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Bacterial and Fungal Diversity in the Starter Production Process of Fen Liquor, a Traditional Chinese Liquor

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Fermented foods and beverages are important parts of human diet. Fen liquor, a Chinese liquor is a fermented beverage that uses a traditional fermentation process. Starters are the main microbial source and also provide nutrients for microorganisms during fermentation. In this study, starters of Fen liquor were produced through a complex traditional fermentation process. To investigate the community structure and the composition of microorganisms in the starter production process, bacterial 16S rRNA and fungal internal transcribed spacer (ITS) regions were sequenced using clone libraries and pyrosequencing, respectively. There was much higher diversity among the bacteria than among the fungi in the starter production process. Bacteria on the surface of the starters belonged mostly to the *Lactobacillaceae* family, while members of the *Bacillacae* family were dominant in the interior of the samples that lacked access to air and water. In the fungi population, diversity was high only in the raw material. In all other samples, nearly all of the fungal sequences were from *Pichia kudriavzevii*, a member of the *Saccharomycetaceae* family. Nearly all samples showed similar fungal community structures, indicating that there was little change in the fungal community. To the best of our knowledge, this is the first report to reveal the whole process of the starter production of Chinese traditional liquor. The findings obtained in this study provide new insights into understanding the composition of the microbial community during the traditional Chinese liquor starter production process and information about the production process control and monitoring.

Keywords: Fen liquor, starter production, bacteria, fungi, diversity

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Introduction

The production of Fen liquor, which is the typical representative of Chinese light-flavor liquor, has a long history and utilizes unique technology. After thousands of years of domestication, microorganisms involved in Fen liquor production have formed a specific community structure based on the geographical and climatic conditions in Shanxi province in China. The quality of Fen liquor is closely related to the composition of the microbial communities during the production process. The changes in the microbial communities during the "solid cylinder separation fermentation" process produce most of the metabolic fermentation characteristics of Fen liquor (Zhang, 2000; Han *et al*., 2009).

The production of Fen liquor in modern times still uses traditional fermentation technology, which involves numerous steps. Microorganisms perform two main functions in the production of Fen liquor: the starter production and the actual fermentation process using the starter and steamed broomcorn (Li *et al*., 2011). The starter acts as the main microbial source and also provides nutrients for microorganisms that produce an array of enzymes with various biochemical properties. The production of the starter is a very complex process that takes nearly seven months. The starter is made from a mixture of barley, wheat, and peas, which are stirred together with the addition of water. After quenching, this mixture is formed into approximately 3-kg firm, rectangular, parallelepiped-like bricks, and cultivated in special rooms. The starter bricks remain in the room for approximately one month, in which they go through six stages of treatment. During the first stage, the starter bricks are covered by straw mats and the temperature of the room rises slowly to approximately 40°C until mold is observed on the surface of starter bricks. This stage usually lasts for more than two days, depending on the season. Next, the straw mats are removed and the starter bricks are turned over several times. This second stage lasts between two and five days. The third stage is the main stage for microbial growth and lasts for five to six days. The starter bricks must be turned over every day to maintain a temperature suitable for the growth of microorganisms. During the fourth stage, the room temperature reaches its highest point, and the air humidity is very low. Workers must turn over the starters every day for three days. Starter bricks are turned over every two days during the fifth stage, which lasts for three or four days. After this stage, only around 1 cm depth from the surface of the starter remained wet, and the room temperature is slowly dropped to approximately 31–32°C over five or six days. During the final stage, the temperature in the central parts of the starters drops slowly over three or four days.

All six stages usually take approximately 26–28 days to complete. Following the complex production of the starters, the drying process lasts from three to six months, and then the starters are ready to be used for liquor fermentation.

During the production of the starters, no microorganisms are intentionally added for the liquor fermentation process. The microbial communities of the starter have been studied in a lot of previous works (Thanh *et al.*, 2008; Wang *et al.*, 2008; Shi *et al.*, 2009; Jung *et al.*, 2012). But most of them focused on the finished product, the knowledge of the microbial communities during production process of the starter is still unknown. During the entire production process of the starter, the composition of microbial communities and abundance of microorganisms must undergo complex changes. In this case, a new method, rather than the traditional ones such as cell culture and colony counting, which have been proved to be helpless, should be used to explore the variation and structure of microbial communities. Molecular biology techniques provide precise insight into microbial diversity and a rapid, high-resolution description of microbial communities by targeting ribosomal genes (Amann and Ludwig, 2000). The high-throughput pyrosequencing technique produces a very large number of reads for many different samples during a single run by using bar-coded primer sets (Hamady *et al.*, 2008).

In this study, the diversity of bacterial and fungal communities in the starter production process was detected using molecular methods. Bacterial diversity was analyzed using sequencing of the 16S rRNA gene clone library. Fungal diversity was analyzed using pyrosequencing of the internal transcribed spacer (ITS) region. The abundance of bacteria and fungi in samples from throughout the starter production process was compared by quantitative real-time PCR (qPCR). The results of this study helped us to further understand the community structure and composition of microorganisms during the starter production process.

