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## Environmental factors at different scales: a review of their effects on spontaneous fermentation Chinese Baijiu and related mechanisms

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## ABSTRACT

Environment serves as the pivotal medium to produce fermented food, with fluctuations in environmental factors exerting a profound impact on the modulation of fermentation microbial communities. Such shifts are crucial for the distinctiveness of fermented food flavor and the variability in quality. Chinese liquor (Baijiu) is one of the typical representatives of spontaneous fermented food. In this review, the multifaceted relationship between regional environmental attributes and the fermentation dynamics of Baijiu was examined, with a spotlight on the strong-flavor, sauce-flavor, and light-flavor varieties. It reveals the influence of regional environmental factors and brewing environmental factors on microbial function and metabolism, which results in the formation of unique flavor characteristics of Baijiu. The 9 main factors affecting the microecology of Baijiu fermentation were further explored, including environmental sensitivity, microbial interactions, biogeographic patterns, and key abiotic factors such as temperature and humidity. Environmental factor management is crucial for controlling microbial community in fermentation. Intelligent detection of the fermentation system is combined with artificial intelligence to realize the digitalization of Baijiu fermentation, with a view to further studying the environmental mechanism or quantitative control relationship of natural fermentation, improving the environmental stability of natural fermentation, and promoting the mechanization and intelligence of fermentation production.

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## 1. Introduction

In most traditional fermented food production processes, various microorganisms spontaneously colonize and enrich on raw materials under natural conditions, gradually constructing a relatively stable flora structure, and jointly creating a unique ecological environment<sup>[1]</sup>. Among the diverse spectrum of fermented beverages, Chinese Baijiu stands out with its millennia-old heritage, alongside world-famous distilled

spirits such as vodka and whisky<sup>[2]</sup>. The essence of Baijiu lies in its unique spontaneous fermentation driven by a complex and dynamic interplay of microorganisms, which thrive in the solid-state fermentation environment<sup>[3-4]</sup>.

A previous study has reported that microbiota on wine-grape surfaces<sup>[5]</sup> was correlated with environmental factors (geographical factors and specific climate features). Similarly, Baijiu fermentation is also affected by numerous environmental factors, such as temperature<sup>[6]</sup>, geographical factors<sup>[7]</sup>, and environmental microbiota<sup>[8]</sup>. Even the same flavor of Baijiu, produced using the same process in different regions, exhibits distinct characteristics. These variations underscore the crucial role of both the environment and microorganisms in food fermentation,

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presenting technical challenges for the industrial production of spontaneous fermentation to ensure consistent product quality.

This review provides an overview of the relationship between regional environmental factors and the ecology of fermentation systems, examining the impact of these factors on various aspects of the composition and function of microbial communities. The analysis extends to how these factors influence the fermentation of the main flavor types of Baijiu—strong-flavor, sauce-flavor, and light-flavor—and emphasizes the geographical nuances that define Baijiu's identity. It aims to provide a valuable reference and inspiration for the control, mechanization, and automation of spontaneous fermented food production, thereby promoting technological progress and industrial upgrading of the brewing industry.

## 2. Different types of Baijiu and their regional environmental characteristics

Different flavors of Baijiu showed obvious regionalism, and strong-flavor Baijiu (STFB), sauce-flavor Baijiu (SAFB), and light-flavor Baijiu (LFB) comprised the 3 dominating categories<sup>[9]</sup>. These flavor differences are not only influenced by human factors such as local Baijiu-making techniques, raw material selection and microbial communities, but also due to natural factors such as the unique climate and water sources of each producing region.

### 2.1 STFB producing area

Chinese STFB is the most widely distributed, and its typical distilleries are mainly concentrated in the latitude zone of 28°N to 30°N, adjacent to the Yangtze River<sup>[7]</sup>. Among them, the Sichuan and Jianghuai River Basin regions are undoubtedly the most representative brewing areas. The Sichuan region is well known for its warm and humid climate, which tends to produce heavy and rich flavor characteristics of Luzhou-flavor<sup>[10]</sup>. Luzhou, located in the humid subtropical climate zone (105°27'13.80"E, 28°53'6.90"N), its unique geographical and climatic conditions play a crucial role in the brewing of Luzhou-flavor Baijiu. In contrast, the climate in the Jianghuai Valley is cooler, which is more conducive to brewing light and delicate Baijiu<sup>[10]</sup>.

By analyzing the microbial composition of typical STFB environmental samples from different regions, it was found that the dominant bacterial groups were mainly composed of 12 bacterial genera and 13 fungal genera, and the saccharifying (*Aspergillus* and *Rhizopus*) and fermentation microbiota (*Lactobacillus*, *Candida*, and *Pichia*) groups were two particularly prominent groups<sup>[11-12]</sup>. There were also significant differences in microbial composition among regions, such as the enrichment of actinomycetes *Kroppenstedtia* in the Henan province region and yeast *Debaryomyces* in the Sichuan Province region<sup>[13]</sup>.

### 2.2 SAFB producing area

In China, the main producing areas for the famous SAFB include Maotai Town and Xishui Town in Guizhou Province, Erlang Town in Sichuan Province, and Beijing and Tianjin<sup>[7,14-15]</sup>, typically located within a latitude range of 27°N to 28°N<sup>[7]</sup>. Moutai town, located in Guizhou Province (27°81'N, 106°41'E), has relatively unique

climate conditions<sup>[16]</sup> and soil environment<sup>[17]</sup>, which together shape a unique microbial ecology. The bacterial community in Moutai Town showed diversity and stability throughout the different seasons, with 8 dominant classes identified, including Betaproteobacteria (2.06%) and *Flavobacterium* (5.32%), which were identified as dominant bacteria in Baijiu-making environments for the first time. In contrast, Beijing and Tianjin regions have a temperate monsoon climate zone, with hot and rainy summers and cold and dry winters.

