Emirates Journal of Food and Agriculture. 2023. 35(3): 189-196
doi: 10.9755/ejfa.2023.v35.i3.3039
http://www.ejfa.me/

RESEARCH ARTICLE

Analysis of microbial diversity in Muscat Hamburg wine and Shine-Muscat wine

Yao Shumin*, Zhang Mengqi, Yu Qike, Liu Yuxin, Ma Shiqiang, Zhao Shengnan, Wu Qiuyu, Ma Daikun

School of Life Sciences, Qufu Normal University, Qu Fu 273165, Shandong, China

ABSTRACT

Different microbes in grapes lead to different flavors of wine. There are many kinds of wine grapes. In this article, the microorganisms in the wine made by the Muscat Hamburg grape and the Shine-Muscat grape are analyzed by using high-throughput sequencing technology. The results indicate that there are 8 species of dominant bacteria phylum which are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Epsilonbacteraeota, Fusobacteria and Chloroflexi, and 13 species of dominant bacteria genus in Muscat Hamburg wine. There are 7 species of dominant bacteria phylum which are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Eps ilonbacteraeota, Fusobacteria and 14 species of dominant bacteria genus in Shine-Muscat wine. The dominant fungi phylum of the two varieties of wine are Ascomycota, Basidiomycota and Zygomycota. There are 2 species of dominant fungi genus in Muscat Hamburg wine and 9 species of dominant fungi genus in Shine-Muscat wine. At the genus level, bacteria are more abundant than fungi in the dominant genera of microorganisms in these two varieties of wine, and the diversity at the genus level was higher than that at the phylum level.

Keywords: Bacteria diversity; Fungi diversity; High-throughput sequencing technology; Wine grape

INTRODUCTION

Wine in China has a huge consumer market, in terms of wine consumption, China is currently the world's fifth-largest wine market (Kim, 2020). Wine is generally produced by fermentation of wine grapes and their epidermis microorganisms. Its flavor is influenced by a variety of factors such as vineyard management, soil type, light, precipitation, grape microbiome and so on (Angela et al., 2010; Wei et al., 2018; Xu et al., 2019; Tempere et al., 2018). The grape microbiota which affects the health of the vine, wine fermentation and flavor, aroma and quality of the finished wine is complex (Angela et al., 2010) and includes filamentous fungi, yeast,bacteria and so on (Liu et al., 2020). Among them, the yeast is responsible for spontaneous alcoholic fermentation (Eder et al., 2019). The challenges and opportunities presented by the contribution of the wine microbiota to the production of high-quality wine are staggering (Belda et al., 2017).

In recent years, the research on epidermal microorganisms of wine grapes has been increasing. In China, Zhang et al. (2017) analyzed the microbial community of wine grape epidermis of different varieties in Shacheng area and concluded that grape variety is the most important factor affecting the microbial community. Zhang et al. (2017) analyzed the diversity of epidermal bacteria of summer black seedless grape, which provided some reference for biological control of fruits and vegetables. Zhao et al. (2021) studied the diversity of Cabernet Sauvignon grapes and found that there were differences in the dominant microorganisms of wine grapes from different producing areas and the differences of genus level were higher than those of phylum level. In addition, Zhang et al. (2021), Zhang et al. (2021), Ding et al. (2021) and others also conducted research on grape epidermal microorganisms. Abroad, Ghosh et al. (2015) studied the microbial diversity during the early stage of alcohol fermentation on grapes from South African vineyards, and the results showed that ARISA can be used to study the microbial diversity and dynamic changes during grape fermentation. Ranade et al. (2021) carried out an analysis of the diversity of epidermal grape microorganisms, providing new information on the applicability of these microorganisms to plant growth, crop protection and bioremediation. In addition, many researchers such as Setati et al. (2012) also investigated the microbial diversity of the grape skin.

***Corresponding author:**
Yao Shumin, School of Life Sciences, Qufu Normal University, Qu Fu 273165, Shandong, China. **E-mail:** Yaoshumin299@163.com

Received: 29 August 2022; **Accepted:** 15 February 2023

Although the research on the microorganisms of wine grapes has gradually increased, there are relatively few studies on the microbial communities in wine made from different varieties of grapes. Therefore, this article takes the wine made from two different varieties of grapes, Muscat Hamburg wine and Shine-Muscat wine, as the research object to study the differences in microbial communities in wine made from different varieties of grapes.

MATERIALS AND METHODS

Main reagents and instruments

The main reagents include QIAGEN's DNeasy PowerSoil Kit, Takara's Tks Gflex DNA Polymerase, Gene Tools, TENP buffer and PCR amplification system, etc.

The main instruments include Eppendorf's desktop high-speed centrifuge and pipettes, Bio-rad's PCR machines, Tanon's electrophoresis and gel imager, Axygen's centrifuge tubes, etc.

