



Can we control microbiota in spontaneous food fermentation? – Chinese liquor as a case example

Qun Wu^{a,d}, Yang Zhu^b, Cheng Fang^{a,d}, Rene H. Wijffels^{b,c}, Yan Xu^{a,*}

^a Key Laboratory of Industrial Biotechnology, Ministry of Education; State Key Laboratory of Food Science and Technology; School of Biotechnology, Jiangnan University, Wuxi, Jiangsu 214122, China

^b Bioprocess Engineering, Wageningen University and Research, P.O. Box 16, 6700AA Wageningen, Netherlands

^c Faculty Biosciences and Aquaculture, Nord University, N-8049, Bodø, Norway

^d Suqian Industrial Technology Research Institute of Jiangnan University, Suqian, Jiangsu, 223800, China

ARTICLE INFO

Keywords:

Fermented food
Flavour compound
Microbiota
Spontaneous fermentation
Chinese liquor

ABSTRACT

Background: Fermented foods, with a history as long as the human civilization, form an indispensable constituent in our daily life. However, most fermented foods are produced by spontaneous fermentation, and the fermentation processes are still uncontrolled. To ensure consistent food quality, it is of paramount importance to understand and control the spontaneous food fermentations.

Scope and approach: In spontaneous food fermentations, metabolic activity of a succession of complex microbiota results in desired flavour that is the key criterion to decide consumers' preference. Therefore, flavour compound formation by microbial metabolism can be used as the control target in spontaneous food fermentations. However, relatively little is known about the complexity of the microbiota associated with the flavour compound formation. Therefore, in this review by using Chinese liquor as a model system, we present key biotechnological aspects of the microbiota crucial for flavour of fermented foods, including the driving forces for the microbiota succession, flavour compound formation, and the regulation of flavour compound formation.

Key findings and conclusions: Core microbiota, associated with flavour compound formation, can be identified and eventually used to construct a synthetic (i.e. designed) microbiota. Meanwhile, key environmental factors, affecting the core microbiota, can also be identified and controlled to regulate the synthetic microbiota. Furthermore, modelling can be used to predict, optimize and control the flavour compound formation by the synthetic microbiota, so that such spontaneous food fermentations can become controllable, with the ultimate goal to monitor, control, and improve the quality, productivity and safety of fermented foods.

1. Introduction

Human history and civilization are closely accompanied by numerous fermented foods including cheese, wine, bread, soy sauce, yogurt, vinegar and many others (Johnson, 2016; Steinkraus, 1983). These fermented foods are usually produced by spontaneous fermentations with complex microbiota from the raw materials and the environment (Hesseltine, 1965; Wood, 2012). The microbiota converts different substrates to various desired compounds, including a variety of flavour compounds. Flavour compounds, here referring to the volatile and non-volatile compounds related to smell and taste (Bertuzzi, McSweeney, Rea, & Kilcawley, 2018; Ulla, Antti, Oskar, & Mari, 2016), play important roles in the consumer perception of fermented foods.

During each stage of a given traditional food fermentation, different microorganisms play their corresponding roles that involve initially microbial growth to produce various enzymes, then the hydrolysis of carbohydrates and proteins, followed by various complex biochemical processes catalysed by the enzymes to form desired flavour compounds. Therefore, studying the association of microbiota with flavour compound formation is essential to understand, control and improve food fermentations efficiently for food quality, productivity and safety. Unfortunately, most spontaneous food fermentations are practiced without the design and control of the microbiota.

Recently, assembly and function of microbiota are addressed in microbiome studies (Chen, Chen, & Lei, 2017). However, the studies remain describing rather than understanding the association of the

* Corresponding author. School of Biotechnology, Jiangnan University, Wuxi, Jiangsu, 214122, China.

E-mail address: yxu@jiangnan.edu.cn (Y. Xu).

<https://doi.org/10.1016/j.tifs.2021.02.011>

Received 1 July 2020; Received in revised form 3 February 2021; Accepted 6 February 2021

Available online 15 February 2021

0924-2244/© 2021 The Authors.

Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

dynamic microbiota succession with flavour compound formation. Three questions need to be answered. First, what determines the microbiota composition and its dynamic succession? Second, what are the roles of the microbiota in forming flavour compounds? Third, how the microbiota can be regulated and controlled for the optimal or desired flavour compound formation? Answering these questions would give a knowledge-based directional improvement and innovation of traditional food fermentation.

The microbial complexity of food fermentations ranges from a few core microorganisms (e.g. for the fermentation of soy sauce) to hundreds of microorganisms (e.g. Chinese liquor). The fermentation of Chinese liquor (called Baijiu in Chinese) is an ideal model to study fermented foods. So far more than 300 flavour compounds have been observed in Chinese liquor (Xu & Ji, 2012), probably with the most abundant flavour compounds among all fermented foods. It is produced via a spontaneous solid-state fermentation of cereals (mainly sorghum) by microbiota including filamentous fungi, yeasts and bacteria, all of them are supposed responsible for the production of such complex composition of flavour compounds (Jin, Zhu, & Xu, 2017). In addition, the unique environmental factors also contribute to the dynamic succession of the microbiota for the liquor fermentation. However, a scientific understanding is badly needed to understand, monitor and control the microbiota for flavour compound formation.

Therefore, we critically address here the available knowledge about this complex microbiota with flavour compound as a criterion, mainly the driving force of the microbiota succession, the flavour compound formation and the regulation of responsible microbiota, with Chinese liquor fermentation as a case example. Chinese liquor fermentation is so

complex that once its microbiota can be controlled, the principles should be easily applicable to similar or less complex food fermentations. Furthermore, we indicate new study directions on controlling microbiota in food fermentations, to benefit the revolution, standardization and modernization of many other spontaneous food fermentations.

2. Microbiota and driving forces in liquor processing stages

2.1. Microbiota and driving forces in starter (Daqu) fermentation

Unlike some western fermented foods using single or a few strains as defined starter, Chinese liquor fermentation still inherits the use of undefined multi-species starter, Daqu (a sort of Koji). Daqu is also used for making many other traditional oriental fermented foods, such as rice wine and vinegar (Jin et al., 2017; Zhu & Tramper, 2013). This type of starter is produced by a spontaneous solid-state fermentation without any inoculum. Thus, the first step is to characterize the role of Daqu in liquor fermentation. As shown in Fig. 1 (left), Daqu contributes not only functional microbial communities, but also their products. For example, filamentous fungi, such as *Aspergillus*, *Rhizomucor* and *Rhizopus*, produce many hydrolytic enzymes, including α -amylase and glucoamylase (Wang, Wu, Xu, & Sun, 2020). Yeasts, such as *Saccharomyces* and *Pichia*, produce flavour compounds. Bacteria, such as *Bacillus* and *Lactobacillus*, produce acids and acetoin (Zhang, Wu, & Xu, 2013). Thus, Daqu enabling the growth and enrichment of the complex microbiota, is a complex bioactive agent to provide hydrolytic enzymes, microbiota and flavour compounds (Zheng et al., 2014). Understanding the microbiota and driving forces in starter

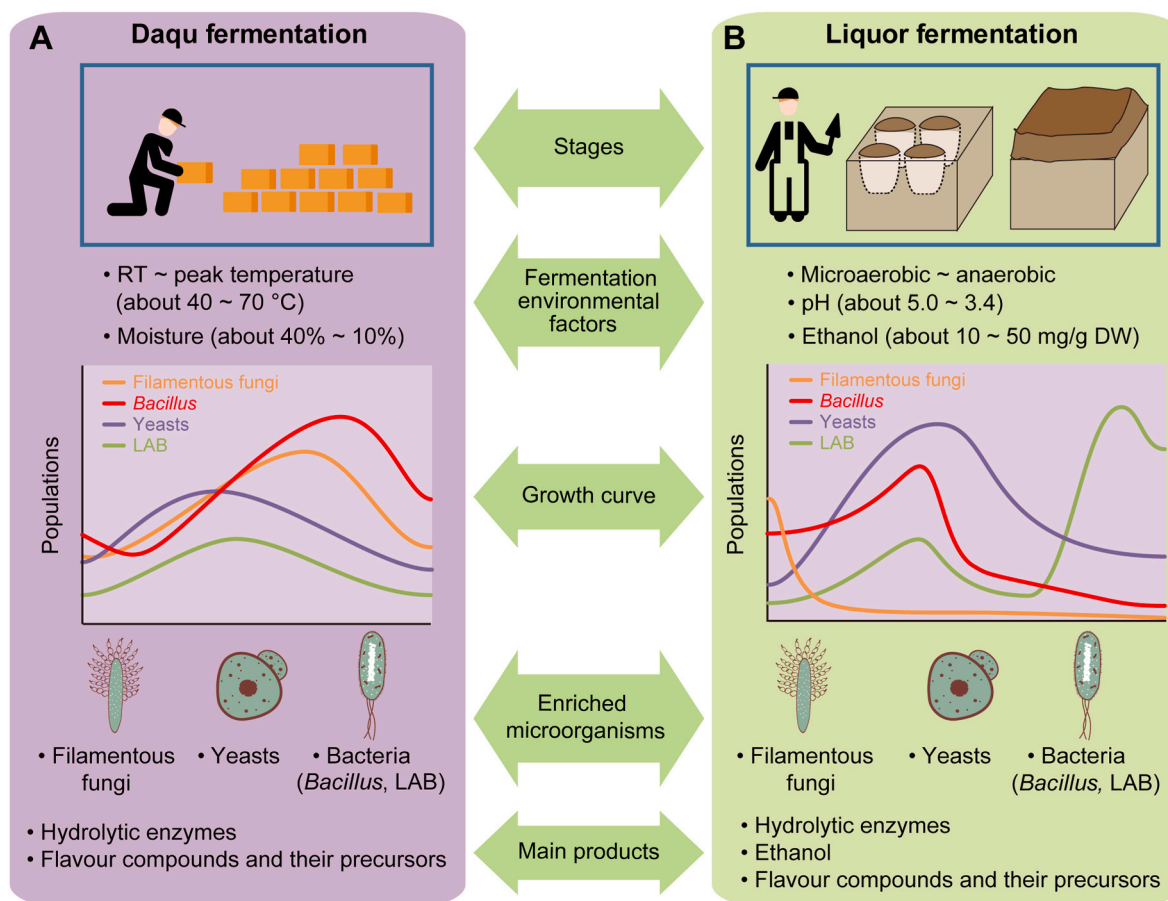


Fig. 1. Schema for Chinese liquor making with two main stages including Daqu fermentation (A) and liquor fermentation (B). Daqu fermentation is mainly influenced by temperature and moisture, to enable microbes to grow and to produce hydrolytic enzymes, flavour compounds and their precursors. Liquor fermentation is characterized by microaerobic to anaerobic fermentation with low pH and high ethanol content. The microbiota produces hydrolytic enzymes, ethanol, flavour compounds and their precursors. RT: room temperature; LAB: lactic acid bacteria; DW: dry weight.

preparation is a premise to control the quality of Daqu.