### 2.3 LFB producing area

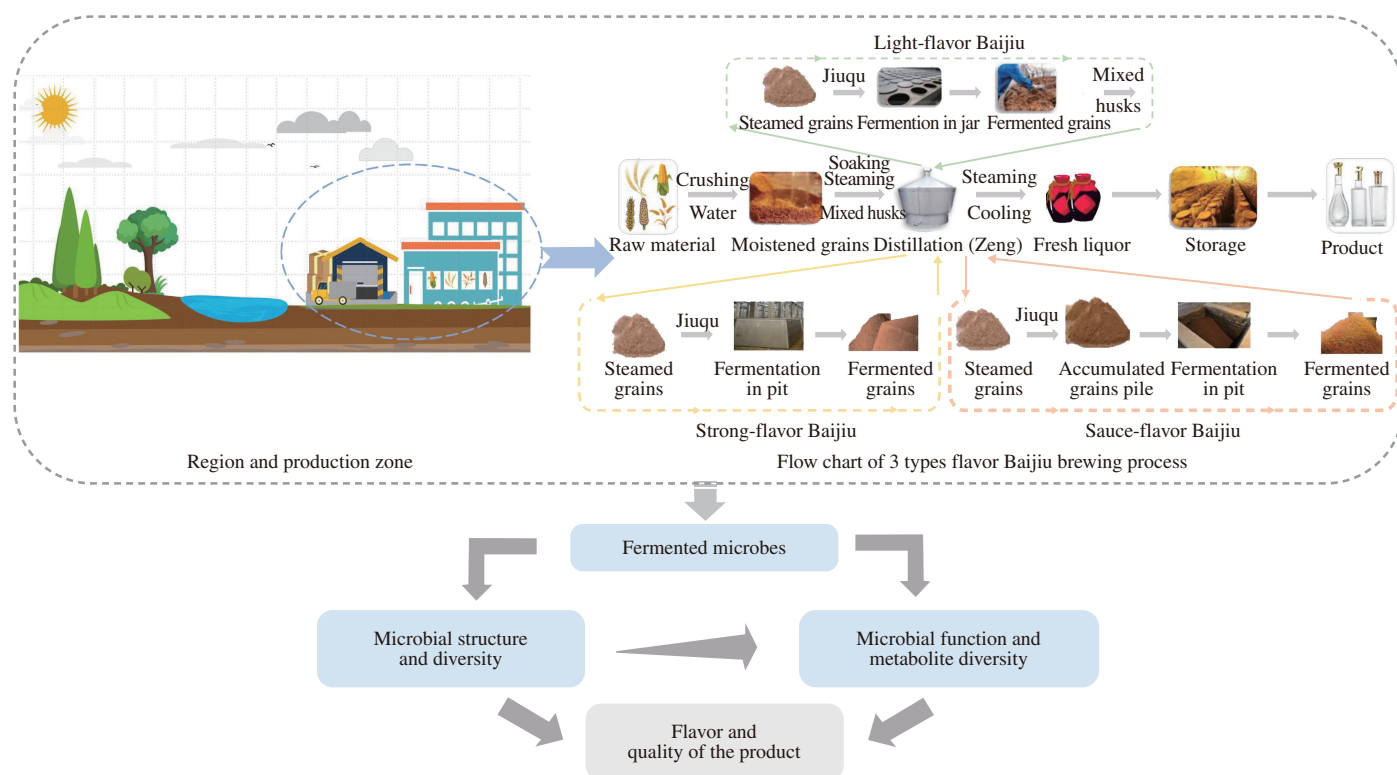
Typical LFB distilleries are located mainly within a latitude range of 36°N to 37°N<sup>[7]</sup>. Fenyang City, in the hinterland of Shanxi Province, is the largest base to produce the Chinese LFB, with a warm temperate continental monsoon climate and 4 distinct seasons. Approximately 400 years ago, due to the migration of merchants from Shanxi to Hubei Province, the unique light-flavor Daqu and ground-pot fermentation process were developed, and a new variety of Hanfenjiu was bred in Hubei Province<sup>[18]</sup>. Although both Fenjiu and Hanfenjiu belong to the LFB category, they show obvious differences in flavor and taste. Fenjiu is characterized by its outstanding floral, fruity, and pea aroma, giving people a fresh and pure feeling. In contrast, Hanfenjiu shows the obvious aroma of wheat and dried dates, with a slightly richer taste.

## 3. Relationship between environmental factors and Baijiu fermentation

In 1934, Baas Becking first proposed the microbial biogeography hypothesis, "everything is everywhere, but the environment selects"<sup>[19]</sup>. Given the high diversity of microbial communities and their functional redundancy, microbial biogeography and regional characteristics are closely interrelated, although they do not guarantee direct causality<sup>[13]</sup>. Microbial succession will profoundly affect the change of habitat environment, and conversely, the change of environmental factors will also react to microorganisms, and have a profound impact on their community structure, function, and metabolite distribution. The brewing of Chinese Baijiu is precisely dependent on the complex fermentation process of these microorganisms in the naturalized environment. As shown in Fig. 1, the microecology of Baijiu fermentation involves various environmental factors directly or indirectly exposed to grain raw materials in the operation process, including regional characteristics, production workshop conditions, operating tools, and operating habits of workers. To achieve more accurate control of Baijiu product quality, more and more studies have begun to focus on the relationship between environmental heterogeneity at different scales and microorganisms in the fermentation system (Table 1).

### 3.1 Fermentation microorganisms derived from the environment

In most spontaneous fermentations, microorganisms in microbial pools such as air, water, soil, and tools<sup>[9,20-21]</sup> are usually artificially or naturally isolated or enriched to become fermentation microorganisms<sup>[22]</sup>. Similarly, controlled by production technology,



**Fig. 1** Overview of environment (regional environment and brewing process environment) effect on microbial diversity and Baijiu quality. Flow chart of 3 flavor Baijiu brewing process was redrawn from Liu et al.<sup>[2]</sup>.

various microorganisms from air, water, tools, operators, and Daqu (fermentation starter) participate in the fermentation process, forming a unique fermentation microbial community, ultimately used to produce Baijiu<sup>[9]</sup>. It has been reported that bacteria from the environment have a significant influence on the composition and abundance of bacteria in the fermentation system at the early stage of fermentation<sup>[23]</sup>. Daqu was the primary source of bacteria responsible for SAFB fermentation, followed by the air (*Pseudomonas* sp.), soil (*Weissella cibaria*, *Bacillus sonorensis*), and sorghum samples (for example, *W. cibaria*, *B. sonorensis*, and *B. subtilis*), while the majority of the fungi were from Daqu and sorghum (for example, *Penicillium decumbens*, *Cryptococcus gastricus*, and *Saccharomyces* sp.)<sup>[24]</sup>. Microorganisms in the environment and raw materials are effectively enriched into Daqu during the production process<sup>[4]</sup>. Especially during the production of medium-temperature Daqu, the environment (such as indoor floors and tools) becomes the main source of fungal communities, contributing most of *Saccharomycopsis fibuligera*, *Pichia kudriavzevii*, *Rhizopus oryzae* and so on, while raw materials contributed mainly to the bacterial community<sup>[22]</sup>.

### 3.2 Changes in environmental factors affect microbial structure and diversity

The relationship between microbial community structure and environmental factors is significant and complex. Even when the same microbiome is inoculated, due to differences in environmental factors, the fermentation process will still form a completely different microbial ecology. Natural environmental factors such as temperature, relative humidity, precipitation, and wind speed show significant differences between regions, and these changes not only

profoundly affect the diversity and functional complexity of the local microbial pools, but also especially play a prominent role in shaping the dominant microbiome<sup>[17]</sup>. In addition, environmental conditions within the fermentation system, such as temperature, oxygen concentration, and availability of nutrients (such as oligosaccharides, monosaccharides, short peptides, and amino acids), drive microbial succession, further shaping the unique flavor and quality of fermented products<sup>[25]</sup>.

