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Research on the Dynamics and Distribution Patterns of Environmental Microorganisms in the Production Process of Strong-Flavour Baijiu Distillery Workshops

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Abstract: In the workshop environment for mechanized Baijiu production, the dynamics of microbial communities are primarily influenced by both the production process and environmental control measures. Effective control of microbial populations and assurance of product quality can be achieved through environmental management and the implementation of automated production processes. To provide a theoretical foundation for the regulation and management of environmental microorganisms, this study employed high-throughput sequencing technology to continuously monitor microbial communities throughout a complete production cycle, from resumption of operations to cessation of production. The following conclusions were drawn from the analysis of the raw data using Origin, SPSS, and R. In the production workshop of strong-flavor Baijiu, Aspergillus, Ralstonia, Dechloromonas, Bacillus, Sphingomonas, Methylobacterium-Methylorubrum and Stenotrophomonas are the dominant microbial species in the environment. As production progresses normally, the microbial community gradually stabilizes. During continuous production, the fermentation area (F area) exhibits greater stability compared to the spreading and drying area (T area). During continuous production, the fermentation area (F area) exhibits greater stability compared to the spreading and drying area (T area). The microbial community in the environment is correlated with environmental humidity, the number of open cellars, and the frequency of steaming operations throughout the production process.

Keywords: Strong-flavor Baijiu; Environmental Microorganisms; Microbial Co-occurrence Network; High-throughput Sequencing.

1. Introduction

Baijiu is a distilled spirit primarily made from grains, using Daqu, Xiaoqu, Fuqu (wheat-based fermentation starters), enzyme preparations, and yeast as saccharifying and fermenting agents. It undergoes processes including steaming, saccharification, fermentation, distillation, aging, and blending [1]. The production of Baijiu from grain raw materials involves a complex natural multi-strain solid-state fermentation process. Traditional Baijiu fermentation occurs in an open environment, making it highly susceptible to environmental factors [2]. The entire Baijiu fermentation system encompasses both the environmental microecology and the fermentation microecology. The environmental microecology includes all environments that directly or indirectly interact with the fermentation process, such as air, equipment surfaces, pipelines, workers, floors, and walls[3]. Microorganisms from these environments enter the nutrient-rich mash substrate via media like air and tools, where they grow, metabolize, and proliferate. This microbial activity significantly influences the stability of the Baijiu brewing system and the quality of the final product[4]. Numerous studies have demonstrated that, despite using identical fermentation materials and processes, fermented foods produced in different environments exhibit significant variability. Variations in the external production environment can lead to changes in the metabolic profiles of flavor compounds in the fermenting mash. The initial microbial communities in the fermenting mash differ significantly across different environments, which further results in variations in microbial succession patterns and metabolic activities within the fermentation system[5]. Strong-flavor Baijiu is fermented in sealed pits. Although the raw materials are steamed before entering the pits, they can easily introduce microorganisms from operating tools, workshop air, workshop floors, and other environmental sources during the cooling and transfer process into the fermentation pits. These microorganisms grow, metabolize, and proliferate in the nutrient-rich mash substrate, thereby affecting the stability of Baijiu brewing to some extent. Differences in environmental microorganisms across regions and seasons directly or indirectly influence the fermentation microecology, leading to variations in the fermentation process and product quality. Understanding the impact of environmental microorganisms on fermentation microecology is a critical step in regulating and optimizing modern Baijiu fermentation[6,7]. The relative stability of environmental microorganisms is crucial for the production and brewing of Baijiu. In modern mechanized production workshops, equipment maintenance is conducted periodically, resulting in temporary production halts. Previous studies have demonstrated that the environmental microorganisms in Daqu production workshops differ before and after resuming production, which can impact the quality of Daqu[8]. This study investigates the changes in environmental microorganisms within strong-aroma Baijiu production workshops following the resumption of normal operations after a halt, as well as the differences in environmental microorganisms between the fermentation area and the cooling and spreading area within the workshop.

Baijiu, a traditional Chinese distilled spirit, has a brewing history spanning several thousand years. This rich heritage has been preserved and refined over generations by artisans who have honed the craft through empirical knowledge and