The solid-state fermentation of Daqu undergoes in an open environment, and is initiated by microbes that are naturally present in raw materials and the environment. Raw materials are the main contributor of bacterial communities for Daqu fermentation, including *Staphylococcus*, *Weissella*, *Leuconostoc*, and *Lactobacillus*. The environment is the main sources of the fungal communities, including *Saccharomycopsis*, *Pichia*, *Rhizopus*, and *Aspergillus* (Du, Wang, Zhang, & Xu, 2019). Different environmental conditions drive the microbiota dynamics in Daqu fermentation. As Daqu preparation is a solid-state fermentation, temperature and moisture are the two most important environmental factors. The inner temperature increases to the peak (ranging from 40 to 70 °C) due to bioheat accumulation, and decreases to the room temperature due to reduced metabolic activity and heat transfer (Jin et al., 2019, 2020). The peak temperature inside Daqu plays an important role in the final diversity of microbiota (Zheng et al., 2015). Lowering the fermentation temperature decreases the relative abundances of thermotolerant microbes (Xiao et al., 2017). Accordingly, the moisture content decreases from approximately 40%–10% during Daqu preparation (Li, Liang, Lin, Feng, & Luo, 2015; Zheng et al., 2014). Those strains tolerating low water activity are dominant at the end of fermentation, such as *Aspergillus*, *Rhizopus* (Wang, Ban, & Qiu, 2018) and *Bacillus* (Li et al., 2015). Understanding the effect of temperature and moisture on the microbiota dynamics would facilitate the control and improvement of the microbiota for optimal liquor fermentation with desired flavour.

2.2. Microbiota in liquor fermentation

Ethanol, flavour compounds and many their precursors are produced in liquor fermentation stage (Fig. 1, right). Grains mixed with Daqu powder are put into underground pits/pots and sealed for fermentation for 30–60 days (Jin et al., 2017). Daqu provides a large part of fungal community including *Pichia*, *Saccharomycopsis* and *Aspergillus*. The environment, during this stage of fermentation, contributes a large part of bacterial community, including *Lactobacillus*, *Bacillus* and *Weissella* (Wang, Du, Zhang, & Xu, 2018).

The assembled microbiota undergoes a typical succession profile during this spontaneous process. Filamentous fungi, dominated by *Aspergillus*, present in the early period. Yeasts, dominated by *Saccharomyces*, *Pichia* and *Saccharomycopsis*, present from the initial to middle period. Bacteria present in the whole process. *Lactobacillus* becomes the most abundant bacterial genus after *Bacillus* and non-*Lactobacillus* lactic acid bacteria, such as *Weissella* (Song, Du, Zhang, & Xu, 2017; Wang, Du, et al., 2018).

This stage is characterized by the shift of microaerobic to anaerobic fermentation, increase of ethanol content and decrease of pH. These changes play different roles in different periods of liquor fermentation. The decrease of oxygen leads to the dramatic decline of filamentous fungi in the early period of the fermentation (Chen, Wu, & Xu, 2014). In addition, microbiota is affected by ethanol produced by yeast in the early period whereas by lactic acid produced by lactic acid bacteria in the late period. Consequently, the shift of oxygen, ethanol and acid (pH) drives the succession of the microbiota (Song et al., 2017).

3. Microbiota and flavour compound formation in Chinese liquor making

3.1. Main flavour compounds in Chinese liquor

Although various flavour compounds only account for about 1%–2% (v/v) in liquor, they play crucial roles in liquor quality, including smell and taste. To date, more than 1000 volatile compounds have been detected in Chinese liquor (Jin et al., 2017; Liu & Sun, 2018). Among them, more than 300 compounds contributing to the flavour of liquor are identified, including esters, alcohols, aldehydes, ketones, acids,

pyrazines, furans, phenols, benzene derivatives, sulphur compounds, lactones, terpenes and other compounds (Chen, Sha, Qian, & Xu, 2017; Fan, Xu, & Zhang, 2007; Liu & Sun, 2018; Wang, Hu, et al., 2016; Xu & Ji, 2012). Table 1 lists the main flavour compounds in main types of Chinese liquor.

Esters are the most abundant flavour compounds in Chinese liquor (Fang, Du, Jia, & Xu, 2019). They can be produced from esterification with acids and alcohols by lipase. Some esters can also be produced by alcohol acetyl transferase, such as ethyl acetate (Zhang et al., 2020). Alcohols are important flavour compounds in Chinese liquor and mainly produced from amino acids and carbohydrates by yeasts (Fang et al., 2019). Short and medium-chain fatty acids are the main organic acids in Chinese liquor. Among them, butyric acid and hexanoic acid could be produced via reverse β -oxidation pathway (Zhu et al., 2017). Pyrazines generally exhibit a nutty, roasted, and toasted aroma (Zhang, Cao, Tong, & Xu, 2019). Among them, 2,3,5,6-tetramethylpyrazine is formed from acetoin and ammonia via a nonenzyme-catalysed reaction (Zhang et al., 2019). 2,5-Dimethylpyrazine is formed from the cyclization and oxidation of aminoacetone. 2,3,5-Trimethylpyrazine can be formed from acetoin and aminoacetone as precursors (Zhang et al., 2019). Terpenes mainly exhibit floral aroma, and a variety of terpenes have been identified in Chinese liquor (Wang, Hu, et al., 2016). β -Damascenone has a strong odour intensity (Wang, Hu, et al., 2016). It can be produced by biotransformation from glycosidic precursors in raw materials (Wu, Zhu, Wang, & Xu, 2015). Volatile sulphur compounds are also key flavour compounds in Chinese liquor. Thirteen volatile sulphur compounds are identified, and 2-furfurylthiol, methanethiol, dimethyl trisulphide, ethanethiol and methional present relatively high odour activity values (Chen, Sha, et al., 2017). 2-Furfurylthiol can be generated from furfural and L-cysteine with microbial and chemical reaction (Shen, Liu, Wu, & Xu, 2020). Methanethiol is generated from methionine catalysed by methionine γ -lyase, or cystathionine γ -lyase (Liu et al., 2017).

Most flavour compounds and their precursors are associated with

Table 1
Main flavour compounds in main types of Chinese liquor.

Groups	Main flavour compounds	Descriptor
Esters	Ethyl acetate	Pineapple
	Ethyl lactate	Fruity
	Ethyl butyrate	Pineapple
	Ethyl pentanoate	Apple
	Ethyl isovalerate	Fruity
	Ethyl hexanoate	Fruity
	Ethyl heptanoate	Fruity
	Ethyl 2-phenylacetate	Rosy, honey
Alcohols	2-Phenylethyl acetate	Floral
	2-Methyl-1-propanol	Malty
	3-Methyl-1-butanol	Malty
	β -Phenylethyl alcohol	Floral
Acids	Acetic acid	Vinegar, acid
	Butanoic acid	Sweaty, rancid, cheesy
	Hexanoic acid	Sweaty, rancid, cheesy
	Heptanoic acid	Rancid
	2-Methylpropanoic acid	Sweaty, rancid, cheesy
Aldehyde	3-Methylbutanoic acid	Sweaty, rancid, cheesy
	(E)-2-Octenal	Green
Pyrazine	2,3,5,6-Tetramethylpyrazine	Nutty, roasted, toasted
	2,3,5-Trimethylpyrazine	Nutty, roasted, toasted
	β -Damascenone	Floral, honey
	Phenol	Smoky
	Sulphur compounds	4-Methylguaiacol
2-Furfurylthiol		Roasted sesame seeds
Methanethiol		Rotten cabbage, burnt rubber
Dimethyl trisulphide		Onion, sulphurous
	Ethanethiol	Onion, rubber
	Methional	Cooked potato

References (Chen, Sha, et al., 2017; Fan et al., 2007; Fang et al., 2019; Jia et al., 2020; Jin et al., 2017; Liu & Sun, 2018; Wang, Hu, et al., 2016; Wang, Yang, Zhao, Zhang, & Su, 2019; Xu & Ji, 2012; Zhang et al., 2019):

microbial metabolism, i.e. either catalysed by enzymes produced by the microbiota, or transferred from precursors generated through microbial activity, confirming the essential role of microbiota in flavour compound formation.

3.2. Flavour compound formation by the microbiota

Like almost all spontaneous food fermentations, how microbiota influences the flavour compound formation in Chinese liquor fermentation is poorly understood, due to the complex compositions and dynamic succession of the microbiota. Kong et al. (Kong, Wu, Zhang, & Xu, 2014) found *Pichia anomala* (renamed as *Wickerhamomyces anomalus*) is responsible for several important flavour compounds in Chinese liquor, by covariation analysis of metabolic profiles and yeast communities in Chinese liquor fermentation. Similarly, key flavour compound producers could be identified in spontaneous fermentation. Recently, meta-omics methods are also applied to reveal the *in situ* metabolic activity (Song et al., 2017; Wang, Wu, Xu, & Sun, 2018). Genera *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces* and *Lactobacillus* are the dominant contributors of flavour compound formation (Song et al., 2017). In addition to these culture-independent methods, the culture-dependent method, as an important complementary method, is still required to validate the microbial metabolic activity predicted via the culture-independent methods. Table 2 illustrates the dominant microorganisms and their biological activities in main types of Chinese liquor fermentations.