Experimental samples description

The experimental samples come from a large-scale winery in Qufu City, Shandong Province (35° 59' latitudes and 116° 98' longitudes). The study samples are collected from wine made from grapes of two varieties, Muscat Hamburg (M) and Shine-Muscat (Y). Among them, the samples of each variety of wine were randomly selected, namely M1, M2, M3 and Y1, Y2, Y3.

Genomic DNA extraction

Genomic DNA is extracted by DNA extraction kit, and then using agarose gel electrophoresis and NanoDrop2000 to detect the concentration of DNA. The DNA extraction and high-throughput sequencing process was performed by OEBIOTECH.

PCR amplification of bacterial 16S rRNA and fungal ITS

The first round of PCR amplification is performed using Takara's Tks Gflex DNA Polymerase to ensure efficiency and accuracy. Bacterial 16s rRNA gene specific primers 347F (5 '- CCTACGGRRBGCASCAGKVRVGAAT - 3') and 802R(5 '- GGACTACNVGGGTWTCTAATCC - 3') are used to amplify V3 - V4 region. The ITS region is amplified by fungi ITS 1F(5 '-CTTGGtCATTTagagGaAGtaA-3') and ITS 2R(5 '-GCTGCGTTCTTCATCGATGC-3'). After 26 cycles of amplification, the PCR product is obtained and then the quality is checked. The products qualified in the first round of PCR amplification are amplified in the second round, and the amplification cycles are seven times.

Gene Tools are used to compare the concentration of each PCR product (ng/μL), and then PCR products of each group are mixed according to the principle of equal quality. Then the SanPrep column DNA gel recovery kit is used to recover PCR mixed products. Finally, the Illumina MiSeq sequencing platform is commissioned to conduct high-throughput sequencing analysis of PCR products.

Data processing and analysis

(i) Remove the sequences whose length is less than 50bp, splice the obtained paired sequences, and remove the low-quality sequences to obtain the clearance tag sequence. Then the optimal sequence with high quality and reliability is obtained by processing the cleared tag sequence(Bolger et al., 2014; Magoč et al., 2011; Caporaso et al.,2010; Edgar et al., 2011)

(ii) QIIME software is used to cluster Tags information at 97% similarity level to obtain OTU composition in each sample at each classification level(Bowen et al., 2012; Wang et al., 2018; Liu et al., 2019).

(iii) According to the comparison results of OTU and Silva databases for each sample, species annotation is performed to obtain community structure composition and abundance information at each classification level.

(iv) R software is used to calculate and analyze the diversity index.

RESULTS

Sample sequencing information

Through the Illumina MiSeq sequencing analysis of the microorganisms in the wine made by Muscat Hamburg grape and Shine-Muscat grape, 328299 original sequences of bacterial 16S rRNA gene V3−V4 regions are obtained. After optimizing the data, 237,842 valid sequences are obtained, and the valid sequences account for 72.45%. OTU clustering is carried out at 97% similarity level, and 26248 valid OTUs are obtained. 380,785 original sequences are obtained from fungal ITS region. After optimizing the data, 371155 valid sequences are obtained, and the valid sequences account for 97.47%. OTU clustering is carried out at 97% similarity level, and the valid OTUs are 8422. Detailed data are shown in the Table 1.

Analysis of OTU in samples

A total of 11097 bacteria OTU are detected in both wine (Fig. 1a). A total of 8521 bacteria OTU are detected in Muscat Hamburg wine, of which 3304 are unique bacteria OTU. In Shine-Muscat wine, there are 7793 bacteria OTU, and 2576 bacteria OTU are unique. There are 5271 bacteria OTU shared by the two varieties of wine.

At the same time, a total of 3711 fungi OTU are detected in both wine (Fig. 1b). A total of 3082 fungi OTU are detected in Muscat Hamburg wine, of which 1114 are unique fungi

Table 1: Sequencing information of two grape samples

Sample	Raw data sequence		Valid tags sequence		Percentage of valid tags sequence		OTU_counts	
	bacteria	fungi	bacteria	fungi	bacteria	fungi	bacteria	fungi
M1	41873	65375	28505	64165	68.07%	98.15%	4942	2376
M2	66605	71017	49434	70653	74.22%	99.49%	4357	537
M3	41155	69148	28277	68089	68.71%	98.47%	4898	2035
Y1	44545	59607	30736	53916	69.00%	90.45%	5008	2253
Y2	62885	60655	43903	59929	69.81%	98.80%	3544	831
Y3	71236	54983	56987	54403	80.00%	98.95%	3499	390

