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Evaluation of bacterial diversity during fermentation process: a comparison between handmade and machine-made high-temperature Daqu of Maotai-flavor liquor

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Abstract

Purpose: High-temperature Daqu is a traditional fermentation starter that is used for Chinese Maotai-flavor Baijiu production. Although the bacteria in high-temperature Daqu are known to be responsible for developing the quality and flavor of Baijiu during the fermentation process, there is little information on the properties of the bacteria during the fermentation of high-temperature Daqu, especially machine-made high-temperature Daqu. This has limited the development of the Maotai-flavor Baijiu industry, particularly with regard to the mechanized production of Maotai-flavor Baijiu.

Methods: Illumina MiSeq high-throughput sequencing was applied to study bacterial compositions during the fermentation of handmade and machine-made high temperatures.

Results: The results show that bacterial diversity in machine-made Daqu was similar but higher than that in handmade Daqu at the end of fermentation, and there was no significant difference between the methods with regard to the dominant genera and their dynamic changes during fermentation. *Rhizobium*, *Bacillus*, *Thermoactinomyces*, *Weissella*, *Lactobacillus*, and *Saccharopolyspora* were the dominant genera during the fermentation of both Daqus, although the relative abundance of these dominant genera differed between the two methods. Interestingly, the machine-made Daqu contained a higher relative abundance of *Bacillus* than handmade Daqu at all fermentation times. *Bacillus* is the most important functional bacteria in the fermentation of Maotai-flavor Baijiu, suggesting that mechanical-molding methods could be applied to industrial Maotai-flavor Daqu production.

Conclusion: These results suggest that mechanical-molding methods could be applied to industrial Maotai-flavor Daqu production, which could be helpful for industrial Maotai-flavor Baijiu production and the development of fermentation technology.

Keywords: Maotai-flavor Daqu, Fermentation, Bacterial diversity, Handmade, Machine-made

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Introduction

Traditional Chinese liquor (Baijiu), alongside brandy, whiskey, vodka, gin, and rum, has been classed as one of the six world-renowned distilled spirits (Liu and Sun 2018). It is usually divided into five categories, soy sauce aroma, strong aroma, fragrant, sweet honey, and others, based on typical aromatic characteristics. Among these, the soy sauce aroma (also known as Maotai-flavor) accounts for 3% of Chinese Baijiu production and contributes to 15 and 40% of Chinese Baijiu sales and profit, respectively, with an annual output of more than $150 billion. Maotai-flavor Baijiu is the most typical and highest quality Baijiu because of the complex processes involved in its production (Xiu et al. 2012). Famous for its highly complex, sweet, and refreshing flavor, Maotai-flavor Baijiu is also the most popular distilled Baijiu in China (Wu et al. 2012). Its characteristics are mainly derived from high-temperature Daqu—a traditional fermentation starter (Wang et al., 2017a, b, c, d).

Daqu, the saccharification-fermentation starter of traditional Chinese Baijiu, is mainly composed of diverse microbes, functional enzymes, and flavor compounds (Tang et al. 2019). As the primary microbial and enzyme source for Chinese Baijiu fermentation, it can determine the quality of the final Baijiu products. Daqu can be classified into three types based on the fermentation temperature: low-temperature (LT, 40–50 °C), medium-temperature (MT, 50–60 °C), and high-temperature (HT, 60–65 °C) (Li et al. 2016). Daqu, especially HT Daqu, has a unique and dynamic combination of microbiota, and the dominant microbial flora in HT Daqu is bacteria, rather than fungi, as these microbes are better adapted to high temperatures (Zheng and Han. 2016).

The traditional preparation process of HT Daqu contains three main stages, as shown in Fig. 1: (i) water infiltration of wheat and grinding, (ii) shaping into bricks, and (iii) fermentation (approximately 40 days). The shaping stage, also called the stepping starter stage, involves pressing the raw material into brick shapes to preserve the microorganisms and their metabolites, and it is a critical process in the production of HT Daqu. In this stage, two brick-molding methods can be used, namely, manual or mechanical. The mechanical molding methods can decrease the influence of operators, labor costs, and production time compared with manual processes. Although there are many advantages with mechanical molding compared with traditional manual methods, there is always the question of whether the microbial structure of Daqu produced by the mechanical process is comparable to that produced by traditional manual methods.

During Maotai-flavor Baijiu fermentation, the functional bacteria and their metabolites in HT Daqu contribute to the production of aromatic compounds (Fan et al. 2011; Fan et al. 2007; Wang et al., 2017a, b, c, d; Wu and Xu 2013), which are critical for the flavor and quality of the resultant Baijiu. The bacterial communities of HT Daqu of Maotai-flavor liquor have been extensively investigated in several studies (Wang et al. 2008; Liang et al. 2015; Wang et al., 2017a, b, c, d; Tang et al. 2019), but most of the studies focused on the finished Daqu product. During the entire fermentation process of HT Daqu, the composition and abundance of bacterial communities must undergo complex changes. Although the bacterial communities in HT Daqu of roasted sesame-like flavor liquor have been studied during HT Daqu fermentation (Su et al. 2015), bacteria involved in the HT Daqu of Maotai-flavor liquor have not been studied, and the two kinds of HT Daqu are very different. The characteristics of bacterial communities in the fermentation processes of machine-made HT Daqu and HT Daqu of Maotai-flavor liquor are still unknown. In particular, the bacterial community structures involved in the fermentation processes of mechanical and manual Daqu production have never been compared. This has limited the development of the Maotai-flavor Baijiu industry, particularly with regard to the mechanized production of Maotai-flavor Baijiu. In this study, we analyzed and compared the microbial community structure and the vital microorganisms in the manual and mechanized HT Daqu fermentation processes using Illumina MiSeq analysis.