(1) Filamentous fungi

Filamentous fungi act as both the saccharifying agents and flavour compound producers. At present, work mainly focuses on the dominant genera of *Aspergillus* and *Rhizopus*. *Aspergillus oryzae* strain isolated from liquor fermentation process produces 2463 U/g glucoamylase and 1491 U/g α -amylase cultured on wheat bran for 72 h (Chen et al., 2014). *A. oryzae* CGMCC 6264 presents saccharification activity and can be used to regulate yeast metabolism. It is also a producer of flavour compounds, including esters, alcohols and acids (Wu, Chen, & Xu, 2015). *Aspergillus hennebergii* strain produces proteases that are responsible for producing varieties of flavour compounds, including alcohols, esters, aldehydes, benzene derivatives and pyrazines (Huang, Wu, & Xu, 2014). *Rhizopus microspores* is identified to be the contributor of glucoamylase by metaproteomics analysis (Wang, Wu, et al., 2018). *Rhizopus chinensis* CCTCCM201021, isolated from Daqu, produces lipase that is important to form esters from alcohols and acids (Sun, Xu, & Wang, 2009). In addition, its draft genome sequence analysis suggests large varieties of genes encoding important enzymes, including 493 glycoside hydrolases, 168 proteases and 121 lipase/esterase genes (Wang, Wu, Xu, & Li, 2013). The rich hydrolase compositions suggest the importance of diversity of filamentous fungi in liquor making (Chen et al., 2014), because they are the main producers of various enzymes that are directly involved in flavour compound formation.

(2) Bacteria

Bacillus is an important flavour compound producer. *Bacillus licheniformis* MT-B06 is reported to produce 3-hydroxy-2-butanone, 2,3-butanediol and several pyrazines during the fermentation (Yang et al., 2020). *B. licheniformis* CGMCC 3962 produces compounds that exhibit soy sauce flavour, the key flavour of Moutai-flavour liquor (Wu & Xu, 2012). *B. licheniformis* CGMCC 3961, 3962 and 3963 produce the non-volatile compound lichenysin (Wu, Zhang, Peng, & Xu, 2015) that significantly decreases the headspace concentrations of off-flavour compounds, such as 4-methylphenol (Zhang, Wu, & Xu, 2014). *Bacillus amyloliquefaciens* 1-45 and *Bacillus subtilis* 2-16 are identified as efficient producers of surfactin (Zhi, Wu, Du, & Xu, 2016; Zhi, Wu, & Xu, 2017) that inhibits the off-flavour compound geosmin-producing

Table 2
Important microbes and their metabolites in Chinese liquor making.

Microbes	Species	Metabolic products	References
Filamentous fungi	<i>A. oryzae</i>	Glucoamylase, α -amylase, esters, alcohols and acids	(Chen et al., 2014; Wu, Chen, & Xu, 2015)
	<i>Paecilomyces variotii</i>	Glucoamylase, α -amylase	Chen et al. (2014)
	<i>R. chinensis</i>	Lipase, glycoside hydrolase, protease	(Sun et al., 2009; Wang et al., 2013)
Yeasts	<i>A. hennebergii</i>	Acid proteases	Huang et al. (2014)
	<i>S. cerevisiae</i>	Ethanol, alcohols, esters, terpenoids, sulphur compounds	(Liu et al., 2017; Wu, Xu, & Chen, 2012; Wu, Zhu, et al., 2015)
	<i>Z. bailii</i>	Ethanol, alcohols, aldehyde and ketones	(Wu et al., 2012; Xu et al., 2017)
	<i>P. kudriavzevii</i> (<i>I. orientalis</i>)	Acids, terpenoids	(Wu et al., 2012; Wu, Zhu, et al., 2015)
	<i>S. pombe</i>	Ethanol, alcohols, acids, esters	(Chen, Wu, & Xu, 2013; Wu et al., 2012)
	<i>S. fibuligera</i>	Glucoamylase, α -amylase, alcohols, esters	Ma et al. (2019)
Bacteria	<i>W. anomalus</i>	Esters	(Fan et al., 2019; Zha, Sun, Wu, et al., 2018)
	<i>B. licheniformis</i>	2,3,5,6-Tetramethylpyrazine, acids, lichenysin, soy sauce aroma	(Wu & Xu, 2012; Wu, Zhang, et al., 2015; Yang et al., 2020; Zhang et al., 2013)
	<i>B. subtilis</i>	2,3,5,6-Tetramethylpyrazine, surfactin	(Zhang et al., 2019; Zhi et al., 2016)
	<i>B. amyloliquefaciens</i>	Surfactin	(Zhi et al., 2016; Zhi et al., 2017)
	<i>L. homohiochii</i>	Acids, alcohols	Zhang, Du, Wu, and Xu (2015)
	<i>L. buchneri</i>	Acids, alcohols	Zhang et al. (2015)

Streptomyces spp. in liquor fermentation (Zhi et al., 2016).

Lactobacillus is correlated with different flavour compounds. Meta-transcriptomic analysis indicated that *Lactobacillus* is active in transcribing genes involved the biosynthesis of flavour compounds and their precursors, including esters and acids (Du, Wu, & Xu, 2020).

(3) Yeasts

The dominant yeast species in liquor fermentation include *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Zygosaccharomyces bailii*, *Saccharomycopsis fibuligera* and *W. anomalus*. One isolated *S. cerevisiae* strain can produce 13 terpenoids by both *de novo* and biotransformation pathways (Wu, Zhu, et al., 2015). *S. cerevisiae* MT1 simultaneously utilizes various sugars, a beneficial trait to efficiently use various substrates. Genomic sequence of *S. cerevisiae* MT1 contains 145 specific genes, resulting in its unique metabolic activity (Lu, Wu, Zhang, & Xu, 2015). Among them, *MEL1* and *MAL63* encode enzymes uniquely utilizing melibiose and maltose. The high transcriptions of *HXT5* and *HXT13* are related with the simultaneous utilization of various sugars

(Lu et al., 2015). These properties would enrich our knowledge about the role of *S. cerevisiae* in alcoholic beverage fermentations. *S. cerevisiae* is also related with the formation of volatile sulphur compounds. *S. cerevisiae* JZ1093 produces 3-(methylthio)-1-propanol and dimethyl disulphide (Liu et al., 2017). *S. cerevisiae* G20 forms 2-furfurylthiol with furfural and L-cysteine as precursors, and proteins Str3p and Cys3p can cleave the cysteine-furfural conjugate to release 2-furfurylthiol (Zha, Sun, Wu, Yin, & Wang, 2018).

Non-*Saccharomyces* are also reported to produce flavour compounds. *P. kudriavzevii*, as one of the dominant species in almost all Chinese liquor fermentations (Jin et al., 2017; Liu & Sun, 2018), is a major source of higher alcohols and acids. *Z. bailii* is a dominant species in soy sauce aroma type liquor fermentation. *Z. bailii* MT15 uniquely produces 19 flavour compounds, including 13 alcohols, 2 acids, 1 ketone, 1 aldehyde, and 2 other flavour compounds (Xu, Zhi, Wu, Du, & Xu, 2017). It is identified as a yeast interspecies hybrid with genome size of 20.19 Mb. Twelve genes related to amino acid metabolism and transport, show higher expression levels at 37 °C, compared to that at 30 °C (Xu et al., 2017). *S. fibuligera*, as a dominant species in most strong and light aroma type liquor fermentations (Jin et al., 2017; Liu & Sun, 2018), is a higher producer of glucoamylase and α -amylase among yeasts. In addition, it is also an excellent producer of flavour compounds, especially alcohols and esters (Ma, Sui, Zhang, Hu, & Liu, 2019). *W. anomalus*, as a dominant species in light aroma type liquor fermentation, is characterized by an excellent producer of ethyl esters (Zha, Sun, Wu, et al., 2018).

The above reveals the contribution of microbes to various flavour compounds formation in a complex multi-species fermentation. Through their complementary contribution, different dominant microbial species facilitate the unique flavour diversity of Chinese liquor.

3.3. Effect of microbial interactions on flavour compound formation

Microbial interaction is crucial for the microbiota structure and will influence the microbial metabolism, hence the final flavour compound formation (Ciani et al., 2016). Understanding the effect of microbial interactions on flavour compound formation is important for regulation of flavour compound formation. *S. cerevisiae* plays important roles in ethanol and flavour compound production, and interacts vigorously with other microbes. Here we illustrate the interactions between *S. cerevisiae* with other flavour compound producers in liquor fermentation.

(1) Interaction between *Saccharomyces cerevisiae* and filamentous fungi

Filamentous fungi, with *A. oryzae* as a representative, produce hydrolases to degrade starch into fermentable sugars, and *S. cerevisiae* simultaneously utilizes sugars to produce ethanol and flavour compounds. *A. oryzae* and *S. cerevisiae* co-exist in the early stage of liquor fermentation. Regulating the coordination of saccharification and fermentation is crucial to control ethanol and flavour compound production (Wu, Chen, & Xu, 2015). Trial is done to regulate this coordination by changing the inoculation ratio of *A. oryzae* and *S. cerevisiae* (Wu, Chen, & Xu, 2015). In addition, different filamentous fungi produce multiple saccharifying enzymes, and these enzymes present a synergistic effect on ethanol production (Wang, Wu, et al., 2020), implying that more filamentous fungi could be involved to alter the microbial interaction.

(2) Interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts

The interaction between *S. cerevisiae* and non-*Saccharomyces* exists in many food fermentations (Ciani et al., 2016). *S. cerevisiae* inhibits non-*Saccharomyces* yeasts in Chinese liquor fermentation, similar to wine fermentation. For example, *S. cerevisiae* inhibits the growth of

W. anomalus (Zha, Sun, Wu, et al., 2018) and *Issatchenkia orientalis* (renamed as *P. kudriavzevii*) (Wu, Ling, & Xu, 2014). The inhibition might be resulted from the metabolites produced by *S. cerevisiae*, such as ethanol (Wu, Kong, & Xu, 2016). Although *S. cerevisiae* inhibits the growth of non-*Saccharomyces* yeasts, the metabolism of the latter is not inactivated in the co-culture. For example, mixed culture of *S. cerevisiae* and *W. anomalus* produces more ethyl acetate and 2-phenylethanol than the single culture of either of the two (Zha, Sun, Wu, et al., 2018). Therefore, regulating the interaction between *S. cerevisiae* and non-*Saccharomyces* yeasts can regulate flavour compounds production in liquor fermentation because each yeast has its own metabolites.

