



Deciphering the core microbes and their interactions in spontaneous *Baijiu* fermentation: A comprehensive review

Jiamu Kang^{a,b,c}, Xiaoning Huang^{a,d}, Rengshu Li^{a,b}, Yuandi Zhang^{a,b}, Xiao-Xue Chen^{a,b,*}, Bei-Zhong Han^{a,b,*}

^a Beijing Laboratory for Food Quality and Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China

^b Key Laboratory of Precision Nutrition and Food Quality, Department of Nutrition and Health, China Agricultural University, Beijing, China

^c School of Food Science and Engineering, Hainan University, Haikou, China

^d Department of Bioengineering, University of Illinois Urbana-Champaign, Urbana, IL, USA



ARTICLE INFO

Keywords:

Baijiu
Microbiome
Microbial interaction
Synthetic microbiota
Fermented foods

ABSTRACT

The spontaneous *Baijiu* fermentation system harbors a complex microbiome that is highly dynamic in time and space and varies depending on the *Jiugu* starters and environmental factors. The intricate microbiota presents in the fermentation environment is responsible for carrying out various reactions. These reactions necessitate the interaction among the core microbes to influence the community function, ultimately shaping the distinct *Baijiu* styles through the process of spontaneous fermentation. Numerous studies have been conducted to enhance our understanding of the diversity, succession, and function of microbial communities with the aim of improving fermentation manipulation. However, a comprehensive and critical assessment of the core microbes and their interaction remains one of the significant challenges in the *Baijiu* fermentation industry. This paper focuses on the fermentation properties of core microbes. We discuss the state of the art of microbial traceability, highlighting the crucial role of environmental and starter microbiota in the *Baijiu* brewing microbiome. Also, we discuss the various interactions between microbes in the *Baijiu* production system and propose a potential conceptual framework that involves constructing predictive network models to simplify and quantify microbial interactions using co-culture models. This approach offers effective strategies for understanding the core microbes and their interactions, thus beneficial for the management of microbiota and the regulation of interactions in *Baijiu* fermentation processes.

1. Introduction

Alcohol fermentation, one of humanity's earliest food-processing techniques, holds immense significance in the development of world civilization. Notably, alcoholic beverages have had a profound impact on the social, economic, and political aspects of human society (Dietler, 2006). Ancient China is renowned for its unique methods of alcohol fermentation (Liu et al., 2019). Alcoholic beverages have played a vital role in China's civilization history, occupying a significant position in the cultural and spiritual lives of the Chinese people. *Baijiu*, which is translated as Chinese liquor, is one of the oldest distilled alcoholic beverages with a 2000-year history. Alongside brandy, whisky, vodka, rum, and gin, *Baijiu* is recognized as one of the world's six most renowned spirits (Jin, Zhu, & Xu, 2017; Zheng & Han, 2016). According to the data from the China alcoholic drinks association and the National

Bureau of Statistics of China, in 2023, the annual output of large-scale *Baijiu* enterprises was 4.492 million kiloliters, while the industry's total sales revenue reached 756.3 billion Yuan (RMB).

Unlike Western alcoholic beverage production, where yeast is added to sugar solutions for fermentation, *Baijiu* fermentation is performed by solid/ semi-solid/ liquid-state fermentation and involves a complex microecosystem with diverse microbiota (Jin et al., 2017). This process relies on a specialized saccharifying and fermenting starter called *Jiugu* (*Daqu*, *Xiaoqu*, and *Fuqu*), unique environmental microbiota, and various innovative regional fermentation techniques. Complex microbial communities endow fermented products with unique flavor characteristics, leading to the high international reputation of *Baijiu* (Liu & Sun, 2018; Zheng & Han, 2016). Given the significant impact of the brewing microbiota on *Baijiu* flavor quality, extensive research has focused on discerning species variation during *Baijiu* fermentation.

* Corresponding authors at: P.O. Box 398, 17 Qinghua East Rd., Beijing 100083, China.

E-mail addresses: chen.xx@cau.edu.cn (X.-X. Chen), hbz@cau.edu.cn (B.-Z. Han).

Technological advancements have successfully addressed the limitations of traditional culture-dependent methods, resulting in a clear understanding of both the structural composition of microbial communities and the metabolic processes of functional microorganisms during *Baijiu* fermentation (Jin et al., 2017; Kang, Xue, Chen, & Han, 2022d; Wu, Zhu, Fang, Wijffels, & Xu, 2021; Zou, Ye, & Zhang, 2018).

To provide a comprehensive overview of *Baijiu* brewing microbiome research, a dataset of relevant studies from the past decade was collected using the Web of Science. The VOSviewer software tool was then used to extract key terms from the dataset's title and abstract fields. Co-occurrence network analysis of the resulting dataset (Fig. 1) revealed 228 terms, 2 clusters, 13,224 links, and a total of 179,108 link strengths, highlighting two main aspects of the research: the fermentation microbiome (green line) and flavor science (blue line). However, while there is extensive focus on the microbial diversity and succession dynamics in the spontaneous fermentation of *Baijiu*, research remains mostly descriptive, providing limited insight into the interaction mechanism (Tu et al., 2022; Wang, 2022). The present research on the *Baijiu* brewing microbiome lacks sufficient investigation of microbial interactions and community assembly in distinct habitats. The knowledge gap presents a major challenge to comprehending the enigma of *Baijiu* brewing and applying effective fermentation management strategies.

As in any natural ecosystem, the microorganisms in fermentations demonstrate a range of interactions, regardless of whether they were brought in the substrate or employed as a starter culture (Ivey, Massel, & Phister, 2013). These intricate microbe-microbe interactions are believed to be fundamental in achieving the desired product qualities. Additionally, microbial interactions are mediated by numerous physiological and molecular mechanisms (Smid & Lacroix, 2013). *Baijiu* brewing necessitates the cooperation of several microbial species, each producing desired compounds that are taken up, used, and transformed into new chemical compounds vital to other species in the community (Kang et al., 2022d; Lin, Du, Wang, Wu, & Xu, 2022; Wu et al., 2021). Despite recent advancements in identifying the diversity, function, and community regulation of microbial species associated with *Baijiu* fermentation (Gao, Zhou, & He, 2022; Tu et al., 2022; Wang, 2022). There remains a critical knowledge gap regarding the specific role of core microbes, their intricate interactions, and their impact on *Baijiu* quality and fermentation process efficiency. This knowledge gap highlights the urgent requirement to clarify the specific core microbes and their interspecific interactions that play a crucial role in *Baijiu* brewing.

Our review not only aims to elucidate the core microbiotas, their functions, and their interactions in *Baijiu* fermentation but also proposes a novel framework strategy for dissecting complex microbial interactions in this complex process. By doing so, we provide unique insights and innovative directions for the fermentation industry, enhancing the understanding and optimization of *Baijiu* production.

2. Overview of core microbiota and function in *Baijiu* fermentation

Baijiu can be categorized into twelve types based on flavor characteristics: sauce-flavor, strong-flavor, light-flavor, rice-flavor, *feng*-flavor, sesame-flavor, miscellaneous-flavor, medicine-flavor, *te*-flavor, *chi*-flavor, *laobaigan*-flavor, and *fuyu*-flavor *Baijiu* (Zheng & Han, 2016). The unique flavor of your favorite *Baijiu* is likely attributed to its humble microbiota. It is generally accepted that the diverse flavor profiles of *Baijiu* are linked to its distinct microbiome. The flavor of *Baijiu* emerges from the collaborative metabolic activities of a complex microbial community. The type, quantity, distribution, and succession of microbes during fermentation directly impact the flavor's quality. Variations in raw materials, *Jiugu* types, fermentation containers, and production methods, influenced by the natural environment and geographical location, result in distinct flavors with distinct characteristics and styles (Liu & Sun, 2018; Tan et al., 2022; Tu et al., 2022). Consequently, the management of microbiota for the purpose of stabilizing fermentation quality is an essential process for improving the *Baijiu* quality.

Many researchers in the past have utilized pure culture technology to explore microbial diversity in *Baijiu* fermentation, leading to the accumulation of significant microbial strain resources in various laboratories. However, due to the enormous range of microorganisms involved in *Baijiu* production and the complex succession of microbial communities, traditional culture methods alone fall short in fully elucidating the intricate microbial dynamics. In recent years, the advancement of molecular biotechnology, high-throughput sequencing, *meta*-omics technologies, and multi-omics techniques has replaced traditional culture methods and become widely adopted for studying multi-species fermentation in *Baijiu* production (Kang et al., 2022d). Novel strategies have been developed to cultivate microorganisms that were previously uncultivable using conventional methods, leading to the revival of culturomics (Xu et al., 2020). Pure culture-based studies, ranging from monocultures to synthetic microbiota, have the potential to uncover the

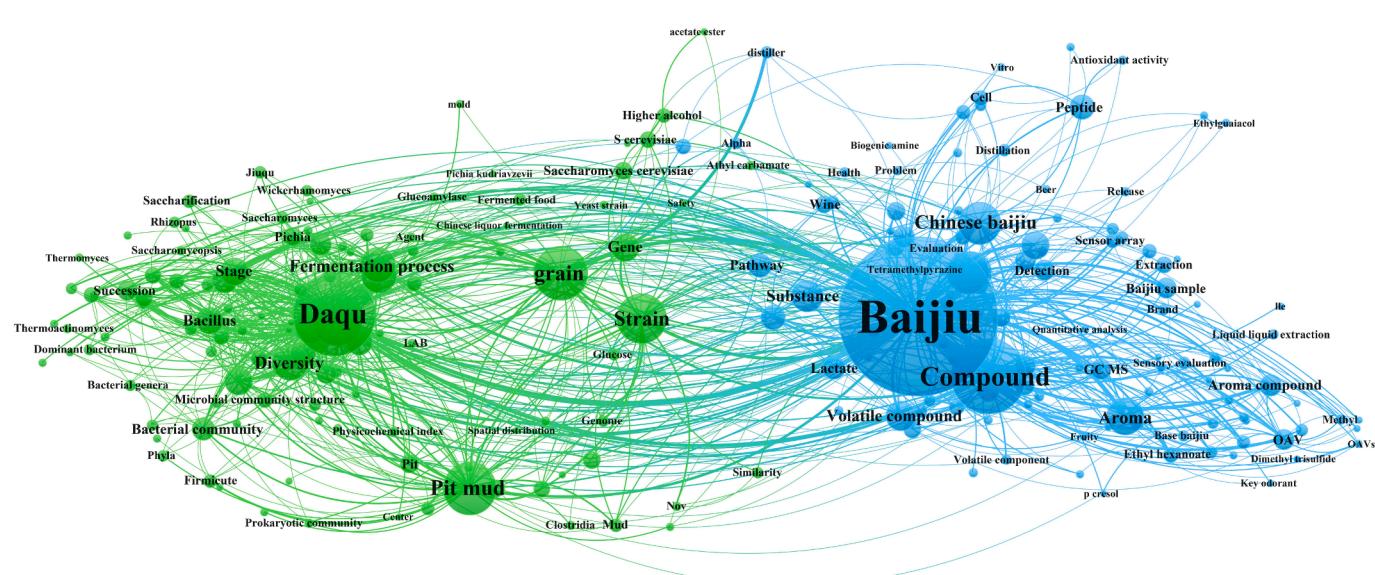


Fig. 1. The bibliometric analysis entailed the utilization of the Visualization of Similarities (VOS) viewer tool to generate a network diagram representing the co-occurrence of key terms related to *Baijiu* research.

interspecific interactions and fermentation dynamics of core microorganisms (Kang et al., 2022d).

Defining and quantifying the core fermenting microbiome is a fundamental step in mining tractable microbiota as models for research. Beyond the dominant microbes with large biomass and major energy transformations, core microbiome can orchestrate microbial communities to perform ecosystem processes, and drive community composition and function regardless of their abundance (Neu, Allen, & Roy, 2021). Core assignments based on operational taxonomic units/ampli-con sequence variants (OTUs/ASVs) have been criticized, and core assignments based on function have been suggested as an alternative (Custer, Gans, van Diepen, Dini-Andreote, & Buerkle, 2023). We describe five core microbiome categories in *Baijiu* ecological fermentation system according to the description of Risely (2020). The abundance core microbiome refers dominant microbes with high relative or absolute abundance only, which is also the most common defining method in *Baijiu* microbiome research (Hu et al., 2021; Kang et al., 2022b; Kang et al., 2022e). The common core microbiome is comprised of the most widely distributed microbes within various fermentation elements (Du, Wu, & Xu, 2020; Kang et al., 2021). The temporal core microbiome uncovers microbes that are temporally stable or predictable during fermentation (Zhang et al., 2021b). The ecological core microbiome can be described as microbiota that have a significant degree of impact on the microbial ecological structure (Zhang, Tan, Wei, Du, & Xu, 2022; Zhang et al., 2021c). In addition, we highlight a core microbiome with native ecological adaptation that consists of indigenous microbes commonly found in *Baijiu* ecosystems (He et al., 2019; Pang et al., 2021; Tan et al., 2022; Wang et al., 2020b). A functional core microbiome aims to identify microbes and their genomes that play a role in important metabolic pathways in fermentation systems (Huang et al., 2020; Pan et al., 2022; Wang, Wu, Xu, & Sun, 2020a). This is the ultimate objective of many studies employing the core microbiome idea. Although the core microbiome described by several approaches may exhibit variations in composition and function, we highlight a group of core microbes that are widely distributed and are vital for *Baijiu* fermentation. Therefore, the strategy for defining the core microbiome should be selected based on the goal of the research and the appropriate microecosystem for *Baijiu* brewing.

In order to gain a deeper understanding of spontaneous fermentation in *Baijiu* production, we have conducted a review of recent studies and compiled a comprehensive list of the core microbes reported in various types of fermentation systems used for the *Baijiu* production (Table 1). Our analysis reveals that lactic acid bacteria, *Bacillus* spp., yeasts, and filamentous fungi are widely found microorganisms in *Baijiu* fermentation (Fig. 2). Furthermore, it has been observed that *Clostridium* spp. are commonly in the strong-flavor *Baijiu* ecosystem and is recognized as a significant contributor to flavor profiles. The roles of these microorganisms in the *Baijiu* fermentation process are further discussed.

2.1. Lactic acid bacteria

Lactic acid bacteria (LAB) are Gram-positive, low-GC, acid-tolerant, non-sporulating, non-respiring rods or cocci, which produce lactic acid as the primary metabolic product of carbohydrate fermentation (Fig. 2). Some LAB are homofermentative, producing lactic acid as the primary byproduct of glucose fermentation, while others are heterofermentative, also generating carbon dioxide and ethanol (Liu, Han, & Zhou, 2011). LAB are the predominant bacteria used in *Baijiu* fermentation due to their physiological characteristics, such as substrate consumption, metabolic capacities, reduction of unfavorable ingredients, and production of aroma or flavor (Huang et al., 2021; Zhao et al., 2020). *Lactobacillus* genus is one of the core members of the microbial community in various *Baijiu* fermentation, becoming the most dominant microorganism during mid and late fermentation of fermented grains, with *Lactobacillus acetotolerans* being particularly dominant. Although initially challenging to cultivate, repeated subculturing on standard

Table 1
Core microbes (genus level) during fermentation of different types of *Baijiu*.

Types	Main elements	Main microbes	References
Sauce-flavor <i>Baijiu</i>	<i>Daqu</i>	LAB: <i>Enterococcus</i> , <i>Pediococcus</i> , and <i>Lactobacillus</i> ; Other bacteria: <i>Staphylococcus</i> and <i>Bacillus</i> ; Filamentous fungi: <i>Trichoderma</i> , <i>Aspergillus</i> , and <i>Thermomyces</i> ; Yeasts: <i>Candida</i> ; LAB: <i>Pediococcus</i> and <i>Lactobacillus</i> ; Other bacteria: <i>Bacillus</i> , <i>Lentibacillus</i> , <i>Kroppenstedtia</i> , <i>Oceanobacillus</i> , and <i>Virgibacillus</i> ; Filamentous fungi: <i>Thermomyces</i> , <i>Byssochlamys</i> , <i>Thermoascus</i> , and <i>Aspergillus</i> ; Yeasts: <i>Pichia</i> and <i>Saccharomyces</i> ;	Jin et al. (2019)
Stacking fermentation		LAB: <i>Pediococcus</i> and <i>Lactobacillus</i> ; Other bacteria: <i>Bacillus</i> , <i>Lentibacillus</i> , <i>Kroppenstedtia</i> , <i>Oceanobacillus</i> , and <i>Virgibacillus</i> ; Filamentous fungi: <i>Thermomyces</i> , <i>Byssochlamys</i> , <i>Thermoascus</i> , and <i>Aspergillus</i> ; Yeasts: <i>Pichia</i> and <i>Saccharomyces</i> ;	Wang, Huang, and Huang (2021a) and Hao et al. (2021)
Solid-state fermentation in the pit	<i>Daqu</i>	LAB: <i>Lactobacillus</i> ; Yeasts: <i>Pichia</i> and <i>Saccharomyces</i> ;	Zhang et al. (2021c)
Strong-flavor <i>Baijiu</i>		LAB: <i>Lactobacillus</i> , <i>Weissella</i> , and <i>Pediococcus</i> ; Other bacteria: <i>Saccharopolyspora</i> , <i>Thermoactinomyces</i> , <i>Proteus</i> , <i>Serratia</i> , and <i>Bacillus</i> ; Filamentous fungi: <i>Thermoascus</i> , <i>Thermomyces</i> , and <i>Aspergillus</i> ; Yeasts: <i>Saccharomyces</i> and <i>Issatchenkia</i> ;	Ma et al. (2022b)
Solid-state fermentation in the pit		LAB: <i>Lactobacillus</i> and <i>Weissella</i> ;	Qian et al. (2021) and Hu et al. (2020)
Pit mud		Other bacteria: <i>Bacillus</i> and <i>Acetobacter</i> ; Filamentous fungi: <i>Aspergillus</i> and <i>Thermoascus</i> ; Yeasts: <i>Saccharomyces</i> , <i>Kazachstania</i> , and <i>Torulaspora</i> ;	Qian et al. (2021)
Huangshui		Bacteria: <i>Hydrogenispora</i> , <i>Proteiniphilum</i> , and <i>Clostridium</i> ; Yeast: <i>Kazachstania</i> ;	Gao, Wu, and Zhang (2020)
Light-flavor <i>Baijiu</i>	<i>Daqu</i>	Bacteria: <i>Lactobacillus</i> ;	Pang et al. (2018) and Fan et al. (2020)
Solid-state fermentation in the earthenware jars		LAB: <i>Lactobacillus</i> and <i>Leuconostoc</i> ; Other bacteria: <i>Streptomyces</i> , <i>Saccharopolyspora</i> , and <i>Bacillus</i> ; Filamentous fungi: <i>Rhizopus</i> ; Yeasts: <i>Saccharomyces</i> , <i>Pichia</i> , and <i>Wickerhamomyces</i> ;	Huang et al. (2020) and

(continued on next page)

Table 1 (continued)

