Analysis of microbial diversity and succession during Xiaoqu Baijiu fermentation using high-throughput sequencing technology

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Abstract
In this study, high-throughput sequencing (HTS) was used to compare and analyze the microbial diversity and succession during the brewing process of xiaoqu Baijiu. A total of 34 phyla and 378 genera of bacteria, as well as four phyla, 32 genera of fungi were detected. At the phylum level, Firmicutes, Proteobacteria, Ascomycota, and Mucoromycota were the dominant groups. During the brewing process of xiaoqu Baijiu, the dominant bacteria were Weissella and unidentified Rickettsiales within the first 2 days of brewing, followed by Lactobacillus at 3 days until to the end of brewing. The dominant fungi were Rhizopus, Saccharomyces, and Issatchenkia. The relative abundance of Rhizopus decreased with the extension of brewing time, while the relative abundance of Saccharomyces increased, and Saccharomyces became the dominant species at the second day of brewing. This study revealed the diversity and changes of the microbial community during the brewing process of xiaoqu Baijiu, providing theoretical support and laying a foundation for future study on the contribution of microbial metabolism during brewing of xiaoqu Baijiu, thereby promoting the development of xiaoqu Baijiu industry.

KEYWORDS
Baijiu, high-throughput sequencing, microbial diversity, microbial succession, Xiaoqu

1 INTRODUCTION

Chinese liquor (Baijiu) is one of the oldest solid-state fermented, distilled spirits [1]. There are some challenges during traditional solid-state Baijiu brewing process [2].

It is difficult to monitor fermentation parameters because the containers are usually set underground. Meanwhile, the parameters (such as temperature, pH) are often not uniform. In addition, it is also difficult to apply modern automatic production lines to the production of Baijiu, because fermentation, distillation and storage are batch operations during the production of Baijiu [2].

According to the aroma components, Baijiu can be categorized into three major types, sauce-flavor Baijiu, strong-flavor Baijiu, and light-flavor Baijiu [3]. Xiaoqu
Baijiu is an important type of light-flavor Baijiu in China, which is produced using xiaoqu, one of the major types of jiuqu starter used in Baijiu production. Xiaoqu is usually made from rice, rice bran and wheat bran as the raw material, which is artificially inoculated with Rhizopus and yeast [4, 5]. Xiaoqu Baijiu had a short brewing period and a higher yield compared with Daqu Baijiu [5].

In early studies, microbial diversity was mostly investigated using traditional microbial culture methods. With the rapid development of molecular biotechnology in recent years, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and high-throughput sequencing (HTS) technology have become the standard approaches for the analysis of microbial community diversity [6]. HTS is a simple technique that generates large amount of data [7]. HTS technology has been increasingly applied to study the diversity and structure of the microbial community during the Daqu Baijiu brewing process, including Daqu, the mud used to seal the fermentation pit, and fermented grains [8–11]. Microorganisms produce a majority of flavored substances during the brewing of Baijiu [12]. Therefore, there is an increasing interest in revealing the characteristics of the microbial community and the important influence of its changes on Baijiu brewing [13]. Bacterial flora was found to significantly influence the production and quality of sauce-flavored and strong-flavored Baijiu [7, 14]. However, few studies investigated the microbial diversity and community structure of light-flavor Baijiu [15], especially xiaoqu Baijiu. Studies of the microbial diversity in xiaoqu Baijiu mainly focused on xiaoqu from different production areas in China, which possess different bacterial compositions [6, 16, 17]. Physicochemical indexes and different metabolites of xiaoqu Baijiu, such as metabolism of carbohydrates, cofactors, vitamins, and amino acids, were closely correlated with microbiota of xiaoqu [18, 19]. Lactobacillus, Bacillus, Acinetobacter and Gluconobacter were dominant bacteria in xiaoqu from Hubei and Sichuan provinces of China [20]. Lactobacillus, Leuconostoc and Saccharomyces, Rhizopus, Aspergillus were the most abundantly present bacterial and fungal genera in Zangqu [21]. However, there is little research focused on the microbial community structure and diversity during the xiaoqu Baijiu brewing process. The abundance of Bacillus was the highest in the early stage of fermentation, while abundance of the lactic acid bacteria (LABs) increased to the highest with the progress of fermentation [22]. In this study, the structure and diversity of fungi were not investigated. Klebsiella, Saccharomyces, and Rhizopus were dominated in Sichuan xiaoqu Baijiu fermented grains [23]. The result was different from the results reported before [22], which maybe due to the region or the variety of xiaoqu used. The numbers of yeasts and bacteria were significantly higher in new mechanical technology than that in traditional technology at the peak of xiaoqu Baijiu fermentation [24]. The results indicated that brewing technology also influenced the microbial diversity and succession.