(3) Interaction between *Saccharomyces cerevisiae* and bacteria

Bacillus is a dominant bacterial genus in the early period of liquor fermentation. When *S. cerevisiae* and *B. licheniformis* coexist, the growth of *B. licheniformis* is significantly inhibited, but the growth of *S. cerevisiae* keeps stable. Interestingly, ethanol production increases in the co-culture compared with the single culture of *S. cerevisiae* (Meng et al., 2015). The increase of ethanol is important for yeast to inhibit bacteria. In addition, 12 flavour compounds uniquely produced by *S. cerevisiae*, are significantly correlated with the inoculation size of *B. licheniformis* (Meng et al., 2015).

Lactobacillus is a dominant bacterial genus in liquor fermentation. The synergetic effect is observed elsewhere between *S. cerevisiae* and *Lactobacillus*. For example, *S. cerevisiae* produces growth factors such as carbon dioxide, to stimulate growth of *Lactobacillus sanfranciscensis* in a sourdough-like environment (Sieuwert, Bron, & Smid, 2018). *S. cerevisiae* benefit the survival of *Lactobacillus plantarum* and *Lactococcus lactis* by secreting amino acids in the co-culture with nitrogen-rich medium (Ponomarova et al., 2017). A synergistic effect on sulphur metabolism is also observed in their co-culture. *S. cerevisiae* is a producer of 3-(methylthio)-1-propanol and dimethyl disulphide, but *Lactobacillus buchneri* could not produce these two flavour compounds. Their co-culture significantly enhances the production of these two compounds by *S. cerevisiae*, where *L. buchneri* shows high transcriptional activity in methyl cycle that can enhance the regeneration of methionine, the precursor of 3-(methylthio)-1-propanol and dimethyl disulphide (Liu et al., 2017).

For Chinese liquor fermentation, many positive effects of microbial interactions on flavour compounds formation exist. Although several interactions have been studied, many remain undiscovered, especially the interactions among all the species in the fermentation. It is important to reveal the exact roles of these interactions during a spontaneous food fermentation, and subsequently, to make control and improve such fermentations possible.

4. Regulation and control of flavour compound formation by microbiota

For all spontaneous food fermentation processes, three categories of factors drive the successive dynamic profile of microbiota, namely the initial environmental factors, the initial microbiota, and the fermentation processing (Fig. 2). By regulating these factors, microbiota can be controlled to obtain desired product flavour.

4.1. Initial environmental factors

The initial environmental factors play a vital role in driving the microbial succession. Most environmental factors are reasonably controllable at the initial stage of the process, including moisture, pH, oxygen and temperature, as shown in Fig. 2. Therefore, we suggest that a predictable model could be constructed associating these initial environmental factors with liquor quality and productivity, the former in fact the flavour compounds. Using this model, we can evaluate how these key environmental factors affect the microbiota that determines

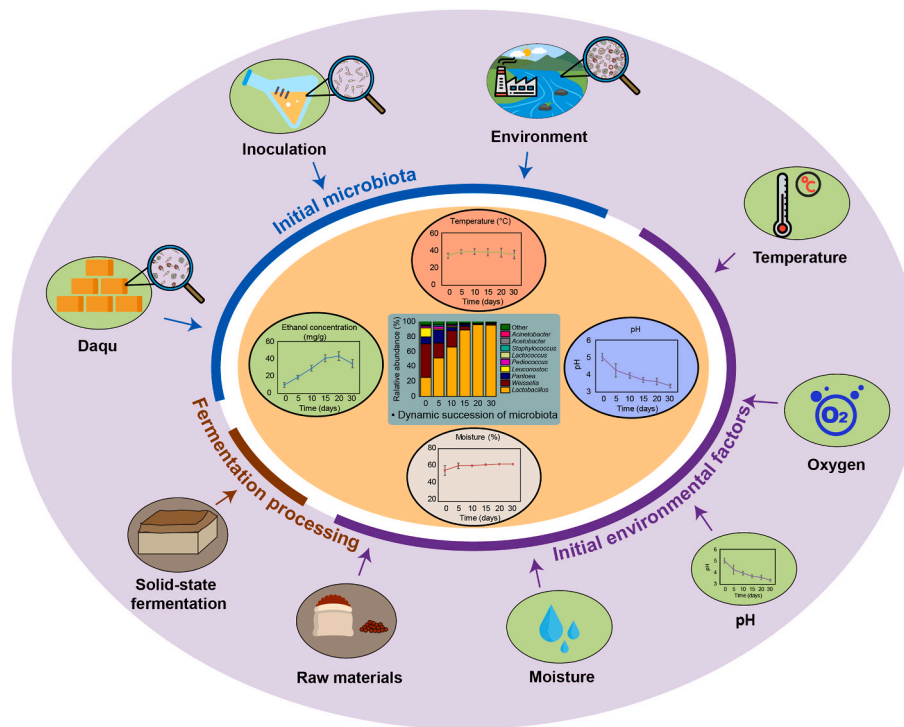


Fig. 2. Factors driving the dynamic succession of microbiota in liquor fermentation. There are three categories of factors, including the initial environmental factors, the initial microbiota, and the fermentation processing.

the liquor quality (flavour) and productivity, and subsequently optimize the flavour compounds profile by adjusting the controllable initial parameters. However, constructing a reliable model requires big data collected from numerous different fermentation batches. This would be realized with the combination of different disciplines, including microbiology, biotechnology and mathematics.

In addition to those controllable factors, raw materials, as the fermentation substrate, also play essential roles in liquor fermentation. Cereals, as the main raw materials, provide various types of sugars, organic acids, amino acids and other microelements for liquor fermentation. The yeast-assimilable nitrogen is identified as key nutrient parameter in wine fermentation (Englezos et al., 2018), and key amino acids are limited in malt beverage fermentation (Nsogning, Fischer, & Becker, 2018). However, the effect of raw materials on the microbiota in Chinese liquor fermentation is more complex, still needs to be uncovered. First, raw materials provide the nutrients that affect the growth and metabolism of the microbiota. Raw materials can affect the single cellular metabolism (Wu, Zhu, et al., 2015), as well as microbiota dynamics and flavour compound formation, due to a complex of nutrient composition in cereal materials. Fermentation with more cereal types leads to a slower microbial succession rate compared with single cereal fermentation, and the slow microbial succession rate is beneficial for the microbial enrichment and diversity maintenance (Tan, Zhong, Zhao, Du, & Xu, 2019). Therefore, it is important to find key elements and their functions in the substrate to enable the regulation of the nutrient composition. Second, raw materials, partly as a source of microbiota, contributes most bacteria for Daqu fermentation. For wine fermentation, grape-associated microbiota presents biogeographic pattern associated with regional and climatic factors (Bokulich, Thorngate, Richardson, & Mills, 2014). Similarly, raw materials that also partly contribute microbiota for Daqu fermentation, can be controlled by factors such as production region, season and climate if the microbial and nutrient composition can be evaluated.

4.2. Initial microbiota

Traditional food fermentations initiate spontaneously with a microbiota present in the environment. Various western food fermentations have developed single or defined starters to replace the natural microbiota (Johansen, 2018; Wache et al., 2018). However, liquor fermentation is initiated by a more complex microbiota source, including microbiota from both the natural environment and the undefined starter, Daqu. Daqu is generally considered as a main part for providing microbiota for liquor fermentation with 9.1%–27.4% of the bacterial community, and 61.1%–80.0% of the fungal community (Wang, Du, et al., 2018). The environment contributes 62.6%–90.9% of the bacterial community and 20.0%–38.9% of the fungal community to liquor fermentation (Wang, Du, et al., 2018). However, the environment itself is often hard to control, because the environmental microbiota is susceptible to the environmental conditions, such as the climate and location. Therefore, inoculation becomes an efficient way to regulate the initial microbiota. *Bacillus*, *Pediococcus*, *Wickerhamomyces* and *Saccharomyces* have been inoculated in Daqu fermentation to regulate the initial microbiota (Li et al., 2017; Wang et al., 2017). The regulation of the microbiota in Daqu improves the flavour compounds profile. In addition to the regulation of Daqu, some beneficial strains can also be fortified at the liquor fermentation stage. For example, *Bacillus* inoculation at the initial stage of liquor fermentation improves flavour compounds profile (Zhang et al., 2013). Therefore, the regulation of microbiota at both the Daqu preparation and liquor fermentation can regulate the initial microbiota for desired flavour compound formation.

4.3. Fermentation processing

Temperature, moisture, oxygen, pH (or acid) and ethanol content influence microbiota during liquor fermentation process. The factors can be theoretically controlled (Liu & Sun, 2018). In the industrial practice, the change of Daqu shape can cause variation of its specific volumetric weight that subsequently affects Daqu moisture and acidity (Wang, Wu, et al., 2018). These factors can also be influenced by the cell growth and

metabolism of the microbiota. For example, in Daqu fermentation, microbial metabolic activity can produce heat to increase the temperature that in turn limits further optimal microbial growth and metabolism. In addition, yeast growth and metabolism can be inhibited by acids produced by lactic acid bacteria that similarly can be inhibited by ethanol produced by yeasts (Jin et al., 2020). Understanding the relationships among all these factors, will help regulate and control the microbiota for desired flavour compound formation (Jin et al., 2017).

