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ORIGINAL ARTICLE

High-throughput sequencing of the microbial diversity of roasted-sesame-like flavored Daqu with different characteristics

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Abstract

The purpose of this experiment was to analyze the microbial community diversity in three Daqu samples displaying different characteristics in the same Daqu fermentation chamber. A high throughput sequencing technique was used to detect the microbial abundance and diversity in these Daqu samples. Of the three samples, the microbial diversity in the Black sample (sample B) was significantly higher than in the other two. At the genus level, *Saccharopolyspora*, *Bacillus*, *Lentibacillus*, *Staphylococcus*, *Kroppenstedtia*, and *Thermoactinomyces* were the primary bacterial groups in the sesame-flavored liquor, while *Thermomyces*, *Thermoascus,* and *Aspergillus* represented the main fungal groups. In sample B, the dominant bacteria were *Thermoactinomyces*, *Saccharopolyspora*, and *Pseudomonas*. In the White sample (sample W), *Thermoactinomyces* was the most abundant, followed by *Saccharopolyspora* and *Lentibacillus*. *Staphylococcus* dominated in the Yellow sample (sample Y), followed by *Bacillus* and *Kroppenstedtia*. Regarding the fungi in the three samples, *Thermomyces* accounted for 93.70% in sample B, and *Aspergillus* dominated in sample W, while the *Thermoascus* and *Aspergillus* content were similar in the sample Y. This study examined the microbial diversity in liquor Daqu with different sesame flavors, providing a foundation for microbial regulation, while investigating the relationship between flavored liquor compounds and microorganisms.

Keywords Sesame flavored liquor · Daqu · Microbial composition · Community diversity · High-throughput sequencing

Introduction

Baijiu is one of the world's best-selling distilled spirits (Liu et al. 2018). According to flavor types, there are 11 traditional Chinese liquors, which includes a sauce flavor, a strong flavor, and light flavor. Sesame-flavored liquor is an innovative form of traditional liquor in China. In terms of production, it integrates strong (Wang et al. 2008), light, and sauce flavors by combining flavor materials and style characteristics. This is typically representative of Shandong liquor, displaying uniquely "fragrant, soft and mellow" qualities (Wu et al. 2017; He et al. 2019). Daqu plays an essential role in the production of fermented products, such as liquor and vinegar (Tang et al. 2019; Liu et al. 2017). The quality of Daqu is directly related to the high-temperature fermentation process, which can reach 65 °C (Xie et al. 2020).

The quality of the Daqu determines the quality of the liquor and significantly affects the flavor and taste of baijiu (Liu et al. 2017; Huang et al. 2017). Daqu is produced via the complex solid-state fermentation of grains and bran and is prepared by grounding and shaping the grains before treatment (Li et al. 2015), after which they are placed in the Daqu fermentation room at a temperature of 55–70 °C. The fermentation process takes about 50 days, while the incubation lasts one month (Zheng et al. 2014a, b). Because the entire process occurs in the Daqu fermentation room, the microbial system is derived from the air, the raw materials, and the placement environment. The microorganisms and their metabolites are responsible for the unique flavor of the liquor

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(Huang et al. 2017; Wang et al. 2017a, b). The various flavors of Daqu present different microbial communities (Yao et al. 2015). For example, Yao et al. found that the *Bacillus* and *Lactobacillus* numbers in the sauce flavored Daqu liquor were closely related to pyrazine and esters, while *Aspergillus* was associated with pyrazine, esters, and aromatics (Yao et al. 2015; Jin et al. 2019); Li et al. found that the thermophilic bacterium *Laceyella sacchari* FBKL4.010 could produce a key enzyme affecting pyrazine metabolism while uncovering its related gene (Li et al. 2019). In addition, Chai et al. revealed that two synthetic pathways of butyrate (butyrate kinase and butyryl-CoA: acetate CoA-transferase), a compound that denotes the key aroma of strong-flavored liquor, were inseparable from microorganisms (Chai et al. 2019).

Although advanced technological innovation has significantly increased the industrial production of liquor Daqu, it is difficult to control this production process without fully understanding its characteristics (Liu et al. 2017). Therefore, a more extensive comprehension regarding the production of Daqu using natural fermentation may prove more conducive to the development of related technology (Fan et al. 2020). For this experiment, three kinds of sesame-flavored liquor Daqu exhibiting different properties are selected from the same storage batch in a single Daqu fermentation chamber. The diversity of the microbial communities of the three types of Daqu are compared and analyzed using high-throughput sequencing technology. This work promotes an understanding of the similarities and differences between the microbial compositions of different sesame-flavored liquor Daqu while providing a scientific basis for microbial control during the fermentation process of sesame-flavored liquor in the future.

Materials and methods

Sample collection

The Daqu samples were collected from the high-temperature (55–65 °C) fermentation room at the Shandong Bandaojing Co. Ltd, Shandong Province, China, after which they were stored at 4 °C. In the Daqu fermentation chamber, the temperatures in the up layer (white Daqu sample, 35–45 °C), middle layer (yellow Daqu sample, 45–55 °C), and low layer (black Daqu sample, 55–65 °C) were different, resulting in a change in the color of the Daqu. Samples were collected from the different temperature layers to obtain the upper white Daqu (sample W), the middle yellow Daqu (sample Y), and the lower black Daqu (sample B). These three samples were collected from different areas of the same Daqu fermentation room (Supplementary Fig.1). Furthermore, the three samples were randomly collected, each with three parallel samples.

DNA extraction

Here, 2.0 g from each Daqu sample was used for the extraction of the total DNA using the Fast DNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) following the manufacturer's instructions.

