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RESEARCH NOTE

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Microbiological Analysis of *Dongchimi*, Korean Watery Radish *Kimchi*, at the Early and Mid-phase Fermentation

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Abstract During *dongchimi* fermentation at 5 and 25°C, the pH lowered slowly and reached 4.03 at 5°C after 30 days, whereas it lowered dramatically and reached 3.59 at 25°C after 2 days. The predominant bacteria were *Leuconostoc* (*Leu.*) *mesenteroides* at 25°C until day 2 which changed into *Lactobacillus* (*Lb.*) *plantarum* at day 3, analyzed by a culture dependent method with partial 16S rRNA gene sequencing, whereas *Leu. mesenteroides* occupied predominantly at 5°C until day 7. In a culture-independent method using a polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) with partial 16S rRNA gene sequencing, *Lb. algidus* was predominant at 5°C until day 7 and *Lb. plantarum* occupied predominantly at 25°C until day 3, which is different from the results of the culture based method, indicating the both methods need to be combined for accuracy. Based on the culture-dependent method, *Leu. mesenteroides* might be responsible for the early and mid-phase of *dongchimi* fermentation.

Keywords: *dongchimi*, *kimchi* fermentation, *Leuconostoc mesenteroides*, denaturing gradient gel electrophoresis (DGGE)

Introduction

Kimchi is Korean traditional fermented vegetable where lactic acid bacteria (LAB) are mainly involved (1,2). *Dongchimi* is a kind of *kimchi* and usually consumed in winter in Korea. To produce *dongchimi*, radish, garlic, fish sauce, and fruits are used as the main ingredients with plenty of water. Generally, CO_2, lactate, and other organic acids are produced during *dongchimi* fermentation (3). In previous papers, physicochemical properties and cell counts were investigated during the fermentation (3-6), however, major bacterial composition was rarely investigated. The analysis of microbial community is important to understand the fermentation profile and to develop starter culture for commercial product. In this study, we analyzed the microbial communities of *dongchimi* at the early and mid-phase of the fermentation, recognized as palatability, by a culture-dependent method combined with partial 16S rRNA gene sequencing. Concomitantly, a culture-independent method using a polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was applied to this analysis. The PCR-DGGE technique enables us to analyze the microbiota in samples by one-step and has been used to study the microbial communities in fermented foods such as sausages, *shochu* (Japanese fermented food), *pozol* (Mexican fermented maize dough), and *kimchi* (7-10).

Materials and Methods

Cultivation of microorganisms Freshly manufactured *dongchimi*, containing radish, garlic, *wakegi*, pepper, and salt water as main ingredients, was got from a local factory and stored at 5 and 25°C, respectively, for fermentation. Aliquots of the samples were taken periodically and their pH values were measured. They were subsequently diluted with peptone buffer (0.85% NaCl, 0.01% peptone, 0.003% KH_2PO_4, and 0.006% Na_2HPO_4) and total aerobic bacteria and LAB were cultured on plate count agar (PCA, Difco Laboratories, Sparks, MD, USA) and MRS agar (Difco), respectively.

Identification of the colonies Appropriate number of colonies, randomly selected from PCA plates, was subjected to PCR for partial 16S rRNA gene amplification using primers 338f (5'-actccgacgggaggcagcag-3') and 518r (5'-attaccgcggctgctgg-3') spanning the V3 region of the 16S rRNA gene (8,10,11). The PCR was performed with a premix (Bioneer Co., Daejeon, Korea) composing 2.5 U of *Taq* DNA polymerase, 250 μM of each dNTP, 10 mM of Tris-HCl (pH 9.0), 40 mM of KCl, and 1.5 mM of $MgCl_2$ per 50 μL reaction under conventional cycle condition. The nucleotide sequences of the PCR products were analyzed for identification of isolates. Homology search of the DNA sequences was performed by the Basic Local Alignment Search Tool (BLAST) at GenBank, National Center for Biotechnology Information (NCBI). Morphological and biochemical tests such as Gram-staining, catalase, and oxidase activities were also performed for further evidences.

