Analysis of Microbial Diversity in Low -Temperature Fermented Kimchi

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Kimchi, is a Korean-traditional fermented vegetable food, has a major situation in Korean food style. The microbial diversities of kimchi were analyzed by many research groups for last decades using culture dependent methods to build a standard index in kimchi fermentation. It was confirmed that major microorganisms responsible for kimchi fermentation are lactic acid bacteria (LAB) and yeasts are known to be role for softening of kimchi texture and off-flavor. The major genus and species of LAB isolated and identified from kimchis were known as *Leuconosctoc mesenteroides*, *Leuconostoc dextranicum*, *Leuconostoc citreum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *and Streptococcus faecalis*. But it has somewhat controversy whether these microorganisms occupied major partition in real environment of kimchi, since the environment of media used in isolation and identification of kimchi fermented microorganisms was differ from that of kimchi.

In recent years, the attention has been drawn toward specific DNA probes and rRNAs, whose genes contain highly conserved regions. To recover of the limit on culture dependent method for determination of environmental microbial diversity, they used culture independent method using total RNA and DNA directly extracted from environments.

In generally, it was thought that the kimchi fermented at winter was more delicious than kimchies fermented at other seasons. It would be caused by the fermentation temperature, and temperature could be closely related to the ecology of fermented microorganisms.

So we tried to investigate the distribution of microorganisms in kimchi fermented at 4℃ by culture independent methods, including determination of microbial fingerprints by denaturing gradient gel electrophoresis (DGGE), quantification of microbial taxa with 16S rRNA-targeted oligonucleotide probes, and 16S ribosomal DNA gene sequencing. Kimchi samples were taken every 5 days over the fermentation periods (for 60 days) to extract total DNA and RNA for culture independent microbial diversity analysis. Touchdown polymerase chain reaction was performed to amplify the V3 region of 16S rRNA gene for DGGE analysis. Sequencing results of partial 16S rDNA amplicons from DGGE profiles revealed that lactic acid bacteria (LAB) were dominants in kimchi fermentation. Especially, *Weissella koreensis*, *Lactobacillus sakei*, *and Leuconostoc gelidum* were major microorganisms in low temperature fermented kimchi. *Weissella koreensis* steadily exited throughout the whole fermentation period, *Lactobacillus sakei* showed up from 10th day, and

Leuconostoc gelidum from 30th day. In the hybridization analysis with 16S rRNA-targeted genus specific probes, lactic acid bacteria were also identified as a dominant microorganisms in kimchi fermentation. *Lactobacillus* sp., *Weissella* sp., and *Leuconostoc* sp. were determined as major lactic acid bacteria related to kimchi fermentation, but *Lactococcus* sp. and *Streptococcus* sp. were not detected.

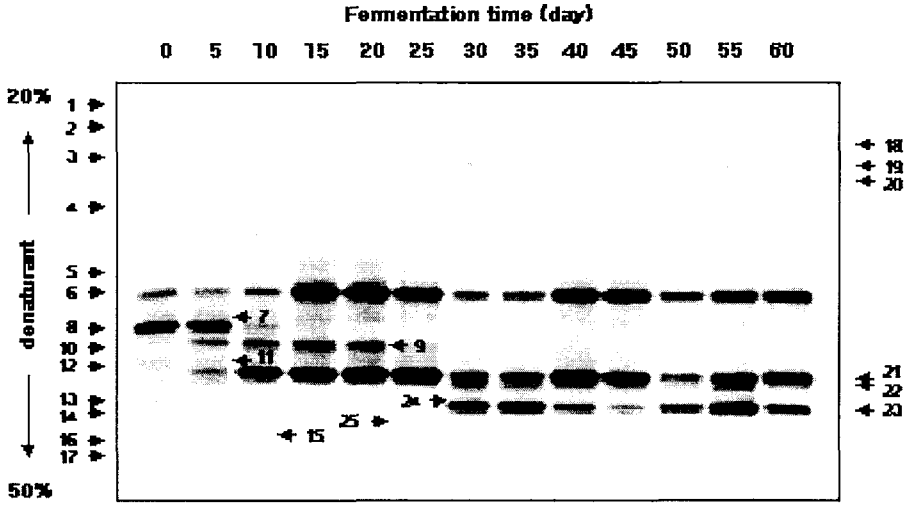

Fig. 1. DGGE profile of 16S-rDNA fragment from kimchi samples. Arabic numbers indicates amplicons.

