Chinese Baijiu - Finding a channel to design a defined starter culture

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Qu (a starter composed of multiple microbes, enzymes, and nutrients) is essential for initiating Chinese baijiu fermentation and is usually prepared in an open system to enrich the starter complex with microorganisms from the local environment. However, with the challenge of increasing manufacturing and labor costs, traditional spontaneous fermentation cannot meet the growing industrial needs for standardization and modernization. Nowadays, the development of a synthetic microbiota built up from selected and cultured microorganisms enables the repeatable, standardized production of fermented foods. The use of such synthetic microbiota to convert raw materials into foods can hopefully reproduce the smells and tastes of traditional products. This review critically summarizes the properties of traditional qu and discusses the potential of a defined synthetic microbiota to revolutionize the production of such fermentation starters for future baijiu production. The prospects and challenges in dealing with the identification, selection, cultivation, and incorporation of microbes into such synthetic microbiota (or new ecological complexes) are specifically related to developing a fully defined and effective mixed-starter culture for use in traditional fermented food production are detailed.

KEYWORDS

Baijiu
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Qu
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1. INTRODUCTION

Baijiu is the national liquor of China and is warmly welcomed by consumers in the Orient [1,2]. Chinese people manufacture baijiu via solid-state fermentation and use qu (a sort of equivalence to koji) as a starter [3]. The preparation of qu is partly a spontaneous process, relying on an enriched collection of microorganisms drawn in from the local environment. The microbes release enzymes to convert substrates into fermentable sugars and other nutrients required for efficient fermentation [4].

However, this spontaneous process faces challenges related to climate change, raw materials consistency, and technical adjustments, which ultimately affect the consistency and stability of resultant products [1-3]. These drawbacks need to be overcome by introducing modern standards and developing a defined qu via a validated, sustainable, and better-controlled industrial process [5,6] (Figure 1). It should be possible to provide a better definition of qu when

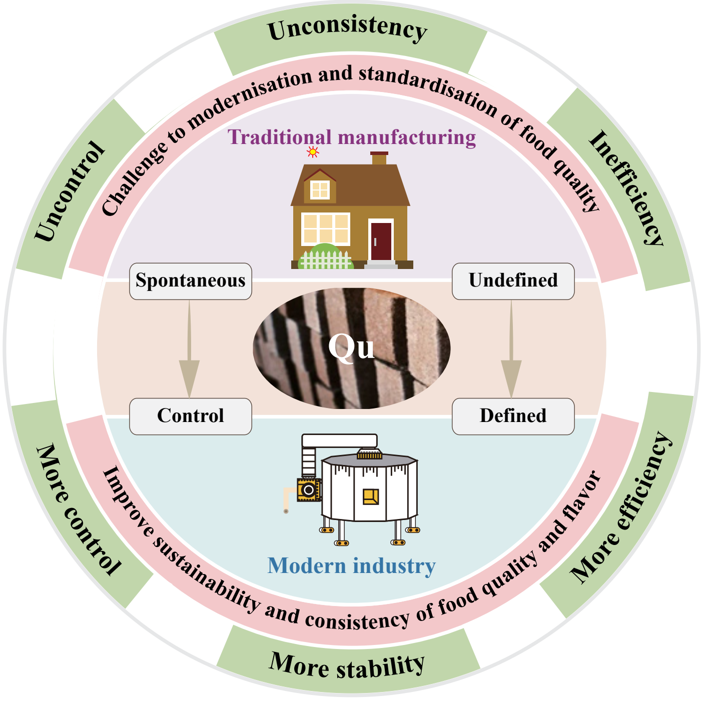


FIGURE 1 Schematic diagram from traditional spontaneous fermented Qu to modern defined starter.

the responsible microbes, critical enzymes, and formative mechanisms are identified in the traditional qu starters currently in use. For a long time, researchers explored the definition of qu employing traditional culture-dependent and modern culture-independent approaches [1-4]. However, defined qu can only be realized when the following posed questions are answered; “What is the composition and function of the microorganisms and enzymes that are present?”, “What is the formative mechanism of microbial communities in the traditional process?” and “How do we construct the community to recreate the metabolism of autochthonous microbiota?” Resolving these questions would provide a significant advance in creating a defined qu for baijiu fermentation and present a knowledge-based improvement to developing defined starters for other food fermentations.

Herein, we critically address the research progress related to traditional qu and illustrate the feasibility of realizing a defined qu for modern industrial production. Moreover, we discuss the potential of synthetic microbiota (new and defined ecological populations) to revolutionize the future of a designated starter for Chinese baijiu fermentation.