PCR-DGGE Whole genomic DNA from each sample was purified by the Blood & Cell Culture DNA Maxi kit (Qiagen Co., Hilden, Germany) according to manufacturer's instruction. Partial 16S rRNA gene sequences from the whole genomic DNA were amplified with the premix using primers gc338f (5'-cgcccgccgcgcgcggcgggcggggcggggg cacgggggggactccgacgggaggcagcag-3') and 518r under conventional cycle condition except for annealing temperature, to which a touchdown PCR method was

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applied for reducing non-specific amplicons. In other words, the initial annealing temperature was 65°C and the temperature was decreased by 1°C every second cycle until 55°C and additional 10 cycles at 55°C were carried out. The PCR products were separated by DGGE using DCode Universal Mutation Detection System (Bio-Rad Co., Hercules, CA, USA). The samples were loaded into 8%(w/v) polyacrylamide gel with gradient denaturant of 10-60% urea-formamide [7 M urea and 40%(v/v) formamide] in 1× TAE buffer. The unit was operated at 60 V for 30 min and then 200 V for 3 hr, followed by gel staining with SYBR Green I (Roche Co., Penzberg, Germany) for 30 min and the gel image was captured by a digital camera combined with a transilluminator (Takara Co., Shiga, Japan). The DNA bands on the gel were excised with sterile scalpel and the DNAs were eluted in 30 μL of sterile water at 4°C overnight. PCR amplicons for partial 16S rRNA gene were produced by the method described above using the eluted DNAs as templates with primers 338f-518r and sequenced by a biotech company (Macrogen Co., Seoul, Korea) and homology search was performed at the BLAST.

Results and Discussion

During the *dongchimi* fermentation, the pH lowered slowly and reached 4.03 at 5°C after 30 days, whereas it lowered fast and reached 3.59 at 25°C after 2 days. The LAB cell count was the highest as 6.4×10^8 CFU/mL at day 2 of 25°C and the count at 5°C slowly increased and reached 1.3×10^7 CFU/mL after 30 days (Fig. 1). Predominant bacteria were *Leuconostoc* (*Leu.*) *mesenteroides* at 5°C until day 7, mid-phase of the fermentation, followed by *Lactobacillus* (*Lb.*) *plantarum, Lb. brevis, and Lb. curvatus*. In the case of 25°C, *Leu. mesenteroides* was also predominant until day 2, mid-phase of the fermentation, and the predominant bacteria changed into *Lb. plantarum* at day 3 (Table 1). These results agree with other data from *kimchi* fermentations, described in previous studies (12-14). In general, *Leu. mesenteroides* is recognized as predominant bacteria at the early and mid-phase, recognized as palatability, during *kimchi* fermentation and then the predominant bacteria change into *Lb. plantarum*, which is acid-tolerant and causes acidification of *kimchi*. However, in our PCR-DGGE analysis combined with partial 16S rRNA gene sequencing, the predominant bacteria at both temperatures were different from the results of the culture-dependent methods. Namely, *Lb. algidus* was predominant at 5°C until day 7 and *Lb. plantarum* was predominant at 25°C until day 3 (Fig. 2). Surprisingly, we could not detect *Leu. mesenteroides*, predominant bacteria in the culture-dependent methods, which could be a controversial matter. In a previous report, the authors mentioned the responsible bacteria for *kimchi* fermentation were *Weissella confusa*, *Leu. citreum*, *Lb. sakei*, and *Lb. curvatus*, determined by culture-independent molecular technique PCR-DGGE combined with 16S rRNA gene sequencing (15). Like our case, *Leu. mesenteroides* was not detected in the study. However, the authors did not introduce culture-dependent methods to the study for the comparison. So, it might be difficult to determine whether the PCR-DGGE results represent the *kimchi* fermentation. In a recent paper, the