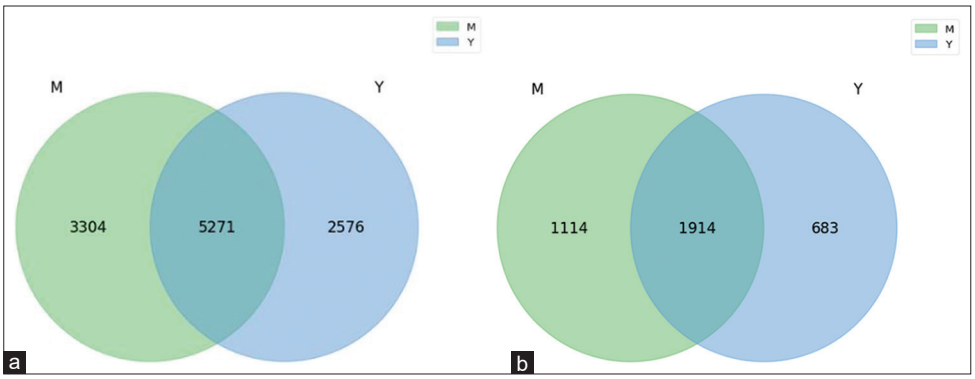

Fig 1. OTU numbers of bacteria (a) and fungi (b). Different colors represent the samples of different varieties of wine. The overlapping parts represent the common OTUs.

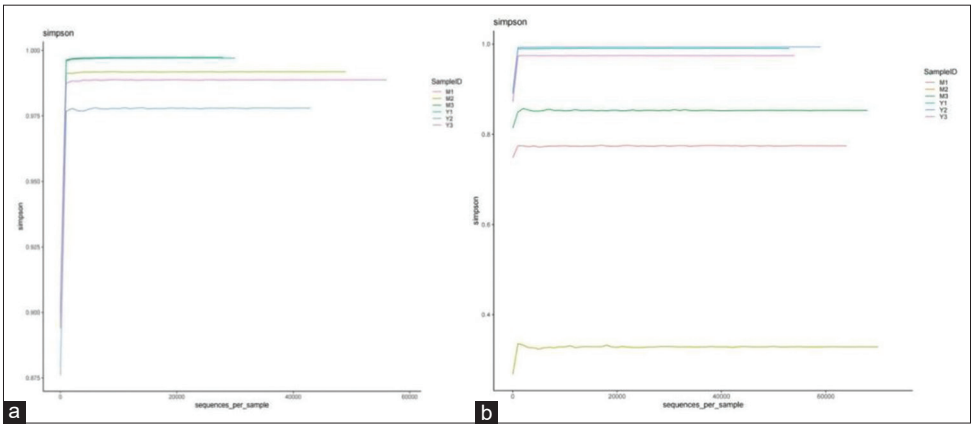

Fig 2. Rarefaction curves of bacteria (a) and fungi (b). The curves begin to flatten, indicating that the sample depth in this study is sufficient. Different colors represent the samples of different varieties of wine.

OTU. In Shine-Muscat wine, there are 2597 fungi OTU, and 683 fungi OTU are unique. There are 1914 fungi OTU shared by the two varieties of wine.

As shown, in the two varieties of wine microbial flora, the total number and unique number of bacteria OTU are more than those of fungi. The total number of OTUs of bacteria and fungi in Muscat Hamburg wine is higher than that in Shine-Muscat wine.

Alpha diversity analysis

Rarefaction curve

The rarefaction curve can be used to compare the abundance of different sample species and indicate whether the amount of sequencing data of the sample is reasonable(Zhao et al., 2021). When the curve tends to be flat, it means that the amount of sequencing data is reasonable. The curves of bacterial 16s rRNA gene and fungal ITS region in the two varieties of wine under the condition of 97% similarity show that when the bacterial Simpson diversity index reaches above 0.915 and the fungi Simpson index reaches a certain value, the curves begin to flatten, indicating that the sample depth in this study is sufficient.

Sample alpha diversity index

Coverage refers to the coverage of each sample library. The higher the value is, the higher the probability that the

sequence in the sample is detected, while the probability that the sequence is not detected is lower. Shannon and Simpson indices are commonly used to reflect the a-diversity of microorganisms(Wang et al., 2020). The larger Shannon value is, the higher community diversity is. For Simpson diversity index, we use its formula here: $D=1-\Sigma Pi2$. As can be seen from the formula that the larger the Simpson value, the higher community diversity. Chao1 is the species richness index, and the larger the value is, the higher the richness is.

The microbial flora of the two varieties of wine are analyzed under 97% similarity(Table 2). The Coverage index of bacterial coverage of the samples reaches 98%, indicating that the measured OTU number could basically reflect the real situation of bacterial samples in the two varieties of wine. The bacterial diversity index in the two varieties of wine shows that the largest chao1 index is Muscat Hamburg wine, indicating that the bacteria richness in Muscat Hamburg wine is higher than Shine-Muscat wine. The highest Shannon index is found in Muscat Hamburg wine, indicating a high diversity of bacteria in Muscat Hamburg wine. In a word, the richness and diversity of bacteria in Muscat Hamburg wine are higher than those in Shine-Muscat wine.

The Coverage index of fungal coverage of the samples reaches 99%, indicating that the measured OTU number could basically reflect the real situation of fungal samples in the two varieties of wine. As the fungal diversity index shows, the Chao1 index of the Muscat Hamburg wine is higher, indicating that the fungal richness of the Muscat Hamburg wine was higher than that of the Shine-Muscat wine. The higher Shannon and Simpson indices are found in Shine-Muscat wine, indicating the high fungal diversity in Shine-Muscat wine.