meticulous practice[9]. Traditional baijiu production methods are characterized by long production cycles, which can extend from months to years depending on the specific type and quality requirements. These methods also feature low levels of mechanization, high labor intensity, and suboptimal working conditions, all contributing to significantly reduced production efficiency. Historically, baijiu production relied heavily on manual labor and empirical knowledge passed down through generations[10]. The brewing process involved numerous intricate steps, each requiring careful attention and expertise. For instance, the fermentation stage alone could take several weeks to months, during which workers had to manually monitor and adjust parameters such as temperature, humidity, and microbial activity. This labor-intensive approach not only limited production capacity but also posed challenges in maintaining consistent product quality. With the advancement of industrial mechanization and technological innovation, the baijiu industry has progressively shifted towards automation and intelligent manufacturing. Modern breweries now employ advanced machinery and automated systems to streamline production processes, enhance efficiency, and improve product consistency. However, mechanical equipment requires periodic maintenance and repair, during which time the brewing workshops temporarily cease operations. This downtime can disrupt the delicate balance of the fermentation environment, potentially affecting the microbial communities that play a crucial role in the brewing process. Numerous studies have demonstrated that environmental microorganisms can enter the fermentation system via various vectors, including air, equipment, and personnel[11]. These microorganisms, both beneficial and harmful, can significantly influence the flavor profile and quality of the final product. For example, certain strains of yeast and bacteria are essential for initiating and maintaining the fermentation process, while others may introduce off-flavors or spoilage if not properly controlled. This study investigates the dynamic changes in environmental microorganisms during the transition from production suspension to resumption and subsequent continuous production. By monitoring these changes, researchers aim to better understand how interruptions in the production cycle affect the microbial ecology of the brewing environment. This knowledge can help optimize maintenance schedules, minimize disruptions, and ultimately enhance the overall efficiency and quality of baijiu production. Through this research, the industry can continue to evolve, balancing tradition with innovation to meet modern demands while preserving its cultural significance.

2. Materials and Methods

2.1. Sample Collection

Collection period: December 10, 2023 to December 30, 2023

Sampling location: Samples were collected from a mechanized strong-flavor Baijiu production workshop in a certain area of Luzhou City, Sichuan Province. Environmental samples were taken from the air and walls in different areas of the workshop. All samples from the same area on the same day were combined into one sample.

Airborne microorganism sampling: Five sampling points were selected in each area, which were 2 meters away from the wall and 1.5 meters above the ground in the interior of the wine-making workshop, and where there was no personnel movement. A medium-flow sampler was used with a controlled flow rate of 80 L/min and a sampling time of 50 minutes. The glass fiber filter membrane was placed in a 10 mL sterile cryotube and stored in a -80 ℃ refrigerator.

Wall microbial sampling: At 1.5 meters above the ground, randomly select 5 positions. Use one cotton swab soaked in sterile 0.03 mol·L-1 phosphate buffered saline (Phosphate Buffered Saline, PBS) or physiological saline sampling solution. Within the specification board, swipe the swab back and forth horizontally and vertically for 5 times each, and rotate the swab accordingly (mark the sampling points to prevent damage).

2.2. Sample DNA Extraction and PCR Amplification

To obtain the nucleic acid profiles of the microbial community, the CTAB method was employed to extract total DNA from the samples. The highly variable V3-V4 region of bacterial 16S rRNA was amplified using primers 338F (5'-barcode+ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTATCTAAT-3'). The ITS1 region of fungal DNA was amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3'). The PCR amplification protocol was as follows: initial denaturation at 98°C for 5 minutes, followed by 25 cycles (30 cycles for ITS): denaturation at 98°C for 30 seconds, annealing at 53°C for 30 seconds (55°C for 45 seconds for ITS), extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes; the reaction was held at 12°C upon completion.

2.3. High-throughput Amplicon Sequencing and Bioinformatics Analysis

The purified amplicons were sequenced and analyzed on the Illumina NovaSeq (PE250 paired-end sequencing) platform following the standard protocol of Shanghai Pageno Biotechnology Co., Ltd.

The raw data were first processed using qiime cutadapt trim-paired to remove primer sequences and discard reads that failed to match the primers. Subsequently, qiime dada2 denoise-paired was employed to perform quality control, denoising, sequence merging, and chimera removal for each library individually. After completing denoising for all libraries, the ASV feature sequences and ASV tables were merged, and singleton ASVs (i.e., ASVs with a total sequence count of only 1 across all samples) were removed as part of the default operation. Finally, an R script was used to statistically analyze the length distribution of high-quality sequences across all samples.

2.4. Environmental Factor Monitoring

Environmental factors such as temperature and humidity were continuously monitored at each sampling point. The production status in the workshop on the day of each sampling was also recorded.

2.5. Statistical Analysis

Data processing and analysis were conducted using Origin, R and Spss.

3. Results and Discussion

3.1. The Composition and Diversity of Bacterial Communities Within the Environmental Microbiome of The Wine-Making Workshop