Amplicon library preparation and sequencing

The bacteria were identified via PCR amplification of the v3-v4 region of the 16S rRNA gene, using the 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Klindworth et al. 2013). An 8 bp barcode was added to the 5′ end of the 806R reverse primer for sample differentiation. The identification of the fungi occurred via PCR amplification in the ITS1 region, and the primers were ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′). An 8 bp barcode was added to the 5′ end of the ITS1F forward primer for sample differentiation. The reaction volume was 50 µL, which comprised 27 µL ddH_2O, 2 µL (5 µM) each of the forward and reverse primers, 2.5 µL (10 ng) of template DNA, 5 µL (2.5 mM) of deoxynucleoside triphosphates, 10 µL of 5 × FastPfu buffer, 0.5 µL of bovine serum albumin and 1 µL of TransStart FastPfu Polymerase (TransGen, Beijing, China). Bacterial amplification occurred via predenaturation at 95 °C for 5 min, after which each thermal cycle was denatured at 94 °C for 30 s, annealed at 55 °C for 35 s, and elongated at 72 °C for 30 s. After 30 cycles, the final extension step was performed at 72 °C for 8 min. Furthermore, the predenaturation of the fungi occurred at 95 °C for 5 min, after which each thermal cycle was denatured at 94 °C for 30 s, annealed at 54 °C for 40 s, and elongated at 72 °C for 30 s. After 30 cycles, the final extension step was performed at 72 °C for 8 min. The PCR products were purified using a gel recovery kit (Life Technology, USA), while Qubit3.0 (Life Technology, USA) was used for quantitative analysis. The purified amplicons were pooled in equimolar concentrations, and library-specific sequencing adapters were added via a NEBNext Ultra (NEB#e7370S/ L) assay, following the manufacturer's instructions. Dual index sequencing of the paired-end 250 bp reads was run on an Illumina HiSeq2500 instrument (Illumina, San Diego, CA, USA).

Sequence data processing and statistics

The original data obtained from the machine were filtered using NGS toolkit software (Patel et al. 2012) according to the default parameter values to remove low-quality reads.

The coincidence pairs were merged using FLASH software. Split_libraries_fastq.py in the QIIME software (Magoč et al. 2011) was used to separate the data according to the barcode at the 5′ end of the primer to obtain each sequence. The mothur software (Caporaso et al. 2010) was used to edit the barcodes (Schloss et al. 2009) and primers in the tags. The sequences were filtered and denoised to remove chimeras, after which they were classified using the Bayesian method against a database derived from the RDP 16S rRNA reference, db128 (Cole et al. 2014). Non-16S sequences (e.g., unknown, archaea, chloroplast, mitochondria, and cyanobacteria) were removed (Xie et al. 2020).

The edited sequence was divided into OTUs at a distance of 97% using the VSEARCH software (Rognes et al. 2016). The representative OTU sequences were denoted by the most abundant sequences in each OTU. The OTU table was simplified to the minimum sequence number between samples to directly compare the alpha and beta diversity values of each flora. Principal coordinate analysis (PCA) based on the UniFrac distance matrix (estimates the similarity according to the distance between samples), as well as two-way permutational multivariate analysis of variance (PERMANOVA) based on the Bray – Curtis dissimilarity was used to assess the variances in the compositions of the microbial communities using mothur and the R package vegan (version 2.2).

Results

Quality control of the sequencing data

The raw high-throughput sequencing data were submitted to the NCBI database under the BioProject number PRJNA612757. A total of 1.55 M raw sequences was obtained after sequencing, while 1.45 M available reads were acquired after the quality control process. After filtering out the nontarget fragments, a total of approximately 0.43 M bacterial 16S rDNA fragment sequences (ranging from 9387 to 86,575) and 1.02 M fungi ITS DNA fragment sequences (ranging from 71,752 to 188,576) were obtained. Figure 1 shows that regardless of whether it was bacteria or fungi, the rarefaction curve based on the OTU number, approaching the saturation plateau, suggested that the sequencing depth in this research was adequate to represent the microbial diversity in the samples, indicating that the selected sample was representative of different sesame-flavored Daqu.

The microbial diversity in the three Daqu samples

The diversity indices, including the number of OTUs, ACE, Chao, Shannon, and Simpson values, are shown in

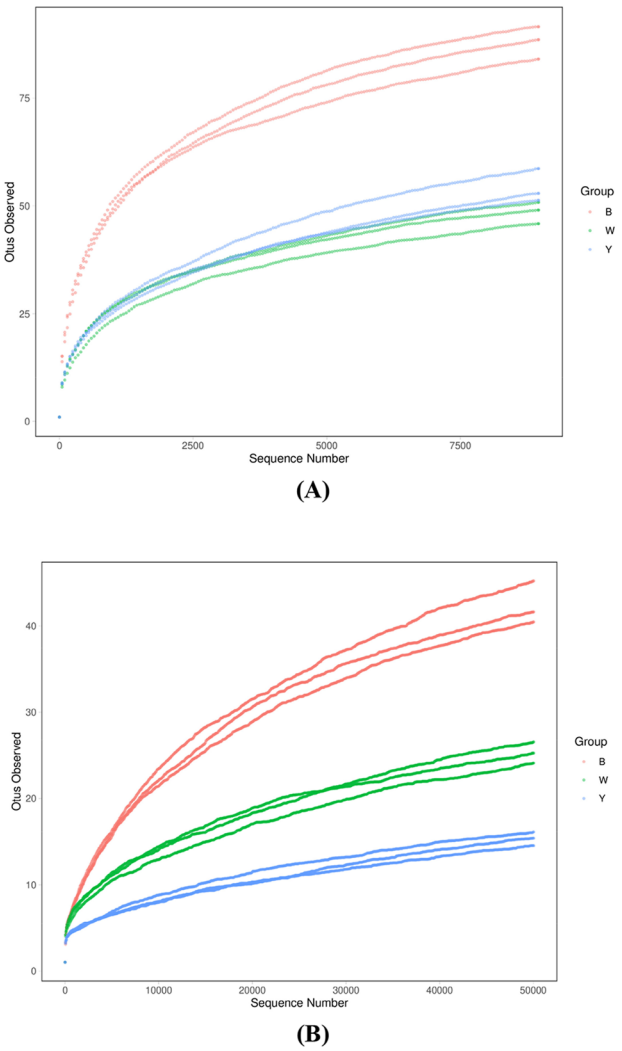


Fig. 1 The rarefaction curves. **a** Bacteria, and **b** Fungi. *B* black Daqu sample, *W* white Daqu sample, *Y* yellow Daqu sample. Each sample has three parallels based on the number of OTUs. The curves are close to equilibrium, indicating the representativity of the Daqu samples

