Microbial Floral Dynamics of Chinese Traditional Soybean Paste (Doujiang) and Commercial Soybean Paste

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Introduction

Soybean paste (doujiang), a traditional Chinese fermented soybean food, is naturally fermented by various microorganisms. It is also called huangdoujiang, huangjiang, dajiang, and dadoujiang [3]. Soybean paste, sauce, douchi, and fu-ru (sufu) are four traditional Chinese fermented soybean foods [4]. Soybean paste is a highly flavorful and delicious fermented food. As people become too busy to make their own traditional soybean paste, a growing demand for this product has created a market for mass-produced commercial soybean paste. There are now soybean paste factories in at least 20 cities. The craft of commercial soybean paste has been simplified and the fermentation time of commercial soybean paste has been shortened. The taste and quality of commercial soybean pastes do not compare with the traditional fermented soybean pastes.

Many microorganisms are involved in the production of soybean paste. Many researchers have reported that the predominant microorganisms involved in soybean paste production are mold, yeasts, and bacteria [14, 26]. Yoo et al. [25] reported that Bacillus subtilis and Bacillus licheniformis play important roles during the fermentation of doenjang, a traditional Korean soybean paste. A researcher from Nigeria has reported that Bacillus subtilis, Bacillus licheniformis, and Bacillus pumilus are the predominant bacterial species found in the traditional fermented soybean paste, Soy-daddawa. The genus Bacillus can degrade the protein and starch in the raw material, along with Aspergillus. It inhibits the growth of Staphylococcus spp. and promotes the growth of

Traditional soybean paste from Shandong Liangshan and Tianyuan Jiangyuan commercial soybean paste were chosen for analysis and comparison of their bacterial and fungal dynamics using denaturing gel gradient electrophoresis and 16S rRNA gene clone libraries. The bacterial diversity results showed that more than 20 types of bacteria were present in traditional Shandong soybean paste during its fermentation process, whereas only six types of bacteria were present in the commercial soybean paste. The predominant bacteria in the Shandong soybean paste were most closely related to Leuconostoc spp., an uncultured bacterium, Lactococcus lactis, Bacillus licheniformis, Bacillus spp., and Citrobacter freundii. The predominant bacteria in the Tianyuan Jiangyuan soybean paste were most closely related to an uncultured bacterium, Bacillus licheniformis, and an uncultured Leuconostoc spp. The fungal diversity results showed that 10 types of fungi were present in the Shandong soybean paste during the fermentation process, with the predominant fungi being most closely related to Geotrichum spp., an uncultured fungal clone, Aspergillus oryzae, and yeast species. The predominant fungus in the commercial soybean paste was Aspergillus oryzae.

Keywords: Traditional soybean paste, commercial soybean paste, PCR-DGGE, clone library, microbial diversity
probiotic species, such as those in the genus Lactobacillus [15]. Previous studies of microorganisms in soybean paste have mainly used separation and culture methods. The limitation of these techniques is that only microorganisms that can be cultured have been identified and studied. In addition, there has been a variety of different technical soybean paste microorganism types in soybean paste, which has made further study difficult.

It is recognized that traditional plate isolation methods have many disadvantages, such as restricting the microbial diversity that can be studied, loss of species, and unclear species structure. For the research presented here, samples were studied using microbial molecular ecology techniques, which can reveal the original microbial diversity of soybean paste more directly and credibly.

Denaturing gradient gel electrophoresis (DGGE) has been used in microbial ecology to determine nucleotide sequences, differences in PCR-amplified regions of the bacterial 16S rRNA gene, and the eukaryotic 26S rRNA gene and has proven to be a valuable approach for monitoring community dynamics in relation to environmental factors in several ecosystems [2, 5, 6, 8, 16]. Some studies utilized DGGE for investigating microflora in fermented soybean pastes [10]. However, comparing the difference of the bacterial and fungal dynamics in the traditional soybean and commercial soybean pastes during the soybean paste fermentation process has not been previously reported.

