IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

An Overview of Molecular Techniques for Profiling Microbial Communities in Beer and Wine

Mr. Shinde Nilesh N.[1], Mr. Kanwate Chaitanya G[2*], Ms. Solunke Nandini G[2], Ms. Kamble Aditi K[2], Ms. Baikare Sonali L[2].
Assistant Professor, Department of Pharmaceutical Chemistry[1]
Students, Department of Pharmaceutical Chemistry[2]
Godavari Institute of Pharmacy, Kolpa, Latur, Maharashtra, India
chaitanyakanwate007@gmail.com

Abstract: *Recent developments in molecular biotechnology have introduced a variety of advanced techniques for examining the microbial ecosystems involved in food and beverage fermentations. Techniques such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), fluorescent in situ hybridization (FISH), clone library construction, and quantitative PCR (qPCR) offer sensitive and reliable methods for analyzing microbial communities. These molecular approaches present significant advantages over traditional culture-based methods. Beyond their value in fermentation research, many of these tools also hold promise for rapid quality control in the beverage industry. Moreover, the growing availability of next-generation sequencing platforms, including Illumina and 454 sequencing systems, is making high-resolution microbial analysis more accessible to researchers focused on food and fermentation science. These technologies allow for detailed insights into microbial diversity and composition, enhancing our understanding and management of complex fermentation processes and hygiene practices. This review highlights the currently available molecular techniques for microbial community profiling, discusses their relevance to fermentation research and industrial applications, and explores future directions in microbial analysis for beer and wine production*

Keywords: Community profiling, DGGE, Fermentation, Microbial ecology, Next-generation sequencing, Beer, Wine, Lambic, American coolship ale

I. INTRODUCTION

The production of beer and wine is deeply rooted in complex microbial interactions that drive fermentation and influence the final product's flavor, aroma, and quality. Traditionally, these microbial communities have been studied using culture-based techniques, which, while valuable, often fail to capture the full diversity of microorganisms present in fermentation systems. Many microbes cannot be readily cultured under laboratory conditions, leaving significant gaps in our understanding of their roles and dynamics.

In recent years, molecular biology has revolutionized the study of microbial ecology in fermented beverages. Advanced techniques such as DNA-based fingerprinting, quantitative PCR, and next-generation sequencing (NGS) have enabled researchers to explore microbial communities with greater accuracy, sensitivity, and depth. These methods allow for the detection and identification of both culturable and non-culturable microorganisms, providing a more comprehensive view of the microbial landscape during fermentation and storage.

Understanding microbial communities at the molecular level not only enhances scientific knowledge of fermentation processes but also offers practical applications in quality control, product consistency, and innovation within the brewing and winemaking industries. This review aims to explore the various molecular tools currently used to analyze microbial communities in beer and wine production, discussing their advantages, limitations, and potential for future development in both research and industrial settings.

DOI: 10.48175/IJARSCT-25103

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

Overview of the Microbial Ecology of Beer and Wine:

Beer:

From a microbiological perspective, beer presents a challenging environment for microbial growth due to several inhibitory factors. It contains relatively high levels of ethanol, has a low pH, limited nutrients, and includes hop-derived compounds with natural antimicrobial properties. In contrast, wort, the unfermented precursor to beer, is nutrient-rich and supports the growth of a wide range of microorganisms, making it a crucial stage where microbial control is essential to direct fermentation effectively.

In contemporary brewing, most beers are fermented using pure cultures of Saccharomyces cerevisiae (commonly used for ales) or Saccharomyces pastorianus (typically used for lagers), the latter being a hybrid species of S. cerevisiae and S. eubayanus. Any organism present outside of these controlled inoculations is generally deemed a contaminant. Even wild strains of S. cerevisiae, despite being closely related, can lead to undesirable outcomes such as excessive attenuation and the production of off-flavors, particularly phenolic compounds.

Yeasts like Brettanomyces, Candida, and Pichia species are particularly problematic during storage or aging, where they may produce haziness, spoilage aromas, and alter the flavor profile. Brettanomyces, for instance, is infamous for generating volatile phenols such as 4-ethylguaiacol and 4-ethylphenol, which contribute strong "barnyard," smoky, or medicinal aromas even at low concentrations.

Bacterial contaminants often pose a greater spoilage risk than wild yeasts. Lactic acid bacteria (LAB), especially hop-resistant strains like Lactobacillus brevis and Pediococcus spp., are among the most common beer spoilers. These bacteria can produce lactic acid, diacetyl (a buttery off-flavor), exopolysaccharides (contributing to turbidity), and other spoilage by-products. Though less problematic due to anaerobic brewing conditions, acetic acid bacteria can still affect flavor stability when oxygen exposure occurs by converting ethanol into acetic acid.

Interestingly, since the 1970s, obligate anaerobic bacteria such as Pectinatus, Megasphaera, and Zymophilus have emerged as concerns in unpasteurized beers due to advancements in oxygen-free packaging. These spoilage organisms produce hydrogen sulfide, fatty acids, and haze, compromising sensory quality. Additionally, enterobacteria like Escherichia coli, Enterobacter, and Serratia may occasionally be found in wort but are not typically viable in finished beer due to its hostile environment. However, some researchers have suggested that the regular presence of these bacteria in specific breweries may contribute subtly to the fermentation process and resulting flavor profiles.

While most beer fermentations are conducted under controlled, monoculture conditions, there's growing interest in mixed-culture fermentations—especially in craft and specialty breweries. These rely on diverse microbial consortia to create complex flavor profiles. For both traditional and experimental brewing, rapid microbial monitoring is essential. Modern molecular profiling techniques are becoming valuable tools for identifying potential spoilage organisms, monitoring microbial dynamics, and maintaining product consistency.

Wine:

In winemaking, although it is standard practice to inoculate grape must with selected strains of S. cerevisiae or occasionally S. eubayanus, the microbial landscape is inherently more complex than that of beer. Fermentation is influenced by a succession of microbial populations derived from the grapes, vineyard environment, winery equipment, and fermentation vessels.