In the present study, we investigated the changes of microbial community structure and diversity during xiaoqu Baijiu brewing. It is helpful to understand the contribution of various microorganisms to the quality of xiaoqu Baijiu. Pure cultured xiaoqu was used to better understand the source and role of functional bacteria in xiaoqu Baijiu by deep mining of functional taxa. Understanding the microbial community structure and microbial succession during xiaoqu Baijiu brewing will provide a scientific basis for quality control and flavor enhancement of xiaoqu Baijiu.

2 | MATERIALS AND METHODS

2.1 | Sample preparation and collection

Samples were collected from the distillery of Wangxian Co. Ltd., Chongqing, China. The pure culture xiaoqu was prepared by inoculating wheat bran with a 7:2:3:1 mixture of Aspergillus niger, Rhizopus nigricans, R. oryzae, and Aspergillus oryzae at a ratio of 0.4% of the weight of raw materials. The aroma-producing S. cerevisiae was added according to 0.5% of the weight.

After soaking, steaming, cooling, mixing with xiaoqu, stacking and saccharification for 24 h, sorghum was put into the pit and sealed with mud. The pit was made of
cement, and the brewing cycle was 7 days (Figure 1). The microbial species and abundance changed greatly in the early stage of brewing, and tended to stabilize in the later stage. Therefore, the samples were taken at the end of the stacking brewing stage (Day 0), everyday during the first three days (Day 1, Day 2, Day 3), and on the fifth and last day (Day 5, Day 7) in the Baijiu brewing stage. The samples were taken at 30 cm below the surface of fermented grains. The samples were frozen in liquid nitrogen immediately after collection, and then stored at -80°C for subsequent DNA extraction.

2.2 Determination of pH, temperature, moisture content, and ethanol concentration

Samples of grains were stirred in distilled water for 30 min and then pH were measured with a pH meter (Mettler Toledo Co., Ltd. Switzerland). Temperature was monitored by an electronic thermometer during fermentation. Samples of grains were baked in an oven until a constant weight reached and measured for moisture content. Samples of grains and water were distilled for determination of alcohol content [23].

2.3 DNA extraction and HTS

Genomic DNA of the samples was extracted using the CTAB method [22]. The purity and concentration of the DNA were assessed by agarose gel electrophoresis. A suitable DNA sample was diluted with nuclease-free water to 1 ng/µL.

The V4 hypervariable region of bacterial 16S rDNA was amplified using the primer pair 515F/806R (515F: GTGYYCAGCMCCGCGGTAA, 806R: GGACTACNVGGGTWTCTAA). The fungal ITS1 hypervariable region was amplified using the primer pair 1737F/2043R (1737F: GGAAGTAAAGCTGTAACAGG, 2043R: GCTGCGTTCTTCATCATCGATGC).

All PCR products were purified and used for library construction using the TruSeq® DNA PCR-Free Sample Preparation Kit. After the library was qualified by qubit and Q-PCR, samples were loaded onto the Illumina NovaSeq6000 platform (Novogene Co., Ltd. China) for HTS. The raw data has been uploaded to NCBI SRA (BioProject ID: PRJNA687145).