Table 1. The number of OTUs, ACE, and Chao indices describe the total number of species. The total number of bacterial and fungal species in sample B exceeded that of both sample Y and sample W, while the bacterial level in sample W was lower than in sample Y. However, the fungal levels exhibited the opposite trend. The Shannon and Simpson indices describe species diversity. Unlike the Shannon index, the higher the Simpson index value, the lower the community diversity. Table 1 shows that the bacterial diversity of sample B was the highest, while the fungal diversity was the lowest. The bacterial diversity in samples W and Y was similar, but the fungal diversity in sample W was significantly higher than that of sample Y.

Table 1 Species richness estimator and diversity index

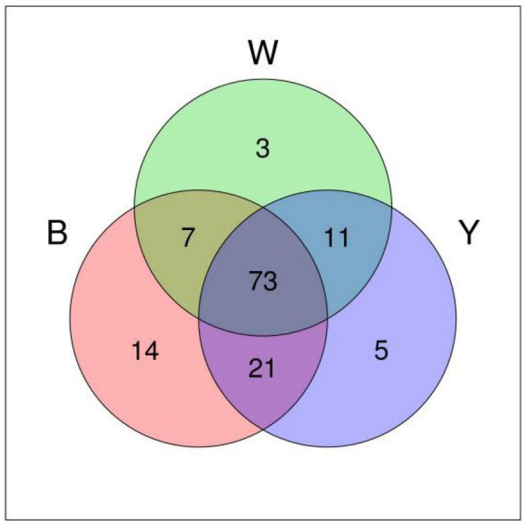
	Group	Num of OTUs[1]	Ace	Simpson	Shannon	Chao[2]
Bacteria	B	86.33 ± 5.69^{a}	98.98 ± 5.74^{a}	0.14 ± 0.01^{c}	2.56 ± 0.08^{a}	94.26 ± 5.33^{a}
	W	47.33 ± 1.53^{b}	79.77 ± 3.91^{b}	0.38 ± 0.03^{a}	1.56 ± 0.07^{c}	68.24 ± 8.55^{b}
	Y	52.33 ± 7.02^{b}	67.27 ± 12.53^{b}	0.27 ± 0.01^{a}	1.78 ± 0.01^{b}	69.40 ± 16.84^{b}
Fungi	B	37.33 ± 2.87^{a}	73.21 ± 28.08^{a}	0.88 ± 0.01^{a}	0.34 ± 0.01^{c}	59.75 ± 20.39^{a}
	W	20.67 ± 2.08^{b}	35.61 ± 8.50^{a}	0.40 ± 0.01^{c}	1.1 ± 0.02^{a}	28.67 ± 5.10^{b}
	Y	13.33 ± 2.52^{c}	73.55 ± 81.45^{a}	0.63 ± 0.01^{b}	0.71 ± 0.02^{b}	26.33 ± 8.62^{b}

1 The number of OTUs in each group reflects microbial richness; 2 Nonparametric statistical predictions of the total OTU richness; Values with superscript letters a, b, c, and d are significantly different across columns ($p < 0.05$)

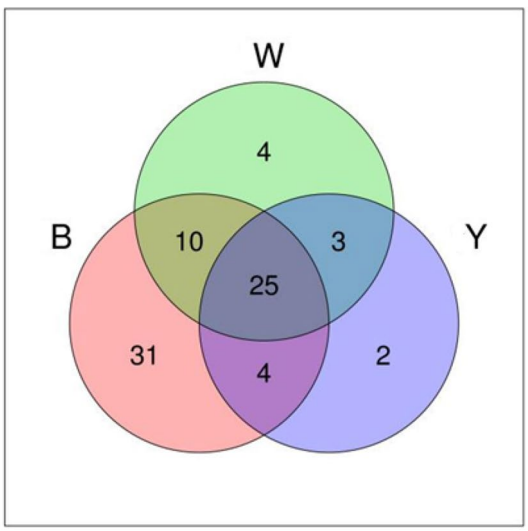
A Venn diagram was created based on the samples (Fig. 2), which was used to investigate whether exclusively shared OTUs existed. At a 97% similarity level, the number of bacterial OTUs in sample B, sample W, and sample Y was 115, 94, and 110, while the number of fungal OTUs was 70, 42, and 34, respectively. The number of OTUs in sample B was the highest for both bacteria and fungi. Although 73 bacterial OTUs and 25 fungal OTUs were evident in the three samples, each specimen presented its own unique bacterial and fungal OTUs. Similarly, the independent bacterial and fungal OTUs in sample B far exceeded that of the other two samples. The proportion of independent bacterial OTUs in samples B, W, and Y was 12.2, 3.2, and 4.5%, while that of the independent fungal OTUs was 44.3, 9.5, and 5.9%, respectively. *Thermoactinomyces*, *Sphingomonas*, *Saccharopolyspora*, *Kroppenstedtia*, and *Bacillus* denoted the primary bacteria in the sesame flavored liquor Daqu, while *Thermomyces*, *Aspergillus*, *Sagenomella*, and *Thermoascus* signified the dominant fungi. However, the microbial content differed in the individual samples.