This study was designed to analyze the microbial diversity differences between traditionally fermented soybean paste and commercial soybean paste, to identify the microbial composition diversity in the two types of soybean paste by DGGE, and to construct a 16S rRNA gene clone library. Furthermore, the floral dynamics during fermentation were analyzed by DGGE. The factors influencing the formation of quality traditional soybean paste were also assessed. It is expected that this study will provide the basis for improving the production quality of commercial soybean paste.

Materials and Methods

Shandong Liangshan Traditional Soybean Paste

Fermented soybean paste made by farmers from Liangshan County, in Jining City of Shandong Province, was chosen as a representative sample for traditional soybean paste. This soybean paste was produced during the cold season, namely during December through March of the next year. Production was performed mainly as follows: Maize (25%) and soybeans (75%) were mixed with approximately 60% (v/v) water, fried, and pulverized. Finally, the mixture was pressed against a wood frame that measured 30 cm in width, 60 cm in length, and 10 cm in height. After the wood frame was removed, the paste block was transversely cut with a knife into 30 cm × 10 cm × 15 cm blocks, which were fermented for approximately 65 to 70 days within a paper package. The paste block was then scrubbed and broken into small pieces after removing the paper wrapper. The paste was then adjusted to a moisture content of approximately 80% using 18–20% saline water (called xiajiang).

The paste was put into a jar and stirred once a day with a wooden spoon. The soybean paste was edible when it was no longer producing bubbles and after salt was added according to taste preferences. During the fermentation process, direct sampling of the soybean paste blocks was performed at 0, 15, 30, 45, 60, and 90 days. Strip samples of the cross-section of the paste block sawed from the surface to the center were used for these analyses.

Tianyuan Jiangyuan Commercial Soybean Paste

Fermented soybean paste made by the Tianyuan Jiangyuan (TYJY) Food Factory of Liubiju Food Company Limited in Beijing was chosen as a representative sample for commercial soybean paste. Production was performed mainly as follows.

Excellent quality soybeans were soaked, steamed, and then evenly mixed with flour in a ratio of 65% soybean to 35% flour. Koji was inoculated at 0.15%. Koji was made at 30°C for 2 days through ventilation. Then, the prepared mature koji was shifted into the fermentation pool (3 m × 2 m × 1.7 m). An approximately 20% saline solution was added into the fermentation pool (i.e., for every 100 kg of a soybean mixture, approximately 60 kg of a saline solution was added). The solid-state stage of fermentation then began.

The early fermentation period lasted from 0 to 7 days after the material was put into the fermentation pool. The fermentation temperature during this period was 40°C to 42°C. The material was thoroughly stirred once every 3 days. The metaphase lasted from 7 to 15 days, during which the temperature was maintained between 42°C and 45°C, and the material was thoroughly stirred once every 5–6 days. During the last stage, lasting 15 to 30 days, 8–10% (mass ratio) solid salt was added to the fermentation pool. The mature soybean paste was packaged after it was ground through a steel mill. During the fermentation process, a multipoint sampling method of the soybean paste was performed at 0, 2, 7, 14, 22, and 32 days and these samples were used for analysis.

Denaturing Gradient Gel Electrophoresis and Sequencing

The genomic DNA was extracted from each sample using the benzyl chloride method [27], and the extracted DNA was used as the template for PCR amplification. The primers used for DGGE were 357F-GC (forward, 5’-CCTACGGAGGCGACGAG-3’; E. coli positions, 341-357, attached to a GC-clamp (5’-CGCCCGCCGCGGCGGGCCGACCCCCCGG-3’) at the 5’-terminus) and 517R (reverse, 5’-ATTACGGCTGCTGG-3’; E. coli positions, 517-534) [22]. Primers were purchased from Sangon Biotech Co., Ltd., Beijing, China. The initial DNA denaturation was performed
at 95°C for 10 min, followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 48°C for 1 min, and elongation at 72°C for 1 min 10 sec, with a final elongation step at 72°C for 5 min. Fungal 26S rRNA gene D1-D2 region PCR amplification was performed using the same PCR amplifier. Primer sequences for the amplification of a fungal DNA fragment encoding the 26S rRNA were NL1 (forward, 5'-GGC TAC CTT GTT ACG ACT T-3') and LS2 (reverse, 5'-ATTCCC AAACAACTCGACTC-3'); S. cerevisiae positions, 266-285) [7, 11]. During PCR, an initial denaturation was performed at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 45 sec, and elongation at 72°C for 1 min. A final extension period of 7 min at 72°C was carried out after the 35 cycles. The products were examined by electrophoresis on a 2% agarose gel.