2.4 Data analysis

The raw HTS data, with reads spliced using FLASH software [25], was processed using QIIME [26]. Final effective data was obtained through tag interception and length filtering, and then clustered into operational taxonomic units (OTUs) with 97% sequence identity using VSEARCH software [27, 28]. The OTU sequences were annotated via database comparisons using Mothur, and the relative content of OTUs in each sample was calculated [29]. Shannon diversity and Chao1 richness were calculated on the basis of the OTUs [30]. A map of bacterial community composition was drawn using R software (R Foundation for Statistical Computing, Vienna, Austria).
3 | RESULTS

3.1 | Physicochemical indicators of fermented grains

The pH tended to decrease during fermentation (Table 1). The temperature first increased from 22 to 35°C from day 0 to day 3 and maintained to day 5, then decreased to 31°C at day 7. The water content increased rapidly from day 0 to day 3. The ethanol content first increased rapidly before day 5 and then increased slowly till to the end of fermentation.

3.2 | Microbial abundance and OTUs diversity

In order to study the species composition of each sample, sequences with 97% identity were clustered into OTUs and annotated. A total of 1609 OTUs based on the bacterial 16S rRNA V4 region and 57 OTUs based on the fungal ITS1 region were obtained by clustering. A total of 34 phyla, 378 genera and 215 species of bacteria, as well as four phyla, 32 genera, and 44 species of fungi were annotated after searching each OTU against the taxonomic database.

The OTUs of bacteria in all samples from different stages of the xiaoqu Baijiu brewing process were quantified and compared. A Venn diagram was created to investigate potential exclusively shared OTUs. As shown in Figure 2, at a 97% similarity level, 60 bacterial and 14 fungal OTUs were found in all of the six samples. The results suggested that the fungal community is less complex than the bacterial community in the brewing process of xiaoqu Baijiu. The number of unique OTUs decreased during brewing.

The community abundance and diversity indexes, including Shannon, Simpson, ACE and Chao1, were calculated to assess the abundance and diversity of microbial OTUs (Table 2). The Chao1 and Shannon indexes of each sample were used to evaluate the richness and diversity describe the alpha-diversity of the microbial community, respectively. The Chao1 index was positively correlated with the microbial community abundance. The average coverage of all samples exceeded 0.99, indicating that the identified sequences were representative of the actual community in the sample. The ACE and Chao1 values decreased on the first day of brewing, and then gradually increased, reaching the highest value on the fifth day of brewing. The Shannon and Simpson indexes were used to estimate the microbial diversity of the samples. Both indexes reached the highest values at the second day of brewing, while bacterial diversity was highest on day 7 in the study reported [23]. The Shannon diversity of traditional xiaoqu group ranged from 2.27 to 3.52, higher than the results of Dong et al. [22]. Higher Shannon index values indicate greater microbial diversity. This indicates that microbial diversity was highest at the second day of brewing in this study. Generally, the abundance and diversity of bacteria in the xiaoqu Baijiu samples decreased with prolonged brewing time.

For fungi, the ACE index increased at the first day, and decreased on the second day. The Chao1 reflects the community richness, and its highest value of 38.6 was observed on the first day of brewing. The Shannon index was lowest at the beginning of brewing and reached the maximum at the third day of brewing, while fungal diversity was highest on day 1 and lowest on day 7 in the study reported before [23]. The Simpson index can also be used to estimate the microbial diversity of the sample, and it was also the lowest at the beginning of brewing (Table 2).