#### 3.2.1 Regional environmental factors

Regional environmental factors, including climatic conditions, geographical differences, and temporal and spatial factors, have direct or indirect effects on the diversity and composition of microbial communities. The changes among these environmental factors are interrelated, forming a complex and coordinated control mechanism. According to the study of Li et al.<sup>[14]</sup>, there was a significant negative correlation between sunshine duration and humidity ( $P = -0.857$ ), and air pressure was negatively correlated with temperature and sunshine duration ( $P = -0.893$ ,  $P = -0.857$ ), while sunshine duration was positively correlated with temperature ( $P = -0.679$ ). The study showed that temperature, air pressure, and daylight duration significantly influenced the dominant fungi in SAFB brewing, with temperature being a key factor in the succession of *Wallemia mellicola*, which contributes to SAFB's unique flavor<sup>[14]</sup>. Yuan et al.<sup>[13]</sup> further revealed the indirect influence mechanism of climate factors on CSFB flavor. Results showed that climate factors such as mean temperature, mean wind speed, mean relative humidity, and mean precipitation affected the microbial community structure and flavor characteristics of fermented grains by regulating the fungal diversity

**Table 1**

Relevant researches on the environmental factors of Chinese Baijiu fermentation in recent years.

| Research object | Baijiu flavor types | Main variables                                         | Analysis factors                                                                                                                                                                     | Analysis of microbial function            | Reference |
|-----------------|---------------------|--------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|-----------|
| Daqu            | STFB                | Seasons                                                | Physiochemical properties (moisture, water activity, acidity, and pH)                                                                                                                | -                                         | [85]      |
| Daqu            | STFB                | Daqu production stages                                 | Environmental parameters (temperature, humidity, and CO <sub>2</sub> concentration in the Qu-room); Physiochemical properties (moisture content and total acidity)                   | PICRUSt2 prediction                       | [34]      |
| Daqu            | STFB                | Daqu temperature during production                     | Physiochemical properties (temperature, moisture, titratable acidity)                                                                                                                | -                                         | [76]      |
| Daqu            | SAFB                | Seasons                                                | Climatic factors (temperature, sunshine, precipitation, humidity, and pressure)                                                                                                      | PICRUSt2 prediction                       | [16]      |
| Daqu            | STFB and SAFB       | Peak temperature of Qu                                 | Physiochemical properties (moisture, total titratable acid, raw starch, reducing sugar contents, saccharification power, the common soluble monosaccharide and uronic acid contents) | Metagenomic and metaproteomic             | [75]      |
| Daqu            | LFB                 | Peak temperature of Qu                                 | -                                                                                                                                                                                    | PICRUSt2 prediction                       | [78]      |
| Daqu            | LFB                 | Produced regions                                       | -                                                                                                                                                                                    | -                                         | [18]      |
| PM              | STFB                | Cellar ages (5, 7, 10, 30 and 50 years)                | Physiochemical properties (moisture content, pH, humus, available phosphorous (AP), NH <sub>4</sub> <sup>+</sup> -N, Fe, Ca, Mg, organic acids)                                      | PICRUSt2 prediction                       | [29]      |
| PM              | STFB                | Cellar spatial heterogeneity                           | Physicochemical factors (moisture, pH, humic acid, NH <sub>4</sub> <sup>+</sup> -N, the total acid, lactate, and Olsen-P)                                                            | -                                         | [28]      |
| PM              | STFB                | Cellar ages (2, 3, 15, 20, and 50 years)               | Physicochemical factors (moisture, pH, total acid, NH <sub>4</sub> <sup>+</sup> -N, AP, organic acids (acetic acid, lactic acid, butanoic acid, and hexanoic acid) )                 | PICRUSt2 prediction                       | [30]      |
| PM              | STFB                | Cellar ages (40 and 400 years)                         | Physicochemical properties (moisture, pH, humic matter, NH <sub>4</sub> <sup>+</sup> -N, AP, available potassium (AK), organic acids (caproic, acetic, and butyric acid))            | -                                         | [27]      |
| PM              | SAFB                | PM quality                                             | Physicochemical factors (pH, AP, AK, total nitrogen (TN), total carbon (TC), humus, amino acid nitrogen (AAN) and urease)                                                            | -                                         | [35]      |
| FG              | STFB                | Regional heterogeneity                                 | -                                                                                                                                                                                    | Metatranscriptomic                        | [13]      |
| FG              | STFB                | Sorghum species                                        | Physicochemical (temperature, moisture content, pH, reducing sugar content, starch content)                                                                                          | PICRUSt2, metagenomic, and metaproteomics | [92]      |
| FG              | STFB                | Cellar ages (2, 20, 30 years)                          | Physicochemical factors (moisture, total titratable acidity, starch, and reducing sugar)                                                                                             | -                                         | [40]      |
| FG              | SAFB                | Spatio-temporal heterogeneity of stacking fermentation | Physicochemical factors (starch, moisture, titratable acidity, reducing sugar and pH)                                                                                                | PICRUSt2 prediction                       | [38]      |
| FG              | SAFB                | Temporal heterogeneity of stacking fermentation        | Physicochemical factors (moisture, total titratable acidity, starch, and reducing sugar)                                                                                             | -                                         | [39]      |
| FG              | SAFB                | Initial temperature of stacking fermentation           | Physicochemical factors (temperature, moisture, pH, titratable acidity, reducing sugar, alcohol, acetic acid, and lactic acid)                                                       | -                                         | [36]      |
| FG              | SAFB                | Temporal heterogeneity of fermentation rounds          | -                                                                                                                                                                                    | -                                         | [15]      |
| FG              | LFB                 | Seasons                                                | Climate data (atmospheric pressure, daily average temperature, relative humidity, precipitation, wind speed, and sunshine)                                                           | -                                         | [6]       |
| FG              | LFB                 | Initial moisture content of the fermented grains       | Physicochemical factors (moisture content, total titratable acidity, starch content, reducing sugar content, amino nitrogen, ammonia, acetic acid, lactic acid, and ethanol)         | PICRUSt2 prediction                       | [82]      |
| FG              | LFB                 | Workshop (4 and 23 years)                              | Physicochemical factors (ethanol and organic acid content)                                                                                                                           | -                                         | [8]       |

Note: FG, fermented grains; - represents no relevant information.

in the environment. Among them, local ambient temperature, as a key factor, can directly affect the initial temperature of fermented grains, thereby further shaping the microbial community and flavor characteristics<sup>[11]</sup>. Low-temperature conditions usually have a negative impact on microbial diversity, which in turn causes seasonal differences in microbial structure<sup>[6,26]</sup>. For example, the microbial

diversity of sauce-flavor Daqu showed obvious changes across seasons, with the highest microbial diversity in summer and the lowest diversity in winter<sup>[16]</sup>. Specifically, high temperature favors the growth of *Bacillus* while not favoring the growth of *Oceanobacillus*. The average relative abundance of *Bacillus*, which plays an important role in the formation of sauce flavor in SAFB production, was highest

in summer-produced Daqu ( $(34.91 \pm 0.20)\%$ ) and lowest in winter-produced Daqu ( $(8.64 \pm 0.04)\%$ ).

Regarding geographical characteristics, the wine microbiota provides a useful reference for us<sup>[5]</sup>. Similarly, Tan et al.<sup>[7]</sup> also observed that fungi genera at different latitudes showed significant differences in the Baijiu fermentation process. In addition, the predominant fungal species in PM in different regions may be influenced by regional geographical climate, soil, and physical and chemical properties of cellars. The moisture content of high-quality PM is usually maintained between 40% and 50%, directly impacting pH, humus content, and the growth state of microbial communities<sup>[27]</sup>. In contrast the PM humidity range in Xinjiang is relatively low, ranging from 28.0% to 38.7%, significantly below the normal moisture content range<sup>[28]</sup>. This humidity difference is closely related to the unique geographical and climatic characteristics of Xinjiang. As the region with the least precipitation and the highest evaporation in China, Xinjiang's special climatic conditions have a significant impact on the humidity of PM. In the case of Xinjiang's PM, regardless of their depth, the fungal communities could be divided into 3 dominant fungal phyla: Ascomycota (78.47%), Basidiomycota (10.07%), and Mortierellomycota (9.77%)<sup>[28]</sup>.

