

Research Progress on Influencing Factors of Microbial Community Succession during Fermentation of Nongxiangxingdaqu

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Abstract: As a saccharation starter culture for liquor fermentation, Daqu is prepared and produced in an open environment. The microorganisms in Daqu mainly come from raw materials and production environment. In the process of Daqu production, environmental conditions such as humidity and temperature in the Qu chamber are changed by repeatedly opening and closing doors and Windows and removing straw curtains to affect the succession and community composition of Daqu microbial community. In this way, the changes of fermentation environment can be adjusted to promote the growth and metabolism of beneficial microorganisms and gradually form Daqu unique microorganisms. This paper reviews the research progress of Daqu microbial community, including Daqu microbial community, the influence of environmental factors on microbial community, and the interaction between microbial communities.

Keywords: Nongxiangxing Daqu; Daqu microorganism; Environmental factors; Microbial interaction

1. Introduction

Baijiu is a traditional distilled spirit made from the fermentation of grains and has a history of more than 2000 years. Together with brandy, whiskey, vodka, rum and gin, it is called one of the world's six distilled spirits. The brewing of baijiu mainly consisted of two processes, namely, making Qu and fermented grains. Daqu is a starter in liquor fermentation process, which is mainly made of wheat (or ingredients barley, peas, sorghum, etc.) as raw materials. Under open operating conditions, Daqu is naturally inoculated with microorganisms in raw materials, air, sites, utensils and mixing water, etc., and fermented by controlling production technological conditions. In the process of koji making, the microorganisms gradually introduced into Daqu form a complex multifunctional microbial community and secrete a variety of enzymes and compounds. These compounds are the precursors of liquor flavor, and enzymes are mainly involved in liquefaction, saccharification, protein decomposition and cellulose degradation during liquor fermentation^[1]. There are many ways to classify Daqu. Generally, Daqu is divided into high temperature Daqu (60 ~ 65°C), medium temperature Daqu (50 ~ 60°C) and low temperature Daqu (40 ~ 50°C) according to the difference of top temperature.^[2] With the continuous development of the production technology of Qu making, there are also different types of Daqu according to the technology of qu making, such as traditional Daqu, pure Daqu, strengthened Daqu and stand Qu^[3]. Medium-high temperature Daqu wine is the main kind of Chinese liquor, its body style is typical, occupies a dominant position in the national liquor market^[4]. Daqu is usually prepared in an open environment. Therefore, it is easy to be affected by environmental or human factors, and its quality is difficult to get absolute stability^[5]. The preparation process of Daqu needs to go through several steps, such as moistening grain, crushing, stirring, shaping, accumulation culture, mature storage, etc^[6]. Figure 1.

NongxiangxingDaqu provides abundant enzyme, bacteria and substance systems for liquor brewing. *Aspergillus*, *Rhizopus*, *Bacillus*, *Lactobacillus*, *Wickerhamomyces* and *Saccharomycopsis* in Daqu^{[7][8]} Saccharification enzyme, liquefaction enzyme and protease produced by microorganisms can degrade large molecular substances such as starch and protein, and further transform them into volatile flavor substances such as alcohol, ester, acid and pyrazine, thus giving liquor rich aroma^[9]. In the process of koji making, microorganisms interact with each other and with the environment, and eventually form Daqu microbial flora, which participates in the subsequent brewing of liquor^[10]. It was found that the proportion of bacteria and fungi in fermented grains of Daqu was 81.5% and 79.2%,

respectively^[11]. Therefore, the diversity and content of Daqu microorganisms play an important role in the quality formation of Luzhou-flavor liquor^[12].



Figure 1: Daqu production process

As the main fermentation period of Daqu, the warming period is the key link affecting the quality of Daqu, and the microbial changes are the most significant at this time. Because temperature, humidity, and water in the koji making process lead to changes in microbial diversity and produce different substances in metabolism, this can be regarded as changes in microbial ecosystem and corresponding responses and functions caused by environmental factor stress^[13]. The fermentation process of Daqu is a dynamic process in which a variety of microbial communities interact together. This process is related to the continuously changing environmental conditions. Under the interaction of environmental factors such as temperature, water content, pH and acidity, the microbial succession rate and the interaction between microorganisms will change in the fermentation process of Daqu. More and more is now known about the microbial composition of various types of Daqu fermentation.

2. Microbial composition of Daqu

Through the long-term study of Daqu microbial community, scholars found that the microorganisms in Luzhou flavor Daqu mainly include mold, yeast and bacteria^{[9][14]}. It mainly includes filamentous fungi such as *Aspergillus*, *Rhizopus*, *monascus*, *Rhizomucor* and *Mucor*, yeast fungi such as *Pichia*, *Candida*, *Polyspora*, *Isasae* and yeast, and bacteria such as *Bacillus*, *Lactobacillus* and *Contococcyces*^{[15][16]}.