The DGGE analysis was performed with the Dcode system (Bio-Rad Laboratories, Hercules, CA, USA) according to references [21, 22]. A total of 13 µl of PCR product was applied to a 1-mm-thick, 6%–12% (w/v) polyacrylamide gradient gel in a 0.5× TAE electrophoresis buffer with 20%–60% denaturant gradient (where 100% is defined as 7 M/l urea with 40% formamide). Electrophoresis was performed at a constant voltage of 200 V and a temperature of 61°C for 5 h.

The gels were stained with SYBR Green I. The bands on the DGGE gels were observed under 302nm UV light using the AlphaImager 2200 Imaging System (Alpha Innotech, USA). The DGGE gels were observed under 302nm UV light using the AlphaImager 2200 Imaging System (Alpha Innotech, USA). The DGGE gels were observed under 302nm UV light using the AlphaImager 2200 Imaging System (Alpha Innotech, USA). The DGGE gels were observed under 302nm UV light using the AlphaImager 2200 Imaging System (Alpha Innotech, USA).

### Cloning and Sequence Analysis of the 16S rRNA Gene

According to the results from the DGGE analysis, the microbial species from the 30 d sample of Shandong traditional soybean paste and the 7 d sample of TYJY commercial soybean paste were the most diverse during the fermentation process. The 16S rRNA gene amplicons from these two samples were used as templates for the construction of a 16S rRNA gene clone library [21]. A total of 200 white colonies were randomly picked and screened by running a DGGE profile as described above. The clones that produced a single band with different melting positions were selected for sequence analysis. The insert DNA fragments were sequenced using the primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3'), 907R (5'-CCC CGT CAA TTC TCT TTA GGT T-3') and 1492R (5'-GGC TAC CTT GAT ACG ACT T-3') [9]. The sequence information was then imported into the CLUSTALX software program for assembly and alignment [19]. A phylogenetic tree was constructed using the neighbor-joining method [27].

### Nucleotide Sequence Accession Numbers

The sequences obtained in this study were deposited in GenBank under accession numbers FJ195758 to FJ195788 (16S rRNA gene clones), FJ195789 to FJ195794 and EU360131 to EU360151 (16S rRNA gene PCR-DGGE), and FJ195795 to FJ195805 (26S rRNA gene PCR-DGGE).

### Results

#### DGGE Analysis of Bacterial Communities During the Shandong Traditional Soybean Paste Fermentation

DGGE profiles of microbial communities from traditional soybean paste are shown in Fig. 1. Overall, 21 bacterial bands were recorded, corresponding to 21 species. Each band was sequenced and compared with reference sequences available in GenBank using a BLAST search [1].

![Fig. 1. DGGE profiles of bacteria from Shandong soybean paste sampled at different times during fermentation.](image)