Materials and methods

Sample collection

HT Daqu samples were provided by a famous Maotai-flavor Baijiu distillery in Guizhou province, China (27° 81'N; 106° 41′ E). Two batches of Daqu in fermentation workshops were selected to collect samples (workshops H and M). Workshop H used manual molding of Daqu bricks, whereas Workshop M used mechanical molding. The same raw materials and fermentation conditions for Daqu production were used in the two batches of Daqu in the fermentation workshops. Based the on preliminary data, the 0th (bricks placed in Daqu room), 7th (first turning of Daqu bricks), 14th (second turning of Daqu bricks), and 40th (end of fermentation) days are essential for the fermentation process; therefore, samples were collected on days 0, 7, 14, and 40 of fermentation. As shown in Table 1, Daqu samples produced by hand were labeled H0 (fermented for 0 days), H7 (fermented for 7 days), H14 (fermented for 14 days), and H40 (fermented for 0 days). Similarly, Daqu produced by machinery was named M0, M7, M14, and M40. At each sampling event, nine samples were collected from the upper, middle, and lower parts of the Daqu room, specifically, near the door, in the middle of the Daqu room, and near the window. To represent a sample of the whole fermentation

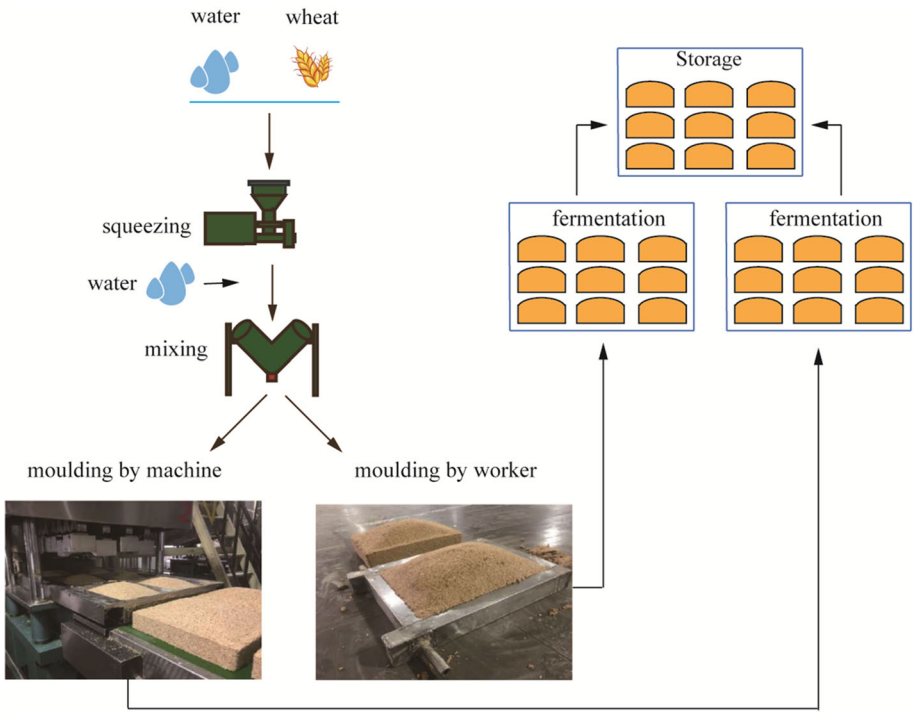

Fig. 1 Process diagram for the production of high-temperature Daqu. Wheat is mixed with 10% (w/w) water and squeezed, and then broken into three or four pieces to adequately release the starch. Then, 40% (w/w) water is added to the wheat, and the mixture is shaped by hand or machine. After the mixture are shaped to bricks weighing 4 to 5 kg each, the bricks are placed in a Daqu room, namely a fermentation chamber, stacked into 4 to 5 layers and fermented for 40 days. Microorganisms, mostly bacteria, are provided by the wheat, water, and air. During the fermentation, workers cover the bricks with rice straw to maintain heat for microbial growth and flavor compound formation. Daqu is turned over to lower the temperature, involving the first turning of Daqu bricks (the 7th day of fermentation) and second turning of Daqu bricks (the 14th day of fermentation). Daqu needs to mature during storage for about 6 months before use

room, the nine samples were then thoroughly mixed to provide a representative sample for each time point. Two parallel samples were collected from two Daqu fermentation rooms for each sample type at each sampling time point. Then, parallel samples at each time point are thoroughly mixed and analyzed. Finally, all samples were transferred to the laboratory on ice and analyzed within 3 h.

