Yan et al. AMB Express (2022) 12:79
https://doi.org/10.1186/s13568-022-01418-6

AMB Express

ORIGINAL ARTICLE

Open Access

# Microbial succession and exploration of higher alcohols-producing core bacteria in northern Huangjiu fermentation

Yi Yan[1,2], Leping Sun[1,2], Xuan Xing[1,2], Huijun Wu[1,2], Xin Lu[3], Wei Zhang[4], Jialiang Xu[1,2*] and Qing Ren[1,2*]

**Abstract**

Higher alcohols (HAs) are abundant compounds that provide important flavors in Huangjiu, but they also cause hangover. Previous studies have shown the production of HAs to be related to yeast, but the correlations between HAs and other microorganisms are rarely reported. In this study, we detected changes in levels of HAs and microbial dynamics during the Huangjiu fermentation process. Relationships were characterized using Pearson's correlation coefficient. The functional core HA-producing bacteria were selected by bidirectional orthogonal partial least squares (O2PLS). The result showed that 2-methyl-1-propanol, phenethyl alcohol and 3-methyl-1-butanol were the principle HAs present at high levels. *Lactococcus* and *Saccharomyces* were predominant at the genus level of bacteria and fungi, respectively. A total of 684 correlations between HAs and microorganisms were established. Five genera were screened as functional core HA-producing bacteria. Our findings might provide some new inspiration for controlling the content of HAs, enhancing international prestige and market expansion of Huangjiu.

**Keywords:** Microbiota, Higher alcohols, Core bacteria, Huangjiu, Fermentation

## Introduction

Huangjiu is a traditional Chinese alcoholic beverage with unique flavor and high nutritional value (Chen and Xu 2010). At the moment, there have been many varieties of Huangjiu all over China, based on different raw materials, sugar content, production process and geographical regions. Therefore, the volatile compounds and the dynamics of microbial community are quite different. Overall, Huangjiu is produced by three major procedures: selection and soaking of raw materials, followed by alcoholic fermentation, finally comes to post-process treatment (Yang et al. 2020). The usage of *Broomcorn millet* as the basic raw material in northern Huangjiu is the result of its high yields in northern China, while glutinous rice is preferred in southern Huangjiu, which is regionally determined (Han et al. 2019). After continuously simmering in a large saucepan, water evaporation accelerates, also with the enhancement of color. In Shaoxing Huangjiu (a typical representative of southern Huangjiu), the status of *Broomcorn millet* should maintain burning instead of scorching, and this is helpful for coloring in subsequent fermentation process. The color of Huangjiu originates from the natural color of grain, especially *Broomcorn millet* from northern Huangjiu, totally different from the caramel-added yellow rice wine in southern Huangjiu. In addition, the high-temperature gelatinization of the glutinous rice is responsible for the coloring of southern Huangjiu, followed by the steaming process, which might increase the risk of contamination when improper gelatinization. The alcoholic fermentation is very critical in Huangjiu brewing, when the nutrients in raw materials mentioned above are transformed to the aroma and flavors with the help of the microorganisms in the fermentation starters of Qu. In some studies, saccharification is also referred to as primary

*Correspondence: xujialiang@btbu.edu.cn; renqing@th.btbu.edu.cn
[1] School of Light Industry, Beijing Technology and Business University, Beijing, China
Full list of author information is available at the end of the article

fermentation while alcoholic fermentation is known as secondary fermentation (Chen et al. 2021). Qu could be classified into yellow Wheat Qu, red Hong Qu and yellow Hong Qu. Wheat Qu is produced in an open environment with nonsterilized, raw wheat as the material. It is a fermentation starter containing various microorganisms and enzyme systems that determine the taste and quality of Huangjiu (Chen and Xu 2013). The microbes during Huangjiu fermentation have been attracting researchers' interests all along. Thanks to the rapid development of high-throughput sequencing (HTS) and culturomics, large amounts of species have been detected, identified and isolated (Cai et al. 2018; Chen et al. 2020; Liang et al. 2020; Liu et al. 2019, 2020a, 2018, 2020b; Ren et al. 2020). For example, during the fermentation of Shaoxing mechanized Huangjiu, the results showed that bacteria of *Saccharopolyspora*, *Staphylococcus*, *Lactobacillus*, *Streptomyces*, *Actinopolyspora* and *Amycolatopsis*, fungi of *Saccharomyces* and *Aspergillus* are the dominant microorganisms (Liu et al. 2019). In our previous study, *Bacillus*, *Weissella*, *Streptomyces*, *Aeromonas* and *Blautia* were the dominant bacteria in the Huangjiu fermented from corn (Ren et al. 2020). *Saccharomyces cerevisiae* was identified as the predominant yeast during the fermentation of Shaoxing Huangjiu, and the other 10 most abundant bacteria genera were *Bacillus*, *Lactococcus*, *Leuconostoc*, *Staphylococcus*, *Pseudomonas*, *Weissella*, *Thermoactinomyces*, *Saccharopolyspora*, *Enterobacter* and *Lactobacillus* according to Zhang et al.'s and Liu et al.'s work (Liu et al. 2015; Zhang et al. 2012). The analysis of rice wine koji from Hubei province and Sichuan province in China indicated that the main bacterial genera were *Weissella*, *Lactobacillus*, *Lactococcus*, *Bacillus*, *Enterococcus* and *Cronobacter* (Zhao et al. 2020b). Generally, there could be great differences in microbial composition and dynamic changes of different raw materials and fermentation starters.

The aroma of Huangjiu is the key factor determining its sensory quality, which includes massive volatile and non-volatile flavor compounds, also affected by raw materials and fermentation starter mentioned above (Chen et al. 2019; Jiao et al. 2017). The volatile aroma components attribute most to the style and quality of Huangjiu (Jiang et al. 2020a; Sun et al. 2018). Alcohols, esters, acids, aldehydes and various heterocyclic compounds are the main trace components of the volatile compounds (Hu et al. 2019; Yang et al. 2020). The alcohols are composed of ethanol, methanol, n-butanol and n-propanol, providing the sweetness and flavor, also function as the precursors of esters, which consist of ethyl lactate, ethyl acetate and ethyl formate (Wang et al. 2022; Xu et al. 2015). The higher alcohols (HAs) in the complex flavor system serve as important aromatic and organoleptic compounds (Li et al. 2018a). HAs also called fusel oils or fusel alcohols, mainly refer to alcohols possessing more than two carbons, containing isopropyl alcohol, allyl alcohol, 2-methyl-1-propanol, 3-methyl-1-butanol and phenethyl alcohol and much more in fermented wine (Huang et al. 2017; Liu 2015). The current findings suggested that moderate amounts of HAs could enrich the coordinated flavors of wine, but the excess might produce adverse effects on health. HAs contribute to the aromatic complexity of wine at concentrations below 300 mg/L, whereas they are considered to have a negative impact on wine quality at concentrations exceeding 400 mg/L (Cameleyre et al. 2015). There have been studies showing that hangover might be enhanced since the HAs would last longer inside of body when the length of the carbon chains in HAs increased (Greenberg, 1970). Some researchers also found that the effects of hangover differed after consuming vodka (without HAs) and whisky (with HAs) (Bonte and Volck 1978; Cheng 2019; Gou et al. 2016; Murphree et al. 1967; Tian et al. 2017). Previous studies have indicated that HAs are formed by microbial metabolism, either from amino acids in feedstock via the Ehrlich pathway or directly from sugar degradation, also known as Harris pathway (Gonzalez and Morales 2017; Sun and Xiao 2018). α-ketonic acids originated from glycolysis and TCA cycle by glucose could further react with $-NH_2$ producing amino acids, along with the formation of HAs with the help of pyruvate decarboxylase and dehydrogenase in Harris pathway (Lilly et al. 2006; Sun et al. 2019). Whereas in Ehrlich pathway, α-ketonic acids are generated from amino acids derived from the protein in *Saccharomyces*. After decarboxylation and reduction, higher alcohols with one carbon less than the original amino acids were accumulated. Valine could produce isobutanol, leucine → isopentanol and phenylalanine → phenylethanol were similar (Lei et al. 2013; Yu et al. 2006). What's more, during the synthesis process of lipids (including the reaction of acids and glycerol for the formation of esters) and the alcoholic catabolism, fatty alcohols are generated from fatty acids initially (Rizzo et al. 1987). In other words, straight chain alcohols with more than 6 carbon atoms are produced from lipid oxidation products, also known as fatty alcohols. The synthesis of fatty alcohols relies on the reduction of activated forms of fatty acyl-CoA/fatty acyl-ACP catalyzed by a fatty acyl reductase (FAR) or the reduction of free fatty acids catalyzed by an enzyme carboxylic acid reductase (CAR) or the reduction of fatty acids to fatty aldehyde through an acyl-protein intermediate, which has been proved appearing in *Photobacterium phosphoreum* (Krishnan et al. 2020).

The contents and varieties of HAs depend on many factors, ranging from 0.1 to 0.7% in relation to ethanol produced (Pietruszka et al. 2010). Yeasts, especially *Saccharomyces cerevisiae*, are considered to be most responsible for the biosynthesis of HAs among the microbe (Li et al. 2018a; Liu et al. 2021; Pires et al. 2014; Tian et al. 2013). Previously, the study of the formation of HAs mostly focused on the only known HA-producing microbe *Saccharomyces* (Cameleyre et al. 2015; Furdikova et al. 2017; Li et al. 2018b; Ma et al. 2017; Zhang et al. 2015). However, the relationships between other microorganisms and HAs have not been fully understood owing to the complex community structure. The study of bacterial function on HAs generation receives less concern (Tian et al. 2022), and this is the reason why we pay attention to the bacteria related to this process.

