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Role of jeotgal, a Korean traditional fermented fish sauce, in microbial dynamics and metabolite profiles during kimchi fermentation

Min Young Jung, Tae-Woon Kim, Changsu Lee, Joon Yong Kim, Hye Seon Song, Yeon Bee Kim, Seung Woo Ahn, Ju Seok Kim, Seong Woon Roh, Se Hee Lee*

Microbiology and Functionality Research Group, World Institute of Kimchi, Gwangju 61755, Republic of Korea

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ABSTRACT

We investigated the effects of jeotgal (fermented fish sauce) on kimchi fermentation, with or without saeu-jeot and myeolchi-jeot. Bacterial community analysis showed that *Leuconostoc*, *Weissella*, *Lactobacillus*, and *Tetragenococcus* were the dominant genera; however, their succession depended on the presence of jeotgal. *Leuconostoc gasicomitatum* was the dominant species in kimchi without jeotgal, whereas *Weissella koreensis* and *Lactobacillus sakei* were the dominant species in kimchi with myeolchi-jeot and saeu-jeot, respectively. Metabolite analysis, using ^{1}H NMR, showed that the amounts of amino acids and gamma-aminobutyric acid (GABA) were higher in kimchi with jeotgal. Increases in acetate, lactate, and mannitol contents depended on fructose consumption and were more rapid in kimchi with jeotgal. Moreover, the consumption of various amino acids affected the increase in kimchi LAB. Thus, the role of jeotgal in kimchi fermentation was related to enhancement of taste, the amino acid source, and the increases in levels of functional metabolites.

1. Introduction

Kimchi is the most well-known traditional fermented food consumed in Korea and has become a popular food product worldwide because of its health benefits and nutritional properties (Park et al., 1999). The raw materials used for preparation of kimchi can be classified into three groups: major vegetables (Chinese cabbage and radishes), seasonings (red pepper, garlic, ginger, leek, and onion), and optional ingredients (e.g., jeotgal) (Cheigh & Park, 1994; Koo et al., 2016).

Jeotgal is a type of fermented fish sauce consumed in Korea and is typically produced by fermentation of highly salted (20–30% [w/w]) sea animals, such as whole fish, fish roe, internal organs of fish, and shellfish. Among the types of jeotgal used as an additive to improve the taste or flavour of kimchi, myeolchi-jeot (salted anchovy) and saeu-jeot (salted tiny shrimp) are the most popular seafoods in Korea (Koo et al., 2016). These fish sauces have various endogenous enzymes derived from the muscle and/or digestive tract of raw sea animals (Jiang, Moody, & Chen, 1991). These enzymes promote the fermentation of salted fish and increase the production of abundant amino acids, even under conditions of high salinity (Jiang et al., 1991; Kim, Sung, Han, Kang, & Jeong, 1996; Sila, Nasri, Bougatef, & Nasri, 2012; Yongsawatdigul, Rodtong, & Raksakulthai, 2007). Based on these characteristics of jeotgal, it is often consumed by itself or added to other fermented foods, such as kimchi, to improve the taste, texture, and flavour of the food and enhance the fermentation activity (Koo et al., 2016). To our knowledge, kimchi is the only fermented food in the world that has a recipe using fermented fish sauce, based on animal raw materials, for the fermentation of vegetable raw materials.

The production (and quality) of kimchi is closely related to the microbial (lactic acid bacteria [LAB]) community and metabolite activity. Previous studies have revealed the relationships between microbial succession and metabolite changes during the fermentation of kimchi and jeotgal (Jung, Lee, Chun, & Jeon, 2016; Jung et al., 2011; Lee, Jung, & Jeon, 2014). However, the microbial succession and metabolite changes occurring, following the addition of jeotgal during kimchi fermentation, have not yet been investigated.