5. Perspectives and conclusions

Chinese liquor, like many other spontaneously fermented foods, serves our daily demand for quality of life, although the production of these fermented foods is rather based on empirical experience and skills inherited from generation to generation than fully understood scientific principles. Spontaneously fermented foods like Chinese liquor are produced through an uncontrolled process with inevitably fluctuations in quality and productivity. What exactly happens during the process remains a mystery in the black box (Jin et al., 2017). Therefore, industrial

sector of traditional fermented foods faces the challenge of standardization and modernization because of the increasing awareness and concern of product quality and safety. With the advances of biotechnology and concerted effort of scientists, to clarify the mystery becomes more feasible and realistic. Here, we propose a roadmap as described in Fig. 3 how we can transform the uncontrolled and mysterious liquor fermentation to a rationale and controlled process, with the emphasis on microbiota with flavour compound formation as the targeted criterion.

As shown in Fig. 3, one direction is to find the core microbiota for flavour compound formation and the other is to find the key factors influencing the core microbiota. Although these two directions can be advanced separately, the outcome must be integrated to realize the ultimate controlled liquor fermentation using a designed microbiota for desired flavour compound profiles.

5.1. Identification and isolation of core microbiota

- (1) Identification of core microbiota with statistical and meta-omics methods

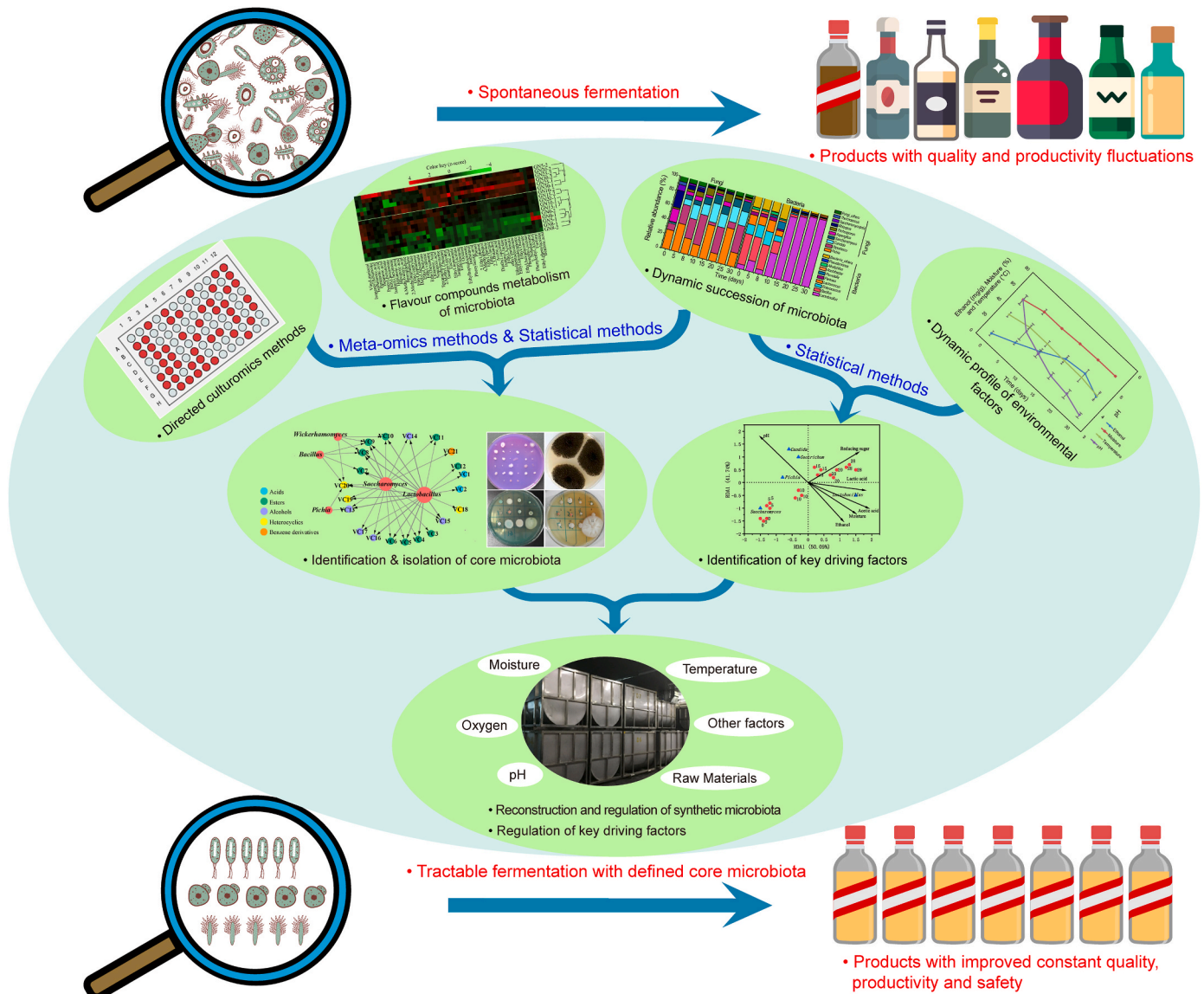


Fig. 3. Roadmap for controlling microbiota in modernization and standardization of spontaneous food fermentations. The first step is to identify the core microbiota influencing flavour compound formation and the environmental factors influencing the core microbiota. Thereafter, a controlled, tractable and defined microbiota will be possible to make the fermentation products with constant quality, productivity and safety.

It seems mission impossible to identify core microbiota responsible for flavour compound formation in such a complex, spontaneous and dynamic multi-species fermentation process like Chinese liquor fermentation. However, recent efforts open the possibility by various methods or combination thereof. As shown in Fig. 3 (left), we address first the route how to identify core microbiota that is the most important prerequisite to control the fermentation process.

Conventional methods to identify core microbiota are laborious, time-consuming, by including or excluding one or more microorganisms in a defined culture and observing the generation or disappearance of certain flavour compounds. Statistical methods used recently to identify core microbiota are more powerful and efficient. Successful examples include the use of partial least squares regression to identify yeast communities responsible for flavour compound formation of light aroma type liquor (Kong et al., 2014) and two-way orthogonal partial least squares to identify responsible filamentous fungi, yeasts and bacteria for flavour compound formation in cereal vinegar fermentation (Wang, Lu, et al., 2016). In addition to statistical methods, meta-omics can be used to identify core microbiota for flavour compound formation in spontaneous food fermentations (Fig. 3, left), such as liquor fermentation (Song et al., 2017) and other food fermentations (Weckx, Van Kerrebroeck, & De Vuyst, 2019).

The study on microbiota is mainly at genus or species levels. However, due to the diversity of strains within species (Duan et al., 2018), it is now going further to strain level identification. Presently, several computational approaches are presented to reveal the strain profile from the metagenomic data of the microbiota, such as StrainEst (Albanese & Donati, 2017). This strain-level analysis will shed light on the strain diversity in the microbiota, it will facilitate the strain identification and characterization (Ercolini, 2017). Therefore, the strain level analysis should be further applied in microbiota from food fermentation process.

(2) Isolation of core microbiota at strain level

The diversity of strain in certain species is shown by the culture method (Daughtry, Johanningsmeier, Sanozky-Dawes, Klaenhammer, & Barrangou, 2018; Kong, Wu, & Xu, 2017). Isolation of the best performed strain within the core microbiota is still challenging due to the strain diversity. In this context, we suggest a new strategy defined as directed culturomics that combines the culturomics method and the directed isolation method to effectively obtain microbes with excellent fermentation performance. The culturomics method can be used to obtain a large collection of strains (Lagier et al., 2016) by designing the medium and culture methods. A medium composition predicted based on microbial genome sequences with the Known Media Database is suggested (Oberhardt et al., 2015). Various culture methods can also be used, such as the 'ichip' method (Berdy, Spoering, Ling, & Epstein, 2017). After cultivation, a high-throughput method is used to identify the large collection of clones, such as the MALDI-TOF MS (De Roos, Verce, Aerts, Vandamme, & De Vuyst, 2018; Miescher Schwenninger, Freimueller Leischfeld, & Gantenbein-Demarchi, 2016). Furthermore, for the directed isolation, the fermentation performance associating with industrial application should be emphasized (Giri, Sen, Saha, Sukumaran, & Park, 2018), including stress tolerance, flavour compound production and safety assessment (Johansen, 2018). The culture-independent methods, such as the strain level phylogenomic analysis, would provide a clue for the isolation. For example, the specific gene sequences can be used as the targets for isolation of the strain with certain properties (Chai et al., 2019).

5.2. Identification and regulation of key environmental driving factors

Traditional liquor fermentation is in general without any control of environmental parameters like temperature, moisture, pH and oxygen supply (microaerobic or anaerobic). For microbial growth and metabolism, the above-mentioned parameters play crucial roles in altering

the outcomes of the process. Therefore, to identify the key environmental factors affecting the microbiota, is another prerequisite to control and improve the process (Fig. 3, right).

Several environmental factors have been studied already. However, more potential factors should be uncovered for controlling them. For example, the factors associated with the seasonal fluctuation and the regional difference are the main drivers for the temporal and spatial difference of the microbiota (Bokulich et al., 2014; Van Reckem et al., 2019; Wang, Xiong, et al., 2020), and they are urgently needed to be revealed. After the identification of the key factors, work can be focused on the optimization of these key environmental factors in food fermentations (Perez-Burillo, Gimenez, Rufian-Henares, & Pastoriza, 2018; Stavropoulou, Van Reckem, De Smet, De Vuyst, & Leroy, 2018). With the increasing accumulation of biological information and available techniques, it is more and more feasible to control such environmental factors.

5.3. Synthetic microbiota for a tractable microbiota

Fermentation with designed synthetic microbiota can be an efficient way to regulate and control the liquor fermentation with desired flavour. Cheese rind has been applied as model microbiota for community reconstruction to provide a tractable microbiota system (Wolfe, Button, Santarelli, & Dutton, 2014). It gives a reference for synthetic microbiota for different fermented foods that the dynamic of microbiota can be reproduced *in vitro*. This opens the possibility to construct a reproducible and tractable microbiota system for high-efficient and quality food fermentation.