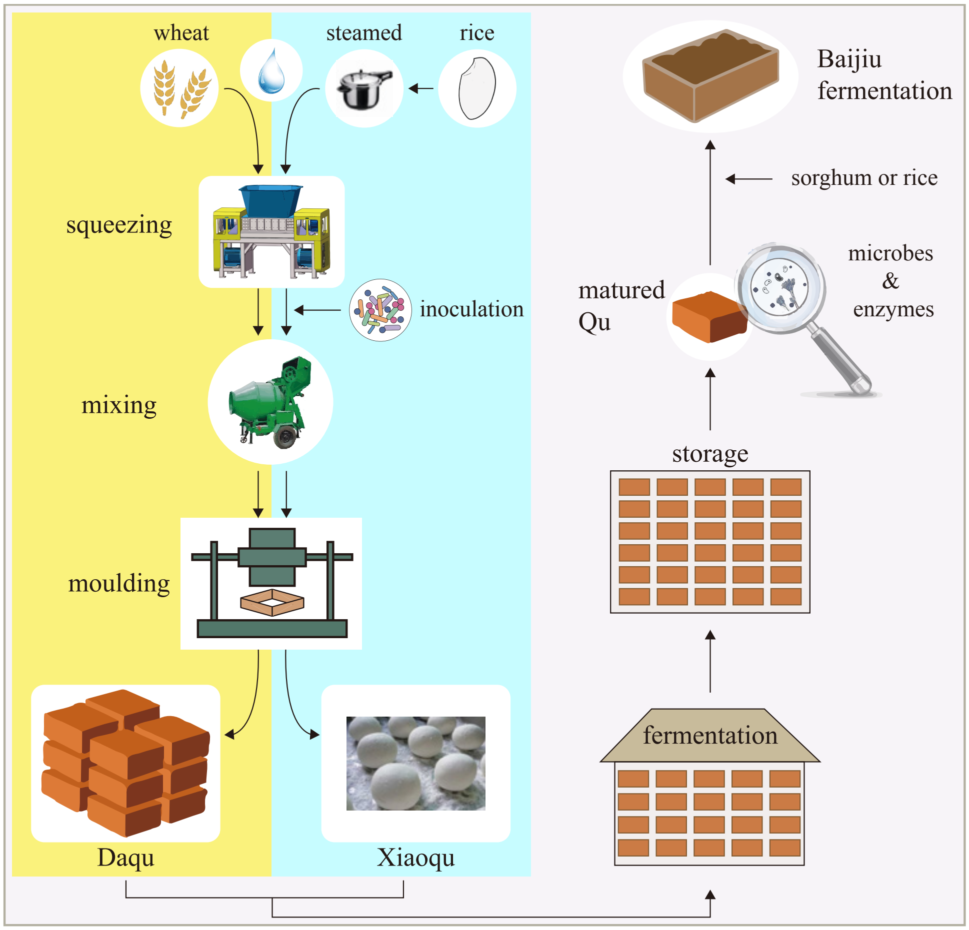


FIGURE 2 Schema of traditional Qu production for Chinese baijiu fermentation.

2. QU: A STARTER FOR BAIJIU FERMENTATION

Chinese baijiu generally uses traditional qu (incorporating a multitude of generally undefined microbial species) as a fermentation starter, unlike western fermented foods driven by defined starters (composed of a single species or a few microbiological strains) [4,5]. Meanwhile, qu is also used to ferment other traditional Oriental fermented foods, like vinegar, soy sauce, rice wine, etc.[5]. This typical starter is mainly made of cereals (wheat, rice, soybean, etc.), and produced under a spontaneous solid-state fermentation in an open system [1]. Moreover, qu is also assigned the names, Daqu, Xiaoqu, and Fuqu, based on raw materials, inoculations, fermented parameters, and their utilization in different liquors [1-4]. For example, daqu, in the shape of a brick with a larger size, is mainly made of raw wheat and produced under a spontaneous fermentation without specific inoculations. Daqu is always classified into three types by the fermented temperature: high-temperature daqu (60-70 °C), medium-temperature daqu (50-60 °C), and low-temperature daqu (40-50 °C) [3,4]. Meanwhile, xiaoqu, in the shape of a ball of relatively small size, is always made of steamed rice and inoculated with functional strains, then fermented in an open system [1]. In comparison, fuqu is composed of a known set of pure cultures produced under modern mechanized conditions by inoculating functional strains in brans [1]. Overall, the traditional production of qu includes ingredient pretreatment, shaping, incubation in a qu-making house, and maturation during storage (Figure 2). During preparation, qu gradually becomes a matured microbial ecosystem with abundant microorganisms, enzymes, and metabolites [2]. This starter then provides the functional microorganisms and their products (enzymes, flavors, or precursors) to fermentation to shape the flavor of the different baijiu[3]. Thus, an understanding of the microbiota in the starter is a key to the quality-controlled use of the qu and to developing and characterizing fully defined forms of the other qu-type starters.