The diversity and composition of the microbial communities in the three Daqu samples

The Mantel test calculation showed a strong correlation ($r = 0.9887$, $p < 0.001$) between the Bray – Curtis dissimilarity matrices (based on the bacterial genera level classification and OTUs). Figure 3a shows the distribution comparison rules of the phyla of the three sample bacterial species. Overall, Firmicutes was the most abundant in the three samples, specifically in sample Y (90.70%). Proteobacteria (25.57%) was significantly higher in sample B than in samples W (1.63%) and Y (2.13%). Actinobacteria (23.17%) was substantially higher in sample B than in samples W (14.77%) and Y (7.17%), while Firmicutes were considerably lower in sample B (50.60%). As shown in Fig. 3b, the bacteria in the three samples were different at the genus level. The main bacteria in sample B included *Thermoactinomyces* (29.47%), *Saccharopolyspora* (17.60%), *Pseudomonas* (10.60%), and *Kroppenstedtia* (7.57%). The bacteria in sample W were



(A)



(B)

Fig. 2 A Venn diagram showing the shared and unique OTUs at 97% identity among the Black Daqu, White Daqu, and Yellow Daqu samples, with OTU abundance < 1% removed

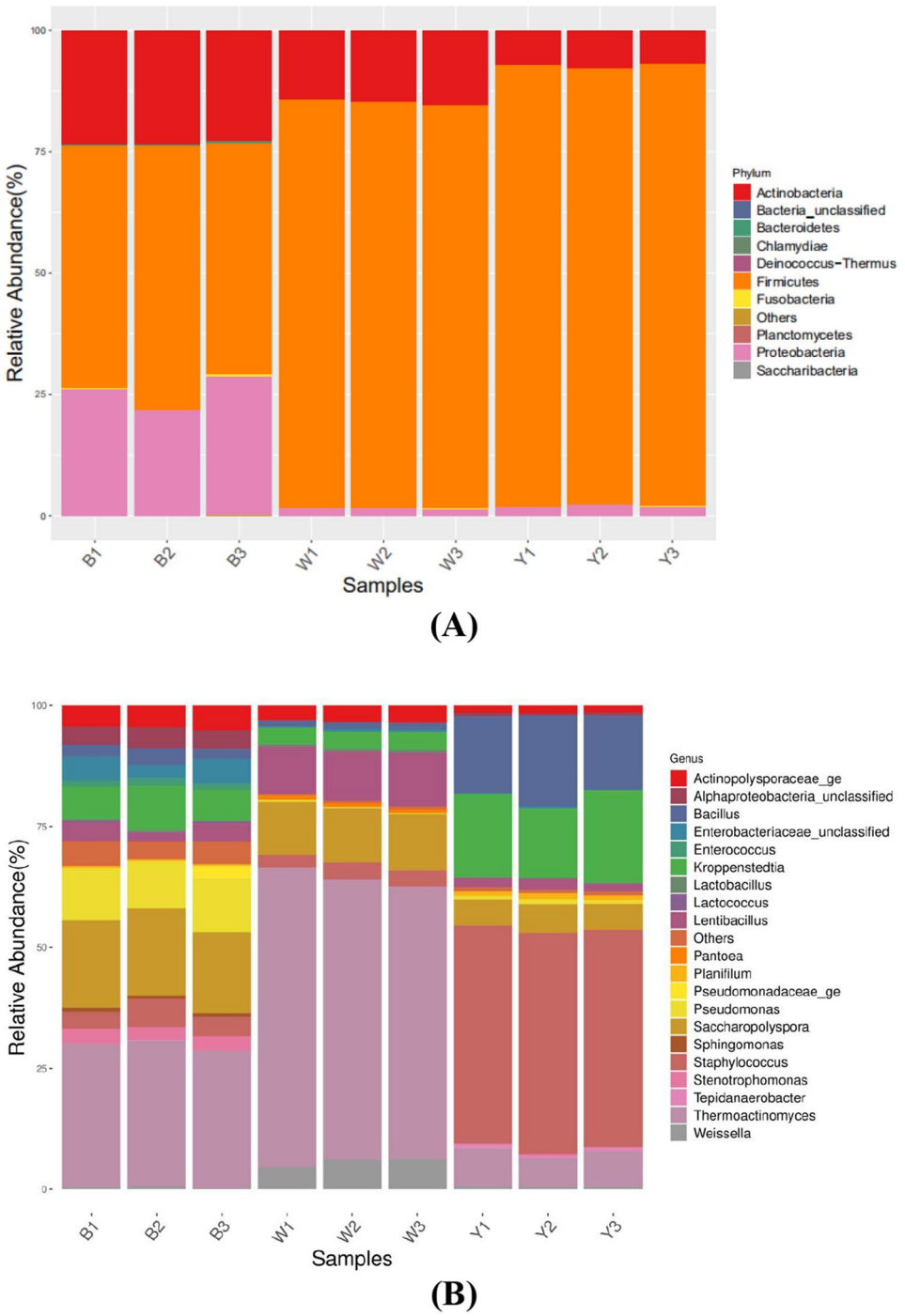


Fig. 3 The distribution of the predominant bacteria in the Black Daqu, White Daqu, and Yellow Daqu samples (three biological replicates in each group). The relative abundance in each sample at the phylum level (**a**) and the genus level (**b**)

mainly *Thermoactinomyces* (58.63%), *Saccharopolyspora* (11.33%), and *Lentibacillus* (10.43%). Sample Y primarily included *Thermoactinomyces* (7.20%), *Staphylococcus* (45.20%), *Kroppenstedtia* (16.87%), and *Bacillus* (16.63%) (Table 2).

As shown in Fig. 4a, the fungi in the three samples were uniformly distributed at the phylum level, with Ascomycetes as the most abundant, while some Basidiomycetes and Zygomycetes were also apparent. The Ascomycetes level was 99.8% in sample B, 99.9% in sample W, and nearly 100% in sample Y. Figure 4b shows the variation in the three samples at the fungal genus level. *Thermomyces* (93.70%) dominated in sample B, while *Thermomyces* (54.60%), *Aspergillus* (28.23%), and *Sagenomella* (13.90%) were the primary fungi in sample W. Furthermore, *Thermomyces* (78.07%) was the most abundant in sample Y, while the disparity between *Thermoascus* (11.10%) and *Aspergillus* (10.17%) was minimal (Table 3).

