Pyrosequencing-Based Analysis of the Bacterial Community in Korean Traditional Seafood, Ojingeo Jeotgal

Jaejoon Jung†, Sungjong Choi†, Che Ok Jeon‡, and Woojun Park*†

1Department of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, Republic of Korea
2Department of Life Science, Chung-Ang University, Seoul 156-756, Republic of Korea

Fermented food has held an important place in human diet, and it is widely produced and consumed in a variety of countries [18]. Because the fermentation process is entirely driven by the metabolic actions of microorganisms, there have been numerous studies performed to understand the relationship between microorganisms and food fermentation via the isolation of novel species [12, 21], genome sequencing of food isolates [10], and analysis of metabolites and the microbial community [9, 15, 17]. General features of food fermentation can be summarized from those studies as follows: (i) Biochemical characteristics of fermented food such as pH and metabolite profiles are significantly different from the beginning of the fermentation. (ii) The succession of the bacterial community results in a simpler composition and a predominant species appears. The predominant species in the fermented food is considered more competitive than other minor groups of microorganisms, regarding the efficient utilization of nutritional sources, resistance to diverse stresses, and competition with other microbial communities [20].

Jeotgal fermentation is dependent upon a diverse microbial community, although a detailed understanding of its microbial composition is limited to a relatively small number of jeotgal. Pyrosequencing-based bacterial community analysis was performed in fermented squid, ojingeo jeotgal. Leuconostoc was identified as the predominant bacterial genus, with Bacillus and Staphylococcus also accounting for a large proportion of the bacterial community. Phylogenetic analysis with 16S rRNA genes of Leuconostoc type species indicated that L. citreum- and L. holzapfelii-like strains could be the major Leuconostoc strains in jeotgal. High concentrations of NaCl were thought to be an important factor determining the makeup of the bacterial community in the fermented squid; however, a genomic survey with osmotic stress-related genes suggests the existence of more complex factors selecting the dominant bacterial species in fermented squid.

Keywords: Staphylococcus, Leuconostoc, ojingeo jeotgal, pyrosequencing-based bacterial community analysis

Received: May 8, 2013
Accepted: July 11, 2013
First published online July 15, 2013
*Corresponding author
Phone: +82-82-3290-3067;
Fax: +82-2-953-0737;
E-mail: wpark@korea.ac.kr
†These authors contributed equally to this work.
pISSN 1017-7825, eISSN 1738-8872
Copyright © 2013 by The Korean Society for Microbiology and Biotechnology

Jeotgal is a Korean traditional fermented food. There are many types of jeotgal depending on the main ingredient, such as squid, shrimp, oyster, fish, and fish eggs. They are seasoned with various kinds of vegetables, red pepper, ginger, sesame, and garlic. The most important feature is the high concentration of salt (10–20%) [8]. Culture-dependent bacterial community analysis of jeotgal determined that the genera Staphylococcus, Bacillus, Halomonas, and Kocuria were major constituents of the jeotgal microbial community. Viable bacterial cell counts of these communities were approximately 10^3–10^5 colony-forming units per gram [8]. The growth of Staphylococcus and Virgibacillus isolated from jeotgal was confirmed in high-NaCl condition [8]. Culture-independent analysis of the bacterial community of our unpublished data and other previous studies indicated that lactic acid bacteria were also frequently dominant. Metabolite analysis of jeotgal and jeotgal-containing fermented foods indicated that sugars and amino acids, including fructose, glucose, alanine, glycine, and valine, were of major metabolites and their concentrations were changed during the fermentation processes [11].

The aims of this study were to investigate the bacterial
community of Korean traditional fermented squid, *ojingeo jeotgal* by culture-independent methods. Any microbiological analysis has not been investigated in fermented squid. To achieve our research purpose, we analyzed the bacterial community of fermented squid via pyrosequencing of the 16S rRNA gene.