3. MICROBIOTA IN TRADITIONAL QU

3.1. MICROORGANISMS

The spontaneous fermentation of qu is a process that propagates microorganisms from the raw materials and the local environment. Therefore, the composition of the microbial community determines the quality of the matured qu [7]. Thus, understanding the compositions of microbial communities in traditional qu provides the basis for improving quality by subsequently adjusting the structure of the microbial community and developing fully defined starter formulations. Since the 20th century, microbiologists have applied culture-dependent methods to isolate and identify strains from qu samples [8-12]. Up to now, hundreds of microorganisms have been cultured by scientists, with the presence of bacteria, molds, and yeasts [8-12]. *Bacillus spps*, lactic acid bacteria (*Lactobacillus*, *Pediococcus* and *Weissella*), *Acetobacter*, *Clostridium*, *Pantoea*, *Enterobacter*, and *Acinetobacter* species always appear in the bacterial communities, whereas the molds (*Aspergillus*, *Mucor*, *Rhizopus*, *Monascus* and *Trichoderma* species) and the yeasts (like *Saccharomyces*, *Pichia*, *Candida*, *Saccharomycopsis*, *Wickerhamomyces* and *Schizosaccharomyces*) are dominant [8-12]. In addition, researchers have succeeded in isolating various thermophilic or acidic-, alcoholic-tolerant strains from the preparations of qu, under environmental extremes, including high temperature, moisture, acidity, and alcoholic conditions [13-17]. A new species of *Thermoactinomyces daqus* was isolated from a high-temperature daqu used for a typical strong-flavor baijiu production, and its genomic information was well-studied to illustrate the thermophilic nature and mechanisms of stress tolerance of this unique strain [13]. Many unique microbes, including a thermophilic bacterium (*Scopulibacillus daqui* sp nov.), a facultatively alkaliphilic species (*Franconibacter daqui* sp nov.), heat resistant Enterobacteriaceae and Bacilli, have been isolated from qu samples, and the interactions between these strains and the local environment have been well-studied [14-17].

This knowledge of the strains found to be present contributes to understanding the formation of special microbiota in traditional qu and could be developed and utilized in food fermentations and other industries. As knowledge of the growing number of strains isolated and identified grows, it becomes more promising that the mystery behind traditional qu can be unraveled. However, culture-dependent analysis is not enough to reveal a complete understanding of microbial communities, owing to the complexity and species diversity of the microbial ecosystem in traditional qu [1-4].

In facing this challenge, culture-independent methods (PLFA, PCR-DGGE, PCR-SSCP, RISA, and high

TABLE 1 Microbial community members in traditional Qu.

NAME	CLASS	MAIN MICROBES		
		MOLDS	BACTERIA	YEASTS
Daqu	High-temperature Daqu (60-70 ºC) [18-21]	*Aspergillus, Lichtheimia, Mucor, Rhizomucor, Rhizopus, Thermoascus, Thermomucor, Thermomyces*	*Bacillus, Brevibacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Weissella, Kroppenstedtia, Pediococcus, Microbacterium, Rubellimicrobium, Saccharopolyspora, Staphylococcus, Streptomyces, Thermoactinomyces*	*Candida, Hanseniaspora, Hansenula, Pichia, Saccharomyces*
	Medium-temperature Daqu (50-60 ºC) [22-28]	*Absidia, Aspergillus, Lichtheimia, Rhizopus, Rhizomucor*	*Bacillus, Enterobacter, Lactobacillus, Leuconostoc, Kroppenstedtia, Staphylococcus, Saccharopolyspora, Pantoea, Pediococcus, Weissella*	*Candida, Hyphopichia, Pichia, Saccharomyces, Saccharomycopsis, Trichosporon*
	Low-temperature Daqu (40-50 ºC) [29-31]	*Absidia, Aspergillus, Rhizomucor, Rhizopus*	*Acetic acid bacteria, Bacillus, Enterobacteriales, Lactobacillus, Staphylococcus, Streptomyces, Weissella*	*Pichia, Saccharomyces, Saccharomycopsis, Wickerhamomyces, Zygosaccharomyces*
Xiaoqu [32-36]		*Absidia, Aspergillus, Rhizopus*	*Acetic acid bacteria, Acinetobacter, Corynebacterium, Deinococcus, Lactobacillus, Pediococcus, Streptococcus, Weissella, Xanthomonas*	*Hansenula, Pichia, Saccharomyces, Saccharomycopsis*
Fuqu [66-70]		*Rhizopus, Aspergillus niger, Aspergillus oryzae, Aspergillus albicans*	*Bacillus licheniformis*	*Saccharomyces cerevisiae, ester yeasts*

throughput sequencing analysis) have provided an efficient set of approaches to aid in discovering the global features of microbial communities, and now an increasing number of species have been identified in the community of qu [18-36].

Different qu exhibits unique characteristics in the composition of the microbial community, based upon the regulations of the manufacturing process, the fermentation ecology, and geographical features (Table 1, Figure 3). The microbial responses to the local environment may be the driving force behind the formation of the microbiota [37]. For example, microbial dispersion on substrates could be used to distinguish the microbial assembly of daqu or xiaoqu. The dispersals of plant-commensal microbes (like *Bacillaceae*, *Enterobacteriaceae*, and plant-associated lactic acid bacteria) on raw materials, could drive the microbial formation of daqu as its preparation originates from the use of raw grains. On the other hand, the selection and inoculation of competitive microbes, which are adapted to the host, should shape the community of xiaoqu [38]. In addition, the adaptability to extreme environments may direct the selection of microbiota in the same qu. For example, the *Bacillus* and *Aspergillus* strains (which are tolerant of high-temperature and low moisture conditions) can survive within such extreme conditions and are thus selected to become the dominant species within the community as compared with the community populations in low-temperature to high-temperature daqu [18-31]. Therefore, understanding the correlations between microbial populations as adapted to specific local environments may also provide a potential avenue to regulate microbiota formation for developing a defined qu.