The combined use of Illumina Miseq sequencing of the 16S rRNA gene and proton nuclear magnetic resonance (^{1}H NMR) is the most comprehensive and powerful method for monitoring of diverse microorganisms and multiple compounds, particularly in fermented foods (Jeong et al., 2013). Therefore, in this study, we applied these methods, in combination with monitoring of bacterial succession and metabolite profiles during kimchi fermentation, with or without jeotgal (myeolchi-jeot and saeu-jeot). Our results are expected to contribute to the current understanding of the role of jeotgal in kimchi fermentation and to facilitate the reliable production of high-quality kimchi.

* Corresponding author at: Microbiology and Functionality Research Group, World Institute of Kimchi, 86, Kimchi-ro, Nam-gu, Gwangju 61755, Republic of Korea.
E-mail address: leesehee@wikim.re.kr (S.H. Lee).

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2. Materials and methods

2.1 Jeotgal kimchi preparation and sampling

Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) was soaked in 15% (w/v) solar salt solution for 10 h, manually washed three times with water, and then drained of excess water. Two types of kimchi with jeotgal (saeu-jeot or myeolchi-jeot, with 7.3% [w/v] salt), were prepared, using the following ratios: salted cabbage:ground jeotgal:ground garlic:ground ginger:ground onion:green onion:ground radish:glutinous rice porridge:red pepper powder:water = 70:4:3.75:1.2:1.85:2.4:4.5:1.2:4.5:6.6. Two types of kimchi without jeotgal were prepared using the same ingredients in the same ratio, but water or 7.3% saline was used instead of jeotgal as a control. The four prepared kimchi samples were dispensed into three polyethylene plastic bags in 5-kg portions for triplicate analysis and fermented at 5 °C for 40 days. Kimchi soups (liquid parts of kimchi) were periodically sampled, and their large particles were filtered, using a sterile stomacher filter bag (Whirl-Pak; Nasco, WI, USA). The filtered kimchi samples were centrifuged (12,000 rpm for 10 min at 4 °C), and separated pellets and supernatants were stored at −80 °C for microbial community and metabolite analyses, respectively. The kimchi samples were labelled as "CK" for kimchi without jeotgal, "NK" for kimchi with salinity adjusted with salt instead of jeotgal, "SK" for kimchi with saeu-jeot, and "MK" for kimchi with myeolchi-jeot.

2.2. Physicochemical analysis

The pH was measured with a pH meter (Orion 3-Star; Thermo Scientific, USA). The titratable acidity was obtained by titration with 0.1 N NaOH to pH 8.3 and estimated as equivalents of lactic acid (1 ml of 0.1 N NaOH amounted to 0.009 g equivalents of lactic acid) (Ramakrishnan, Goveas, Prakash, Halami, & Narayan, 2014). NaCl concentrations in kimchi samples were measured using a PAL-SALT 4250 digital salt meter (ATAGO, Shiba-koen, Japan).

2.3. Enumeration of microorganisms

The filtered kimchi soup was serially diluted with sterilized 0.85% saline solution to determine viable bacteria counts. Bacterial counts were measured using 3M Petrifilm count plates (3M-UK; Bracknell, Berkshire, UK) as culture-based approaches targetting total aerobes, LAB, coliform, and yeast, according to the manufacturer's instructions as follows: 3M Petrifilm Rapid Aerobic Count Plates for aerobic mesophilic bacterial counts; 3M Petrifilm Lactic Acid Bacteria Count Plates for LAB; 3M Petrifilm *Escherichia coli*/Coliform Count Plates for coliform counts; and 3M Petrifilm Yeast and Mold Count Plates for yeast. Total aerobic bacteria and coliform bacteria/LAB were incubated at 30 °C for 1–2 or 2–3 days, respectively. Colonies were counted from 3M films, on which 30–300 colonies appeared, and were reported as log CFU/ml.