Materials and Methods

Sampling

The raw materials of a starter and a newly formed brick were sampled. The starter (dimensions 27×18×5 cm) was freshly produced during the summer of 2008. During the whole production process, a total of 12 stages were analyzed. For the starter brick of each stage, samples were collected from the surface, interior, and the center of the starter. Scrapings to a depth of 0.5 cm were initially taken from the entire surface of the starter brick. The starters were then broken apart, and samples were taken from the central parts of the bricks. After the central samples were removed, samples were removed from the interior of the starter bricks. The interior was defined as an area at least 1 cm from the surface and 1 cm from the central part of the brick. The raw materials were defined as stage S1, the sample taken from the newly formed starter brick was defined as S2, the six stages of production in the room were defined as stages S3-S8, and the six months of the starter storage were defined as stages S9-S14. The detail information of each sampling stage was described in Table 1.

DNA extraction and quantitation

Samples were stored at -20°C prior to DNA extraction. Total DNA was extracted from 0.2 g of each sample, as previously described (Schmidt *et al.*, 1991; Li *et al.*, 2011). The concentration of extracted DNA was determined using a NanoDrop 3300 (Thermo Fisher, USA).

Bacterial 16S rRNA gene and fungal ITS region amplification and sequencing, classification analysis, and phylogenetic analysis

The experimental procedures and primer sets for the amplification and sequencing of bacterial 16S rRNA genes and fungal ITS regions were similar to those previously described for the Fen liquor fermentation process (Li *et al.*, 2011). The classification and phylogenetic analyses described by Li *et al.* (2011) were used in this study.

Table 1. **The sampling stages in this study**

Process of making starters[a]		Stage name	Sampling time
Raw material		S1	2008-6-12
Making of starter bricks		S2	2008-6-12
Incubation of starter bricks in room	Stage I	S3	2008-6-13
	Stage II	S4	2008-6-16
	Stage III	S5	2008-6-20
	Stage IV	S6	2008-6-26
	Stage V	S7	2008-7-02
	Stage VI	S8	2008-7-04
Drying process of starter bricks	After one month	S9	2008-8-12
	After two months	S10	2008-9-13
	After three months	S11	2008-10-13
	After four months	S12	2008-11-13
	After five months	S13	2008-12-12
	After six months	S14	2009-1-13

[a] The details of each stage were shown in the description of starter making process in the introduction part.

Quantitative real-time PCR

Three parts of S3, S7, and S11 of the starter production process were selected for investigation of the abundance of bacteria and fungi as previously described (Li *et al.*, 2011).

Community analysis

The coverage percentages were determined using the rarefaction estimator in the Mothur program v.1.17.2 (Schloss *et al.*, 2009) and Analytic Rarefaction software v 1.3 (http://www.uga.edu/strata/software/Software.html). Microbial community similarity analyses were conducted by the unweighted pair group method with arithmetic averages (UPGMA) clustering using online UNIFRAC program (Lozupone and Knight, 2005), which measures the molecular evolutionary distances of the sequences and is able to compare the relationships among microbial communities.

Nucleotide sequence accession numbers

The sequences of each OTU of the 16S rRNA gene sequences of the bacteria in this study were available in GenBank under the accession numbers KC563212–KC565455, and the ITS1 region sequences of the fungi were available in SRA under the accession number SRA052669.1.

Results

Analysis of OTU richness

The estimated OTU numbers (according to the rarefaction method) were generated to allow for 3% sequence dissimilarities of the bacterial 16S rRNA gene sequences (Fig. 1C) or different taxonomic annotations of the fungal ITS1 regions (Fig. 4C). In total, there was greater diversity among the bacteria than among the fungi. The bacterial diversity was higher in surface samples than in the other samples. The bacterial diversity in surface samples was minimal from stages S1 to S5, but there was significant diversity observed in the later stages. In the interior samples, the bacterial diversity increased slowly from stages S4 to S8, as well as S10 to S13, but there was little diversity in stages S9 and S14. The central samples, like the interior samples, showed the