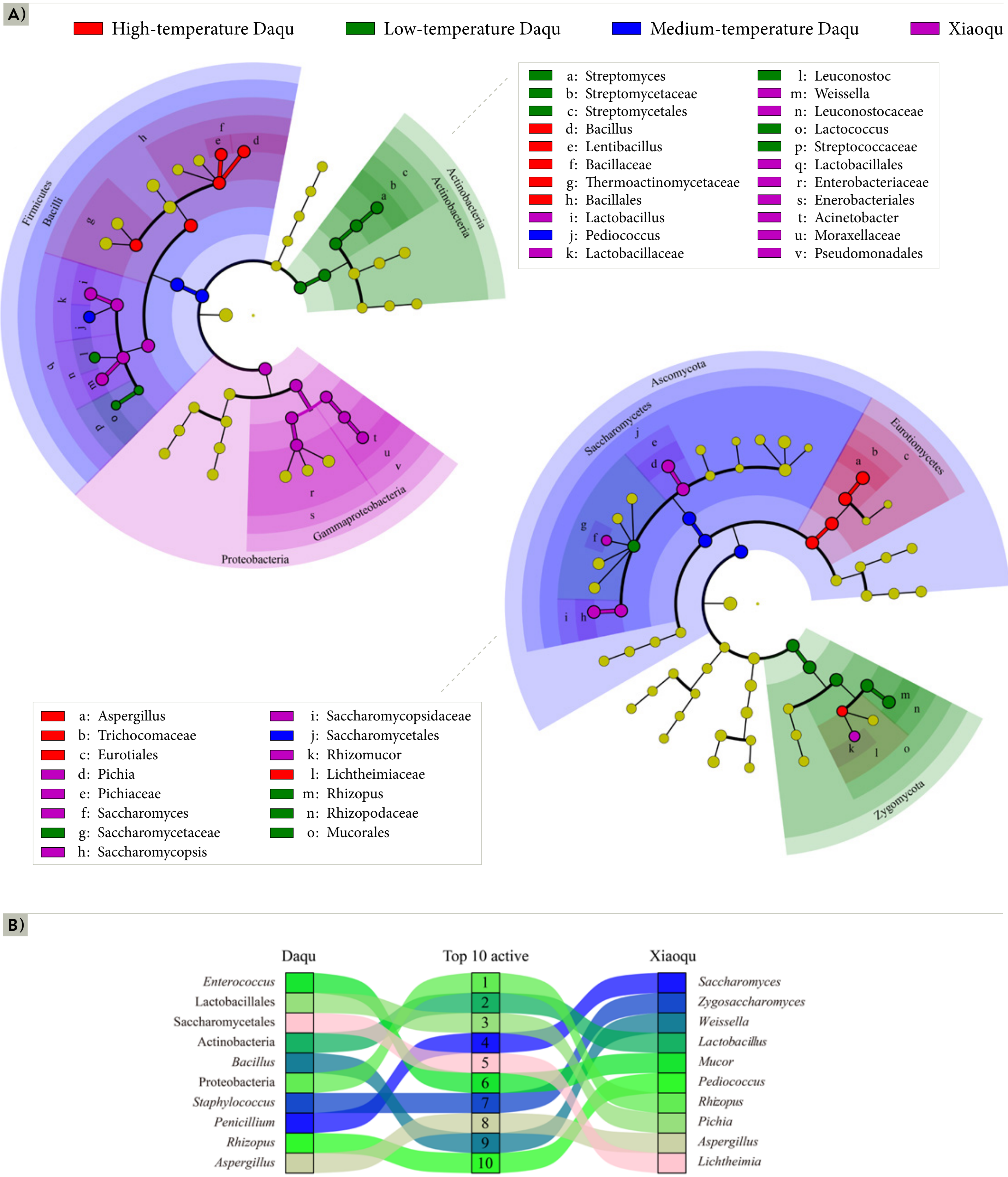
3.2. ENZYMES

In the preparations of qu, enriched microorganisms release abundant enzymes via their respective metabolic activities[1,3]. Researchers also utilize culture-dependent or culture-independent methods to reveal the enzyme profiles of qu [1-5]. In short, current knowledge indicates the complexity and diversity of enzyme profiles in this special bio-system of qu [39]. Previous studies generally isolated functional strains from qu samples, and then examined the characteristics of enzymes released under such culture-dependent conditions [40-42]. The isolated filamentous fungi always present vigorous activity with respect to various glucosidases, hydrolyzing polysaccharides to release oligosaccharides, monosaccharides (the fermentable sugars included), or flavors [40-42]. For example, an isolated *Aspergillus sydowii* F5 strain exhibits high activity of α-galactosidase under optimal conditions [43]. A *Rhizopus microsporus var. tuberosus* strain (isolated from daqu) can release extracellular fibrinolytic enzymes, and the purified enzyme has been well-studied [44]. Besides filamentous fungi, the plant-commensal bacteria also release hydrolases as the requirement for their penetration into raw materials [37]. *Bacillus* and *Streptomyces* species, isolated from low-temperature daqu used for light-style baijiu fermentation, can produce extracellular alpha-amylase and glucoamylase, like *Bacillus cereus* H17 and *Bacillus licheniformis* H55 [11]. Given the complexity and diversity of enzyme profiles, the culture-dependent analysis of single strains has not yet provided a comprehensive description of the full spectrum of enzymatic profiles and potential of qu.

Furthermore, the profile of the metabolic activities of microbial species isolated and studied under cultured conditions cannot represent the characteristics of particular strains present *in situ* [7,38]. To resolve this dilemma, modern metatranscriptomic and metaproteomic approaches provide an efficient approach to reveal the global feature of enzyme profiles within qu matrices[7,38,39,45,46]. For example, relevant metatranscriptomic studies indicate that approximately 1,000 carbohydrate-active enzymes are potentially expressed in the medium-temperature daqu, and the active Mucorales and Bacillales are responsible for the expression of key enzymes associated with polysaccharide hydrolysis and flavor generation [39,46]. In addition, the respective high-temperature stage contributes to the expression of thermostable enzymes and promotes the activity of such enzymes for flavor production [39]. Moreover, several thermostable enzymes (like fungal alpha-amylase, endoglucanase) have been purified from daqu [47-49]. These studies reveal the enzyme profiles and their interactions with the local environment in the preparations of qu. On this basis, the metaproteomic studies further strengthen our understanding of the essential features of the