The earliest research on Daqu microorganisms was culturable, mainly focusing on the isolation, identification, quantity change, physiological and biochemical detection of Daqu microorganisms. Chen Lin et al^[17] By isolating and screening the microorganisms in Huangtai Jiuqu, 73 strains were obtained. It was found that the bacteria in Luzhou-flavor Huangtai Jiuqu were abundant in quantity and variety, and *Bacillus* was the dominant bacteria. Luo Hubo et al^[18]. isolated and purified 69 strains of fungi from Luzhou-flavor Daqu by means of culturable and screening bacteria. Through identification and analysis, they found that the fungal groups of Luzhou-flavor Daqu mainly included *Aspergillus*, *Monascus*, *Rhizomorphiza*, *Transverticillus*, *Penicillium* and *Penicillium*.

Culturable technology can only study a very small number of microorganisms and cannot reflect the composition of the entire Daqu microbial community. Therefore, with the development of modern molecular biology technology, Denaturing gradient gel electrophoresis (PCR-DGGE), single chain conformation polymorphism (PCR-SSCP), phospholipid fatty acid analysis (PLFA), fluorescence in situ hybridization (FISH), high-throughput sequencing, metagenomics and macrotranscriptomics have been gradually applied in the study of Daqu preparation and liquor brewing microorganisms^[19-22]. Luo Huibo et al.^[23] used PCR-SSCP (single chain conformation polymorphism) technology to study the changes of prokaryotic microbial community structure in the fermentation process of Luzhou-flavor Daqu, and the results showed that the community structure of the yeast samples in different fermentation periods was similar, but also had polymorphism. Different microbial communities have complex ecological effects of coordination and restriction. Huang Xiaoning et al.^[24] used culturable and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) to analyze and identify the microbial community composition of different flavors of Daqu in the same distillery. The results showed that the number of *Bacillus licheniformis* was absolutely dominant in the two varieties of Daqu *Mucor umbelliferum* and *Paecilomyces mutans* were predominant.

High-throughput sequencing technology has the advantages of high throughput, rapid accuracy and low cost^{[25][26]}, which can reflect the distribution of microorganisms in Daqu more comprehensively and provide more comprehensive and reliable microbial information^[27]. Wu Shukun et al^[28]. analyzed the differences in microbial community structure of Luzhou Daqu in different regions of Sichuan Province, and found that *Staphylococcus*, *Weisella*, *Bacillus*, *Lactobacillus*, *Kroppenstedtia*, *pyroactinomyces* and

Saccharomyces were the dominant fungi. Omics techniques such as metagenomics and macrotranscriptomics can more accurately reflect the microbial function and physiological or transcriptional activity in samples, so they are also applied to study the microbial function and function of Daqu. Zhang et al.^[29] studied the differences in microbial communities and functional genes between Daqu and trikoqu by metagenomic sequencing. The results showed that the community composition of Daqu and trikoqu was significantly different at the species level, but the functional genes were similar. Through these multiomics techniques, the distribution and composition of Daqu microbial community were revealed from different perspectives.

3. Function of Daqu microbial community

In recent years, with the study of Daqu microbial community and its function, people have gradually deepened their understanding of the composition and function of Daqu microbial community. In the open fermentation process, Daqu was naturally inoculated and assembled microflora from the surrounding fermentation environment, so Daqu was rich in bacteria, mold, yeast, actinomyces and other microorganisms^[30]. These microorganisms play an important role in the fermentation process of Daqu. For example, bacteria, as the most important microorganism in the brewing process of liquor, plays an important role in improving the flavor and quality of liquor, and has been the focus and hot spot of research^[31]. As a class of brewing microorganisms, yeast is not only the dominant force in liquor fermentation process, but also its aroma production performance is crucial to the quality and characteristics of liquor with different flavors^[32]. In the process of Daqu fermentation, molds produce protease, lipase, saccharifying enzyme, cellulase and other rich and diverse enzyme systems, which have the ability of aldehyde and esterification, and can grow mycelium, which is conducive to water volatilization and substance utilization of Qu core, and plays a crucial role in the maturation process of Daqu fermentation^[33]. Actinomyces have various metabolic activities, including hydrolyzing starch, cellulose, protein and pectin in raw grains, and producing various secondary metabolites^[34]. The main functional microorganisms in Daqu play an important role in liquor flavor quality.

At present, metagenomics, metaproteomics, metproteomics and metabolomics technologies are widely applied in the field of liquor, as well as omics technologies or the combination of multiple microbiome technologies are important ways to reveal functional microorganisms and in-situ metabolic characteristics of koji^[35]. Song et al^[36]. determined the core microbial community of Daqu, Maotai-flavor liquor, by high-throughput amplified sequencing, ultra-high performance liquid chromatography and headspace solid phase microextraction-gas chromatography-mass spectrometry. They found that in the first stage of Daqu fermentation, the bacteria with strong ability to produce ethanol was schistose, while in the second stage, the core functional microorganism that produced a large amount of lactic acid and acetic acid was lactic acid bacteria. Huang et al.^[37] studied the microorganisms and functions of Luzhou flavor Daqu by macrotranscriptomics technology, and found that fungi were the most abundant and active microorganisms, with a total of 932 carbohydrate active enzymes, including 9 highly expressed co-active families and 10 proteins. Tian et al^[38]. used metagenomics to analyze the diversity of bacterial community in four batches of Shilixiang liquor Daqu and determine the influence of fermentation process on microorganisms. The results showed that after 30 days of fermentation, the richness and diversity of microorganisms in four batches of Daqu samples increased, and the main bacteria were choriomyces, lactobacillus, pneumococcus and staphylococcus. The proportion of pseudomonas decreased; Through the prediction of functional gene composition, it was proved that the bacterial population of Shilixiang liquor Daqu mainly had the functions of metabolism, genetic information processing and environmental information processing.