DNA extraction and PCR amplification for 16S rRNA sequence

Genomic DNA was extracted using the Soil DNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's instructions. The V4 region of 16S rDNA was amplified using universal bacterial primers F (5′-GTGCCAGCMGCCGCGG-3′) and R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR was carried out with 20 μL reactions containing 0.8 μL of the forward primer, 0.8 μL of reverse primer, 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.4 μL of FastPfu Polymerase, 2 μL of template DNA, and 10 μL of sterile ddH_2O. The following PCR conditions were used: pre-denaturation at 95 °C for 5 min, then 27 cycles at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. Subsequently, the PCR products were detected with 2% agarose gel electrophoresis and recovered using a gel extraction Kit (AXYGEN Co.). The amplicon library was prepared using a kit (Illumina, San Diego, CA, USA), and high-throughput sequencing was performed using an Illumina HiSeq platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd, China).

Sequence data analysis

After sequencing, all the generated raw sequences were processed by QIIME pipeline (Caporaso et al. 2010). Firstly, each sequence was classified to remove tags, primers, and junction sequences; sequences with lengths of less than 110 bp, a fuzzy base greater than 2 bp, and average mass less than 30 bp were removed; the high-quality sequences were obtained by removing chimeras by UCHIME and operated using UCLUST with 97% similarity, and the alpha diversity indices (the rarefaction, Chao1 richness, Ace richness estimators, Simpson and Shannon diversity indices) were calculated.

Table 1 Microbial community richness and diversity indices of the 16S rRNA sequences for clustering at 97% sequence similarity from all samples of Daqu H and Daqu M

Sample ID	Production method	Fermentation days	OTU	Ace	Chao	Shannon	Simpson	Coverage
H0	Hand	0 days	139	147	151	2.82	0.1088	0.9995
H7		7 days	110	135	135	2.32	0.1748	0.9991
H14		14 days	109	124	120	1.61	0.4711	0.9993
H40		40 days	78	95	92	1.45	0.527	0.9994
M0	Machine	0 days	130	140	147	2.57	0.1472	0.9994
M7		7 days	123	139	146	2.77	0.111	0.9993
M14		14 days	88	100	97	1.92	0.326	0.9995
M40		40 days	82	99	99	2.16	0.2013	0.9994

Statistical analysis

Alpha diversity (rarefaction curves, Ace indices, Chao 1 richness, Shannon diversity index, and Simpson index) and Good's coverage index were calculated by QIIME (version 1.7.0) (Caporaso et al. 2010; Cao et al. 2017). Beta diversity was analyzed using R software. Hierarchical clustering was used to evaluate the similarity of the bacterial communities present among the Daqu produced by hand (Daqu H) and Daqu produced by machine (Daqu M) fermentation samples (Tian et al. 2017). Linear discriminant analysis (LDA) effect size (LEfSe) was used to analyze the differences in the microbiomes between the Daqu H and M samples during fermentation (Kozik et al. 2017). Non-metric multidimensional scaling (NMDS) was used to reveal differences between and within groups (Carmen Portillo et al. 2016).

Results and discussion

Overall analysis of Illumina MiSeq sequencing

A total of 526,728 valid reads and 859 OTUs were obtained from all samples. Daqu H and M tended to have the same number of OTUs during fermentation (Table 1). The number of OTUs decreased from 139 to 78 with Daqu H fermentation time, whereas the number of OTUs decreased from 130 to 82 with Daqu M fermentation time. All the rarefaction curves based on the OTUs of 97% similarity tended to reach saturation (Fig. 2), indicating that our sequencing depth met the requirements for sequencing and analysis.

The total OTUs calculated by the Chao 1 estimator decreased from 151 to 92 with Daqu H fermentation time, but the total OTUs calculated by Chao 1 estimator tended to fluctuate during the Daqu M fermentation. After the 40-day fermentation, the total number of OTUs of fermentated Daqu M calculated by the Chao 1 estimator was higher than that of fermented Daqu H, indicating that the final Daqu product from the mechanized method (M40) had higher species richness than that produced by hand (H40), which was also demonstrated by the ACE indices (Table 1) and consistent with the differences in bacterial counts. The Shannon diversity index (positively correlated with alpha diversity index) and Simpson index (negatively correlated with alpha diversity) were used to evaluate the abundance of each species distributed in a community. During fermentation, the Shannon indices were different between the fermented Daqu H and M. Shannon index declined with Daqu H fermentation time but tended to fluctuate during Daqu M fermentation. After fermentation for 40 days, the Shannon index of Daqu M was higher than that of Daqu H; therefore, the species diversity of the final machine-made Daqu (M40) was higher than that of the handmade product (H40), which was validated by the Simpson index and Chao-1 estimator shown in Table 1. All samples had high coverage (99.9%).