enzyme profiles in the qu bio-system [7,38]. So far, more than 2,000 enzymes have been identified in samples of qu, with these enzymes being expressed by about 200 fungal or bacterial genera, including *Lactobacillus*, *Aspergillus*, *Pichia*, *Saccharomyces*, *Rhizopus*, and so on [7,38,46]. According to annotations in GO, KEGG or other databases, these identified enzymes are associated with the hydrolysis of polysaccharides (allowing for a controlled release of fermentable sugars), ethanol metabolism, and flavor generation [7,38,46].

In short, current knowledge indicates that glycosidases are always the most abundant enzymes in the qu starters and determine the efficiency of polysaccharide hydrolysis and metabolite production during fermentation [38]. The expression of enzymes may be determined by the selection of materials and the control of parameters. For example, the diversity of glycosidases in daqu is significantly higher than that of xiaoqu, as the raw materials for daqu production present a more complex mixture of substrates[39,46].

FIGURE 3 Microbial distribution in traditional Qu. (A) Microbial structure in high temperature Daqu, medium temperature Daqu, low temperature Daqu and Xiaoqu. (B) Dominant functional microorganisms in high temperature Daqu, medium temperature Daqu, low temperature Daqu and Xiaoqu.



The abundant glycosidases are mainly expressed by the temperature- and desiccation-tolerant strains (like *Aspergillus*, *Bacillus*, etc.) [39,46]. By comparison, xiaoqu contains specific hydrolases (like α-amylase, glucoamylase, etc.), and the steaming process exposes more starch sites for hydrolysis[7,38]. These enzymes are mainly expressed by the inoculated and competitive strains (like *Rhizopus*, *Aspergillus*, *Rhizomucor*, etc.) in xiaoqu [7,38]. Besides the species, the cooperation of glycosidases is identified as a key to sustaining efficient saccharification and fermentation in the baijiu fermentation [38]. For example, the synergistic effect of α-amylase and glucoamylase could enhance starch hydrolysis and ethanol production by regulating fermentable sugars' formation to affect the core strains' metabolism in the baijiu fermentation [38,50]. Thus, this active mode could help baijiu producers better understand and manage the enzyme profile of qu or develop a defined qu for enhancing ethanol and flavor production in baijiu fermentation.

Although, as noted above, thousands of enzymes are identified already in the qu starters, there are still lots of unidentified enzymes, which hampers us in the deeper exploration of microbial resources in various fermented foods. The emergence of a specific database for other traditional fermented foods would provide for a more efficient approach to explore and utilize the outstanding resources available concerning such foods. This could enable the application of such knowledge towards defined qu production, as will the development of new and superior biotechnology techniques.