The general recipe for fermented squid is as follows; the skin of the squid was peeled off and 10% (w/w) coarse salt was spread on the squid to ripen for 1 day. After ripening of the squid, it was cut into pieces and seasoned with 3% (w/w) fish sauce, 3% (w/w) red pepper powder, 3% (w/w) sesame, 3% (w/w) scallion, 4% (w/w) crushed garlic, 10% (w/w) chopped pear, 12% (w/w) chopped white radish, and 14% (w/w) starch syrup. The mixed ingredients were fermented at room temperature for 5 days and stored at 4°C. Because there could be subtle differences in the recipe by regions, we purchased *jeotgal* samples from five different manufacturers in Seoul, Korea and mixed them into a sample.

To analyze the culture-independent microbial community of fermented squid, the total genomic DNA was extracted twice from a pellet obtained from 50 ml of fermented squid filtrate using a NucleoSpin Soil kit (Macherey-Nagel, Germany) according to manufacturer instructions, and the concentrations of the extracted genomic DNA were measured with a Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, USA). The V1 to V3 hypervariable regions of the bacterial 16S rRNA gene from the genomic DNA were amplified using primers V1-27F (5'-CCTATCCCCTGTGTCCTTGGCAGTC-TCAG-AC-GAGTTTGATCMTGGCTCAG-3') and V3-541R (5'-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-TCAGATG-AC-WTTACCGCGGCTGCTGG-3' and 5'-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-ATAGCTCTCG-AC-WTTACCGCGGCTGCTGG-3') [2, 16]. All of the polymerase chain reactions (PCR) were conducted in a MyCycler Thermal Cycler (Bio-Rad, USA) in 20 µl volumes containing 30 ng of genomic DNA, 50 pmol of each primer, and Han-Taq (Genenmed, Republic of Korea) polymerase. The following PCR protocol was used: 94°C for 90 sec (1 cycle); 94°C for 45 sec, 55°C for 45 sec, and 72°C for 45 sec (30 cycles); and 72°C for 5 min (1 cycle). Pyrosequencing of the PCR Product was performed by Macrogen (Korea) using a 454 GS FLX Titanium system (Roche, Germany). To assess bacterial species richness estimators, diversity indices and rarefaction curves, we applied the pyrosequencing pipeline (http://pyro.cme.msu.edu) of the Ribosomal RNA Database Project (PCR) [3].

A total of 566 reads resulted from a single run. The Shannon and Chao1 indices were 2.45 and 42, respectively, which were close to the previously determined bacterial diversity from other fermented seafoods [1, 16, 18, 19]. Although the number of reads from the pyrosequencing data was small, a rarefaction curve approached to a plateau, indicating that pyrosequencing results reflected the composition of the whole bacterial community (Fig. 1). The phylogenetic classification of bacterial phyla and genera from the fermented seafood is summarized in Fig. 2. Six phyla – Proteobacteria (37.1%), Firmicutes (32.9%), Actinobacteria (14.6%), Cyanobacteria (13.3%), Bacteroidetes (1.8%), and Deinococcus-Thermus (0.4%) – were identified. The predominant genus was *Leuconostoc* (37.6%), followed by *Bacillus* (11.0%), *Staphylococcus* (9.9%), and *Psychrobacter* (8.0%). *Leuconostoc* was frequently identified as a predominant genus of another Korean traditional fermented food, *kimchi* (fermented Chinese cabbage), and used as a fermentation starter culture in a commercial product [11]. It is of note that the bacterial community of *kimchi* was usually taken up by only a small number of genera such as *Leuconostoc*, whereas *jeotgal* often contained a number of minor groups of bacteria [8]. Therefore, squid fermentation was considered as a result of a more complicated interaction between bacterial communities.

Since the PCR did not assign the sequence reads to the species level, we performed phylogenetic analysis of *Leuconostoc*-assigned reads with the previously described *Leuconostoc* type species, as it is the predominant genus and frequently identified in other fermented foods. *Leuconostoc*-associated reads were extracted from the total sequencing data and aligned with 16S rRNA gene sequences of *Leuconostoc* type species. Unaligned sequences were trimmed and 450 bp was used for a neighbor-joining phylogenetic

![Fig. 1. Rarefaction curve of the bacterial community from fermented squid.](image)