Table 2 The predominant species of Daqu bacteria in each sample

OTU ID	*B* (%)	*W* (%)	*Y* (%)
Actinopolysporaceae	4.33	3.33	1.50
Saccharopolyspora	17.60	11.33	5.50
Bacillus	2.70	1.40	16.63
Lentibacillus	3.10	10.43	1.87
Staphylococcus	4.40	3.20	45.20
Kroppenstedtia	7.57	3.50	16.87
Thermoactinomyces	29.47	58.63	7.20
Weissella	0.37	5.67	0.43
Pseudomonas	10.60	0.23	0.83
Stenotrophomonas	2.93	0.03	0.20
Lactobacillus	0.37	0.00	0.07

PCA (Principal coordinate analysis) showed significant differences between the microbial communities of the three Daqu samples (Fig. 5). Although the dominant bacterial (top 20) and fungal genera (top 12) were almost identical in each sample, the microbial abundance was significantly different between the three groups. The statistical differences between the microbial populations in the two groups were identified using bidirectional PERMANOVA based on Bray Curtis similarity.

Discussion

The metabolic activity of the Daqu microbe is essential to the unique flavor of sesame flavored liquor (He et al. 2020), a traditional Chinese drink. High-temperature fermentation is responsible for the main characteristics of sesame-flavored liquor Daqu. During the production process of Daqu, most thermophilic yeasts and molds were eliminated by increasing the temperature, while the microorganisms formed a microbial community dominated by high-temperature bacteria (Yi et al. 2019). This study analyzed the microbial communities of three kinds of sesame-flavored liquor Daqu with different properties. The microbial communities of samples B, W, and Y were similar, as were their physical and chemical indices (the data has not been published yet). The determination of the microbial communities will provide a foundation for the correlation between the brewing of sesame-flavored liquor and the Daqu microorganisms, as well as its physicochemical properties. Furthermore, it will be useful in follow-up research involving the unique flavor compounds and optimization of sesame-flavored liquor.

In 2015, Yao et al. used a culturally based method to separate sesame flavored Daqu samples by employing traditional separation techniques. The dominant bacterial genera that were evident during the drying stage were *Bacillus* sp. (23.15%), *Thermoactinomyces* sp. (15.68%) and *Lactobacillus* sp. (7.47%). (Yao et al. 2015). In 2017, Sha et al. demonstrated that *Pantoea*, *Weissella*, *Lactobacillus*, and *Thermoactinomyces* were the dominant bacterial genera via an independent culture method using a 16S rDNA clone library (Sha et al. 2017). In 2019, Fan et al. used high-throughput techniques to reveal that the dominant bacteria in sesame-flavored Daqu samples were *Bacillus* (26.9%), and *Thermoactinomyces* (12.1%), while the dominant fungi were *Thermomucor*, *Absidia*, and *Gregarina* (Fan et al. 2020). In the same year, based on simulated fermentation experiments, Fan et al. performed a comprehensive analysis of three sesame-flavored Daqu of different grades, evaluating the physical and chemical indices, volatile compounds, microbial community diversities and their correlation. The results differed significantly from those acquired in this study in terms of the diversity and abundance of the microbial communities. Firmicutes were the most abundant bacterial phylum in all the Daqu samples, followed by Proteobacteria. At the genus level, the detection results of the three samples were completely different. The first dominant genera in the three samples were *Kroppenstedtia* (18.9%), *Thermoactinomyces* (25.8%), and *Weissella* (18.0%), respectively. *Lactobacillus* was the only dominant genus with a relative abundance of more than 1% shared by the three samples. The eukaryotic microorganism communities in all the Daqu samples were similar. Ascomycota and Zygomycota represented the dominant eukaryotic communities. The fungal genera consisted mainly of *Absidia*, and *Thermomucor* (Fan et al. 2019). According to a study by Xie et al., *Kroppenstedtia*, *Lactobacillus*, *Weissella*, *Lentibacillus*, *Bacillus*, and *Saccharopolyspora* represented the primary bacterial groups in the high-temperature Daqu of the Chinese sesame-flavored liquor (Xie et al. 2020).

The traditional Chinese Maotai-flavored liquor Daqu is fermented at high temperatures of up to 60–70 °C (Jin et al. 2017). Based on the previous research, the dominant bacteria in Maotai Daqu were *Thermoactinomyces*, *Saccharopolyspora*, *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, and *Weissella*, while molds were represented by A*spergillus*, *Monascus*, *Thermoascus*, and *Thermomyces*, and the yeasts were *Saccharomyces* and *Pichia* (Wang et al. 2008; Jin et al. 2017; Zheng et al 2014a, b; Tang et al. 2017; Li et al. 2014). During the fermentation process, the local temperature of sample B was the same as that of the high-temperature Daqu, and the dominant bacteria were *Thermoactinomyces Saccharopolyspora*, *Pseudomonas*, *Kroppenstedtia*, *Staphylococcus*, *Actinopolysporaceae*, *Lentibacillus*, *Stenotrophomonas*, *Bacillus*, and *Lactobacillus*. The most abundant strains in sample B were similar to high-temperature Daqu and included *Thermoactinomyces*, *Saccharopolyspora*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, and *Weissella*. The Luzhou-flavored liquor Daqu was fermented at a medium temperature of 50–60 °C (Jin et al. 2017). According to the