4. Influence of environment on the structure of Daqu microflora

The occurrence of microbial succession in Daqu is due to the adaptation of microorganisms to the external environment or the specific environmental conditions established by the microbial community in the environment, and the adaptive choice made by microorganisms in the face of the highly variable environmental conditions in Daqu House. Microbial succession in the koji making process is closely related to environmental factors including temperature, acidity, humidity, etc.^[39]. WU et al^[40]. found that the change of Beta diversity of dominant microorganisms in Daqu microbial community was significantly related to temperature. Zhu et al.^[41] studied the correlation between environmental factors and Daqu microorganisms through Sperman correlation network, and the results showed that moisture, CO₂ and humidity were significantly positively correlated with Kosakonia, Kodamaea, Epicoccum,

Candida and other fungi. It was negatively correlated with high temperature actinomyces, Saccharopolyspora and Acinetobacter. Therefore, most fungi are abundant in the early fermentation period. However, humidity, CO₂ and water were negatively correlated with most bacteria. For example, the dominant bacteria belonged to thermoascomyces and were negatively correlated with environmental humidity and CO₂ concentration, so the abundance of bacteria was higher in the late fermentation period. Environmental filtration (that is, the environment is suitable for the colonization or survival of certain microbial communities) is an important selection process during fermentation [42]. For example, lactic acid bacteria and yeast can maintain a dominant position at low temperature in the early stage of Daqu fermentation, possibly due to their advantages in the production of acids, alcohols and antibiotics, as well as competition for resources and ecological niche [43]. With the increase of fermentation temperature, Bacillus is more competitive than S. aureus in the fermentation process, secreting amylase and protease to convert starch and protein into glucose and amino acids, and promoting the formation of precursor flavor compounds [44]. This is temperature-driven selection based on the thermal tolerance characteristics of species, which plays a crucial role in the formation of microbial structure and metabolism [45]. Environmental factors have a strong influence on the growth, metabolism and succession of fermentation microflora. Many studies have shown that the driving factors of growth, metabolism and succession of microbial community are acidity, moisture and temperature [1]. Zheng et al. [46] analyzed the correlation between the evolution of microbial diversity and environmental factors such as temperature, pH, acidity and water during koji preparation, and found that temperature and pH were positively correlated with microbial community structure in the middle stage of fermentation. There was a positive correlation between water content and microbial community structure at the end of fermentation. At the same time, other studies have shown that temperature factors affect the composition of microbial community in finished koji by regulating the succession rule of microbial community in the early stage of Daqu fermentation [47-49].

5. Effect of microbial interaction on Daqu microflora

The succession of microbial community was mainly influenced by the interaction between environmental factors and microorganisms, which together promoted the evolution of microbial community in the fermentation process of Luzhou-flavor Daqu. In the process of solid fermentation, the interaction between microorganisms determines the structure of microbial community to some extent [50]. Hu [51] et al. showed that there was a potential synergistic relationship between the members of this genus: Clostridia, Bacteroidia, Methanobacteria and Methanomicrobia in the process of liquor brewing through the analysis of symbiotic patterns. The synergistic interactions between microorganisms may be beneficial to the stability of microbial community structure and ecosystem, while the Lactobacillus, pedicoccus, and streptococcus may disrupt this stability through the production of lactic acid or various bacteriins. Ma [1] et al. found that the succession of Daqu microbial community was mainly driven by the interaction between temperature, humidity, CO₂, O₂, acidity, humidity and biomarkers with lactic acid bacteria, yeast and acetate bacillus, and the final Daqu microbial community structure was formed.

6. Conclusion and Prospect

Daqu fermentation is a solid-state fermentation process of mixed bacteria, which is affected by environmental conditions and the interaction between microorganisms. Current studies mainly focus on the change rule of Daqu microorganisms in the fermentation process and the influence of environment on them, the effect of microbial interaction on Daqu succession, or the difference of metabolic function. However, matter is the front end of information flow, and metabolism is the end. In the middle, protein and transcription processes are crossed, which cannot reflect the complete metabolic process. Therefore, in the future, multi-omics technologies such as metagenomics, metproteomics, metagenomics and other means will be assisted to explore the factors affecting the microorganisms and functions in Daqu fermentation process.

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