Types	Main elements	Main microbes	References
Rice-flavor Baijiu	Xiaoqu	<i>Lichtheimia</i> , and <i>Rhizopus</i> ; Yeasts: <i>Saccharomyces</i> and <i>Pichia</i> ; LAB: <i>Weissella</i> , <i>Pediococcus</i> , and <i>Lactobacillus</i> ; Other bacteria: <i>Acetobacter</i> ; Filamentous fungi: <i>Rhizopus</i> ; Yeast: <i>Saccharomyces</i> . LAB: <i>Lactobacillus</i> ; Filamentous fungi: <i>Rhizopus</i> ; Yeast: <i>Saccharomyces</i> . LAB: <i>Lactobacillus</i> ; Filamentous fungi: <i>Rhizopus</i> ; Yeast: <i>Saccharomyces</i> . LAB: <i>Lactobacillus</i> ; Filamentous fungi: <i>Rhizopus</i> ; Yeast: <i>Saccharomyces</i> ; LAB: <i>Lactobacillus</i> ; Other bacteria: <i>Sphingobacterium</i> , <i>Brevibacterium</i> , <i>Acinetobacter</i> , <i>Saccharopolyspora</i> , and <i>Streptomyces</i> ; Filamentous fungi: <i>Rhizopus</i> , <i>Pseudoeurotium</i> , and <i>Aspergillus</i> ; Yeasts: <i>Naumovozyma</i> , <i>Saccharomyces</i> , and <i>Pichia</i> ; LAB: <i>Lactobacillus</i> and <i>Pediococcus</i> ; Other bacteria: <i>Bacillus</i> and <i>Streptomyces</i> ; Filamentous fungi: <i>Rhizopus</i> , <i>Thermoascus</i> , <i>Aspergillus</i> , and <i>Pseudoeurotium</i> ; Yeasts: <i>Naumovozyma</i> , <i>Saccharomycopsis</i> , <i>Pichia</i> , <i>Candida</i> , and <i>Saccharomyces</i> ; Bacteria: <i>Bacillus</i> , <i>Kroppenstedtia</i> , <i>Thermoactinomyces</i> , <i>Streptomyces</i> , <i>Lentibacillus</i> , and <i>Staphylococcus</i> ; Filamentous fungi: <i>Thermomyces</i> , <i>Thermoascus</i> , and <i>Aspergillus</i> ; Yeasts: <i>Saccharomyces</i> and <i>Hansenula</i> ; LAB: <i>Lactobacillus</i> , <i>Weissella</i> , and <i>Leuconostoc</i> ; Other bacteria: <i>Bacillus</i> , <i>Cellulosimicrobium</i> , <i>Acetobacter</i> , and <i>Citrobacter</i> ; Yeasts: <i>Isatchenka</i> , <i>Wickerhamomyces</i> , <i>Candida</i> , and <i>Saccharomyces</i> ; Bacteria: <i>Bacillus</i> , <i>Thermoactinomyces</i> , <i>Kroppenstedtia</i> , and <i>Saccharopolyspora</i> ; Filamentous fungi: <i>Aspergillus</i> , <i>Thermomyces</i> , <i>Rhizopus</i> ,	Hu et al. (2021)
Feng-flavor Baijiu	Daqu	Zhang and Yan (2019)	
Sesame-flavor Baijiu	Daqu and Fuqu	Chen et al. (2020a)	
Miscellaneous-flavor Baijiu	Daqu	Hu et al. (2017)	

Table 1 (continued)

Types	Main elements	Main microbes	References	
		Solid-state fermentation	and <i>Monascus</i> ; Yeasts: <i>Candida</i> ; LAB: <i>Lactobacillus</i> ; Other Bacteria: <i>Bacillus</i> , <i>Virgibacillus</i> , <i>Acetobacter</i> , and <i>Thermoactinomyces</i> ; Filamentous fungi: <i>Paecilomyces</i> , <i>Thermomyces</i> , <i>Thermoascus</i> , and <i>Monascus</i> ; Yeasts: <i>Saccharomyces</i> , <i>Issatchenka</i> , and <i>Zygosaccharomyces</i> ; Various bacteria; Filamentous fungi: <i>Monascus</i> , <i>Rhizopus</i> , <i>Penicillium</i> , and <i>Geotrichum</i> ; Various yeasts; LAB: <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Weissella</i> , and <i>Enterococcus</i> ; Other bacteria: <i>Acetobacter</i> , <i>Enterobacter</i> , <i>Kroppenstedtia</i> , <i>Oceanobacillus</i> , and <i>Bacillus</i> ; Filamentous fungi: <i>Aspergillus</i> ; Yeasts: <i>Pichia</i> and <i>Saccharomyces</i> ; LAB: <i>Lactobacillus</i> and <i>Enterococcus</i> ; Other bacteria: <i>Bacillus</i> and <i>Acetobacter</i> ; Yeasts: <i>Saccharomyces</i> , <i>Candida</i> , <i>Pichia</i> , and <i>Wickerhamomyces</i> ; LAB: <i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Weissella</i> ; Other bacteria: <i>Bacillus</i> , <i>Acetobacter</i> , <i>Acinetobacter</i> , <i>Klebsiella</i> , and <i>Gluconobacter</i> ; Filamentous fungi: <i>Aspergillus</i> , <i>Rhizomucor</i> , and <i>Rhizopus</i> ; Yeasts: <i>Saccharomycopsis</i> and <i>Saccharomyces</i> ; LAB: <i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Weissella</i> ; Other bacteria: <i>Streptomyces</i> ; Filamentous fungi: <i>Rhizopus</i> and <i>Aspergillus</i> ; Yeasts: <i>Saccharomyces</i> , <i>Pichia</i> , and <i>Candida</i> ; LAB: <i>Lactobacillus</i> and <i>Weissella</i> ; Other bacteria: <i>Streptomyces</i> ; Filamentous fungi: <i>Rhizopus</i> , <i>Mucor</i> , and <i>Aspergillus</i> ; Yeasts: <i>Saccharomycopsis</i> and <i>Issatchenka</i> ; LAB: <i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Weissella</i> ; Filamentous fungi: <i>Rhizopus</i> and <i>Aspergillus</i> ;	Liu and Miao (2020)
	Medicine-flavor Baijiu	Daqu and Xiaoqu	An and Ma (2008)	
	Te-flavor Baijiu	Daqu	Chen et al. (2020b)	
	Chi-flavor Baijiu	Xiaoqu	Hong et al. (2021) and Li et al. (2020)	
	Laobaigan-flavor Baijiu	Daqu	Wang et al. (2020a) and Wang et al. (2018a)	
		Solid-state fermentation	Ma et al. (2020a)	
		Solid-state fermentation	Ma et al. (2020b)	

(continued on next page)

Table 1 (continued)

Types	Main elements	Main microbes	References
Fuyu-flavor Baijiu	Daqu and Xiaoqu	Yeast: <i>Saccharomyces</i> , <i>Issatchenka</i> , and <i>Candida</i> ; Bacteria: <i>Bacillus</i> , <i>Pantoea</i> , <i>Staphylococcus</i> , and unclassified Enterobacteriaceae; Filamentous fungi: <i>Rhizopus</i> ; LAB: <i>Weissella</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , and <i>Lactobacillus</i> ; Other bacteria: <i>Staphylococcus</i> , unclassified Enterobacteriaceae, and <i>Bacillus</i> ; Filamentous fungi: <i>Rhizopus</i> and <i>Aspergillus</i> ; Yeast: <i>Saccharomyces</i> , <i>Candida</i> , and <i>Pichia</i> ;	Kang et al. (2021)
	Solid-state fermentation	LAB: <i>Weissella</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , and <i>Lactobacillus</i> ; Other bacteria: <i>Staphylococcus</i> , unclassified Enterobacteriaceae, and <i>Bacillus</i> ; Filamentous fungi: <i>Rhizopus</i> and <i>Aspergillus</i> ; Yeast: <i>Saccharomyces</i> , <i>Candida</i> , and <i>Pichia</i> ;	Kang et al. (2022e)
Huangshui		LAB: <i>Lactobacillus</i> ; Filamentous fungi: <i>Aspergillus</i> ; Yeast: <i>Pichia</i> and <i>Kazachstania</i> ;	Kang et al. (2022c)

bacteriological media improves the cultivability of *L. acetotolerans* (Deng et al., 2014). In *Baijiu* fermentation, the ideal LAB strains for flavor development should not only be able to thrive in low pH and high ethanol fermentation conditions but also possess enzyme production capabilities and the ability to utilize sugar (Huang et al., 2021). Free-living LAB strains often experience acid stresses due to their production of organic acids (Gong, Ren, & Xu, 2020).

The rapid increase of *Lactobacillus* in relative abundance signifies a change in community composition and succession during strong-flavor *Baijiu* fermentation (Tan, Zhong, Zhao, Du, & Xu, 2019). The absence

of *L. acetotolerans* may be a significant factor contributing to the fermentation lag and reduced ester content (Pang et al., 2018). *Lactobacillus* also can be considered as an antimicrobial agent during alcoholic fermentation, while lactic acid creates a favorable environment for the development of aroma and fragrance (Du et al., 2020; Zhang, Du, & Xu, 2021a). The addition of indigenous *Lactobacillus* spp. to the fermentation process significantly alters the flavor profile of light-flavor *Baijiu* (Pang et al., 2021). *Weissella* genus, within the Leuconostocaceae family, consists of 19 recognized species. They are heterogeneous fermenters, producing carbon dioxide, D(+) or D(−)- and L(+)lactate, and acetic acid as the main end products of sugar metabolism (Fusco et al., 2015). During the summer solid-state saccharification phase, *Weissella* shows a rapid rise followed by a slight decrease, while remaining dominant throughout the winter, with a negative correlation to ambient temperature (Kang et al., 2022e). *Pediococcus* spp. have been found to synthesize excessive diacetyl, exopolysaccharides, and biogenic amines (Wade, Strickland, Osborne, & Edwards, 2019). Guan, Lin, Chen, Ou, and Zhang (2020) found that a significant negative correlation between *Pediococcus* and moisture, acidity, and alcohol during the fermentation of fermented grains in the production of strong-flavor *Baijiu*. Huang et al. (2021) found that *L. plantarum* strains exhibit higher ethanol and acid tolerances, while *Weissella* strains show relatively weak ethanol tolerance. Fortified inoculation of LAB strains into the native complex community enhance the concentration of flavor compounds, likely due to increased levels of ester precursors like lactic acid (Huang et al., 2021).

2.2. *Bacillus* spp.

Bacillus species are widely present in the production of fermented foods as aerobic, spore-forming, heat-resistant, rod-shaped bacteria. They possess the ability to hydrolyze protein and starch and metabolize various flavor components in *Baijiu* fermentation (Fig. 2). The production of organic acids varies during the fermentation of a liquid culture of *Daqu* by different *Bacillus* species (Yan, Zheng, Chen, Han, & Han, 2013). Moreover, *Bacillus* plays an important role in reducing lactic acid accumulation (Li, Li, Wang, Yang, & Zhao, 2017). *Bacillus* genera are

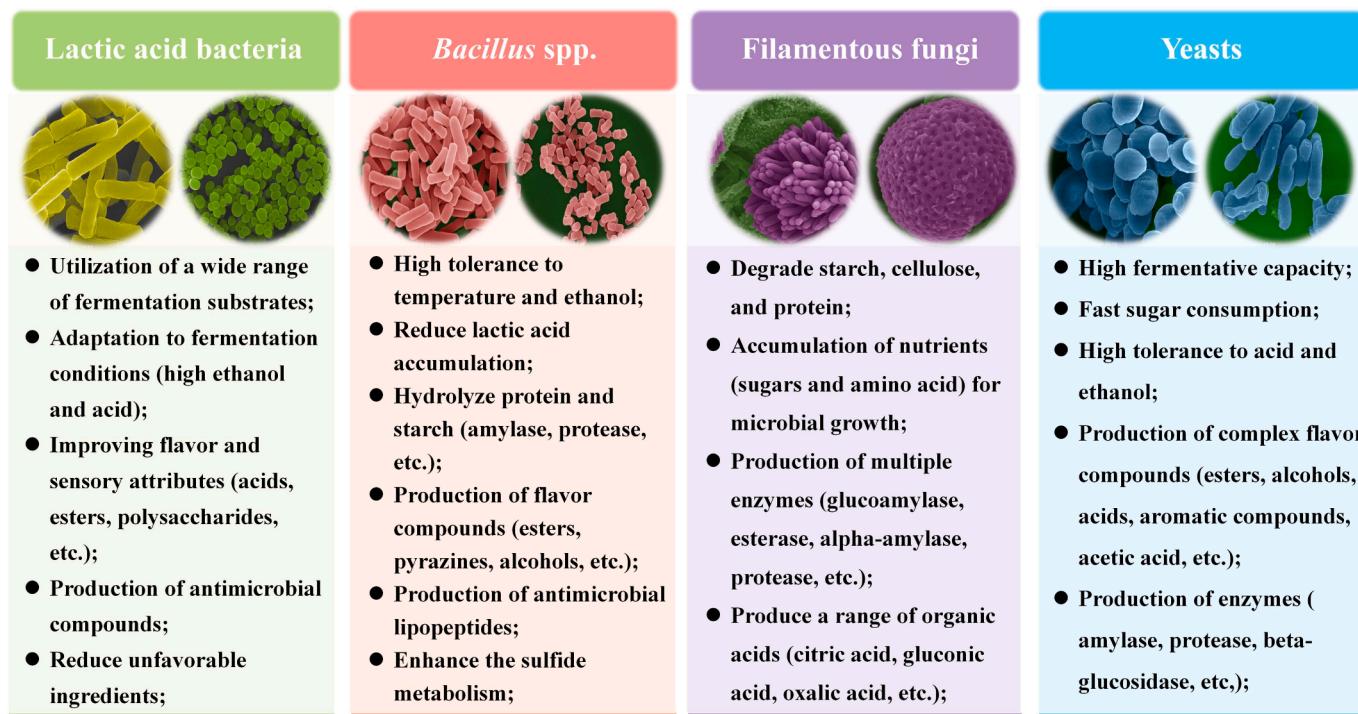


Fig. 2. Fermentation characteristics of core microbes in *Baijiu* ecological brewing system.

detected as major microbes in different types of *Daqu* (Kang, Chen, Han, & Xue, 2022a; Zheng, Tabrizi, Robert Nout, & Han, 2011). *Bacillus* spp. not only impact the generation of amylolytic and proteolytic enzymes during fermentation but also influence the composition of the microbial community through the production of antimicrobial lipopeptides (Li, Fernandez, Vederas, & Ganzle, 2023).

Several *B. licheniformis* strains isolated from *Daqu* demonstrate high ethanol tolerance, potentially attributed to the production of cell macrofibers and organized filamentous growth in response to ethanol stress (Li et al., 2018). The exceptional fermentation performance of *Bacillus* has prompted investigations into the biofortification of *Daqu* with *Bacillus* for *Baijiu* production. Bioaugmentation with *Bacillus velezensis* and *Bacillus subtilis* significantly increases the content of volatile compounds, including esters, pyrazines, and alcohols (He et al., 2019). In addition, *Bacillus* inoculation influences the microbial community structure and physicochemical and enzymatic properties of *Daqu*, leading to altered amylase and esterase activities and enriched pyrazines and flavor profiles (Wang et al., 2017a; Xu et al., 2022). *Bacillus* also plays a role in enhancing the sulfide metabolism, including pyrazines production (Shen, Liu, Wu, & Xu, 2020). In addition, *Bacillus* originating from wheat plays a regulatory and promotional role in microbial succession and metabolite accumulation during high-temperature *Daqu* incubation (Zhang, Kang, Han, & Chen, 2024).

2.3. Filamentous fungi

The fermentation of carbon sources by fungi for food and beverage production is one of the oldest and economically significant biotechnologies (Venturini Copetti, 2019). Filamentous fungi and yeast play crucial roles in different fermentation phases of *Jiuqu* and *Baijiu*, with the former breaking down macromolecules into sugars and amino acids that can be utilized by yeasts and bacteria (Fig. 2). Filamentous fungi are widely present in fermentation systems of 12 flavor types of *Baijiu*. Simultaneously, yeasts are responsible for alcohol and flavor compound production. Analysis of culturable molds in *Daqu* and environmental samples from seven fermentation rounds of sauce-flavor *Baijiu* revealed the dominance of certain molds such as *Lichtheimia*, *Mucor*, *Aspergillus*, *Monascus*, *Rhizomucor*, *Paecilomyces*, and *Schizophyllum* in the fermentation environment. Additionally, *Lichtheimia*, *Aspergillus*, *Penicillium*, *Phanerochaete*, and *Thermoascus* are the most prevalent molds in *Daqu* (Zhu & Huang, 2020).

During the open fermentation process, there is a significant interaction between macromolecules and molds in the environment. The increase in fermentation temperature leads to a selection of the microbiota in *Daqu*, resulting in the migration and enrichment of beneficial molds from the environment, raw materials, and external sources (Zhu & Huang, 2020; Zhu & Huang, 2021). *Jiuqu* is responsible for the majority of carbohydrate hydrolase production in *Baijiu* fermentation. α -amylase and glucoamylase from *Aspergillus*, *Rhizomucor*, and *Rhizopus* contribute to starch hydrolysis and ethanol production (Wang et al., 2020a). *Lichtheimia ramosa*, identified by Huang et al. (2020), is associated with cellulase and α -amylase production during the early stages of light-flavor *Baijiu* fermentation. Zhu et al. (2022) highlighted that molds involved in the saccharification stage primarily originate from *Jiuqu*, with some molds potentially coming from the surrounding environment. *Aspergillus* spp. dominate the fermentation process. While most isolated molds exhibit saccharification enzymes and cellulase production, their protease and pectinase production are limited. Most molds show high glucoamylase and protease pectinase activities, whereas their other enzyme activities are relatively low (Zhu et al., 2022).

2.4. Yeasts

Yeasts are eukaryotic microorganisms found in various ecological niches, including water, soil, air, and the surfaces of plants and fruits. Their most fascinating habitat is the latter, as they actively participate in

fermentation and the breakdown of ripe plant and fruit materials. Yeasts have the necessary nutrients and substrates in natural settings to carry out their metabolic and fermentation activities efficiently (Maicas, 2020). Yeasts are essential and prevalent in all 12 flavor-type *Baijiu* brewing. However, during *Baijiu* brewing, yeasts face environmental stressors such as ethanol stress, acid stress, and temperature stress, which can lead to reduced fermentation efficiency or cell death (Chen et al., 2022b). *Baijiu* fermentation systems serve as the primary reservoir of indigenous yeasts, and researchers have identified numerous key yeast species in the microecology of various *Baijiu* production (Fig. 2 and Table 1). These yeasts are categorized into *Saccharomyces* and non-*Saccharomyces* types, each playing distinct roles. Among them, *Saccharomyces cerevisiae* is considered the most important yeast species for alcoholic fermentation due to its ability to ferment various sugars into ethanol (Li et al., 2018). *S. cerevisiae* is a model organism for physiology, genetics, and molecular biology, and its genome was the first eukaryotic genome to be entirely sequenced. Duan et al. (2018) revealed that domesticated populations of *S. cerevisiae* originated in China. In *Baijiu* fermentation, *S. cerevisiae* is responsible for producing most of the higher alcohols. In yeast cells, the aromatic molecule arises from the biosynthetic or the Ehrlich pathway. In the Ehrlich pathway, the breakdown of branched-chain amino acids produces branched-chain alcohols such as isobutanol, isoamylol, active amyl alcohol, and β -phenyl ethanol (Li et al., 2017).