The influence of temporal and spatial factors on microbial communities should not be ignored<sup>[29-30]</sup>. During the same period, the microbial communities of different batches may show some similarity, but the differences in the bacterial communities of different periods can lead to significant changes in the flavor of fermented products<sup>[31]</sup>. The study revealed that in the 23-year workshop, a variety of bacterial genera were enriched at the initial stage of fermentation, including *Lactobacillus* (11.73% average relative abundance), *Bacillus* (20.78%), *Pseudomonas* (6.13%), *Kroppenstedtia* (10.99%), *Weissella* (16.64%), *Pantoea* (3.40%), as well as fungal genera such as *Pichia* (55.10%), *Candida* (1.47%), *Aspergillus* (10.66%), *Saccharomycopsis* (22.11%), and *Wickerhamomyces* (3.35%). However, in the 4-year workshop, changes in the environmental flora led to a significant adjustment in the abundance of some microorganisms. For example, the abundance of *Bacillus* (5.74%), *Weissella* (6.64%), *Pichia* (33.91%), and *Aspergillus* (7.08%) decreased, while the abundance of *Pseudomonas* (17.04%), *Kroppenstedtia* (13.31%), *Pantoea* (11.41%), and *Candida* (16.47%) increased<sup>[8]</sup>. This showed that the microbial community structure within the workshop varied significantly over different working years. As cellar age increased (0–25 years), the microbial community structure within PM evolved, eventually stabilizing to form a relatively balanced state with high microbial diversity. Notably, the study also found that the abundance of *Lactobacillus* on the floor and tool surfaces of the old workshop was significantly higher than that of the new workshop<sup>[8]</sup>, further emphasizing the importance of spatio-temporal factors in the composition of microbial communities. In addition, Yuan et al.<sup>[13]</sup> also reported that microbial communities in Chinese STFB fermented grains and their environment (air and ground) have significant regional characteristics, among which the regional characteristics of fungi are more obvious than those of bacteria. This reaffirms the complexity and diversity of regional environmental factors that influence microbial communities.

In general, the influence of regional environment on microbial communities is significant and complex, but there is still a lack of research on how to make good use of regional characteristics to

better guide production. For example, based on clear local dominant microorganisms, combined with clear key brewing microbiome, targeted microbial community construction. Of course, this also requires capturing key environmental factors and quantifying the strength of their influence on fermentation system factors and microorganisms. On the other hand, regional characteristics can be defined, the construction of workshop and fermentation system can be optimized, and the role of natural environmental factors can be reduced.

### 3.2.2 Brewing environmental factors

A close correlation exists between the microbial community structure of the fermentation system and fermentation microecology<sup>[16]</sup>. Subtle changes in physicochemical properties during fermentation not only affect microbial succession but also collectively shape a unique micro-ecosystem, creating an ideal growth environment for functional microorganisms in Baijiu brewing<sup>[8]</sup>. A study by Lin et al.<sup>[25]</sup> found that the abundance of *Pichia* and *Lactobacillus* was positively correlated with acidity, amino acid nitrogen, and water content, while *Lactobacillus* was negatively correlated with reducing sugars.

In addition, differences in microbial community composition are often closely related to the characteristic distribution of microorganisms at different temperatures<sup>[32]</sup>. According to the highest inoculation temperature, we can distinguish low-temperature Daqu (45–50 °C), medium-temperature Daqu (50–60 °C), and high-temperature Daqu (60–65 °C). As reported, bacterial communities of low-temperature Daqu were mainly composed of Firmicutes and Actinobacteria, while almost all the fungal communities were *Pichia kudriavzevii*<sup>[33]</sup>. Elevated temperatures (37–42 °C) markedly promoted the proliferation of *P. kudriavzevii*, accounting for *Pichia*'s rapid dominance in SAFB's heap fermentation. Further, the large number of metabolites (including ethanol) produced by *P. kudriavzevii* selectively favors some microorganisms (e.g., *Saccharomyces*) and may be detrimental to others, shaping the fermentation microbial community. During the production of medium-high temperature Daqu, CO<sub>2</sub> and moisture were positively correlated with the growth of most fungi but negatively correlated with *Thermoactinomyces*, *Saccharopolyspora*, and *Acinetobacter*<sup>[34]</sup>. Especially in the production process of high-temperature Daqu, when the maximum temperature exceeds 60 °C, thermophilic microorganisms proliferate. At this time, the dominant microorganisms are mainly bacteria, especially *Bacillus* spp., because fungi are more sensitive to high temperatures<sup>[3]</sup>.

In this complex fermentation system, existing research is also focused on describing system changes (physicochemical indicators, microorganisms, flavor substances) and demonstrating key environmental factors based on correlation. And when these changes are clear, how do we move forward with practical use? The mechanism for change needs to be clarified. On the one hand, we can start from the key microbial population or local microbial population, and use the key fermentation factors to induce fermentation by artificial intervention, so as to find the means to adjust the structure of the target microbial community. On the other hand, starting from a single system, we gradually designed a complex system, built a model, and found the quantitative relationship between fermentation factors and microbial populations.

### 3.3 Changes in environmental factors affect microbial function and metabolism

In the fermentation process, microorganisms not only contribute a rich set of enzyme systems, but also generate numerous flavor compounds and their precursors, adding a unique flavor level to the brewing process<sup>[35]</sup>. Subtle adjustments to environmental factors such as temperature, humidity, and CO<sub>2</sub> concentration can guide these biological processes, altering metabolic pathways and accumulations of metabolites. Studies have shown that in the key stage of Daqu preparation, precise regulation of these environmental factors can effectively promote the active growth and reproduction of microorganisms in Daqu, so as to optimize the metabolic efficiency and accumulation of microbial enzymes during Baijiu fermentation<sup>[34]</sup>. In particular, the activity of key enzymes (EC 1.2.7.11, EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) closely related to acetyl-CoA were significantly higher in summer than in spring and winter<sup>[16]</sup>, indicating the profound impact of seasonal changes on microbial metabolism.

Various parameters of fermented grain, such as temperature, total acidity, moisture, and starch, are the key factors to determine the flavor quality of Baijiu. Higher initial temperature can accelerate the proliferation of *Lactobacillus*, thus accelerating the succession rate of microorganisms, and eventually leading to the generation of flavor substances such as lactic acid and acetic acid<sup>[36]</sup>. Under different temperature conditions, the flavor characteristics of Daqu are also different<sup>[20]</sup>. High-temperature Daqu is characterized by tetramethylpyrazine, guaiacol and other compounds (1,3-butanediol, methyl ester, and acetic acid) as the main flavor components, showing a unique flavor profile. In contrast, the main flavor compounds in medium-temperature Daqu include tetradecanoic acid ethyl ester, guaiacol, pyrazines, phenylethyl alcohol, and caryophyllene<sup>[20]</sup>. In addition, the synergistic effect of acetic acid, lactic acid, as well as hexyl acetate, ethyl acetate, phenylethyl alcohol and other compounds, together constructed the light and pure taste of LFB<sup>[20]</sup>. It is worth noting that the type and content of sugar has a significant impact on microbial interactions. Different glucose profiles will lead to changes in microbial preferences, interactions, and metabolic pathways, which will affect the flavor characteristics of fermentation products<sup>[37-39]</sup>. Therefore, in the fermentation process, the precise regulation of sugar is also one of the important means to achieve flavor customization.