3.3 | Diversity analysis of the bacterial community

At the phylum level, 34 bacterial species were identified in the samples. The top 10 species in abundance and related species are shown in Figure 3. The dominant bacterial phyla was Proteobacteria and Firmicutes (Figure 3A). In the early stage of brewing, the dominant bacteria were Proteobacteria. The proportion of Proteobacteria
Statistics and comparison of OTUs in samples from different stages of the *xiaoqu Baijiu* brewing process. (A) OTUs of bacteria; (B) OTUs of fungi

| Samples | Bacterial Shannon | Simpson | Chao1 | Ace | Fungi Shannon | Simpson | Chao1 | Ace |
|---------|------------------|---------|-------|-----|--------------|---------|-------|-----|
| Day 0   | 2.95             | 0.81    | 179   | 183 | 0.75         | 0.21    | 28    | 30.22 |
| Day 1   | 2.33             | 0.64    | 134   | 135 | 1.57         | 0.57    | 39    | 42.79 |
| Day 2   | 3.52             | 0.81    | 155   | 159 | 1.71         | 0.65    | 28    | 31.70 |
| Day 3   | 2.27             | 0.59    | 146   | 150 | 1.77         | 0.66    | 26    | 27.00 |
| Day 5   | 2.79             | 0.77    | 145   | 176 | 1.62         | 0.65    | 28    | 32.34 |
| Day 7   | 3.06             | 0.77    | 145   | 147 | 1.43         | 0.56    | 28    | 33.20 |

Bacterial community structure of samples at phylum level and genus level. (A) at phylum level; (B) at genus level

A total of 378 bacterial species were detected in *xiaoqu Baijiu* samples at the genus level. The top 20 most abundant genera and related species merged are shown in Figure 3B. Although the dominant bacterial genera (top 20) were almost identical, there were significant differences in bacterial abundance among the samples. The top five genera were *Lactobacillus*, *Weissella*, unidentified *Rickettsiales*, *Bacillus*, and *Pantoea*. The relative abundance of *Weissella* and unidentified *Rickettsiales* accounted for a large proportion in the early stage of brewing and decreased during brewing. The relative abundance of
Bacillus increased before the second day of brewing. The relative abundance of Pantoea did not change much during brewing process. The relative abundance of Lactobacillus did not change much in the first 2 days of brewing, but increased rapidly during the brewing process, and became the dominant bacteria in the late stage of brewing.

3.4 Diversity analysis of the fungal community

At the phylum level, four fungal species were identified in the samples. As shown in Figure 4, the dominant fungi were Ascomycota and Mucoromycota at the phylum level. During the brewing process, the starch content of the substrate progressively decreases, and the relative abundance of Mucoromycota decreased. The community of Ascomycota increased and occupied a dominant position during the brewing process, which indicated that Ascomycota play an important role in the brewing of xiaoqu Baijiu.

At the genus level, a total of 32 fungal species were detected in the xiaoqu Baijiu samples. The top 10 genera and related species are shown in Figure 4B. Rhizopus and Aspergillus were the dominant genera before the material paced into the fermentation pit, but their relative abundance decreased during brewing. The relative abundance of Saccharomyces increased during the entire brewing process and it became the dominant genus at the end of brewing. The relative abundance of Issatchenkia increased at the beginning of the brewing process, but did not change much during late stage of the brewing.

3.5 Sample clustering in bacterial and fungal communities

Principal component analysis (PCA) was used to analyze the changes of relative abundance and diversity of the microbial community on a two-dimensional coordinate chart. The variation in the bacterial communities between samples was found to be 30.55% (PC1) and 25.44% (PC2) with a strong separation by region as shown in Figure 5A. Samples Day 3, Day 5, and Day 7 were close together, indicating that the bacterial composition are similar and more stable. Samples Day 0, Day 1, and Day 2 were far from these, indicating that fermentation process had impact on the bacteria of samples. For the fungal community, the maximal variation values were 35.73% (PC1) and 23.13% (PC2) as shown in Figure 5B. Samples were scattered, and their fungal composition were relatively different, indicating there were some differences in fungal diversity during Baijiu brewing stages.