<table>
<thead>
<tr>
<th>Species</th>
<th>Matching species</th>
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<tbody>
<tr>
<td>a. Weissella cibaria</td>
<td>100%; Weissella cibaria</td>
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<tr>
<td>b. Weissella cibaria</td>
<td>99%; uncultured</td>
</tr>
<tr>
<td>c. uncultured bacterium</td>
<td>82%; Bacillus sp.</td>
</tr>
<tr>
<td>d. uncultured bacilli</td>
<td>97%; Leuconostoc lactis</td>
</tr>
<tr>
<td>e. Leuconostoc lactis</td>
<td>98%; Streptococcus sp.</td>
</tr>
<tr>
<td>f. Streptococcus sp.</td>
<td>90%; Citrobacter freundii</td>
</tr>
<tr>
<td>g. uncultured bacterium</td>
<td>92%; Ureibacillus thermosphaericus</td>
</tr>
<tr>
<td>h. Bacillus licheniformis</td>
<td>98%; Streptococcus sp.</td>
</tr>
<tr>
<td>i. Streptococcus sp.</td>
<td>90%; Bacillus pumilus</td>
</tr>
<tr>
<td>j. uncultured bacterium</td>
<td>100%; Bacillus pumilus</td>
</tr>
<tr>
<td>k. uncultured bacterium</td>
<td>87%; Bacillus sp.</td>
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<tr>
<td>l. Bacillus sp.</td>
<td>82%; Bacillus sp.</td>
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<tr>
<td>m. Bacillus sp.</td>
<td>90%; Bacillus sp.</td>
</tr>
<tr>
<td>n. Bacillus sp.</td>
<td>88%; Bacillus sp.</td>
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<tr>
<td>o. Bacillus sp.</td>
<td>90%; Bacillus sp.</td>
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<tr>
<td>p. Bacillus sp.</td>
<td>82%; Bacillus sp.</td>
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<td>q. Bacillus sp.</td>
<td>91%; Bacillus sp.</td>
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band corresponded to a different species. There were a number of bands in the profiles of Shandong 0 d sample (i.e., raw material). Except for the fact that a band existed for a longer time, the other bands soon disappeared in the subsequent samples. The highest diversity was obtained in the 30 d sample, and the number of bands decreased gradually after 30 days. The bands were concentrated, and the dominant species were identified in the final product. Dynamic changes from every band can be summarized into the following three types. (i) Some bands appeared during the early fermentation period (15 days) and then disappeared, such as bands k, m, and n. Sequence comparison with the GenBank BLAST database showed that these bands corresponded to an unknown bacterial clone and Bacillus spp. These bacteria were mainly involved in the initial substance decomposition. (ii) Some bands remained for a longer time and then gradually disappeared during the fermentation process, such as bands a, r, s, t, and u. Sequences of these bands showed the highest similarity to Weissella cibaria, Bacillus spp., and Clostridium spp., which played a role during the decomposition process. (iii) Some bands existed during the whole fermentation process, such as bands f, g, j, l, o, p, and q. Sequences from these bands most closely matched the bacteria Leuconostoc garciicum, an uncultured bacterial clone, Lactococcus lactis, Bacillus licheniformis, other Bacillus spp., and Citrobacter freundii. These bacteria were dominant during the whole process and played an important role during fermentation. In particular, bands g, j, and l became the predominant three bands in the final soybean paste (90 days), as the fermentation gradually thickened.

The sequence of band j matched that of Lactococcus lactis (100%), which is often found in fermented food, especially in fermented dairy products [12, 13]. Lactic acid bacteria (LAB) could produce lactic acid during the growing process, which can form esters with the metabolites of yeast. It clearly played an important role in the flavor formation of the finished soybean paste. In addition, the peptidoglycans synthesized by LAB help regulate the immune system [23]. The sequence of band l was highly similar to Bacillus licheniformis (98%). Bacillus licheniformis is a common microorganism found in environments undergoing natural organic matter decomposition and is often isolated from traditional foods [14, 20].

**DGGE Analysis of Bacterial Communities During the TYJY Commercial Soybean Paste Fermentation**

DGGE profiles of microbial communities from commercial soybean paste are shown in Fig. 2. There were more species of bacteria than fungi during the fermentation process. In general, the bacteria originally found in the raw material were the dominant species at the beginning of fermentation. Bacterial abundance increased during the later stages of fermentation. The predominant bacterial bands from the 0 d paste samples were bands a, b, e, and f, which contained sequences most similar to Weissella (99%), uncultured Leuconostoc sp. clone, 100%; d. uncultured bacterial clone, 99%; e. Bacillus licheniformis, 98%; and f. Pediococcus acidilactici, 98%.