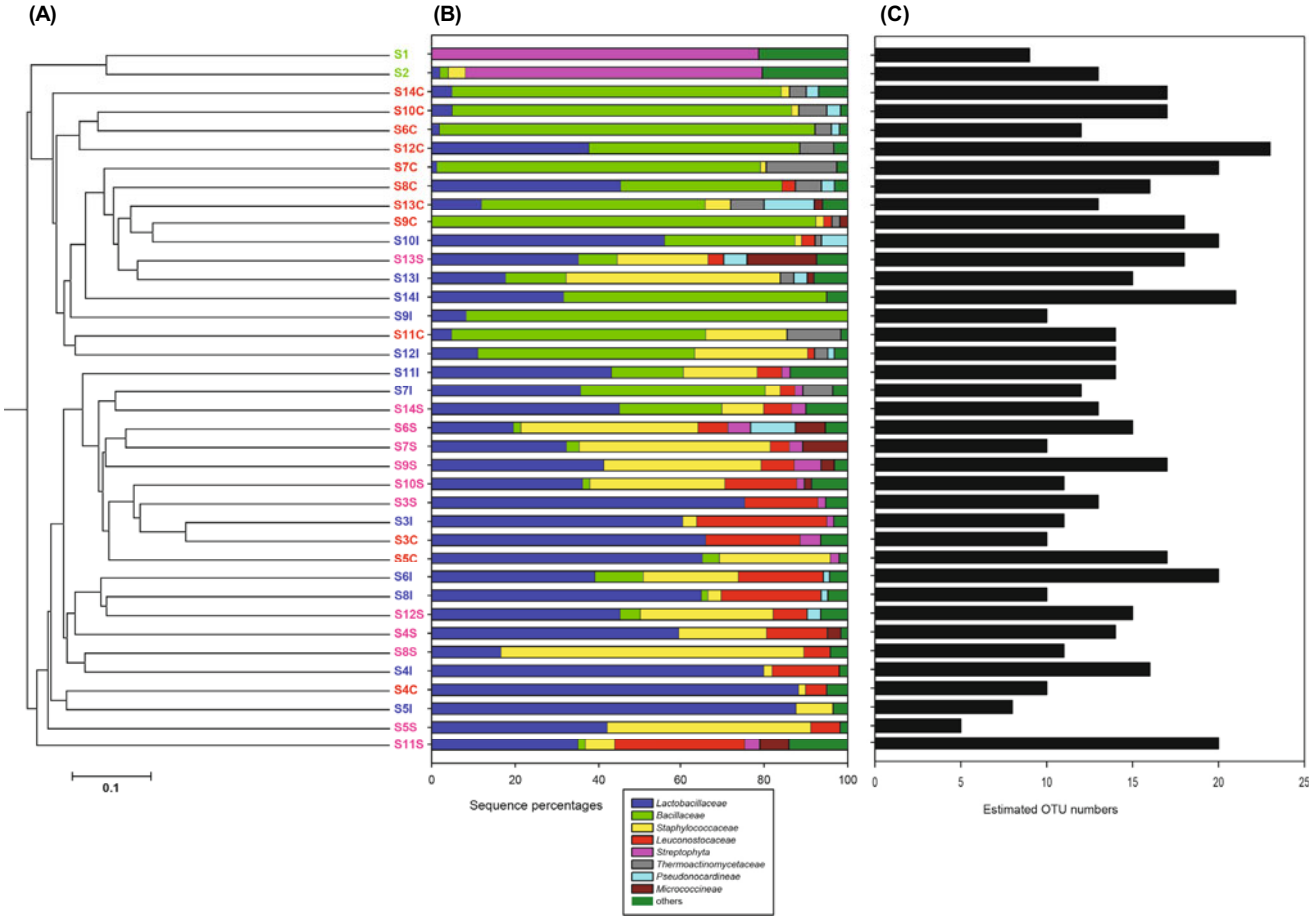

Fig. 1. (A) Relative abundance of bacterial taxa across samples. Samples were clustered by unweighted pair group method with arithmetic mean (UPGMA). The samples were colored according to different sections of the starter brick and stages of the starter production process with S1-2, green; surface, yellow; interior, blue; central, red. "S", "I", and "C" indicate the three parts of the samples (surface, interior, and central, respectively) in S3–S14. (B) The major families (more than 1%) were named in the bottom of the columns. (C) Estimated OTU numbers, according to the rarefaction method, depending on OTUs identified with a 3% cut-off. The raw materials were defined as stage S1, the sample taken from the newly formed starter brick was defined as S2, the six stages of production in the room were defined as stages S3-S8, and the six months of the starter storage were defined as stages S9–S14.