Bacterial community profiles

During fermentation, Daqu produced by the two methods had slightly different bacterial communities at the phylum and class level. As shown in Fig. 3a, nine phyla were detected from eight representative samples of Daqu H and M, but only four phyla were abundant (> 1% abundance of at least one sample on average). Firmicutes were the most abundant phylum and maintained an increasing trend during the entire fermentation process. During fermentation, the proportion of Firmicutes increased from 43.15 to 93.10% and from 27.16 to 97.88% for Daqu H and Daqu M, respectively. The percentage of Firmicutes in Daqu H was significantly higher than that in Daqu M at 0 days fermentation. However, the proportion of Firmicutes in Daqu M was considerably higher than that in Daqu H on the fermentation days following day 0. Proteobacteria was the second most abundant phylum, and its proportions and dynamic successions in Daqu H and M were the exact opposite to those of Firmicutes. The proportion of Proteobacteria decreased from 41.13 to 6.53% and from

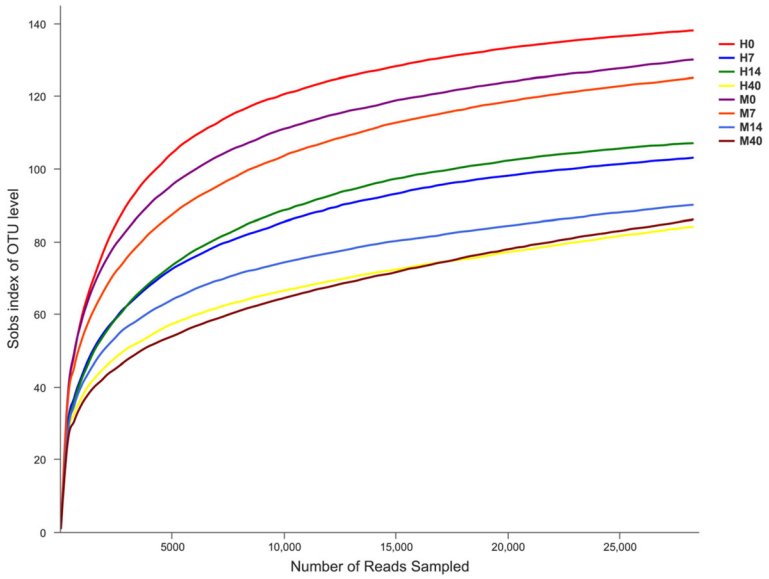

Fig. 2 Rarefaction analysis of the samples. Rarefaction curves of OTUs from different samples clustered at 97% sequence identity

54.26 to 1.50% with fermentation time in Daqu H and Daqu M, respectively. Actinobacteria was the third most abundant phylum, and its proportion tended to fluctuate. In Daqu H, the proportion of Actinobacteria increased from 1.93 to 14.19% at 0 to 14 days of fermentation, then reduced to 0.21% at the end of the fermentation. In Daqu M, the proportion of Actinobacteria increased from 3.08 to 5.06% at 0 to 14 days of fermentation, then reduced to 0.56% at the end of the fermentation period. Cyanobacteria were found almost only at day 0 of the fermentation, accounting for 13.42 and 15.23% of the total microorganisms in Daqu H and M, respectively, and were not detected on the following days of fermentation.

At the beginning of fermentation, the dominant bacteria phyla of Daqu H and M were Firmicutes and Proteobacteria. However, after 14 days of fermentation, both Daqus were dominated by Firmicutes with an abundance of 91.73 to 99.73%. Previous studies found that Firmicutes and Proteobacteria dominated the bacterial communities during the Fen-flavor Baijiu Daqu (LT Daqu) fermentation process (Li et al. 2011) and during Maotai-flavor Baijiu fermentation (Wang et al. 2015). According to these studies, Firmicutes and Actinomycetes were dominant phyla at 0-14 days, whereas Firmicutes were dominant phylum at 14–40 days fermentation.

Among the 15 bacterial classes identified from the eight representative samples of Daqu H and M, only seven bacterial classes were abundant (> 1% abundance of at least one sample on average), as shown in Fig. 3b. During fermentation, Bacilli (relative abundance of 43.12% in Daqu H and 27.1% in Daqu M), Alphaproteobacteria (relative abundance of 31.44% in Daqu H and 22.72% in Daqu M), Gammaproteobacteria (relative abundance of 9.39% in Daqu H and 31.39% in Daqu M), Actinobacteria (relative abundance of 1.94% in Daqu H and 3.08% in Daqu M), and Cyanobacteria (13.42% in Daqu H and 15.19% in Daqu M) were also abundant at the beginning of fermentation (H0 and M0). After fermenting for 7 days (H7 and M7), the relative abundances of three bacterial classes increased in Daqu H and M: Bacilli (relative abundance of 60.62% in Daqu H and 72.98% in Daqu M), Alphaproteobacteria (relative abundance of 12.71% in Daqu H and 15.95% in Daqu M), and Actinobacteria (relative abundance of 16.51% in Daqu H and 5.46% in Daqu M). The relative abundance of Gammaproteobacteria increased to 9.96% in Daqu H, whereas it decreased to 2.48% in Daqu M. Interestingly, Clostridia was only detected in fermented Daqu M samples after 7 days fermentation. After fermenting for 14 days (Fig. 3b, H14, and M14 samples), Bacilli was dominant in both Daqu H and M fermentation samples (relative abundance of 86.19 and 95.44%, respectively). The relative abundances of three bacterial classes decreased in Daqu H and M: Alphaproteobacteria (relative abundance of 6.13% in Daqu H and 1.60% in Daqu M), Gammaproteobacteria (relative abundance of 1.32% in Daqu H and 2.48% in Daqu M), and Actinobacteria (relative abundance of 6.09% in Daqu H and 1.90% in Daqu M). In addition, at the end of the fermentation, Bacilli dominated Daqu H and M