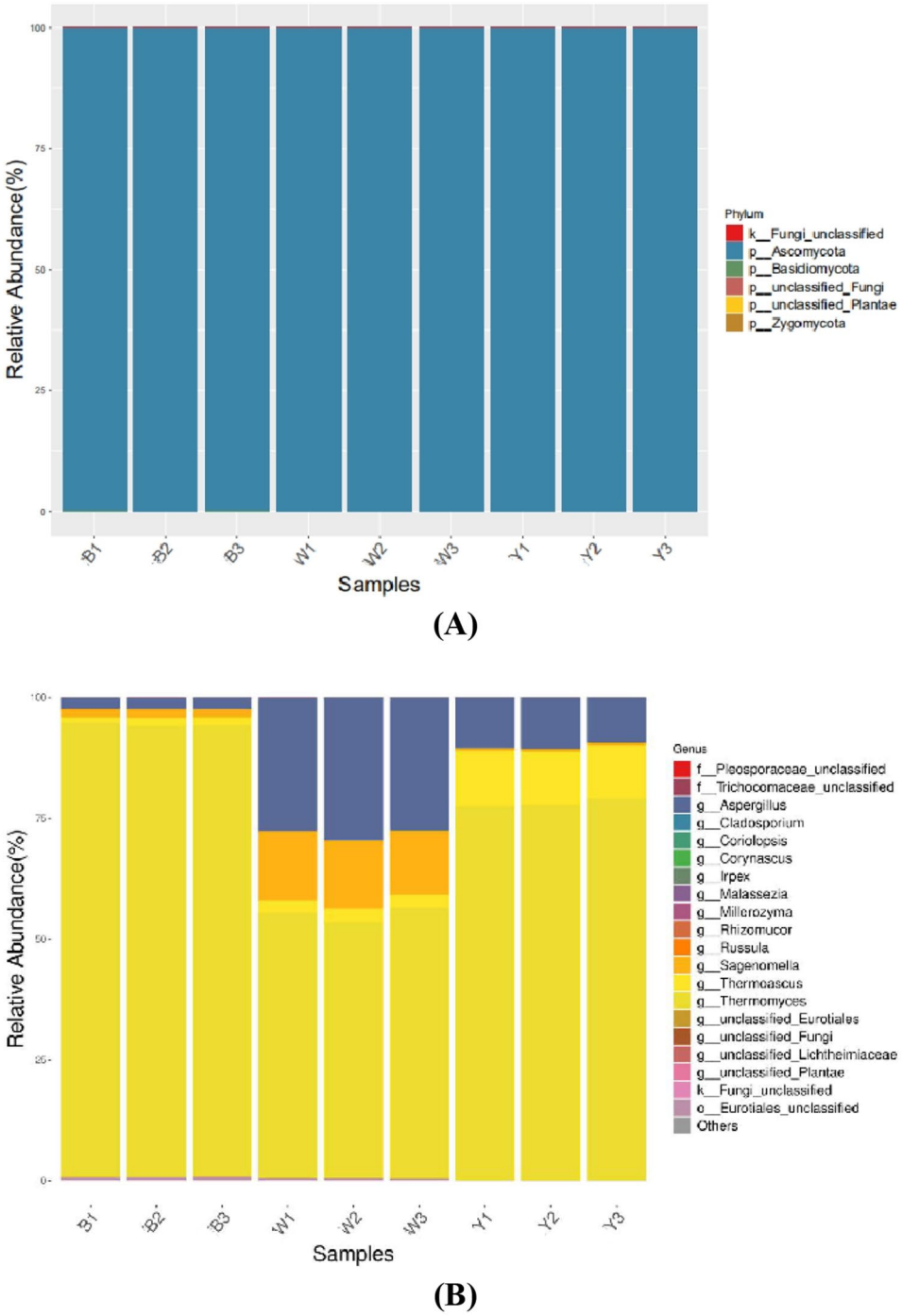


Fig. 4 The distribution of the predominant fungi in the Black Daqu, White Daqu, and Yellow Daqu samples (three biological replicates in each group). The relative abundance in each sample at the phylum level (**a**) and the genus level (**b**)

Table 3 The predominant species of Daqu fungi in each sample

OTU ID	*B* (%)	*W* (%)	*Y* (%)
Thermomyces	93.70	54.60	78.07
Thermoascus	1.37	2.67	11.10
Aspergillus	2.37	28.23	10.17
Sagenomella	1.60	13.90	0.60

previous study, the dominant bacteria in the Luzhou-flavored liquor Daqu include *Weissella*, *Lactobacillus*, *Staphylococcus*, *Pediococcus*, *Bacillus*, *Kroppenstedtia*, *Thermoactinomyces*, *Lactococcus*, and *Enterobacter*. The dominant fungi were *Pichia*, *Saccharomycopsis*, *Aspergillus*, *Rhizopus*, *Lichtheimia*, *Thermomyces*, *Thermoascus*, *Absidia*, and *Geotrichum* (Yang et al. 2017,2018; Du et al. 2019; Gou et al. 2015). The fermentation temperature of sample Y was 45–55 °C, while the dominant bacteria included

