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Changes of microbial community and metabolite in kimchi inoculated with different microbial community starters

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ABSTRACT

Gas chromatography mass spectrometry-based metabolomics and next generation sequencing-based metagenomics were applied to investigate the effect of different microbial community starters (MCSs) on kimchi fermentation. Profiles of metabolites and microbial community in kimchi were remarkably different from those on the first day of fermentation according to MCS used. Kimchi inoculated with MCS5 or MCS10 had relatively higher levels of lactic acid, leucine, propanedioic acid, serine, glycerol, erythritol, and sorbitol but lower levels of butanedioic acid, glyceric acid, myo-inositol, xylose, fructose, and talose compared to control kimchi or kimchi inoculated with MCS1. Microbial communities of kimchi inoculated with MCS1, MCS5, and MCS10 were characterized by high ratios of *Leuconostoc*, *Lactobacillus plantarum*, and *Lactobacillus brevis*, respectively. The microbial community of kimchi formed on the first day of fermentation was stable for 50 days, suggesting that microbial communities containing multiple microorganisms can be used as starters to obtain desired quality of kimchi.

1. Introduction

Kimchi, a fermented vegetable product, is the most traditional food in Korea. It is made through fermentation of Chinese cabbage or radish with minor ingredients such as ginger, garlic, red pepper, onion, fish sauce, and salts (Jung et al., 2012). It is known to have various functions such as anti-cancer, anti-obesity, cholesterol reduction, and antioxidant effects (Park, Jeong, Lee, & Daily, 2014). For a long time, kimchi has been manufactured by spontaneous fermentation without using starter culture in each household. Since various microorganisms originated from raw materials are present in the early stage of fermentation, it is time consuming and difficult to produce uniform quality products (Cheigh, Park, & Lee, 1994; Jung et al., 2012). Due to increasing number of housewives who participate in social activities and nuclear family, there is a growing demand for commercial kimchi products made at factories (Moon, Byun, Kim, & Han, 1995). Generally, when producing kimchi commercially, a starter culture is used to produce kimchi for uniform quality and quick fermentation. Studies related to kimchi starter cultures have been focused on quality changes of kimchi such as extension of the optimal ripening period and improvement of sensory properties, functionality, and safety (Lee, et al., 2015).

Physical and sensory characteristics of kimchi products can vary depending on the starter strain used (Kim & Chun, 2005). Lactic acid bacteria (LAB) such as *Leuconostoc*, *Lactobacillus*, and *Weissella* are identified as main microbes in the microbial community during kimchi fermentation (Jung et al., 2011; Lee et al., 2005; Park, et al., 2012). Therefore, most studies have been done by only inoculating single strains of these (Chang & Chang, 2010; Choi, et al., 2003; Jung et al., 2012; Lee & Lee, 2010). However, even if a single bacterial strain is inoculated as a starter, various bacteria from raw materials are involved in the actual fermentation of kimchi unless they are sterilized. According to a study using *Leu. mesenteroides* as a starter in kimchi, *Leu. mesenteroides* were initially abundant in starter-inoculated samples. However, microbial communities in kimchi became similar as fermentation progresses regardless of starter inoculation (Jung et al., 2012). In addition, since kimchi research was conducted using only cultivable strains, there is a limit to reflect microorganism compositions of actual fermented kimchi (Jung et al., 2011). Namely, inoculated single microorganism is different from the microbial community shown in aged kimchi. To produce tailored kimchi with a specific purpose, it is necessary to use microbial community rather than a single strain as starter culture for kimchi fermentation. Therefore, in the present study,

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different microbial community starters (MCSs) other than a single strain were used as kimchi fermentation starters.

With the development of various technologies for analyzing microbial community in recent years, they also have been utilized in the study of kimchi. Randomly collected commercial kimchi samples have shown different microbial structures during aging with differences in the microbial community of the final product (Kim & Chun, 2005; Park et al., 2012). These results are due to different initial microbial communities in kimchi. Differences in the microbial community of kimchi can also lead to differences in metabolites. Recent studies have reported the relationship between microbial communities and metabolites of kimchi during fermentation (Jeong, Jung, Lee, Jin, & Jeon, 2013; Jeong, Lee, Jung, Lee, et al., 2013; Jeong, Lee, Jung, Choi, & Jeon, 2013; Jung et al., 2012).

To determine the effect of different MCSs on kimchi fermentation, profiles of metabolites and microbial community in kimchi during fermentation were investigated using gas chromatography mass spectrometry-based metabolomics and next generation sequencing-based metagenomics approach. Consequently, the possibility of shortening the fermentation period of kimchi by using aged MCS was determined in this study.

2. Materials and methods

2.1. Preparation of kimchi microbial community starters

Kimchi microbial community starters (MCSs) were prepared by modifying proportions of ingredient mixtures described in a previous study (Park, et al., 2016). Briefly, a seasoning mixture was prepared by mixing tap water, Korean leek, red pepper powder, garlic, and ginger in a ratio of 32:28:22:14:4 (w/w/w/w). This seasoning mixture was then added to Chinese cabbage at a ratio of 1:9 (seasoning mixture:cabbage). Kimchi mixtures were grinded and mixed. The salinity was then adjusted to 2.7%. The mixture was placed in sterilized zipper bags to produce different kimchi microbial community starters (MCSs). MCSs were incubated at 20 °C for 10 days. Samples were taken on the 1st (MCS1), 5th (MCS5), and 10th (MCS10) day of fermentation for use as inoculation starters. MCS1, MCS5, and MCS10 were prepared in advance at 1, 5, and 10 days from the inoculation time, respectively, for use as starters at the same time.