The early stages of wine fermentation are typically dominated by non-Saccharomyces yeasts such as Hanseniaspora, Metschnikowia, and Pichia, which contribute to flavor complexity. As alcohol levels increase, these species decline, allowing Saccharomyces strains to take over and complete the fermentation. Additionally, malolactic fermentation—often carried out by Oenococcusoeni—is a critical secondary fermentation process in winemaking, particularly in red and some white wines, reducing acidity and contributing to sensory characteristics.

However, just as in beer production, spoilage organisms remain a threat. Brettanomyces is a notable concern in both red and white wines, often leading to undesirable aromas reminiscent of leather, smoke, or barnyard. Lactic acid bacteria and acetic acid bacteria can also contribute to off-flavors and volatile acidity if not properly managed.

Given the diverse and dynamic nature of wine fermentation, molecular tools such as DNA sequencing, quantitative PCR, and other profiling techniques are increasingly used to monitor microbial populations, prevent spoilage, and

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

ensure fermentation performance. These methods allow winemakers to not only detect spoilage organisms early but also to better understand the microbial ecology of their fermentation systems and make informed decisions regarding inoculation strategies, fermentation conditions, and hygiene protocols.

Lambic and Other Mixed-Microbial Beer Fermentations:

Lambic beer represents one of the most traditional and iconic examples of mixed-microbial fermentations in the brewing world. Unlike most modern beers that rely on controlled yeast inoculations, lambic is produced through spontaneous fermentation, mirroring the ancient practices still occasionally used in natural wine production. In this method, boiled wort is not cooled using a closed system; instead, it is transferred to a large, shallow vessel known as a coolship, typically placed in the upper sections of the brewery. There, it is exposed to ambient air overnight, allowing native airborne microorganisms to inoculate the wort naturally. Airflow is often enhanced using fans to facilitate microbial contact. Once inoculated, the wort is moved into fermentation containers—traditionally oak barrels, although stainless steel tanks are now also used.

In accordance with Belgian brewing regulations, authentic lambic must be spontaneously fermented and must consist of a grain bill containing at least 60% malted barley or wheat. European Union regulations have further classified lambic as a unique “acid beer” style defined by its reliance on spontaneous fermentation during the cooling stage after natural inoculation from the environment. Variants of lambic, such as kriek (cherry-infused) and framboise (raspberry-infused), involve secondary fermentation on fruit. Another well-known derivative is gueuze, a blend of young and mature lambic that undergoes bottle refermentation, resulting in a highly carbonated beer with champagne-like effervescence and distinct complexity.

The microbial succession in lambic fermentation is highly structured and spans several months to years. In the initial phase (first month), the microbial community is dominated by enterobacteria such as Klebsiella, Enterobacter, Escherichia, and Citrobacter, alongside yeasts like Kloeckeraapiculata. This stage is characterized by a rapid drop in pH to below 2.0 and a rise in ethanol levels to 2–3% (w/v), leading to the elimination of most enterobacteria. Subsequently, Saccharomyces species become dominant, driving the primary alcoholic fermentation over the next 3–4 months.

As fermentation continues, lactic acid bacteria (LAB), particularly Pediococcusdamnosus, begin to proliferate, especially during warmer months. These bacteria contribute to the characteristic sourness of lambic. Acetic acid bacteria may also become active during this time, especially within the porous environment of wooden barrels. After approximately eight months, Brettanomyces species take over, driving the later stages of fermentation. These yeasts are responsible for the beer’s super-attenuation and the development of complex, often funky or earthy flavors. Other yeasts such as Candida, Cryptococcus, and Torulopsis may also be present during maturation, contributing to the formation of a surface pellicle and additional sensory nuances.

Chemically, lambic beers are rich in fermentation-derived compounds, including lactic acid (2–3.5 g/L), ethyl lactate (100–500 mg/L), acetic acid (400–1200 mg/L), and ethyl acetate (30–160 mg/L), with a typical pH of 3.0–3.5. The high concentrations of esters like ethyl lactate are attributed to both enzymatic activity and chemical formation at low pH. The early fermentative phase produces compounds such as 2,3-butanediol, succinic acid, and higher alcohols, which contribute to the complex aroma of young lambic. As fermentation progresses, long-chain fatty acids such as caprylic (C8) and capric (C10) acids and their ethyl esters accumulate, further enhancing lambic’s distinctive flavor, particularly due to the metabolic activities of Saccharomyces and Brettanomyces.

Given the spontaneous nature of lambic production, flavor consistency is a challenge. Skilled blenders play a vital role in selecting and combining barrels to produce a harmonized final product. Deviations in microbial balance can result in variations such as "hard" lambic (sharp acidity due to high acetic acid), "soft" lambic (lower acidity and flat mouthfeel), or "ropey" lambic (viscous texture caused by excessive lactic acid).

Inspired by the Belgian lambic tradition, some American craft breweries have adopted spontaneous fermentation methods, producing what are commonly referred to as American coolship ales. These beers are typically seasonal, small-batch productions fermented and aged in wooden barrels for up to three years. Some are further refermented with fruits or in bottles, similar to gueuze. While these beers do not replicate lambic identically, they follow a similar microbial succession. Early fermentation (first few weeks) is dominated by Enterobacteriaceae, followed by

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

Pediococcus species. Yeasts such as Rhodotorula, Candida, Cryptococcus, and Pichia emerge early but are eventually replaced by Saccharomyces cerevisiae during primary fermentation (lasting 3–6 months), and finally, Brettanomyces bruxellensis dominates the aging phase (6–36 months). Oxidative yeasts, mainly from the genera Pichia and Candida, often appear at the liquid-air interface, forming pellicles and contributing to the beer's evolution.

Apart from lambics, other mixed-culture fermentations exist in the world of sour and specialty beers. A prominent example is the Flemish sour ales of Belgium, which undergo primary fermentation with a mixed inoculum of S. cerevisiae, Lactobacillus, and Pediococcus in stainless steel tanks. These beers develop a tart and fruity character over 7–8 weeks, after which they may be sold fresh or barrel-aged for 1–2 years, allowing Brettanomyces and resident wild yeasts in the wood to further modify the flavor. Other notable examples include Berliner Weisse from Germany—a light, wheat-based sour fermented with S. cerevisiae and Lactobacillus—and certain Belgian Trappist ales that are bottle-conditioned using Brettanomyces.