3.3. FORMATION OF MICROBIOTA

The solid-state fermentation of qu is a spontaneous process in an open environment, and the formation of its microbiota is shaped by microbes from the raw materials and the local environment [3-5]. Raw materials mainly contribute bacterial communities (like lactic acid bacteria, Bacillales, etc.) to the qu community. In contrast, the local environment is the primary contributing source of the fungal communities (like Mucorales, Saccharomycetales, etc.) for the qu ecological community [51]. In the preparation of qu, different factors drive the formation of the microbiota and the enzyme profiles in this unique solid-state fermentation; manufacturing parameters (raw material, inoculation, process-control, etc.) and environmental factors (season, weather, location, transport, and storage) [3]. For example, the selections and pretreatment (raw or steaming) of substrate materials (wheat, rice, etc.) determine the compositions of the community, especially the species of lactic acid bacteria, Bacillales, and Mucorales (Figure 3A). In addition, the shape and size of qu also drive the dynamics of microbial communities and the production of their respective enzymes by regulating the moisture transfer and acidity variations within the system [7]. Besides the manufacturing parameters, the key environmental factors (temperature, moisture, acidity, etc.) also drive the microbiota dynamics in qu fermentation [52-54]. For instance, the accumulation of bioheat results in a peak inner temperature, affecting moisture transfer and the metabolic activity of microbes [52,53]. The peak temperature thus plays a vital role in the diversity of the microbial community and contributes to the abundance of thermotolerant microbes (like *Bacillus*, *Aspergillus*, etc.) [54].

Thus, all these noted studies indicate the key factors associated with the formation of microbiota in the preparations of qu. Moreover, modeling of microbial communities, with details of environmental factors included, has been implemented to try and predict the dynamics of the activities of the various microbes and the formation of their metabolites in the solid-state fermentation of qu. With the reminder that the production of qu starters themselves are subject to substantial fermentation activity to render them suitable for subsequent baijiu production [55-57]. It provides a new perspective to study the correlations between microbial formation and environmental changes in the traditional production of qu. Therefore, understanding the effects of multiple factors on the formation of the microbiota would facilitate better control and improvement of the overall microbial consortium for developing defined qu starters.

4. EFFECTS OF QU ON BAIJIU FERMENTATION

Qu is both a starter and a part of the raw materials for Chinese baijiu fermentation. The microorganisms from qu contribute to alcoholic fermentation and flavor generation in the primary baijiu fermentation process [35,36]. Qu provides a large part of the fungal communities (more than 60%) and a small part of the bacterial communities (approximately 20%) to fermentation [58]. These microorganisms from qu, therefore, play crucial roles in the metabolic activities important to baijiu production: (1) filamentous fungi can secrete abundant hydrolases, which act to release fermentable sugars, amino acids, and fatty acids from nutritional macromolecular components (such as starch, protein, and fat), (2) *Saccharomyces cerevisiae* and non-*Saccharomyces cerevisiae* strains contribute to the generation of alcohols and esters during baijiu fermentation, (3) bacteria (like Bacillales, lactic acid bacteria, etc.) are the main contributors to the production of acids, alcohols, ketones, and various other aroma compounds [1-5].

Besides microorganisms, qu itself, by nature of its constituents, is also a main contributor of enzymes to the baijiu fermentation. These enzymes play essential roles in the solid-state fermentation process of baijiu production [1]. Qu thus also provides a large contribution of enzymes (more than 60%, from the various microbial species) such as glycosidases to the baijiu fermentation mashes. The optimal combination of glycosidases could enhance ethanol production in the fermentation process [38]. These facts strengthen the understanding of the effects of glycosidases on baijiu fermentation. However, more attention should be paid to the effects of other enzymes from qu upon baijiu fermentation activities.

In summary, conclusions regarding the effects of qu on baijiu fermentation have been made based solely on an understanding of the relative abundance of microorganisms and enzymes present. Full knowledge of the quantification of the qu starter contributions on baijiu fermentation is still needed. With the development of newer analytical techniques, researchers could better qualify and quantify the contributions to baijiu fermentations and utilize such information as the criteria to realize a fully defined starter.

5. PROGRESS TO A DEFINED STARTER

As a further reminder, the solid-state fermentation of traditional qu occurs under an open environment system. Thus, baijiu producers always face the challenge in maintaining stability and achieving quality consistency in batch production [59]. As noted, previous studies indicate that the compositions of microorganisms and enzymes are associated with the quality and function of qu [7,60]. Therefore, it is now more important to monitor and control the formation of qu within modern baijiu production facilities and the distilling communities where the spirit products are made. This will then answer our question as to how to develop a defined qu starter best and most efficiently and how it might apply to the different styles or types of baijiu produced.

5.1. FORTIFIED QU

Poorly controlled conditions, microbes present within raw materials, and the environment currently drive the formation of the ecological populations of microorganisms during the spontaneous fermentation production stage of qu starter formation. The deliberate inoculation of some of the typical autochthonous strains may thus be efficient for improving the quality of traditional qu by optimizing the starter microbiome [61-65]. In developing better-defined starters, isolated strains of Bacillales, Lactobacillales and Saccharomycetales are always selected as the candidates to build a fortified qu[61-63]. In practice, fortified qu could partially improve the structure of the microbial community and the efficiency of ethanol production in baijiu fermentation [64,65]. However, the formation of the microbial community in the preparation of fortified qu, is regulated by the ecological constraints and correlations between the inoculated strains and the autochthonous microbes, and via local environmental conditions [62]. Thus, this process of fortification by implementing the addition of functional strains faces the challenge of uncertainties of other variables involved regarding regional or seasonal differences in the populations of the localized organisms, to possible instability or a lack of robustness with respect to the autochthonous microbiota - synergistic and antagonistic considerations in play, and to potentially poorly controlled fermentations [61-62]. Thus, the consistent construction of a starter qu of defined population and quality will require implementing a more efficient and refined approach under more appropriate and stringent standards of microbiological quality control to prevent unwanted microorganisms from gaining access to the qu starter matrix.