With the development of HTS and bioinformatics, statistical and mathematical models have been applied to predict the role of microbiota and the function of the community in the fermentation of food (Cao et al. 2017; Ercolini 2013; Jagadeesan et al. 2019). The Pearson correlation coefficient (r) has been applied to analyze relationships between microbial genera and metabolites and thereby correlate the microbiota with volatile compounds in the fermentation of Baijiu (Wang et al. 2017). In addition, bidirectional orthogonal partial least squares (O2PLS) has been applied to select functional core microbiota by comparing the comprehensive importance of microbiota correlated with certain flavors (Wang et al. 2016).

In this study, we adopted the Pearson correlation coefficient (r), correlation networks and O2PLS to model the relationship between microbial genera and HAs. Five functional core bacteria were correlated with HAs in the process of Huangjiu fermentation. Detection of the changes in HAs and the dynamics of microorganisms of northern Huangjiu, as well as prediction of their relationships and speculation regarding methods to control HAs to increase flavor or reduce hangover, are presented in this study. Our findings might provide some new inspiration for controlling the content of HAs, enhancing international prestige and market expansion of Huangjiu.

## Materials and methods

### Sampling

Semisolid-state fermentation was carried out in Bei-Zong Huangjiu winery's experimental pit located in Hebei province, China, under a constant temperature of 25 °C. Huangjiu was fermented using *Broomcorn millet* grain as feedstock. The fermentation process followed was according to the conventional fermentation method used in wineries. Wheat Qu and amyloglucosidase were added as starter before fermentation. Wheat Qu was made in an open environment in August 2017. The period of fermentation was 10 days. Three parallel Huangjiu mash samples were collected every two days during the fermentation stages of 0, 2, 4, 6, 8 and 10 days in October 2017. A total of 18 samples were collected. Each sample, approximately 300 g, was divided into 3 parts: 200 g of each sample was used for quality control and HAs testing, approximately 5 g of each sample was used for isolation of strains, and the remainder of each sample was placed in a separate sterile bottle and stored at − 80 °C until DNA extraction. Dry ice was used to maintain low temperature during transportation.

*Broomcorn millet*→Soaking→Steaming→10 days fermentation→Filtering and sterilizing

### Quality control of the fermentation process

The National Standard Method GB/T13662-2018 was employed to determine acidity and alcohol content to evaluate Huangjiu quality, providing reference standards for the detailed operating procedures. A 200 g sample was centrifuged at 4000 r/min for 20 min, and the supernatant was collected. A sample (100 mL) of the supernatant was rotary evaporated, 95 mL of the distillate was collected, and water was added to 100 mL. The alcohol content was measured with an alcohol meter. Acidity of the supernatant during the fermentation process was detected by titration. The dinitro salicylic acid (DNS) method was employed to determine the reducing sugar content with glucose as a reference standard. A 1.0 mL liquid sample was diluted 10 times, and then 1.0 mL of the diluted solution and 1.0 mL of DNS solution were mixed and kept in a boiling water bath for 5 min. The mixture was diluted by distilled water to 10.0 mL when it was cooled to room temperature, and its absorbance at 540 nm was read (Guan et al. 1999).

### Qualitative and semiquantitative analysis of higher alcohols

HAs in each sample were analyzed by headspace solid phase microextraction gas chromatography mass spectrometry (HS-SPME/GC-MS) (Jiang et al. 2020b; Ma et al. 2017; Zhao et al. 2020a). For HS-SPME/GC-MS analysis, each Huangjiu sample (0.5 mL) was placed in a 15-mL solid phase micro-extraction (SPME) glass vial together with 5.44 mL ethanol (15% volume fraction) and 60 μL of the internal standard 2-octanol (8800 μg/L). The 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) extraction fiber was inserted into the vial. The sample vials were bathed in 50 °C water and ultrasonic extracted for 45 min. After extraction, fiber was inserted into the injection port of the gas chromatography mass spectrometry (GC–MS) system and thermally desorbed at 250 °C for 5 min.

The analysis was carried out on a Shimadzu-QP2010 Plus-GCMS. Each enriched compound was analyzed by DB-wax column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The operating condition for GC was as follows: high-purity helium (purity > 99.999%) as the carrier gas without split flow, flow rate at 1.0 mL/min, and temperature of injection port at 250 °C. The column oven temperature program began with 40 °C for 3 min, increased at a rate of 6 °C/min up to 100 °C, and then increased at a rate of 10 °C/min up to 230 °C for 7 min. The temperature of the injector and detector was 250 °C, the temperature of the ion source was 230 °C, the ionization mode was electronic ionization (EI), the EI emission current was 50 μA, and the EI ion energy was 70 eV. The chromatograms were recorded by monitoring the total ion currents in the range of 33–400 mass.

HA content in Huangjiu was calculated by substituting peak area of HA and internal standard detected by GC–MS into Eq. (1).

$$C = 12A_c/A_{is} \times C_{is} \tag{1}$$

In the equation: C: content of HA in Huangjiu, μg/L; $C_{is}$: content of the internal standard in sample, μg/L; $A_c$: peak area of HA in Huangjiu; $A_{is}$: peak area of the internal standard.

## DNA extraction, PCR amplification and Illumina MiSeq sequencing

Microbial DNA was extracted from the samples by the E.Z.N.A.® soil DNA kit (OMEGA Bio-tek, America). The quality of genomic DNA quality was detected by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified by thermocycler polymerase chain reaction (PCR). The thermocycler PCR system process was set at: 95 °C for 5 min; 25 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 40 s; followed by extension at 72 °C for 10 min. Primers were 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′). The internal transcribed spacer ITS1 region of fungi was amplified by PCR with a system process set at: 95 °C for 2 min; 30 cycles at 95 °C for 30 s, 61 °C for 30 s, and 72 °C for 45 s; and extension at 72 °C for 10 min. Primers were ITS1 (5′-AxxxCTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-BGCTGCGTTCTTCATCGATGC-3′) (Buee et al. 2009). The final DNA concentration and purity were determined using a NanoDrop 2000 UV–vis spectrophotometer. The amplicons were then pooled in equimolar concentrations into a single tube in preparation for paired-end sequencing (2 × 300 bp) (Ren et al. 2015) on an Illumina MiSeq platform according to standard protocols provided by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The fungal and bacterial sequence data reported in this study have been archived in the Sequence Read Archive (SRA) database with the accession number SRP303298, SRP303296, respectively.

## Processing of sequencing data

Quality filtration by Trimmomatic eliminated unqualified samples, and reads were merged by fast length adjustment of short reads (FLASH) under the following principle: first, the reads were truncated at any site receiving an average quality score < 20, setting a 50-bp sliding window. Second, according to the overlap relationship between paired-end DNA sequencing (PE) reads, the pairs of reads were merged into a sequence, with a minimum overlap length was 10 bp. Third, the maximum mismatch ratio allowed for the overlap area of the paired-end sequence was 0.2, and the reads containing ambiguity bases were deleted. Finally, primers were removed by allowing two nucleotide mismatches (Magoc and Salzberg 2011).

Operational taxonomic units (OTUs) clustering was performed on all sequences with 97% identity cutoff by UPARSE software (version 7.1). Chimeric sequences were identified and deleted by UCHIME. The taxonomy of the 16S rRNA gene sequence was analyzed by the ribosomal database project (RDP) Classifier algorithm against the Silva (SSU123) 16S rRNA database with 70% confidence threshold (Cole et al. 2009; Gurevich et al. 2013; Klindworth et al. 2013). The taxonomy of each ITS gene sequence was analyzed by Unite (Release 6.0) (Koljalg et al. 2013). Alpha rarefaction was performed in QIIME (version 1.7.0) using Chao1 to estimate species abundance (Caporaso et al. 2010). Species richness was estimated by the number of unique operational taxonomic units (OTUs). The Simpson index of OTU was calculated. The greater the value of the Simpson index, the lower the diversity of the community (Hill et al. 2003). Network analysis was used for correlating species abundance information between different samples to obtain the coexistence relationship and the interaction of species in the same environment.

## Prediction of correlation between microorganisms and HAs

Correlations between the microbial genera and HAs were established by Pearson correlation coefficient (r) to predict the relationship of the microbiota with the HA composition. The P value was adjusted by false discovery rate (FDR) using the Benjamini–Hochberg method. The cutoff for P and for the adjusted P value was set at 0.05. The correlation network was constructed by all significant associations and displayed using R programming language.

### Exploring HA-producing core bacteria in northern Huangjiu

To further integrate the microorganisms and HA data, O2PLS analysis was performed. Datasets were preprocessed as described by Bylesjö et al. (Bylesjo et al. 2007). In this method, both datasets were mean-centered by feature element, and the HA data were scaled to unit variance for each resolved peak. In addition, both datasets were scaled to an equal total sum of squares of 1. Finally, the O2PLS model was built by the OmicsPLS package of R34. A permutation test was performed to establish a threshold for identifying the most influential variables. The normalization of matrix involves subtracting the mean of each column and then dividing by the standard deviation. Datasets were reshuffled 1000 times and the O2PLS model was established for each permutation. Significance level (α) was set as 0.05 for the two datasets. Latent variables were the lower and upper α/2 quantiles of the loading values (Rodriguez et al. 2018). Further statistical and graphical analyses were performed in Excel and R software.