![Fig. 2. DGGE profiles of bacteria from TYJY soybean paste sampled at different times during fermentation.](image)

**a.** Weissella cibaria, 99%; **b.** uncultured bacterial clone, 96%; **c.** uncultured Leuconostoc sp. clone, 100%; **d.** uncultured bacterial clone, 99%; **e.** Bacillus licheniformis, 98%; and **f.** Pediococcus acidilactici, 98%.
isolated from fermented food, especially in dairy products [13]. This bacterial species played an important role in the flavor formation of the finished soybean paste.

The bacterial species in Shandong soybean paste were more abundant than in commercial soybean paste during the fermentation process. The predominant bacteria from the Shandong soybean paste sampled during fermentation included relatives of Leuconostoc garciun, uncultured bacteria, Lactococcus lactis, Bacillus licheniformis, Bacillus spp., and Citrobacter freundii. The predominant bacteria from the TYJY soybean paste included relatives of uncultured bacteria, Bacillus licheniformis, and uncultured Leuconostoc. Furthermore, the bacteria found in the TYJY soybean paste were also found in the Shandong traditional soybean paste. However, there were more abundant bacterial species in the naturally fermented soybean paste. This is likely one of reasons why traditional soybean paste has a better flavor quality than the commercial soybean paste.

Construction of a 16S rRNA Clone Library and Analysis of the Bacterial Composition of the Shandong and TYJY Soybean Pastes

Based on the results of the DGGE, the bacterial species from the 30 d sample of Shandong traditional soybean paste and the 7 d sample of TYJY commercial soybean paste were used to construct 16S rRNA gene clone libraries. The sequencing results obtained from these libraries were consistent with the PCR-DGGE results in that the bacterial diversity and abundance in the Shandong traditional soybean paste were greater than in the TYJY commercial soybean paste. For the Shandong clone library, 200 clones with a correct 16S rRNA gene insertion were obtained from the colony PCR-DGGE screen, and 22 distinct clones were selected and named Shandong-clone-1 through Shandong-clone-22. For the TYJY clone library, 104 clones were screened by PCR-DGGE and had a correct 16S rRNA gene insertion, and nine distinct clones were selected and named TYJY-clone-1 through TYJY-clone-9.

The genes from the 16S rRNA clone libraries were sequenced and analyzed using BLAST. A phylogenetic tree was constructed (Fig. 3). The clone library for the 30 d Shandong soybean paste sample can be summarized into eight groups, whereas the clone library for the 7d TYJY soybean paste sample can be summarized into six groups. Shandong-clone-3, -7, -9, -11, -15, -17, and -22 clustered together and accounted for 34% of the total number of clones in the library. These sequences formed the largest group, but their relationships were highly diverged from the rest of the sequences in the phylogenetic tree. Shandong-
clone-1, -5, -19, and -20 clustered together and accounted for 20% of the total clones. They were most closely related to *Pseudomonas* spp. Shandong-clone-4, -13, -14, and -16 clustered together and accounted for 22% of the total clones. Analysis showed that they had a relatively close genetic relationship with *Achromobacter* spp. Shandong-clone-6 formed an independent group, which accounted for 4% of the total clones. It had a close genetic relationship with *Brevundimonas diminuta* (99%). TYJY-clone-5 formed an independent group, which accounted for 7% of total clones in the day 7 TYJY library. It was most related to a species of uncultured bacteria (99%). TYJY-clone-1 and -9 clustered together and accounted for 13% of the total clones. They were most closely related to species of *Weissella*. Shandong-clone-12 and -18, as well as TYJY-clone-7, clustered together and accounted for 6% and 7% of the total clones in their respective libraries. They were most closely related to species of *Bacillus*. Shandong-clone-10 and TYJY-clone-3 clustered into one group, which belonged to a different species of LAB. Shandong-clone-10 was most closely related to *Pediococcus acidilactici* (98%) and accounted for 4% of its library. TYJY-clone-3 was most closely related to *Lactobacillus plantarum* (100%) and accounted for 20% of its library. Shandong-clone-21 and TYJY-clone-8 clustered together and were most closely related to *Enterococcus* spp. (99%). Shandong-clone-2 and -8, as well as TYJY-clone-2, -4, and -6, clustered together and accounted for 6% and 46% of the total clones in their respective libraries. They were most closely related to *Leuconostoc* spp. (99%).