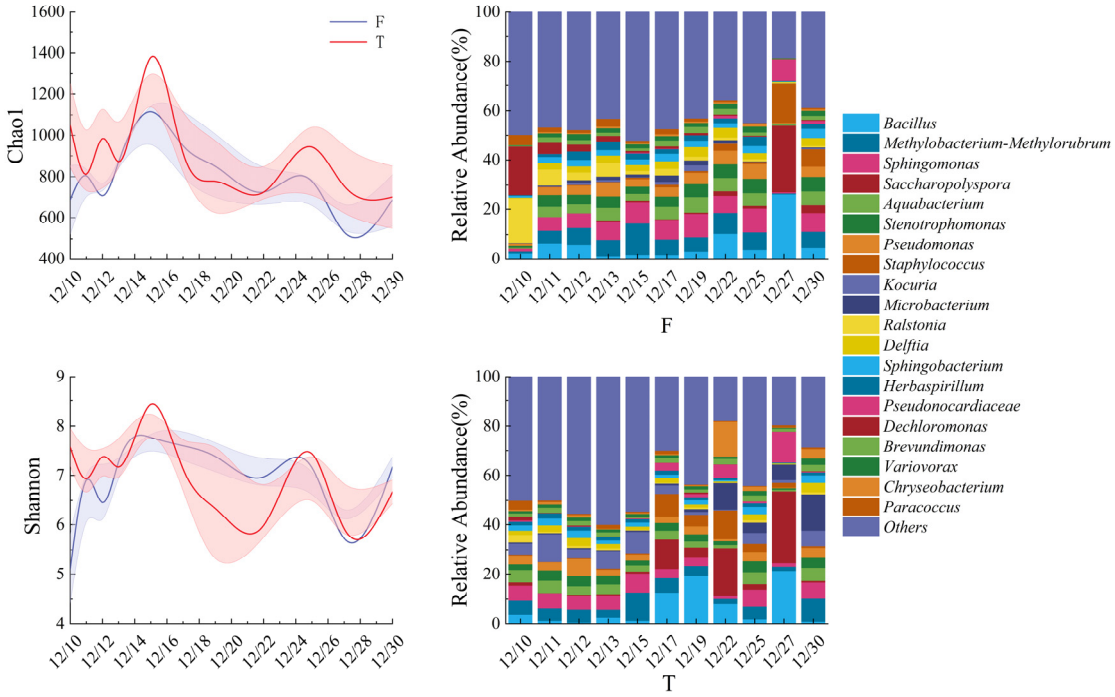

Figure 1. The α-diversity index and relative abundance of bacterial species within the workshop environment

The changes in environmental microorganisms during the production process of strong-flavor Baijiu in the brewing workshop were analyzed using 16S rDNA amplicon sequencing. The results demonstrated that the coverage rate for all samples exceeded 97%, indicating adequate sequencing depth for subsequent analyses. At the genus level, a total of 982 bacterial genera were identified. Figure 1 illustrates the top 20 species with the highest relative abundance, while the remaining species are categorized as "others". On the day before resumption of work, Ralstonia and Dechloromonas were the dominant species in the fermentation area (F aera) environment. However, their abundance decreased as production resumed and progressed. Bacillus, Methylobacterium-Methylorubrum, Sphingomonas, Aquabacterium, and Stenotrophomonas are the dominant species in the workshop following the resumption of normal production. The dominant bacterial species in the T area are largely similar to those in the F area, but their relative abundances differ. As shown in the figure, during the turning-over stage (12/27), both areas exhibit fewer species compared to normal production, and the community composition structure shows significant differences. The dominant bacterial species in both regions have undergone substantial changes. Notably, Saccharopolyspora, Staphylococcus, and Pseudonocardiaceae have emerged as the new dominant taxa. These species are all dominant in the fermented grains. It is hypothesized that this shift occurs because the microbial community from the wine cellar's fermented grains is transferred to the workshop environment during the turning process[12]. However, while Lactobacillus is a dominant bacterial genus in the wine grains, its relative abundance in the workshop environment is relatively low [13]. This suggests that most of the Lactobacillus in the wine grains likely originates from Daqu and cellar mud, contributing to a more stable fermentation system.

The α-diversity of environmental microorganisms during this production cycle (from the start of this round to the start of the next round) was quantified using the Chao1 index for richness and the Shannon index for diversity. Figure () characterizes the α-diversity of bacterial communities in environmental samples from the winemaking workshop. The Chao1 index indicates that, as work and production resume and stabilize, the richness of environmental microorganisms in both the T and F areas of the brewing workshop initially increases, subsequently decreases, and eventually stabilizes. Microbial diversity was characterized using the Shannon index. The results showed that in area F, bacterial diversity initially increased and then stabilized, whereas in area T, it exhibited more pronounced fluctuations. During the pit-turning stage, both areas experienced a significant decline in bacterial diversity. The microbial community in the F area environment is relatively stable during normal production. In the seven days prior to resuming work, the bacteria that increased were predominantly of very low abundance and miscellaneous species. It is hypothesized that this increase occurred because, after resuming operations, workers and equipment began to enter and operate normally, introducing some low-abundance miscellaneous bacteria from outside the brewing workshop into the workshop. This likely caused certain disturbances to the microecology of the F area. However, as production proceeds normally, the resilience of environmental microorganisms in the winemaking workshop

helps maintain the microecology of the core fermentation area in a stable state. In the production of strong-flavored Baijiu, airborne microorganisms can enter the fermentation system through air circulation. Therefore, the stability of the F area environment contributes to the stability of the fermentation system within the cellar [14].