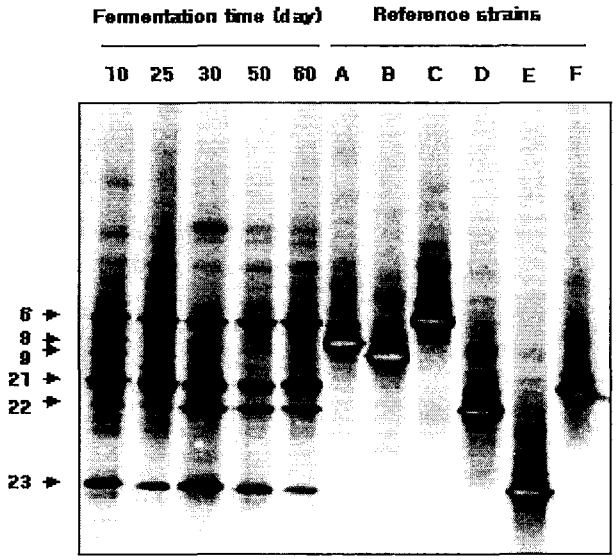

Fig. 2. DGGE profile of 16S-rDNA fragments from reference strains and kimchi samples. Lanes: A, *Leuc. citreum* KCTC 3526^T; B, *Leuc. mesenteroides* subsp. *mesenteroides* KCTC 3505^T; C, *W. koreensis* KCTC 3621^T; D, *Leuc. pseudomesenteroides* KCTC 3652^T; E, *Leuc. gelidum* KCTC 3527^T; F, *Lb. sakei* KCTC 3603^T. Numbers of bands are same to numbers in Table 1.

Table 1. Identification of the amplicons obtained from DGGE analysis of kimchi samples

Amplicon[a]	Closest relatives[b]	% identity	Accession no.
1	*Leuc. citreum/pseudomesenteroides*	98%	AY241941
2	*Leuc. citreum /pseudomesenteroides*	98%	AY241942
3	*Leuc. mesenteroides*	97%	AY241948
4	*Leuc. pseudomesenteroides*	96%	AY241949
5	*Leuc. mesenteroides*	96%	AY241952
6	*W. koreensis*	100%	AY241937
7	*W. koreensis*	94%	AY241946
8	*Leuc. citreum*	100%	AY241943
9	*Leuc. mesenteroides*	98%	AY241944
10	*Uncultured bacterium*	97%	AY241945
11	*Unclturred gamma proteobacterium*	96%	AY241953
12	Arctic sea ice bacterium	98%	AY241954
13	Arctic sea ice bacterium	97%	AY241955
14	*Lb. fuchensis / Lb. sakei*	97%	AY241950
15	*Lb. fuchensis / Lb. sakei*	97%	AY241951
16	Uncultured bacterium	95%	
17	*Leuc. pseudomesenteroides*	95%	
18	*W. koreensis*	97%	AY241934
19	*Leuc. gelidum*	99%	AY241935
20	*Leuc. pseudomesenteroides*	98%	AY241936
21	*Lb. sakei*	99%	AY241938
22	*Leuc. pseudomesenteroides*	97%	AY241939
23	*Leuc. gelidum/inhae*	99%	AY241940
24	*Leuc. gelidum/inhae*	98%	AY241947
25	*Lb. curvatus*	97%	AY241956

[a]Bands were extracted from DGGE gel shown fig. 1.

[b]Percentage of identical nucleotides between the sequences retrieved from the DGGE gel and the closet relative found in Genbank or RDP. Comparison was made using partial 16S rDNA sequences only (around 180 bases, corresponding to the region sequenced.

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