4 DISCUSSION

This study applied HTS to reveal the microbial diversity and succession during xiaoqu Baijiu fermentation. The bacterial dynamics were similar with the report of Dong et al. [22]. The increase of relative abundance of LAB was mainly due to Lactobacillus in the late stage of fermentation. However, Weissella was the predominant LAB in the early stage of xiaoqu Baijiu brewing in our study while Lactococcus was the predominant LAB in the report of Dong et al. [22]. Bacillus exhibited higher abundance only at the second day of fermentation in our study, which was also different with reported before. Klebsiella was not found in top 20 most abundant genera (Figure 3B), while it is the dominant microorganism during brewing of Sichuan Xiaoq Baijiu [23]. Saccharomyces and Issatchenkia were the dominant fungal in the late stage of fermentation (Figure 4B), while Pichia kudriavzevii was more active than S. cerevisiae in the study of Hu et al. [24]. Different microbial diversities during xiaoqu Baijiu brewing process might be attributed to three factors, Xiaoqu, fermentation conditions and environment. There are similarities and differences between our study and previous studies, which provide guidance for xiaoqu Baijiu brewing.
improvement of Xiaoqu, optimization of fermentation conditions and environment are directions to improve the quality of Xiaoqu Baijiu.

*Bacillus* in the starter can secrete various hydrolyses, including amylases, proteases, and lipases, which hydrolyze macromolecular substrates and produce specific flavor compounds such as acetate, higher alcohols, diacetyl, pyrazines and aromatic compounds, improving the sensory experience and conferring health benefits [31]. Moreover, a synergistic interaction between *Bacillus* and *Aspergillus* leads to a richer spectrum of aromatic compounds [11]. Addition of *Bacillus* in Xiaoqu provide a way to improve the flavor of Xiaoqu Baijiu. *Pantoea* is an important heterotrophic bacterium that is often found in brewing. Although the abundance of *Acetobacter* and *Gluconobacter* was not high during the brewing process, they can produce acetic acid as a precursor of ethyl acetate, which is a major flavor component of Xiaoqu Baijiu [23].

*Lactobacillus*, *Weissella*, *Pediococcus*, *Leuconostoc*, and *Lactococcus* are LABs. The increase trend of Firmicutes in the process of Baijiu brewing was mainly related to the increase in abundance of LABs [31,32]. As facultatively anaerobic or aerotolerant bacteria [33], LABs have an important role in regulating the bacterial community during Baijiu brewing. LABs also predominate during the brewing stage of sauce-flavored, strong-flavored, and light-flavored Baijiu [7, 14, 32]. Lactic acid and acetic acid, produced by LABs, can react with ethanol to produce ethyl lactate and ethyl acetate, which are important flavor components of Xiaoqu Baijiu [30]. However, too much ethyl lactate causes an undesirable aroma in light-flavored Baijiu. Lactic acid, produced with the increase of LABs, reduces the pH of the fermented grains, thereby greatly influencing the community structure [30] and inhibiting the propagation of spoilage bacteria [34]. A negative correlation between LABs and other prokaryotic groups was observed during the brewing process [30]. LABs also possess different enzymes such as glucosidases, esterases and proteases, which play a critical role in the formation of volatile compounds in fermented foods [35, 36]. *Lactobacillus* significantly enhanced the generation of 3-(methylthio)-1-propanol and dimethyl disulfide when cocultured with *S. cerevisiae*, though it could not produce these two compounds. In addition, the related gene transcriptions in *S. cerevisiae* and *Lactobacillus* were significantly enhanced [37]. The type, quantity and dynamic change of LABs species are crucial determinants of the flavor of Baijiu. LABs may be introduced from the environment of the Xiaoqu Baijiu brewing workshop during sorghum cooling, because no LABs are added to Xiaoqu. In a previous study, the abundance of LABs on the surface of the ground and tools in the brewing workshop was indeed found to be very high [38].

Molds and yeasts are mainly responsible for saccharification and alcoholic brewing during the principal production of Baijiu [39]. *Rhizopus* and *Aspergillus* are important functional microorganisms in many fermented foods [16]. The relative abundance of *Aspergillus* and *Rhizopus* decreased due to the continuous increase of the alcohol and acid concentrations, as well as the lack of oxygen. *R. oryzae* has a high amylase production capacity, and is often used to hydrolyze starch from sorghum in *koji* and Baijiu brewing [40]. *Rhizopus* also affects Baijiu flavor by producing glycerol, lactic acid, enzymes and volatile compounds, such as ethanol, 2-methyl-1-butanol, acetoin, and acetaldehyde, which are associated with the aromatic and alcohol flavors of Baijiu.