3.2. The Composition of Fungal Communities Among Environmental Microorganisms in The Wine-Making Workshop

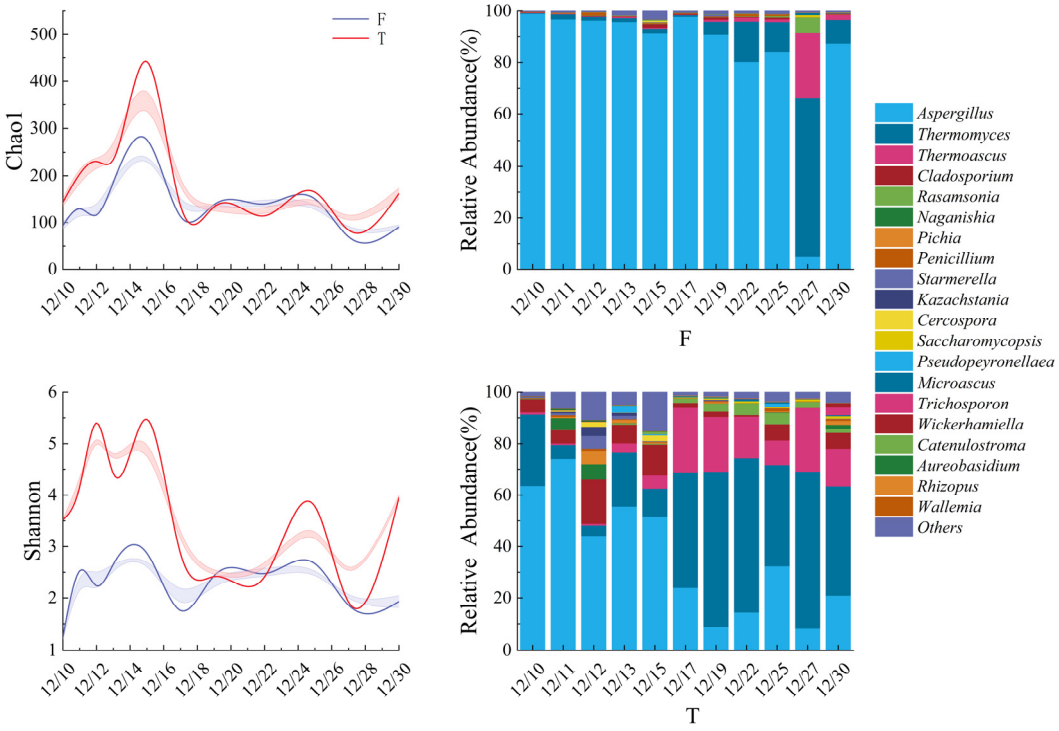

Figure 2. The α-diversity index and relative abundance of fungal species within the workshop environment

A total of 455 fungal genera were detected at the genus level. Among these, Aspergillus, Thermomyces, and Thermoascus were the predominant fungal genera in the F aera. Among these, Aspergillus is the dominant fungal genus throughout the production process, while the relative abundance of Thermomyces exhibits an increasing trend as operations resume and proceed normally. During the pit-turning period, Thermomyces, Thermoascus, and Rasamsonia became the dominant fungal genera in the fermentation zone. The fungal richness detected in the T area was significantly higher than that in the fermentation zone at the genus level. Aspergillus, Thermomyces, Thermoascus, and Cladosporium are the predominant fungal genera in this region. Although Aspergillus remains one of the predominant fungal genera in the T zone, its relative abundance is significantly lower than that in the F zone and gradually decreases with ongoing production. Meanwhile, the relative abundance of Thermomyces and Thermoascus gradually increases as production continues normally.

Figure 2 characterizes the α-diversity of bacterial communities in environmental samples collected from the winemaking workshop. The Chao1 index indicates that as operations resume and proceed normally, the fungal richness in the equipment spreading area (T) and the fermentation core area (F) of the brewing workshop initially increases, then decreases, and gradually stabilizes. Prior to resuming work, the microbial community richness in area T was significantly higher than that in area F. However, after seven days of continuous production, once the Chao1 index of the microbial community stabilized, no significant difference in fungal richness was observed between the two areas. The fungal diversity was characterized using the Shannon index. Results showed that in the early stage of resumption (7 days prior to resuming operations), the species diversity in the equipment spreading and drying area (T) was significantly higher than that in the fermentation core area (F). However, as continuous production progressed, this difference gradually diminished. After production ceased on day 30, a significant difference re-emerged. The Shannon index of fungi in Area F showed minimal variation as operations resumed and proceeded normally, while in Area T, there was a significant downward trend. Meanwhile, both the Chao1 index and Shannon index of the bacterial community were higher than those of fungi, suggesting that bacteria may dominate the microbial community in the strong-flavor Baijiu brewing workshop.

The internal environment of the workshop is influenced by Baijiu production and brewing activities, leading to a certain degree of stability in the microbial population. Although microbial richness increases during the early stage of resumption, as brewing activities proceed normally, the overall environmental microbial community gradually stabilizes. Both richness and diversity peak on the fifth day of resumption (Day 15). Therefore, based on the similarity of fungal and bacterial communities, the production cycle is divided into two phases: the resumption period (seven days prior to and including the first week of resumption) and the continuous production period (after seven days of resumption).