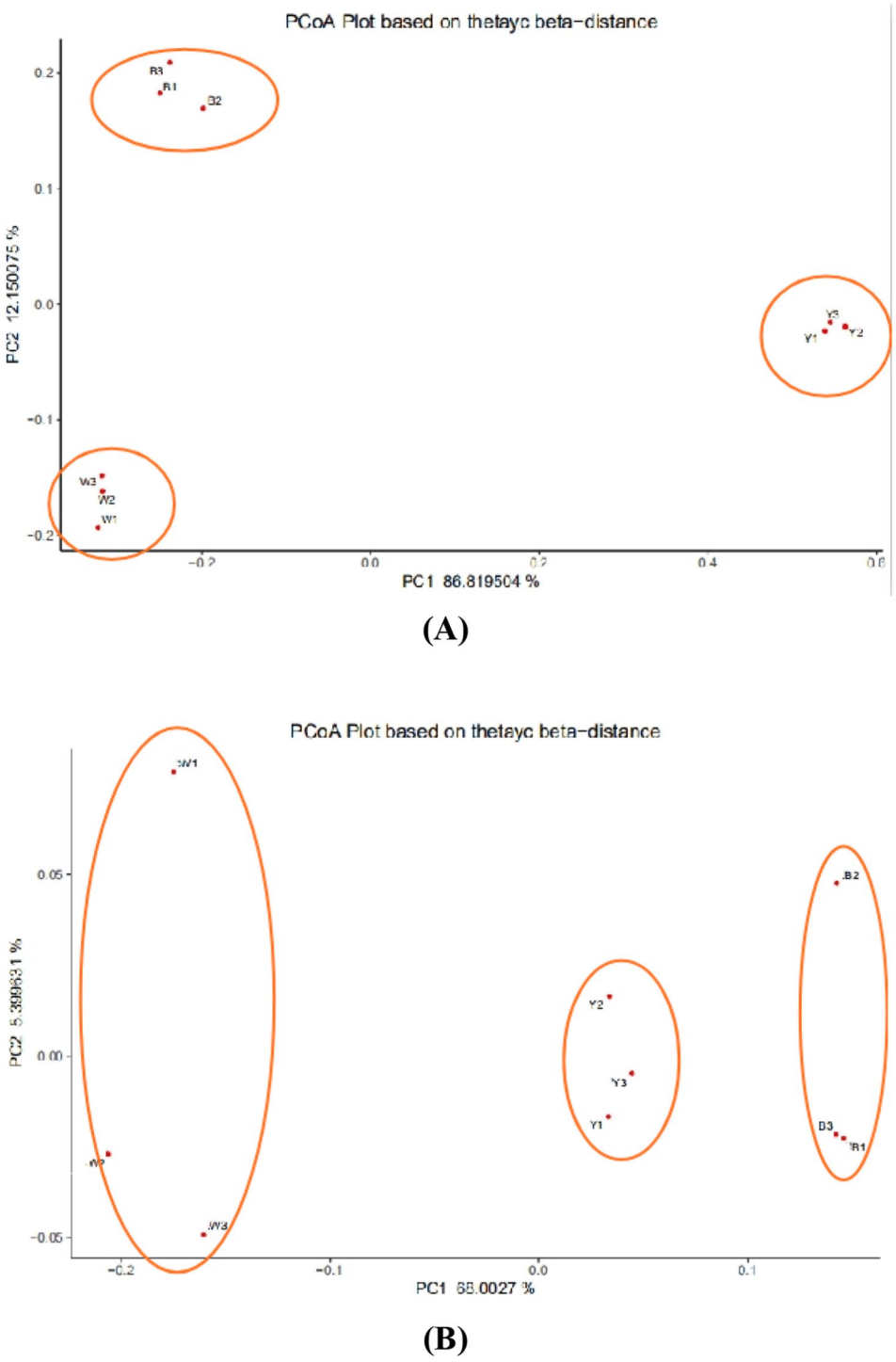


Fig. 5 The clustering in the Black Daqu sample, White Daqu sample, and Yellow Daqu sample (three biological replicates in each group), obtained via Principal Coordinate Analysis (PCoA) and based on the Thetayc-beta distance. The first two principal coordinates are plotted on the *x* and *y* axes, respectively (representing 98.9% of the total variation). The distance between two points represents the similarity between two samples

Staphylococcus, *Kroppenstedtia*, *Bacillus*, *Thermoactinomyces*, *Saccharopolyspora*, *Lentibacillus*, *Actinopolysporaceae*, *Pseudomonas*, *Weissella*, *Stenotrophomonas*, and *Lactobacillus*. The same strains were found in sample Y and the medium temperature Daqu, and included *Staphylococcus*, *Kroppenstedtia*, *Bacillus*, *Thermoactinomyces*, *Saccharopolyspora*, *Weissella*, and *Lactobacillus*. The light-flavored Daqu was fermented at a low temperature of 40–50 °C (Jin et al. 2017), while the primary bacteria were represented by *Pantoea*, *Klebsiella*, *Lactobacillus*, *Bacillus*, *Kroppenstedtia*, *Staphylococcus*, *Weissella*, *Acinetobacter*, and *Lentibacillus*, and the primary fungi were *Saccharomycopsis*, *Pichia*, and *Aspergillus* (Wang et al. 2017a, b; Fan et al. 2018; Zhang et al. 2014). The fermentation temperature of

sample W was 35–45 °C, and the dominant bacteria were *Thermoactinomyces*, *Saccharopolyspora*, *Lentibacillus*, *Weissella*, *Kroppenstedtia*, *Actinopolysporaceae*, *Staphylococcus Bacillus*, *Pseudomonas*, and *Stenotrophomonas*. The bacterial strains in sample W and the low-temperature Daqu were the same, and were represented by *Bacillus*, *Kroppenstedtia*, *Staphylococcus*, *Weissella*, and *Lentibacillus*.

High-throughput sequencing revealed the presence of primarily three types of bacteria in the three sesame-flavored liquor Daqu samples, namely Firmicutes, Actinobacteria, and Proteobacteria, of which Firmicutes was the dominant bacteria. According to the previous research reports, as well as the results of this study, *Thermomyces*, *Thermoascus*, A*spergillus*, and *Sagenomella* were the dominant fungi in sesame-flavored Daqu. Furthermore, *Actinopolysporaceae*, *Saccharopolyspora*, *Bacillus*, *Lentibacillus*, *Thermoactinomyces*, *Kroppenstedtia*, and *Aspergillus* were abundant in all three types of Daqu. Significant differences were evident in the content of four bacterial genera, namely *Weissella*, *Stenotrophomonas*, *Pseudomonas*, and *Lactobacillus*, as well as three fungal genera, namely *Thermomyces*, *Thermoascus*, *Sagenomella*. The compositions and concentrations of the volatile compounds, such as esters, alcohols, and acids, substantially influenced the unique flavor and quality of the liquor (Liu et al. 2020). Minimal studies exist regarding the unique fried sesame flavor substances of sesame-flavored liquor. Hu et al. found that pyrazines and other nitrogen-containing heterocyclic compounds and sulfur compounds (notably methionol) are important contributors to the aroma of sesame flavor baijiu (Hu et al. 2017). Several studies have shown that the combination of pyrazines (trimethylpyrazine and tetramethylpyrazine), furans, and phenolic compounds denote the primary flavor substances in sesame-flavored liquor (Qi et al. 2009; Sun et al. 2018). Various common flavor compounds were present in the sesame-flavored liquor samples, such as ethyl hexanoate, ethyl pentanoate, ethyl butanoate, ethyl furoate, ethyl 4-methylpentanoate, 2-furfurylthiol, 3-methyl-1-butanol, methional, hexanoic acid, butanoic acid, 3-methylbutanal, 2-acetyl furan, and trimethyl pyrazine (Liu et al. 2018; Wu et al. 2014).