In the dimensions of time and space, complex metabolic processes lead to significant flavor changes. Practical experience shows that the Baijiu brewed in the aged cellar is superior to that in the new cellar in taste and quality, and its microbial flora is more abundant and stable<sup>[40]</sup>. The evolution and stability of this microbial community are crucial for the formation and maintenance of Baijiu flavor. Comparing the distribution of volatile compounds in fermented grains from aged cellars and fresh cellars, we observed significant differences in the concentration and type of alcohols, aldehydes, esters and aromatic compounds<sup>[40]</sup>. In particular, in the new workshop (4-year), dynamic changes in the microbial community resulted in an increase in acetic acid, lactic acid, malic acid, and ethyl acetate, and a decrease in ethyl lactate during fermentation<sup>[8]</sup>. As cellars age, specific microbial beneficial to the brewing process gradually screened and accumulated in the cellar's environment. In addition, the long-term systematic

fermentation process enhances the adaptability of microorganisms<sup>[40]</sup>, thereby improving the fermentation capacity of the PM, the metabolic reactions within the PM become more complex. At large spatial scales, climate variations have direct or indirect effects on the regional distribution pattern of Baijiu flavor. The microbiota also exhibits geographical variations at the functional level<sup>[13]</sup>. This unique biogeographical distribution pattern may be one of the important reasons for the regional characteristics of Baijiu.

Based on all the above analysis, the entire production process plays a decisive role in the quality of the final product. From the selection of production time, the selection of fermentation raw materials and fermenters, to the aging treatment of the base liquor, each step is a process of optimization and regulation of the microbial system, aiming at obtaining high-quality base Baijiu.

## 4. Main factors affecting the microecology of Baijiu fermentation

### 4.1 Environmental sensitivity of microorganisms

Fig. 2 shows 9 typical influencing factors, and 4 of which are closely related to the characteristics of microorganisms themselves. In exploring environmental sensitivity, we observed significant differences in the response of bacteria and fungi to changes in the external environment. Many studies have indicated that fungal community, as an indispensable part of food fermentation system, is particularly sensitive to local climate conditions and closely related to regional characteristics of food. For example, in the brewing process of Chinese Baijiu, local climate temperature becomes a key factor in predicting the abundance of dominated genera, such as *Saccharomyces*, *Pichia*, and *Candida*<sup>[6]</sup>. In contrast, bacterial communities exhibit higher phylogenetic diversity and taxonomic conversion rates when exposed to frequent environmental disturbances. This property makes bacterial communities more complementary in their ecological niche<sup>[41]</sup>. Therefore, it can be inferred that bacterial communities may have richer functional redundancy than fungal communities, which makes them more able to withstand environmental changes while maintaining their versatility<sup>[42]</sup>.



Fig. 2 An overview of main factors affecting the microecology of Baijiu brewing.

**Table 2**

Relevant researches on interaction relationships of microorganisms in Chinese Baijiu fermentation system in recent years.

| Microorganism                                                                                                                                                                        | Interaction relationships | Effect or detailed mechanism                                                                                                                                                                                                 | Reference |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <i>S. fibuligera</i> and <i>Wickerhamomyces anomalus</i>                                                                                                                             | Synergistic effect        | The stability of the environment was enhanced. <i>S. fibuligera</i> normally propagated before ethanol fermentation and produced extracellular proteolytic enzymes; <i>W. anomalus</i> produced various esters and aromatics | [14]      |
| <i>Clostridium</i> and <i>Methanobacterium</i>                                                                                                                                       | Synergistic effect        | The production capacity of caproic acid and ethyl caproate was improved. Interspecies hydrogen transfer                                                                                                                      |           |
| <i>Saccharomyces</i> and <i>Lactobacillus</i>                                                                                                                                        | Synergistic effect        | Sulfur compounds are produced by methionine recycling                                                                                                                                                                        | [12]      |
| <i>Bacillus</i> species and <i>Streptomyces sampsonii</i>                                                                                                                            | Antagonistic effect       | Inhibit the growth of <i>Streptomyces sampsonii</i> and reduce the production of geosmin producers                                                                                                                           |           |
| Lactic acid bacteria and spoilage microorganisms                                                                                                                                     | Antagonistic effect       | Ensure the growth of functional microorganisms, saccharification and alcohol fermentation. LAB strains produce metabolites and/or their antimicrobial products                                                               | [46]      |
| <i>Bacillus amyloliquefaciens</i> and <i>Saccharomyces cerevisiae</i>                                                                                                                | Antagonistic effect       | The growth of <i>S. cerevisiae</i> was inhibited                                                                                                                                                                             |           |
| <i>Pichia kudriavzevii</i> and <i>B. amyloliquefaciens</i> and 3 other fungal species ( <i>Monascus purpureus</i> , <i>Aspergillus oryzae</i> and <i>Byssoschlamys spectabilis</i> ) | Antagonistic effect       | The growth of <i>B. amyloliquefaciens</i> and 3 other fungal species ( <i>Monascus purpureus</i> , <i>Aspergillus oryzae</i> and <i>Byssoschlamys spectabilis</i> ) was inhibited                                            | [26]      |
| <i>Pichia</i> and filamentous fungi                                                                                                                                                  | Antagonistic effect       | <i>Pichia</i> secrete toxins or organic acids that are lethal to other sensitive yeast and filamentous fungi                                                                                                                 |           |
| <i>Pichia membranaefaciens</i> and <i>Bacillus amyloliquefaciens</i>                                                                                                                 | Antagonistic effect       | The stability of the environment was enhanced. <i>B. amyloliquefaciens</i> were inhibited                                                                                                                                    |           |
| <i>Bacillus amyloliquefaciens</i> and <i>Candida humilis</i>                                                                                                                         | Antagonistic effect       | The growth of <i>C. humilis</i> was inhibited. <i>Bacillus</i> secreting iturin A or bacillomycin D to inhibit the growth of <i>Candida</i>                                                                                  | [8]       |
| <i>Pichia</i> and <i>Saccharomyces</i>                                                                                                                                               | Synergistic effect        | It can improve the metabolism of <i>S. cerevisiae</i> in the later stage of fermentation, thereby reducing the production of higher alcohols                                                                                 | [25]      |
| <i>B. subtilis</i> and <i>Aspergillus</i>                                                                                                                                            | Antagonistic effect       | The growth of <i>Aspergillus</i> was inhibited                                                                                                                                                                               |           |
| <i>Monascus</i> and <i>B. subtilis</i>                                                                                                                                               | Synergistic effect        | Through close metabolic cooperation for producing higher contents of cellulases or $\beta$ -glucosidase which catalyzed more hydrolysis processes                                                                            | [43]      |
| <i>Lactobacillus</i> and methanogenic                                                                                                                                                | Antagonistic effect       | Lactic acid produced by <i>Lactobacillus</i> is not conducive to the production of hydrogen and caproic acid, which results in the inhibited growth of methanogenic                                                          | [29]      |

## 4.2 Microbial interaction

Interspecific interactions between microbial communities are widespread, and these interactions can have both positive and negative effects<sup>[43]</sup>. In the Baijiu fermentation system, these interactions are mainly manifested as nutrient and space competition, cell-cell contact mechanisms, and influence by metabolites (such as acids, ethanol, and volatile organic compounds (VOCs))<sup>[21,44]</sup> (Table 2).

