

Analysis of the Bacterial Composition During *Kochujang*, a Korean Traditional Fermented Hot Pepper-soybean Paste, Fermentation

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Abstract In this study we analyzed the dynamic changes in microbiota composition during *kochujang* fermentation at 30°C. During fermentation, the viable cell counts slowly increased and reached 3.2×10^7 for aerobic bacteria, 8.3×10^3 for yeast, and 1.4×10^3 CFU/mL for fungi after 60 days. Bacilli were found to be the most dominant microorganisms throughout the fermentation process. Using the culture dependent method *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus amyloquelaciens* were found to be the main species during the early stages of fermentation; however, *Bacillus pumilus* and *Bacillus stearothermophilus* became the most dominant species during the late stage of fermentation. In contrast, when the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method was used *Bacillus ehimensis* was found to be the dominant species during the early stage of fermentation and *Bacillus megaterium*, *B. pumilus*, *B. subtilis*, and *B. licheniformis* were dominant in the late stages. These results indicate various other *Bacillus* species rather than just *B. subtilis* and *B. licheniformis* might be involved in the fermentation of *kochujang*.

Keywords: *kochujang*, fermentation, *Bacillus* species, denaturing gradient gel electrophoresis

Introduction

Kochujang is a traditional fermented food and a very famous hot sauce in Korea. Various methods for manufacturing *kochujang* have been developed in Korea and these different methods generally involve using the following ingredients; red pepper powder, soybean, glutinous rice, salt, and water (1). In a previous paper, 10 different bacterial genera, *Bacillus*, *Corynebacterium*, *Enterococcus*, *Flavimonas*, *Flavobacterium*, *Gemella*, *Pasteurella*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* were found in traditional *kochujang* where *Bacillus subtilis* was the dominant species (2). In addition to bacterial strains, yeast and fungi were also found in *kochujang* during fermentation. Jin *et al.* (3) analyzed the microbial composition of a traditional *kochujang* and found that the genus *Bacillus* constituted 97% of the bacterial compositions, where *Bacillus licheniformis* was found to be the most dominant species. In addition, *Zygosaccharomyces pseudorouxii* and *Aspergillus oryzae* were determined to be the most dominant yeast and fungi species, respectively.

Determining the microbial composition of fermented foods is very important for understanding the fermentation profile and providing valuable information for the commercial production of fermented foods. In the past, the methods used to analyze microbial composition have been dependent on culture based techniques; however, these methods are time-consuming, laborious, and uneconomical. In this respect, the use of a culture-independent molecular

technique, such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) in combination with 16S rRNA gene sequencing, would be a powerful and superior tool to analyze the microbial composition from complex matrices such as fermented foods (4-7). In this study we used this molecular technique as well as a culture-dependent method using API kit to analyze the dynamic changes in microbiota composition during *kochujang* fermentation although these methods still have limitations to discriminate one *Bacillus* sp. from another in species level (8).

Materials and Methods

***Kochujang* fermentation** Freshly prepared *kochujang* from a factory (Sunchang MoonOkRae Food) in the Sunchang province, which is famous for its Korean traditional sauces such as *kochujang*, *doenjang* (a fermented soybean paste), and *cheonggukjang* (9), was used for the experiments. After preparation, it was immediately stored at 30°C, which temperature is for the fermentation of commercial *kochujang*.

Culture-dependent method To determine the microbial composition of the fermented *kochujang* samples using the culture-dependent method, the samples (1 g) were diluted by 10 fold with a peptone buffer (0.85% NaCl, 0.01% peptone, 0.003% KH₂PO₄, and 0.006% Na₂HPO₄) and then spread on a plate count agar (PCA; Difco, Sparks, MD, USA) plate to determine the aerobic bacterial composition, an MRS agar (Difco) plate to assess the lactic acid bacteria and a 3M petrifilm (St. Paul, MN, USA) to determine the yeast and fungi. Viable cell counts were measured by counting the colonies on the plates after incubation for 24

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cellular autolysis in the PCR-DGGE method and media selectivity in the culture-dependent method may be problematic for analyzing microbial compositions from food samples. In addition, they emphasized the importance of using a combination of both methods for a more accurate analysis. Currently, neither method can correctly analyze the microbiota from complex samples such as

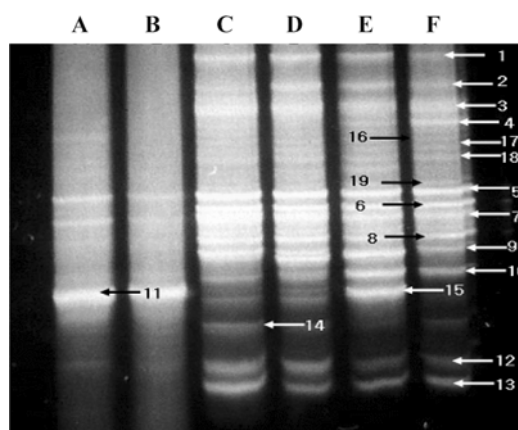


Fig. 2. DGGE analysis of partial 16S rRNA gene fragments from the microbiota of *kochujang* samples at 30°C. Day 0 (A), 7 (B), 14 (C), 21 (D), 28 (E), and 60 (F). The DNA bands correspond to partial 16S rRNA gene products.

fermented foods; however, it would be highly beneficial to develop powerful tools based on molecular techniques that can resolve the current problems associated with analyzing complex samples.

In general, it has been established that *B. licheniformis* and *B. subtilis* are the prevailing bacteria that exist during the fermentation of commercial and traditional *kochujang* (2,3). However, based on our results various other *Bacillus* species, such as *B. amyloquefaciens*, *B. megaterium*, *B. pumilus*, *B. stearothermophilus* as well as *B. licheniformis* and *B. subtilis*, could also be the main species in *kochujang* fermentation. However, we cannot conclusively state that these results represent the microbiota composition during *kochujang* fermentation since many different varieties of *kochujang* are manufactured by various methods in Korea.

Kochujang is a representative Korean hot sauce and a very important ingredient in many foods in Korea (11). As the result, there have been many Korean food researchers that have studied the physicochemical, microbiological, and sensory properties of *kochujang* (2,3,9,12,13). Particularly, understanding the microbial composition during fermentation is quite important because *kochujang* is a fermented food that is controlled by the microorganisms that exist during fermentation. By better understanding the microbial composition we can attain useful information about the correlation between the fermentation process and microbial composition. Despite this, the analysis of microbial composition during fermentation has been rarely studied. In this study, we systematically analyzed the major microorganisms of a traditional *kochujang* during

fermentation using a culture-dependent and a culture-independent method. To the best of our knowledge, this is the first report that used the PCR-DGGE method to analyze the microbiota of *kochujang* during fermentation.

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