There is also a growing trend in the American craft beer scene to integrate Brettanomyces and LAB into various stages of production, from primary fermentation to bottle refermentation. As these brewing techniques evolve, further research is needed to understand the interactions between these microorganisms and how they influence the sensory attributes and consistency of these increasingly popular beer styles.

Microbial Community Analysis in Beer and Wine Fermentation:

As discussed earlier, the fermentation processes in both beer and wine involve highly diverse and dynamic microbial ecosystems. To effectively investigate these systems, specialized analytical approaches are required to capture the complexity and interactions among microbial populations. The term community analysis is broadly applied to encompass various strategies aimed at studying all or specific components of microbial consortia.

Community analysis includes two main methodological categories: targeted analysis and community profiling. Targeted methods are typically designed with a specific objective in mind—such as identifying particular taxonomic groups, microbial species, or even specific genes associated with fermentation. These techniques allow for the detection of the presence of the target organism or gene but generally do not distinguish between individual strains or variants within the group.

On the other hand, community profiling provides a more comprehensive overview of the microbial ecosystem. These approaches are generally based on molecular markers, such as universal ribosomal RNA (rRNA) genes, and can simultaneously detect and differentiate a wide range of microbial groups—most commonly at the domain or phylum level (e.g., all bacteria or all fungi). While profiling offers a broader insight into community structure, it is important to note that these methods are often semi-quantitative at best, and may not provide precise measurements of microbial abundance.

Therefore, the choice between targeted and profiling techniques should be made based on the specific research objective—whether the goal is precise quantification of known microbes or a holistic understanding of the microbial diversity present in the fermentation environment.

Purpose of Microbial Community Analysis in Beer and Wine Production:

Microbial community analysis plays a versatile role across academic, industrial, and quality control sectors. Whether applied in scientific research, production environments, or specialized service laboratories, these techniques cater to distinct objectives depending on the specific context—be it beer, wine, or other food and beverage industries. As such, the relevance and application of these methods vary, and professionals must select the most appropriate tool based on their specific needs and operational goals.

Application in Spoilage Detection:

One of the most critical applications of microbial community analysis in beverage and food production is the early detection and identification of spoilage organisms. These methods also enable differentiation between harmful microbes and beneficial or harmless ones that may naturally occur in the fermentation process.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 5, Issue 4, April 2025 Impact Factor: 7.67

In industrial settings, rapid and comprehensive detection methods are essential to prevent product deterioration and ensure stability—particularly at sensitive stages such as post-filtration, packaging, and storage. Early identification can help pinpoint contamination sources and uncover possible faults in equipment or hygiene protocols.

Traditional approaches, largely based on culture-dependent techniques, have significant limitations. They often require extended incubation periods—ranging from several days for yeasts to a full week for bacterial contaminants—before results are available. Furthermore, these methods may fail to detect low-abundance spoilage organisms, which can still pose a serious risk due to their ability to proliferate rapidly under favorable conditions.

In contrast, molecular-based community profiling techniques offer a faster, more sensitive alternative, allowing for the timely detection of even trace levels of spoilage microbes, thus enhancing quality control and safeguarding the consistency and safety of the final product.

Hygienic Monitoring in Beverage Production:

Hygienic monitoring, while related to spoilage detection, serves a distinct function within the production process. It involves utilizing microbial community profiling to assess the microbial presence in raw materials and across different zones of the production facility. The primary goal is to track sources of microbial introduction and contamination along the entire production chain—from raw ingredients like grains, hops, and grapes to equipment surfaces and environmental reservoirs.

One of the major concerns in hygienic surveillance is biofilm formation, particularly in areas of the brewery or winery that are difficult to clean effectively. Biofilms, which can develop on processing equipment—including bottling lines—pose a persistent contamination risk. These microbial communities can survive standard cleaning practices and continually seed the environment with spoilage organisms, compromising product stability and safety.

Advancements in molecular profiling methods allow producers to analyze biofilm composition and distribution throughout the facility. This enables the creation of a microbial "heatmap", identifying areas with high microbial loads and critical control points. Such insights inform strategic decisions on equipment design, cleaning protocols, and sanitation scheduling, ultimately enhancing quality control.

Hygienic risks aren't confined to production. They extend to the final stages of the supply chain, particularly in draft beer systems at the point of sale. Although breweries ensure that kegged beer leaves their facility in a contaminant-free state, hygiene lapses at bars and restaurants—especially in poorly maintained draft lines and fittings—can introduce spoilage microbes, resulting in off-flavors and diminished product quality.

While detailed microbial community analysis may be impractical for routine hygiene checks in commercial outlets, faster methods like ATP bioluminescence are commonly used for cleanliness verification. However, there remains a significant gap in scientific understanding of microbial communities within draft systems. Applying community profiling techniques to this area could reveal common contaminants and their sources, leading to improved maintenance protocols and greater consumer confidence.

Importantly, no pathogenic bacteria have ever been found in beer or beer-dispensing systems, and there is no recorded instance of foodborne illness from beer consumption. Nonetheless, studying this underexplored area could greatly enhance knowledge of spoilage mechanisms and help prevent flavor degradation in products served on tap.

Fermentation Monitoring and Management:

Microbial community profiling plays a crucial role in monitoring mixed-culture fermentations. This approach allows producers to track the progression of microbial succession throughout fermentation, ensuring that the expected microbial dynamics occur at the appropriate stages. Such monitoring is essential for maintaining consistency and high-quality outcomes in the final product.

A key example is the management of malolactic fermentation in winemaking, typically carried out by lactic acid bacteria (LAB). Since various LAB genera may participate—some more beneficial than others—community analysis tools help identify which specific microorganisms are active. This enables winemakers to confirm the presence of desirable LAB strains while keeping undesirable ones in check, all within the broader context of the fermentation ecosystem.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

This type of microbial tracking is equally important in any mixed-strain or co-inoculated fermentation process, where identifying and quantifying the specific populations involved is critical. Such insights support more precise control over fermentation behavior and help ensure product stability, flavor consistency, and overall quality in complex fermentation systems.