3.3. Trends in Environmental Microbial Communities During the Resumption of Work and Production

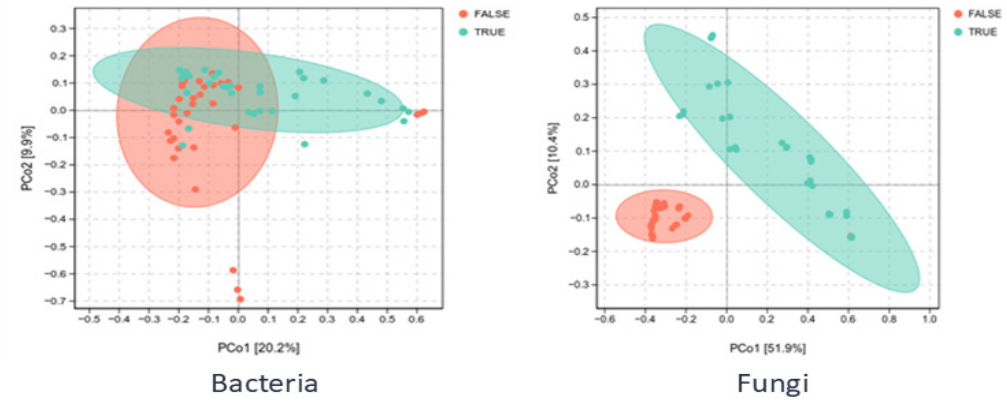

Figure 3. The principal coordinate analysis (PCoA) plot of bacterial and fungal communities within the workshop environment

At the genus level, PCoA analysis was conducted across different regions to further elucidate the β-diversity differences in microbial community structure between the F and T areas within the workshop. As shown in Figure 3, the bacterial communities in area F are primarily distributed in the first and third quadrants, whereas those in area T are predominantly found in the first and second quadrants. Despite the considerable distance between the two areas, the distances among points within area F are relatively short. This suggests that bacterial communities in area F exhibit greater stability during the production process. As production proceeds normally, the distance between the bacterial communities in the two areas gradually decreases, and their similarity increases. In contrast, the fungal communities in the two areas remain more distant with no overlapping regions. The F aera is primarily distributed in the third quadrant, whereas the T aera is mainly distributed across the first, second, and fourth quadrants. As production progresses, the fungal communities in the F aera exhibit minimal variation. In contrast, the T aera shows significant differences in fungal community structure following the normal resumption of work. The F area exhibits greater stability compared to the T area. This may be attributed to the fact that, in the brewing process, the T area, which serves as the spreading and cooling zone, undergoes frequent steaming of grains and continuous spreading, cooling, and yeast addition. Consequently, high-temperature-resistant advantageous microorganisms from the yeast gradually accumulate in the T area environment.

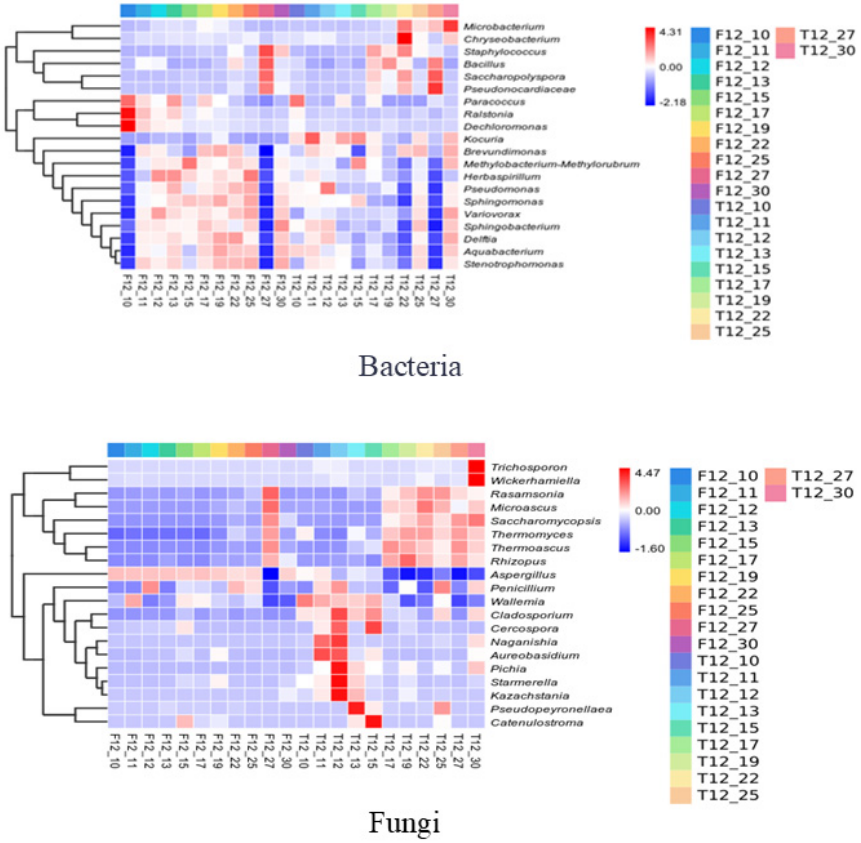

Figure 4. Heatmap of cluster analysis for the top 20 most abundant fungal and bacterial taxa in the workshop environment