The main microorganisms found in these samples were related to species of *Leuconostoc*, *Lactobacillus*, *Pseudomonas*, and *Bacillus*. *Leuconostoc* and *Lactobacillus* both belong to the LAB group, which consists of bacteria that can produce lactic acid during their growth and metabolism. They also produce esters using metabolites from yeast. The LAB played an important role in flavor formation. Peptidoglycan synthesized by LAB can also modulate the immune system [26]. These results show that the proportion of LAB in the clone library for the TYJY sample was much higher than that in the clone library for the Shandong sample. The difference in raw materials between the two soybean pastes had a direct relationship on the LAB proportion in the fermented sample. Shandong soybean paste is a traditional fermented soybean paste. Its accessory ingredient is corn powder (25%) because the Shandong province is rich in corn. In contrast, soybean plantings in Shandong cover a relatively small area. Zhao [28] thought that adding corn powder made the flavor sweeter than pure soybean paste.

The fermentation process is carried out under natural conditions, and the fermentation period of the soybean paste is rather long (approximately 100 days). The microbes in this soybean paste come from the environment. The main accessory ingredient for the TYJY commercial soybean paste is flour (35%). The addition of koji can shorten the production period (approximately 30 days). The microbes of the koji are the main microorganisms found in the fermentation process.

**PCR-DGGE Analysis of the Fungal Communities During the Shandong Traditional Soybean Paste Fermentation**

DGGE profiles of fungal communities from traditional soybean paste are shown in Fig. 4. Ten species of fungi were detected during the Shandong soybean paste fermentation. Fungal composition was relatively simple in the 0 d raw material sample. There were some miscellaneous fungal bands and the main band d was most closely related to *Geotrichum* spp. This species was found until the later period of fermentation when it decreased in abundance. Fungi were abundant during mid-fermentation (15 to 45 days). The predominant bands during this period were bands d, f, g, h, and j. The quantity and species of fungi

![Fig. 4. DGGE profiles of fungi from Shandong soybean paste sampled at different times during fermentation.](image)

decreased during the late period of fermentation (60 to 90 days). Fungal bands became fainter at these time points and faintest in the final soybean paste. Bands a and c were detected in the 15 d samples. The sequence of band a was most closely related to *Mucor circinelloides* (95%), whereas the sequence of band c most closely matched *Candida krisii* (98%). These bands gradually disappeared in the samples taken after 15 days. Bands f and g appeared in the 15 d sample and then were found in all subsequent samples. The sequence of band f was most closely related to yeast spp. (95%), whereas the sequence of band g most closely matched an uncultured fungal clone (97%). Bands h and i appeared in the 30 d samples. The band h sequence most closely matched *Candida* spp. (95%), and the band i sequence was most similar to *Fusarium oxysporum* (98%). These bands became fainter after the 60 d samples. The band j sequence was related to *Aspergillus oryzae* (99%) and was present throughout the fermentation process. However, the abundance of band J at 0 d was low and became progressively fainter after 15 days, Bacteria can produce various proteases, amylase, glucoamylase, and other enzymes, which help break down the protein that is present in the raw materials into peptones, polypeptides, and amino acids. Starch is degraded into dextran and other smaller sugars, such as glucose and maltose [24]. Bands b and e appeared only in the 45 d samples. The sequence of band b was most closely related to *Monascus* spp. (98%), and the sequence of band e most closely matched *Pichia wickerhamii* (97%). These may be infectious microbes present during the fermentation process.