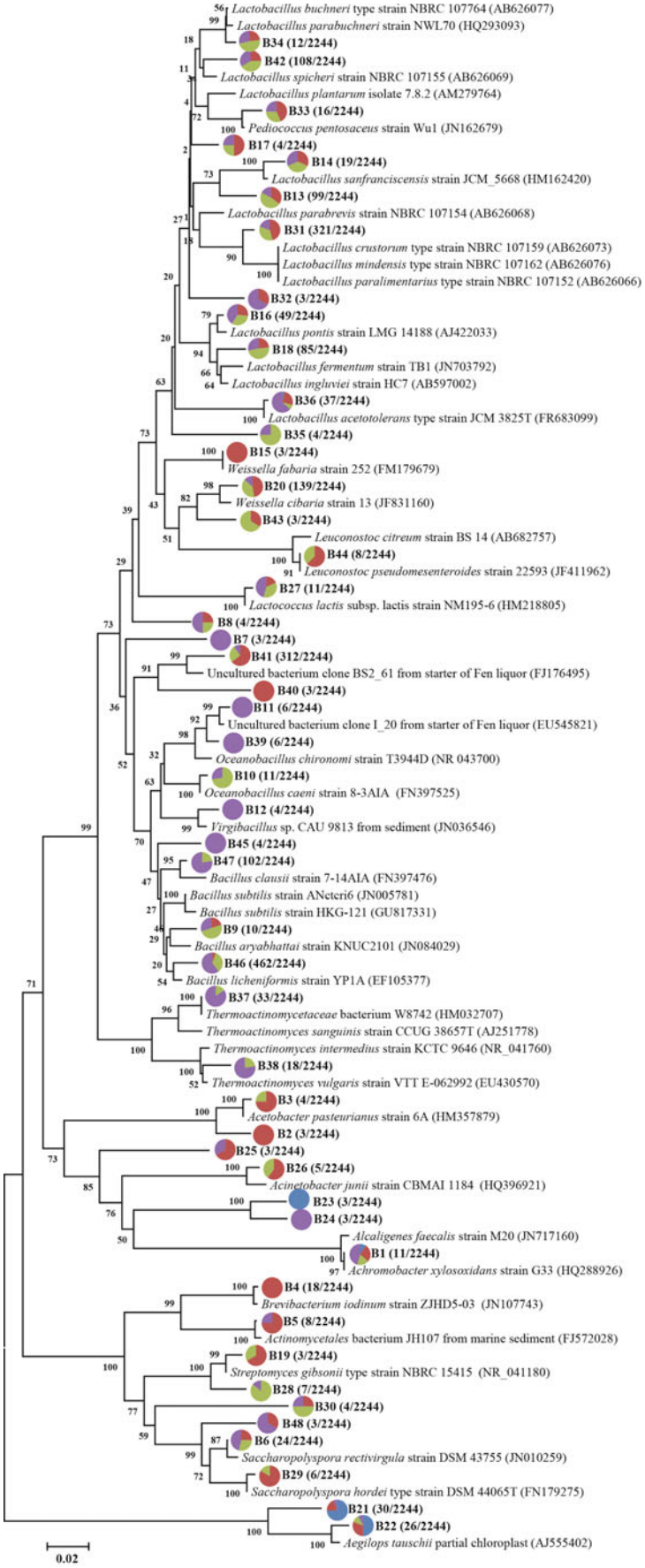

Fig. 2. Phylogenetic tree of the 16S rRNA gene sequences and their phylogenetic relatives. The tree was calculated with the maximum likelihood method. Numbers on nodes indicate bootstrap values (1,000 resamplings). The scale bar indicates 0.02 nucleotide substitutions per nucleotide position. The OTUs obtained in this study are shown in boldface. The pie before the OTU names indicates the percentages in different stages of the OTU with S1-2, blue; surface, red; interior, green; central, purple.

lowest diversity in stage S9. However, in the opposite trend from the interior samples, the diversity in the central samples decreased from stages S4 to S7 and increased from stages S9 to S14. The fungal diversity remained low at every stage except stage S1.

Diversity of bacteria

An examination of 2244 clones of the 16S rRNA gene sequences (the sequence number was 59±10) indicated that the bacteria participating in fermentation process were distributed among eight phyla, including *Firmicutes*, *Actino-*