5.2. PURE CULTURES

The term fuqu is applied to pure cultures, yet these are still only partially defined starters. They are made via the inoculation of functional strains into brans and prepared using mechanized equipment [66,67]. The unique species of Mucorales, Bacillales, and Saccharomycetales have been selected to develop a pure culture (Table 1). In the baijiu fermentation, these pure cultures provide outstanding ethanol production and efficient utilization of raw materials [68-70]. However, the liquors produced by pure cultures are still imperfect in both taste and aromatic qualities due partly to the loss of metabolites from poorly active, undesirable, and possibly decaying microorganisms in the fermentation [67,68]. Meanwhile, the current combined community is susceptible to being disturbed by the presence of the "ecologically new" microorganisms within the preparations of pure cultures due to an overall decreased microbial diversity [7].

Invasion by undesired microorganisms creates competition with endogenous microorganisms and dramatically affects the structure and function of pure cultures [70]. Therefore, constructing a stable and sustainable community (enough to resist the disturbing effects of the undesired microorganisms) is a goal of developing an efficient defined starter.

6. PERSPECTIVES AND CONCLUSIONS

Chinese baijiu and other traditional fermented foods are part of our daily life, although their production is based on practical experience and skills passed down through the generations [71, 72]. Qu and relevant starters are the essential ingredients to improve the quality of spontaneous solid-state fermentation. However, the traditional production of qu represents a poorly controlled process with the inevitable fluctuations of quality and productivity. Therefore, industrial production of a defined starter would increase the ability to improve the quality and safety of baijiu or fermented foods in the future. Herein, with the advances of biotechnology and research, this review proposes a roadmap to establish a rational and controlled process for developing a defined qu or starter in the industry (Figure 4). The goal of constructing a synthetic microbiota is to realize a defined qu for baijiu with the desired flavor profile.