**FIGURE 5** Principal component analysis (PCA) of bacteria and fungi at the genus level. (A) PCA of bacteria; (B) PCA of fungi.
and 3-methyl-1-butanol [41]. *Aspergillus* produce a wide spectrum of proteolytic and other hydrolytic enzymes, playing an important role in the saccharification of starch and protein hydrolysis in sorghum [42]. *Aspergillus* can also promote the synthesis of certain esters, improving the flavor of *Baijiu*. This is the reason why *Rhizopus* and *Aspergillus* were added in *xiaoqu*.

With its strong ethanol brewing capacity under anaerobic conditions, *Saccharomyces* is responsible not only for the alcohol yield but also for the flavor and taste features of *xiaoqu Baijiu* [16,43]. *Saccharomyces* also interacts with other microorganisms such as molds and bacteria, improving the quality of *xiaoqu Baijiu*. Since *Saccharomyces* can tolerate anaerobic conditions and high ethanol concentrations, it is unsurprising that its relative abundance increased during the *xiaoqu Baijiu* brewing process. *Issatchenkia*, a yeast that is abundantly found in soil and fermented grains, can produce ethanol and ethyl acetate, and resistant to acid, high temperatures and ethanol. It is a functional species in the brewing process of *Baijiu*, and was found in many samples of *Daqu* and fermented grains used for the production of *Baijiu* with different flavor types [38, 44].

*Baijiu* can maintain its character due to the similar microbial communities of different batches in the same period. The difference of microbial communities in different workshops or periods may cause the different flavor profiles even if the exact same processing methods were used [13]. The abundance and diversity of microorganisms such as bacteria, yeasts and molds in fermentation starters, more conducive to the production of flavor compounds, is crucial for the production of high-quality *Baijiu* [45]. These factors are influenced by changes of the microenvironment, such as reduction of substrate, decrease of pH, increase of alcohol concentration, anaerobic conditions, as well as biotic and abiotic interactions [46, 47].

Because *xiaoqu* and *xiaoqu Baijiu* are produced in an open environment, the contained microorganisms are derived not only from the starters, but also from the environment encountered in the process of material pretreatment, including the water, machines, tools, ground and air in the material pretreatment room [4,48]. Consequently, the quality of *Baijiu* depends on a well-balanced microbial community derived from both the production environment and the starter. Microorganisms such as *Lactobacillus*, *Bacillus*, and *Saccharomyces* are thought to be introduced into sorghum from the brewing workshop environment during cooling and enter the alcoholic brewing stage [38]. *Lactobacillus* and *Saccharomyces* were the predominant bacterial and fungal genera at the later stages of brewing, and they are closely related to the formation of various flavors during *xiaoqu Baijiu* brewing.

A more comprehensive understanding of the microbial diversity and microbial succession during the brewing process of *xiaoqu Baijiu* was obtained. The microbial community structure and diversity showed a microbial succession during the *xiaoqu Baijiu* brewing process. This study expands our understanding of the microbial community diversity of *xiaoqu Baijiu* during the brewing process and provides useful information for future in depth studies of the microbial diversity and quality improvement of *xiaoqu Baijiu*.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to the publication of this paper.

DATA AVAILABILITY STATEMENT

All data generated or analyzed in this study are included in this paper. The datasets can be found in online repositories. The names of the repositories and accession number can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA687145.