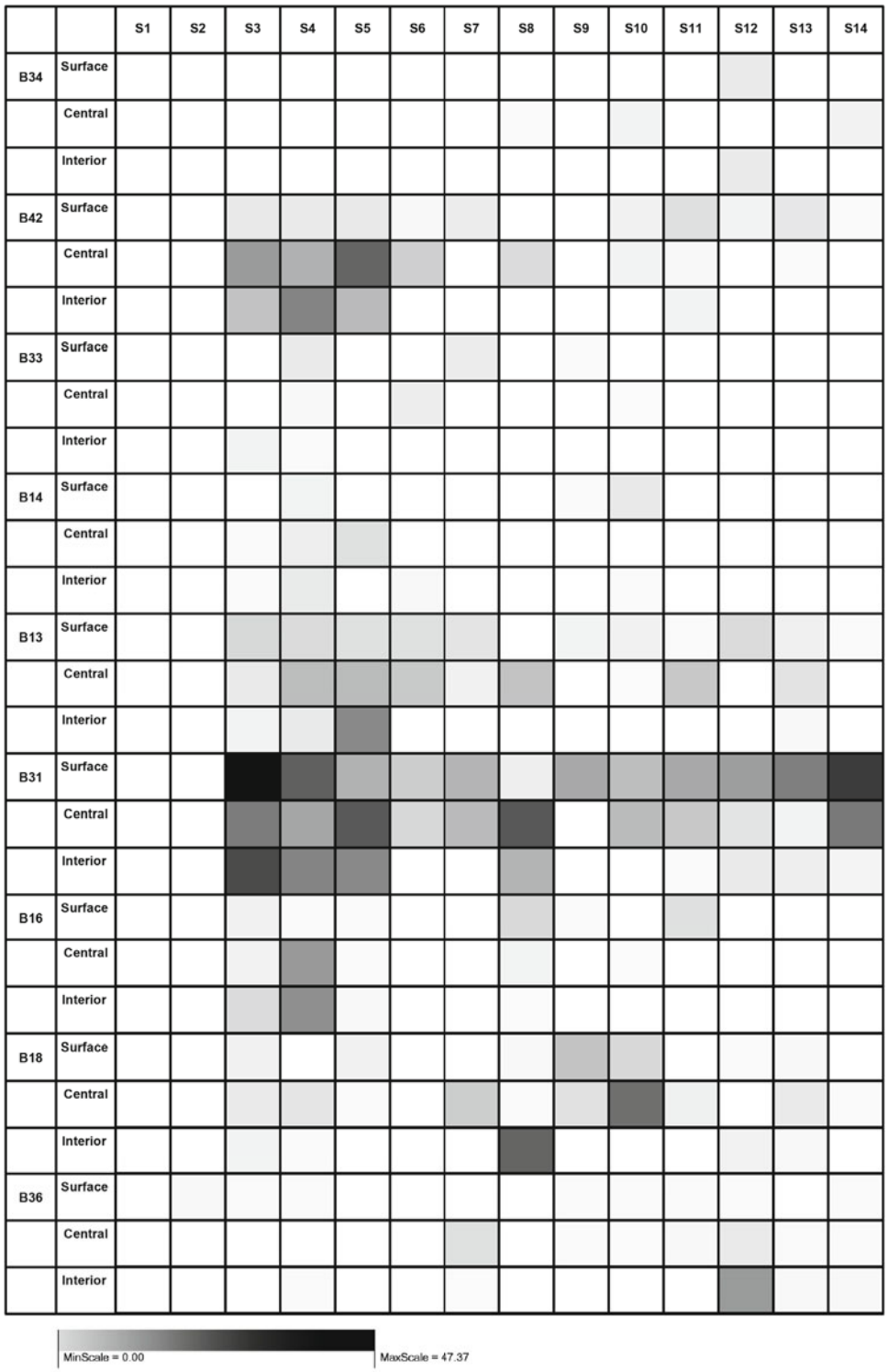

Fig. 3. The sequence percentages of OTUs in the family *Lactobacillaceae* during the starter production process. The abbreviations of the fermentation stages are the same as in Fig. 1. The names of OTUs were similar with Fig. 2. The depth of the color indicates the sequence percentage of each OTU. The percentages of OTUs less than 5% were not shown.

bacteria, and *Proteobacteria*. Nearly 90% of the 16S rRNA gene sequences belonged to the phylum *Firmicutes*, of which, approximately 35% of sequences could be matched to members of the family *Lactobacillaceae*, and 28% represented members of the family abundance family was *Bacillaceae*.

The UPGMA clustering among 38 samples from 14 stages of the bacteria in starter production is shown in Fig. 1A. The bacterial communities could be grouped into three clusters according to abundance of specific bacteria. The bacterial communities in stages S1 and S2 were distinct from those of other stages and mainly consisted of the 16S rRNA gene sequences of chloroplast. The family *Bacillaceae* accounted for the majority of the bacteria in most central samples and some of interior samples. Bacteria from the other samples, including some interior samples, a few central samples, and most of the surface samples were members of the family *Lactobacillaceae* (Fig. 1B).

A total of 225 OTUs were achieved based on a 3% cutoff. Singletons and doubletons were removed, and the remaining 48 OTUs were used to construct the phylogenetic tree (Fig. 2). The sequence numbers and the distribution in different samples are indicated using the number and pie in the phylogenetic tree. Most of sequences in S1 and S2 were similar to the 16S rRNA gene sequence of chloroplast from *Pisum sativum* and *Oryza rufipogon*. About half of the OTUs were detected in all three sections of the starters. The OTUs detected only in the surface or central parts were very abundant. The most abundant OTU was OTU B46, which represented approximately 21% of the 16S rRNA gene sequences and showed high similarity to *Bacillus licheniformis*. Approximately 14% of the sequences belonged to the second most abundant OTU B31, which was similar to bacteria of the genus *Lactobacillus*. Half of the OTUs with more than 10 sequences were members of the *Lactobacillaceae* family.

The family *Lactobacillaceae* was the most important bacteria during the Fen liquor fermentation process (Li *et al.*,