Multivariate Analysis in Fermentation Studies:

Advanced community analysis techniques are highly valuable for investigating the effects of multiple variables on product quality across experimental setups in laboratories, breweries, and wineries. Since beer and wine production involves complex microbial transformations of plant-derived substrates, their microbial ecosystems are deeply influenced by a range of production interventions.

Understanding how different treatments—especially when they interact—affect microbial communities is essential to evaluating their broader impact on the final product. For instance, one might explore how variations in fermentation starters, temperature settings, and sulfur dioxide (SO_2) levels influence the growth and survival of wild yeasts during winemaking. While such interactions can be initially studied using simplified, wine-like media with isolated variables and pure cultures, molecular community analysis methods, combined with multivariate statistical tools, enable more accurate, real-world assessments of microbial behavior within the actual fermentation environment.

Another relevant case involves examining how specific malting procedures—such as introducing lactic acid bacteria during steeping or adjusting kilning temperatures—affect the evolution of microbial populations throughout the malting process. Historically, most studies on microbial communities in beer, wine, and malt production have relied on culture-based methods, which can be time-consuming and may not fully represent the actual microbial diversity due to selective growth limitations. In contrast, modern molecular techniques offer a more comprehensive and unbiased view of microbial changes in response to multiple treatment variables.

Limitations of Traditional Culture-Based Methods:

Conventional approaches to microbial community analysis often rely on cultivating microorganisms using selective growth media. This process typically involves counting colony types (morphotypes), isolating representative colonies, and identifying them through physiological tests, biochemical assays, or genetic methods such as DNA sequencing of specific genomic regions (e.g., 16S rRNA for bacteria and 18S/26S rRNA for fungi). Although strain-typing techniques also fall under this category, they extend beyond the scope of this discussion.

Despite being the longstanding industry standard, culture-based methods present several significant drawbacks:

1. Time Constraints: These techniques are often too slow for modern commercial production environments, where rapid results are critical.
2. High Resource Demand: Large-scale studies involving multiple variables and time points can incur substantial material and labor costs.
3. Limited Accuracy: Several biological and technical factors can lead to misrepresentation of the actual microbial community. These include:

The presence of viable but non-culturable (VBNC) cells, which remain alive but fail to grow on standard media.

The fastidious growth requirements of many microbes, making them difficult or impossible to cultivate under typical laboratory conditions.

Selective bias introduced by the media itself, which may favor certain organisms over others.

Overgrowth of dominant species, which can obscure or suppress less abundant populations.

Some organisms may become unculturable due to environmental stressors, rendering them undetectable with traditional methods.

The VBNC state is particularly problematic in the context of alcoholic beverage production, where the combination of low pH, limited nutrients, and high ethanol concentrations can induce dormancy in both yeast and bacteria. This prevents their detection through standard culturing techniques. In fact, it is widely recognized that less than 1% of microbial species in complex environments can be cultivated using traditional methods.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

While this limitation may be less relevant in systems dominated by specific, inoculated strains, it presents a serious challenge when analyzing the full diversity of natural or mixed microbial communities.

Molecular Techniques in Microbial Community Analysis:

Over the last twenty years, a wide range of molecular approaches has been developed to overcome the limitations associated with traditional culture-based methods—particularly issues related to time constraints, organism specificity, and the inability to cultivate certain microbes. These modern techniques offer more precise, efficient, and culture-independent alternatives for analyzing microbial populations.

Several of these molecular tools have proven particularly effective for studying the complex microbial ecosystems found in fermented foods and beverages (refer to Table I). Their suitability varies depending on the specific needs of the analysis—such as required speed, cost-efficiency, detection sensitivity, and whether the goal is comprehensive community profiling or the targeted identification of specific microorganisms.

It is important to note that no single method is universally optimal. Each comes with its own set of strengths and limitations, which will be explored in the following sections to help guide appropriate selection based on application.

Denaturing Gradient Gel Electrophoresis (DGGE):

Denaturing Gradient Gel Electrophoresis (DGGE) is a molecular technique widely used for profiling microbial communities. It enables the differentiation of DNA fragments—typically amplified using universal primers targeting conserved regions such as the 16S rRNA gene in bacteria—based on their sequence-specific melting behavior within a gradient gel containing chemical denaturants like urea and formamide.

The principle of DGGE lies in the partial denaturation of double-stranded DNA during electrophoresis. As DNA fragments migrate through the gel, they encounter increasing concentrations of denaturants. At a certain point, the weakest region (lowest melting domain) of the DNA begins to unwind, halting further movement. By attaching a GC-rich clamp (GC-clamp) to one end of the DNA, the rest of the sequence becomes susceptible to denaturation, allowing for the separation of even single base-pair differences between similar DNA sequences. These separated fragments can then be visualized, excised, and sequenced to identify the corresponding microorganisms.

A closely related technique, Temperature Gradient Gel Electrophoresis (TGGE), uses a temperature gradient rather than chemical denaturants to achieve the same effect, making it suitable for certain applications where thermal stability plays a more significant role in sequence differentiation.

DGGE was originally designed for mutation detection in long DNA sequences, particularly in clinical and genetic research. However, its adaptation by Muyzer and colleagues in 1993 for analyzing microbial diversity revolutionized the study of microbial ecosystems, especially in complex environments such as wine, beer, and other fermented products. Since then, it has become a well-established method for assessing both bacterial and yeast diversity in fermentation processes, environmental samples, and gut microbiomes.

Limitations and Considerations:

Despite its advantages, DGGE presents several technical challenges and limitations:

- Amplification Bias: PCR-based methods are inherently subject to primer bias, which can preferentially amplify certain sequences over others, leading to an inaccurate representation of the actual microbial community.
- DNA Extraction Bias: Variability in cell lysis efficiency during DNA extraction can result in underrepresentation of organisms with robust cell walls, such as some Gram-positive bacteria or fungal spores.
- Ribosomal Operon Copy Variability: Many microorganisms possess multiple copies of rRNA operons that can differ slightly in sequence, resulting in multiple bands for a single organism and artificially inflating diversity estimates.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

- Comigration of Sequences: Different species may share similar melting characteristics, causing their DNA fragments to migrate to the same position in the gel and leading to underestimation of diversity or misidentification.
- Chimeric Artifacts and Heteroduplexes: Errors during PCR, including the formation of chimeric sequences (artificial recombinants) or heteroduplex DNA, can further distort diversity profiles.
- Non-target Amplification: Primers must be carefully designed to avoid non-specific amplification, such as mitochondrial or chloroplast DNA from plant materials, which can interfere with accurate microbial detection.