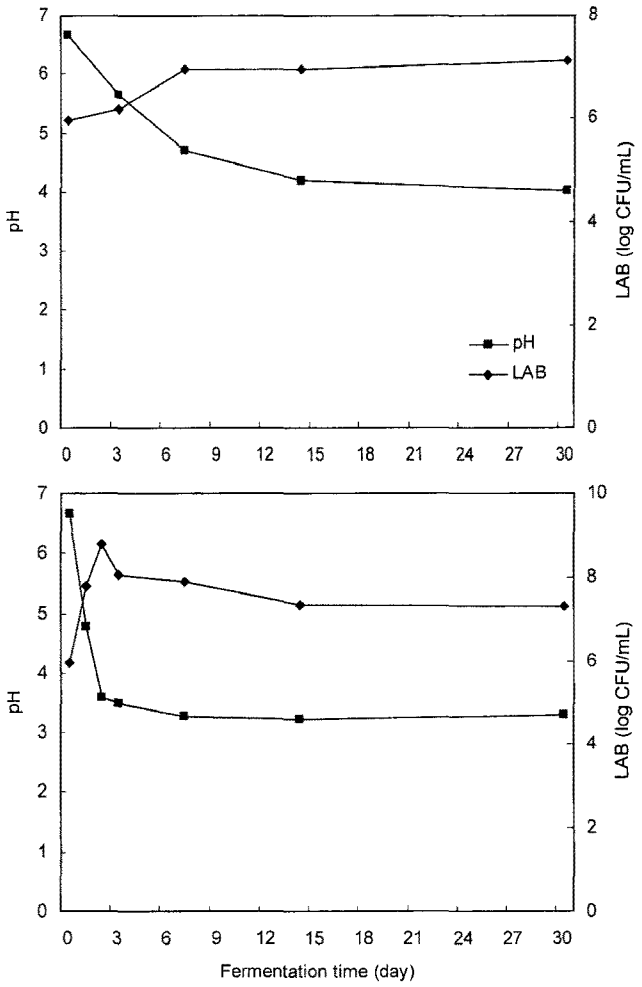

Fig. 1. Changes of pH and LAB cell counts during *dongchimi* fermentations at 5 (A) and 25°C (B).

Table 1. Identification of isolates from *dongchimi* samples

Temperature	Time (day)	Identification[1)]	Number of colonies
5°C	0[2)]	*Leuconostoc mesenteroides*	22
		Lactobacillus plantarum	6
		Lactobacillus curvatus	1
	3	*Leuconostoc mesenteroides*	22
		Lactobacillus plantarum	2
	7	*Leuconostoc mesenteroides*	34
		Lactobacillus plantarum	3
		Lactobacillus brevis	2
25°C	1	*Leuconostoc mesenteroides*	40
		Lactobacillus plantarum	4
		Lactobacillus brevis	3
	2	*Leuconostoc mesenteroides*	13
		Lactobacillus plantarum	5
	3	*Lactobacillus plantarum*	11
		Leuconostoc mesenteroides	5

[1)]The bacteria were identified by a culture-dependent method combined with partial 16S rRNA gene sequencing.
[2)]The result of day 0 is for both temperatures 5 and 25°C.

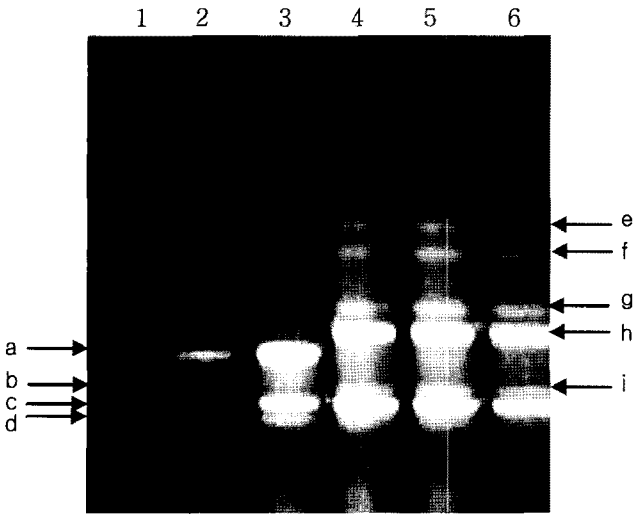

Fig. 2. DGGE analysis of partial 16S rRNA gene fragments from microbiota of *dongchimi* samples at 5 and 25°C. Day 0 (lane 1), 3 (lane 2), and 7 (lane 3) at 5°C; day 1 (lane 4), 2 (lane 5), and 3 (lane 6) at 25°C. The DNA bands correspond to partial 16S rRNA gene products. The DNA sequences of the products were best matched with those of *Lactobacillus algidus* (a), *Lactobacillus curvatus* (b, c), *Lactobacillus intestinalis* (d), *Lactobacillus plantarum* (e, g, h), *Lactobacillus* sp. (f), and *Streptococcus difficile* type strain (i).

results of both methods for microbial composition from a traditional Italian cheese were different from each other. In relation to the difference, the authors indicated the permanent bacterial DNA from cell autolysis for the culture-independent method and selectivity of media for the culture-dependent method and highlighted the importance of combination of the both methods for the study of complex microbial communities from food matrix (16). We also suggest that traditional culture based method and molecular techniques should be combined to get more reasonable and informative results for microbial compositions from foods. Besides the fermentation experiments, we purchased commercial *dongchimi* products in palatability (pH 4.0-4.2) and analyzed their microbial communities by the culture-dependent method used in this study. The results showed the predominant bacteria were *Leu. mesenteroides* followed by *Lactobacillus* spp. including *Lb. curvatus*, *Lb. brevis*, and *Lb. plantarum* in all products (data not shown), which supports our fermentation experiments.