## Results

### Quality control of the fermentation process

Experimental data for quality control are shown in Fig. 1. The data indicated that content of alcohol and acid increased with the fermentation stage, and alcohol content increased most during the first two days of fermentation. During the fermentation period of the 2nd to the 4th days, alcohol content increased significantly in the fermenter. From the 4th to the 10th day of fermentation, alcohol content increased slightly and stabilized.

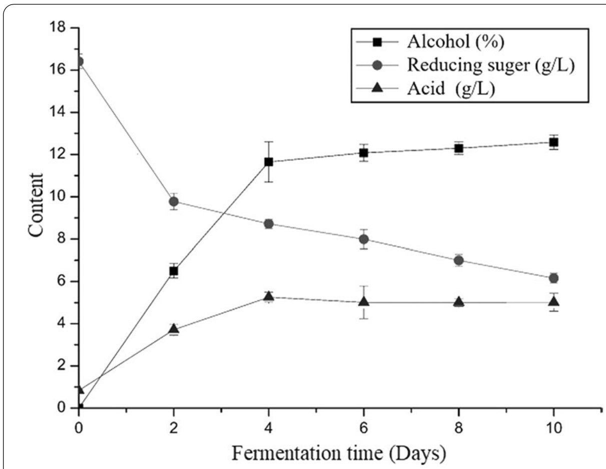


**Fig. 1** The changes in alcohol, reducing sugar and acid during fermentation of northern Huangjiu. Square indicates the content of alcohol. Circle indicates the content of reducing sugar. Triangle indicates the content of acid

During the fermentation process, reducing sugar content decreased with fermentation time. In the first two days of fermentation, reducing sugar content dropped sharply and coincided with a trend of rising alcohol content. From the 2nd to the 10th day, the content of reducing sugar gradually decreased and tended to stabilize. The acid content first increased and then tended to plateau. Finally, the acid content was approximately 5 g/L, within the standard of corruption. All of the above indicators meet the national standard GB/T-13662-2008 for Huangjiu. Detailed data are attached in Additional file 1: Table S1.

### Qualitative and semiquantitative analysis of higher alcohols

A total of 23 kinds of HAs were detected in the samples over 10 days (Table 1). HAs with high content (> 1.000 mg/L detected at one time) included 2-methyl-1-propanol, phenethyl alcohol, 1-triacontanol, 3-methyl-1-butanol, 1-hexacosanol, 2-o-decyl-threitol, 2-ethyl-2-methyl-tridecanol, 2-furanmethanol, 2-phenoxy-ethanol and 2-(2-butoxyethoxy)-ethanol. In addition, 1-dodecanol and 1-hexadecanol were steady. The total concentration of HA exhibited an upward trend and reached the maximum of 31.39 mg/L on the 10th day. However, only eleven kinds of HAs were detected on the last day of fermentation.

### Fungal diversity during the fermentation process in northern Huangjiu

The high-throughput sequencing results showed that 678,552 sequencing fragments in 18 Huangjiu samples met the quality control requirements, and 7 OTUs were obtained after clustering (Additional file 1: Table S2). According to the results from comparison with the database, there were 2 phyla, 4 classes, 4 orders, 5 families and 5 genera of fungi at each classification level. The average of three parallel samples was shown in Fig. 2. *Saccharomyces* occupied an absolute predominance in all samples at the genus level. A small amount of *Thermoascus* and *Aspergillus* were also found in the samples (Fig. 2).

To analyze the fungal community diversity of the samples, alpha-diversity index analysis was performed. The result of alpha-diversity index analysis showed that the Simpson index value was same at different fermentation stages (Additional file 1: Figure S1). There was no difference in the fungal community diversity of the samples.

### Bacterial diversity during the fermentation process in northern Huangjiu

The high-throughput sequencing results showed that 817,386 sequencing fragments in 18 Huangjiu samples met the quality control requirements, and 580

**Table 1** The changes of HA during the fermentation of northern Huangjiu

| HA (μg/L) | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 |
|---|---|---|---|---|---|---|
| Phenylethyl Alcohol | 3622.04 ± 41.20 | 1044.49 ± 27.29 | 1514.35 ± 58.50 | 2186.36 ± 67.92 | 3636.99 ± 65.38 | 3935.78 ± 26.14 |
| 2-methyl-1-Propanol | ND | 33.79 ± 1.66 | 57.62 ± 2.02 | 273.63 ± 10.84 | 489.67 ± 5.50 | 16,280.09 ± 759.33 |
| 3-methyl-1-Butanol | ND | 570.81 ± 5.09 | 816.92 ± 11.53 | 3896.08 ± 135.25 | 4922.36 ± 22.90 | 3830.97 ± 93.43 |
| 2-phenoxy-Ethanol | 1360.17 ± 29.66 | 251.37 ± 8.64 | 66.17 ± 1.44 | 254.19 ± 7.46 | 439.94 ± 12.84 | 924.99 ± 14.49 |
| 1-Triacontanol | ND | 175.23 ± 4.79 | 40.41 ± 0.60 | 10,605.38 ± 703.62 | 3509.15 ± 128.55 | 1040.19 ± 70.22 |
| 1-Hexacosanol | ND | 343.02 ± 19.80 | 100.64 ± 0.71 | 6555.62 ± 217.16 | 2045.77 ± 32.04 | 1250.21 ± 64.71 |
| 1-Dodecanol | ND | 96.87 ± 1.95 | 19.54 ± 0.92 | 444.37 ± 31.65 | 873.75 ± 24.19 | 391.80 ± 4.39 |
| 1-Hexadecanol | ND | 19.48 ± 0.40 | 80.71 ± 2.16 | 518.09 ± 23.46 | 925.01 ± 8.90 | 305.18 ± 3.88 |
| 2-O-decyl-Threitol | 3669.00 ± 34.11 | 46.34 ± 1.99 | 128.94 ± 3.39 | 434.45 ± 15.03 | 731.42 ± 5.80 | 634.47 ± 20.50 |
| 2-ethyl-2-methyl-Tridecanol | ND | 12.68 ± 1.42 | 13.28 ± 0.12 | 1023.48 ± 52.55 | 2513.16 ± 26.41 | 1653.45 ± 38.04 |
| 2-(2-butoxyethoxy)-Ethanol | ND | 93.29 ± 1.77 | 95.33 ± 2.56 | 493.28 ± 6.34 | 772.57 ± 20.62 | 1143.66 ± 45.92 |
| 6-Methyl-1-octanol | ND | ND | ND | 517.20 ± 16.00 | ND | ND |
| 2-Furanmethanol | 3550.90 ± 42.75 | 118.16 ± 2.40 | 70.06 ± 1.16 | ND | ND | ND |
| Octaethylene glycol | 542.35 ± 26.68 | 57.55 ± 2.43 | 25.39 ± 2.39 | ND | ND | ND |
| 9,12-Octadecadien-1-ol | ND | 464.43 ± 13.11 | 186.01 ± 7.77 | ND | ND | ND |
| 2-ethyl-1-Hexanol | ND | 54.63 ± 3.49 | 58.92 ± 0.18 | ND | ND | ND |
| 2-Isopropyl-5-methyl-1-heptanol | ND | 27.80 ± 1.29 | 60.70 ± 0.45 | ND | ND | ND |
| 3,7-dimethyl-1,6-Octadien-3-ol | ND | 46.97 ± 1.39 | 16.98 ± 0.73 | ND | ND | ND |
| 3-(methylthio)-1-Propanol | ND | 47.58 ± 0.49 | 16.58 ± 0.32 | ND | ND | ND |
| 1-Heptadecanol | ND | 33.45 ± 0.68 | 24.83 ± 0.62 | ND | ND | ND |
| 1-Undecanol | ND | ND | 28.77 ± 0.65 | ND | ND | ND |
| 5-methyl-2-(1-methylethenyl)-4-Hexen-1-ol | ND | 19.47 ± 0.19 | ND | ND | ND | ND |
| 4-methyl-1-Heptanol | ND | 13.29 ± 0.22 | ND | ND | ND | ND |

Every value was expressed as means standard error (n = 3)

*ND* no detection

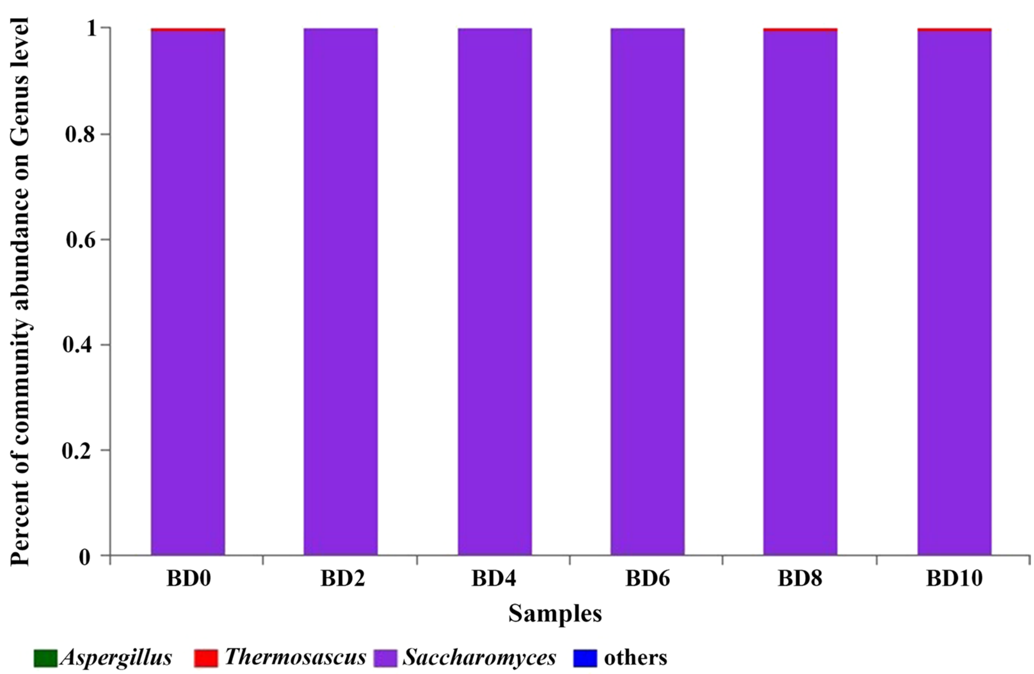


**Fig. 2** Fungal diversity during the fermentation process in northern Huangjiu at genus level. BD means "Beizong Huangjiu fermentation Day"

OTUs were obtained after clustering (Additional file 1: Table S3). The results from comparison with the database indicated that there were 27 phyla, 49 classes, 103 orders, 178 families and 335 genera of bacteria at each classification level. The average of three parallel samples was shown in Fig. 3. At the genus level, *Lactococcus* occupied predominance in samples (Fig. 3). Bacterial abundances showed obvious diversity in different fermentation stages.