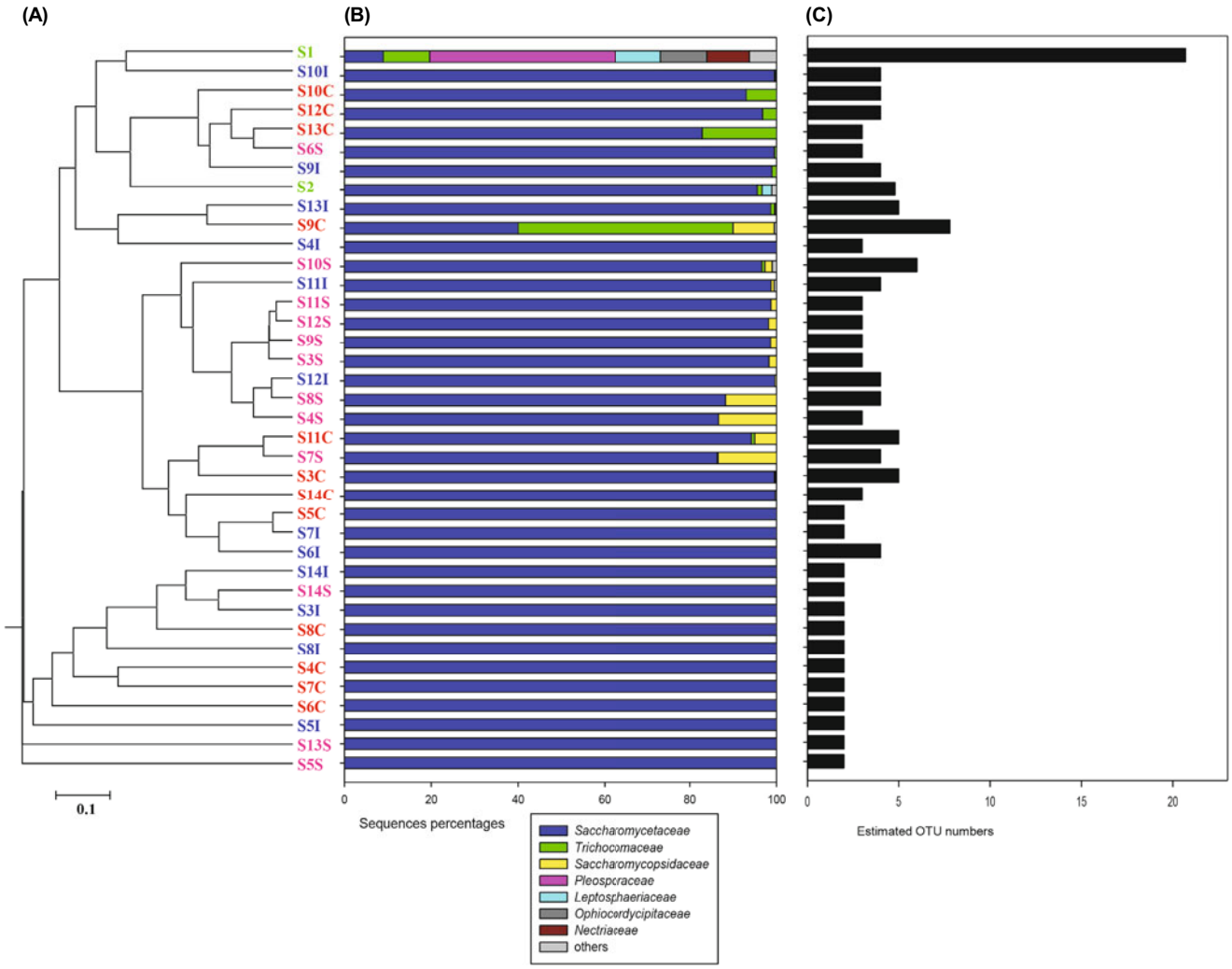

Fig. 4. (A) Relative abundance of fungal taxa across samples. Samples were clustered by UPGMA. The samples were colored according to different sections of the starter brick and stages of the starter production process with S1-2, green; surface, yellow; interior, blue; central, red. (B) The major families (more than 1%) were named in the bottom of the columns. The abbreviations of the fermentation stages are the same as in Fig. 1. "S", "I", and "C" at the end of abbreviations of the fermentation stages indicate the three parts of the samples (surface, interior, and central, respectively). (C) Estimated OTU numbers, according to the rarefaction method, depending on OTUs identified with the same taxonomic annotations.

2011), so the detailed change of OTUs in this family during the 14 stages is shown in Fig. 3. No *Lactobacillaceae* sequences were detected in S1 and only one sequence was detected in S2. The most abundant OTU in the family *Lactobacillaceae* was OTU B31, which showed high similarity to *L. crustorum*, *L. mindensis*, and *L. paralimentarius*. This OTU was found in a higher proportion in surface samples than in interior or central samples. However, in all three types of samples, percentages of this OTU were higher in the early stages of the starter production and in the last month of storage. The second most abundant OTU, B42, showed similarity to *L. spicheri*, and was more frequently detected in early stages in the interior and central parts of the starter brick and was detected in nearly equal amounts throughout the different stages in the surface samples. The OTU B13 was detected more often in the surface and interior samples and only rarely in the central samples.

Diversity of fungi

The fugal diversity was relatively low. As Fig. 4 shows, approximately 97% of the ITS region sequences could be assigned to the family *Saccharomycetaceae*. According to the BLAST results, nearly all sequences in the family *Saccharomycetaceae* showed more than 99% similarity to *Pichia kudriavzevii*. Only 1% of the sequences belonged to the families *Saccharomycopsidaceae* and *Trichocomaceae*. The ITS region sequences in different samples showed similar taxonomic profiles. In stage S1, only 9% of the sequences were similar to *P. kudriavzevii*, but this percentage increased in later samples. The fungal diversity was high only in stage S1 and was very low in other samples. In all of the surface samples, more than 85% of the sequences belonged to *P. kudriavzevii*, and the percentages were only slightly lower in S4, S7, and S8. Greater than 98% of the sequences were *P. kudriavzevii* in all interior samples. In the central samples from S3–S8, and S14, the results were similar to those of the interior samples, while in S9–S13, the percentages decreased. The decrease was most dramatic in S9. According to the fungal communities, the samples could be grouped into two clusters. One cluster consisted of S3-S8 and the last stage, S14. The second cluster consisted of all other samples.

Table 2. The copy numbers of the bacterial 16S rRNA gene and fungal ITS1 region during three stages of the starter production process

Stage[a]	The bacterial 16S rRNA gene copy numbers (/g sample)	The fungal ITS1 region copy numbers (/g sample)
S3S	$4.394 \pm 0.004 \times 10^7$	$3.692 \pm 0.009 \times 10^7$
S3I	$5.421 \pm 1.252 \times 10^7$	$5.061 \pm 0.024 \times 10^6$
S3C	$1.559 \pm 0.005 \times 10^7$	$2.159 \pm 0.003 \times 10^6$
S7S	$2.283 \pm 0.014 \times 10^6$	$9.209 \pm 0.011 \times 10^6$
S7I	$7.658 \pm 1.069 \times 10^5$	$3.809 \pm 0.004 \times 10^6$
S7C	$1.764 \pm 0.185 \times 10^7$	$1.444 \pm 0.009 \times 10^7$
S11S	$1.498 \pm 0.010 \times 10^6$	$5.913 \pm 0.007 \times 10^6$
S11I	$1.167 \pm 0.239 \times 10^6$	$5.798 \pm 0.000 \times 10^6$
S11C	$1.034 \pm 0.122 \times 10^6$	$2.927 \pm 0.002 \times 10^5$

[a] The abbreviations of the fermentation stages are the same as in Fig. 1. "S", "I", and "C" at the end of S3, S7, and S11 indicate the three parts of the samples (surface, interior, and central, respectively).