In conclusion, the bacterial richness and diversity of Muscat Hamburg wine are higher than those of Shine-Muscat wine, while the fungus richness and diversity of Muscat Hamburg wine are higher and lower than those of Shine-Muscat wine.

Sample beta diversity analysis

PCA is performed on the bacterial flora in the two varieties of wine and the PCA chart is drawn. In PCA chart, the closer the samples are to each other, the more similar they are. M1 and M3 are distributed in the second quadrant, indicating that the bacteria in M1 and M3 are more similar. Y2 and Y3 are distributed in the fourth quadrant, indicating that the bacteria are more similar in Y2 and Y3. Y1 and M2 are distributed in the first and third quadrants respectively, indicating that the bacteria in Y1 and M2 are quite different (Fig. 3a). Through PCA chart, PC1 and PC2 are two characteristic values that cause sample differences. It can be seen that PC1 and PC2 contribute 28.14% and 18.85% to the sample differences, which are the sources of bacterial differences.

It can be seen from the PCA chart(Fig. 3b), M1 and M3 are distributed in the second quadrant, indicating that the fungi in M1 and M3 are more similar. M2, Y2 and Y3 are distributed in the third quadrant, indicating that the fungi are more similar in M2,Y2 and Y3. Y1 is distributed in the fourth quadrants, indicating that the fungi in Y1 is quite different from the other five samples. It can be seen that PC1 and PC2 contribute 34.82% and 21.21% to the sample differences, which are the sources of fungal differences.

Community structure and correlation analysis

Analysis of community structure based on phylum level

At the phylum level, high-throughput analysis of the bacterial flora is performed. Analysis of the microbial community structure shows that there are 8 species of dominant bacteria phylum which are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Epsilonbacteraeota, Fusobacteria and Chloroflexi in Muscat Hamburg wine. There are 7 species of dominant bacteria phylum which are Bacteroidetes, Firmicutes, Pr oteobacteria, Actinobacteria, Acidobacteria, Epsilonbact eraeota and Fusobacteria in Shine-Muscat wine (Fig. 4a).

High-throughput analysis of fungal flora is performed. Analysis of the microbial community structure shows that the dominant fungi phylum of the two varieties of wine are Ascomycota, Basidiomycota and Zygomycota (Fig. 4b).

Analysis of community structure based on genus level

At the genus level, high-throughput analysis of the bacterial flora is performed. Analysis of the microbial community structure shows that there are 13 species of dominant bacteria genus in Muscat Hamburg wine and 14 species of dominant bacteria genus in Shine-Muscat wine. There were 6 species of dominant bacteria flora which are *Bacte roides*, *Lactobacillus*, *Lachnospiraceae*_NK4A136_group, *Stre*

Table 2: Microbial diversity index of the two varieties of wine

Sample	Coverage		Chao1		Shannon		Simpson		Observed-species	
	bacteria	fungi	bacteria	fungi	bacteria	fungi	bacteria	fungi	bacteria	fungi
M	0.98	0.99	4940.16	1829.91	9.56	4.62	0.99	0.65	4349.10	1611.37
Y	0.99	1.00	3983.31	844.52	9.29	8.33	0.99	0.99	3398.00	829.70

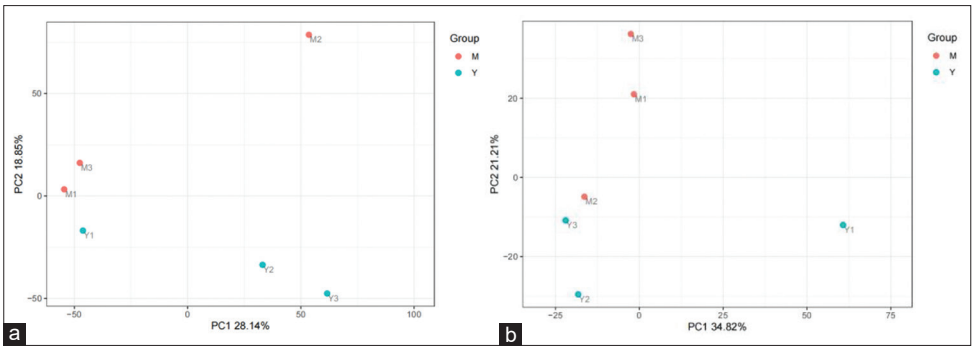

Fig 3. Comparison of microbial community in different samples. (a) Bacteria, (b) Fungi. PCA analysis uses variance decomposition to reflect the differences between multiple data sets on a two-dimensional coordinate plot. Different colors represent the samples of different varieties of wine.