Cluster analysis of the top 20 most abundant microbial species was performed, as shown in Figure 4. At the bacterial genus level, the abundance of bacteria in the F region was significantly higher than that in the T region. On the day before resuming work, the relative abundance of Paracoccus, Ralstonia, and Dechloromonas in area F was notably higher. However, as brewing production proceeded normally, the relative abundance of these three genera gradually decreased. These three bacterial strains, known for their environmental pollution control capabilities, possess the functions of inhibiting algal growth and removing heavy metals from soil. Furthermore, it can effectively prevent the invasion of pests and pathogens[15,16]. Their presence ensures that the workshop remains free from contamination by miscellaneous bacteria and heavy metals during the shutdown period. As production resumes and the workshop environment changes, they gradually lose their competitive advantage and are progressively replaced by the dominant microbial strains associated with Baijiu fermentation[17,18]. The bacterial species, including Brevundimonas, Methylobacterium-Methylorubrum, Herbaspirillum, Pseudomonas, Sphingomonas, Variovorax, Sphingobacterium, Delftia, Aquabacterium, and Stenotrophomonas, exhibited a trend of gradually increasing their relative abundance in Area F as production resumed normally. After reaching a certain level, their relative abundance stabilized. This indicates that following a period of resumed production, the microbial community in Area F reached a relatively stable state. Meanwhile, in the T area, the trend of bacterial genus changes was opposite to that observed in Area F during the resumption of work. As production resumed, the relative abundance of the aforementioned bacteria decreased, while the relative abundance of Microbacterium, Chryseobacterium, Staphylococcus, Pseudonocardiaceae, Bacillus, and Saccharopolyspora significantly increased. During the pit-turning stage (12/27), the bacterial community structure in Area F exhibited significant fluctuations, with a sharp increase in the relative abundance of dominant species such as Staphylococcus and Bacillus in the fermented grains. In contrast, the bacterial community structure in Area T remained relatively stable during the pit-turning period. However, on the day of cleaning (12/25), the bacterial community structure in Area T also showed fluctuations and gradually converged with that of Area F. After the production halt (12/30), the differences in the bacterial community structures between Area F and Area T progressively diminished.

At the genus level, the relative abundance of fungi in Area T was significantly higher than that in area F. The fungal community structure in area F remained relatively stable, with Aspergillus consistently maintaining a high relative abundance and showing little variation. Aspergillus is a key fungal genus in Daqu [19], playing a crucial role in producing amylases and other hydrolases that degrade starch and proteins in raw materials. In contrast, in area T, the relative abundance of Aspergillus gradually decreased as the brewing process progressed. This may be attributed to the lower heat tolerance of Aspergillus compared to extreme thermophiles such as Thermomyces and Thermoascus, which reduces its competitive advantage in the environment and consequently leads to a relative decrease in abundance [20]. Meanwhile, in the area T, the abundance of six fungal genera—Rasamsonia, Microascus, Saccharomycopsis, Thermomyces, Thermoascus, and Rhizopus—gradually increased during the production process. In contrast, the abundance of other fungal genera, including Penicillium, Wallemia, Cladosporium, Cercospora, Naganishia, Aureobasidium, Pichia, Starmerella, Kazachstania, Pseudopeyronellaea, and Catenulostroma, decreased as production progressed. During the pit-turning stage in the F area, the fungal community structure underwent significant changes, with Rasamsonia, Microascus, Saccharomycopsis, Thermomyces, Thermoascus, and Rhizopus replacing Aspergillus as the dominant genera. Unlike bacterial communities, the fungal communities in the two areas did not converge after the shutdown. Additionally, the abundance of Trichosporon and Wickerhamiella in the T area increased significantly. The latter can facilitate the formation of alcohols and esters during the fermentation process [21].

3.4. The Impact of Environmental Factors on Microbial Communities

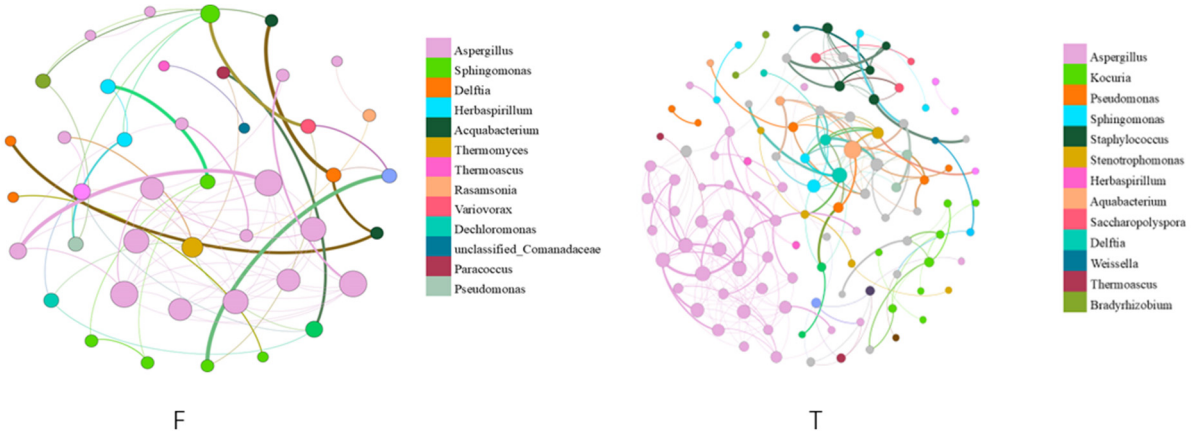

Figure 5. The co-linear network diagram of environmental microorganisms in regions F and T