Quantitative analysis of bacteria and fungi in different sections of samples

Three sections of S3, S7, and S11 were selected to measure the copy numbers of the 16S rRNA gene and the ITS region (Table 2). Overall, the copy numbers of the 16S rRNA gene and the ITS region decreased during the starter production process while these values first increased and then decreased for the central sections of the samples. In S3 and S11, the copy numbers among the surface samples were higher than the copy numbers in central samples. However, in stage S7 the highest copy numbers were observed in the central samples.

Discussion

The microbial communities in fermented foods have received attention and been increasingly studied in recent years. Researchers have analyzed different types of fermented foods such as fermented seafood (Roh *et al.*, 2010), kimchi (Chang *et al.*, 2008), traditional fermented mustard (Chao *et al.*, 2009), and traditional Vietnamese alcohol fermentation starters (Thanh *et al.*, 2008) using rRNA gene sequence analysis to investigate microbial community diversities. Chinese liquor is produced using traditional methods and one of the most popular methods involves fermentation processes of grains in solid form utilizing starter cultures, which consist of crude combinations of microorganisms. The starters supply the microbial source and nutrients for the liquor fermentation process. The changes in the microbial community during the starter production process were complex and it is critical to understand the functional microorganisms during the fermentation process. In this study, a total of 14 stages throughout the starter production process were sampled, as described in Table 1. For stages S3-14, three parts of the starter bricks were analyzed to investigate the full range of microbial community diversity.

The bacterial community showed various profiles in different sample types. The samples taken from the raw materials used in the starter production had clustered together in which most sequences were similar to the 16S rRNA gene sequences of chloroplast. The other samples had different patterns of bacterial diversity, depending on which section of the starter brick from which they were taken. The surface samples and central samples showed different types of bacterial communities. The interior samples were removed from an area between the surface and central samples and displayed features of both sample types. This distribution of bacteria was in agreement with the physical properties of the samples. The central samples were not suitable for the growth of bacteria except spore-forming bacteria because of less access to water and air and because of a higher temperature in the central parts of the starter bricks.

Different results were observed when the samples of the bacterial and fungal communities were clustered. In the bacteria population, most of the samples were divided into several clusters. These clusters were based mainly upon different locations in the starter bricks that have different physical and chemical properties. In the fungi population, S1 showed a different community with other samples, which was consistent with the composition of the bacterial com-

munity. Other samples showed similar community structures indicating that there are few changes in fungal communities. There were no significant changes in microbial community structures for either bacteria or fungi in different stages of the starter production.

In the bacteria population, the most abundant family could group to 12 OTUs. The sequence of OTU B31 was similar to some *Lactobacillus* species. This OTU was also similar to the uncultured *Lactobacillus* sp. Clone Al2-6c 16S rRNA gene sequences detected in alfalfa fermentation (Wang *et al.*, 2006), indicating that this OTU correlates with fermentation processes using plants raw materials. The distribution of this OTU in the samples suggested that this OTU grew in early stages of the starter production. Although the percentage decreased during the six months of storage in the production of the starter, it increased during the final month in the starter production process. The OTUs B42 and B13 that are related to *Lactobacillus* spp. were detected in higher percentages in the early stages in the interior and central samples but were found in equal amounts during all stages in the surface samples. This result indicateed that these OTUs grew from the surface to the interior of the starter bricks in the early stages. In the fungi population, *P. kudriavzevii* was undoubtedly the most important fungi in the starter production process, as well as throughout the whole fermentation process. This result was confirmed by the fungal diversity study on the fermentation process (Li *et al.*, 2011).

The composition of the microbial community during the fermentation process of Fen liquor was reported recently (Li *et al.*, 2011). In Li's study, nearly all of the 16S rRNA gene sequences belonged to the family *Lactobacillaceae*, while more than three families accounted for the fungal communities. Interestingly, the bacterial and fungal community diversities showed differences in the starter production process. Some aspects of the major bacterial and fungal types were similar in that study and our study. The most abundant bacteria in the starter samples were of the family *Lactobacillaceae*, which also played an important role during the fermentation process. Nearly all of the ITS region sequences of starter samples belonged to the family *Saccharomycetaceae*, which were also the major fungal community in the fermentation process. There were also notable differences in microbial community composition. In the bacteria population, there were three shared OTUs in both the starter production and the fermentation process that matched to the family *Lactobacillaceae*: OTU B13, B34, and B36. None of these were abundant in the starter samples. The most abundant OTU in the fermentation process was similar to *Lactobacillus acetotolerans* while only 37 sequences (2%) belonging to this OTU were detected in the interior and central parts of the starter samples and none in the surface parts. Therefore, the possible effect of the bacteria that were only detected in the starter samples should receive more attention. If those types of bacteria do not affect the fermentation process, inhibiting the unwanted bacteria and promoting the useful bacteria is a very necessary step in improving the quality of fermentation. The detailed microbial communities formed during the entire starter production process in this study provide us with more information than previous studies that focused only on the finished starter products (Shi *et al.*, 2009; Zheng *et al.*, 2012). In those studies, *Bacillus* spp. were the most abundant bacteria while the results in this study showed that *Bacillus* spp. were mostly detected in the central samples only. The combination of the starter production and fermentation process also showed that *Lactobacillus* spp. were actually the most important bacteria in the starters. The only fungal OTU in starter samples was similar to *P. kudriavzevii* and was one of the seven main OTUs in the fermentation process. Furthermore, the family *Trichocomaceae*, which was thought to play an important role in the food fermentation process, was detected more in the central samples of the later stages, indicating that this fungus might not be important in the fermentation process. This result was confirmed by the fungal diversity study of the fermentation process. However, the microorganisms that were not detected in the fermentation process but were dominant in the starter production process might still play important roles in changing enzyme or substrate activities during the starter production process. The findings obtained by both our study and the fermentation process study provide new insights into understanding the microbial community as well as the function of the microbes during the traditional Chinese liquor production process and also provide us with information about the production process control and monitoring of the fermentation process.