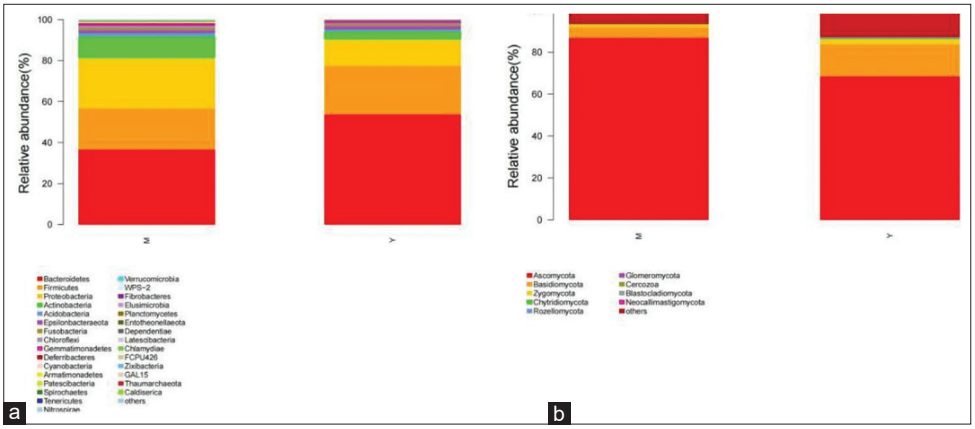

Fig 4. Community structure analysis diagram at phylum level of bacteria (a) and fungi (b). Bars indicate samples, different colors indicate different annotation information, and others indicate all species except Top. There are 8 species of dominant bacteria phylum which are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Epsilonbacteraeota, Fusobacteria and Chloroflexi in Muscat Hamburg wine. There are 7 species of dominant bacteria phylum which are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Epsilonbacteraeota and Fusobacteria in Shine-Muscat wine. The dominant fungi phylum of the two varieties of wine are Ascomycota, Basidiomycota and Zygomycota.

ptococcus, *Alloprevotella* and *Escherichia-Shigella* shared by the two varieties of wine at the genus level (Fig. 5a).

High-throughput analysis of the fungal flora is performed. Analysis of the microbial community structure shows that there are 2 species of dominant fungi genus in Muscat Hamburg wine and 9 species of dominant fungi genus in Shine-Muscat wine. There are 2 species of dominant fungal flora which are *Fusarium* and *Monographella* shared by the two varieties of wine at the genus level (Fig. 5b).

Heatmap diagram

Based on the Spearman rank correlation coefficient, a heatmap is used to analyze the genus correlation between bacteria and fungi. As can be shown that there are correlations between the different genera of the bacterial flora, and there are significant positive correlations among the three genera of *Alipis*, *Ruminococcaceae*-UCG-014 and *Rikenellaceae*_RC9_gut_group. There is also a significant positive correlation between *Prevotella* 9 and *Escherichia-Shigella* (Fig. 6a). As for fungi, there is a significant positive correlation between *Hygrocybe* and *Phaeomycocentrospora* (Fig. 6b).

DISCUSSION

Differences in the microbial dominant flora between two varieties of wine

The results indicate that there are 8 species of dominant bacteria phylum in Muscat Hamburg wine and 7 species of dominant bacteria phylum in Shine-Muscat wine. There are 7 species of dominant bacteria flora which are Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Epsilonba cteraeota and Fusobacteria shared by the two varieties of wine at the phylum level. Among them, the Bacteroides account for the highest proportion, which account for 53.62% of Shine-Muscat wine and 36.43% of Muscat Hamburg wine. The unique dominant bacterial phylum in Muscat Hamburg wine is Chloroflexi. In these two varieties of wine, the dominant fungal phylum are Ascomycota, Basidiomycota and Zygomycota, which are consistent with the research results of (Zhang et al., 2017). The highest proportion of these three phyla is Ascomycota, which accounts for 86.74% of Muscat Hamburg wine and 68.36% of Shine-Muscat wine.

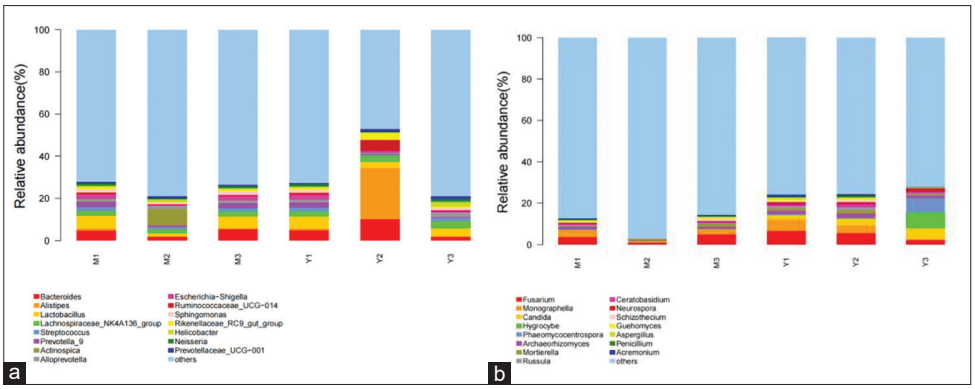

Fig 5. Community structure analysis diagram at genus level of bacteria (a) and fungi (b). Bars indicate samples, different colors indicate different annotation information, and others indicate all species except Top. Species annotation is based on comparative results of OTU and Silva database for each sample to obtain community structure composition at each classification level.