Alpha-diversity index analysis was used to analyze bacterial community diversity. The result of alpha-diversity index analysis showed that the Simpson index value was clearly different in different fermentation stages (Additional file 1: Figure S2). The diversity of the bacterial community decreased significantly from the 0th to the 2nd day and rose gradually from the 2nd to the 8th day. Samples of the 2nd and the 4th days showed the highest values of the Simpson index, indicating that bacterial community diversity in these two samples were minimal. At the beginning of fermentation, bacterial community diversity was greatest.

Network analysis was performed to obtain the coexistence relationship and the interaction of species. Network analysis of bacterial dynamics during Huangjiu fermentation at the genus level showed that *Lactococcus*, which was the most frequent bacterial genus, was negatively correlated with most bacterial genera. *Escherichia-Shigella* only established correlation with *Lactococcus*. *Spongiimonas*, *Vibrio*, *Photobacterium* and *Pediococcus* built positive correlations with each other and were not associated with other bacteria.

### Correlation between microorganisms and HAs

The correlations between the microbial genera and HAs were tested by Pearson correlation coefficient (r), $r > 0.95$ was considered as a robust correlation (Wang et al. 2017). The results showed that HAs exhibited 684 correlations with 271 genera of microorganisms. Microorganisms with high abundance ($\geq 1\%$) were correlated with octaethylene glycol, 2-o-decyl-threitol, 2-furanmethanol, 2-ethyl-1-hexanol, 1-hexadecanol, 1-heptadecanol and 1-dodecanol. Additionally, 2-methyl-1-propanol, 3-methyl-1-butanol, 3-(methylthio)-1-propanol, phenethyl alcohol and 2-phenoxy-ethanol, which are always found in Huangjiu, were correlated with 33 genera, 6 genera, 7 genera, 12 genera and 19 genera of microorganisms, respectively. Additionally, 1-triacontanol, 1-hexacosanol, 2-o-decyl-threitol and 2-ethyl-2-methyl-tridecanol with higher content were correlated with 22 genera, 21 genera, 121 genera and 9 genera of microorganisms, respectively. The P values were adjusted by

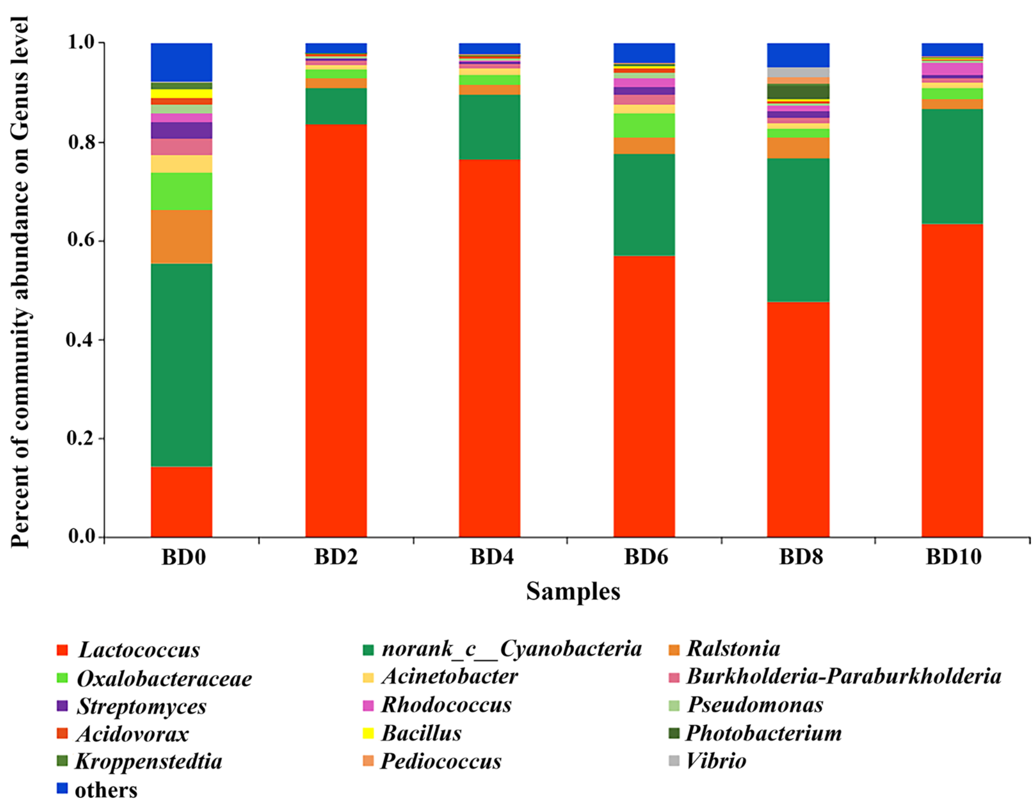


**Fig. 3** Bacterial diversity during the fermentation process in northern Huangjiu at genus level. BD means "Beizong Huangjiu fermentation Day"

FDR for precise association. (Fig. 4 and Additional file 1: Table S4).

## Exploring HA-producing core bacteria in northern Huangjiu

An O2PLS model was used to study the HA-producing functional core microbiota in Huangjiu. A total of 59 genera were selected as significant HA-producing microbiota. Among them, *Lactobacillus* and *Weissella* etc. had upper quantiles. *Thermoascus*, *Podosphaera paludibaculum* and others had lower quantiles (Additional file 1: Table S5). The correlations between the significant microbiota and HAs were built; 53 genera of significant microbiota built 86 correlations with 12 HAs ($p \leq 0.05$). Among them, 2-methyl-1-propanol established the most correlations and was related to 31 genera of microorganisms (Additional file 1: Table S6). For a bacterial genus to be considered functional core HA-producing bacteria in Huangjiu, two criteria needed to be met: (i) its abundance had to be high (top 20%), and (ii) it had to be correlated with stable HAs (was detected $\geq 5$ times). Based on these, five genera (*Lactobacillus*, *Neisseria*, *Staphylococcus*, *Thauera* and *Bifidobacterium*) were selected as functional core HA-producing bacteria in Huangjiu. They were related to the production of 2-methyl-1-propanol, 1-hexacosanol,