Non-*Saccharomyces* yeasts, on the other hand, have limited ethanol-producing capabilities but significantly impact *Baijiu*'s flavor profiles through their metabolite profiles. *Saccharomyopsis fibuligera*, an amylolytic yeast, dominates in *Jiuqu* starters and the early stages of *Baijiu* fermentation. It plays a critical role in converting starch into fermentable sugars, providing a substrate for *S. cerevisiae* and other microorganisms to produce alcohol and flavor compounds. This yeast secretes two main amylases, α -amylase and glucoamylase, which are responsible for starch breakdown (Wang et al., 2021c). *Pichia*, *Wickerhamomyces*, and *Candida* are involved in the production of aromatic compounds such as esters, acids, aldehydes, and higher alcohols during solid-state fermentation of *Baijiu*. *Pichia* is the predominant fungal genus throughout the fermentation process of sauce-flavor *Baijiu*, exhibiting heat resistance and strong competitiveness (Zhang et al., 2021b). It contributes to accelerated fermentation and helps maintain yeast diversity and ethanol production within the microbial community (Zhang et al., 2021a). *Wickerhamomyces anomalus* is known for its production of ethyl acetate, which adds a fruity, sweet aroma to the final product (Li et al., 2018). Bioaugmentation with *W. anomalus* can alter the ester content and other aromatic compound profile, affecting the microbial structure and interaction networks (Wang et al., 2020b). *Candida*, which shows seasonal variation in abundance, has been associated with the production of phenethyl alcohol, an important small signal molecule for microbial community control (Jin et al., 2019; Kang et al., 2022e).

Schizosaccharomyces spp. play significant roles in *Baijiu* fermentation, including acetic acid control, high ethanol production, and synthesis of 3-hydroxypropionic acid. They also help reduce the levels of harmful compounds that can degrade product quality, such as ethyl carbamate (Du, Song, Zhang, Nie, & Xu, 2021). Additionally, *S. pombe* possesses unique resistance to 10 g/L acetic acid in laboratory cultures and reduces the average concentration of acetic acid from 9.62 to 6.55 g/kg in fermented grains during the manufacture of sauce-flavor *Baijiu* (Song, Du, Zhang, Nie, & Xu, 2019). The absence or appearance of *S. pombe* caused the content divergence in 227 aroma-related metabolites, especially in ethanol, butanol, and pentanoic acid (Du et al., 2021). *Kazachstania* spp., specifically *K. turicensis* and *K. humilis*, have been observed to exhibit distinct growth characteristics and functions during *Baijiu* fermentation, with differences in organic acid production, ethanol production, and the synthesis of specific aroma compounds (Yi et al., 2021).

2.5. Other core microbe—*Clostridium* spp.

Clostridium, a Gram-positive bacterium, is an anaerobic or obligate anaerobic organism with a range of metabolic activities. These activities include the conversion of starches, proteins, and purines into organic acids (such as acetic, butyric, and caproic acid), as well as alcohols, CO₂, and H₂. *Clostridium* is known to be a characteristic microorganism involved in the brewing of strong-flavor *Baijiu* and important contributor to the major flavor compounds found in *Baijiu*, such as ethyl caproate (Zou et al., 2018). In the brewing fermentation cellar for strong-flavor *Baijiu*, pit mud serves both as a source of inoculum and a habitat for microbes (Tao et al., 2014). *Clostridium* is identified as one of the core genus in the microbial community of pit mud (Zou et al., 2018), and it has been utilized for inoculation in pit mud or fermented grains to enhance the flavor and quality of *Baijiu* (Guan et al., 2023; Sun et al., 2022a). Numerous studies have shown that *Clostridium* is significantly enriched in aged pit mud, with percentages ranging from 1.12 to 12.71 % in 1-year-old to 50-year-old pit mud (Ren et al., 2024; Tao et al., 2014; Zheng et al., 2015). Therefore, the presence of *Clostridium* is considered an important biomarker for evaluating the quality of pit mud (Mao et al., 2023).

The natural maturation process of pit mud microbiota during the *Baijiu* brewing process is extremely slow, requiring a minimum of 20 years of uninterrupted domestication to produce high-quality *Baijiu* (Mao et al., 2023). Consequently, research on artificial foundation pit mud (APM) has gained prominence. Zhou et al. (2024) examined the impact of fortified pit mud and found that inoculation with *Caproicibacterium lactatfermentan* led to increased production of fatty acids and ethyl esters in the short term. However, long-term use resulted in a decrease in these compounds. Moreover, there are interplays between *Clostridium* species, hydrogen-producing *Clostridium*, methanogenic archaea, and other microorganisms such as *Bacillus*, which can enhance the production of caproic acid and maintain the stability of the strong-flavor *Baijiu* ecosystem (Zou et al., 2018).

3. Source of microbes that drive the *Baijiu* fermentation

Microbiome data mining is aimed at estimating the proportions of microorganisms in a community from various source environments. However, tracing the sources of microbiota poses several challenges. To address this, various strategies have been developed to analyze the contribution of potential source settings to a community sample accurately and efficiently. One unique tool, SourceTracker, utilizes a Bayesian methodology to estimate the origin of microorganisms by employing community structure and similarities between sink communities and potential source settings (Knights et al., 2011). Du, Wang, Zhang, and Xu (2019) conducted a SourceTracker analysis to identify the fungal communities in *Daqu*, such as *S. fibuligera*, *P. kudriavzevii*, *Rhizopus oryzae*, *Sterigmatomyces elviae*, *Aspergillus flavus/oryzae*, *Hyphopichia burtonii*, and *Lichtheimia corymbifera*, originating mainly from *Daqu*-making tools and indoor ground. Bacterial communities in *Daqu* primarily originated from raw materials, such as Gammaproteobacteria, Alphaproteobacteria, and Bacilli. The contribution of wheat to the bacterial community differed between the inner and outer parts of fresh or mature *Daqu*, while *Staphylococcus*, *Saccharopolyspora*, and *Kroppenstedtia* were main bacteria in *Daqu* sourced from wheat (Zhang et al., 2021d). Zhou et al. (2021) found that bacterial communities in new *Daqu* are primarily contributed by raw materials (89.3 %), followed by straw mats (5.6 %). Fungal communities, on the other hand, mainly originate from outdoor ground (55.18 %) and indoor roof (23 %) in new *Daqu*.

During alcoholic fermentation, the contributions of different sources to the bacterial and fungal communities were explored. Cool grain and rice husk were major contributors to the bacterial community during saccharification, while the saccharification fungi primarily sourced from the *Qu* starter (Kang et al., 2021). The starter primarily provides the

saccharification microbiota, while environmental microorganisms dominate the microbial fermentation community. Specifically, 79.5 % and 59.3 % of the bacteria in the saccharification microbiota originated from single (pure) and mixed (traditional) species starters, respectively. Within the pure starter group, 73.5 % of the bacteria came from the fermentation environment. During the fermentation stage, the pure starter and traditional starter accounted for approximately 50.0 % and 48.0 % of the fungi in the saccharification community, respectively. On the other hand, the environment contributed 95.2 % and 98.5 % of the fungal community in the pure starter and traditional starter groups, respectively (Shen et al., 2021). Wang, Du, Zhang, and Xu (2018b) indicated that environments (outdoor ground, indoor ground, tools, and other unknown environments) contributed 62.61 to 90.90 % of bacterial communities and 20.00 to 38.94 % of fungal communities to fermentation. In contrast, *Daqu* contributed 9.10 to 27.39 % of bacterial communities and 61.06 to 80.00 % of fungal communities. Zhang et al. (2021b) reported that *Daqu* supplied 95.6 % of the bacteria and 28.10 % of the fungi to heap fermentation of sauce-flavor *Baijiu* production, whereas the surroundings (indoor ground and tools) supplied 71.9 % of the fungal populations (mostly *Pichia*).

Tan et al. (2019) reported that different proportions of bacteria are given by pit mud in fermented grains made from various raw materials. *Clostridiales* is the most abundant enriched bacterial order from pit mud in all fermented grains, and Group F, which employs sorghum, corn, wheat, rice, and glutinous rice as fermentation raw materials, has the highest ratio of that among the three groups. Zhao et al. (2022) found that *Daqu* (72.66 %) and pit mud (24.54 %) are the main sources of the abundant fungal taxa, while pit mud (65.92 %) and air (27.31 %) are the main sources of the rare fungal taxa for fermented grains.

While SourceTracker has proven to be valuable, its computational cost limits its application primarily to small to medium-sized datasets. As an alternative, the Fast expectation–maximization microbial source tracking (FEAST) system offers scalable estimation of microbial contributions from thousands of possible sources (Shenav et al., 2019). Overall, the use of SourceTracker has provided insights into the origin and migration of microorganisms in *Baijiu* brewing systems. Both raw materials and the environment contribute to the microbiome of *Baijiu* brewing, with *Daqu* provides significant microbial resource for alcoholic fermentation. Environmental microorganisms undergo significant enrichment during the raw material pretreatment stage, thus playing a role in the solid-state fermentation process. However, accurate source tracing and assessment throughout the entire fermentation process remain limited. To further understand the complex nature of *Baijiu* brewing microorganisms and improve traceability, a multi-genomic approach is necessary to study their origin, migration principles, and contributions in the brewing microecology.

4. Microbial interaction in *Baijiu* fermentation systems

Microbial interaction is the major driver for the assembly and functioning of complex microbial communities (Liu et al., 2020a). Ecological interactions in nature between microbial pairs can be characterized using six possible distinct patterns (Fig. 3), including neutralism, commensalism, amensalism, competition, mutualism, and parasitism (Smid & Lacroix, 2013). Microbiology's most-studied interaction is beneficial mutualism. Commensalism is an interaction form where one party benefits and the other does not. Commensalism and mutualism can also include microorganisms consuming inhibitory metabolites. However, amensalism is a partnership where one microbe is disadvantaged and the other neither gains nor is injured. Predator-prey or parasitism occurs when one member benefits and another member is disadvantaged (i.e., bacteria and viruses) (Zuniga, Zaramela, & Zengler, 2017).

Interactions patterns, such as cooperation or commensalism, are ubiquitous in microbial communities and are found to be essential in shaping succession of microbial ecosystems (Liu et al., 2020a). For

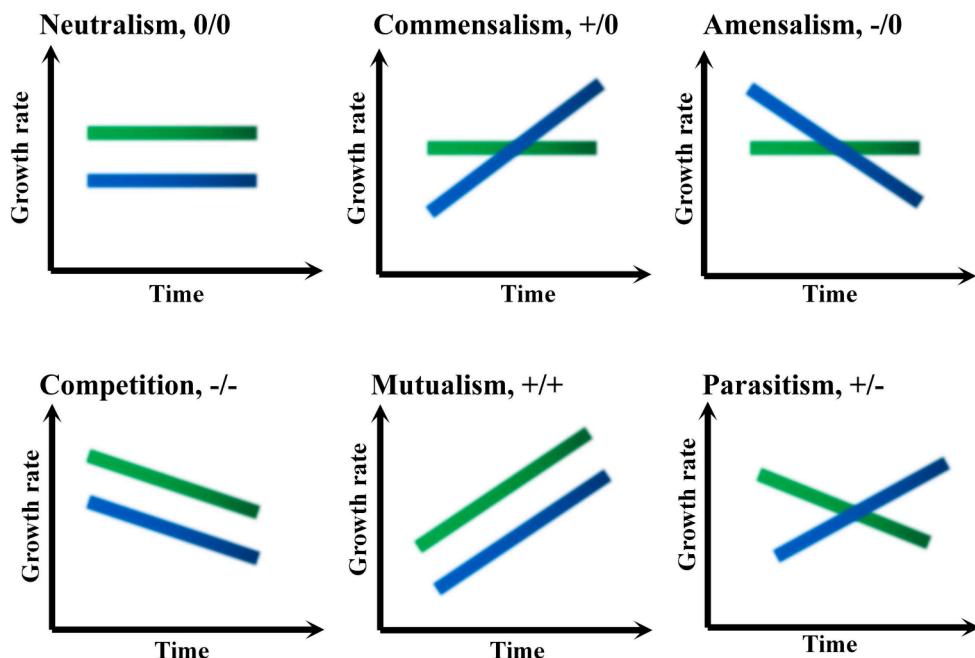


Fig. 3. The conceptual model of ecological interaction between microbial pairs in nature. The green and blue lines each represent a species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

instance, commensalism between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* during yogurt fermentation (Arioli et al., 2017); and between LAB in kefir fermentation to sequentially colonize milk (Blasche et al., 2021). The microbial interaction in *Baijiu* fermentation is also the main determinant of the final product. To unravel the interaction between individual species in *Baijiu* fermentation, an understanding of the role of individual microbes within the community was firstly needed. However, in multi-species fermentation systems with complex microbial interactions, the characteristics of individual species are sometimes quite different. Therefore, dissecting the interaction patterns also offer insight into microbial function identification for *Baijiu* microbes.

Fig. 4 depicts the interaction network between bacterium (pink), filamentous fungus (gray), yeast (blue), and phage (green), as well as their potential driver factors including initial microbial community, fermentation parameters, and biotic and abiotic factors. In addition, we describe and summarize the findings for yeast–yeast interactions, bacterium–yeast interactions, bacterium–bacterium interactions, filamentous fungus–filamentous fungus interactions, filamentous fungus–bacteria interactions, and filamentous fungus–yeast interactions, as well as bacterium–phage interactions during *Baijiu* fermentation, mainly focusing on microbial growth performance and metabolite formation.

4.1. Yeast-yeast interactions

Baijiu fermentation involves various yeast species exhibiting numerous interaction patterns, which contribute to ethanol production and flavor formation. Some yeasts growth simultaneously during fermentation, and some show an antagonistic relationship with each other. The competition between *S. cerevisiae* and non-*Saccharomyces* yeasts has been reported many times, for example, *S. cerevisiae* inhibits the growth of *W. anomalus* (Zha, Sun, Wu, Yin, & Wang, 2018) and *Issatchenkia orientalis* (Wu, Ling, & Xu, 2014) when co-cultured in simulated *Baijiu* fermentation, that positively affecting aroma formation. *S. cerevisiae* enhance *W. anomalus* production of ethyl acetate at an inoculation ratio of 3:1 in liquid and simulated solid-state *Baijiu* fermentation, although they compete for nutrients (Fan et al., 2019a).

Furthermore, β-phenethyl alcohol and phenethyl acetate, which are also *Baijiu* flavor compounds are increased simultaneously (Fan et al., 2019b). The findings are consistent with the co-culture results performed by Zha et al. (2018). In mixed fermentation experiments, the combination of *W. anomalus* and *S. cerevisiae* produced more ethanol and ethyl acetate in a 1:1 ratio. Moreover, the primary metabolites' accumulation could influence microbial interactions, and adding L-phenylalanine to the fermentation medium enhanced the final product flavor (Zha et al., 2018). The functional cooperation displayed by the genera *Zygosaccharomyces*, *Pichia*, *Schizosaccharomyces*, and *Saccharomyces* proved critical in maintaining a high level of 6-phosphofructokinase expression. The supplementation of several metabolic pathways reflects such functional cooperation (Zhang et al., 2022). Deng, Du, and Xu (2020) demonstrated that the synergistic effect of *P. kudriavzevii* and *S. cerevisiae* significantly improved the lactic acid tolerance of *S. cerevisiae* monoculture. *P. kudriavzevii* reduced the lactic acid concentration at first, thereby facilitating *S. cerevisiae* to grow. Transcription analysis found that co-culture resisted acid stress mainly through lactate catabolism, H⁺ extrusion, and glycerol transportation (Deng et al., 2020).

In summary, through yeast-yeast interaction, some metabolites produced by one yeast species can promote the metabolism of other yeasts to produce more esters. The interaction between *S. cerevisiae* and non-*Saccharomyces* during alcoholic fermentation is proven to be species or strain-specific, so the indigenous functional strains' screening and selection are vital for *Baijiu* quality enhancement. Furthermore, the microbial interaction could be a guide during functional yeasts identification, avoiding strain mutation under long-term stress. Meanwhile, various environmental factors, such as acid, temperature, and ethanol, may act as key determinants in microbial interactions. Yeast interactions enable the population to collectively regulate gene expression in response to environmental factors, leading to changes in the microbial structure dynamics.

4.2. Yeast-bacterium interactions

The interactions between yeast and bacteria during fermentation directly affect *Baijiu* quality. Various studies have reported that there are

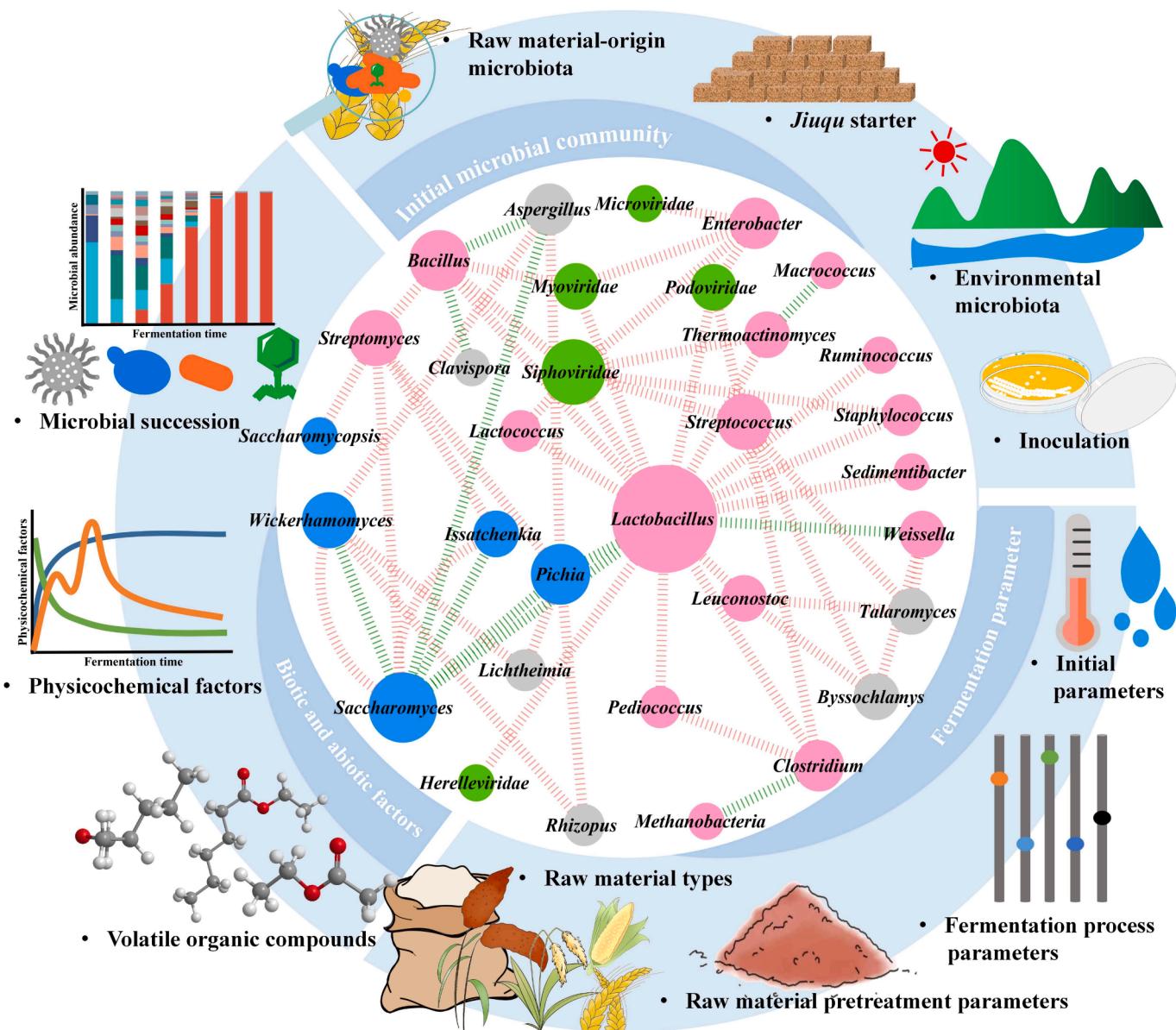


Fig. 4. Microbial interaction network in *Baijiu* brewing microecology and its driving factors. For the interaction partner, there show two possible outcomes: positive (green) and negative (red), the size of the network nodes represents the number of interaction relationships. The pink, gray, blue and green circles represent bacteria, filamentous fungi, yeast, and phage, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interactions involving many species. Tang et al. (2019) found that *Lactobacillus* was positively correlated with *Pichia* but negatively correlated with other microbes in *Xiaoqu*. During medium-temperature *Dagu* fermentation, a higher co-occurrence incidence among the species from yeasts and LAB were indicated (Du et al., 2019). During light-flavor *Baijiu* fermentation, the co-occurrence network showed that *Lactobacillus* and *Saccharomyces* are mainly negatively correlated with other dominant microbes. However, they showed a positive correlation with each other (Wang, Wu, Nie, Wu, & Xu, 2019).