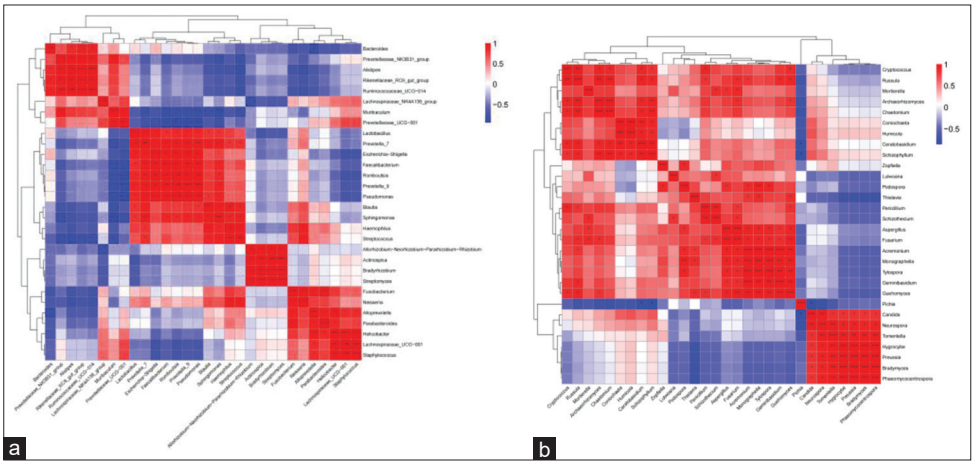

Fig 6. Microbial correlations at the genus level of bacteria (a) and fungi (b). Red is positive correlation and blue is negative correlation. The color shades indicate the level of correlation.

The results indicate that there are 13 species of dominant bacteria genus in Muscat Hamburg wine and 14 species of dominant bacteria genus in Shine-Muscat wine. There are 6 species of dominant bacteria flora which are *Bacteroides*, *Lactobacillus*, *Lachnospiraceae*_NK4A136_ group, *Streptococcus, Alloprevotella* and *Escherichia-Shigella* shared by the two varieties of wine at the genus level. The highest proportion of these 6 genera is *Lactobacillus*, which accounts for 3.68% of Muscat Hamburg wine and 3.89% of Shine-Muscat wine.

The results indicate there are 2 species of dominant fungi genus in Muscat Hamburg wine and 9 species of dominant fungi genus in Shine-Muscat wine. There are 2 species of dominant fungal flora which are *Fusarium* and *Monographella* shared by the two varieties of wine at the genus level. The highest proportion of these 2 genera is *Fusarium*, which accounts for 3.05% of Muscat Hamburg wine and 4.79% of Shine-Muscat wine. In addition, the unique dominant bacteria genera of Shine-Muscat wine from high to low are *Candida*, *Hygrocybe*, *Phaeomycocentrospora* and *Archaeorhizomyces*.

Effects of microbiome on wine taste

The unique dominant bacterial phylum in Muscat Hamburg wine is Chloroflexi which is a thermophilic bacteria and one of the most abundant groups in anaerobic reactors. It can oxidize hydrogen and carbon monoxide to provide energy and plays an important role in the decomposition of organic matter (Mendes et al., 2015; Kadnikov et al., 2021; Bovio-Winkler et al., 2021). Bacteroidetes which are strictly anaerobic bacteria exist widely in anaerobic reactors. They are mainly related to substrate hydrolysis and acid production, and may also be related to terpenoid synthesis (Zhang et al., 2021; Fu et al., 2021).

The Ascomycota and Basidiomycota microbes in both wine are the dominant species in the soil and can decompose most organic matter and plant debris(Hou et al., 2019).

The highest proportion of dominant bacteria flora shared by the two varieties of wines at the genus level is *Lactobacillus* which is a probiotic genus. Lactic acid bacteria is one of the representative bacteria of *Lactobacillus*. It is

used in fermentation can improve food quality, promote the production of phenols, amino acids, polypeptides and other substances (Guo et al., 2021; Sun et al., 2021; Quan et al., 2021). The highest proportion of dominant fungi flora shared by the two varieties of wines at the genus level is *Fusarium*. It is a fruiting contaminating fungus that affects crop yields and can also produce deoxynibutenol (DON), which is potentially harmful to animal and human health (Meng et al., 2021; Chen et al., 2021). Therefore, it may damage the flavor of wine.