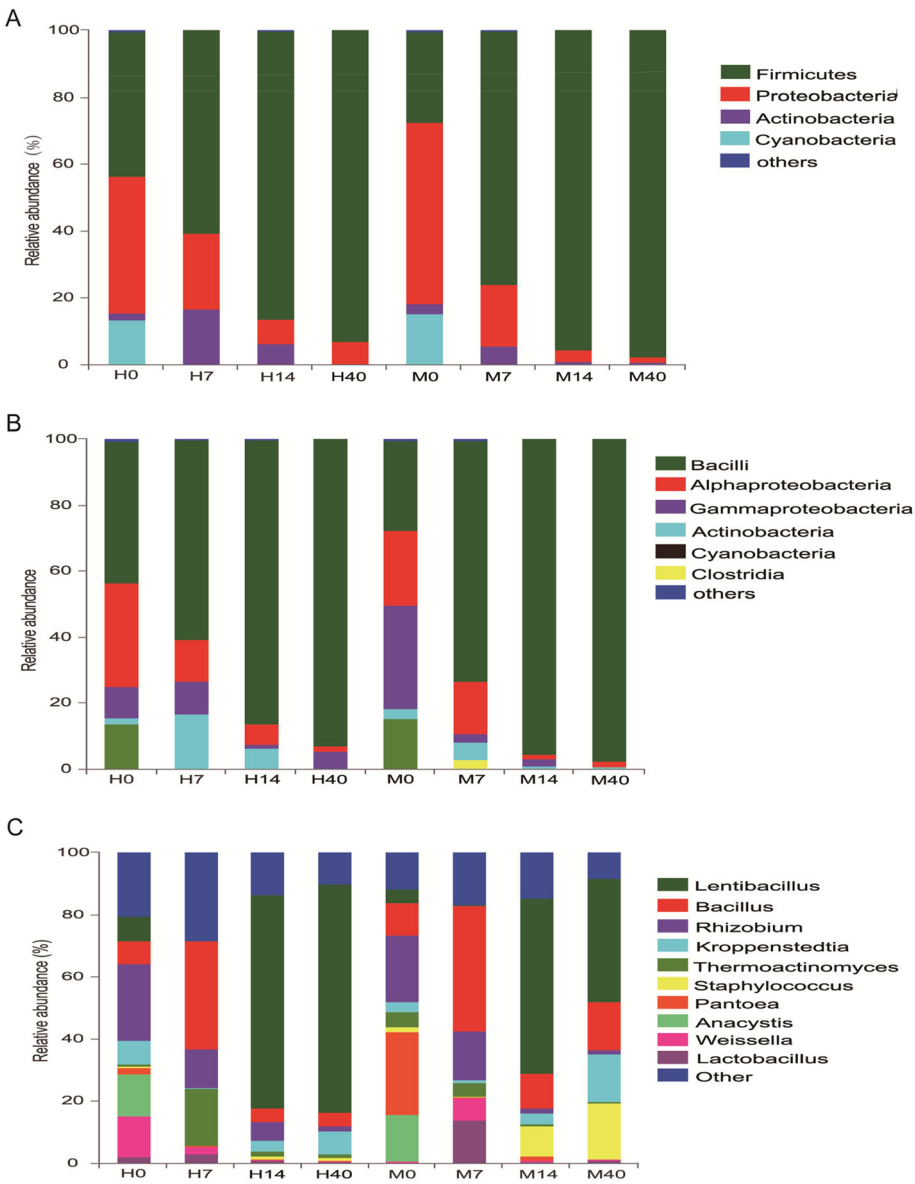

Fig. 3 Relative abundance of bacterial community compositions at phylum (**a**), classes (**b**), or genus (**c**) levels in Daqu H and Daqu M samples during incubation

(relative abundances of 93.06 and 97.71%) (Fig. 3b, H40 and M40 samples). In order to move genetic elements to produce heat resistance in *Bacillus*, the $spoVA^{2mob}$ operon is enriched during the Daqu incubation process, conveying high heat resistance to *Bacillus* for the Daqu incubation process and resulting in *Bacillus* being the main bacterial species in HT Daqu (Wang et al. 2018). The relative abundance of *Alphaproteobacteria* in Daqu H and M decreased to 1.56 and 1.46%, respectively, but Clostridia was only detected in incubated Daqu H samples. Therefore, there was no significant difference between Daqu H and M with regard to the main bacterial classes and their abundance patterns during fermentation.