In this study, the microbial diversity in the three Daqu varieties was distinctly different, affecting the liquor flavor obtained as a result. *Weissella* and *Lactobacillus* are both typical lactic acid bacteria (LAB) found in liquor Daqu. They can produce lactic acid to provide a substrate for the esterification reaction of yeast, while the ethyl lactate produced by esterification can improve the flavor of the liquor (He et al. 2019; Liu et al. 2020). The *Weissella* was positively correlated with pentanoic acid and 2-methoxy-5-methylphenol (Liu et al. 2019). The ethyl carbamate (EC; $C_2H_5OCONH_2$) that naturally exists in fermented foods and beverages, displays carcinogenic and mutagenic effects. The synergistic effect of LAB and yeast can effectively control the level of EC precursors during solid fermentation (Du et al. 2017). Some scholars believe that this synergistic relationship between LAB and yeast during liquor fermentation has not been fully explored. The interaction between LAB with yeast can effectively promote the development of the liquor industry while contributing to the modernization of traditional fermentation technology (Liu et al. 2020). In addition, the synthesis of exopolysaccharides and oligosaccharides by LAB with compounds containing free amino groups during the Maillard reaction can reduce ketones, aldehydes, and heterocyclic compounds, which denote the primary sources of liquor flavor (Xie et al. 2020).

Bacillus is a heat-resistant microorganism commonly found in mature Daqu. A variety of hydrolases, including amylase protease lipase, cellulase, glucanase, are secreted to facilitate the hydrolysis of macromolecules and the production of flavor compounds during the brewing process (He et al. 2019; Yao et al. 2015; Wang et al. 2017a, b; Zhao et al. 2019). One study showed that inoculating *Bacillus licheniformis* into Daqu significantly improved the amylase action while decreasing the activity of glucose amylase, and lipase (Wang et al. 2017a, b). Furthermore, ethyl octanoate, ethyl hexanoate, hexyl hexanoate, hexanol, and hexanoic acid were positively correlated with *Bacillus* (He et al. 2020). *Lentibacillus* and *Bacillus* are closely related genetically (Xie et al. 2020). As the metaproteomic data show, some *Lentibacillus* secrete a variety of proteases with a strong ability to metabolize amino acids (Zhang et al. 2018). Changes in the microbial communities, such as in *Kroppenstedtia* during liquor fermentation lead to an increase in acetic acid, lactic acid, malic acid, and ethyl acetate, while decreasing the ethyl lactate levels (Wang et al. 2017a, b). *Thermoactinomyces* can also produce lipase and phosphatase, while *Saccharopolyspora* plays a vital role in compound volatility (Jin et al. 2019). Although *Kroppenstedtia* and *Saccharopolyspora* represent the main sesame-flavored Daqu microbes, not much research exists regarding their microbial diversity and biological functions, necessitating further investigation.

The saccharification, liquefaction, and ester production in liquor are related to fungi (Fan et al. 2020). *Aspergillus* denotes an important filamentous fungus during liquor fermentation, able to produce a variety of enzymes for the formation of starch saccharification, protein hydrolysis, and flavonoids, thus affecting the flavor of the liquor (Chen et al. 2014; Machida et al. 2008). A study involving Maotai-flavored liquor showed that *Aspergillus* was positively correlated not only with pyrazines, but also with esters and some aromatic compounds (Jin et al. 2019). *Thermoascus* and *Thermomyces* produce heat-resistant enzymes that degrade carbohydrates efficiently (McClendon et al. 2012).

In this study, the diversity in the microbial communities of three different sesame-flavored Daqu samples were characterized using high-throughput sequencing technology.

This technique facilitated a better understanding of the differences between the microbial communities in Daqu while allowing the detection of various microbial species that was not previously possible using the culture method. *Saccharopolyspora*, *Bacillus*, *Lentibacillus*, *Staphylococcus*, *Kroppenstedtia*, and *Thermoactinomyces* were all dominant in the three Daqu samples, but the abundance values varied significantly for each strain. *Thermomyces* represented the primary fungi in the three types of Daqu, while a more significant difference was evident for the *Aspergillus* and *Thermoascus* content. In general, the microbial diversity and abundance in sample B were significantly higher than in samples W and Y. Daqu is central to liquor brewing, providing raw materials, enzymes, and microorganisms. The study of the microbial communities in different Daqu provides a foundation for examining the relationship between flavor compounds and microorganisms in liquor. At the same time, it provides a scientific basis for the microbial regulation of sesame-flavored Daqu and the industrialization of the winemaking process.

Conclusion

This study reveals the presence of 134 bacterial Daqu OTUs in the evaluated samples, which include the phyla Firmicutes, Proteobacteria, and Actinobacteria. *Thermoactinomyces*, *Saccharopolyspora*, *Pseudomonas*, *Kroppenstedtia*, *Lactobacillus*, *Weissella*, *Bacillus*, and *Lentibacillus* represent the central bacterial communities in Chinese sesame-flavored liquor Daqu. Furthermore, 79 fungal OTUs are present in the samples, primarily representing the phylum Ascomycota, while *Thermomyces*, *Thermoascus*, *Aspergillus*, and *Sagenomella* denote the dominating fungi in the sesame-flavored Daqu. The three samples that are assessed in this study are collected in the same culture conditions. Moreover, the analysis of the three types of Daqu exhibiting different microbial characteristics is central to this experiment.

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Author contributions XW, RJ, WC and XG designed and participated in all experimental procedures, performed data analysis, and drafted the manuscript. ML and FY participated in the Daqu samples collection and preparation. YY and YL supervised the study and critically revised the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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