Network analysis of symbiotic patterns in microbial communities not only aids in interpreting the complex structure of these communities along spatial or temporal gradients but also elucidates potential interactions among microorganisms. In network analysis, closely related microorganisms are clustered into modules [22]. By partitioning these modules and characterizing their key genes, the initial symbiotic network can be simplified into a few

tightly interconnected modules. To evaluate the interactions and symbiotic patterns of microbial communities under changing environmental conditions, a genus-level co-occurrence network analysis was conducted on the environmental microbial communities within the workshop. Additionally, Mantel tests were used to analyze the relationships between each module and various environmental factors, including environmental conditions and production status [23].

As shown in Figure 5, the co-occurrence network of the F area was clustered into 11 modules. Among these, bacteria accounted for 52.37% and fungi for 47.63% of the total nodes. The network comprised 42 nodes and 76 edges. The largest module, Module 9, contained Actinobacteria, representing 40.48% of all nodes. Additionally, fungi predominantly formed a small-world structure between Modules 9 and 10, with connections primarily internal to these modules. Based on this co-occurrence network analysis, Mantel tests were conducted to examine the relationships between microbial species in the F area and environmental factors as well as production conditions. In the T region, all nodes of the co-occurrence network were clustered into 18 modules. The co-occurrence network of environmental microorganisms in this region comprises 116 nodes and 249 edges. Among these, bacteria account for 56.52% of the total nodes, while fungi account for 43.48%. Bacteria exhibit a higher diversity of species and genera but have relatively weak correlations, whereas fungi show fewer species but stronger module associations and correlations. The microbial co-occurrence network diagram reveals that the environmental microorganisms in the F area have weaker correlations compared to those in the T area, which exhibit stronger correlations. This difference may be attributed to the sealed fermentation process of Baijiu within the cellar pools. Except during the stages of entering and exiting the cellar, the interaction between the internal microecology and environmental hygiene is minimal, and external workshop microorganisms do not significantly influence the internal fermentation microecosystem. In the T area, the high-temperature working environment, particularly during steaming grains, results in heat-sensitive strains lacking competitiveness. Additionally, during the spreading and cooling processes as well as yeast addition, the dominant microbial strains from the fermentation system are introduced into the surrounding environment through production activities.

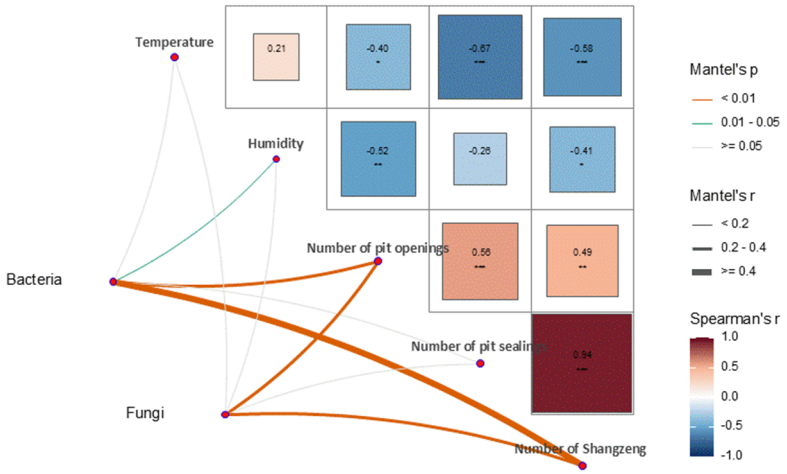

Figure 6. Mantel test of environmental microorganisms and environmental factors within the workshop