**PCR-DGGE Analysis of the Fungal Communities During the TYJY Commercial Soybean Paste Fermentation**

DGGE profiles for TYJY commercial soybean paste fungal communities are shown in Fig. 5. The fungal composition was simple throughout the fermentation. Commercial soybean paste had fewer infectious microbes. Fungi from the 0 d raw material sample disappeared in the 2 d sample and represented the infectious fungi that were found in the raw material. Lane 1 shows the DNA of *Aspergillus oryzae* 3024, which was isolated from the mold starter for the commercial soybean paste. It had two bands, g and h. The mold starter was added to the 2 d sample during the fermentation process. The profiles for the 2 d samples were consistent with *Aspergillus oryzae*. Except for the 0 d samples, the main bands were g and h. The band containing *Aspergillus oryzae* gradually faded as the fermentation time increased, showing that the main effects of this fungus occur in the early and middle stages of fermentation. *Aspergillus oryzae* is the predominant microorganism found during the commercial fermentation process. It produces amylase and proteases during the earlier fermentation period. Protein and starch in the raw material are decomposed into small-molecule substances, such as polypeptides, amino acids, glucose, maltose, and fructose [24]. Some of these substances are used by other microbes, making them part of the reaction matrix, while others remain as nutrients in the fermented food.

**Discussion**

Both of these soybean paste preparations used soybean as the primary raw material, but their production technology, accessory ingredients, and fermentation microorganisms were not identical. The ratio of soybean and flour was 65% to 35% in the TYJY raw material, whereas the ratio of soybean and corn was 75% to 25% in the Shandong raw material. The high starch content of the TYJY was beneficial for the growth and reproduction of LAB, which was consistent with the DGGE results. These differing factors accounted for the main reasons the traditional and commercial soybean paste preparations differ in their flavor and quality. Therefore, the stability of the commercial product is better than that of the traditional soybean paste. The
microorganisms in the Shandong soybean paste are more abundant than those in the commercial soybean paste. Shandong soybean paste is made of natural fermentation. More microbes are involved in the fermentation. This is one of reasons why the flavor and quality of traditional soybean paste are better than that of commercial soybean paste. The bacterial composition of both soybean pastes is more abundant than that of the fungi. According to the results of the DGGE and 16S rRNA gene clone libraries, the predominant bacteria found in the fermentation process are Bacillus spp. and Lactobacillia spp., including primarily Bacillus subtilis, Bacillus pumilus, Bacillus amyloliquefaciens, Bacillus licheniformis, and others. Some Bacillus spp. exist throughout the entire fermentation process. Pseudomonas spp. can decompose protein and lipid. Thus, the Pseudomonas spp. may help fungi to decompose the protein during the fermentation period, but sometimes it is considered to cause food spoilage [18]. They cooperate, along with Aspergillus, in decomposing the proteins and starches found in the raw material. At the same time, these bacteria have antagonistic actions against pathogens such as Staphylococcus aureus. They can also promote the growth of the probiotic species such as lactic acid bacteria [14]. This may play an important role in the fermentation process, which is consistent with the research from Korea on traditionally fermented soybean paste and the research from Nigeria on traditional fermented bean products called soy-daddawa [25]. Lactic acid bacteria are important members of the fermentation process [15]. The main lactic acid bacteria are Pediococcus, Lactobacillia, Lactococcus, Leuconostoc, Weissella, and others. Lactic acid bacteria play a major role in the later period of fermentation. They can convert sugar, malic acid, and citric acid into lactic acid and other substances, and they thus can produce flavor compounds in conjunction with the yeast [14].

Fungal species in the Shandong soybean paste are more abundant than those in the TYJY soybean paste during the fermentation process. Aspergillus oryzae is the single fungal spp. found in the TYJY soybean paste during fermentation, whereas a variety of fungi are detected in the Shandong soybean paste. The predominant fungi are Aspergillus oryzae, Candida spp., yeast spp., an uncultured fungal clone, Geotrichum spp., Mucor circinelloides, and others. Yeast and lactic acid bacteria produce flavor compounds during the late fermentation period. Geotrichum spp. can yield proteases, which cooperate with Aspergillus oryzae in the decomposition of proteins in the raw materials before the mid-fermentation period. Mucor circinelloides, which can be isolated from traditionally fermented food [14], can saccharify starch, along with Aspergillus oryzae. It also can decompose protein into flavor compounds during the production of sufu and doucii. These microbes have not been detected in commercial soybean paste and may therefore be one of the reasons why traditional soybean paste has a better flavor than that of commercial soybean paste.

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