This study provided detailed information about microbial community composition and diversity, furthering the understanding of an important step of traditional Chinese liquor fermentation process and showing the rich microbial diversity in the production process of the starter. The production of the starter is completed by several types of microbes and different stages had different a niche, so different microbes might play different roles in each stage of this process. However, in this study, the quantity and taxonomic information about microbes was based on the sequences from a clone library and pyrosequencing and the bias in experiments made it relative only for the microbial description. Therefore, these results only reflect the microbial communities to a certain extent. Furthermore, the analysis by amplification of the rRNA gene might not reflect activated microbes, so RNA might be better target to demonstrate microbial community and composition in the process of the starter production in future studies. However, the room where the starters were produced was not sterile; therefore, the monitoring of microbes in the room is necessary in any further studies.

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References

Amann, R. and Ludwig, W. 2000. Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. *FEMS Microbiol. Rev*. **24**, 555–565.

Chang, H.W., Kim, K.H., Nam, Y.D., Roh, S.W., Kim, M.S., Jeon, C.O., Oh, H.M., and Bae, J.W. 2008. Analysis of yeast and archaeal population dynamics in kimchi using denaturing gradient gel electrophoresis. *Int. J. Food Microbiol.* **126**, 159–166.

Chao, S.H., Wu, R.J., Watanabe, K., and Tsai, Y.C. 2009. Diversity of lactic acid bacteria in *suan-tsai* and *fu-tsai*, traditional fermented mustard products of taiwan. *Int. J. Food Microbiol.* **135**, 203–210.

Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J., and Knight, R. 2008. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat. Methods* **5**, 235–237.

Han, S., Lei, Z.H., Li, Q., Lu, L.H., and Zhao, L.Q. 2009. Study on the cultured microbial community and the metabolism regulation during the brewing process of the Fen liquor. *Food Fermentation Industries* **35**, 9–13. (Chinese)

Jung, M.-J., Nam, Y.-D., Roh, S.W., and Bae, J.-W. 2012. Unexpected convergence of fungal and bacterial communities during fermentation of traditional Korean alcoholic beverages inoculated with various natural starters. *Food Microbiol*. **30**, 112–123.

Li, X.R., Ma, E.B., Yan, L.Z., Meng, H., Du, X.W., Zhang, S.W., and Quan, Z.X. 2011. Bacterial and fungal diversity in the traditional chinese liquor fermentation process. *Int. J. Food Microbiol.* **146**, 31–37.

Lozupone, C. and Knight, R. 2005. Unifrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228–8235.

Roh, S.W., Kim, K.-H., Nam, Y.-D., Chang, H.-W., Park, E.-J., and Bae, J.-W. 2010. Investigation of archaeal and bacterial diversity in fermented seafood using barcoded pyrosequencing. *ISME J.* **4**, 1–16.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., and *et al.* 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541.

Schmidt, T.M., DeLong, E.F., and Pace, N.R. 1991. Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. *J. Bacteriol.* **173**, 4371–4378.

Shi, J.H., Xiao, Y.P., Li, X.R., Ma, E.B., Du, X.W., and Quan, Z.X. 2009. Analyses of microbial consortia in the starter of fen liquor. *Lett. Appl. Microbiol.* **48**, 478–485.

Thanh, V.N., Mai, L.T., and Tuan, D.A. 2008. Microbial diversity of traditional vietnamese alcohol fermentation starters (*banh men*) as determined by PCR-mediated DGGE. *Int. J. Food Microbiol.* **128**, 268–273.

Wang, X.F., Haruta, S., Wang, P., Ishii, M., Igarashi, Y., and Cui, Z.J. 2006. Diversity of a stable enrichment culture which is useful for silage inoculant and its succession in alfalfa silage. *FEMS Microbiol. Ecol.* **57**, 106–115.

Wang, C.L., Shi, D.J., and Gong, G.L. 2008. Microorganisms in daqu: A starter culture of chinese maotai-flavor liquor. *World J. Microbiol. Biotechnol.* **24**, 2183–2190.

Zhang, Q.H. 2000. The evolution history and development trend of distillate liquor in China. *Liquor-Making Sci. Tech.* **102**, 25–31. (Chinese)

Zheng, X.W., Yan, Z., Han, B.Z., Zwietering, M.H., Samson, R.A., Boekhout, T., and Robert Nout, M.J. 2012. Complex microbiota of a Chinese "fen" liquor fermentation starter (fen-daqu), revealed by culture-dependent and culture-independent methods. *Food Microbiol.* **31**, 293–300.