2.2. Kimchi manufacturing

Kimchi used in this experiment was manufactured with the same materials and the methods as MCS. Kimchi mixtures were inoculated with 10% (w/w) of MCS1 (one-day aged kimchi), MCS5 (five-days aged kimchi), and MCS10 (ten-days aged kimchi), respectively. Control kimchi was manufactured without inoculation of starter for spontaneous fermentation. Each sample was subjected to five replications. Kimchi samples were stored at 20 °C for 24 h for active fermentation. They were then placed at 4 °C for 50 days. Sampling was performed on 0, 1, 5, 20, and 50 days after fermentation for analyses. The overall workflow of this experiment is shown in Supplementary Fig. 1.

2.3. Microbial community analysis

To estimate copy number of 16S rRNA gene in total bacteria during kimchi fermentation, total genomic DNA was extracted using PowerSoil® DNA Isolation Kit (Cat. No. 12888, MO BIO) according to the manufacturer's instructions. Only one sample representing the experimental group was analyzed. For each sample, V3-V4 regions of 16S rRNA gene were amplified by polymerase chain reaction (PCR) using 16S Amplicon PCR Forward Primer (5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16S Amplicon PCR Reverse Primer (5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC). Input gDNA (12.5 ng) was amplified with 16S V3-V4 primers. Final products were normalized and pooled using PicoGreen. Sizes of libraries were verified using LabChip GX HT DNA High Sensitivity Kit (PerkinElmer, Waltham, MA, USA). Pooled PCR products were sequenced using MiSeq™ platform (Illumina, San Diego, CA, USA).

After sequencing was completed, FASTQ file of MISeq raw data was generated using MCS software (MiSeq Control Software v2.2) and bcl2fastq (v1.8.4). PhiX sequence was removed through BWA. Paired-end reads obtained from the MiSeq system were merged using FLASH software (Magoč & Salzberg, 2011). Sequencing errors such as a low quality sequences, ambiguous sequence, and chimera sequence were trimmed by using CD-HIT-OUT (Li, Fu, Niu, Wu, & Wooley, 2012). OTU was formed by clustering over 97% of similar sequences. Taxonomic assignment was carried out using BALSTN (v2.4.0) with reference DB (NCBI 16S Microbial) and organism information of the most similar subject (Zhang, Schwartz, Wagner, & Miller, 2000). Taxonomy was not defined when query coverage of the best hit that matched the DB was less than 85% and the identity of the match area was less than 85%. Various microbial community comparisons were also performed using QIIME (v1.8) (Caporaso et al., 2010). Through this, shannon index and inversed simpson index were obtained. Alpha diversity was obtained by rarefaction curve. Chao1 index showing the richness and diversity of OUT was also confirmed. Beta diversity was obtained based on Weighted UniFrac distance.

2.4. Sample derivatization and GC–MS analysis

Sample derivatization protocol and GC–MS analysis conditions were similar to those described in a previous study (Mastrangelo, Ferrarini, Rey-Stolle, García, & Barbas, 2015) with slight modifications. Briefly, kimchi sample was centrifuged (Union 55R, Hanil, Seoul, Korea) at 13,000 rpm for 5 min. Then 1 mL of sample supernatant was lyophilized and 100 μL of O-methoxyamine hydrochloride in pyridine solution (15 mg/mL) was added followed by vortex-mixing for 10 min. The reaction was incubated at 25 °C for 16 h and then derivatized using 100 μL of BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide) containing 1% TMCS (trimethylchlorosilane) at 70 °C for 1 h. Next, cooling was carried out in the dark at 25 °C for 1 h and 600 μL of methyl stearate in heptane (10 ppm) was added as internal standard. Finally, the supernatant was used for analysis after centrifugation at 13,000 rpm for 15 min. To monitor the analytical variability, quality control (QC) samples were prepared by pooling equal volumes (approximately 10 μL) of each sample prior to the derivatization process. QC samples were analyzed every 20 samples throughout GC/MS run. One milliliter of heptane was added into an eppendorf tube as a blank sample. The remaining steps were the same as those used for kimchi pretreatment.

A Shimadzu single quadrupole GC–MS QP2020 (Kyoto, Japan) was used for metabolites analysis. Parameters of the apparatus were as follows: injection volume, 2 μL with a 50:1 split ratio; carrier gas (He), 1 mL/min; injection temperature, 250 °C; interface temperature, 280 °C; ion source temperature, 230 °C; and electron impact (EI) voltage, 70 eV. A fused silica capillary column (Rtx-5MS, 30 m × 0.25 mm × 0.25 μm, Restek, USA) was used for separation. The oven temperature was set at 60 °C for 1 min. It was then increased at 10 °C/min to 300 °C, and then held for10 min. The mass range for full-scan mode was set to 50–550 *m/z* with a scan speed of 2 scans/s. GC–MS data were acquired using Shimadzu GC solution software.

2.5. Data processing and statistical analysis

Raw GC–MS data were converted into netCDF format files using Shimadzu GC–MS Postrun Analysis software and subsequently processed using XCMS (https://xcmsonline.scripps.edu). Parameters consisted of the default Centwave method. They were: signal/noise threshold, 2; mzdiff, 0.1; integration methods, 1; prefilter peaks, 3; prefilter intensity, 100; mzwid, 0.25; minfrac, 0.5; and bandwidth, 3.