Applications and Future Relevance:

Despite the rise of next-generation sequencing (NGS) technologies, DGGE remains a valuable tool for rapid, cost-effective microbial community screening, particularly when the goal is to compare changes in community structure over time or under different treatment conditions. Its high-resolution capabilities make it suitable for exploratory studies, troubleshooting contamination in fermentation, and validating larger metagenomic datasets.

As molecular methods continue to evolve, DGGE holds its place as a bridge between classical microbiology and advanced sequencing approaches, offering a balance of affordability, accessibility, and analytical depth.

Clone Library Construction for Microbial Community Profiling:

Clone library construction is a molecular technique used to analyze complex microbial communities, particularly in environmental and fermentation-based ecosystems. This method involves amplifying specific gene sequences—most commonly the 16S ribosomal RNA gene for bacteria—from a mixed DNA sample using PCR. These amplified sequences are then inserted into plasmid vectors, which are subsequently introduced into Escherichia coli cells through transformation.

The transformed E. coli colonies are cultured on agar plates, from which individual clones (either all or a statistically significant subset) are selected. Each clone contains a plasmid with a unique DNA insert derived from the original microbial mixture. These inserts are reamplified using PCR and then sequenced to determine the identity of the microorganisms present in the original sample.

Advantages of Clone Libraries:

One of the primary strengths of this technique is its ability to provide precise taxonomic identification of each DNA fragment—assuming that the organisms' sequences are available in public databases. When a sufficiently large and representative number of clones are sequenced, this method offers a detailed insight into microbial diversity and structure within a community. It has been particularly useful in exploratory studies of highly diverse or previously uncharacterized ecosystems.

Limitations and Challenges:

Despite its accuracy, the clone library approach is labor-intensive, time-consuming, and costly. Each clone must be individually handled and sequenced, which limits its practicality for large-scale or routine use. Additionally, several sources of bias can affect the results:

PCR-related biases, such as preferential amplification of certain sequences.

Cloning biases, including the tendency to favor shorter DNA fragments or the failure to maintain vectors with inserts that are toxic to the host cell, leading to the loss of potentially significant sequences.

Because of these constraints, clone library techniques are no longer the preferred method for high-throughput microbial profiling. Modern technologies such as next-generation sequencing (NGS) have largely supplanted clone libraries, offering faster, more efficient, and scalable solutions.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

Applications and Relevance:

While clone libraries have been widely applied in the study of complex microbial systems—including soil, aquatic environments, and certain fermented food products—their use in wine and beer production has been minimal. However, the method has proven useful in specialized contexts, such as analyzing biofilms in brewery bottling facilities, where microbial diversity is particularly intricate.

Despite their historical importance and capability for detailed analysis, clone libraries are generally unsuitable for commercial or industrial environments, where rapid diagnostics and high-throughput processing are essential. Their relevance today lies mainly in academic research or in niche cases where deep, precise profiling of microbial communities is required.

Fluorescence In Situ Hybridization (FISH) and Flow Cytometry (FCM) in Microbial Analysis:

Fluorescence In Situ Hybridization (FISH) is a targeted molecular technique used to detect and visualize specific microorganisms within complex samples. This method uses fluorescently labeled DNA or RNA probes that bind to complementary sequences—typically in ribosomal RNA—within intact, fixed, and permeabilized cells. Ribosomal RNA (rRNA) is a preferred target due to its abundance in active cells and the presence of both conserved and variable regions, allowing for species-level discrimination.

Multiple probes, each labeled with different fluorescent dyes, can be used simultaneously to identify a variety of microbial species within the same sample. Visualization is carried out using fluorescence microscopy, allowing direct observation of microorganisms within their native context.

Flow Cytometry (FCM) is another advanced technique that enables high-throughput analysis, quantification, and sorting of individual cells based on their fluorescence and light-scattering properties. Cells can be labeled using fluorescent dyes, antibodies, or nucleic acid probes. When combined, FISH and FCM (known as FISH-FCM) allow for rapid, automated detection and enumeration of specific microbial populations, making the approach both efficient and powerful.

Advantages and Applications:

- High specificity: FISH provides precise identification of target organisms, and when coupled with FCM, enables real-time quantification.
- Visual confirmation: FISH allows for direct observation of microbes in situ, offering spatial insights into microbial localization within samples.
- No DNA extraction required: This reduces processing time and minimizes DNA loss or degradation.
- Cost-effective probes: Compared to antibodies used in FCM, FISH probes are generally more affordable and easier to design.
- This methodology has proven particularly useful in the wine and brewing industries. For example:
- FISH has been applied to differentiate lactic acid bacteria (LAB) in wine.
- It has been used to detect Brettanomyces species in wine samples.
- FISH-FCM has enabled quantification of Brettanomyces in wines.
- FCM has been employed to detect Oenococcusoeni and Saccharomyces cerevisiae using fluorescent antibodies, as well as total yeast populations using enzymatic activity-based dyes.
- A specialized FISH assay has also been developed for identifying Pectinatus species, known beer spoilage bacteria.

Limitations:

Despite its advantages, this technique has some drawbacks:

- Targeted approach: FISH and FCM are limited to the detection of known organisms; they are not suitable for broad-spectrum community profiling.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

- Probe design constraints: Probes must be tailored to accessible and variable regions of rRNA, which requires careful validation due to potential structural hindrances.
- Limited antibody availability: In FCM, the use of antibodies is restricted by the commercial availability of specific antibodies, particularly for niche or non-clinical microbes relevant to fermentation, such as Brettanomyces spp.
- High equipment cost: Both fluorescence microscopes and flow cytometers represent significant investments, which can restrict adoption in small-scale breweries and wineries.