Among the 90 bacterial genera identified from eight representative samples, only five bacterial genera were abundant (with over 1% average abundance) at the beginning of fermentation (day 0), as shown in Fig. 3b: *Rhizobium* (relative abundance of 24.74% in Daqu H and 21.35% in Daqu M), *Bacillus* (relative abundance of 6.97% in Daqu H and 10.14% in Daqu M), *Lentibacillus* (relative abundance of 7.96% in Daqu H and 4.23% in Daqu M), *Pantoea* (relative abundance of 3.55% in Daqu H and 28.54% in Daqu M), and *Kroppenstedtia* (relative

abundance of 7.57% in Daqu H and 3.20% in Daqu M). After fermenting for 7 days, six genera (*Rhizobium*, *Bacillus*, *Thermoactinomyces*, *Weissella*, *Lactobacillus*, and *Saccharopolyspora*) were enriched in Daqu H and M; the relative abundance of *Rhizobium* in Daqu H and M decreased to 10.62 and 14.72% respectively, while the relative abundance of *Bacillus* in Daqu H and M increased significantly to 37.89 and 40.48%, respectively. The other four bacterial genera were enriched (with over 1% average abundance) for the first time in this period, as shown in Fig 3b: *Thermoactinomyces* (relative abundance of 16.90% in Daqu H and 3.99% in Daqu M), *Weissella* (relative abundance of 2.37% in Daqu H and 7.55% in Daqu M), *Lactobacillus* (relative abundance of 3.05% in Daqu H and 14.61% in Daqu M), and *Saccharopolyspora* (relative abundance of 9.94% in Daqu H and 3.05% in Daqu M). After fermenting for 14 days, five genera (*Rhizobium*, *Bacillus*, *Lentibacillus*, *Kroppenstedtia*, and *Oceanobacillus*) were enriched in Daqu H and M; the relative abundance of *Rhizobium* in Daqu H and M decreased to 5.56 and 1.51%, respectively, and the relative abundance of *Bacillus* in Daqu H and M significantly decreased to 4.07 and 12.11%, respectively. The other three bacterial genera were enriched (with over 1% average abundance) for the first time in this period, as shown in Fig. 3b: *Lentibacillus* (relative abundance of 68.44% in Daqu H and 49.34% in Daqu M), *Kroppenstedtia* (relative abundance of 3.58% Daqu H and 3.23% Daqu M), and *Oceanobacillus* (relative abundance of 4.28% in Daqu H and 12.43% in Daqu M). Interestingly, *Lactobacillus* was barely detected after 14 days of fermentation. Moreover, *Lentibacillus* was dominant in both Daqu H and M (relative abundance of 72.19 and 40.16%, respectively; Fig. 3b) at the end of fermentation. In addition, the relative abundance of the other four species of bacteria was over 1% on average: *Rhizobium* (relative abundance of 1.48% in Daqu H and 1.09% in Daqu M), *Bacillus* (relative abundance of 3.84% in Daqu H and 14.89% in Daqu M), *Kroppenstedtia* (relative abundance of 7.55% in Daqu H and 14.63% in Daqu M), and *Oceanobacillus* (relative abundance of 1.82% in Daqu H and 4.87% in Daqu M).

During fermentation, the dominant genera (with over 1% average abundance) detected in both types of Daqu were *Rhizobium*, *Bacillus*, *Lentibacillus*, *Pantoea*, *Kroppenstedtia*, *Thermoactinomyces*, *Weissella*, *Lactobacillus*, *Saccharopolyspora*, and *Oceanobacillus*, which differs from those in Luzhou-flavor Baijiu Daqu (MT Daqu) fermentation, where the dominant genera were *Weissella*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Staphylococcus*, and *Enterobacter* (Yang et al. 2018). Among the dominant genera (with over 1% average abundance) during the fermentation of Daqu H and M, *Bacillus*, *Lactobacillus*, and *Weissella* have been reported in various flavor Baijiu fermentation processes (Wang et al., 2017b; Zhang et al. 2005; Li et al. 2011; Wang et al., 2017a, b, c, d; Wang et al. 2008), and they are also the main functional bacterial populations in Baijiu fermentation. *Bacillus* can metabolize protease, amylase, and other hydrolases and can also metabolize pyrazines, volatile acids, and aromatic and phenolic compounds; these substances make important contributions to the formation and quality of the flavor of Maotai-flavor Baijiu (Xiu et al. 2019; Meng et al. 2015). The relative abundance of *Bacillus* tended to fluctuate in Daqu H and M, but the relative abundance of *Bacillus* in Daqu M samples was higher than that in Daqu H during the entire fermentation process. In addition, *Lactobacillus* and *Weissella* can metabolize lactic, acetic, and other organic acids, which provide synthetic precursors for flavor substances in Baijiu fermentation (Wang et al., 2017a, b, c, d; Zhang et al. 2005; Li et al. 2011), and these organic acids also inhibit non-acid-resistant bacteria, such as *Pseudomonas*. The relative abundance of *Lactobacillus* was higher in Daqu H than in Daqu M at 0 days incubation, but the relative abundance of *Lactobacillus* was significantly higher in Daqu M than Daqu H after fermenting for 7 days. In addition, the relative abundance of *Lactobacillus* was higher in Daqu M than in Daqu H at the end of the fermentation, indicating the relative abundance of *Lactobacillus* was higher in the final Daqu products produced by machinery (M40) than in those produced by hand (H40). The relative abundance of *Weisseria* was significantly higher in Daqu H than in Daqu M at 0 days fermentation, whereas the relative abundance of *Weisseria* was higher in Daqu M than in Daqu H after incubating for 7 days. However, these two bacteria classes were barely detected in Daqu H and M in the days following fermentation day 7. The difference in the abundance of these main functional strains in Daqu H and M samples may affect the quality of the Daqu and, thus, the quality of the final Baijiu product. *Lentibacillus*, *Thermoactinomyces*, and *Kroppenstedtia* have been found in Daqu (Liang et al. 2015; Yao et al. 2012; Hu et al. 2017; Guo et al. 2017), but their functions have not been reported in detail. *Saccharopolyspora* has been detected in a variety of Daqu that can metabolize and produce important bioactive substances, such as antibiotics, vitamins, enzymes, algal growth factors, cellulose degradation promoting factors, and immunosuppressants (Meng et al. 2015).