CONCLUSION

Through the analysis of microorganisms in Muscat Hamburg wine and Shine-Muscat wine by high-throughput sequencing technology, it can be concluded that:

(i) In terms of OTU differences, the total number and specificity of bacteria are more than that of fungi. The total OTUs of bacteria and fungi in Muscat Hamburg wine was higher than that in Shine-Muscat wine. (ii) In terms of diversity and richness, the bacterial richness and diversity of Muscat Hamburg wine are higher than that of Shine-Muscat wine, while the fungal richness of Muscat Hamburg wine is higher than that of Shine-Muscat wine, but the diversity is lower than that of Shine-Muscat wine. (iii) In terms of community structure and correlation, there are 7 species of dominant bacteria flora which are Bacte roidetes, Firmicutes, Proteobacteria, Actinobacteria, Ac idobacteria, Epsilonbacteraeota and Fusobacteria shared by the two varieties of wine at the phylum level. In these two varieties of wine, the dominant fungal phylum are Ascomycota, Basidiomycota and Zygomycota. There are 6 species of dominant bacteria flora which are *Bacteroides*, *Lactobacillus*, *Lachnospiraceae*_NK4A136_group, *Streptoc occus*, *Alloprevotella* and *Escherichia-Shigella* shared by the two varieties of wine at the genus level and 2 species of dominant fungal flora which are *Fusarium* and *Monographella* shared by the two varieties of wine at the genus level.

ACKNOWLEDGEMENTS

Thank Mrs. Yao for her earnest teaching, encouraging us to explore and innovate, and encouraging us to explore true knowledge. Thank my team members for their help in this experiment. Without them, this experiment will not be completed successfully.

Thanks to the support of Qufu grape breeding base for the experiment, the grapes they provided laid the foundation for the success of the experiment. I sincerely thank all the teachers, classmates and friends who have helped me.

Author contributions

Yao Shumin*; write, review and edited. Zhang Mengqi; Chart arrangement and analysis, write the first draft of the article, methods. Yu Qike;write the first draft of the article, chart analysis. Liu Yuxin;write and edite, methods, sorting table. Ma Shiqiang;write and edite, format modification,sorting table. Zhao Shengnan;write and edite, format modification. Wu Qiuyu; write and edite, format modification. Ma Daikun;write and edite, format modification.

REFERENCES

Belda, I., J. Ruiz, A. Esteban-Fernandez, E. Navascues, D. Marquina, A. Santos and M. V. Moreno-Arribas. 2017. Microbial contribution to wine aroma and its intended use for wine quality improvement. Molecules. 22: 189.

Bolger, A. M., M. Lohse and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 30: 2114-2120.

Bovio-Winkler, P., A. Cabezas and C. Etchebehere. 2021. Database mining to unravel the ecology of the phylum Chloroflexi in methanogenic full scale bioreactors. Front. Microbiol. 11: 603234.

Bowen, J. L., H. G. Morrison, J. E. Hobbie and M. L. Sogin. 2012. Salt marsh sediment diversity: A test of the variability of the rare biosphere among environmental replicates. ISME J. 6: 2014-2023.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7: 335-336.

Ding, Y., R. Wei, Y. Song, H. Li and H. Wang. 2021. Research status of the diversity of natural microbial community and metabolic enzyme system in grape ecosystem. J. Microbiol. China. 48: 2837-2852.

Eder, M. L. R. and A. L. Rosa. 2019. Yeast diversity in Vitis non-vinifera ecosystems. Rev. Argent. Microbiol. 51: 278-283.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27: 2194-2200.

Fu, J. X., L. Chen, S. Z. Yang, Y. Z. Li, L. Jin, X. P. He, L. He, X. L. Ao, S. L. Liu, A. P. Liu, Y. Yang, B. C. Ma, X. W. Cui, S. J. Chen and L. K. Zou. 2021. Metagenome and analysis of metabolic potential of the microbial community in pit mud used for Chinese strong-flavor liquor production. Food Res. Int. 143: 110294.

Ghosh, S., B. Bagheri, H. H. Morgan, B. Divol and M. E. Setati. 2015. Assessment of wine microbial diversity using ARISA and cultivation-based methods. Ann. Microbiol. 65: 1833-1840.

Guo, L., J. Wang, T. Li and X. Bai. 2021. Possible mechanism of action of hyperglycemia caused by partial pancreatectomy on gut floras in a rat model. Chin. Gen. Pract. 24: 4234-4240.

Hou, F., R. Wang, A. Salman, X. Gao and S. Guo. 2021. Variations of soil microbial communities along a valley bottom of the loess plateau and the in-fluencing factors. China Environ. Sci. 39: 4350-4359.

Kadnikov, V. V., A. V. Mardanov, A. V. Beletsky, M. A. Grigoriev, O. V. Karnachuk and N. V. Ravin. 2021. Thermophilic Chloroflexi dominate in the microbial community associated with coal-fire gas vents in the Kuznetsk Coal Basin, Russia. Microorganisms. 9: 948.

Kim, W. 2020. A study on the analysis of the imported wine market in China. J. Chin. Stud. 73: 733-757.