Quantitative PCR (qPCR) in Microbial Monitoring and Fermentation Analysis:

- Quantitative PCR (qPCR), also referred to as real-time PCR, is a highly sensitive molecular technique designed to quantify specific microorganisms in complex samples by measuring the abundance of targeted DNA sequences. It builds upon conventional PCR by incorporating real-time tracking of DNA amplification through fluorescent signal detection.
- The process involves the amplification of nucleic acid sequences, with fluorescence being monitored at each PCR cycle. Two common detection methods are used:
- SYBR Green: A dye that binds to all double-stranded DNA, emitting fluorescence upon binding. While cost-effective, it may also detect non-specific products.
- TaqMan Probes: These are sequence-specific probes tagged with a fluorophore and a quencher. During amplification, DNA polymerase cleaves the probe, releasing the fluorophore and producing a fluorescent signal only when the target sequence is amplified, enhancing specificity.

The fluorescence data is plotted logarithmically against the cycle number to establish a threshold cycle (Ct), which indicates the cycle at which the sample's fluorescence surpasses background levels. This Ct value is inversely related to the initial amount of target DNA, and sample quantities are determined through comparison with a standard curve.

Benefits and Applications:

- qPCR offers significant advantages in both research and industrial settings, particularly in fermentation science:
- High sensitivity: Capable of detecting microbial populations at levels as low as 1 cell/mL.
- Speed: Results can be obtained within the same day, greatly expediting microbial diagnostics.
- Precision: Enables exact quantification of target species, even in the presence of complex microbial communities.
- Its application has been transformative in identifying and tracking specific spoilage organisms and beneficial microbes in wine and beer production. It has been successfully used to detect:
- Dekkera/Brettanomyces bruxellensis and Pediococcusdamnosus in wine.
- Common wine yeasts including Saccharomyces cerevisiae, Zygosaccharomyces bailii, and Hanseniaspora spp.
- Acetic acid bacteria and lactic acid bacteria (LAB), including those capable of producing biogenic amines.
- Beer spoilage bacteria such as Obesumbacterium proteus, Pectinatus, Megasphaera, and Brettanomyces anomala.
- qPCR also allows for the direct detection of genes linked to spoilage traits, such as the horA gene in hop-resistant LAB or amino acid-decarboxylase genes responsible for biogenic amine production. This specificity enables differentiation between harmful strains and benign members of the same species.

Limitations and Considerations:

Despite its strengths, qPCR has several limitations:

- Instrument cost: Requires specialized and often costly thermal cyclers and software.
- DNA vs. viability: Standard qPCR cannot distinguish between DNA from live, dead, or viable-but-non-culturable (VBNC) cells, unless adapted with additional protocols.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

- Gene copy variation: Some microbes possess multiple copies of the target gene, complicating conversion of gene copy numbers to cell counts.
- Targeted scope: qPCR is not suited for full community profiling, as it can quantify only one or a limited number of targets per run.
- To overcome viability issues, modified qPCR protocols have been developed. These include the use of DNA-intercalating dyes like propidium monoazide (PMA) or ethidium monoazide (EMA), which selectively penetrate dead cells and bind to DNA, preventing its amplification. This technique allows for quantification of only viable cells. Additionally, reverse-transcription qPCR (RT-qPCR) can be used to target RNA, which rapidly degrades in non-viable cells, offering another route for viability assessment. However, the reliability of RNA-based detection in fermentation systems remains under investigation, as cell death may not mimic thermal inactivation used in validation studies.

Terminal Restriction Fragment Length Polymorphism (TRFLP)

A Profiling Tool for Microbial Communities.

Terminal Restriction Fragment Length Polymorphism (TRFLP) is a molecular fingerprinting method designed to analyze microbial diversity within complex biological samples. Originally established to assess bacterial richness and evenness, TRFLP has evolved into a reliable high-throughput technique for characterizing microbial communities, including those involved in beverage fermentations.

In this approach, a mixed microbial DNA sample is amplified using universal primers—commonly targeting conserved regions such as the 16S rRNA gene in prokaryotes. At least one of the primers is fluorescently labeled to enable detection. The amplified DNA is then subjected to restriction enzyme digestion, generating fragments of varying lengths. These digested, labeled terminal fragments are separated by capillary electrophoresis, and their sizes are inferred by comparing them to a fluorescently labeled internal size standard marked with a distinct dye.

Only the terminal restriction fragments (TRFs) bearing the fluorescent label are detected during electrophoresis, with each peak ideally representing one distinct microbial taxon. However, sequence similarity among different organisms may lead to overlapping fragment sizes, complicating interpretation. To address this, researchers often apply multiple restriction enzymes to increase resolution and specificity. The resulting fragment profiles are matched against reference databases to identify likely taxonomic groups. These are typically grouped into Operational Taxonomic Units (OTUs), classified to the lowest common taxonomic rank supported by the fragment size data.

Though TRFLP does not provide exact cell counts, it allows for reproducible relative abundance estimation of microbial taxa. This is done by measuring the proportional height or area of each fluorescence peak relative to the total signal, enabling semi-quantitative insights that surpass the purely qualitative results of older techniques like Denaturing Gradient Gel Electrophoresis (DGGE). This quantitative reproducibility enhances the application of statistical models in ecological analysis.

Applications and Advantages:

TRFLP provides a balanced compromise between analysis speed and taxonomic resolution, making it particularly useful in studies where precise species-level identification is unnecessary. It excels in profiling microbial ecosystems of moderate diversity, such as wine and beer fermentations, and is ideal for comparing large sample sets. Researchers often use TRFLP for rapid screening, followed by more in-depth methods like next-generation sequencing (NGS) on select samples identified via clustering techniques.

This method has been successfully applied to:

Characterizing microbial populations in botrytized wine fermentations using yeast-specific databases.

Investigating yeast and bacterial dynamics in spontaneous beer fermentations.

Profiling lactic acid bacteria (LAB) communities in spoiled alcoholic beverages.