REFERENCES

1. Zheng X, Han B, Baijiu (白酒). Chinese liquor: history, classification and manufacture. *J Ethn Foods*. 2016;3:19-25.
2. Ye H, Wang J, Shi J, et al. Automatic and intelligent technologies of solid-state fermentation process of *Baijiu* production: applications, challenges, and prospects. *Foods*. 2021;10:680.
3. Liu H, Sun B. Effect of fermentation processing on the flavor of *Baijiu*. *J Agr Food Chem*. 2018;66:5425-5432.
4. Jin G, Zhu Y, Xu Y. Mystery behind Chinese liquor fermentation. *Trends Food Sci Tech*. 2017;63:18-28.
5. Gou M, Wang H, Yuan H, et al. Characterization of the microbial community in three types of fermentation starters used for Chinese liquor production. *J Inst Brew*. 2015;121:620-627.
6. Wu H, Zhang S, Ma Y, et al. Comparison of microbial communities in the fermentation starter used to brew *Xiaoqu* liquor. *J Inst Brew*. 2017;123:113-120.
7. Yang J, Dou X, Ma Y. Diversity and dynamic succession of microorganisms during *Daqu* preparation for Luzhou-flavour liquor using second-generation sequencing technology. *J Inst Brew*. 2018;124:498-507.
8. Xie M, Lv F, Ma G, et al. High throughput sequencing of the bacterial composition and dynamic succession in *Daqu* for Chinese sesame flavour liquor. *J Inst Brew*. 2020;126:98-104.
9. Yan S, Chen X, Guang J. Bacterial and fungal diversity in the traditional Chinese strong flavour liquor *Daqu*. *J Inst Brew*. 2019;125:443-452.
10. Ling Y, Li W, Tong T, et al. Assessing the microbial communities in four different Daqu by using PCR-DGGE, PLFA, and Biolog Analyses. Pol J Microbiol. 2020;69:1-11.

11. He G, Huang J, Zhou R, Wu C, Jin Y. Effect of fortified Daqu on the microbial community and flavor in Chinese strong-flavor liquor brewing process. Front Microbiol. 2019;10:56.

12. Xiao C, Lu Z, Zhang X, et al. Bioinformatics driver shaping the microbial community of medium-temperature Daqu. Appl Environ Microbiol. 2017;83:e01550-e0157.

13. Pang X, Han B, Huang X, et al. Effect of the environment microbiota on the flavour of light-flavour Baijiu during spontaneous fermentation. Sci Rep. 2018;8:3396.

14. Yang F, Chen L, Liu Y, et al. Identification of microorganisms producing lactic acid during solid-state fermentation of Maotai flavour liquor. J Inst Brew. 2019;125:171-177.

15. Huang X, Fan Y, Lu T, et al. Composition and metabolic functions of the microbiome in fermented grain during light-flavor Baijiu fermentation. Microorganisms. 2020;8:1281.

16. Tang Q, He G, Huang J, et al., Characterizing relationship of microbial diversity and metabolite in sichuan Xiaoqu. Front Microbiol. 2019;10:696.

17. Wang C, Tang J, Qiu S. Profiling of fungal diversity and fermentative yeasts in traditional Chinese Xiaoqu. Front Microbiol. 2020;11:2103.

18. Zhao C, Su W, Mu Y, Mu Y, Jiang L. Integrative metagenomics-metabolomics for analyzing the relationship between microorganisms and non-volatile profiles of traditional Xiaoqu. Front Microbiol. 2021;11:e617030.

19. Wang B, Wu Q, Xu Y, Sun B. Synergistic effect of multiple saccharifying enzymes on alcoholic fermentation for Chinese Baijiu production. Appl Environ Microbiol. 2020;86:e00013-e00020.

20. Wang J, Zhong Q, Yang Y, et al. Comparison of bacterial diversity between two traditional starters and the Round-Koji-Maker starter for traditional cantonese Chi-Flavor liquor brewing. Front Microbiol. 2018;9:1053.

21. Ma W, Geng X, Jia F, et al. Investigation of microbial composition and functional characterization of Zangqu using high throughput sequencing. Lwt. 2021;136:101342.

22. Dong W, Yang Q, Liao Y, et al. Characterisation and comparison of the microflora of traditional and pure culture Xiaoqu during the Baijiu liquor brewing process. J Inst Brew. 2020;126:213-220.