2.4. DNA extraction and PCR amplification for MiSeq sequencing

Total genomic DNA from the kimchi pellet was extracted using a MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) through mechanical lysis, chemical lysis, and DNA purification. The DNA quality was measured using PicoGreen and a Nanodrop instrument. Input gDNA (10 ng) was amplified by PCR. For MiSeq sequencing, bacterial genomic DNA amplification was performed using primers targetting the V3 to V4 hypervariable regions of the 16S rRNA gene. 341F (5′-TCGTCGGCAGCGTC-AGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3′; the underlined sequences indicate the target regions; AGATGTGTATAAGAGACAG is the adaptor sequence) (Fadrosh et al., 2014; Shin et al., 2016). The PCR products were purified using a QIA Quick PCR Purification Kit, visualized on 1% agarose gels, and adjusted to equal concentrations. Paired-end sequencing was performed by Macrogen, Inc. (Seoul, Korea), using an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

2.5. Analysis of bacterial succession during kimchi fermentation

Generated MiSeq reads were processed using CLcommunity software (v.3.46). Obtained sequencing reads underwent a quality check, and those with a low quality score (average score < 25) were trimmed by Trimmomatic 0.32 (Bolger, Lohse, & Usadel, 2014). MiSeq forward and reverse reads were then paired using PandaSeq v.2.9 (Masella, Bartram, Truszkowski, Brown, & Neufeld, 2012) with default parameters, and unjoined reads were filtered out by an in-house script. The chimeric sequences were removed by the bellerophone method, and the taxonomic classification of each read was assigned, based on the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net) (Kim et al., 2012). The high-quality sequences were normalized to lowest number of reads (46,826 reads) by randomly selecting reads from the sequencing of fasta files, using the Mothur programme (https://www.mothur.org/). The original and normalized sequencing reads were clustered into operational taxonomic units (OTUs), using the CD-HIT programme. The richness and diversity of samples were determined by Ace, Chao1 estimate of richness (Chao, 1987), Shannon-Weaver index (Shannon, 1997), and Simpson index (Hill, 1973) at a distance of 3%. Taxonomic assignments of the high-quality sequencing reads derived from kimchi samples were performed and visualized at the genus level, using CLcommunity software v.3.46 (ChunLab, Inc., Korea). LAB sequencing reads, assigned at the genus level by CLcommunity software, were further classified at the species level, using the local BLAST (Standalone MEGABLAST) programme, based on the nucleotide (nt) database (July 2016), as previously described (Han, Lee, Jeong, Jeon, & Hyun, 2017).

2.6. Analysis of metabolite changes during kimchi fermentation

Metabolite profiling analysis, including carbohydrates, organic acids, nitrogen compounds, and amino acids, was performed in duplicate, using ^{1}H NMR spectroscopy, as described previously (Lee, Jung, & Jeon, 2015). Briefly, five millilitres of each of the respective kimchi supernatants obtained by centrifugation were adjusted to pH 6.0 and then lyophilized. The freeze-dried powder samples were suspended in five millilitres of 99.9% D_2O (deuterium oxide; Sigma-Aldrich, USA) with 0.5 mM 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS; Sigma-Aldrich, USA). After centrifugation at 13,000 rpm for 5 min, one millilitre of supernatant was transferred into a 5 mm NMR tube. ^{1}H NMR spectra of kimchi samples were acquired at 25 °C on a Varian Inova 600-MHz NMR spectrometer (Varian Inc., Palo Alto, CA, USA), using the standard PRESAT pulse sequence. The NMR spectra were collected into 32 k data points with a spectral width of 9,615 Hz. All kimchi ^{1}H NMR spectra were manually phased and baseline-corrected, using VnmrJ 3.2 software. The NMR spectral intensities were reduced into integral bin areas (buckets) of equal width (0.04 ppm) over the range of 0.5–10.0 ppm, and the buckets were normalized to the intensity of the DSS signal at 0 ppm. Identification and quantification of targetted individual metabolites from the ^{1}H NMR spectra of kimchi samples were performed, by the Chenomx NMR suite programme (v. 8.3; Chenomx, Canada), using 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard. The programme mapped the ^{1}H NMR spectra result with the reference library 9 (600-MHz compounds) (Jung et al., 2012; Lee et al., 2009).

2.7. Statistical analysis

Total ^{1}H NMR spectra for multivariate statistical analyses were