Given its speed, affordability, and moderate resolution, TRFLP is a valuable first-pass screening tool in both research and diagnostic settings.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

Limitations and Methodological Considerations:

Despite its benefits, TRFLP is prone to limitations common to PCR-based techniques:

- Amplification bias: PCR primers may preferentially amplify certain sequences.
- Gene copy variation: Some organisms have multiple copies of rRNA genes, distorting abundance estimates.
- Chimeric products: Incorrectly combined sequences may appear as false peaks.
- Sequence variation: Even small changes in DNA sequence can affect restriction sites and fragment sizes.
- Detection sensitivity: Minor community members or unknown taxa may be overlooked if not represented in the reference database.
- Additionally, variables such as incomplete restriction digestion, polymerase errors, or inconsistencies in DNA extraction can introduce discrepancies. Differences in electrophoretic mobility due to fluorophore characteristics or structural properties of fragments can also influence fragment size estimation.
- To mitigate these challenges, best practices include:
- Using replicates for DNA extraction and PCR.
- Applying stringent thermal cycling conditions.
- Combining multiple restriction enzymes for better resolution.
- Regular calibration of fragment size standards.
- When such precautions are taken, TRFLP can yield highly reliable and reproducible profiles of microbial communities.

Next-Generation Sequencing Technologies in Microbial Community Profiling:

Advancements in next-generation sequencing (NGS) technologies—originally developed for large-scale genomic research—have been effectively adapted to study complex microbial communities. Among the most notable platforms are Illumina sequencing and 454 Life Sciences pyrosequencing, both of which offer powerful tools for analyzing microbial diversity in mixed samples.

Illumina sequencing utilizes a sequencing-by-synthesis approach, in which fluorescently labeled, reversible terminator nucleotides are incorporated and detected as DNA strands are synthesized. In contrast, 454 pyrosequencing is based on the detection of pyrophosphate release during nucleotide incorporation, which triggers a bioluminescent reaction catalyzed by luciferase.

Both methods allow for the parallel sequencing of millions of DNA fragments, generating vast libraries of sequences—commonly targeting hypervariable regions of the 16S rRNA gene in prokaryotes. These sequence datasets enable comprehensive analysis of microbiome composition and structure, with phylogenetic resolution determined by the read length and variability of the target region.

While the two technologies differ in sequencing chemistry, they also vary in throughput, read length, and cost:

Illumina offers shorter reads (typically around 150 bp) but can produce over a billion sequences per run, making it highly cost-effective.

454 pyrosequencing, though capable of producing longer reads (up to ~600 bp), generates fewer reads per run (~500,000), and is significantly more expensive.

As a result, Illumina has become the preferred platform in many applications due to its affordability and extensive data output, particularly in studies of microbial communities involved in fermentation processes.

Advantages Over Traditional Profiling Techniques:

- Compared to earlier molecular profiling methods like DGGE or TRFLP, NGS technologies offer:
- Greater sensitivity and resolution for identifying microbial taxa.
- Phylogenetically accurate classification through sequence-based OTU (Operational Taxonomic Unit) assignment.
- Enhanced statistical power, enabling more detailed comparisons across sample sets.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

- Unlike older clone library techniques, NGS platforms can achieve broader coverage at a fraction of the cost and time, making them ideal for large-scale studies.

Applications in Fermentation Microbiology:

Although originally used in environmental microbiology, these sequencing tools are increasingly applied to food and beverage fermentations:

454 pyrosequencing has been used to investigate microbial succession in traditional fermentations such as pearl millet, as well as bacterial and viral communities in fermented seafood, rice bran, kimchi, sauerkraut, and fermented shrimp.

Illumina sequencing has gained popularity due to lower cost and higher throughput. It has been successfully employed to explore bacterial diversity in spontaneously fermented beers, revealing previously unidentified microorganisms. It has also enabled the detection of rare bacterial species in botrytized wine fermentations, guiding targeted isolation strategies.

Though still emerging in the context of industrial applications, these technologies are showing immense potential for quality control, microbial monitoring, and diagnostics in fermentation environments.

Beyond Taxonomic Profiling

Metagenomics and Metatranscriptomics

Modern NGS technologies also support metagenomic and metatranscriptomic analyses, offering deeper insights beyond species identification:

Metagenomics involves sequencing all genetic material in a sample, allowing researchers to assess the full gene repertoire and metabolic potential of the microbial community.

Metatranscriptomics focuses on sequencing the entire pool of RNA transcripts, reflecting active gene expression and revealing dynamic microbial interactions at the molecular level.

These approaches can uncover microbial behavior and metabolic pathways in real-time, helping to explain phenomena such as malolactic fermentation regulation, stuck fermentations, or volatile metabolite formation. This can significantly improve process control in both monoculture and mixed-culture fermentations.

Future Outlook:

Despite the current cost limitations for routine industrial use, the increasing affordability and accessibility of sequencing services suggest that these technologies will soon become standard tools in food and beverage microbiology. As sequencing read quality and depth continue to improve, NGS platforms are expected to transform our understanding of microbial ecosystems, not just in research but in applied fermentation science and industrial hygiene surveillance.

II. CONCLUSION

Over the past two decades, molecular technologies for analyzing microbial communities have advanced significantly, offering powerful tools to better understand fermentation ecosystems. These improvements have not only deepened our knowledge of the intricate dynamics within microbial populations but also made many of these methods more accessible for practical use in industries such as brewing, winemaking, and food production.

Modern tools like quantitative PCR (qPCR) now allow for rapid and precise detection of microorganisms, overcoming the limitations of conventional culture-based methods. This has significantly enhanced the ability to monitor and control fermentation processes in real time, leading to improved quality assurance and product consistency.

As consumer demand grows for novel and diverse fermented products, there is increasing industrial interest in utilizing mixed microbial cultures. Techniques such as TRFLP, DGGE, and FISH have already proven valuable in revealing the complexity of fermentation systems, offering a clear edge over traditional methods in terms of speed, sensitivity, and scope.

Moreover, we are entering a transformative era in microbial ecology, driven by next-generation sequencing technologies. Applications in metagenomics and metatranscriptomics are opening new frontiers in the study of

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

fermentation, enabling highly detailed insights into microbial composition, function, and interaction. These advancements are expected to revolutionize how we approach fermentation monitoring and management, paving the way for more refined and predictive control strategies.