23. Wang M, Zhao Q, Chang S, Yang J. Analysis of the microbial community structure during brewing of Sichuan Xiaoqu Baijiu. J Am Soc Brew Chem. 2019;77:210-219.

24. Hu Y, Yang Q, Chen D, et al. Study on microbial communities and higher alcohol formations in the fermentation of Chinese Xiaoqu Baijiu produced by traditional and new mechanical technologies. Food Res Int. 2021;140:109876.

25. Mago T, Salzberg S. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27:2957-2963.

26. Caporaso J, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-336.

27. Edgar R. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460-2461.

28. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016;4:e2584.

29. Schloss P, Westcott S, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75:5573-5541.

30. Tao Y, Li J, Rui J, et al. Prokaryotic communities in pit mud from different-aged cellars used for the production of Chinese strong-flavored liquor. Appl Environ Microbiol. 2014;80:2254-2260.

31. Zhang Y, Zhu X, Li X, et al. The process-related dynamics of microbial community during a simulated fermentation of Chinese strong-flavored liquor. BMC Microbiol. 2017;17:196.

32. Fan G, Fu Z, Sun B, et al. Roles of aging in the production of light-flavored Daqu. J Biosci Bioeng. 2019;127:309-317.

33. Günzel M. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr Opin Food Sci. 2015;2:106-117.

34. Lv X, Weng X, Zhang W, Rao P, Ni L. Microbial diversity of traditional fermentation starters for Hong Qu glutinous rice wine as determined by PCR-mediated DGGE. Food Control. 2012;28:426-434.

35. Song Z, Du H, Zhang Y, Xu Y. Unraveling core functional microbiota in traditional solid-state fermentation by high-throughput amplicons and metatranscriptomics sequencing. Front Microbiol. 2017;8:1294.

36. Wang Z, Lu Z, Shi J, Xu Z. Exploring flavour-producing core microbiota in multispecies solid-state fermentation of traditional Chinese vinegar. Sci Rep. 2016;6:26818.

37. Liu J, Wu Q, Wang P, et al. Synergistic effect in core microbiota associated with sulfur metabolism in spontaneous Chinese liquor fermentation. Appl Environ Microbiol. 2017;83:e01475-e01417.

38. Wang X, Du H, Zhang Y, Xu Y. Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. Appl Environ Microbiol. 2018;84:e02369-e02317.

39. Cheng P, Fan W, Xu Y. Quality grade discrimination of Chinese strong aroma type liquors using mass spectrometry and multivariate analysis. Food Res Int. 2013;54:1753-1760.

40. Ghosh B, Lahiri D, Nag M, Dash S, Ray R. Biocharacterization of purified isoamylase from Rhizopus oryzae: learning from the history of Koji mold and exploration of its future. DNA Res. 2008;15:173-183.

41. Machida M, Yamada O, Gomi K. Genomics of Aspergillus oryzae: learning from the history of Koji mold and exploration of its future. DNA Res. 2014;6:26621.

42. Wu Q, Ling J, Xu Y. Starter culture selection for making Chinese sesame-flavored liquor based on microbial metabolic activity in mixed-culture fermentation. Appl Environ Microbiol. 2014;80:4450.

43. Chai L, Xu P, Qian W, et al. Profiling the Clostridia with butyrate-producing potential in the mud of Chinese liquor fermentation cell. Int J Food Microbiol. 2019;297:41-50.
46. Wang H, Zhang X, Zhao L, Xu Y. Analysis and comparison of the bacterial community in fermented grains during the fermentation for two different styles of Chinese liquor. *J Ind Microbiol Biot.* 2008;35:603-609.

47. Li X, Ma E, Yan L, et al. Bacterial and fungal diversity in the traditional Chinese liquor fermentation process. *Int J Food Microbiol.* 2011;146:31-37.

48. Pang X, Huang X, Chen J, et al. Exploring the diversity and role of microbiota during material pretreatment of light-flavor *Baijiu.* *Food Microbiol.* 2020;91:103514.

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