Although LAB contribute to the formation of aromatic compounds, the accumulation of lactic acid could also inhibit ethanol production during fermentation. Conversely, the environmental adaptability and growth of LAB are enhanced through its co-culture with *S. cerevisiae* (Mounier et al., 2008). Chen et al. (2022a) found that co-cultivation of LAB with *S. cerevisiae* enhances the synthesis of esters, whereas co-cultivation of *L. acetotolerans* or *Leuconostoc mesenteroides* with *P. anomala* enhances the production of esters and alcohols. LAB have a

modest inhibitory effect on the growth of yeast, while yeast significantly inhibits the growth of LAB in a co-culture system (Chen et al., 2022a). Besides esters, sulfur compounds also play an important role in *Baijiu* fermentation. *Saccharomyces* and *Lactobacillus* were correlated with sulfur metabolism by determining both transcript abundance and distribution frequency of each genus in *Baijiu* fermentation. This prediction for sulfur metabolism was proved using an *S. cerevisiae* JZ109 and *Lactobacillus buchneri* JZ-JN-2017 co-culture. Their co-culture significantly increased the production of 3-(methylthio)-1-propanol and dimethyl disulfide. Additionally, co-culture significantly enhanced the gene expression of precursor methionine for sulfur compound production by *S. cerevisiae* and the methyl cycle of *L. buchneri*. This study demonstrated a synergistic interaction between *Saccharomyces* and *Lactobacillus* for sulfur compound production (Liu et al., 2017).

Geosmin, an earthy off-aroma compound, is primarily synthesized by *Streptomyces* spp. during *Baijiu* fermentation, significantly impacting the quality of the final product. *Streptomyces sampsonii* demonstrates potent

antibiotic activity against various microorganisms involved in *Baijiu* production, such as *S. cerevisiae*, *P. anomala*, *I. orientalis*, and *S. fibuligera*, with the identified antibiotics belonging to the heptaene macrolide compound class (Du, Lu, & Xu, 2015). The *Daqu* microbes have inherently developed a distinct community structure. A study by Du, Wei, Zhang, and Xu (2022) highlighted that indigenous functional microorganisms, such as *S. cerevisiae* Y8-2 and *P. fermentans* Y1B-2, exhibit the ability to restrict the growth of *Streptomyces albus*. This indicates their efficacy as biocontrol agents in minimizing *Streptomyces* contamination and geosmin production during *Daqu* fermentation. Moreover, the fungal community plays a critical role in the initial stages of fermentation, with the dynamic between *Pichia* and *Streptomyces* transitioning from indirect promotion to direct inhibition upon introduction of geosmin-suppressing microorganisms (Du et al., 2022). During *Baijiu* fermentation, *Bacillus* sp. is inhibited by *S. cerevisiae*, while *S. cerevisiae* is barely influenced by *B. licheniformis*. The co-culture influenced several flavor compounds produced by *S. cerevisiae*, including fatty acids, their corresponding esters, and terpenes. Although *P. membranifaciens* and *B. amyloliquefaciens* are not efficient aroma producers in their monocultures, their addition alleviated competition among *S. cerevisiae*, *I. orientalis*, and *B. licheniformis*, and altered their growth rates and aroma production (Wu et al., 2014).

Yeast–bacterium interaction is a complex field of study prevalent in fermentation. Possible symbiotic mechanisms between yeast and bacteria include the formation of physical complexes, nutritional interactions, cross-feeding, antibiosis, signaling-based interactions, and horizontal gene transfer (Alvarez-Perez, Lievens, & Fukami, 2019). Numerous metabolites or signals from yeasts/bacteria that are involved in stimulating or inhibiting bacteria/yeasts are still unidentified. Subsequent studies could elucidate the key factors or metabolites that prompt these interactions. Moreover, the ultimate objective could center on uncovering how these metabolites or factors can be utilized for *Baijiu* production through the screening or engineering of strains and the regulation of fermentation conditions to guarantee successful *Baijiu* fermentation.

4.3. Bacterium–Bacterium interactions

Despite the fact that *Baijiu* is produced by alcoholic fermentation, bacterial strains such as LAB, particularly *Lactobacillus* and *Pediococcus*, dominate at a later stage of alcoholic fermentation, whereas *Bacillus* dominates in mature *Daqu*. A previous study on co-occurrence networks demonstrated that bacteria have a higher degree of network connectivity compared to fungi (Huang et al., 2017; Kang et al., 2022b); thus, bacteria's intra- and interspecies interactions should not be ignored.

Huang et al. (2017) compared the microbiome of medium- and high-temperature *Daqu* microbiome during *Daqu* fermentation. They found that at the early stage of fermentation, a substantial majority of co-occurring taxa are related to *Weissella* and *Lactobacillus*, while at the middle stage, they are connected to *Thermoactinomyces* and *Macroccoccus*. Jin et al. (2019) demonstrated that microbial interaction is stronger in the core than on the surface in *Daqu*. The co-exclusion of *Lactobacillus* with *Thermoactinomyces* are likely related to the highly reproducible succession of medium-temperature *Daqu* microbiota (Yang et al., 2021). *B. subtilis*, and *B. amyloliquefaciens* isolated from *Daqu* showed an antagonistic interaction with *Streptomyces sampsonii*. The antimicrobial substances-lipopeptides produced by *Bacillus* could inhibit *Streptomyces* and geosmin production (Zhi, Wu, Du, & Xu, 2016).

The pit mud microbial community is also an important reservoir for strong-flavor *Baijiu* brewing. *Clostridium* is in high co-occurrence with the Methanobacteria, which is also predominant in pit mud, but has significantly robust negative correlations with LABs, such as *Lactobacillus*, *Pediococcus*, and *Streptococcus* (Hu, Du, Ren, & Xu, 2016). Wang, Du, and Xu (2017b) found that *Lactobacillus* was negatively related to *Clostridium*, *Ruminococcus*, *Sedimentibacter*, *Syntrophomonas*, *Thermoactinomyces*, *Leuconostoc*, *Pediococcus*, *Staphylococcus*, *Bacillus*, and

Lactococcus in fermentation grains. In contrast, p-cresol, as the main off-odor and toxic compound in the strong-flavor *Baijiu*, is mainly produced by *Clostridium* in pit mud. *Clostridium* is negatively correlated with *Lactobacillus* (Du, Liu, Wang, & Xu, 2017). In a study by Sun et al. (2022a), it was observed that co-culturing two pit mud clostridial strains, *Clostridium fermenticellae* (JN500901) and *Novisynthrophicoccus fermenticellae* (JN500902), led to an increase in the accumulation of short- and medium-chain fatty acids. Their findings suggested that formic acid produced by *C. fermenticellae* stimulated the Wood-Ljungdahl pathway of *N. fermenticellae*, resulting in an enhanced production of acetic acid. Subsequently, the acetic acid is converted to butyric acid and caproic acid by *C. fermenticellae* through reverse β-oxidation (Sun et al., 2022a).

Bacteria are widespread in the microecology of *Baijiu* brewing, with each species influencing the growth and survival of the others. The impact of these bacterial interactions on the brewing microbiota varies. Multiple interactions, such as reciprocity, exploitation, and competition, exist among bacterial species in the same habitat, and as environmental factors vary, so do the relationships among bacteria. As a strategy for fermentation management, intra- or inter-specific bacterial interactions can be reasoned. Thus, mutually restrictive microbial interactions in fermentation drive the microbial and metabolic succession and determine the quality of *Baijiu*. That is also why traditional starters could not be replaced by artificial construction microbiota yet.

4.4. Filamentous fungus–filamentous fungus interactions

Filamentous fungi degrade raw materials such as sorghum and wheat into fermentable sugars by providing multiple enzymes, such as α-amylase, glucoamylase esterase, and proteases, which become aromatic substrate precursors for bacteria and yeast and conduct alcoholic fermentation for *Baijiu*. There are numerous species of filamentous fungus in the microecology of *Baijiu* brewing, and their roles vary. Environmental filamentous fungi have a crucial role in the microbiome assembly of *Jiuqu* and the initial stages of alcoholic fermentation. Filamentous fungi undeniably partake in complex interspecific interactions.

Wang et al. (2021b) isolated 75 mold strains from *Daqu*, and further screened out an *Aspergillus niger* strain with high glucoamylase production, and investigated its interaction with the dominant *Rhizopus oryzae* (high protease production) in *Daqu*. They found that under various inoculation ratios, the enzyme activities, organic acids, and flavor profiles formed by the interaction of the two strains are significantly distinct. In addition, rice bran fermented with *Rhizopus oligosporus* and *Monascus purpureus* has more phenolics and antioxidants, while vanillic, caffeic, and 4-hydroxybenzoic acids increase following rice bran fermentation (Abd Razak, Abd Rashid, Jamaluddin, Sharifudin, & Long, 2015). These studies have demonstrated that the interaction of filamentous fungi can positively affect enzyme production, the release of phenolic compounds, and the formation of aroma compounds. Currently, there is also a shortage of studies on the interaction between filamentous fungi. It is important to explain the interaction between some famous mold strains for the study regarding the bioaugmentation of *Jiuqu*.

4.5. Bacterium–filamentous fungus interactions

In the study conducted by Du et al. (2019), the process of strong-flavor *Daqu* manufacturing was monitored, revealing a negative correlation between thermotolerant filamentous fungi, specifically *Talaromyces* and *Byssochlamys*, and bacteria such as *Weissella*, *Leuconostoc*, and *Streptococcus*. Additionally, a negative relationship was observed between these fungi and *B. amyloliquefaciens*, as well as the majority of LAB.

As fermentation proceeds, the environment seems harsh for filamentous fungi with relatively low pH, high acidity and ethanol concentration, and low oxygen. It was not suitable for the survival of

filamentous fungi. This could explain the results obtained by co-occurrence analysis, where filamentous fungi and bacteria were negatively correlated with LAB. However, the interaction benefits from fungi should not be ignored. The interaction between filamentous fungi and bacteria can promote bacteria performance. The *Aspergillus* and *Bacillus* co-culture had more *Bacillus*-produced flavor compounds (Wang et al., 2017a). Furthermore, it has been previously reported that filamentous fungi can create physical networks for motile bacteria dispersal in solid-state fermentation, which promotes the growth of other motile over non-motile bacterial members in the community (Zhang, Kastman, Guasto, & Wolfe, 2018). We speculate that filamentous fungi in *Daqu* or alcoholic fermentation could also act as a superhighway for various bacteria to move and communicate. In a laboratory-scale simulation of fermentation, benzyl alcohol generated by *Rhizopus microspores* significantly promote the growth of *Lactobacillus fermentum*. The addition of benzyl alcohol enhances the activation of alanine, aspartate, and glutamate metabolism as well as arginine production in order to promote the growth of *L. fermentum* (Hao et al., 2022). Thus, volatile organic compounds are important driving factors to affect the bacterium–filamentous fungus interactions and the flavor formation in *Baijiu* production.

4.6. Filamentous fungus–yeast interactions

While filamentous fungi and yeast are crucial in the initial and final stages of *Baijiu* production, the interaction between them remains poorly understood. The limited evidence showed that from co-occurrence analysis in *Daqu*, filamentous fungi, such as *Lichtheimia ramosa*, *R. oryzae*, and *A. flavus/oryzae*, are negatively related with *P. kudriavzevii* and *W. anomalus* (Du et al., 2019). During *Baijiu* fermentation, saccharification rate and flavor metabolites are affected by the interaction of *S. cerevisiae* and *A. oryzae*. When fermented at certain inoculum, *A. oryzae* produces large quantities of alcohols and esters compared to yeast only (Wu, Chen, & Xu, 2015). Also, Zhang et al. (2021a) identified the pairwise negative interactions between *Pichia* and filamentous fungi using the sauce-flavor *Baijiu* fermentation system as a model. They demonstrated that the antifungal activity of *Pichia* may be accomplished by volatile organic compounds, primarily 2-phenylethanol, by merging findings from an *in situ* community with reconstructions of an *in vitro* community. In addition, they found that a beneficial impact of this antagonistic interaction on the fermentation community.

4.7. Bacterium–phage interactions

Phages, the most abundant and diverse biological entities on Earth, have a significant impact on bacterial hosts. Bacteria-phage relationships in nature are complex and have wide-ranging effects beyond particular pairwise interactions (Diaz-Munoz & Koskella, 2014). Fermented foods provide a suitable environment for phages due to their rich and diverse microbial populations. Phages can either hinder fermentation by affecting the growth and metabolism of core fermenting microbes or enhance the fermentation process and improve food safety, with potential probiotic properties in modern fermented foods (Maske et al., 2021). The contamination of phages can pose a major challenge in processes requiring bacterial growth (Samson & Moineau, 2013). Natural phage populations serve as obligatory parasites, agents of horizontal gene transfer, drivers of bacterial evolution, and mediators of species competition, performing ecological functions such as direct regulation of bacterial populations and indirect impacts on non-host populations (Diaz-Munoz & Koskella, 2014; Paillet & Dugat-Bony, 2021).

The mere presence of bacteriophages in microbial cultures is insufficient to influence fermentation processes. The probability of fermentation failure depends on the microbial composition of the starter, with greater diversity reducing the risk of failure (Maske et al., 2021; Spus

et al., 2015). Disturbances caused by phages significantly impact the dynamics and metabolism of microbial communities in fermented beverages, particularly in relation to temperature. Variation in sugar consumption, organic acid formation, and the production of volatile organic compounds has been reported (Ledormand et al., 2022). Kang et al. (2022a) found that low-, medium-, and high-temperature *Daqu* are dominated by the *Genomoviridae*, *Mimiviridae*, and *Siphoviridae*, and *Parvoviridae* families, respectively. Among these, *Siphoviridae* has the highest number of hosts, with *Lactobacillus* being the dominant bacterial genus infected by *Siphoviridae* phages. Additionally, *Microviridae*, *Myoviridae*, *Podoviridae*, and *Siphoviridae* can use *Enterobacter* as a host, while *Myoviridae* and *Siphoviridae* phages can infect *Bacillus* (Kang et al., 2022a). Du et al. (2023) reported that interactions among members of the microbial community, including viruses, significantly influence the microbial structure during sauce-flavor *Baijiu* fermentation. Temperature and ethanol content are correlated with the viral community.

Previous studies have shown that interactions between bacteria and phages shape bacterial genomes, maintain diversity within and between bacterial populations, stabilize and influence microbial communities, and have far-reaching effects beyond the microbial community itself (Diaz-Munoz & Koskella, 2014). Although research on phages during *Baijiu* brewing is still in its early stages compared to bacterial and fungal communities and functions, exploring the distribution characteristics of phage communities will be crucial for managing the fermentation process and regulating the succession of functional microbes. For instance, studying *Streptomyces* phages in *Daqu* could be a strategy for controlling geosmin, while regulating *Lactobacillus* phages may help avoid excessive acid production during fermentation. Therefore, the advancement of computational, experimental, and comparative methods will unveil the extent and complexity of phage involvement in microbial communities.

5. Quorum sensing and microbial community interactions

Quorum-sensing (QS) microbes release chemical signal molecules, called autoinducers, whose concentration depends on cell-population density (Waters & Bassler, 2005). These molecules regulate gene expression and behavior in microorganisms, such as biofilm formation, bioluminescence, and secretion of virulence factors (Albuquerque & Casadevall, 2012; Papenfort & Bassler, 2016). Gram-negative bacteria use N-acylated-L-homoserine (AHL) as autoinducers, while Gram-positive bacteria use processed oligo-peptides (AIP). An interspecies communication system called autoinducer-2 (AI-2), a furanoyl borate diester quorum sensing system, is found in Gram-negative and Gram-positive bacteria (Whiteley, Diggle, & Greenberg, 2017). QS systems have also been described in fungi, including aromatic alcohols compounds in *Candida* and phenyl ethanol and tryptophol in *S. cerevisiae* (Albuquerque & Casadevall, 2012). Since QS is crucial for cellular communication and coordination, researchers have shown great interest in understanding how microbes interact using QS.

While QS is not extensively discussed in *Baijiu* fermentation, its significance has been demonstrated in other fermented foods like kimchi, sourdough, dairy products, and wine, suggesting a role for QS in food fermentation. The *luxS* gene involved in AI-2 synthesis has been identified in species from fermented food communities (Moslehi-Jenabian, Gori, & Jespersen, 2009; Qian, Kando, Thorsen, Larsen, & Jespersen, 2015). Different AI-2 signaling intensities in kimchi samples are found to affect the balance of microbiota (Park, Shin, Lee, & Holzapfel, 2016). In cocoa bean fermentation, metagenomic sequencing predicted an interspecific QS system in dominant genera with quorum quenching (QQ) potential, affecting bacterial dominance and maintenance in fermentation (Almeida et al., 2020). Similar findings were observed in rice wine fermentation, indicating QS-based communication between microbes had a role in rice wine fermentation (Hong et al., 2016). In the wine industry, the aromatic alcohols 2-phenylethanol, tryptophol and tyrosol, are shown to be regulated in *S. cerevisiae* as autoinducers, which would regulate the microbial interactions in fermentation, affecting

wine quality (Avbelj, Zupan, & Raspot, 2016).

Considering the progress of the QS system in fermented foods, it is plausible to speculate that QS exists in *Baijiu* fermentation as a means of microbial social communication. LAB, *Bacillus*, and *Saccharomyces* are common in *Daqu* and fermented grains, and aromatic alcohols are widely detected during *Baijiu* fermentation (Zhang et al., 2021a). Furthermore, biofilm formation during *Daqu* making may also be regulated by QS (Fan, Huang, Chen, & Han, 2020). Future *Baijiu* fermentation research should integrate “Omics” data with QS-related databases to explore the beneficial role of QS in maintaining and controlling the microbiome and regulating social interactions between microbes. In addition, horizontal gene transfer and mutation allow viruses and bacteria to update their genetic information together. Bacteria can hinder phage infections through QS mechanisms, while phages can manipulate bacterial QS systems to evade host defenses (Wang, Dai, Wang, Wang, & Tang, 2022). Therefore, understanding the processes of bacteria and phage interactions mediated by QS systems is vital for controlling *Baijiu* fermentation.

6. A framework for dissecting microbial interactions during *Baijiu* fermentation

Omics tools have significantly expanded our understanding of microbial diversity in *Baijiu* fermentation systems. However, surprisingly little is known about the complexity of the *Baijiu* brewing microbiome. The system exhibits high species diversity, limited cultivability, and challenges in simulating its environment. Microbes engage in intricate interactions with other members of their microbiota and their environments. Given the numerous species and potential interactions, deciphering the functions of the microbiome can be challenging. To overcome these obstacles, studying tractable microbial communities is crucial to understanding microbiome assembly and function (Kang et al., 2022d; Wu et al., 2021). Understanding microbial interactions is a fundamental step in dissecting, controlling, and manipulating the microbiome. Previous studies on microbial communities in *Baijiu*

fermentation have mainly focused on correlating core microbes with environmental variables or metabolites as abiotic interactions (Hao et al., 2021; Liu & Miao, 2020; Pang et al., 2020). While these studies have shed light on the contribution of environmental variables to microbial community structure and the role of individual species in metabolite production, they have provided limited insights into competitive levels or mutualistic interactions among coexisting species, including niche specialization and labor partition. Intraspecific and interspecific interactions play critical roles in driving community coexistence and stability, which are essential in understanding microbiome assembly. In this context, we propose a three-step framework (Fig. 5) for inferring microbial interspecies interactions based on microbial monoculture and co-culture behavior, as well as coculture growth outcomes: (i) Integrate environmental variables and omics datasets into a network model, (ii) Identify core microbiota from the complex microbiome via cultivation, and (iii) Construct a co-culture model to examine microbe-microbe interspecies interactions.