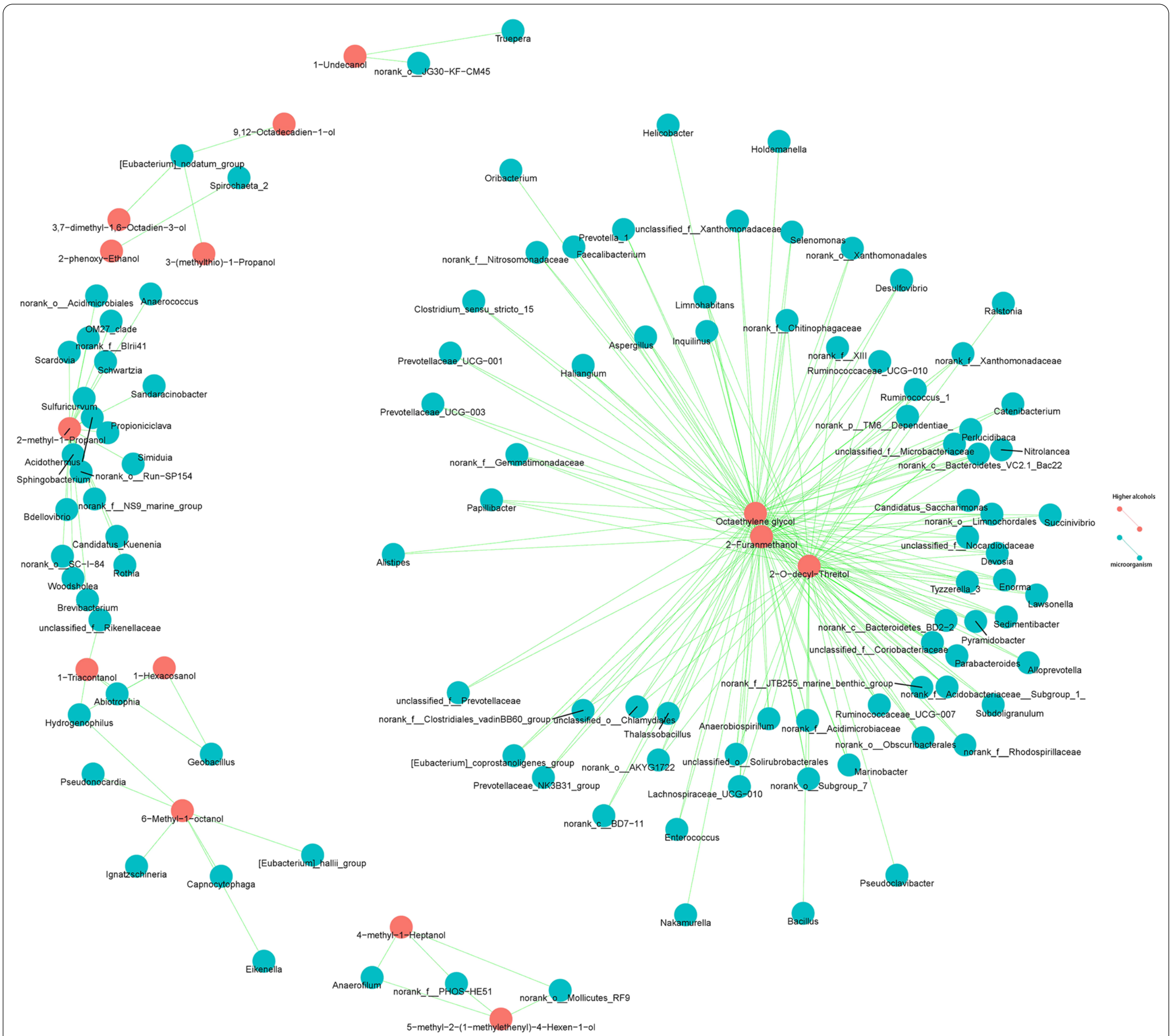


**Fig. 4** Correlation between microorganisms and higher alcohols. The green line indicates correlation was built between HAs and microorganisms; the blue pie indicates microbiota composition; the red pie indicates HA composition

1-triacontanol, 2-phenoxyethanol and phenethyl alcohol (Fig. 5).

## Discussion

Previous studies have shown that HAs in fermentation processes are mainly produced by yeast metabolism, including the degradative metabolic pathway (Ehrlich pathway) and the anabolic metabolic pathway (Harris pathway) (Cheng et al. 2011). In the Ehrlich pathway, amino acids form HAs by the catalysis of transaminase, ketoacid decarboxylase and aldehyde dehydrogenase. For example, the production of phenethyl alcohol, 2-methyl-1-propanol and 3-methyl-1-butanol are related to L-phenylalanine, valine and leucine, respectively (Hazelwood et al. 2008; Lambrechts and Pretorius 2000). Although amino acid contribution to production of HAs was clear, the input from central carbon metabolism was by no means negligible. In the Harris pathway, glucose forms pyruvate by glycolysis. Pyruvic acid enters the amino acid biosynthesis pathway under the catalysis of acetohydroxy acid synthase and forms an α-keto acid intermediate in the final stage of anabolism. Finally, HAs are produced by enzymatic catalysis.

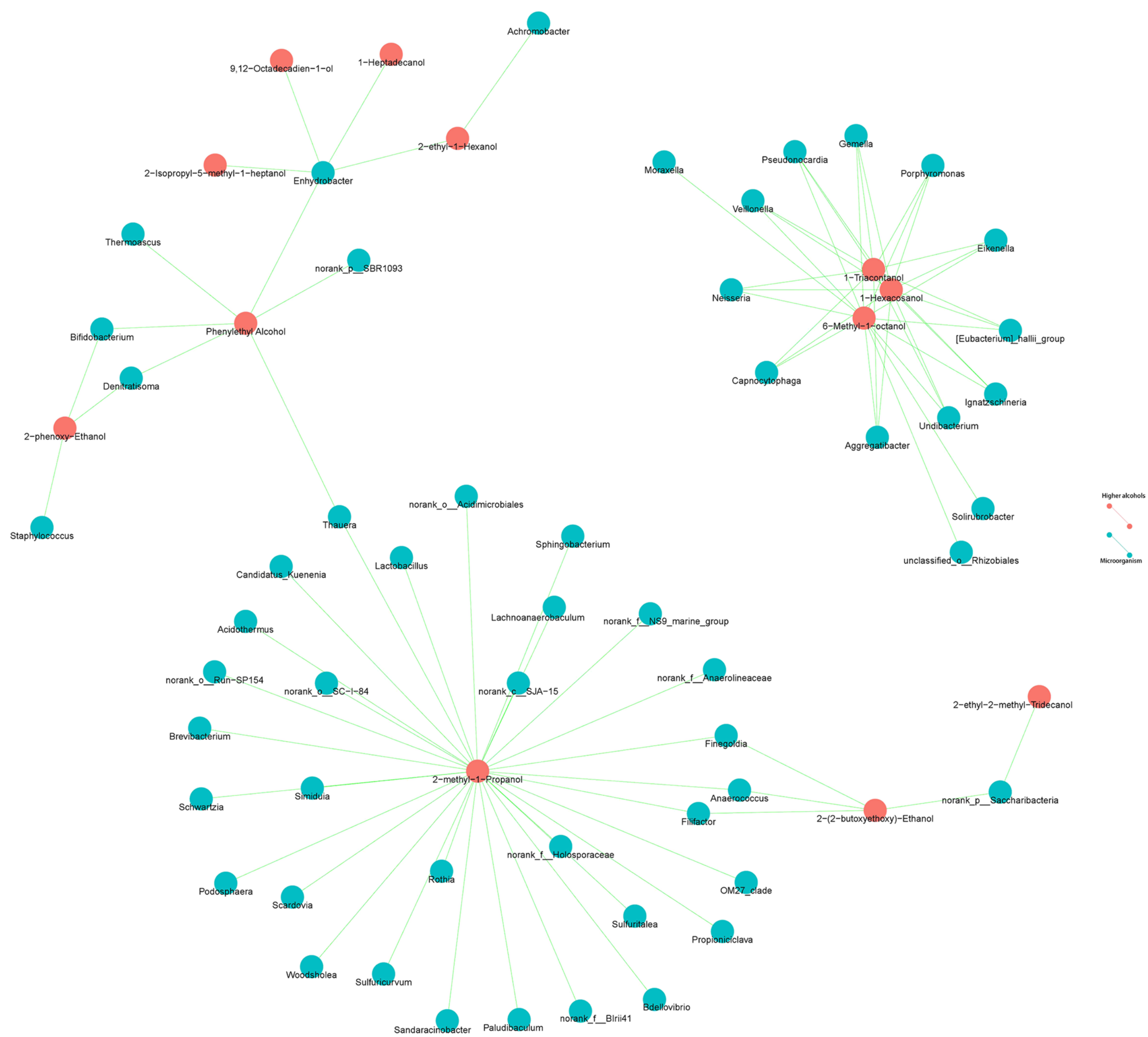


**Fig. 5** Correlation between HA-producing core bacteria and higher alcohols. The green line indicates correlation was built between HAs and microorganisms; the blue pie indicates micro-biota composition; the red pie indicates HA composition

Phenethyl alcohol is one of the important HAs in Huangjiu. It is an aromatic alcohol with rose-like fragrance which is naturally found in essential oils of many flowers and plants (Chen et al. 2017). In addition, phenethyl alcohol can inhibit some gram-negative bacteria and fungi (Fraud et al. 2003). In this study, *Lactococcus* was inhibited in the middle and last stages of fermentation. This may be due to the increase in phenethyl alcohol content from the 2nd to the 10th day. The main metabolic pathways producing phenethyl alcohol are phenylalanine metabolism and biosynthesis of plant secondary metabolites. In the Ehrlich pathway, phenethyl alcohol is synthesized from L-phenylalanine by transamination to phenylpyruvate, followed by decarboxylation to phenylacetaldehyde and reduction to phenethyl alcohol (Etschmann et al. 2002). Previously, various microorganisms including *Cladosporium cladosporioides*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Hansenula anomala* and *Kluyveromyces marxianus* have been reported to be capable of producing phenethyl alcohol (Etschmann et al. 2003; Hui 2006). Comparing the microorganisms that were associated with phenethyl alcohol in this study with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, four microorganisms involved in metabolism of phenethyl alcohol were consistent with those in the KEGG database. They are *Bifidobacterium*, *Bradyrhizobium*, *Lysinibacillus*, *Spirochaeta_2* and *Thauera* (Arai et al. 1999; Diaz et al. 1998; Ferrandez et al. 1997; Hwang et al. 2009; Teufel et al. 2010). In this study, *Denittrasoma*, *Enhydrobacter* and *Thermoascus* were positively correlated with phenylethyl alcohol. These genera are potential producers of phenylethyl alcohol. In addition, *Bifidobacterium* and *Thauera*, which were the HA-producing functional core bacteria, showed positive correlations with phenethyl alcohol. Therefore, they may make the greatest contribution to the production of phenethyl alcohol other than yeast.

2-methyl-1-propanol (isobutyl alcohol) is an important raw material for artificial musk and essential oils (Bauer et al. 2008). It can be biosynthesized from the 2-ketoisovalerate (KIV) biosynthetic pathway and the Ehrlich pathway (Atsumi et al. 2008; Li et al. 2012a, 2012b). Dickinson showed that a single isozyme of pyruvate decarboxylase can form isobutyl alcohol from valine (Dickinson et al. 1998). *Clostridium* is natural producer of 2-methyl-1-propanol, and it can produce quantities of 2-methyl-1-propanol under suitable fermentation conditions (Peralta-Yahya et al. 2012). In this study, the metabolism of 2-methyl-1-propanol was related to 33 genera, such as *Acidothermus*. *Lactobacillus* and *Thauera*, which are HA-producing functional core bacteria and showed negative and positive correlations with 2-methyl-1-propanol, respectively. Therefore, *Thauera* was predicted to contribute to the production of 2-methyl-1-propanol. *Lactobacillus* may have inhibited or decomposed 2-methyl-1-propanol.