Liu, D., Q. L. Chen, P. Z. Zhang, D. L. Chen and K. S. Howell. 2020. The fungal microbiome is an important component of vineyard ecosystems and correlates with regional distinctiveness of wine. mSphere. 5: e00534-2.

Liu, H. Y., J. J. Wang, W. Zhao, Q. Q. Li, Y. Chen, Z. B. Yi, X. Lyu and Z. Y. Huang. 2019. Structure of soil bacteria community and diversity in cotton field and Euphrates poplar forest in the middle and lower reaches of Tarim river basin. J. Microbiol. China. 46: 2214-2230.

Magoč, T. and S. L. Salzberg. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 27: 2957-2963.

Mendes, L. W., S. M. Tsai, A. A. Navarrete, M. de Hollander, J. A. van Veen and E. E. Kuramae. 2015. Soil-borne microbiome: Linking diversity to function. Microb. Ecol. 70: 255-265.

Meng, J., K. Fan, W. Guo, J. Wang, D. Nie and Z. Han. 2021. Isolation and identification of fungi contamination on surface of yellow peach (*Amygdalus persica* L.). Acta Agric. Shanghai. 37: 57-62.

Quan, Q., W. Liu, M. Zuo and J. Zhang. 2021. Advances in the flavor of fruit and vegetable juices fermented by lactic acid bacteria. Food Ferment. Ind. 1-11.

Ranade, Y., I. Sawant, S. Saha, M. Chandrashekar and P. Pathak. 2021. Epiphytic microbial diversity of *Vitis vinifera* fructosphere: Present status and potential applications. Curr. Microbiol. 78: 1086-1098.

Setati, M. E., D. Jacobson, U. C. Andong and F. F. Bauer. 2012. The vineyard yeast microbiome, a mixed model microbial map. PLoS One. 7: e52609.

Sun, Y., S. Zhang, Z. Liu, X. Hu and J. Yi. 2021. Healthy promoting effects of lactic acid bacteria in fermented foods. Food Ferment. Ind. 47: 280-287.

Tempere, S., A. Marchal, J. C. Barbe, M. Bely, I. Masneuf-Pomarede, P. Marullo and W. Albertin. 2018. The complexity of wine: Clarifying the role of microorganisms. Appl. Microbiol. Biotechnol. 102: 3995-4007.

Wang, D., Q. Lin, M. Otkur, H. M. Yang and K. Lou. 2018. Diversity of airborne bacterial communities in sandstorm area of Taklimakan. Acta Microbiol. Sin. 58: 1420-1430.

Wang, W., B. Jialengbieke, X. Hu, J. Xia, Z. Zhang, M. Gu and Y. Wu. 2020. Analysis of the microbial community diversity of soil from wine grape producting area in Xinjiang based on High-throughput Sequencing. J. Xinjiang Agric. Sci. 57: 859-868.

Wei, Y., W. Zou, W. Ma, Y. Yan, Y. Wu and J. Xue. 2018. Microbial diversity of berries, leaves and soil of grapevine plants grown in Xinjiang analyzed by high-throughput sequencing. J. Food Sci. 39: 162-170.

Xu, W. N., X. M. Liu, X. J. Kong, X. Chen, M. Li and C. L. Geng. 2019. Effect of pesticide application on the community structure of wine yeasts during spontaneous fermentation. J. Microbiol. China. 46: 721-728.

Zhang, C., Y. Li, B. Dong, X. Liu, Y. Bai, D. Cao, J. Liu and S. Li. 2021. Effect of alkali treatment on microbial community structure in solid-stateanaerobic digestion of corn straw. Food Ferment. Ind. 1-8.

Zhang, E., R. Zhao, X. Yin, H. Cai, Y. Lu and Z. Luo. 2021. Analysis of fungal community composition of grape surfaces and rhizosphere Soilin different producing areas in Tibet. J. Sci. Technol. Food Ind. 42: 106-111.

Zhang, J. 2021. Study on Microbial Diversity and Establishment of Bacterial Resource Bank in Wine Region of Helan Mountain in Ningxia, (Master Dissertation). Tibet Agricultural and Animal Husbandry University, Tamil Nadu.

Zhang, J., C. Guo, Y. Shang, X. Yang, J. Chen, W. Zhang and C. LIU. 2017. Study on the microbial diversity on the grape surface of marketed Summer Black Seedless variety. J. Light Ind. 32: 20-26.

Zhang, S., X. Chen, Q. Zhong, Z. Huang, Z. Meng, J. Luo, L. Shi and Z. Bai. 2017. Microbial communities on the wine grape surfaces of different cultivars. Bitechnol. Bull. 33: 128-137.

Zhang, X., R. Fan, S. Luo and Y. Chen. 2021. Advances in the mechanism of deoxynivalenol causing anorexia and vomiting. J. Anim. Sci. 57: 24-31.

Zhao, Y., J. Li, W. Xie and H. Zhao. 2021. Microbial diversity of wine grape skins from four different regions in China. J. Food Ferment. Ind. 47: 53-60.