6.1. Interaction model construction

To understand microbiome interactions, it is necessary to employ existing methodologies that can explore microbial diversity, community structure, functioning, and activity. Recent advancements in omics technologies have enabled the characterization of intricate interaction networks within microbial communities. Therefore, integrative, multi-omics approaches and analyses are required to unravel the mechanisms and interactions that influence community composition and dynamics (Kang et al., 2022d). Co-occurrence networks constructed from microbial omics data are commonly used to uncover interactions among community members.

For *Baijiu* fermentation microbiome studies, the networks are all built with similarity-based methods, mainly using either Pearson or Spearman correlations for relative abundance data distribution (Hao et al., 2022; Kang et al., 2022b; Kang et al., 2022c; Liu et al., 2020b; Tan et al., 2019). For absolute abundance dataset, Spearman or Pearson

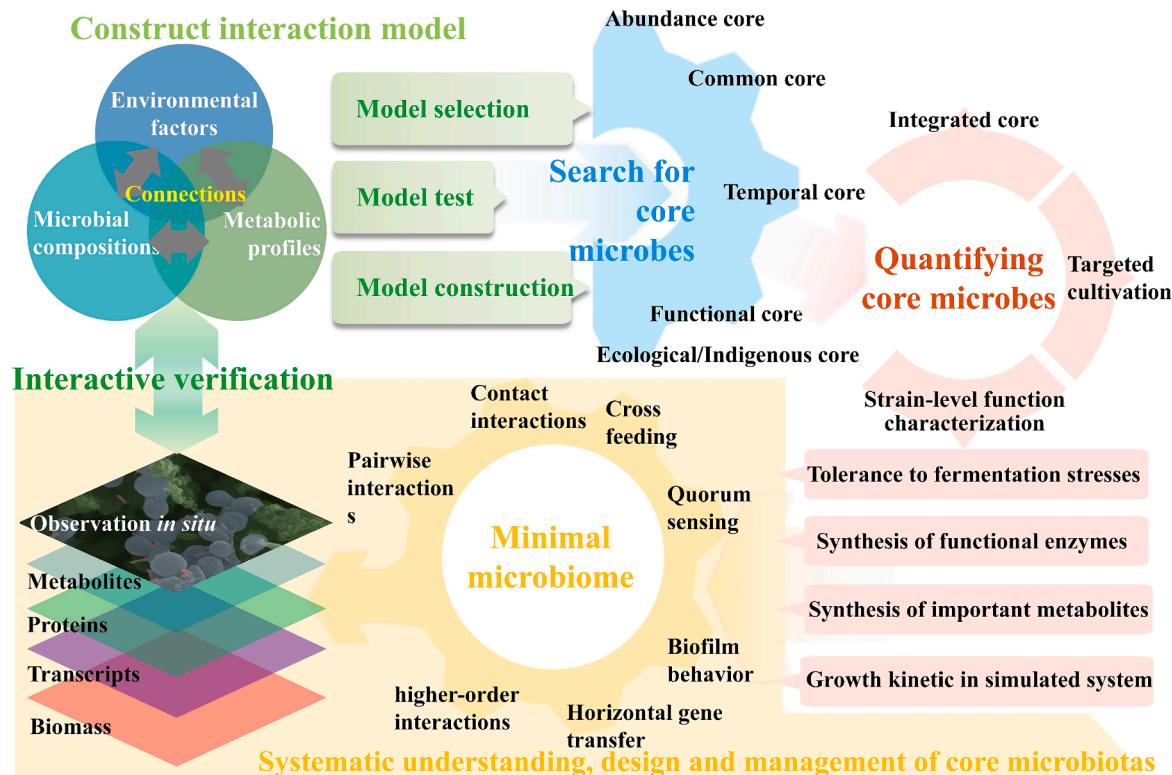


Fig. 5. Integrating experimental data, *in silico* analysis, and validation to dissect the microbial interactions in a simplified community.

correlation coefficients are better suited to detect interactions accurately than other measures (Berry & Widder, 2014). Besides the commonly used correlation coefficients, cutting-edge statistical tools like Sparse correlations for compositional data (SparCC) can be employed to dissect microbial interaction networks from sequencing data. SparCC calculates approximately linear correlations between microbial taxa based on log ratio abundances, and it has proven successful in analyzing human gut sequencing data (Biswas, McDonald, Lundberg, Dangl, & Jovic, 2016; Friedman & Alm, 2012). Du et al. (2023) employed the SparCC method to build a co-occurrence network encompassing core bacteria, fungi, and major viruses involved in the fermentation process of sauce-flavor *Baijiu*. They also revealed that approximately 50 % of the network's connections were associated with viruses, comprising 30.4 % virus–bacterium links and 10.1 % bacterium–fungus links. Phage–bacteria interaction networks are typically represented as bipartite networks (Parente, Zotta, & Ricciardi, 2022). Wang, Gao, and Zhao (2016) utilized CRISPR elements identified from sequencing reads and assemblies to create phage–bacterium infection networks, revealing the effects of specific phages on the microbial community and function.

Another useful method, sparse inverse covariance estimation for ecological association inference (SPIEC-EASI), has been used to study bacteria and fungi associations and has been applied to human lung and skin micro- and mycobiomes (Biswas et al., 2016). SPIEC-EASI constructs microbiome networks using sparse neighborhood and inverse covariance selection algorithms, and it could also be appropriate for analyzing microbial interactions in *Baijiu* fermentation systems (Layeghifard, Hwang, & Guttman, 2017). Lotka-Volterra models are commonly employed to predict interactions. The generalized Lotka-Volterra model uses a logistic model to simulate microbe development and determine the competitive, amensalistic, or predator-prey relationship between two species (Matchado et al., 2021). These models have been applied to study specific microbiomes such as the intestinal community or cheese microbiome to assess species interactions based on growth rate and population dynamics (Marino, Baxter, Huffnagle, Petrosino, & Schloss, 2014; Mounier et al., 2008). Dynamic Bayesian networks and temporal event networks are useful for studying the temporal changes in microbial data (Matchado et al., 2021). Inferring cross-feeding interactions is a crucial area of community research. Tools such as GutCP use metagenomic and metabolomic data along with machine learning optimization techniques to predict empirically untested cross-feeding interactions in the human gut microbiome (Goyal, Wang, Dubinkina, & Maslov, 2021).

Once the compositions and abundances of the core microbiota, along with the topological parameters of the inter-domain and cross-domain interaction network, are identified using interaction models, they can be employed to investigate the relationship between environmental factors. Canonical Correspondence Analysis (CCA), Redundancy Analysis (RDA), and distance-based RDA (db-RDA) are commonly used models to assess the correlation between core microorganisms and environmental factors (Kang et al., 2022e; Wang et al., 2019; Zhang et al., 2021c). In addition, the core microbial modules can be correlated with environmental factors through Mantel tests (Kang et al., 2022a; Ma et al., 2022a; Ren et al., 2024; Zhao et al., 2022). The correlation between the topological features of the microbial interaction network and environmental factors can be characterized using statistical methods such as Pearson correlation (Zhang et al., 2022). However, there is currently a lack of models that directly investigate the interactions of the core microbiota within complex fermentation microbiome systems, especially considering longitudinal changes over time and space, as well as responses to environmental fluctuations. Given the limited availability of sequencing data for *Baijiu* fermentation compared to gut or soil microbiota, it is crucial to develop a robust model that can accurately explain interaction patterns. To achieve this, collaboration among statisticians, bioinformaticians, computer scientists, mathematicians, microbiologists, and microbiome scientists is essential to overcome these challenges (Milligan-McClellan et al., 2022).

6.2. From complex communities to core microbes

Despite employing cutting-edge techniques in *Baijiu* research, it remains challenging to unravel the contributions of individual members in the complex interactions during fermentation. One promising strategy is to simplify the complex microbial system to focus on a few core members, which can help reduce complexity. However, identifying this core microbiome is no easy task. While some suggest that a computerized forecast approach is no longer effective, others argue that culturable core microbes should be emphasized. In Section 2, we outlined five broad definitions of the core microbiome to reduce complexity in *Baijiu* fermentation research and explore the correlation between the microbiome and environmental variables. Employing calculation model remains an ideal strategy to reduce complexity within the complex microbiome of *Baijiu* ecological fermentation. It's important to note that the core microbiome of an investigation is unlikely to include all key microbes. Together, these core members provide insights into multiple layers of microbial organization, revealing ecological and evolutionary mechanisms that influence important microbe interactions (Risely, 2020). Considering the cost of research, this complexity reduction method should be applied following the annotation of *meta*-omics data to identify significant samples from large-scale amplicon datasets. Conversely, functional annotation of *meta*-omics, particularly through logical integration studies, can provide a more accurate understanding of community functions.

Once omics and computational models shed light on the core microbiota within *Baijiu* fermentation habitats of interest, it becomes crucial to establish a lab-scale culture and screening system. Currently, there are two primary methodologies for efficiently isolating microbes. The first is high-throughput isolation and culture, which improves the chances of isolating interesting species. The second is targeted isolation, where species with specific functional traits or taxonomic classes are selectively isolated (Lewis, Tahon, Geesink, Sousa, & Ettema, 2021). High-throughput cultivation technologies, known as culturomics, have successfully cultured over 70 % of human intestinal bacteria. However, isolating certain key species may require more targeted approaches. These methods involve integrating ecology with metagenome-predicted nutritional requirements (Shetty, Smidt, & de Vos, 2019). Chai et al. (2019) used a 16S rRNA gene sequence-guided culture medium prediction strategy to obtain *Clostridium* spp. The KOMODO database accurately predicted 16 media, leading to the isolation of 17 strains of *Clostridium* spp. from fermented grains. Zou, Kou, and Han (2021) established the Chinese *Baijiu* Cultivated Microorganisms database (CMBaijiu, <https://cmbaijiu.i-sanger.com/>), which allows retrieval of cultivable microbe and medium information in the *Baijiu* fermentation system, as well as online uploading of new cultivable microbe and medium information. In-depth information and topic-specific objectives will undoubtedly assist researchers in identifying the most promising cultivation targets (Lewis et al., 2021). Once the target core microbes are obtained, it is recommended to characterize their fermentation performance using a laboratory-scale screening method. The evaluation of technological parameters involves assessing their tolerance to fermentation factor stress (such as temperature, ethanol, and acid), synthesis of functional enzymes, synthesis of important metabolites, and analyzing growth kinetics in simulated fermentation media (Huang et al., 2021; Li et al., 2018).

6.3. Validation of interactions by co-culture model

Using synthetic co-culture consortia is a valuable tool for studying microbial interactions and simplifying community dynamics. These consortia can be manipulated directly and their responses can be accurately measured in controlled laboratory conditions. A reductionist approach can be employed by identifying the key characteristics of natural microbiomes and constructing a minimal core consortium that retains these features. This core consortium captures the essential

metabolism of the indigenous microbiota and studying its functioning and interactions can provide insights into the natural microbiomes. To understand microbial composition and their metabolic changes, community-based assays are necessary. This is because microbial interactions often lead to new physiological activities that cannot be observed in monoculture. Several interactions in co-culture model, such as pairwise interactions, cross-feeding, quorum sensing, biofilm behavior, horizontal gene transfer, and higher-order interactions (HOIs), have been extensively characterized using a combination of microbiological, molecular biology, microscopy methods, and omics techniques.

The most commonly used method for determining microbial growth is the detection of colony forming units (CFU) on selective agar media (Chen et al., 2022a). This technique, based on the universal dilution series method, is time-consuming and some microbes are difficult to cultivate in the laboratory. Additionally, numerous microbes may transition into a viable but non-cultivable state. Contact-type interactions, especially the presence of microbial aggregates in biofilm systems, can strongly influence species quantification results. Alternatively, real-time quantitative PCR (RT-qPCR) allows for rapid measurement of specific microbial biomass within a community (Sun et al., 2022a). However, it cannot distinguish between live and dead cells. Propidium monoazide (PMA) combined with qPCR can be used to analyze the biomass of viable cells in a multi-species fermentation community. However, it has limitations such as changes in cell membrane permeability and false-positive signals (Liu et al., 2021). RNA-based qPCR methods are suitable for detecting specific live cells in a consortium, but they often face challenges in RNA extraction (Magalhaes, Franca, Pereira, & Cerca, 2019). Antibodies, fluorescent dyes, and flow cytometry can also differentiate between viable and non-viable yeast and bacterial populations in simultaneous fermentations (Rodríguez & Thornton, 2008).

Microscopy techniques have significantly advanced the observation of mixed microbial fermentation systems. Scanning electron microscopy is ideal for visualizing microbial morphology and interactions, particularly in bacterial adhesion and biofilm structures (Fan et al., 2020). Transmission electron microscopy enables the visualization of fine details, such as phages, surface features of cells, and bacterial appendages like flagella (Du et al., 2023). However, electron microscopy techniques have limitations when it comes to manipulating individual properties and interactions of different cell surfaces in their natural environments. Atomic force microscopy (AFM) provides this capability (Dufrene, 2004). AFM images and calculated cell surface roughness have revealed changes in the surface roughness of *S. cerevisiae* cells in coculture, influenced by the surrounding *L. plantarum* cells (Liu et al., 2022). Confocal laser scanning microscopy allows for the differentiation of live and dead cells and the examination of microbe distribution and interaction (Sun et al., 2022b). In addition, Muller, Neugeboren, Nelson, and Murray (2014) conducted a study utilizing two fluorescently labeled strains of *S. cerevisiae* yeast on agar surfaces to investigate the interplay between genetic drift and mutualism during spatial expansions. Their findings revealed that strong mutualism hampers genetic demixing, thereby preserving diversity. However, weak or asymmetrical mutualism succumbs to genetic drift, even when it remains beneficial, resulting in slowed growth and reduced diversity.

Omics techniques are crucial for uncovering the dynamic metabolic activities and interspecies interactions within the minimal core microbiome. For example, a comprehensive transcriptome-level investigation of the microbial interaction between *L. plantarum* and *S. cerevisiae* highlighted the involvement of the *lamBDCA* QS system in *L. plantarum*. This system enhances adhesion capability, leading to the aggregation of *L. plantarum* with *S. cerevisiae* (Liu et al., 2022). Transwell system provides a physical barrier between microbial populations while allowing chemical interactions through a shared medium reservoir. Exometabolites, including small compounds, extracellular enzymes, and antibiotics, can be measured using sensitive mass spectrometry in the

reservoir. Biomass measurements, flow cytometry, and transcript analysis may all be used to learn about the community's members and their effects on growth, productivity, and gene regulation (Chodkowski & Shade, 2017). Transwell coculture and transcriptome analysis have provided insights into the metabolite exchange between *C. fermenticellae* and *N. fermenticellae* (Sun et al., 2022a). Shetty et al. (2022) reconstructed a metabolic network using the KEGG ortholog annotations of metatranscriptomics data, and computed pairwise metabolic complementarity and competition using actively expressed genes, hence estimating "active" interspecies interactions. Metaproteomic approaches allow for determination of community structure, identification of *in situ* carbon sources utilized by the community members, and tracking the uptake of labeled substrates (Kleiner, 2019). Combining metagenomic data with functional layer metaproteomic data enhances our understanding of microbial symbiosis and their metabolic processes (Kang et al., 2022d). Mass spectrometry-based metabolomics studies examine microorganism-generated compounds' global relationships. Molecular networking can reveal microorganism-specific compounds. It is much more complex to tease apart shared primary metabolite attribution, feeding studies with labelled substrates and careful quantification will need to be performed. Also, metabolic enrichment strategies identify related metabolites to interpret metabolic regulation from the metabolomic dataset (Bauermeister, Mannochio-Russo, Costa-Lotufo, Jarmusch, & Dorrestein, 2022). It should be noted that current microbial network construction tools often overlook the complexity of higher-order interactions (HOIs) that widely exist in the microbial communities of *Baijiu* brewing (Wu, Zhu, & Xu, 2023). Morin, Morrison, Harms, and Dutton (2022) demonstrated the complex architecture of microbial interactions even within a simple microbiome, and pointed out this knowledge of the mechanical rearrangement of interactions and patterns of HOIs is vital for bottom-up techniques to predict. HOI visualization requires the use of hypergraphs, which are networks with edges connecting more than two nodes. Analyzing and interpreting hypergraphs pose additional challenges in studying HOIs (Faust, 2021).

The synthetic community system, when used in conjunction with in-depth *in situ* investigations, can uncover and explore microbial interactions that may be difficult to observe in natural fermentation systems. This framework can contribute to a better understanding of how core microbiota interactions are established and offer opportunities for manipulating the growth of core fermentation microbiota. To unravel the principles of microbiome assembly and function, it is crucial to study the transferability of mechanistic conclusions across microbiomes and perform intentional cross-system comparisons in spontaneous food fermentation systems.

7. Conclusion

Overall, this review discusses recent advances in research on the origin, composition, function, and interactions of core microbes in the *Baijiu* fermentation microbiome. It also addresses knowledge gaps related to defining the core microbiome and systematically analyzing microbial interactions. The complexity of the microbiota and its function in the ecological brewing system of *Baijiu* present challenges in managing fermentation. The intricate interaction between species makes it difficult to ensure precise control. Currently, most *Baijiu* industrial processes rely on experience-based approaches. Unraveling the core microbes in diverse brewing systems and investigating their interactions under varying environmental conditions can enhance precise manipulation of spontaneous *Baijiu* fermentation.

Microbial interactions that maintain the inherited core microbes during spontaneous fermentation play a crucial role in promoting the accumulation of essential metabolites and controlling undesirable microbiota. To gain a more nuanced understanding of community function, it is essential to identify core microorganisms that perform vital tasks within the larger community. Once the role of a single core species is established, reconstructing simplified microbial communities

can help unravel the interactions of critical metabolic processes that define quality characteristics. It is possible that systematic and artificial simplified microbiomes could serve as model systems, addressing emerging questions related to complex *Baijiu* fermentation systems that are challenging to explore through field observations of larger organisms' communities. Understanding microbial composition and interactions can facilitate intelligent management and industrialization of conventional fermentation processes. The framework of microbial interaction analysis in *Baijiu* fermentation is expected to provide a comprehensive understanding of the fermentation process.

CRediT authorship contribution statement

Jiamu Kang: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Xiaoning Huang:** Writing – review & editing, Writing – original draft, Conceptualization. **Rengshu Li:** Visualization, Methodology. **Yuandi Zhang:** Visualization, Methodology. **Xiao-Xue Chen:** Resources, Investigation. **Bei-Zhong Han:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by The National Natural Science Foundation of China (Grant No. 31972975) and China Alcoholic Drinks Association.