Comparison between Daqu H and Daqu M

The Venn diagram of the common and unique OTUs is used to illustrate the differences and similarities in bacterial species among all samples of Daqu H and M (Fig. 4a). A total of 171 OTUs were observed in all

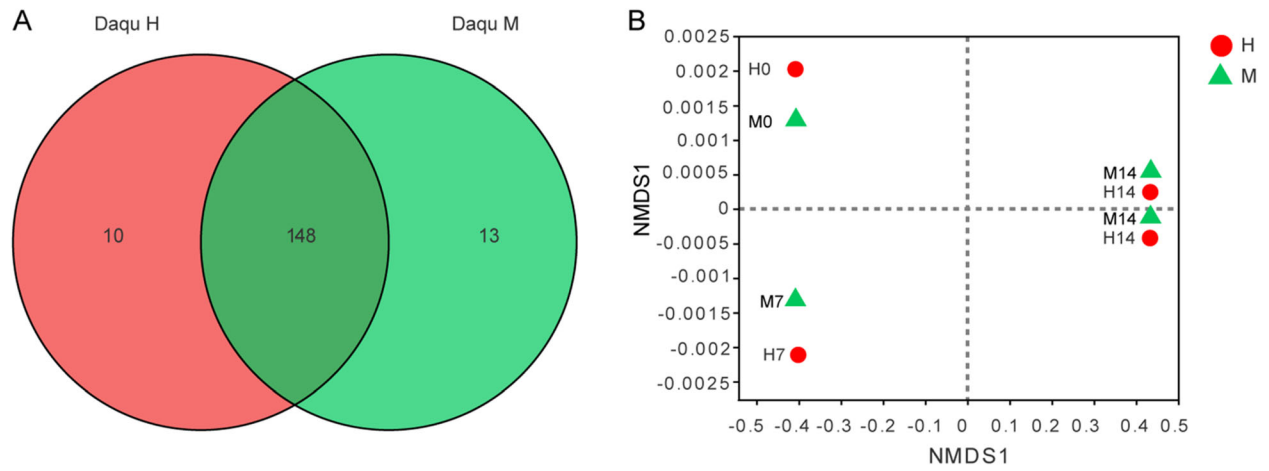

Fig. 4 Bacterial community comparisons and shared OTU analyses across Daqu H and Daqu M samples. **a** Venn diagram showing the unique and shared OTUs in different Daqu during the entire incubation process. **b** Non-metric multidimensional scaling (NMDS) plot generated using Bray distances at the genus level between samples of two types of Daqu

samples of Daqu H and M, 148 of which were common. The number of OTUs unique to individual Daqu types was 10 for Daqu H and 13 for Daqu M, indicating that both Daqus had high similarity in bacterial species during the fermentation process.

In addition, non-metric multidimensional scaling (NMDS) and hierarchical clustering analysis were used to determine which OTUs were differentiated among Daqu H and M. NMDS (genus level similarity) analysis results indicated that the separation of the bacterial communities in the two groups of samples, with samples H0 and M0, H7 and M7, H14 and M14, and H40 and M40, clustered as shown in Fig. 4b. Therefore, the differences in the bacterial communities were not significant in the Daqu H and M during fermentation.

Linear discriminant analysis effect size (LEfSe) analysis was used to determine the significant differences in the bacterial communities between Daqu H and M (Fig. 5a, b). Overall, one order, one family, and two genera were significantly higher in Daqu H samples compared with Daqu M during fermentation; while one class, one order, one family, and one genus were significantly higher in Daqu M samples compared with Daqu H. At the class level, the relative abundance of Betaproteobacteria was significantly higher in Daqu M samples compared with Daqu H during fermentation. Meanwhile, the Neisseriales order was significantly higher in Daqu M samples than in Daqu H samples, while the Pseudomonadales order was significantly higher in Daqu H samples. At the family level, the relative abundances of Chromobacteriaceae were significantly higher in Daqu M samples compared with Daqu H samples, while the relative abundances of Pseudonmonadaceae were significantly higher in Daqu H samples. Finally, at the family level, the relative abundances of *Deefgea* and unclassified *Erysipelotrichaceae* were significantly higher in Daqu M samples than Daqu H samples, while the relative abundances of *Pseudonmonas* and *Frateuria* were significantly higher in Daqu H samples. Among them, *Frateuria* has a strong ability to degrade microcystin. However, *Frateuria* has not been reported to be present in the brewing process of Maotai-flavor Baijiu, and it was detected for the first time in the fermentation of Maotai-flavor Daqu in this study. *Pseudonmonas* is a widely known pathogenic bacteria, but its function in the Baijiu-production process is not clear (Su et al. 2015). The unique bacteria in the Daqu M samples were *Deefgea* and unclassified *Erysipelotrichaceae*. Among them, *Deefgea* is a bacterial genus of the phylum Proteobacteria (Kroppenstedt and Lnsdorfl 2007) that has not previously been reported to be in Baijiu during brewing. Previous research has shown that *Erysipelotrichaceae* abundance is positively correlated with many markers related to carbohydrate digestion, including dietary carbohydrate and fiber content and VFA production (Cox et al. 2013). Its specific function in the fermentation process remains unknown and requires further investigation.