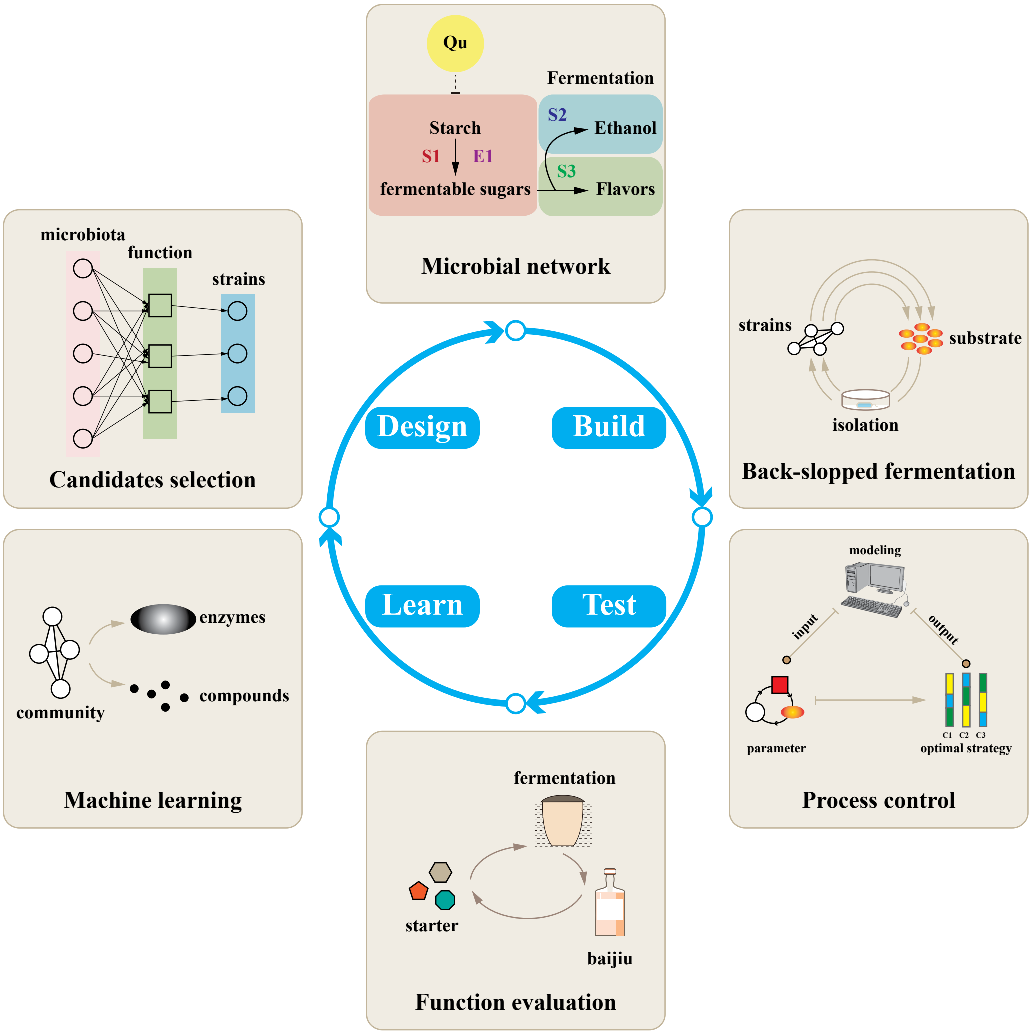


FIGURE 4 Schema of synthetic microbiota to design a defined Qu.

6.1. ISOLATION, SELECTION, AND COMBINATION OF CANDIDATE STRAINS OR ENZYMES

Culture-dependent approaches have led to the isolation of a vast number of strains from traditional sources, and these strains have been well-studied under optimal cultured conditions. Moreover, meta-omics analyses can shed light on the metabolism of specific strains within *in situ* systems. Therefore, the combinations of culture-dependent and culture-independent analyses lead to the proposals for selecting the outstanding strains needed to develop pure cultures. However, the current attempts at using pure cultures limit the selection to one or several functional strains but neglect the role of enzymes that are indispensable for baijiu fermentations. The recent studies reveal that the synergistic effects of multiple enzymes or the combination of strains and enzymes may enhance saccharification, ethanol production, and flavor metabolism in baijiu fermentation. Thus, the future development of defined starters may be selecting the combinations of multiple elements (strains, enzymes, or substrates) as a basis for production.

6.2. IDENTIFICATION, MONITORING AND THE CONTROL KEY DRIVING FORCES

As already illustrated, traditional preparations of qu are generally developed under an uncontrolled environment, and the poorly controlled conditions (like temperature, moisture, acidity, etc.) affect the assembly of the microbial community in this spontaneous process. With the application of systems biology methods, the correlations between environmental factors and microbiota have been established as key driving forces that determine microbiota formation in traditional qu production. Furthermore, this knowledge will potentially regulate the critical factors for further improving and developing a defined starter.

6.3. SYNTHETIC MICROBIOTA TO DESIGN A DEFINED STARTER

Designed synthetic microbiota serve as an efficient approach to develop a defined qu or pure culture mixes [73]. This method has succeeded in allowing the construction of a tractable microbiota *in vitro* system for optimizing traditional food fermentations, like cheese, light-style baijiu, and soybean sauce [73-76]. The tractable microbiota is constructed through strain selection (based on structure, function, and correlations with the local environment), flora combinations, and function evaluation [73-76]. Additionally, the production of these foods may be likened to the production of traditional qu, through the metabolic activities of similar microbiota, the raw materials used, processing and type of end products made. Therefore, this progress in our understanding opens a channel to construct a reproducible and tractable microbiota driving towards standardization and modernization of defined fermentation starters. First, for the design, we select candidate strains based on their roles in the baijiu fermentation and combine the community with/without enzymes to reduce the redundancy of specific pathways. Second, we rely on back-slopped fermentation to domesticate the combined community in selected materials or substances. Third, we monitor the metabolism of the synthetic microbiota (biomass, enzymes, metabolites, etc.) and gather the process parameters (temperature, moisture, acidity, etc.). Fourth, we build the model of the fermentation of designed microbiota to estimate and evaluate the productivity and function of defined starters. Fifth, we attain the optimum combinations of synthetic microbiota from the modeling and develop the group of desired starters. Then we study the effects of desired starters on baijiu fermentation to verify and acquire a more efficient and competitive starter. Sixth, we expect to build a broader database about specific synthetic microbiota and the desired baijiu, to assist in other traditional fermented food production (Figure 4). Therefore, the idea of building qu by a synthetic microbiota approach is worth developing to provide a defined starter, and such starters then need to be proven in practice.

6.4. CONCLUSIONS

Chinese qu or other starters determine the quality and flavors of baijiu or other fermented foods. Traditional spontaneous fermentation of starters (with a diverse collection of microbial species) cannot meet the increasing needs for industrial growth and expectations of quality. The challenge of rising manufacturing and labor costs makes it imperative to standardize and modernize products. There is thus an urgent need to develop a standardized and modernized industrial production program to overcome the drawbacks of uncontrolled fermentation. The design of a tractable microbiota could provide an efficient approach to developing a defined qu for Chinese baijiu fermentation, just as the development of koji helped to realize better-controlled production of high-quality and perfect-tasting Japanese soy sauce and sake [5]. With the fast development of modern technology, a defined qu could be realized and overcome traditional technical limitations, resulting in better quality and consistency of baijiu spirit.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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