3-methyl-1-butanol (isoamyl alcohol) is one of the compounds with the highest potential sensory impact in wines (Gomez-Miguez et al. 2007). It also showed a high proportion in this study. Therefore, controlling the yield of isoamyl alcohol was significant for controlling the content of HA in the fermentation of Huangjiu. Leucine can be metabolized via the Ehrlich pathway to form isoamyl alcohol in *Saccharomyces* (Atsumi et al. 2008). However, the content of isoamyl alcohol did not strictly increase with increasing abundance of yeast (Sun 2012). The production of isoamyl alcohol was the result of a variety of microbial and environmental interactions. In this study, isoamyl alcohol established correlation with 6 genera. It was positively associated with *Cloacibacterium* and negatively associated with *Anaerrotruncus*, *Eubacterium eligens*, *Odoribacter*, *Lachnospiraceae_NK4A136_group* and *norank_f_Erysipelotrichaceae*. However, because of the lack of significance and low abundance, 3-methyl-1-butanol may be most closely related to *Saccharomyces*.

2-phenoxyethanol is an ingredient used for many fragrance products. It is a colorless liquid with a mildly rosy aroma (Scognamiglio et al. 2012). In this study, 2-phenoxyethanol was associated with 19 genera. It was negatively correlated with *Saccharomyces* and *Lactococcus* and positively correlated with *Staphylococcus*, *Denitratisoma* and *Bifidobacterium*, which were significant microorganisms. Furthermore, *Staphylococcus* and *Bifidobacterium* were screened as HA-producing functional core bacteria. Therefore, *Staphylococcus* and *Bifidobacterium* may make a high contribution to the production of 2-phenoxyethanol.

Among the 23 HAs, there were 6 kinds of alkanols, including 1-triacontanol, 1-hexacosanol, 1-dodecanol, 1-hexadecanol, 1-heptadecanol and 1-undecanol. 1-hexacosanol, 1-octacosanol and 1-triacontanol have been studied and discussed extensively. 1-hexacosanol (cerylalcohol) shows anti-fatigue, anti-tumor, immunity, anti-cholesterol, and neuroprotective action and provides nutrition for nerves, greatly reducing the degradation of cholinergic neurons. 1-triacontanol (myricylalcohol) is mostly present in the form of esters in a variety of plants. It is a natural plant growth regulator, which has special regulatory effects on the growth of plants and no toxic effects on humans or animals (Duan et al. 2005). However, alkanols play a negative role in wine's aroma quality (de-la-Fuente-Blanco et al. 2016). Therefore, it is necessary to control the microorganisms that produce alkanols during the fermentation process. There are mainly three pathways for alkanol synthesis in microorganisms: fatty acyl-ACP as substrate, free fatty acids as substrates and fatty acyl-CoA as substrate (Cao et al. 2015; Liu et al., 2013, 2014; Lu et al. 2016). However, the above-referenced studies focused on constructing genetically engineered microorganisms. However, the correlation between alkanols and wild-type strains has rarely been reported. In this study, six alkanols were associated with 83 genera. 1-triacontanol and 1-hexacosanol showed positive correlations with *Neisseria,* a functional core bacterial genus; therefore, *Neisseria* may have contributed substantially to their production.

The type of HA varies little among different Huangjiu wines. Liu et al. (2019) found that β-phenylethanol, isoamyl alcohol and isobutanol were the most abundant HAs in Shaoxing mechanized Huangjiu. It is the same as this study. HAs are important flavor compounds; their sources are mainly studied through metabolic pathway analysis and mathematical model prediction. *Saccharomyces* and non-*Saccharomyces* yeasts (*Pichia mississippiensis* and *Wickerhamomyces anomalus*) have generally been considered to be the main producers of HAs, especially during the prefermentation stage of Huangjiu, based on O2PLS-based correlation analysis (Huang et al. 2018). The results of a flavor metabolic network, which was constructed using the KEGG database and information from the literature, indicated that *Streptomyces*, *Staphylococcus*, *Lactobacillus*, *Aspergillus* and *Choanephora* are potential producers of HAs (Liu et al. 2019). In this study, *Lactobacillus*, *Neisseria*, *Staphylococcus*, *Thauera* and *Bifidobacterium* were selected as functional core bacteria for producing HAs in Huangjiu. *Lactobacillus* and *Staphylococcus* were designated as HA producers under both methods. However, *Neisseria*, *Thauera* and *Bifidobacterium* were bacterial genera without high relative abundance in Shaoxing Huangjiu. Therefore, the differences in HA-producing core bacteria under different analytic methods may be caused by the discrepancy in microbial diversity among different samples.

In this study, a total of 23 HAs were detected. 2-methyl-1-propanol, phenethyl alcohol and 3-methyl-1-butanol were the principle HAs in northern Huangjiu. *Lactococcus* and *Saccharomyces* predominated at the genus level of bacteria and fungi, respectively. 684 correlations between HAs and microorganism were built. In addition, *Lactobacillus*, *Neisseria*, *Staphylococcus*, *Thauera* and *Bifidobacterium* were screened as functional core bacteria for producing HAs in Huangjiu. They were related to the production of 2-methyl-1-propanol, phenethyl alcohol, 2-phenoxy-ethanol, 1-triacontanol and 1-hexacosanol using the Pearson correlation coefficient.

The production of HAs during the fermentation of Huangjiu is the result of a variety of microbial and environmental interactions. However, in addition to yeast, the role of other microorganisms in the production of HAs is not clear. The prediction and verification of the correlations between HAs and microorganisms have great significance for controlling HA content, the selection of fermentation strains and the quality control of Huangjiu. Through the selection or elimination of strains, the types and content of HAs can be controlled, thereby achieving the effect of increasing flavor or reducing the sense of a hangover.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-022-01418-6.

**Additional file 1: Figure S1.** The fungal community diversity of the samples using alpha-diversity index. **Figure S2.** The bacterial community diversity of the samples using alpha-diversity index. **Table S1.** The changes of alcohol, reducing sugar and acid during fermentation in northern Huangjiu. **Table S2.** The high-throughput sequencing of fungal diversity during the fermentation process in northern Huangjiu. **Table S3.** The high-throughput sequencing of bacterial diversity during the fermentation process in northern Huangjiu. **Table S4.** Correlation between HA and microorganisms on genus level. **Table S5.** Significant microbiota for HA-producing. **Table S6.** Correlation between HA and significant microbiota on genus level.

**Acknowledgements**
We sincerely appreciate the great technical help from Professor Xueli Cao with preparing HS-SPME and GC-MS.

**Author contributions**
Methodology, XX and HJW; writing—review and editing, QR, YY and WZ; writing—original draft preparation, JLX and LPS; supervision, XL; funding acquisition, YY and WZ. All authors have read and approved the final manuscript.

**Funding**
This research was funded by Fundamental Research Funding of Beijing Technology and Business University (PXM2020_014213_000017) and the Coarse Cereals and Various Beans Processing Project of the Modern Agricultural Industrial Technology System of Hebei province (HBCT2018070206).

**Availability of data and materials**
All data generated or analyzed in this study have been included in this manuscript and additional file.

## Declarations

**Ethics approval and consent to participate**
This article does not contain any studies with human participants or animals performed by any of the authors. All listed authors have approved the manuscript before submission, including the names and order of authors.

**Consent for publication**
All authors have reviewed the final version of the manuscript and approve it for publication.

**Competing interests**
The authors declare no competing interest.

**Author details**
[1]School of Light Industry, Beijing Technology and Business University, Beijing, China. [2]Key Laboratory of Brewing Molecular Engineering of China Light Industry, Beijing 100048, China. [3]State Key Laboratory for Infectious Disease Prevention and Control, Chinese Center for Disease Control and Prevention, National Institute for Communicable Disease Control and Prevention, Beijing,

China. [4]College of Food Science and Technology, Hebei Agricultural University, Baoding, China.