Based on the co-occurrence network analysis, we conducted Mantel tests to examine the relationships between environmental bacteria and both environmental factors and production conditions, as shown in Figure 6. Through this analysis, we found that environmental bacteria were significantly correlated with humidity ($r < 0.2$, $0.01 < p < 0.05$), the number of open cellars ($0.2 < r < 0.4$, $p < 0.01$), and the number of steaming operations ($r \geq 0.4$, $p < 0.01$). Specifically, variations in humidity have a notable impact on the distribution of bacteria. Although the correlation coefficient is relatively low ($r < 0.2$), the statistical significance remains substantial ($0.01 < p < 0.05$). The number of open cellars and the frequency of loading stills exhibited stronger correlations with bacterial communities, particularly for the latter, where the correlation coefficient reached above 0.4 ($r \geq 0.4$, $p < 0.01$), indicating a more pronounced impact of these production activities on bacterial distribution. For fungi, significant correlations were also observed with both the number of open cellars ($0.2 < r < 0.4$, $p < 0.01$) and the frequency of loading stills ($0.2 < r < 0.4$, $p < 0.01$). This indicates that the changes in fungal populations are strongly correlated with the production conditions at the time. As shown in the graph, the abundance and diversity of fungi vary significantly with production activities, particularly during the processes of opening cellars and loading stills, when both the activity and diversity of fungi are notably influenced. The sources of bacteria in the environment are not limited to the production process but can also be influenced by external environmental factors, particularly humidity. Humidity, as a critical environmental factor, significantly affects the growth and reproduction of microorganisms. Research has demonstrated that in environments with higher humidity levels, the survival rate and reproduction rate of bacteria tend to increase. Therefore, the correlation between humidity and bacterial abundance is a topic worthy of further investigation. Given the minimal temperature variation during this sampling round, it is currently challenging to establish a clear relationship between environmental microorganisms and seasonal changes. To achieve a more comprehensive understanding of the seasonal dynamics of microbial communities, continuous monitoring is essential, particularly under varying environmental conditions such as temperature and humidity, to observe the dynamic shifts in microbial populations.

By comparing the microbial co-occurrence network diagrams of the two regions, it is evident that the fungal

module in the T region exhibits greater complexity and stronger correlations compared to that in the F region. This phenomenon may be attributed to the introduction of Daqu during the mixing process with the mash, which likely introduced additional fungi into the environment, thereby increasing fungal diversity in the T region and enhancing the complexity of interactions among the modules. In contrast, the F region did not undergo this process, resulting in a relatively stable fungal community with a stronger correlation to the number of days since the cellar was opened. This difference highlights the influence of distinct production stages on microbial community structure and provides valuable insights into the mechanisms governing microbial activity during fermentation. In conclusion, through a comprehensive analysis of environmental factors and production conditions, we can gain a clearer understanding of the dynamic patterns of microbial communities under varying conditions. This analysis provides a robust scientific foundation for further optimizing the production process.

4. Summary

During the continuous production process of strong-flavor Baijiu, the richness of environmental microorganisms exhibits an initial increase, followed by a decrease, and eventually stabilizes within a specific range. Specifically, in the early stage of production, as production activities resume and intensify, the number of microorganisms in the environment rapidly increases. This is primarily attributed to the introduction of production equipment, raw materials, and personnel, which introduce a significant influx of microorganisms into the production environment. However, over time, the microbial population gradually decreases and eventually stabilizes at a relatively constant level. To better investigate this phenomenon, the production process was divided into two stages: the resumption period (the 7 days leading up to resumption) and the continuous production period (starting 7 days after resumption). During the resumption period, production activities gradually normalized, while the continuous production period indicated that production had fully stabilized. During this process, bacteria established a dominant presence in the environment.

Studies have demonstrated that the predominant bacteria in the environment primarily include Bacillus, Methylobacterium-Methylorubrum, Sphingomonas, Aquabacterium, and Stenotrophomonas. These bacterial strains exhibit strong adaptability and reproductive capabilities in production environments, enabling them to thrive and play crucial roles under complex conditions. Meanwhile, the dominant fungi primarily include Aspergillus, Thermomyces, and Thermoascus, which also excel in high-temperature and high-humidity production environments. As work and production resume normally, the relative abundance of dominant microbial species in the environment has undergone notable changes. This phenomenon occurs as microorganisms from the liquor fermentation system progressively infiltrate the environment during production, thereby altering its microecological structure. Over time, the microbial communities in the workshop become increasingly similar to those within the fermentation system and achieve greater stability. This change facilitates the reduction of contamination by non-target microorganisms and ensures the stability of fermentation products. Following a production halt, environmental microbial communities gradually revert to their initial state, demonstrating a clear cyclical pattern. By constructing the microbial symbiotic network and conducting Mantel tests, we found that during the production process, the fermentation area (F area) exhibits greater stability compared to the spreading and drying area (T area). The microbial community structure in the fermentation area maintains higher stability during continuous production, likely due to more consistent environmental conditions and more complex and tightly-knit microbial interactions. In contrast, the microbial community in the spreading and drying area (T area) is more susceptible to external environmental factors such as temperature and humidity, resulting in relatively lower stability. In addition, there is a significant correlation between the environmental microorganisms and factors such as environmental humidity, the frequency of cellar openings, and the number of steamer loadings during the production process. For example, in environments with higher humidity, the growth and reproduction rates of microorganisms are accelerated; meanwhile, frequent cellar openings and steamer loadings lead to changes in microbial diversity and abundance.

These conclusions provide critical reference points for production regulation during the resumption period, facilitating the optimization of production processes, enhancement of product quality, and improvement of production efficiency. In summary, through a comprehensive analysis of environmental factors and production conditions, we can gain a clearer understanding of the variation patterns of microbial communities under different conditions, thereby providing a scientific basis for further optimizing production practices. These research findings not only enhance the production quality of strong-flavor Baijiu but also offer valuable insights for the production of other fermented foods.

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