Lactic acid bacteria, one of the most well-studied microorganisms, inhibit the growth of undesirable spoilage microorganisms through substrate competition<sup>[23]</sup> or the production of antagonistic substances<sup>[29]</sup>. They also promote the growth conditions of functional microorganisms by providing essential amino acids and vitamins. Specifically, it was found that the metabolism of *Lactobacillus buchneri* can directly provide free amino acids (i.e., threonine, tyrosine, valine, ornithine, lysine,  $\beta$ -alanine, and aspartic acid)<sup>[45]</sup> for the propagation of other microorganisms<sup>[46]</sup>. *Streptomyces* species, a widespread soil bacterium, are known for their ability to produce antibiotics. These antibiotics can inhibit yeast growth and affect the production of flavor compounds, especially alcohols and esters<sup>[47]</sup>. Interactions and cross-feeding between bacterial species in a community not only affect the adaptability of cells but also alter their susceptibility to antibiotics and other stressors<sup>[48]</sup>. Yu et al.<sup>[49]</sup> demonstrated that microbial species that interact and exchange metabolites within communities are more tolerant to antibiotic exposure. This interaction

and metabolite exchange have also been observed in mixed cultures of yeast and bacteria<sup>[50]</sup>. The coexistence of bacteria and fungi in the same environment is a universal phenomenon, and the exchange of matter and energy inevitably takes place between them. For example, *Bacillus subtilis* is able to attach to the mycelium of fungi in the presence of the fungus *Aspergillus niger*<sup>[51]</sup>, forming complex symbiotic relationships.

In many fermented foods, VOCs play a critical role in determining the quality and commercial value of the product, while their functions in the fermentation microecology remain largely elusive<sup>[44]</sup>. VOCs are typically small compounds (up to C<sub>20</sub>) with a low molecular mass (100–500 Da), low boiling point, and a lipophilic moiety<sup>[52]</sup>. These properties enable VOCs to exhibit significant antimicrobial properties even at low concentrations<sup>[52–53]</sup>. Due to their ability to travel through solid-, liquid-, and gas-phases through evaporation and diffusion, and their capacity to function without direct contact with microorganisms, VOCs are considered ideal antimicrobial agents<sup>[53]</sup>. Notably, VOCs can propagate in the solid-liquid-gas three-phase mode and inhibit mold growth on both substrate and airborne mycelium<sup>[44]</sup>.

The study of microbial interaction is of great significance for building a stable and complex symbiotic fermentation system<sup>[26]</sup>. However, given the complexity and diversity of microbial interactions, their study is often highly dependent on correlation coefficient analysis, such as Spearman's rank correlation. In the study of Baijiu brewing, *in situ* investigations of microbial interactions are rare and challenging

due to the complexity of the fermentation system, which involves the co-culture of multiple species<sup>[54]</sup>. At present, most research on bacteria-fungus interactions is still confined to the laboratory and focuses on interactions between a few microorganisms. In a natural environment with a wide variety of microorganisms and complex external influences, where types and quantities of microorganisms are abundant and external factors are complex, most research results often rely on the use of specific microorganisms to enhance the fermentation process<sup>[15]</sup>. Therefore, in order to fully understand and optimize the Baijiu fermentation system, we need to develop more accurate and comprehensive research methods to deeply explore the interaction of the same and different functional microorganisms and their application in the actual fermentation environment.

### 4.3 Microbial biogeographic patterns

Evidence supporting microorganisms exhibit spatial biogeographic patterns can be grouped into at least 2 categories<sup>[55]</sup>. The first category pertains to the existence of endemic taxa and the non-random relationship between the similarity of taxa. These local taxa are specific to a location, region or habitat type and are therefore not evenly distributed globally. This regional feature is undoubtedly the most intuitive manifestation of microbial biogeography. Similar to larger organisms, some microorganisms exhibit unique adaptations to specific geographic regions and become endemic species<sup>[56]</sup>. Other taxa, such as those adapted to extreme environments like hot springs, exhibit clear preferences for specific habitat types<sup>[57]</sup>. As mentioned above, specific microorganisms are distributed in the natural environment and production environment of different Baijiu producing areas. And divergences in microbiota have been observed in a comparison of fermented samples from the Guizhou province and Beijing city (located in the northern of China)<sup>[15]</sup>. The second type of evidence focuses on detecting patterns of genetic similarity among microbial taxa at different sites. This includes a method to compare genetic diversity based on the principles of population genetics to reveal differences among sites<sup>[57]</sup>. This kind of method is still less used in the study of Baijiu fermentation microorganisms, and the horizontal gene transfer analysis is currently involved<sup>[58]</sup>. However, due to the complex interaction of microorganisms in the fermentation system, the microbial screening results are highly repetitive and time-consuming, so it is difficult to promote related research from the molecular mechanism level. In addition, another approach attempts to establish a correlation between taxonomic similarity and geographical distance. With an increase of geographical distance, the similarity showed a downward trend (i.e., distance attenuation relationship), which not only indicated the differences in microbial composition at different locations, but also showed the spatial autocorrelation of this change (i.e., closely related to spatial distance)<sup>[59]</sup>. This distance decay relationship has been repeatedly verified in a variety of habitats at different taxonomic resolutions<sup>[56,59]</sup>. In SAFB fermentation, as the increase distance from the distillery increased, the dissimilarity between microbial communities also increased, displaying a deterministic-random pattern<sup>[60]</sup>. In particular, the ecological environment 50 m away from the distillery is less affected by the fermentation process, resulting in a lower degree of microbial domestication. The distance-decay relationship further indicates that only a few species are retained across different

environments, with most species disappearing during migration due to environmental pressures<sup>[61]</sup>. This also shows that the proportion of liquor fermentation production affected by the environment is not low. Even if the same production process and raw materials are used, the effect of environmental pressure on microorganisms is still unknown.

However, due to certain limitations, the detection of these patterns is also constrained. For example, not all studies have been able to find clear evidence of microbial biogeographic patterns<sup>[62]</sup>, as some microbial taxa may have global distribution characteristics. Second, biogeographic patterns can exist in the absence of endemism. Finally, the ability to detect a biogeographic pattern, including endemism or a distance-decay relationship, may be limited by taxonomic resolution<sup>[55]</sup>. Therefore, it is necessary to consider these complex factors comprehensively and optimize the research methods when exploring the spatial biogeographical pattern of microorganisms.