Received: 13 December 2021 Accepted: 8 June 2022
Published online: 18 June 2022

## References


Arai H, Yamamoto T, Ohishi T, Shimizu T, Nakata T, Kudo T (1999) Genetic organization and characteristics of the 3-(3-hydroxyphenyl)propionic acid degradation pathway of *Comamonas testosteroni* TA441. Microbiology 145:2813–2820

Atsumi S, Hanai T, Liao JC (2008) Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451:86-U13

Bauer K, Garbe D, Surburg H (2008) Common fragrance and flavor materials: preparation, properties and uses. Wiley, Hoboken

Bonte W, Volck J (1978) Investigations in respect of the problem of alcoholic after-effects. Blutalkohol 15:35–46

Buee M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184:449–456

Bylesjo M, Eriksson D, Kusano M, Moritz T, Trygg J (2007) Data integration in plant biology: the O2PLS method for combined modeling of transcript and metabolite data. Plant J 52:1181–1191

Cai HY, Zhang T, Zhang Q, Luo J, Cai CG, Mao JW (2018) Microbial diversity and chemical analysis of the starters used in traditional Chinese sweet rice wine. Food Microbiol 73:319–326

Cameleyre M, Lytra G, Tempere S, Barbe JC (2015) Olfactory impact of higher alcohols on red wine fruity ester aroma expression in model solution. J Agr Food Chem 63:9777–9788

Cao YX, Xiao WH, Liu D, Zhang JL, Ding MZ, Yuan YJ (2015) Biosynthesis of odd-chain fatty alcohols in *Escherichia coli*. Metab Eng 29:113–123

Cao Y, Fanning S, Proos S, Jordan K, Srikumar S (2017) A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. Front Microbiol. https://doi.org/10.3389/fmicb.2017.01829

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Chen SA, Xu Y (2010) The influence of yeast strains on the volatile flavour compounds of Chinese rice wine. J Inst Brew 116:190–196

Chen S, Xu Y (2013) Effect of 'wheat Qu' on the fermentation processes and volatile flavour-active compounds of Chinese rice wine (Huangjiu). J Inst Brew 119:71–77

Chen XR, Wang ZY, Guo XN, Liu S, He XP (2017) Regulation of general amino acid permeases Gap1p, GATA transcription factors Gln3p and Gat1p on 2-phenylethanol biosynthesis via Ehrlich pathway. J Biotechnol 242:83–91

Chen S, Wang C, Qian M, Li Z, Xu Y (2019) Characterization of the key aroma compounds in aged Chinese rice wine by comparative aroma extract dilution analysis, quantitative measurements, aroma recombination, and omission studies. J Agric Food Chem 67:4876–4884

Chen C, Liu Y, Tian HX, Ai LZ, Yu HY (2020) Metagenomic analysis reveals the impact of JIUYAO microbial diversity on fermentation and the volatile profile of Shaoxing-jiu. Food Microbiol. https://doi.org/10.1016/j.fm.2019.103326

Chen GM, Huang ZR, Wu L, Wu Q, Guo WL, Zhao WH, Liu B, Zhang W, Rao PF, Lv XC, Li N, Sun JY, Sun BG (2021) Microbial diversity and flavor of Chinese rice wine (Huangjiu): an overview of current research and future prospects. Curr Opin Food Sci. https://doi.org/10.1016/j.cofs.2021.02.017

Cheng DD (2019) Research progress on "Headache" caused by craft beer. China Brew 38:12–15

Cheng J, Qin WS, Zhao XJ (2011) Formation and regulation of higher alcohols in wine fermentation. China Brew. https://doi.org/10.1007/s00253-017-8715-5

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM et al (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:D141–D145

de-la-Fuente-Blanco A, Saenz-Navajas MP, Ferreira V (2016) On the effects of higher alcohols on red wine aroma. Food Chem 210:107–114

Diaz E, Ferrandez A, Garcia JL (1998) Characterization of the hca cluster encoding the dioxygenolytic pathway for initial catabolism of 3-phenylpropionic acid in *Escherichia coli* K-12. J Bacteriol 180:2915–2923

Dickinson JR, Harrison SJ, Hewlins MJ (1998) An investigation of the metabolism of valine to isobutyl alcohol in *Saccharomyces cerevisiae*. J Biol Chem 273:25751–25756

Duan QF, Ma LY, Zheng H, Chen XM (2005) A review on research progress of some policosanols. J Chen Ind Forest Pro. https://doi.org/10.1371/journal.pone.0197343

Ercolini D (2013) High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. Appl Environ Microb 79:3148–3155

Etschmann MMW, Bluemke W, Sell D, Schrader J (2002) Biotechnological production of 2-phenylethanol. Appl Microbiol Biot 59:1–8

Etschmann MMW, Sell D, Schrader J (2003) Screening of yeasts for the production of the aroma compound 2-phenylethanol in a molasses-based medium. Biotechnol Lett 25:531–536

Ferrandez A, Garcia JL, Diaz E (1997) Genetic characterization and expression in heterologous hosts of the 3-(3-hydroxyphenyl)propionate catabolic pathway of *Escherichia coli* K-12. J Bacteriol 179:2573–2581

Fraud S, Rees EL, Mahenthiralingam E, Russell AD, Maillard JY (2003) Aromatic alcohols and their effect on Gram-negative bacteria, cocci and mycobacteria. J Antimicrob Chemoth 51:1435-U1438

Furdikova K, Makysova K, Spanik I (2017) Effect of indigenous *S. cerevisiae* strains on higher alcohols, volatile acids, and esters in wine. Czech J Food Sci 35:131–142

Gomez-Miguez MJ, Cacho JF, Ferreira V, Vicario IM, Heredia FJ (2007) Volatile components of Zalema white wines. Food Chem 100:1464–1473

Gonzalez R, Morales P (2017) Wine secondary aroma: understanding yeast production of higher alcohols. Microb Biotechnol 10:1449–1450

Gou JY, Jia ZY, Yan ZK, Du J (2016) Research progress in decreasing the contents of higher alcohols in Baijiu (Liguor). Liquor Mak 43:25–29

Greenberg LA (1970) The appearance of some congeners of alcoholic beverages and their metabolites in blood. Q J Stud Alcohol Suppl 31:20–25

Guan B, Ding Y, Xie L, Long Y (1999) The modification of the DNS method for the determination of reducing sugar. J Wuxi Univ Light Ind. https://doi.org/10.1038/s41598-018-23013-1

Gurevich A, Saveliev V, Vyahhi N, Tesler G (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075

Han HM, Li XS, Geng JZ, Xiao YX, Wu DP, Li M (2019) Research status and prospects of Huangjiu brewing raw materials and production process. Biot Resour 41:87–93

Hazelwood LA, Daran JM, van Maris AJA, Pronk JT, Dickinson JR (2008) The ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. Appl Environ Microb 74:2259–2266

Hill TCJ, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. Fems Microbiol Ecol 43:1–11

Hu K, Jin GJ, Xu YH, Xue SJ, Qiao SJ, Teng YX, Tao YS (2019) Enhancing wine ester biosynthesis in mixed *Hanseniaspora uvarum*/*Saccharomyces cerevisiae* fermentation by nitrogen nutrient addition. Food Res Int 123:559–566

Huang GD, Peng JW, Zhong XF, Shangguan GL, Guo YM, Huang WL, Liang QC (2017) Main higher alcohol contents determination and aroma contribution analysis of semi-dry Shaoxing rice wine. CHN BRW. https://doi.org/10.1016/j.foodres.2020.109238

Huang ZR, Hong JL, Xu JX, Li L, Guo WL, Pan YY, Chen SJ, Bai WD, Rao PF, Ni L et al (2018) Exploring core functional microbiota responsible for the production of volatile flavour during the traditional brewing of Wuyi Hong Qu glutinous rice wine. Food Microbiol 76:487–496

Hui YH (2006) Handbook of food science, technology, and engineering, vol 149. CRC Press, Boca Raton

Hwang JY, Park J, Seo JH, Cha M, Cho BK, Kim J, Kim BG (2009) Simultaneous synthesis of 2-phenylethanol and L-homophenylalanine using aromatic transaminase with yeast ehrlich pathway. Biotechnol Bioeng 102:1323–1329

Jagadeesan B, Gerner-Smidt P, Allard MW, Leuillet S, Winkler A, Xiao YH, Chaffron S, Van der Vossen J, Tang SL, Katase M et al (2019) The use of next generation sequencing for improving food safety: translation into practice. Food Microbiol 79:96–115

Jiang L, Su W, Mu Y, Mu Y (2020a) Major metabolites and microbial community of fermented black glutinous rice wine with different starters. Front Microbiol 11:593

Jiang S, Wang H, He YH, Chen YF, Deng QB et al (2020) Effect of *Saccharomyces cerevisiae* overexpressing acetaldehyde dehydrogenase genes on reducing higher alcohols in Huangjiu. China Brew 39:153–159

Jiao A, Xu X, Jin Z (2017) Research progress on the brewing techniques of new-type rice wine. Food Chem 215:508–515

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glockner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. https://doi.org/10.1093/nar/gks808

Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM et al (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277

Krishnan A, McNeil BA, Stuart DT (2020) Biosynthesis of fatty alcohols in engineered microbial cell factories: advances and limitations. Front Bioeng Biotechnol 8:610936

Lambrechts M, Pretorius I (2000) Yeast and its importance to wine aroma-a review. S Afr J Enol Vitic 21:97–129

Lei H, Li H, Mo F, Zheng L, Zhao H, Zhao M (2013) Effects of Lys and His supplementations on the regulation of nitrogen metabolism in lager yeast. Appl Microbiol Biotechnol 97:8913–8921

Li H, Opgenorth PH, Wernick DG, Rogers S, Wu TY, Higashide W, Malati P, Huo YX, Cho KM, Liao JC (2012a) Integrated electromicrobial conversion of $CO_2$ to higher Alcohols. Science 335:1596–1596

Li SS, Jia XQ, Wen JP (2012b) Improved 2-methyl-1-propanol production in an engineered *Bacillus subtilis* by constructing inducible pathways. Biotechnol Lett 34:2253–2258

Li W, Chen SJ, Wang JH, Zhang CY, Shi Y, Guo XW, Chen YF, Xiao DG (2018a) Genetic engineering to alter carbon flux for various higher alcohol productions by *Saccharomyces cerevisiae* for Chinese Baijiu fermentation. Appl Microbiol Biot 102:1783–1795

Li W, Cui DY, Wang JH, Liu XE, Xu J, Zhou Z, Zhang CY, Chen YF, Xiao DG (2018b) Overexpression of different alcohol acetyltransferase genes with BAT2 deletion in *Saccharomyces cerevisiae* affects acetate esters and higher alcohols. Eur Food Res Technol 244:555–564