References

- Abd Razak, D. L., Abd Rashid, N. Y., Jamaluddin, A., Sharifudin, S. A., & Long, K. (2015). Enhancement of phenolic acid content and antioxidant activity of rice bran fermented with *Rhizopus oligosporus* and *Monascus purpureus*. *Biocatalysis and Agricultural Biotechnology*, 4(1), 33–38. <https://doi.org/10.1016/j.biab.2014.11.003>
- Albuquerque, P., & Casadevall, A. (2012). Quorum sensing in fungi—a review. *Medical Mycology*, 50(4), 337–345. <https://doi.org/10.3109/13693786.2011.652201>
- Almeida, O. G. G. d., Pinto, U. M., Matos, C., Frazilao, D. A., Braga, V. F., von Zeska-Kress, M., & De Martinis, E. C. P. J. F. R. I. (2020). Does quorum sensing play a role in microbial shifts along spontaneous fermentation of cocoa beans? An *in silico* perspective. *Food Research International*, 131, 109034. doi:10.1016/j.foodres.2020.109034.
- Alvarez-Perez, S., Lievens, B., & Fukami, T. (2019). Yeast-Bacterium Interactions: The Next Frontier in Nectar Research. *Trends in Plant Science*, 24(5), 393–401. <https://doi.org/10.1016/j.tplants.2019.01.012>
- An, W.-F., & Ma, Z.-J. (2008). Dynamic Change of Microbes During the Production of Dongjiu. *Liquor-Making Science & technology*, 10(172), 48–53. <https://doi.org/10.13746/j.njjk.2008.10.002>. in Chinese.
- Arioli, S., Della Scala, G., Remagni, M. C., Stuklynyte, M., Colombo, S., Guglielmetti, S., De Noni, I., Ragg, E., & Mora, D. (2017). *Streptococcus thermophilus* urease activity boosts *Lactobacillus delbrueckii* subsp. *bulgaricus* homolactic fermentation. *International Journal of Food Microbiology*, 247, 55–64. <https://doi.org/10.1016/j.ijfoodmicro.2016.01.006>
- Avbelj, M., Zupan, J., & Raspor, P. (2016). Quorum-sensing in yeast and its potential in wine making. *Applied Microbiology and Biotechnology*, 100(18), 7841–7852. <https://doi.org/10.1007/s00253-016-7758-3>
- Bauermeister, A., Mannochio-Russo, H., Costa-Lotufo, L. V., Jarmusch, A. K., & Dorrestein, P. C. (2022). Mass spectrometry-based metabolomics in microbiome investigations. *Nature Reviews Microbiology*, 20(3), 143–160. <https://doi.org/10.1038/s41579-021-00621-9>
- Berry, D., & Widder, S. (2014). Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Frontiers in Microbiology*, 5, 219. <https://doi.org/10.3389/fmicb.2014.00219>
- Biswas, S., McDonald, M., Lundberg, D. S., Dangl, J. L., & Jojic, V. (2016). Learning Microbial Interaction Networks from Metagenomic Count Data. *Journal of Computational Biology*, 23(6), 526–535. <https://doi.org/10.1089/cmb.2016.0061>
- Blasche, S., Kim, Y., Mars, R. A., Machado, D., Maansson, M., Kafkia, E., Milanese, A., Zeller, G., Teusink, B., & Nielsen, J. (2021). Metabolic cooperation and spatiotemporal niche partitioning in a kefir microbial community. *Nature Microbiology*, 6(2), 196–208. <https://doi.org/10.1038/s41564-020-00816-5>
- Chai, L. J., Lu, Z. M., Zhang, X. J., Ma, J., Xu, P. X., Qian, W., Xiao, C., Wang, S. T., Shen, C. H., Shi, J. S., & Zheng-Hong, X. (2019). Zooming in on Butyrate-Producing Clostridial Consortia in the Fermented Grains of *Baijiu* via Gene Sequence-Guided Microbial Isolation. *Frontiers in Microbiology*, 10, 1397. <https://doi.org/10.3389/fmicb.2019.01397>
- Chen, C., Xiong, Y., Xie, Y., Zhang, H., Jiang, K., Pang, X.-N., & Huang, M. (2022a). Metabolic characteristics of lactic acid bacteria and interaction with yeast isolated from light-flavor *Baijiu* fermentation. *Food Bioscience*, 50, Article 102102. <https://doi.org/10.1016/j.fbio.2022.102102>
- Chen, X., Zhang, Y., Yan, Z., Meng, Q., Qi, H., Li, H., & Jia, Z. (2020a). Correlation Analysis of Microbial Community Succession in Fermented Grains of *Xifeng*-Flavor Chinese Liquor with Its Physicochemical Indexes. *Food science*, 41(22), 200–205. <https://doi.org/10.7506/spkx1002-6630-20190910-126>. in Chinese.
- Chen, Y., Li, K., Liu, T., Li, R., Fu, G., Wan, Y., & Zheng, F. (2020b). Analysis of Difference in Microbial Community and Physicochemical Indices Between Surface and Central Parts of Chinese Special-Flavor *Baijiu Daqu*. *Frontiers in Microbiology*, 11, Article 592421. <https://doi.org/10.3389/fmicb.2020.592421>
- Chen, Y., Yang, Y., Cai, W., Zeng, J., Liu, N., Wan, Y., & Fu, G. (2022b). Research progress of anti-environmental factor stress mechanism and anti-stress tolerance way of *Saccharomyces cerevisiae* during the brewing process. *Critical Reviews in Food Science and Nutrition*, 63(33), 1–16. <https://doi.org/10.1080/10408398.2022.2101090>
- Chodkowski, J. L., & Shade, A. (2017). A Synthetic Community System for Probing Microbial Interactions Driven by Exometabolites. *mSystems*, 2 (6), e00129-00117. doi:10.1128/mSystems.00129-17.
- Cui, F.-J., Li, Y.-G., Gao, D.-Y., & Chen, J.-X. (2019). Microbial diversity and its relationships with stress factors in sesame-flavor liquor fermentation process. *Microbiology China*, 46 (10), 2620–2629 (in Chinese). doi:10.13344/j.microbiol.china.180851.
- Custer, G. F., Gans, M., van Diepen, L. T. A., Dini-Andreote, F., & Buerkle, C. A. (2023). Comparative Analysis of Core Microbiome Assignments: Implications for Ecological Synthesis. *mSystems*, 8 (1), e0106622. doi:10.1128/msystems.01066-22.
- Deng, N., Du, H., & Xu, Y. (2020). Cooperative Response of *Pichia kudriavzevii* and *Saccharomyces cerevisiae* to Lactic Acid Stress in *Baijiu* Fermentation. *Journal of Agricultural and Food Chemistry*, 68(17), 4903–4911. <https://doi.org/10.1021/acs.jafc.9b08052>
- Deng, Y., Liu, J., Li, H., Li, L., Tu, J., Fang, H., Chen, J., & Qian, F. (2014). An improved plate culture procedure for the rapid detection of beer-spoilage lactic acid bacteria. *Journal of the Institute of Brewing*, 120(2), 127–132. <https://doi.org/10.1002/jib.121>
- Diaz-Munoz, S. L., & Koskella, B. (2014). Bacteria-phage interactions in natural environments. *Advances in Applied Microbiology*, 89, 135–183. <https://doi.org/10.1016/B978-0-12-800259-9.00004-4>
- Dietler, M. (2006). Alcohol: Anthropological/Archaeological Perspectives. *Annual Review of Anthropology*, 35(1), 229–249. <https://doi.org/10.1146/annurev.anthro.35.081705.123120>
- Du, H., Chen, B., Fu, W., Yang, F., Lv, X., Tan, Y., Xi, X., Wang, L., & Xu, Y. (2023). Composition and function of viruses in sauce-flavor *baijiu* fermentation. *International Journal of Food Microbiology*, 387, Article 110055. <https://doi.org/10.1016/j.ijfoodmicro.2022.110055>
- Du, H., Liu, B., Wang, X., & Xu, Y. (2017). Exploring the microbial origins of p-cresol and its co-occurrence pattern in the Chinese liquor-making process. *International Journal of Food Microbiology*, 260, 27–35. <https://doi.org/10.1016/j.ijfoodmicro.2017.08.016>
- Du, H., Lu, H., & Xu, Y. (2015). Influence of geosmin-producing *Streptomyces* on the growth and volatile metabolites of yeasts during chinese liquor fermentation. *Journal of Agricultural and Food Chemistry*, 63(1), 290–296. <https://doi.org/10.1021/jf503351w>
- Du, H., Song, Z., Zhang, M., Nie, Y., & Xu, Y. (2021). The deletion of *Schizosaccharomyces pombe* decreased the production of flavor-related metabolites during traditional *Baijiu* fermentation. *Food Research International*, 140, Article 109872. <https://doi.org/10.1016/j.foodres.2020.109872>
- Du, H., Wang, X., Zhang, M., Nie, Y., & Xu, Y. (2019). Exploring the impacts of raw materials and environments on the microbiota in Chinese *Daqu* starter. *International Journal of Food Microbiology*, 297, 32–40. <https://doi.org/10.1016/j.ijfoodmicro.2019.02.020>
- Du, H., Wei, J., Zhang, X., & Xu, Y. (2022). Biocontrol of Geosmin Production by Inoculation of Native Microbiota during the *Daqu*-Making Process. *Fermentation*, 8 (11), 588. <https://doi.org/10.3390/fermentation8110588>
- Du, R., Wu, Q., & Xu, Y. (2020). Chinese Liquor Fermentation: Identification of Key Flavor-Producing *Lactobacillus* spp. by Quantitative Profiling with Indigenous Internal Standards. *Applied and Environmental Microbiology*, 86 (12), e00456-00420. doi:10.1128/AEM.00456-20.
- Duan, S. F., Han, P. J., Wang, Q. M., Liu, W. Q., Shi, J. Y., Li, K., Zhang, X. L., & Bai, F. Y. (2018). The origin and adaptive evolution of domesticated populations of yeast from Far East Asia. *Nature Communications*, 9(1), 2690. <https://doi.org/10.1038/s41467-018-05106-7>
- Dufrene, Y. F. (2004). Using nanotechniques to explore microbial surfaces. *Nature Reviews Microbiology*, 2(6), 451–460. <https://doi.org/10.1038/nrmicro905>
- Fan, G., Teng, C., Xu, D., Fu, Z., Liu, P., Wu, Q., Yang, R., & Li, X. (2019a). Improving Ethyl Acetate Production in *Baijiu* Manufacture by *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* Mixed Culture Fermentations. *BioMed research international*, 2019, 1470543. <https://doi.org/10.1155/2019/1470543>

- Fan, G. S., Teng, C., Xu, D., Fu, Z. L., Minhazul, K. A. H. M., Wu, Q. H., Liu, P. X., Yang, R., & Li, X. T. (2019b). Enhanced production of ethyl acetate using co-culture of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*, 128(5), 564–570. <https://doi.org/10.1016/j.jbiosc.2019.05.002>
- Fan, Y., Huang, X., Chen, J., & Han, B. (2020). Formation of a mixed-species biofilm is a survival strategy for unculturable lactic acid bacteria and *Saccharomyces cerevisiae* in *Daqu*, a Chinese traditional fermentation starter. *Frontiers in Microbiology*, 11, 138. <https://doi.org/10.3389/fmicb.2020.00138>
- Faust, K. (2021). Open challenges for microbial network construction and analysis. *The ISME Journal*, 15(11), 3111–3118. <https://doi.org/10.1038/s41396-021-01027-4>
- Friedman, J., & Alm, E. J. (2012). Inferring correlation networks from genomic survey data. *PLOS Computational Biology*, 8(9), e1002687.
- Fusco, V., Quero, G. M., Cho, G. S., Kabisch, J., Meske, D., Neve, H., Bockelmann, W., & Franz, C. M. (2015). The genus *Weissella*: Taxonomy, ecology and biotechnological potential. *Frontiers in Microbiology*, 6, 155. <https://doi.org/10.3389/fmicb.2015.00155>
- Gao, L., Zhou, J., & He, G. (2022). Effect of microbial interaction on flavor quality in Chinese *baijiu* fermentation. *Frontiers in Nutrition*, 9. <https://doi.org/10.3389/fnut.2022.960712>
- Gao, Z., Wu, Z., & Zhang, W. (2020). Effect of Pit Mud on Bacterial Community and Aroma Components in Yellow Water and Their Changes during the Fermentation of Chinese Strong-Flavor Liquor. *Foods*, 9(3), 372. <https://doi.org/10.3390/foods9030372>
- Gong, L., Ren, C., & Xu, Y. (2020). GlnR negatively regulates glutamate-dependent acid resistance in *Lactobacillus brevis*. *Applied and Environmental Microbiology*, 86(7), e02615–e02619. <https://doi.org/10.1128/AEM.02615-19>
- Goyal, A., Wang, T., Dubinkina, V., & Maslov, S. (2021). Ecology-guided prediction of cross-feeding interactions in the human gut microbiome. *Nature Communications*, 12 (1), 1335. <https://doi.org/10.1038/s41467-021-21586-6>
- Guan, T., Lin, Y., Chen, K., Ou, M., & Zhang, J. (2020). Physicochemical Factors Affecting Microbiota Dynamics During Traditional Solid-State Fermentation of Chinese Strong-Flavor *Baijiu*. *Frontiers in Microbiology*, 11, 2090. <https://doi.org/10.3389/fmicb.2020.02090>
- Guan, T., Wu, X., Hou, R., Tian, L., Huang, Q., Zhao, F., Liu, Y., Jiao, S., Xiang, S., Zhang, J., Li, D., Luo, J., Jin, Z., & He, Z. (2023). Application of *Clostridium butyricum*, *Rummeliibacillus suwonensis*, and *Isatchenkia orientalis* for *Nongxiangxing baijiu* fermentation: Improves the microbial communities and flavor of upper fermented grain. *Food Research International*, 169. <https://doi.org/10.1016/j.foodres.2023.112885>
- Hao, F., Tan, Y., Lv, X., Chen, L., Yang, F., Wang, H., Du, H., Wang, L., & Xu, Y. (2021). Microbial Community Succession and Its Environment Driving Factors During Initial Fermentation of Maotai-Flavor *Baijiu*. *Frontiers in Microbiology*, 12, Article 669201. <https://doi.org/10.3389/fmicb.2021.669201>
- Hao, H., Yan, R., Miao, Z., Wang, B., Sun, J., & Sun, B. (2022). Volatile organic compounds mediated endogenous microbial interactions in Chinese *baijiu* fermentation. *International Journal of Food Microbiology*, 383, Article 109955. <https://doi.org/10.1016/j.ijfoodmicro.2022.109955>
- He, G., Dong, Y., Huang, J., Wang, X., Zhang, S., Wu, C., Jin, Y., & Zhou, R. (2019). Alteration of microbial community for improving flavor character of *Daqu* by inoculation with *Bacillus velezensis* and *Bacillus subtilis*. *LWT-Food science and technology*, 111, 1–8. <https://doi.org/10.1016/j.lwt.2019.04.098>
- Hong, L., Fu, G., Liu, T., Chen, Y., Wu, S., Cai, W., Xie, Z., & Wan, Y. (2021). Functional microbial agents enhance ethanol contents and regulate the volatile compounds in Chinese *Baijiu*. *Food Bioscience*, 44, Article 101411. <https://doi.org/10.1016/j.fbio.2021.101411>
- Hong, X., Chen, J., Liu, L., Wu, H., Tan, H., Xie, G., Xu, Q., Zou, H., Yu, W., Wang, L., & Qin, N. (2016). Metagenomic sequencing reveals the relationship between microbiota composition and quality of Chinese Rice Wine. *Scientific Reports*, 6, 26621. <https://doi.org/10.1038/srep26621>
- Hu, X., Du, H., Ren, C., & Xu, Y. (2016). Illuminating Anaerobic Microbial Community and Cooccurrence Patterns across a Quality Gradient in Chinese Liquor Fermentation Pit Muds. *Applied and Environmental Microbiology*, 82(8), 2506–2515. <https://doi.org/10.1128/AEM.03409-15>
- Hu, X. L., Wang, K. L., Chen, M. G., Fan, J. H., Han, S. N., Hou, J. G., Chi, L., Liu, Y. P., & Wei, T. (2020). Profiling the composition and metabolic activities of microbial community in fermented grain for the Chinese strong-flavor *Baijiu* production by using the metatranscriptome, high-throughput 16S rRNA and ITS gene sequencings. *Food Research International*, 138, Article 109765. <https://doi.org/10.1016/j.foodres.2020.109765>
- Hu, Y., Dun, Y., Li, S., Fu, B., Xiong, X., Peng, N., Liang, Y., & Zhao, S. (2017). Changes in microbial community during fermentation of high-temperature *Daqu* used in the production of Chinese '*Baiyunbian*' liquor. *Journal of the Institute of Brewing*, 123(4), 594–599. <https://doi.org/10.1002/jib.455>
- Hu, Y., Lei, X., Zhang, X., Guan, T., Wang, L., Zhang, Z., Yu, X., Tu, J., Peng, N., Liang, Y., & Zhao, S. (2021). Characteristics of the Microbial Community in the Production of Chinese Rice-Flavor *Baijiu* and Comparisons With the Microflora of Other Flavors of *Baijiu*. *Frontiers in Microbiology*, 12, Article 673670. <https://doi.org/10.3389/fmicb.2021.673670>
- Huang, X., Fan, Y., Lu, T., Kang, J., Pang, X., Han, B., & Chen, J. (2020). Composition and Metabolic Functions of the Microbiome in Fermented Grain during Light-Flavor *Baijiu* Fermentation. *Microorganisms*, 8(9), 1281. <https://doi.org/10.3390/microorganisms8091281>
- Huang, X., Fan, Y., Meng, J., Sun, S., Wang, X., Chen, J., & Han, B.-Z. (2021). Laboratory-scale fermentation and multidimensional screening of lactic acid bacteria from *Daqu*. *Food Bioscience*, 40, Article 100853. <https://doi.org/10.1016/j.fbio.2020.100853>
- Huang, Y., Yi, Z., Jin, Y., Zhao, Y., He, K., Liu, D., Zhao, D., He, H., Luo, H., Zhang, W., Fang, Y., & Zhao, H. (2017). New microbial resource: Microbial diversity, function and dynamics in Chinese liquor starter. *Scientific Reports*, 7(1), 14577. <https://doi.org/10.1038/s41598-017-14968-8>
- Ivey, M., Massel, M., & Phister, T. G. (2013). Microbial interactions in food fermentations. *Annual Review of Food Science and Technology*, 4, 141–162. <https://doi.org/10.1146/annurev-food-022811-101219>
- Jin, G., Zhu, Y., & Xu, Y. (2017). Mystery behind Chinese liquor fermentation. *Trends in Food Science & Technology*, 63, 18–28. <https://doi.org/10.1016/j.tifs.2017.02.016>
- Jin, Y., Li, D. Y., Ai, M., Tang, Q. X., Huang, J., Ding, X. F., Wu, C. D., & Zhou, R. Q. (2019). Correlation between volatile profiles and microbial communities: A metabonomic approach to study *Jiang*-flavor liquor *Daqu*. *Food Research International*, 121, 422–432. <https://doi.org/10.1016/j.foodres.2019.03.021>
- Kang, J., Chen, X., Han, B. Z., & Xue, Y. (2022a). Insights into the bacterial, fungal, and phage communities and volatile profiles in different types of *Daqu*. *Food Research International*, 158, Article 111488. <https://doi.org/10.1016/j.foodres.2022.111488>
- Kang, J., Hu, Y., Ding, Z., Ye, L., Li, H., Cheng, J., Fan, L., Zhao, H., Han, B., & Zheng, X. (2021). Deciphering the Shifts in Microbial Community Diversity From Material Pretreatment to Saccharification Process of *Fuyu*-Flavor *Baijiu*. *Frontiers in Microbiology*, 12, Article 705967. <https://doi.org/10.3389/fmicb.2021.705967>
- Kang, J., Hu, Y., Jia, L., Zhang, M., Zhang, Z., Huang, X., Chen, X., & Han, B.-Z. (2022b). Response of microbial community assembly and succession pattern to abiotic factors during the second round of light-flavor *Baijiu* fermentation. *Food Research International*, 162, Article 111915. <https://doi.org/10.1016/j.foodres.2022.111915>
- Kang, J., Sun, Y., Huang, X., Ye, L., Chen, Y., Chen, X., Zheng, X., & Han, B. Z. (2022c). Unraveling the microbial compositions, metabolic functions, and antibacterial properties of *Huangshui*, a byproduct of *Baijiu* fermentation. *Food Research International*, 157, Article 111320. <https://doi.org/10.1016/j.foodres.2022.111320>
- Kang, J., Xue, Y., Chen, X., & Han, B. Z. (2022d). Integrated multi-omics approaches to understand microbiome assembly in *Jiugu*, a mixed-culture starter. *Comprehensive Reviews in Food Science and Food Safety*, 21(5), 1–32. <https://doi.org/10.1111/1541-4337.13025>
- Kang, J. M., Zheng, X. W., Yang, X., Li, H. R., Cheng, J., Fan, L., Zhao, H., Xue, Y. S., Ding, Z. Y., & Han, B. Z. (2022e). Contrasting summer versus winter dynamic microbial communities and their environmental driving factors in the solid-state saccharification process of *Fuyu*-flavor *Baijiu*. *Food Research International*, 154, Article 111008. <https://doi.org/10.1016/j.foodres.2022.111008>
- Kleiner, M. (2019). Metaproteomics: Much More than Measuring Gene Expression in Microbial Communities. *mSystems*, 4 (3), e00115-00119. doi:10.1128/mSystems.00115-19.
- Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., Bushman, F. D., Knight, R., & Kelley, S. T. (2011). Bayesian community-wide culture-independent microbial source tracking. *Nature Methods*, 8(9), 761–763. <https://doi.org/10.1038/nmeth.1650>
- Layeghifard, M., Hwang, D. M., & Guttmann, D. S. (2017). Disentangling Interactions in the Microbiome: A Network Perspective. *Trends in Microbiology*, 25(3), 217–228. <https://doi.org/10.1016/j.tim.2016.11.008>
- Ledermann, P., Desmasures, N., Schlusselhuber, M., Sesboue, A., Ledauphin, J., & Dalmaso, M. (2022). Phages Shape Microbial Dynamics and Metabolism of a Model Community Mimicking Cider, a Fermented Beverage. *Viruses*, 14(10), 2283. <https://doi.org/10.3390/v14102283>
- Lewis, W. H., Tahon, G., Geesink, P., Sousa, D. Z., & Ettema, T. J. G. (2021). Innovations to culturing the uncultured microbial majority. *Nature Reviews Microbiology*, 19(4), 225–240. <https://doi.org/10.1038/s41579-020-00458-8>
- Li, K., Chen, Y., Liu, T., Deng, M., Xu, Z., Fu, G., Wan, Y., Chen, F., & Zheng, F. (2020). Analysis of spatial distribution of bacterial community associated with accumulation of volatile compounds in *Jiugei* during the brewing of special-flavor liquor. *LWT-Food science and technology*, 130, Article 109620. <https://doi.org/10.1016/j.lwt.2020.109620>
- Li, R.-Y., Zheng, X.-W., Zhang, X., Yan, Z., Wang, X.-Y., & Han, B.-Z. (2018). Characterization of bacteria and yeasts isolated from traditional fermentation starter (*Fen-Daqu*) through a 1H NMR-based metabolomics approach. *Food Microbiology*, 76, 11–20. <https://doi.org/10.1016/j.fm.2018.03.015>
- Li, W., Li, J., Wang, Y., Yang, F., & Zhao, H. (2017). Screening and identification of lactic acid-utilizing bacteria from *Fen*-flavor *Baijiu*. *China Brewing*, 36(9), 87–91. <https://doi.org/10.11882/j.issn.0254-5071.2017.09.019>, in Chinese.
- Li, W., Wang, J. H., Zhang, C. Y., Ma, H. X., & Xiao, D. G. (2017). Regulation of *Saccharomyces cerevisiae* genetic engineering on the production of acetate esters and higher alcohols during Chinese *Baijiu* fermentation. *Journal of Industrial Microbiology & Biotechnology*, 44(6), 949–960. <https://doi.org/10.1007/s10295-017-1907-2>
- Li, Z., Fernandez, K. X., Vederas, J. C., & Ganze, M. G. (2023). Composition and activity of antifungal lipopeptides produced by *Bacillus* spp. in *daqu* fermentation. *Food Microbiology*, 111, Article 104211. <https://doi.org/10.1016/j.fm.2022.104211>
- Lin, L., Du, R., Wang, Y., Wu, Q., & Xu, Y. (2022). Regulation of auxotrophic lactobacilli growth by amino acid cross-feeding interaction. *International Journal of Food Microbiology*, 377, Article 109769. <https://doi.org/10.1016/j.ijfoodmicro.2022.109769>
- Liu, F., Mao, J., Kong, W., Hua, Q., Feng, Y., Bashir, R., & Lu, T. (2020a). Interaction variability shapes succession of synthetic microbial ecosystems. *Nature Communications*, 11(1), 309. <https://doi.org/10.1038/s41467-019-13986-6>
- Liu, H., & Sun, B. (2018). Effect of Fermentation Processing on the Flavor of *Baijiu*. *Journal of Agricultural and Food Chemistry*, 66(22), 5425–5432. <https://doi.org/10.1021/acs.jafc.8b00692>
- Liu, H., Tan, G., Chen, Q., Dong, W., Chen, P., Cai, K., Hu, Y., Zhang, W., Peng, N., Liang, Y., & Zhao, S. (2021). Detection of viable and total fungal community in *zaopei* of Chinese strong-flavor *baijiu* using PMA combined with qPCR and HTS based

