food 7, 38–44.

Oren, A., 2000. The order Halobacteriales. In: Dworkin, M. (Ed.), The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community. Springer-Verlag, New York.

Ovreas, L., Forney, L., Daae, F.L., Torsvik, V., 1997. Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Applied and Environmental Microbiology 63, 3367–3373.

Park, K.Y., Cho, E.J., Rhee, S.H., Jung, K.O., Yi, S.J., Jhun, B.H., 2003. Kimchi and an active component, beta-sitosterol, reduce oncogenic H-Ras(v12)-induced DNA synthesis. Journal of Medicinal Food 6, 151–156.

Prakitchaiwattana, C.J., Fleet, G.H., Heard, G.M., 2004. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. FEMS Yeast Research 4, 865–877.

Praphailong, W., Fleet, G.H., 1997. The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts. Food Microbiology 14, 459–468.

Purdy, K.J., Cresswell-Maynard, T.D., Nedwell, D.B., McGenity, T.J., Grant, W.D., Timmis, K.N., Embley, T.M., 2004. Isolation of haloarchaea that grow at low salinities. Environmental Microbiology 6, 591–595.

Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., Cattaneo, P., Comi, G., Cocolin, L., 2005. Culture-dependent and -independent methods to investigate the microbial ecology of Italian fermented sausages. Applied and Environmental Microbiology 71, 1977–1986.

Reeson, A.F., Jankovic, T., Kasper, M.L., Rogers, S., Austin, A.D., 2003. Application of 16S rDNA-DGGE to examine the microbial ecology associated with a social wasp *Vespula germanica*. Insect Molecular Biology 12, 85–91.

Roh, S.W., Nam, Y.D., Chang, H.W., Sung, Y., Kim, K.H., Lee, H.J., Oh, H.M., Bae, J.W., 2007a. *Natronococcus jeotgali* sp. nov., a halophilic archaeon isolated from shrimp jeotgal, a traditional fermented seafood from Korea. International Journal of Systematic and Evolutionary Microbiology 57, 2129–2131.

Roh, S.W., Nam, Y.D., Chang, H.W., Sung, Y., Kim, K.H., Oh, H.M., Bae, J.W., 2007b. *Halalkalicoccus jeotgali* sp. nov., a halophilic archaeon from shrimp jeotgal, a traditional Korean fermented seafood. International Journal of Systematic and Evolutionary Microbiology 57, 2296–2298.

Ross, R.P., Morgan, S., Hill, C., 2002. Preservation and fermentation: past, present and future. International Journal of Food Microbiology 79, 3–16.

Santos, H., da Costa, M.S., 2002. Compatible solutes of organisms that live in hot saline environments. Environmental Microbiology 4, 501–509.

Sharma, S., Szele, Z., Schilling, R., Munch, J.C., Schloter, M., 2006. Influence of freeze–thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Applied and Environmental Microbiology 72, 2148–2154.

Song, Y.O., 2004. The functional properties of kimchi for the health benefits. Journal of Food Science and Nutrition 9, 27–33.

Vreeland, R.H., Straight, S., Krammes, J., Dougherty, K., Rosenzweig, W.D., Kamekura, M., 2002. *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. Extremophiles 6, 445–452.

Xia, S., Shi, Y., Fu, Y., Ma, X., 2005. DGGE analysis of 16S rDNA of ammonia-oxidizing bacteria in chemical–biological flocculation and chemical coagulation systems. Applied and Environmental Microbiology 69, 99–105.

Xu, X.W., Wu, M., Zhou, P.J., Liu, S.J., 2005. *Halobiforma lacisalsi* sp. nov., isolated from a salt lake in China. International Journal of Systematic and Evolutionary Microbiology 55, 1949–1952.

Yamanaka, H., Moriyoshi, K., Ohmoto, T., Ohe, T., Sakai, K., 2007. Degradation of bisphenol A by *Bacillus pumilus* isolated from kimchi, a traditionally fermented food. Applied Biochemistry and Biotechnology 136, 39–51.

Yeates, C., Gillings, M.R., Davison, A.D., Altavilla, N., Veal, D.A., 1998. Methods for microbial DNA extraction from soil for PCR amplification. Biological Procedures Online 1, 40–47.