### 4.4 Dispersal of microorganisms

Dispersal has been extensively studied as a central element in macroecological models of microbial communities<sup>[63]</sup>. Its process is not only affected by random factors, but also regulated by external forces<sup>[64]</sup>. In particular, airborne microbial transport plays a pivotal role in the diffusion process and has a decisive influence on determining diffusion outcomes<sup>[65]</sup>. In the specific environment of the liquor distillery, aerosol transmission and direct contact are common modes of microbial transmission, significantly enhancing the efficiency of microbial transmission from one surface to another. Bacterial and fungal spores float in the air as aerosols and can be naturally inoculated into the workshop to participate in the fermentation process<sup>[24]</sup>. *Pseudomonas* sp. were detected in the air samples around the Moutai production workshop, and these bacteria were found in the air outside both the liquor production area and the fermentation workshop<sup>[24]</sup>, indicating that airborne transmission plays a key role in the distribution of microorganisms. It is worth noting that *Pseudomonas* sp. has the ability to produce amylases, lipases, and proteases<sup>[66]</sup>, which may play a crucial role in the Baijiu fermentation process.

However, during the pit fermentation process, microorganisms showed significant spatial distribution heterogeneity, characterized by uneven distribution. Compared to fungi, bacteria show higher dispersibility during fermentation, mainly due to many bacteria being single-celled with flagellar structures, such as *Lactobacillus acidophilus*<sup>[67]</sup>. In addition, Huangshui, as a waste serofluid formed together with the organic compounds during solid-state fermentation, is not only rich in a variety of bacteria, but also drives the movement of microorganisms and nutrients through its infiltration process further affecting the spatial distribution of microorganisms.

Factors such as the processing and transfer of materials, equipment cleaning, and human and insect activities all accelerate the transfer of microorganisms between these surfaces, including from pits to indoor floors. However, some physical obstacles (such as geographical distance and walls) as well as physical and chemical conditions (such as humidity, temperature, and precipitation) may limit the spread of some microorganisms. Specifically, wind speed which is closely related to air pressure, has a direct effect on the migration and movement of fungi. Since most fungi exist in the environment in the form of spores, changes in wind speed will indirectly affect the germination and regeneration

process of fungi<sup>[68]</sup>, thus having a profound impact on the distribution and dynamics of the entire microbial community.

By introducing novel genetic diversity (alleles and genes), including unknown strains and taxa, dispersal has the potential to profoundly alter microbiome performance<sup>[69]</sup>. Although the early research paradigm generally believed that microorganisms are ubiquitous and widely distributed in various environments<sup>[19]</sup>, with the deepening of microbial ecology research, ecologists have gradually realized that the diffusion capacity of microorganisms is limited<sup>[70]</sup>. Such dispersal limitation can significantly affect the composition and structure of microorganisms in natural communities, which in turn has a profound impact on community function<sup>[71]</sup>. The dispersal of new strains and taxa into the community is a process of gradual accumulation. Over time, the addition of these new elements will gradually bring the community structure to a relatively stable level, and at the same time, favorable mutations within the community will accumulate, allowing the potential for evolutionary adaptation to the new environment to gradually increase<sup>[69]</sup>. As reported, even though there are only minor nucleotide differences between bacterial strains, their rapid evolution has been shown to have significant ecological effects on carbon utilization, interspecies interactions, nutrient partitioning, and overall community productivity<sup>[72]</sup>. Compared to bacteria, fungi may be more susceptible to diffusion limitations due to their unique biology. Such diffusion limitation may be an important factor leading to the geographic diversity observed in fungal communities<sup>[70,73]</sup>. However, the exact rate at which this diffusion phenomenon occurs is still an open scientific question and further research and exploration are needed to reveal the mechanism behind it.

#### 4.5 Temperature

Temperature is a key factor that influences microbial enzyme activity and cell membrane fluidity. Due to the differences in cell structure and biological characteristics among different microorganisms (genomic potential of microorganisms<sup>[74]</sup>), they exhibit varying levels of thermal resistance<sup>[34,75]</sup>. This level of thermal resistance has a significant effect on microbial community composition, especially during production, which is further confirmed by Xiao et al.<sup>[76]</sup>. Different types of microorganisms exhibit varying adaptations to temperature. For example, yeast usually performs best in the optimal growth temperature range of 20–30 °C (*Saccharomyces cerevisiae*, 28–32 °C), while mold can resist higher temperatures by producing spores (*Rhizopus* and *Aspergillus*, 28–35 °C). Bacteria show a wider range of temperature (*Bacillus*, 30–37 °C) adaptation<sup>[4]</sup>, while fungi tend to be more sensitive to heat than bacteria<sup>[3]</sup>. Exposure to temperatures higher than the optimal growth range for yeast can induce heat stress, causing irreversible damage to yeast growth, and subsequently impacting ethanol production<sup>[36]</sup>. In high-temperature Daqu, the microbial community is predominately composed of high-temperature tolerant and thermophilic microorganisms<sup>[77]</sup>. The thermophilic flora, such as *Thermoactinomyces* and *Thermoascus*, produce heat-stable enzymes that contribute to the occurrence of the Millard reaction and the production of aromatic compounds in Daqu<sup>[18,34,78]</sup>. In addition, the high abundance of acetolactate decarboxylase (EC 4.1.1.5) in Daqu produced in summer contributes to the accumulation of acetoin and tetramethylpyrazine<sup>[79]</sup>, thus enhancing the flavor characteristics of Chinese Baijiu, which was related to the significant enrichment of *Bacillus* in Daqu<sup>[16]</sup>.

Over the longer term, the adaptability of microbes to temperature may arise from differences in community composition. Oliverio et al.<sup>[80]</sup> noted that microbial communities may form adaptations to specific temperature conditions through long-term natural selection. In the short term, microorganisms can respond to temperature changes through physiological regulation<sup>[81]</sup>. Over time, however, those species that can thrive at the new temperatures will gradually dominate, leading to species replacement and changes in community composition, which ultimately manifest as an increase in the relative abundance and activity of warm-adapted species<sup>[80]</sup>.

#### 4.6 Moisture content/water activity

Water serves as an important medium for both intracellular and extracellular reactions, facilitates the movement of solutes and cells, and is essential for sustaining life activities. Previous studies have shown that the influence of moisture content/humidity on solid-state fermentation is multifaceted<sup>[82]</sup>.

Firstly, moisture content directly influences the composition of microbial communities by regulating the availability and diffusion of nutrients, oxygen supply, and heat dissipation<sup>[83]</sup>. Secondly, moisture content indirectly influences microbial competition and succession among microorganisms<sup>[82]</sup>. Strains best adapted to specific humidity conditions often dominate the entire ecosystem and shape the flavor characteristics of products through their metabolites<sup>[82]</sup>. Conversely, excessively low water activity and water content are detrimental to the growth and reproduction of strains. The water activity value of spring Daqu was higher than that of summer Daqu, potentially explaining the higher *Saccharomyces* content in spring compared to summer. This balance between yeasts and bacteria was observed<sup>[82]</sup>. As such fluctuations can significantly enhance the survival of microorganisms, especially yeast, under extreme water content variations and promote the production of certain Maillard reaction products<sup>[84]</sup>. The water activity value of Daqu in spring is significantly higher than that in summer, which could partly explain why the *Saccharomyces* content in spring was higher than that in summer<sup>[85]</sup>. Lastly, moisture content directly influences both the yield and quality of the product during solid-state fermentation<sup>[82]</sup>. Given the unique ability of water to dissolve a variety of compounds, it serves as a channel for flavor molecules, and influences the extracellular excretion of aroma, as well as the separation and purification of flavor compounds<sup>[86]</sup>. Therefore, maintaining appropriate moisture content is crucial for successful fermentation and high-quality product production<sup>[82]</sup>.