As described above, microbiota is very important to fermentation foods and has influence on the quality of the products. For this reason, many researchers have tried to know what predominant bacteria exist during the fermentations. In this respect, we tried to analyze the microbial communities at the early and mid-phase of *dongchimi* fermentation by a culture-dependent method combined with partial 16S rRNA gene sequencing and a molecular technique, PCR-DGGE, was also applied to the analysis. From the culture based method, we knew the predominant bacteria were *Leu. mesenteroides* until mid-phase of the *dongchimi* fermentation, which is recognized as the stage of palatability and the bacteria could be starter cultures to produce good taste *dongchimi* products. To explain the different results between culture-dependent and culture-independent method, however, further studies should be carried out. At the moment, this study is incomplete but renders us key information about *dongchimi* fermentation and the first case, to the best of our knowledge, to systemically investigate microbial community of *dongchimi*.

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References

1. Um S, Shin WS, Lee JH. Real-time PCR monitoring of *Lactobacillus sake*, *Lactobacillus plantarum*, and *Lactobacillus paraplantarum* during *kimchi* fermentation. Food Sci. Biotechnol. 15: 595-598 (2006)
2. Lee JH, Kweon DH, Lee SC. Isolation and characterization of an immunopotentiating factor from *Lactobacillus plantarum* in *kimchi*: Assessment of immunostimulatory activities. Food Sci. Biotechnol. 15: 877-883 (2006)
3. Lee HJ, Oh SD. Properties changes of radish variety group of *Altari dongchimi* inoculated with *Leuconostoc citreum* IH22 during fermentation. J. East Asian Soc. Dietary Life 12: 47-54 (2002)
4. Kwon SM, Kim YJ, Oh HI, Jo DH. Physicochemical and microbiological changes in *dongchimi* juice during fermentation with the addition of *Panax ginseng* C.A. Meyer. Korean J. Ginseng Sci. 20: 299-306 (1996)
5. Park JE, Kim HR, Jang MS. Sensory and microbiological properties of *dongchimi* added with *gatt* (*Brassica juncea*). Korean J. Soc. Food Sci. 16: 57-64 (2000)
6. Park JE, Moon SW, Jang MS. Physicochemical properties of *dongchimi* added with *gatt* (*Brassica juncea*). Food Sci. Biotechnol. 14: 21-27 (2005)
7. Comi G, Citterio B, Manzano M, Cantoni C. Evaluation and characterization of *Micrococcaceae* strains in Italian dry fermented sausages. Fleischwirtschaft 72: 1679-1683 (1992)
8. ben Omar N, Ampe F. Microbial community dynamics during production of the Mexican fermented maize dough *pozol*. Appl. Environ. Microb. 66: 3664-3673 (2000)
9. Endo A, Okada S. Monitoring the lactic acid bacterial diversity during *shochu* fermentation by PCR-denaturing gradient gel electrophoresis. J. Biosci. Bioeng. 99: 216-221 (2005)
10. Ampe F, ben Omar N, Moizan C, Wacher C, Guyot JP. Polyphasic study of the spatial distribution of microorganisms in Mexican *pozol*, a fermented maize dough, demonstrates the need for cultivation-independent methods to investigate traditional fermentations. Appl. Environ. Microb. 65: 5464-5473 (1999)
11. Ampe F, Sirvent A, Zakhia N. Dynamics of the microbial community responsible for traditional sour cassava starch fermentation studied by denaturing gradient gel electrophoresis and quantitative rRNA hybridization. Int. J. Food Microbiol. 65: 45-54 (2001)
12. Mheen TI, Kwon TW. Effect of temperature and salt concentration on *kimchi* fermentation. Korean J. Food Sci. Technol. 16: 443-450 (1984)
13. Lee CW, Ko CY, Ha DM. Microfloral changes of the lactic acid bacteria during *kimchi* fermentation and identification of the isolates. Korean J. Appl. Microbiol. Biotechnol. 20: 102-109 (1992)
14. Choi JY, Kim M, Lee JH. Reevaluation of the change of *Leuconostoc* species and *Lactobacillus plantarum* by PCR during *kimchi* fermentation. J. Microbiol. Biotechn. 12: 166-171 (2002)
15. Lee JS, Heo GY, Lee JW, Oh YJ, Park JA, Park YH, Pyun YR, Ahn JS. Analysis of *kimchi* microflora using denaturing gradient gel electrophoresis. Int. J. Food Microbiol. 102: 143-150 (2005)
16. Dolci P, Alessandria V, Rantsiou K, Rolle L, Zeppa G, Cocolin L. Microbial dynamics of Castelmagno PDO, a traditional Italian cheese, with a focus on lactic acid bacteria ecology. Int. J. Food Microbiol. 122: 302-311 (2008)