For the microbiota design, selection of strains can be based on their phylogeny, interaction networks and functions (Vorholt, Vogel, Carlström, & Müller, 2017). The design of synthetic microbiota generally follows the top-down or bottom-up approach. The top-down approach is based on the ecosystem level design, and the bottom-up approach is based on the metabolic activity of the individual members and their interactions (Lawson et al., 2019). We suggest a combination of these two approaches for the design of the synthetic microbiota (Lawson et al., 2019). In addition, a principle should be constructed to evaluate the success of the construction. Besides the final flavour compound compositions, the dynamic profiles of the microbiota, environmental factors and the flavour compound formation should also be revealed to predict the success of the reproducible fermentation (Wang, Wu, Nie, Wu, & Xu, 2019). It is of great significance for the tractable and constant production of various fermented foods if such principle is followed.

5.4. Model construction for predicting and optimizing fermentation performance

Models can be used to understand and improve the fermentation process. For example, a mathematical model is constructed to simulate the starch conversion, the water loss and the temperature development in Chinese liquor fermentation (Jin et al., 2020). Modelling should also be used to predict and optimize the microbial growth and fermentation performance. However, few models are reported to predict microbiota dynamics in Chinese liquor and other spontaneous food fermentations. Modelling of microbiota in the other similar fermentation systems might be an option to start with. Cell growth can be predicted by the environmental factors, such as pH (Akkermans & Van Impe, 2018), time and temperature (Nor-Khaizura, Flint, McCarthy, Palmer, & Golding, 2019). Besides the abiotic environmental factors, certain biotic factors should also be considered, including the initial microbial abundance, lag phase and pair-wise interactions (D'Hoe et al., 2018). Moreover, the model can also be constructed to predict and optimize ethanol and flavour compound formation (Henriques, Alonso-del-Real, Querol, & Balsa-Canto, 2018; Jiang et al., 2019).

It is true that more factors would improve the prediction accuracy of

the model. However, model with the minimum number of independent parameters is still suggested. Because more parameters make it more difficult for the identification and the cross-validation. Thus, the most important parameters should be identified before modelling to decrease the number of the independent parameters. In addition, we still need to notice unknown factors, although their effect on microbiota is still hard to uncover. This requires a complex design for the modelling, and will have an enormous potential for the improvement of food quality.

5.5. Conclusions

Once we can identify both the core microbiota and key factors for the feasibility of designing and constructing synthetic microbiota, we will be able to control the fermentation process for desired product quality and consistency, as shown in the bottom of Fig. 3. There are similar processes like Japanese soy sauce and sake that are realized with better control for product quality and consistency, although these processes originally were also complex multi-species fermentations (Zhu & Tramper, 2013). Moreover, these were realized last century when the technologies and facilities were incompatible with those today of such a fast advance. Spontaneous food fermentations like Chinese liquor fermentation with complex multi-species under uncontrolled environmental conditions need urgently modernization and standardization for safety and quality reasons, but also for the sustainable development. Fast advances in microbial ecology combining food science and biotechnology make these feasible soon.

Declaration of competing interest

The authors declare that they have no conflicts of interest to this work.

Funding

This work was supported by the National Natural Science Foundation of China (31530055), National Key R&D Program of China (2018YFD0400402), Jiangsu Province Science and Technology Project (BE2017705), and national first-class discipline program of Light Industry Technology and Engineering (LITE2018-12).

References

Akkermans, S., & Van Impe, J. F. (2018). Mechanistic modelling of the inhibitory effect of pH on microbial growth. *Food Microbiology*, 72, 214–219. <https://doi.org/10.1016/j.fm.2017.12.007>

Albanese, D., & Donati, C. (2017). Strain profiling and epidemiology of bacterial species from metagenomic sequencing. *Nature Communications*, 8, 2260. <https://doi.org/10.1038/s41467-017-02209-5>

Berdy, B., Spoering, A. L., Ling, L. L., & Epstein, S. S. (2017). *In situ* cultivation of previously uncultivable microorganisms using the ichip. *Nature Protocols*, 12, 2232–2242. <https://doi.org/10.1038/nprot.2017.074>

Bertuzzi, A. S., McSweeney, P. L. H., Rea, M. C., & Kilcawley, K. N. (2018). Detection of volatile compounds of cheese and their contribution to the flavor profile of surface-ripened cheese. *Comprehensive Reviews in Food Science and Food Safety*, 17, 371–390. <https://doi.org/10.1111/1541-4337.12332>

Bokulich, N. A., Thorgate, J. H., Richardson, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America*, 111, E139–E148. <https://doi.org/10.1073/pnas.1317377110>

Chai, L., Xu, P., Qian, W., Zhang, X., Ma, J., Lu, Z., et al. (2019). Profiling the *Clostridia* with butyrate-producing potential in the mud of Chinese liquor fermentation cellar. *International Journal of Food Microbiology*, 297, 41–50. <https://doi.org/10.1016/j.ijfoodmicro.2019.02.023>

Chen, G., Chen, C., & Lei, Z. (2017). Meta-omics insights in the microbial community profiling and functional characterization of fermented foods. *Trends in Food Science & Technology*, 65, 23–31. <https://doi.org/10.1016/j.tifs.2017.05.002>

Chen, S., Sha, S., Qian, M., & Xu, Y. (2017). Characterization of volatile sulfur compounds in Moutai liquors by headspace solid-phase microextraction gas chromatography-pulsed flame photometric detection and odor activity value. *Journal of Food Science*, 82, 2816–2822. <https://doi.org/10.1111/1750-3841.13969>

Chen, L., Wu, Q., & Xu, Y. (2013). Characteristics of low fusel alcohol yielded by *Schizosaccharomyces pombe* in Maotai-flavor liquor-making. *Industrial Microbiology*, 43, 1–6. <https://doi.org/10.1111/j.1472-765x.2012.03294.x>

Chen, B., Wu, Q., & Xu, Y. (2014). Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese Maotai-flavor liquor. *International Journal of Food Microbiology*, 179, 80–84. <https://doi.org/10.1016/j.ijfoodmicro.2014.03.011>

Ciani, M., Capece, A., Comitini, F., Canonico, L., Siesto, G., & Romano, P. (2016). Yeast interactions in inoculated wine fermentation. *Frontiers in Microbiology*, 7, 555. <https://doi.org/10.3389/fmicb.2016.00555>

D'Hoe, K., Vet, S., Faust, K., Moens, F., Falony, G., Gonze, D., et al. (2018). Integrated culturing, modeling and transcriptomics uncovers complex interactions and emergent behavior in a three-species synthetic gut community. *Elife*, 7, Article e3790. <https://doi.org/10.7554/eLife.37900>

Daughtry, K. V., Johanningsmeier, S. D., Sanozky-Dawes, R., Klaenhammer, T. R., & Barrangou, R. (2018). Phenotypic and genotypic diversity of *Lactobacillus buchneri* strains isolated from spoiled, fermented cucumber. *International Journal of Food Microbiology*, 280, 46–56. <https://doi.org/10.1016/j.ijfoodmicro.2018.04.044>

De Roos, J., Verce, M., Aerts, M., Vandamme, P., & De Vuyst, L. (2018). Temporal and spatial distribution of the acetic acid bacterium communities throughout the wooden casks used for the fermentation and maturation of lambic beer underlines their functional role. *Applied and Environmental Microbiology*, 84, 17–e02846. <https://doi.org/10.1128/aem.02846-17>

Duan, S., Han, P., Wang, Q., Liu, W., Shi, J., Li, K., et al. (2018). The origin and adaptive evolution of domesticated populations of yeast from Far East Asia. *Nature Communications*, 9, 2690. <https://doi.org/10.1038/s41467-018-05106-7>

Du, H., Wang, X., Zhang, 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, 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, 20–e00456. <https://doi.org/10.1128/aem.00456-20>

Englezos, V., Cocolin, L., Rantsiou, K., Ortiz-Julien, A., Bloem, A., Dequin, S., et al. (2018). Specific phenotypic traits of *Starmarella bacillaris* related to nitrogen source consumption and central carbon metabolite production during wine fermentation. *Applied and Environmental Microbiology*, 84, 18–e00797. <https://doi.org/10.1128/aem.00797-18>

Ercolini, D. (2017). Exciting strain-level resolution studies of the food microbiome. *Microbial Biotechnology*, 10, 54–56. <https://doi.org/10.1111/1751-7915.12593>

Fang, C., Du, H., Jia, W., & Xu, Y. (2019). Compositional differences and similarities between typical Chinese baijiu and western liquor as revealed by mass spectrometry-based metabolomics. *Metabolites*, 9, 2. <https://doi.org/10.3390/metabo9010002>

Fan, G., Teng, C., Xu, D., Fu, Z., Minhazul, K. A. H. M., Wu, Q., et al. (2019). Enhanced production of ethyl acetate using co-culture of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*, 128, 564–570. <https://doi.org/10.1016/j.jbiosc.2019.05.002>

Fan, W., Xu, Y., & Zhang, Y. (2007). Characterization of pyrazines in some Chinese liquors and their approximate concentrations. *Journal of Agricultural and Food Chemistry*, 55, 9956–9962. <https://doi.org/10.1021/jf071357q>

Giri, S. S., Sen, S. S., Saha, S., Sukumaran, V., & Park, S. C. (2018). Use of a potential probiotic, *Lactobacillus plantarum* L7, for the preparation of a rice-based fermented beverage. *Frontiers in Microbiology*, 9, 473. <https://doi.org/10.3389/fmicb.2018.00473>

Henriques, D., Alonso-del-Real, J., Querol, A., & Balsa-Canto, E. (2018). *Saccharomyces cerevisiae* and *S. kudriavzevii* synthetic wine fermentation performance dissected by predictive modeling. *Frontiers in Microbiology*, 9, 88. <https://doi.org/10.3389/fmicb.2018.00088>

Hesseltine, C. W. (1965). A millennium of fungi, food, and fermentation. *Mycologia*, 57, 149–197. <https://doi.org/10.2307/3756821>

Huang, Y., Wu, Q., & Xu, Y. (2014). Isolation and identification of a black *Aspergillus* strain and the effect of its novel protease on the aroma of Moutai-flavoured liquor. *Journal of the Institute of Brewing*, 120, 268–276. <https://doi.org/10.1002/jib.135>