- on ITS2 region. *BMC Microbiology*, 21(1), 274. <https://doi.org/10.1186/s12866-021-02334-8>
- Liu, J., Huang, T. Y., Liu, G., Ye, Y., Soteyome, T., Seneviratne, G., Xiao, G., Xu, Z., & Kjellerup, B. V. (2022). Microbial Interaction between *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae*: Transcriptome Level Mechanism of Cell-Cell Antagonism. *Microbiology Spectrum*, 10(5). <https://doi.org/10.1128/spectrum.01433-22>. e0143322.
- Liu, J., Wu, Q., Wang, P., Lin, J., Huang, L., & Xu, Y. (2017). Synergistic Effect in Core Microbiota Associated with Sulfur Metabolism in Spontaneous Chinese Liquor Fermentation. *Applied and Environmental Microbiology*, 83 (24), e01475-01417. doi: 10.1128/AEM.01475-17.
- Liu, L., Wang, J., Levin, M. J., Sinnott-Armstrong, N., Zhao, H., Zhao, Y., Shao, J., Di, N., & Zhang, T. (2019). The origins of specialized pottery and diverse alcohol fermentation techniques in Early Neolithic China. *Proceedings of the National Academy of Sciences of the United States of America*, 116(26), 12767-12774. <https://doi.org/10.1073/pnas.1902668116>
- Liu, M.-K., Tang, Y.-M., Guo, X.-J., Zhao, K., Penttiläinen, P., Tian, X.-H., Zhang, X.-Y., Ren, D.-Q., & Zhang, X.-P. (2020b). Structural and Functional Changes in Prokaryotic Communities in Artificial Pit Mud during Chinese *Baijiu* Production. *mSystems*, 5 (2), e00829-00819. doi:10.1128/mSystems.00829-19.
- Liu, P., & Miao, L. (2020). Multiple Batches of Fermentation Promote the Formation of Functional Microbiota in Chinese Miscellaneous-Flavor *Baijiu* Fermentation. *Frontiers in Microbiology*, 11, 75. <https://doi.org/10.3389/fmicb.2020.00075>
- Liu, S.-N., Han, Y., & Zhou, Z.-J. (2011). Lactic acid bacteria in traditional fermented Chinese foods. *Food Research International*, 44(3), 643-651. <https://doi.org/10.1016/j.foodres.2010.12.034>
- Ma, B., Fan, E., Li, Z., Zhang, Y., Guo, Y., Jiang, D., Chen, Y., Guo, X., Xiao, D., & Zhang, Z. (2020a). Microbial diversity and its relationship with flavor compounds in the process of *Daqu* making of *Laobaigan*-flavor *Baijiu*. *Food and Fermentation Industries*, 46(16), 7-16. <https://doi.org/10.13995/j.cnki.11-1802.ts.023902>. in Chinese.
- Ma, B., Fan, E., Li, Z., Zhang, Y., Zhang, Z., Chen, Y., Xiao, D., & Guo, X. (2020b). Study on Relationship Between Brewing Microflora and Microconstituents of *Laobaigan*-Flavor *Baijiu*. *Journal of Food Science and Technology*, 38(2), 20-33. <https://doi.org/10.3969/j.issn.2095-6002.2020.02.004>. in Chinese.
- Ma, S., Luo, H., Zhao, D., Qiao, Z., Zheng, J., An, M., & Huang, D. (2022a). Environmental factors and interactions among microorganisms drive microbial community succession during fermentation of *Nongxiangxing daqu*. *Bioresource Technology*, 345. <https://doi.org/10.1016/j.biortech.2021.126549>
- Ma, S., Shang, Z., Chen, J., Shen, Y., Li, Z., Huang, D., & Luo, H. (2022b). Differences in structure, volatile metabolites, and functions of microbial communities in *Nongxiangxing daqu* from different production areas. *LWT-Food science and technology*, 166, Article 113784. <https://doi.org/10.1016/j.lwt.2022.113784>
- Magalhaes, A. P., Franca, A., Pereira, M. O., & Cercia, N. (2019). RNA-based qPCR as a tool to quantify and to characterize dual-species biofilms. *Scientific Reports*, 9(1), 13639. <https://doi.org/10.1038/s41598-019-50094-3>
- Maicas, S. (2020). The Role of Yeasts in Fermentation Processes. *Microorganisms*, 8(8), 1142. <https://doi.org/10.3390/microorganisms8081142>
- Mao, F., Huang, J., Zhou, R., Qin, H., Zhang, S., Cai, X., & Qiu, C. (2023). Succession of microbial community of the pit mud under the impact of *Daqu* of *Nongxiang Baijiu*. *Journal of Bioscience and Bioengineering*, 136(4), 304-311. <https://doi.org/10.1016/j.jbiose.2023.07.005>
- Marino, S., Baxter, N. T., Huffnagle, G. B., Petrosino, J. F., & Schloss, P. D. (2014). Mathematical modeling of primary succession of murine intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 111(1), 439-444. <https://doi.org/10.1073/pnas.1311322111>
- Maske, B. L., de Melo Pereira, G. V., da Silva Vale, A., Marques Souza, D. S., De Dea Lindner, J., & Soccol, C. R. (2021). Viruses in fermented foods: Are they good or bad? Two sides of the same coin. *Food Microbiology*, 98, Article 103794. <https://doi.org/10.1016/j.fm.2021.103794>
- Matchado, M. S., Lauber, M., Reitmeier, S., Kacprowski, T., Baumbach, J., Haller, D., & List, M. (2021). Network analysis methods for studying microbial communities: A mini review. *Computational and Structural Biotechnology Journal*, 19, 2687-2698. <https://doi.org/10.1016/j.csbj.2021.05.001>
- Milligan-McClellan, K. C., Dundore-Arias, J. P., Klassen, J. L., Shade, A., Kinkel, L. L., & Wolfe, B. E. (2022). Deciphering the Microbiome: Integrating Theory, New Technologies, and Inclusive Science. *mSystems*, 7 (5), e0058322. doi:10.1128/msystems.00583-22.
- Morin, M. A., Morrison, A. J., Harms, M. J., & Dutton, R. J. (2022). Higher-order interactions shape microbial interactions as microbial community complexity increases. *Scientific Reports*, 12(1), 22640. <https://doi.org/10.1038/s41598-022-25303-1>
- Moslehi-Jenabian, S., Gori, K., & Jespersen, L. (2009). AI-2 signalling is induced by acidic shock in probiotic strains of *Lactobacillus* spp. *International Journal of Food Microbiology*, 135(3), 295-302. <https://doi.org/10.1016/j.ijfoodmicro.2009.08.011>
- Mounier, J., Monnet, C., Vallaeys, T., Arditì, R., Sarthou, A.-S., Hélias, A., & Irlinger, F. (2008). Microbial interactions within a cheese microbial community. *Applied Environmental Microbiology*, 74(1), 172-181. <https://doi.org/10.1128/AEM.01338-07>
- Muller, M. J., Neugeboren, B. I., Nelson, D. R., & Murray, A. W. (2014). Genetic drift opposes mutualism during spatial population expansion. *Proceedings of the National Academy of Sciences of the United States of America*, 111(3), 1037-1042. <https://doi.org/10.1073/pnas.1313285111>
- Neu, A. T., Allen, E. E., & Roy, K. (2021). Defining and quantifying the core microbiome: Challenges and prospects. *Proceedings of the National Academy of Sciences of the United States of America*, 118(51). <https://doi.org/10.1073/pnas.2104429118>
- Paillet, T., & Dugat-Bony, E. (2021). Bacteriophage ecology of fermented foods: Anything new under the sun? *Current Opinion in Food Science*, 40, 102-111. <https://doi.org/10.1016/j.cofs.2021.03.007>
- Pan, Y., Wang, Y., Hao, W., Duan, C., Wang, S., Wei, J., & Liu, G. (2022). Metatranscriptomics Unravel Composition, Drivers, and Functions of the Active Microorganisms in Light-Flavor Liquor Fermentation. *Microbiology Spectrum*, 10(3). <https://doi.org/10.1128/spectrum.02151-21>. e0215121.
- Pang, X. N., Chen, C., Huang, X. N., Yan, Y. Z., Chen, J. Y., & Han, B. Z. (2021). Influence of indigenous lactic acid bacteria on the volatile flavor profile of light-flavor *Baijiu*. *LWT-Food science and technology*, 147, Article 111540. <https://doi.org/10.1016/j.lwt.2021.111540>
- Pang, X. N., Han, B. Z., Huang, X. N., Zhang, X., Hou, L. F., Cao, M., Gao, L. J., Hu, G. H., & Chen, J. Y. (2018). Effect of the environment microbiota on the flavour of light-flavor *Baijiu* during spontaneous fermentation. *Scientific Reports*, 8(1), 3396. <https://doi.org/10.1038/s41598-018-21814-y>
- Pang, X. N., Huang, X. N., Chen, J. Y., Yu, H. X., Wang, X. Y., & Han, B. Z. (2020). Exploring the diversity and role of microbiota during material pretreatment of light-flavor *Baijiu*. *Food Microbiology*, 91, Article 103514. <https://doi.org/10.1016/j.fm.2020.103514>
- Papenfort, K., & Bassler, B. L. (2016). Quorum sensing signal-response systems in Gram-negative bacteria. *Nature Reviews Microbiology*, 14(9), 576-588. <https://doi.org/10.1038/nrmicro.2016.89>
- Parente, E., Zotta, T., & Ricciardi, A. (2022). A review of methods for the inference and experimental confirmation of microbial association networks in cheese. *International Journal of Food Microbiology*, 368, Article 109618. <https://doi.org/10.1016/j.ijfoodmicro.2022.109618>
- Park, H., Shin, H., Lee, K., & Holzapfel, W. (2016). Autoinducer-2 properties of kimchi are associated with lactic acid bacteria involved in its fermentation. *International Journal of Food Microbiology*, 225, 38-42. <https://doi.org/10.1016/j.ijfoodmicro.2016.03.007>
- Qian, W., Lu, Z.-M., Chai, L.-J., Zhang, X.-J., Li, Q., Wang, S.-T., Shen, C.-H., Shi, J.-S., & Xu, Z.-H. (2021). Cooperation within the microbial consortia of fermented grains and pit mud drives organic acid synthesis in strong-flavor *Baijiu* production. *Food Research International*, 147, Article 110449. <https://doi.org/10.1016/j.foodres.2021.110449>
- Qian, Y., Kando, C. K., Thorsen, L., Larsen, N., & Jespersen, L. (2015). Production of autoinducer-2 by aerobic endospore-forming bacteria isolated from the West African fermented foods. *FEMS Microbiology Letters*, 362(22), 1-7. <https://doi.org/10.1093/femsle/fnv186>
- Ren, H., Cai, Z., Du, C., Li, Z., Guo, X., Wang, Y., Ma, D., Zhang, B., & Zheng, Y. (2024). Interrelated spatiotemporal variations between bacterial community and physicochemical factors in pit mud of Chinese strong-flavor *Baijiu*. *LWT-Food science and technology*, 192. <https://doi.org/10.1016/j.lwt.2023.115630>
- Risely, A. (2020). Applying the core microbiome to understand host-microbe systems. *Journal of Animal Ecology*, 89(7), 1549-1558. <https://doi.org/10.1111/1365-2656.13229>
- Rodriguez, S. B., & Thornton, R. J. (2008). Use of flow cytometry with fluorescent antibodies in real-time monitoring of simultaneously inoculated alcoholic-malolactic fermentation of Chardonnay. *Letters in Applied Microbiology*, 46(1), 38-42. <https://doi.org/10.1111/j.1472-765X.2007.02265.x>
- Samson, J. E., & Moineau, S. (2013). Bacteriophages in food fermentations: New frontiers in a continuous arms race. *Annual Review of Food Science and Technology*, 4, 347-368. <https://doi.org/10.1146/annurev-food-030212-182541>
- Shen, D., Shen, H., Yang, Q., Chen, S., Dun, Y., Liang, Y., Zheng, J., & Zhao, S. (2021). Deciphering succession and assembly patterns of microbial communities in a two-stage solid-state fermentation system. *Microbiology Spectrum*, 9(2), e00718-e00721. <https://doi.org/10.1128/Spectrum>
- Shen, T., Liu, J., Wu, Q., & Xu, Y. (2020). Increasing 2-furfurylthiol content in Chinese sesame-flavored *Baijiu* via inoculating the producer of precursor l-cysteine in *Baijiu* fermentation. *Food Research International*, 138(Pt A), Article 109757. <https://doi.org/10.1016/j.foodres.2020.109757>
- Shenhai, L., Thompson, M., Joseph, T. A., Briscoe, L., Furman, O., Bogumil, D., Mizrahi, I., Pe'er, I., & Halperin, E. (2019). FEAST: Fast expectation-maximization for microbial source tracking. *Nature Methods*, 16(7), 627-632. <https://doi.org/10.1038/s41592-019-0431-x>
- Shetty, S. A., Kuipers, B., Atashgahi, S., Aalvink, S., Smidt, H., & de Vos, W. M. (2022). Inter-species Metabolic Interactions in an *In-vitro* Minimal Human Gut Microbiome of Core Bacteria. *NPJ Biofilms Microbiomes*, 8(1), 21. <https://doi.org/10.1038/s41522-022-00275-2>
- Shetty, S. A., Smidt, H., & de Vos, W. M. (2019). Reconstructing functional networks in the human intestinal tract using synthetic microbiomes. *Current Opinion in Biotechnology*, 58, 146-154. <https://doi.org/10.1016/j.copbio.2019.03.009>
- Smid, E. J., & Lacroix, C. (2013). Microbe-microbe interactions in mixed culture food fermentations. *Current Opinion in Biotechnology*, 24(2), 148-154. <https://doi.org/10.1016/j.copbio.2012.11.007>
- Song, Z. W., Du, H., Zhang, M. H., Nie, Y., & Xu, Y. (2019). Schizosaccharomyces pombe Can Reduce Acetic Acid Produced by *Baijiu* Spontaneous Fermentation Microbiota. *Microorganisms*, 7(12), 606. <https://doi.org/10.3390/microorganisms7120606>
- Spus, M., Li, M., Alexeeva, S., Wolkers-Rooijackers, J. C., Zwietering, M. H., Abeel, T., & Smid, E. J. (2015). Strain diversity and phage resistance in complex dairy starter cultures. *Journal of Dairy Science*, 98(8), 5173-5182. <https://doi.org/10.3168/jds.2015-9535>
- Sun, H., Chai, L. J., Fang, G. Y., Lu, Z. M., Zhang, X. J., Wang, S. T., Shen, C. H., Shi, J. S., & Xu, Z. H. (2022a). Metabolite-based mutualistic interaction between two novel