Liang Z, Lin X, He Z, Li W, Ren X, Lin X (2020) Dynamic changes of total acid and bacterial communities during the traditional fermentation of Hong Qu glutinous rice wine. Electron J Biotechnol 43:23–31

Lilly M, Bauer FF, Styger G, Lambrechts MG, Pretorius IS (2006) The effect of increased branched-chain amino acid transaminase activity in yeast on the production of higher alcohols and on the flavour profiles of wine and distillates. Fems Yeast Res 6:726–743

Liu SQ (2015) Impact of yeast and bacteria on beer appearance and flavour. In: Hill AE (ed) Brewing microbiology. Woodhead Publishing, Sawston, pp 357–374

Liu AQ, Tan XM, Yao L, Lu XF (2013) Fatty alcohol production in engineered *E. coli* expressing Marinobacter fatty acyl-CoA reductases. Appl Microbiol Biot 97:7061–7071

Liu R, Zhu FY, Lu L, Fu AS, Lu JK, Deng ZX, Liu TG (2014) Metabolic engineering of fatty acyl-ACP reductase-dependent pathway to improve fatty alcohol production in *Escherichia coli*. Metab Eng 22:10–21

Liu SP, Mao J, Liu YY, Meng XY, Ji ZW, Zhou ZL, Ai-lati A (2015) Bacterial succession and the dynamics of volatile compounds during the fermentation of Chinese rice wine from Shaoxing region. World J Microbiol Biotechnol 31:1907–1921

Liu ZB, Wang ZY, Lv XC, Zhu XP, Chen LL, Ni L (2018) Comparison study of the volatile profiles and microbial communities of Wuyi Qu and Gutian Qu, two major types of traditional fermentation starters of Hong Qu glutinous rice wine. Food Microbiol 69:105–115

Liu SP, Chen QL, Zou HJ, Yu YJ, Zhou ZL, Mao J, Zhang S (2019) A metagenomic analysis of the relationship between microorganisms and flavor development in Shaoxing mechanized huangjiu fermentation mashes. Int J Food Microbiol 303:9–18

Liu SP, Hu J, Xu YZ, Xue JB, Zhou JD, Han X, Ji ZW, Mao J (2020) Combined use of single molecule real-time DNA sequencing technology and culture-dependent methods to analyze the functional microorganisms in inoculated raw wheat Qu. Food Res Int. https://doi.org/10.1016/j.foodres.2020.109062

Liu ZB, Wang ZY, Sun JY, Ni L (2020) The dynamics of volatile compounds and their correlation with the microbial succession during the traditional solid-state fermentation of Gutian Hong Qu glutinous rice wine. Food Microbiol. https://doi.org/10.1016/j.fm.2019.103347

Liu SP, Ma DL, Li ZH, Sun HL, Mao JQ, Shi Y, Han X, Zhou ZL, Mao J (2021) Assimilable nitrogen reduces the higher alcohols content of huangjiu. Food Control. https://doi.org/10.1016/j.foodcont.2020.107660

Lu JK, Liu Y, Guo DY, Deng ZX, Liu TG (2016) Production of fatty alcohol in *Komagataella pastoris* by heterologous expression of fatty acyl-CoA reductase. Microbiol China 43:1181–1189

Ma LJ, Huang SY, Du LP, Tang P, Xiao DG (2017) Reduced production of higher alcohols by *Saccharomyces cerevisiae* in red wine fermentation by simultaneously overexpressing BAT1 and deleting BAT2. J Agr Food Chem 65:6936–6942

Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963

Murphree H, Greenberg L, Carroll R (1967) Neuropharmacological effects of substances other than ethanol in alcoholic beverages. Federation Proceedings, Bethesda

Peralta-Yahya PP, Zhang FZ, del Cardayre SB, Keasling JD (2012) Microbial engineering for the production of advanced biofuels. Nature 488:320–328

Pietruszka M, Pielech-Przybylska K, Szopa J (2010) Synthesis of higher alcohols during alcoholic fermentation of rye mashes. Lodz University of Technology, Łódź

Pires EJ, Teixeira JA, Branyik T, Vicente AA (2014) Yeast: the soul of beer's aroma-a review of flavour-active esters and higher alcohols produced by the brewing yeast. Appl Microbiol Biot 98:1937–1949

Ren G, Ren WJ, Teng Y, Li ZG (2015) Evident bacterial community changes but only slight degradation when polluted with pyrene in a red soil. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00022

Ren Q, Sun LP, Sun ZB, Liu QS, Lu X, Li ZP, Xu JL (2020) Bacterial succession and the dynamics of flavor compounds in the Huangjiu fermented from corn. Arch Microbiol 202:299–308

Rizzo WB, Craft DA, Dammann AL, Phillips MW (1987) Fatty alcohol metabolism in cultured human fibroblasts. Evidence for a fatty alcohol cycle. J Biol Chem 262:17412–17419

Rodriguez VM, Padilla G, Malvar RA, Kallenbach M, Santiago R, Butron A (2018) Maize stem response to long-term attack by *Sesamia nonagrioides*. Front Plant Sci. https://doi.org/10.3389/fpls.2018.00522

Scognamiglio J, Jones L, Letizia CS, Api AM (2012) Fragrance material review on 2-phenoxyethanol. Food Chem Toxicol 50:S244–S255

Sun JX (2012) Methods for isoamyl alcohol control in wine. Mod Food Sci Techno 28:1541–1544

Sun ZG, Xiao DG (2018) Review in metabolic modulation of higher alcohols in top-fermenting yeast. Lect Notes Electr En 444:767–773

Sun JY, Yin ZT, Zhao DR, Sun BG, Zheng FP (2018) Qualitative and quantitative research of propyl lactate in brewed alcoholic beverages. Int J Food Prop 21:1351–1361

Sun ZG, Wang MQ, Wang YP, Xing S, Hong KQ, Chen YF, Guo XW, Xiao DG (2019) Identification by comparative transcriptomics of core regulatory genes for higher alcohol production in a top-fermenting yeast at different temperatures in beer fermentation. Appl Microbiol Biot 103:4917–4929

Teufel R, Mascaraque V, Ismail W, Voss M, Perera J, Eisenreich W, Haehnel W, Fuchs G (2010) Bacterial phenylalanine and phenylacetate catabolic pathway revealed. P Natl Acad Sci USA 107:14390–14395

Tian Y, Wang YQ, Wang QY (2013) Research progress of isobutanol biosynthesis. Biot Bull 1:40–44

Tian XM, Zhang SY, Zheng RX, Ming HM, Wei CH, Xia Y (2017) Analysis of headache and dizziness effect after drinking Baijiu and research of the mitigation measure. CHN BRW 36:10–13

Tian S, Zeng W, Fang F, Zhou J, Du G (2022) The microbiome of Chinese rice wine (Huangjiu). Curr Res Food Sci 5:325–335

Wang ZM, Lu ZM, Shi JS, Xu ZH (2016) Exploring flavour-producing core microbiota in multispecies solid-state fermentation of traditional Chinese vinegar. Sci Rep. https://doi.org/10.1038/srep26818

Wang P, Wu Q, Jiang XJ, Wang ZQ, Tang JL, Xu Y (2017) *Bacillus licheniformis* affects the microbial community and metabolic profile in the spontaneous fermentation of Daqu starter for Chinese liquor making. Int J Food Microbiol 250:59–67

Wang Z, Wang S, Liao P, Chen L, Sun J, Sun B, Zhao D, Wang B, Li H (2022) HS-SPME combined with GC-MS/O to analyze the flavor of strong aroma Baijiu Daqu. Foods. https://doi.org/10.3390/foods11010116

Xu E, Long J, Wu Z, Li H, Wang F, Xu X, Jin Z, Jiao A (2015) Characterization of volatile flavor compounds in Chinese rice wine fermented from enzymatic extruded rice. J Food Sci 80:C1476-1489

Yang YJ, Hu WY, Xia YJ, Mu ZY, Tao LR, Song X, Zhang H, Ni B, Ai LZ (2020) Flavor formation in Chinese rice wine (Huangjiu): impacts of the flavor-active microorganisms, raw materials, and fermentation technology. Front Microbiol. https://doi.org/10.3389/fmicb.2020.580247

Yu XH, Chen HX, Wang ZJ, Fang WM (2006) Research progress in influencing factors of higher alcohols metabolic by product of beer yeast. Liquor Mak Sci Techno 11:78–81

Zhang B, Kong LQ, Cao Y, Xie GF, Guan ZB, Lu J (2012) Metaproteomic characterisation of a Shaoxing rice wine "wheat Qu" extract. Food Chem 134:387–391

Zhang CY, Qi YN, Ma HX, Li W, Dai LH, Xiao DG (2015) Decreased production of higher alcohols by *Saccharomyces cerevisiae* for Chinese rice wine fermentation by deletion of Bat aminotransferases. J Ind Microbiol Biotechnol 42:617–625

Zhao N, Zhang YZ, Liu D, Zhang J, Qi YM, Xu JN, Wei XY, Fan MT (2020a) Free and bound volatile compounds in 'Hayward' and 'Hort16A' kiwifruit and their wines. Eur Food Res Technol 5:1–16

Zhao X, Wang Y, Cai W, Yang M, Zhong X, Guo Z, Shan C (2020b) High-throughput sequencing-based analysis of microbial diversity in rice wine koji from different areas. Curr Microbiol 77:882–889

## Publisher's Note