Jia, W., Fan, Z., Du, A., Li, Y., Zhang, R., Shi, Q., et al. (2020). Recent advances in Baijiu analysis by chromatography based technology-A review. *Food Chemistry*, 324. <https://doi.org/10.1016/j.foodchem.2020.126899>

Jiang, J., Liu, Y., Li, H., Yang, Q., Wu, Q., Chen, S., et al. (2019). Modeling and regulation of higher alcohol production through the combined effects of the C/N ratio and microbial interaction. *Journal of Agricultural and Food Chemistry*, 67, 10694–10701. <https://doi.org/10.1021/acs.jafc.9b04545>

Jin, G., Uhl, P., Zhu, Y., Wijffels, R. H., Xu, Y., & Rinzema, A. (2020). Modeling of industrial-scale anaerobic solid-state fermentation for Chinese liquor production. *Chemical Engineering Journal*, 394, 124942. <https://doi.org/10.1016/j.cej.2020.124942>

Jin, G., Zhu, Y., Rinzema, A., Wijffels, R. H., Ge, X., & Xu, Y. (2019). Water dynamics during solid-state fermentation by *Aspergillus oryzae* YH6. *Bioresource Technology*, 277, 68–76. <https://doi.org/10.1016/j.biortech.2019.01.038>

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>

Johansen, E. (2018). Use of natural selection and evolution to develop new starter cultures for fermented foods. *Annual Review of Food Science and Technology*, 9, 411–428. <https://doi.org/10.1146/annurev-food-030117-012450>

Johnson, A. J. (2016). Artisanal food microbiology. *Nature Microbiology*, 1, 16039. <https://doi.org/10.1038/nmicrobiol.2016.39>

Kong, Y., Wu, Q., & Xu, Y. (2017). Comparative studies on the fermentation performance of autochthonous *Saccharomyces cerevisiae* strains in Chinese light-fragrant liquor during solid-state or submerged fermentation. *Journal of Applied Microbiology*, 122, 964–973. <https://doi.org/10.1111/jam.13377>

- Kong, Y., Wu, Q., Zhang, Y., & Xu, Y. (2014). *In situ* analysis of metabolic characteristics reveals the key yeast in the spontaneous and solid-state fermentation process of Chinese light-style liquor. *Applied and Environmental Microbiology*, 80, 3667–3676. <https://doi.org/10.1128/aem.04219-13>
- Lagier, J. C., Khelaifia, S., Alou, M. T., Ndongo, S., Dione, N., Hugon, P., et al. (2016). Culture of previously uncultured members of the human gut microbiota by culturomics. *Nature Microbiology*, 1, 16203. <https://doi.org/10.1038/nmicrobiol.2016.203>
- Lawson, C. E., Harcombe, W. R., Hatzenpichler, R., Lindemann, S. R., Löffler, F. E., O'Malley, M. A., et al. (2019). Common principles and best practices for engineering microbiomes. *Nature Reviews Microbiology*, 17, 725–741. <https://doi.org/10.1038/s41579-019-0255-9>
- Li, P., Liang, H., Lin, W., Feng, F., & Luo, L. (2015). Microbiota dynamics associated with environmental conditions and potential roles of cellulolytic communities in traditional Chinese cereal starter solid-state fermentation. *Applied and Environmental Microbiology*, 81, 5144–5156. <https://doi.org/10.1128/aem.01325-15>
- Li, P., Lin, W., Liu, X., Wang, X., Gan, X., Luo, L., et al. (2017). Effect of bioaugmented inoculation on microbiota dynamics during solid-state fermentation of Daqu starter using autochthonous of *Bacillus*, *Pediococcus*, *Wickerhamomyces* and *Saccharomyces*. *Food Microbiology*, 61, 83–92. <https://doi.org/10.1016/j.fm.2016.09.004>
- Liu, H., & Sun, B. (2018). Effect of fermentation processing on the flavor of Baijiu. *Journal of Agricultural and Food Chemistry*, 66, 5425–5432. <https://doi.org/10.1021/acs.jafc.8b00692>
- 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, 17–e01475. <https://doi.org/10.1128/aem.01475-17>
- Lu, X., Wu, Q., Zhang, Y., & Xu, Y. (2015). Genomic and transcriptomic analyses of the Chinese Maotai-flavored liquor yeast MT1 revealed its unique multi-carbon co-utilization. *BMC Genomics*, 16, 1064. <https://doi.org/10.1186/s12864-015-2263-0>
- Ma, R., Sui, L., Zhang, J., Hu, J., & Liu, P. (2019). Polyphasic characterization of yeasts and lactic acid bacteria metabolic contribution in semi-solid fermentation of Chinese Baijiu (traditional fermented alcoholic drink): Towards the design of a tailored starter culture. *Microorganisms*, 7, 147. <https://doi.org/10.3390/microorganisms7050147>
- Meng, X., Wu, Q., Wang, L., Wang, D., Chen, L., & Xu, Y. (2015). Improving flavor metabolism of *Saccharomyces cerevisiae* by mixed culture with *Bacillus licheniformis* for Chinese Maotai-flavor liquor making. *Journal of Industrial Microbiology & Biotechnology*, 42, 1601–1608. <https://doi.org/10.1007/s10295-015-1647-0>
- Miescher Schwenninger, S., Freimueller Leischfeld, S., & Gantenbein-Demarchi, C. (2016). High-throughput identification of the microbial biodiversity of cocoa bean fermentation by MALDI-TOF MS. *Letters in Applied Microbiology*, 63, 347–355. <https://doi.org/10.1111/lam.12621>
- Nor-Khaizura, M. A. R., Flint, S. H., McCarthy, O. J., Palmer, J. S., & Golding, M. (2019). Modelling the effect of fermentation temperature and time on starter culture growth, acidification and firmness in made-in-transit yoghurt. *LWT-Food Science and Technology*, 106, 113–121. <https://doi.org/10.1016/j.lwt.2019.02.027>
- Nsong'ni, S. D., Fischer, S., & Becker, T. (2018). Investigating on the fermentation behavior of six lactic acid bacteria strains in barley malt wort reveals limitation in key amino acids and buffer capacity. *Food Microbiology*, 73, 245–253. <https://doi.org/10.1016/j.fm.2018.01.010>
- Oberhardt, M. A., Zarecki, R., Gronow, S., Lang, E., Klenk, H. P., Gophna, U., et al. (2015). Harnessing the landscape of microbial culture media to predict new organism-media pairings. *Nature Communications*, 6, 8493. <https://doi.org/10.1038/ncomms9493>
- Perez-Burillo, S., Gimenez, R., Rufian-Henares, J. A., & Pastoriza, S. (2018). Effect of brewing time and temperature on antioxidant capacity and phenols of white tea: Relationship with sensory properties. *Food Chemistry*, 248, 111–118. <https://doi.org/10.1016/j.foodchem.2017.12.056>
- Ponomarova, O., Gabrielli, N., Sevin, D. C., Mulleder, M., Zirngibl, K., Bulyha, K., et al. (2017). Yeast creates a niche for symbiotic lactic acid bacteria through nitrogen overflow. *Cell Systems*, 5, 345–357. <https://doi.org/10.1016/j.cels.2017.09.002>
- 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, 109757. <https://doi.org/10.1016/j.foodres.2020.109757>
- Sieuwerws, S., Bron, P. A., & Smid, E. J. (2018). Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT-Food Science and Technology*, 90, 201–206. <https://doi.org/10.1016/j.lwt.2017.12.022>
- Song, Z., Du, H., Zhang, Y., & Xu, Y. (2017). Unraveling core functional microbiota in traditional solid-state fermentation by high-throughput amplicons and metatranscriptomics sequencing. *Frontiers in Microbiology*, 8, 1294. <https://doi.org/10.3389/fmicb.2017.01294>
- Stavropoulou, D. A., Van Reckem, E., De Smet, S., De Vuyst, L., & Leroy, F. (2018). The narrowing down of inoculated communities of coagulase-negative staphylococci in fermented meat models is modulated by temperature and pH. *International Journal of Food Microbiology*, 274, 52–59. <https://doi.org/10.1016/j.ijfoodmicro.2018.03.008>
- Steinkraus, K. H. (1983). Fermented foods, feeds and beverages. *Biotechnology Advances*, 1, 31–46. [https://doi.org/10.1016/0734-9750\(83\)90299-9](https://doi.org/10.1016/0734-9750(83)90299-9)
- Sun, S., Xu, Y., & Wang, D. (2009). Novel minor lipase from *Rhizopus chinensis* during solid-state fermentation: Biochemical characterization and its esterification potential for ester synthesis. *Biorescience Technology*, 100, 2607–2612. <https://doi.org/10.1016/j.biortech.2008.11.006>
- 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, 108350. <https://doi.org/10.1016/j.ijfoodmicro.2019.108350>
- Ulla, H., Antti, K., Oskar, L., & Mari, S. (2016). Genetic basis of flavor sensitivity and food preferences. In P. Etiévant, E. Guichard, C. Salles, & A. Voilley (Eds.), *Flavor: From food to Behaviors, Wellbeing and Health* (pp. 203–227). Woodhead Publishing.
- Van Reckem, E., Charmpi, C., Van der Veken, D., Geeraerts, W., De Vuyst, L., & Leroy, F. (2019). Exploring the link between the geographical origin of European fermented foods and the diversity of their bacterial communities: The case of fermented meats. *Frontiers in Microbiology*, 10, 2302. <https://doi.org/10.3389/fmicb.2019.02302>
- Vorholt, J. A., Vogel, C., Carlström, C. I., & Müller, D. B. (2017). Establishing causality: Opportunities of synthetic communities for plant microbiome research. *Cell Host & Microbe*, 22, 142–155. <https://doi.org/10.1016/j.chom.2017.07.004>
- Wache, Y., Do, T. L., Do, T. B. H., Do, T. Y., Haure, M., Ho, P. H., et al. (2018). Prospects for food fermentation in South-East Asia, topics from the tropical fermentation and biotechnology network at the end of the AsiFood Erasmus+Project. *Frontiers in Microbiology*, 9, 2278. <https://doi.org/10.3389/fmicb.2018.02278>
- Wang, X., Ban, S., & Qiu, S. (2018). Analysis of the mould microbiome and exogenous enzyme production in Moutai-flavor Daqu. *Journal of the Institute of Brewing*, 124, 91–99. <https://doi.org/10.1002/jib.467>
- Wang, X., Du, H., Zhang, Y., & Xu, Y. (2018). Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Applied and Environmental Microbiology*, 84, 17–e02369. <https://doi.org/10.1128/aem.02369-17>
- Wang, L., Hu, G., Lei, L., Lin, L., Wang, D., & Wu, J. (2016). Identification and aroma impact of volatile terpenes in Moutai liquor. *International Journal of Food Properties*, 19, 1335–1352. <https://doi.org/10.1080/10942912.2015.1064442>
- Wang, Z., Lu, Z., Shi, J., & Xu, Z. (2016). Exploring flavour-producing core microbiota in multispecies solid-state fermentation of traditional Chinese vinegar. *Scientific Reports*, 6, 26818. <https://doi.org/10.1038/srep26818>
- Wang, P., Wu, Q., Jiang, X., Wang, Z., Tang, J., & Xu, Y. (2017). *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 metabolism in Chinese light aroma type liquor produced by solid-state fermentation. *Applied and Environmental Microbiology*, 18–e03090. <https://doi.org/10.1128/aem.03090-18>
- Wang, D., Wu, R., Xu, Y., & Li, M. (2013). Draft genome sequence of *Rhizopus chinensis* CCTCCM201021, used for brewing traditional Chinese alcoholic beverages. *Genome Announcements*, 1, 12–e00195. <https://doi.org/10.1128/genomeA.00195-12>
- Wang, B., Wu, Q., Xu, Y., & Sun, B. (2018). Specific volumetric weight-driven shift in microbiota compositions with saccharifying activity change in starter for Chinese Baijiu fermentation. *Frontiers in Microbiology*, 9, 2349. <https://doi.org/10.3389/fmicb.2018.02349>
- Wang, B., Wu, Q., Xu, Y., & Sun, B. (2020). Synergistic effect of multiple saccharifying enzymes on alcoholic fermentation for Chinese Baijiu production. *Applied and Environmental Microbiology*, 86, e00013–20. <https://doi.org/10.1128/AEM.00013-20>
- Wang, S., Xiong, W., Wang, Y., Nie, Y., Wu, Q., Xu, Y., et al. (2020). Temperature-induced annual variation in microbial community changes and resulting metabolome shifts in a controlled fermentation system. *mSystems*, 5, 20–e00555. <https://doi.org/10.1128/mSystems.00555-20>
- Wang, M., Yang, J., Zhao, Q., Zhang, K., & Su, C. (2019). Research progress on flavor compounds and microorganisms of Maotai flavor baijiu. *Journal of Food Science*, 84, 6–18. <https://doi.org/10.1111/1750-3841.14409>
- Weckx, S., Van Kerrebroeck, S., & De Vuyst, L. (2019). Omics approaches to understand sourdough fermentation processes. *International Journal of Food Microbiology*, 302, 90–102. <https://doi.org/10.1016/j.ijfoodmicro.2018.05.029>
- Wolfe, B. E., Button, J. E., Santarelli, M., & Dutton, R. J. (2014). Cheese rind communities provide tractable systems for *in situ* and *in vitro* studies of microbial diversity. *Cell*, 158, 422–433. <https://doi.org/10.1016/j.cell.2014.05.041>
- Wood, B. J. (2012). *Microbiology of fermented foods: Springer science & Business Media*.
- 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., Kong, Y., & Xu, Y. (2016). Flavor profile of Chinese liquor is altered by interactions of intrinsic and extrinsic microbes. *Applied and Environmental Microbiology*, 82, 422–430. <https://doi.org/10.1128/aem.02518-15>
- 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, 4450–4459. <https://doi.org/10.1128/aem.00905-14>
- Wu, Q., & Xu, Y. (2012). Transcriptome profiling of heat-resistant strain *Bacillus licheniformis* CGMCC3962 producing Maotai flavor. *Journal of Agricultural and Food Chemistry*, 60, 2033–2038. <https://doi.org/10.1021/jf204270h>
- Wu, Q., Xu, Y., & Chen, L. (2012). Diversity of yeast species during fermentative process contributing to Chinese Maotai-flavour liquor making. *Letters in Applied Microbiology*, 55, 301–307. <https://doi.org/10.1111/j.1472-765X.2012.03294.x>
- Wu, Q., Zhang, R., Peng, S., & Xu, Y. (2015). Transcriptional characteristics associated with lichenysin biosynthesis in *Bacillus licheniformis* from Chinese Maotai-flavor liquor making. *Journal of Agricultural and Food Chemistry*, 63, 888–893. <https://doi.org/10.1021/jf5036806>