#### 4.7 pH and acidity

Variations in environmental pH profoundly affect microbial communities, with every 1.5 pH unit change imposing a significant constraints on individual microbes<sup>[6]</sup>. Consequently, pH could drive the diversity, interaction, and stability of the microbial communities<sup>[6]</sup>. It has been reported that the bacterial community structure will undergo significant changes after a high-acidity and low-pH incubation period, showing typical acidophilic and aerobic communities<sup>[87]</sup>. A low-pH environment can effectively inhibit the growth of unnecessary microbes, thus providing a favorable environment for the propagation of lactic acid bacteria and yeast<sup>[31,88]</sup>. However, it is worth noting that the growth of the *Lactobacillus* is inhibited at pH levels below 4.6<sup>[89]</sup>.

While many bacterial taxa exhibit relatively limited pH tolerance, fungi generally have a wider optimal pH range and respond differently to acidity<sup>[88]</sup>.

Two main explanations elucidate how pH affects the diversity and composition of microbial communities<sup>[90]</sup>. Firstly, pH can directly or indirectly affect numerous physical and chemical properties, such as the availability of nutrients, the properties of organic carbon, and the solubility of cationic metals, and these indirect effects can lead to changes in microbial communities. Secondly, *in situ* pH value can directly exert physiological pressure on microorganisms<sup>[91]</sup>, and when the pH value exceeds the suitable range of microorganisms, those hard-to-adapt microbial communities will be inhibited, thus changing the structure and composition of the entire community. Together, these two hypotheses reveal the complexity and multifaceted nature of pH in shaping microbial communities.

#### 4.8 Nutrient substance

Various materials are employed in the production of Baijiu, each containing unique types and concentrations of substances, which ultimately affect the microbial community and function<sup>[92]</sup>, as well as the flavor of production<sup>[37,93]</sup>. Raw materials with high levels of carbohydrates, specific proteins, and tannins can provide a rich source of nutrients for microorganisms<sup>[2]</sup>. Previous studies have examined the impact of cereals type on the fermentation process. For instance, in LFB fermentation experiments using 2 varieties of barley (Heilaoya and Duli Huang), it was found that *Komagataella*, which is closely related to ester compounds, dominated the fermentation process of Hyaloo, while *Pichia*, which is closely related to carbonyl compounds, dominated the fermentation process of Duli Huang<sup>[93]</sup>. Moreover, different varieties of sorghum result in varying glucose profiles, among which glucose, maltose, fructose, and arabinose were significantly correlated with microbial and metabolic diversity during fermentation<sup>[37]</sup>. These sugars have specific effects on microbial growth and metabolism, for example, *Pichia* grew better in environments with higher glucose, while *Komagataella* prefers environments rich in fructose<sup>[93]</sup>. Sugars, especially glucose and maltose, play a key role in regulating microbial interactions between *Saccharomyces* and *Lactobacillus*<sup>[94]</sup>. Bacteria and fungi utilize distinct pathways to metabolize glucose, fructose, and arabinose for ethanol production<sup>[11]</sup>. Importantly, arabinose is a key pentose in the ethanol metabolism of *S. cerevisiae*<sup>[95]</sup>. Research has revealed that arabinose can extensively regulate the cellular metabolic pathway and stimulates gene expression related to sugar transport and transcriptional regulation in fungal strains<sup>[96]</sup>. Therefore, microbial preference and metabolism varied with different sugar profiles, which might explain how the sugar profile can regulate microbial diversity.

#### 4.9 Oxygen

Dissolved oxygen, a key factor in the fermentation system, profoundly impacts the growth and metabolic processes of microorganisms, thereby determining their relative abundance in the system<sup>[97]</sup>. Natural gaps within the piled fermented grains facilitate the flow of dissolved oxygen, enriching the accumulated grain pile with dissolved oxygen<sup>[15]</sup>. During the initial stage of fermentation in the cellars, when microbial activity is not yet at its peak, dissolved

oxygen levels are relatively high. However, with the gradual vigorous growth of microorganisms, their consumption of dissolved oxygen also increases, resulting in a significant decrease in dissolved oxygen levels during fermentation<sup>[79]</sup>.

### 5. Perspective and summaries

Despite research on regional and flavor variations in Baijiu, the specific impacts of environmental factors on fermentation and their optimization for efficiency are not well understood. The complex dynamics between microbes and their environment are challenging to quantify. In order to accurately quantify and regulate the effects of these environmental factors, we urgently need to conduct more systematic and in-depth research, we propose the following considerations.

#### 5.1 Simulation and optimization of fermentation processes

The domain of spontaneous fermentation, represented by Chinese Baijiu, encompasses challenges such as raw material selection and proportioning, process control, and production environment management. To enhance efficiency, simulated fermentation research can be carried out. Computational biology offers a robust framework for simulating microbial succession and predicting shifts in microbial diversity across varying conditions. This approach not only forecasts the emergence of flavor compounds under different fermentation scenarios but also lays a theoretical foundation for optimizing the fermentation process to achieve desired flavor profiles. Moreover, by constructing a virtual fermentation platform, we can expedite the screening and refinement of fermentation parameters, leveraging computer simulations to complement traditional experimental methods.

#### 5.2 Development and utilization of regional microbial resources

The distinctive geographical characteristics of Chinese liquor are significantly influenced by the diversity of regional microbial resources. To effectively utilize the potential of these microorganisms, systematic geographic surveys can be conducted to analyze their distribution and patterns. The establishment of a resource bank rich in regional microbial strains can provide a wide variety of strains for liquor production. Adapting the production process to the unique attributes of these regional microorganisms and investigating whether the regional characteristics of the product can be enhanced. It is also crucial to understand the interactions between microorganisms and their environments, as this can help determine how environmental conditions can be manipulated to promote the proliferation of beneficial microorganisms.

#### 5.3 Intelligent control technology for fermentation environment

Intelligent production is becoming the core trend of modern manufacturing industry. Integrating sensor technology for real-time monitoring of fermentation parameters such as temperature, humidity,

pH, and dissolved oxygen minimizes disruptions from sampling while enabling precise process control. Meanwhile, machine learning models, such as neural networks and deep learning, are developed to better predict the development trend and potential problems of the fermentation process. By harnessing big data analytics, we can aggregate and scrutinize the vast datasets generated during fermentation. This data-driven approach uncovers underlying patterns and trends, facilitating more informed decision-making and refining the fermentation process for optimal outcomes.

## 6. Conclusion

Although spontaneous fermented food production has a long history and profound practical experience, there are still many challenges to achieve precision production. Environmental factor management is an important way to regulate the dynamics of microbial communities. To improve the environmental stability of spontaneous fermentation, it is necessary to quantify the interaction between key influencing factors and microorganisms to achieve automatic control and standardized production.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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