Conclusion

The present study is the first to compare the bacterial diversity between mechanized and handmade Maotai-flavor Daqu using an Illumina MiSeq high-throughput sequencing. The main bacteria genera were

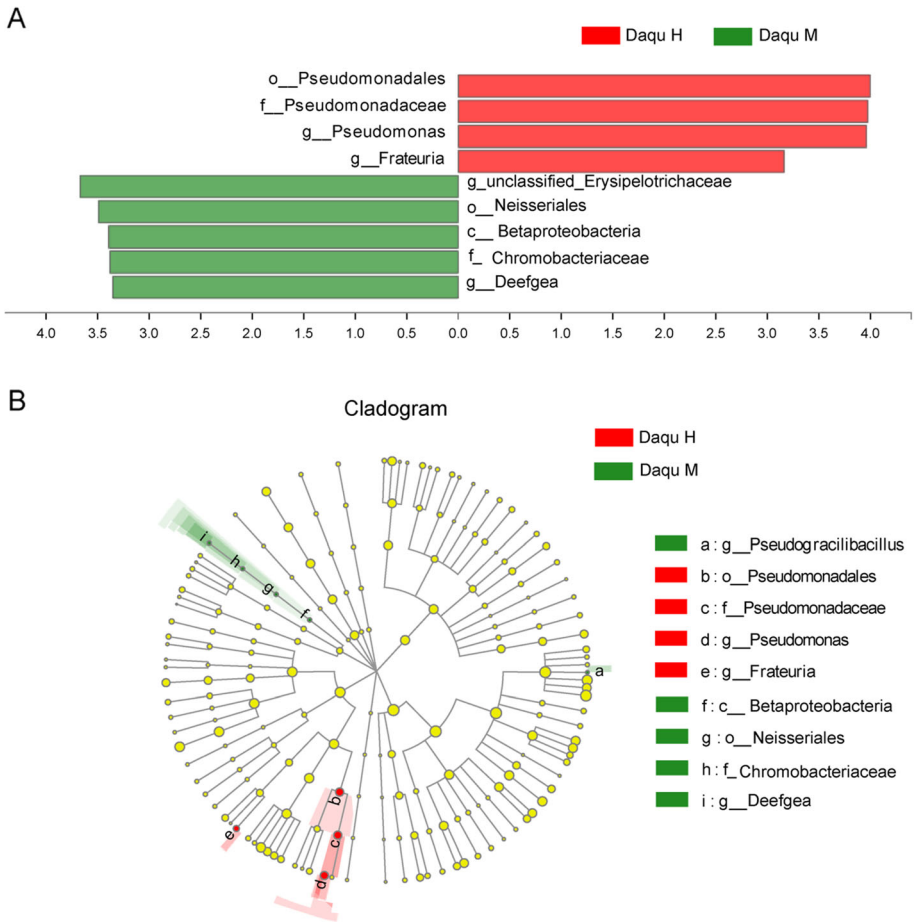

Fig. 5 Key phyla of bacteria in Daqu H and Daqu M samples during incubation, as determined by LEfSe. **a** Indicator bacterial groups with LDA values higher than 2.0 within the two types of Daqu. **b** Cladogram indicating the phylogenetic distribution of microbial species associated with the two types of Daqu

Rhizobium, *Bacillus*, *Thermoactinomyces*, *Weissella*, *Lactobacillus*, and *Saccharopolyspora* in Daqu H and M samples during fermentation. However, the two different types of Daqu harbored different and specific microbial communities and abundances, which might be due to the differences in the shape and density of the Daqu H and M bricks. Daqu M contained a higher relative abundance of *Bacillus* than Daqu H at all fermentation times. Furthermore, the bacteria diversity was higher in the final Daqu M than the final Daqu H product, which suggests that the mechanized methods could be successfully applied to industrial Maotai-flavor Daqu production. Therefore, our findings provide useful information on the mechanized production of Maotai-flavor Daqu. The mechanical molding methods can decrease the influence of operators and reduce labor costs and production time compared with the manual methods; at the same time, they can greatly improve production efficiency and product stability, which will help to progress the production of Maotai-flavor Baijiu. However, further research is needed to increase our knowledge of Daqu production, as well as other aspects that may be affected by machine production, such as the fungal communities, functional enzymes, and flavor compounds during fermentation.

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Authors' contributions
Qiancheng Zuo analyzed the data and prepared the manuscript. Min Guo designed and performed the experiments. Qiancheng Zuo and Yongguang Huang contributed to the experimental design, manuscript revision, and overall support of this study. All authors read and approved the final manuscript.

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Availability of data and materials
The authors declare that all materials and data are available.

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

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Competing interests
The authors declare that they have no conflict of interest.

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