- Wu, Q., Zhu, W. A., Wang, W., & Xu, Y. (2015). Effect of yeast species on the terpenoids profile of Chinese light-style liquor. *Food Chemistry*, 168, 390–395. <https://doi.org/10.1016/j.foodchem.2014.07.069>
- Xiao, C., Lu, Z., Zhang, X., Wang, S., Ao, L., Shen, C., et al. (2017). Bio-heat is a key environmental driver shaping the microbial community of medium-temperature Daqu. *Applied and Environmental Microbiology*, 83, 17–e01550. <https://doi.org/10.1128/aem.01550-17>
- Xu, Y., & Ji, K. (2012). Moutai (Maotai): Production and sensory properties. In *Alcoholic beverages* (pp. 315–330). Elsevier.
- Xu, Y., Zhi, Y., Wu, Q., Du, R. B., & Xu, Y. (2017). *Zygosaccharomyces bailii* is a potential producer of various flavor compounds in Chinese Maotai-flavor liquor fermentation. *Frontiers in Microbiology*, 8, 2609. <https://doi.org/10.3389/fmicb.2017.02609>
- Yang, F., Liu, Y., Chen, L., Li, J., Wang, L., & Du, G. (2020). Genome sequencing and flavor compound biosynthesis pathway analyses of *Bacillus licheniformis* isolated from Chinese Maotai-flavor liquor-brewing microbiome. *Food Biotechnology*, 34, 193–211. <https://doi.org/10.1080/08905436.2020.1789474>
- Zhang, L., Cao, Y., Tong, J., & Xu, Y. (2019). An alkylpyrazine synthesis mechanism involving L-threonine-3-dehydrogenase describes the production of 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine by *Bacillus subtilis*. *Applied and Environmental Microbiology*, 85, 19–e01807. <https://doi.org/10.1128/AEM.01807-19>
- Zhang, Y., Du, H., Wu, Q., & Xu, Y. (2015). Impacts of two main lactic acid bacteria on microbial communities during Chinese Maotai-flavor liquor fermentation. *Microbiology China*, 42, 2087–2097. <https://doi.org/10.13344/j.microbiol.china.150120>
- Zhang, S., Guo, F., Yan, W., Dong, W., Zhou, J., Zhang, W., et al. (2020). Perspectives for the microbial production of ethyl acetate. *Applied Microbiology and Biotechnology*, 104, 7239–7245. <https://doi.org/10.1007/s00253-020-10756-z>
- Zhang, R., Wu, Q., & Xu, Y. (2013). Aroma characteristics of Moutai-flavour liquor produced with *Bacillus licheniformis* by solid-state fermentation. *Letters in Applied Microbiology*, 57, 11–18. <https://doi.org/10.1111/lam.12087>
- Zhang, R., Wu, Q., & Xu, Y. (2014). Lichenysin, a cyclooctapeptide occurring in Chinese liquor Jiannanchun reduced the headspace concentration of phenolic off-flavors via hydrogen-bond interactions. *Journal of Agricultural and Food Chemistry*, 62, 8302–8307. <https://doi.org/10.1021/jf502053g>
- 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, 189–195. <https://doi.org/10.1016/j.jbiosc.2018.02.010>
- Zha, M., Sun, B., Yin, S., Mehmood, A., Cheng, L., & Wang, C. (2018). Generation of 2-furfurylthiol by carbon-sulfur lyase from the Baijiu yeast *Saccharomyces cerevisiae* G20. *Journal of Agricultural and Food Chemistry*, 66, 2114–2120. <https://doi.org/10.1021/acs.jafc.7b06125>
- Zheng, X., Yan, Z., Nout, M. J. R., Boekhout, T., Han, B., Zwietering, M., et al. (2015). Characterization of the microbial community in different types of Daqu samples as revealed by 16S rRNA and 26S rRNA gene clone libraries. *World Journal of Microbiology and Biotechnology*, 31, 199–208. <https://doi.org/10.1007/s11274-014-1776-z>
- Zheng, X. W., Yan, Z., Nout, M. J., Smid, E. J., Zwietering, M. H., Boekhout, T., et al. (2014). Microbiota dynamics related to environmental conditions during the fermentative production of Fen-Daqu, a Chinese industrial fermentation starter. *International Journal of Food Microbiology*, 182–183, 57–62. <https://doi.org/10.1016/j.ijfoodmicro.2014.05.008>
- 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>
- Zhi, Y., Wu, Q., & Xu, Y. (2017). Genome and transcriptome analysis of surfactin biosynthesis in *Bacillus amyloliquefaciens* MT45. *Scientific Reports*, 7, 40976. <https://doi.org/10.1038/srep40976>
- Zhu, Y., & Tramper, J. (2013). Koji - where east meets west in fermentation. *Biotechnology Advances*, 31, 1448–1457. <https://doi.org/10.1016/j.biotechadv.2013.07.001>
- Zhu, X., Zhou, Y., Wang, Y., Wu, T., Li, X., Li, D., et al. (2017). Production of high-concentration n-caproic acid from lactate through fermentation using a newly isolated Ruminococcaceae bacterium CPB6. *Biotechnology for Biofuels*, 10, 102. <https://doi.org/10.1186/s13068-017-0788-y>