- clostridial species from pit mud enhances butyrate and caproate production. *Applied and Environmental Microbiology*, 88(13). <https://doi.org/10.1128/aem.00484-22.e0048422>.
- Sun, X., Xu, Z., Xie, J., Hesselberg-Thomsen, V., Tan, T., Zheng, D., Strube, M. L., Dragos, A., Shen, Q., Zhang, R., & Kovacs, A. T. (2022b). *Bacillus velezensis* stimulates resident rhizosphere *Pseudomonas stutzeri* for plant health through metabolic interactions. *The ISME Journal*, 16(3), 774–787. <https://doi.org/10.1038/s41396-021-01125-3>
- Tan, Y., Du, H., Zhang, H., Fang, C., Jin, G., Chen, S., Wu, Q., Zhang, Y., Zhang, M., & Xu, Y. (2022). Geographically Associated Fungus-Bacterium Interactions Contribute to the Formation of Geography-Dependent Flavor during High-Complexity Spontaneous Fermentation. *Microbiology Spectrum*, 10(5). <https://doi.org/10.1128/spectrum.01844-22.e0184422>
- Tan, Y., Zhong, H., Zhao, D., Du, H., & Xu, Y. (2019). Succession rate of microbial community causes flavor difference in strong-aroma *Baijiu* making process. *International Journal of Food Microbiology*, 311, Article 108350. <https://doi.org/10.1016/j.ijfoodmicro.2019.108350>
- Tang, Q., He, G., Huang, J., Wu, C., Jin, Y., & Zhou, R. (2019). Characterizing Relationship of Microbial Diversity and Metabolite in *Sichuan Xiaoqu*. *Frontiers in Microbiology*, 10, 696. <https://doi.org/10.3389/fmicb.2019.00696>
- Tao, Y., Li, J., Rui, J., Xu, Z., Zhou, Y., Hu, X., Wang, X., Liu, M., Li, D., Li, X., & Björkroth, J. (2014). Prokaryotic Communities in Pit Mud from Different-Aged Cellars Used for the Production of Chinese Strong-Flavored Liquor. *Applied and Environmental Microbiology*, 80(7), 2254–2260. <https://doi.org/10.1128/aem.04070-13>
- Tu, W., Cao, X., Cheng, J., Li, L., Zhang, T., Wu, Q., Xiang, P., Shen, C., & Li, Q. (2022). Chinese *Baijiu*: The Perfect Works of Microorganisms. *Frontiers in Microbiology*, 13, Article 919044. <https://doi.org/10.3389/fmicb.2022.919044>
- Venturini Copetti, M. (2019). Yeasts and molds in fermented food production: An ancient bioprocess. *Current Opinion in Food Science*, 25, 57–61. <https://doi.org/10.1016/j.cofs.2019.02.014>
- Wade, M. E., Strickland, M. T., Osborne, J. P., & Edwards, C. G. (2019). Role of *Pediococcus* in winemaking. *Australian Journal of Grape and Wine Research*, 25(1), 7–24. <https://doi.org/10.1111/ajgw.12366>
- Wang, B., Wu, Q., Xu, Y., & Sun, B. (2020a). Synergistic Effect of Multiple Saccharifying Enzymes on Alcoholic Fermentation for Chinese *Baijiu* Production. *Applied and Environmental Microbiology*, 86(8), e00013–e00020. <https://doi.org/10.1128/AEM.00013-20>
- Wang, H., Huang, Y., & Huang, Y. (2021a). Microbiome diversity and evolution in stacking fermentation during different rounds of *Jiang*-flavoured *Baijiu* brewing. *LWT-Food science and technology*, 143, Article 111119. <https://doi.org/10.1016/j.lwt.2021.111119>
- Wang, J., Gao, Y., & Zhao, F. (2016). Phage-bacteria interaction network in human oral microbiome. *Environmental Microbiology*, 18(7), 2143–2158. <https://doi.org/10.1111/1462-2920.12923>
- Wang, J., Zhong, Q., Yang, Y., Li, H., Wang, L., Tong, Y., Fang, X., & Liao, Z. (2018a). Comparison of Bacterial Diversity Between Two Traditional Starters and the Round-Koji-Maker Starter for Traditional Cantonese *Chi*-Flavor Liquor Brewing. *Frontiers in Microbiology*, 9, 1053. <https://doi.org/10.3389/fmicb.2018.01053>
- Wang, J., Zhu, D., Sun, L., Chen, X., Sun, J., Wang, R., & Xu, N. (2021b). Physiological and Metabolic Characteristics of Molds from *Daqu* of Shanxi Aged Vinegar and Interactions of Excellent Strains. *Journal of Chinese Institute of Food Science and Technology*, 21(4), 79–89. <https://doi.org/10.16429/j.1009-7848.2021.04.010>. in Chinese.
- Wang, J. W., Han, P. J., Han, D. Y., Zhou, S., Li, K., He, P. Y., Zhen, P., Yu, H. X., Liang, Z. R., Wang, X. W., & Bai, F. Y. (2021c). Genetic diversity and population structure of the amylolytic yeast *Saccharomyces fibuligera* associated with *Baijiu* fermentation in China. *Journal of Microbiology*, 59(8), 753–762. <https://doi.org/10.1007/s12275-021-1115-7>
- Wang, L. (2022). Research trends in *Jiang*-flavor *baijiu* fermentation: From fermentation microecology to environmental ecology. *Journal Food Science*, 87(4), 1362–1374. <https://doi.org/10.1111/1750-3841.16092>
- Wang, P., Wu, Q., Jiang, X., Wang, Z., Tang, J., & Xu, Y. (2017a). *Bacillus licheniformis* affects the microbial community and metabolic profile in the spontaneous fermentation of *Daqu* starter for Chinese liquor making. *International Journal of Food Microbiology*, 250, 59–67. <https://doi.org/10.1016/j.ijfoodmicro.2017.03.010>
- Wang, S., Wu, Q., Nie, Y., Wu, J., & Xu, Y. (2019). Construction of synthetic microbiota for reproducible flavor compound metabolism in chinese light-aroma-type liquor produced by solid-state fermentation. *Applied and Environmental Microbiology*, 85(10), e03090-03018. doi:10.1128/AEM.03090-18.
- Wang, W., Fan, G., Li, X., Fu, Z., Liang, X., & Sun, B. (2020b). Application of *Wickerhamomyces anomalus* in Simulated Solid-State Fermentation for *Baijiu* Production: Changes of Microbial Community Structure and Flavor Metabolism. *Frontiers in Microbiology*, 11, Article 598758. <https://doi.org/10.3389/fmicb.2020.598758>
- Wang, X., Du, H., & Xu, Y. (2017b). Source tracking of prokaryotic communities in fermented grain of Chinese strong-flavor liquor. *International Journal of Food Microbiology*, 244, 27–35. <https://doi.org/10.1016/j.ijfoodmicro.2016.12.018>
- Wang, X., Du, H., Zhang, Y., & Xu, Y. (2018b). Environmental Microbiota Drives Microbial Succession and Metabolic Profiles during Chinese Liquor Fermentation. *Applied and Environmental Microbiology*, 84(4), e02369-02317. doi:10.1128/AEM.02369-17.
- Wang, Y., Dai, J., Wang, X., Wang, Y., & Tang, F. (2022). Mechanisms of interactions between bacteria and bacteriophage mediate by quorum sensing systems. *Applied Microbiology and Biotechnology*, 106(7), 2299–2310. <https://doi.org/10.1007/s00253-022-11866-6>
- Waters, C. M., & Bassler, B. L. (2005). Quorum sensing: Cell-to-cell communication in bacteria. *Annual Review of Cell and Developmental Biology*, 21, 319–346. <https://doi.org/10.1146/annurev.cellbio.21.012704.131001>
- Whiteley, M., Diggle, S. P., & Greenberg, E. P. (2017). Progress in and promise of bacterial quorum sensing research. *Nature*, 551(7680), 313–320. <https://doi.org/10.1038/nature24624>
- Wu, Q., Chen, B., & Xu, Y. (2015). Regulating yeast flavor metabolism by controlling saccharification reaction rate in simultaneous saccharification and fermentation of Chinese Maotai-flavor liquor. *International Journal of Food Microbiology*, 200, 39–46. <https://doi.org/10.1016/j.ijfoodmicro.2015.01.012>
- Wu, Q., Ling, J., & Xu, Y. (2014). Starter culture selection for making Chinese sesame-flavored liquor based on microbial metabolic activity in mixed-culture fermentation. *Applied and Environmental Microbiology*, 80(14), 4450–4459. <https://doi.org/10.1128/AEM.00905-14>
- Wu, Q., Zhu, Y., Fang, C., Wijffels, R. H., & Xu, Y. (2021). Can we control microbiota in spontaneous food fermentation? – Chinese liquor as a case example. *Trends in Food Science & Technology*, 110, 321–331. <https://doi.org/10.1016/j.tifs.2021.02.011>
- Wu, Q., Zhu, Y., & Xu, Y. (2023). *Microbial Interaction in Chinese Liquor Fermentation*. Singapore: Springer Nature Singapore Pte Ltd. doi:10.1007/978-981-19-2195-7.
- Wu, X., Jing, R., Chen, W., Geng, X., Li, M., Yang, F., Yan, Y., & Liu, Y. (2020). High-throughput sequencing of the microbial diversity of roasted-sesame-like flavored *Daqu* with different characteristics. *3 Biotech*, 10(11), 502. <https://doi.org/10.1007/s13205-020-02500-1>
- Xu, B., Xu, S., Cai, J., Sun, W., Mu, D., Wu, X., & Li, X. (2022). Analysis of the microbial community and the metabolic profile in medium-temperature *Daqu* after inoculation with *Bacillus licheniformis* and *Bacillus velezensis*. *LWT-Food science and technology*, 160, Article 113214. <https://doi.org/10.1016/j.lwt.2022.113214>
- Xu, J., Sun, L., Xing, X., Sun, Z., Gu, H., Lu, X., Li, Z., & Ren, Q. (2020). Culturing Bacteria From Fermentation Pit Muds of *Baijiu* With Culturomics and Amplicon-Based Metagenomic Approaches. *Frontiers in Microbiology*, 11, 1223. <https://doi.org/10.3389/fmicb.2020.01223>
- Yan, Z., Zheng, X.-W., Chen, J.-Y., Han, J.-S., & Han, B.-Z. (2013). Effect of different *Bacillus* strains on the profile of organic acids in a liquid culture of *Daqu*. *Journal of the Institute of Brewing*, 119(1–2), 78–83. <https://doi.org/10.1002/jib.58>
- Yang, Y., Wang, S. T., Lu, Z. M., Zhang, X. J., Chai, L. J., Shen, C. H., Shi, J. S., & Xu, Z. H. (2021). Metagenomics unveils microbial roles involved in metabolic network of flavor development in medium-temperature *daqu* starter. *Food Research International*, 140, Article 110037. <https://doi.org/10.1016/j.foodres.2020.110037>
- Yi, T., Ling, Y., Rong-Qing, Z., Tao, W., Zong-Wei, Q., & Dong, Z. (2021). Distribution and Function of *Kazachstania* Yeast in the Fermentation of Strong Flavor *Baijiu*. *Biotechnology Bulletin*, 37(6), 108–116. <https://doi.org/10.13560/j.cnki.biotech.bull.1985.2020.1159>, in Chinese.
- Zha, M., Sun, B., Wu, Y., Yin, S., & Wang, C. (2018). Improving flavor metabolism of *Saccharomyces cerevisiae* by mixed culture with *Wickerhamomyces anomalus* for Chinese *Baijiu* making. *Journal of Bioscience and Bioengineering*, 126(2), 189–195. <https://doi.org/10.1016/j.jbiotec.2018.02.010>
- Zhang, H., Du, H., & Xu, Y. (2021a). Volatile organic compound-mediated antifungal activity of *pichia* spp. and its effect on the metabolic profiles of fermentation communities. *Applied and Environmental Microbiology*, 87(9), e02992-02920. doi:10.1128/AEM.02992-20.
- Zhang, H., Tan, Y., Wei, J., Du, H., & Xu, Y. (2022). Fungal Interactions Strengthen the Diversity-Functioning Relationship of Solid-State Fermentation Systems. *mSystems*, 7(4). <https://doi.org/10.1128/msystems.00401-22>, e0040122.
- Zhang, H., Wang, L., Tan, Y., Wang, H., Yang, F., Chen, L., Hao, F., Lv, X., Du, H., & Xu, Y. (2021b). Effect of *Pichia* on shaping the fermentation microbial community of sause-flavor *Baijiu*. *International Journal of Food Microbiology*, 336, Article 108898. <https://doi.org/10.1016/j.ijfoodmicro.2020.108898>
- Zhang, H., Wang, L., Wang, H., Yang, F., Chen, L., Hao, F., Lv, X., Du, H., & Xu, Y. (2021c). Effects of initial temperature on microbial community succession rate and volatile flavors during *Baijiu* fermentation process. *Food Research International*, 141, Article 109887. <https://doi.org/10.1016/j.foodres.2020.109887>
- Zhang, W., Li, J., Rao, Z., Si, G., Zhang, X., Gao, C., Ye, M., & Zhou, P. (2020). Sesame flavor *baijiu*: A review. *Journal of the Institute of Brewing*, 126(3), 224–232. <https://doi.org/10.1002/jib.614>
- Zhang, Y., Kang, J., Han, B.-Z., & Chen, X. (2024). Wheat-origin *Bacillus* community drives the formation of characteristic metabolic profile in high-temperature *Daqu*. *LWT-Food science and technology*, 191. <https://doi.org/10.1016/j.lwt.2023.115597>
- Zhang, Y., Kastman, E. K., Guasto, J. S., & Wolfe, B. E. (2018). Fungal networks shape dynamics of bacterial dispersal and community assembly in cheese rind microbiomes. *Nature Communications*, 9(1), 336. <https://doi.org/10.1038/s41467-017-02522-z>
- Zhang, Y., Shen, Y., Cheng, W., Wang, X., Xue, Y., Chen, X., & Han, B. Z. (2021d). Understanding the Shifts of Microbial Community and Metabolite Profile From Wheat to Mature *Daqu*. *Frontiers in Microbiology*, 12, Article 714726. <https://doi.org/10.3389/fmicb.2021.714726>
- Zhang, Y., & Yan, Z. (2019). Comparison Between traditional Feng-type Koji and New Feng-type Koji. *LIQUOR MAKING*, 46(5), 59–60, 1002-8110(2019)05-0059-02 in Chinese.
- Zhao, L., Wang, Y., Xing, J., Gu, S., Wu, Y., Li, X., Ma, J., & Mao, J. (2022). Distinct succession of abundant and rare fungi in fermented grains during Chinese strong-flavor liquor fermentation. *LWT-Food science and technology*, 163, Article 113502. <https://doi.org/10.1016/j.lwt.2022.113502>
- Zhao, Q.-S., Yang, J.-G., Zhang, K.-Z., Wang, M.-Y., Zhao, X.-X., Su, C., & Cao, X.-Z. (2020). Lactic acid bacteria in the brewing of traditional *Daqu* liquor. *Journal of the Institute of Brewing*, 126(1), 14–23. <https://doi.org/10.1002/jib.593>

- Zheng, Q., Lin, B., Wang, Y., Zhang, Q., He, X., Yang, P., Zhou, J., Guan, X., & Huang, X. (2015). Proteomic and high-throughput analysis of protein expression and microbial diversity of microbes from 30- and 300-year pit muds of Chinese Luzhou-flavor liquor. *Food Research International*, 75, 305–314. <https://doi.org/10.1016/j.foodres.2015.06.029>
- Zheng, X.-W., & Han, B.-Z. (2016). Baijiu (白酒), Chinese liquor: History, classification and manufacture. *Journal of Ethnic Foods*, 3(1), 19–25. <https://doi.org/10.1016/j.jef.2016.03.001>
- Zheng, X.-W., Tabrizi, M. R., Robert Nout, M. J., & Han, B.-Z. (2011). *Daqu* – A Traditional Chinese Liquor Fermentation Starter. *Journal of the Institute of Brewing*, 117(1), 82–90. <https://doi.org/10.1002/j.2050-0416.2011.tb00447.x>
- Zhi, Y., Wu, Q., Du, H., & Xu, Y. (2016). Biocontrol of geosmin-producing *Streptomyces* spp. by two *Bacillus* strains from Chinese liquor. *International Journal of Food Microbiology*, 231, 1–9. <https://doi.org/10.1016/j.ijfoodmicro.2016.04.021>
- Zhou, H., Xu, S., Xu, B., Jiang, C., Zhao, E., Xu, Q., Hong, J., & Li, X. (2024). Effect of *Caproicibacterium lactatifermentans* inoculation on the microbial succession and flavor formation of pit mud used in Chinese *Baijiu* fermentation. *Food Research International*, 175. <https://doi.org/10.1016/j.foodres.2023.113730>
- Zhou, T., He, H., Zhou, Q., Du, H., Cao, R., & Xu, Y. (2021). Exploring the source of microbiota in medium-high temperature *Daqu* based on high-throughput amplicon sequencing. *Food and Fermentation Industries*, 47(16), 66–71. <https://doi.org/10.13995/j.cnki.11-1802/ts.027000>. in Chinese.
- Zhu, L., Yang, Q., Jiang, W., Li, Q., Lin, B., Tang, J., & Chen, S. (2022). Mold communities and enzyme activity characteristics in light-flavor *Xiaoqu Baijiu*. *Food and Fermentation Industries*, 48(7), 70–77. <https://doi.org/10.13995/j.cnki.11-1802-ts.028796>. in Chinese.
- Zhu, Z., & Huang, Y. (2020). Structure and Diversity of Culturable Mold Communities in Different Fermentation Rounds in Main Maotai-Flavored Liquor-Brewing Areas of Maotai Town. *Food science*, 41(22), 184–192. <https://doi.org/10.7506/splx1002-6630-20190918-226>. in Chinese.
- Zhu, Z., & Huang, Y. (2021). Structure and Diversity Analysis of Mold Community in Main Maotai-flavor *Baijiu* Brewing Areas of Maotai Town Using High-throughput Sequencing. *Food science*, 42(8), 150–156. <https://doi.org/10.7506/splx1002-6630-20191227-332>. in Chinese.
- Zou, W., Kou, H., & Han, C. (2021). Construction of the database of cultivated microorganisms for Chinese *Baijiu*. *Acta Microbiologica Sinica*, 61(21), 3829–3835. <https://doi.org/10.13343/j.cnki.wsxb.20210370>. in Chinese.
- Zou, W., Ye, G., & Zhang, K. (2018). Diversity, Function, and Application of *Clostridium* in Chinese Strong Flavor *Baijiu* Ecosystem: A Review. *Journal of food science*, 83(5), 1193–1199. <https://doi.org/10.1111/1750-3841.14134>
- Zuniga, C., Zaramela, L., & Zengler, K. (2017). Elucidation of complexity and prediction of interactions in microbial communities. *Microbial Biotechnology*, 10(6), 1500–1522. <https://doi.org/10.1111/1751-7915.12855>