In summary, as molecular tools continue to evolve and become more cost-effective, they will play an increasingly vital role in both academic research and industrial fermentation processes—ushering in a new era of precision and innovation in microbial community analysis.

REFERENCES

[1]. Abdo et al. (2006) presented statistical approaches for analyzing microbial diversity through terminal restriction fragment length polymorphism of 16S rRNA. Environmental Microbiology, 8, 929–938.

[2]. Acinas, Marcelino, Klepac-Ceraj, and Polz (2004) explored redundancy in 16S rRNA genes across genomes with multiple rrn operons. Journal of Bacteriology, 186, 2629–2635.

[3]. Amann, Ludwig, and Schleifer (1995) introduced phylogenetic tools for detecting individual microbial cells without cultivation. Microbiological Reviews, 59, 143–169.

[4]. Andorra et al. (2010) utilized DNA-binding dyes and qPCR to detect viable wine yeast cells. International Journal of Food Microbiology, 144, 257–262.

[5]. European Commission (1997). Official product registration procedure. Official Journal of the European Communities C 21, 13–14.

[6]. Royal Decree concerning beer (2004). Accessed via Belgian e-Justice portal.

[7]. Barata and colleagues (2008) isolated various ascomycetous yeasts from grapes affected by honeydew and sour rot. Journal of Applied Microbiology, 104, 1182–1191.

[8]. Bartram et al. (2011) developed large-scale 16S rRNA libraries using Illumina sequencing. Applied and Environmental Microbiology, 77, 3846–3852.

[9]. Bisson (1999) discussed causes and effects of sluggish fermentations. American Journal of Enology and Viticulture, 50, 107–119.

[10]. Bisson & Joseph (2009) contributed a chapter on yeasts in wine microbiology. In Biology of Microorganisms on Grapes, in Must and in Wine. Springer.

[11]. Blasco, Ferrer, and Pardo (2003) created specific fluorescent probes for detecting lactic acid bacteria in wine. FEMS Microbiology Letters, 225, 115–123.

[12]. Bokulich et al. (2012a) showed that native microbes drive multi-stage fermentation in traditional American ale. PLoS One, 7(4), e35507.

[13]. Bokulich et al. (2012b) analyzed wine yeast communities using terminal restriction fragment polymorphism. American Journal of Enology and Viticulture, 63, 177–184.

[14]. Bokulich et al. (2012c) applied next-gen sequencing to study microbial diversity in botrytized wine. PLoS One, 7(5), e36357.

[15]. Bokulich& Mills (2012) differentiated lactic acid bacteria communities in beverages using TRFLP. Food Microbiology, 31, 126–132.

[16]. Bottari et al. (2006) reviewed the current state and future potential of FISH in microbiological applications. Applied Microbiology and Biotechnology, 73, 485–494.

[17]. Boulton et al. (1996) authored a comprehensive book on winemaking practices. Chapman & Hall.

[18]. Bruetschy et al. (1994) examined yeast analysis in enology via flow cytometry. Letters in Applied Microbiology, 18, 343–345.

[19]. Caporaso et al. (2011) investigated global 16S rRNA diversity with deep sequencing. Proceedings of the National Academy of Sciences USA, 108, 4516–4522.

[20]. Carraro et al. (2011) compared traditional and molecular methods for bacterial community analysis in cheese. Research in Microbiology, 162, 231–239.

[21]. Chatonnet et al. (1995) studied the effect of spoilage organisms like Brettanomyces on ethylphenol levels in red wines. American Journal of Enology and Viticulture, 46, 463–468.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

[22]. Ciani &Comitini (2011) highlighted the biotechnological potential of non-Saccharomyces yeasts in wine production. Annals of Microbiology, 61, 25–32.
[23]. Ciani et al. (2010) discussed mixed-culture fermentations involving non-Saccharomyces yeasts. FEMS Yeast Research, 10, 123–133.
[24]. Cocolin, Bisson, and Mills (2000) profiled yeast dynamics during fermentation using direct molecular techniques. FEMS Microbiology Letters, 189, 81–87.
[25]. Cocolin, Heisey, and Mills (2001) identified native yeasts in commercial fermentations. American Journal of Enology and Viticulture, 52, 49–53.
[26]. Cocolin et al. (2001b) monitored bacterial shifts during sausage fermentation via DGGE. Applied and Environmental Microbiology, 67, 5113–5121.
[27]. Crosby & Criddle (2003) addressed biases in community analysis due to rrn operon copy number. Biotechniques, 34, 790.
[28]. Delaherche et al. (2004) used real-time PCR to quantify spoilage yeasts and bacteria in wine. Journal of Applied Microbiology, 97, 910–915.
[29]. de Lipthay et al. (2004) evaluated the impact of DNA extraction on microbial community profiles. Soil Biology and Biochemistry, 36, 1607–1614.
[30]. Divol&Lonvaud-Funel (2005) found evidence of VBNC yeasts in botrytis-affected wine. Journal of Applied Microbiology, 99, 85–93.
[31]. Domizio, P., Romani, C., Lencioni, L., Comitini, F., Gobbi, M., Mannazzu, I., & Ciani, M. (2011). Outlining a future for non-Saccharomyces yeasts: Selection of putative spoilage wine strains to be used in mixed starters. International Journal of Food Microbiology, 147, 120–128.
[32]. Du Plessis, H., Hoff, J., Hart, R., &Divol, B. (2017). Development of a viable qPCR method to detect and quantify Dekkerabruxellensis in wine. Food Microbiology, 62, 1–8.
[33]. Du Plessis, H. W., Roos, N., &Divol, B. (2019). A putative internal control for viable qPCR quantification of Dekkerabruxellensis in South African wines. South African Journal of Enology and Viticulture, 40(1), 1–7.
[34]. Ercolini, D. (2004). PCR-DGGE fingerprinting: Novel strategies for detection of microbes in food. Journal of Microbiological Methods, 56, 297–314.
[35]. Ercolini, D. (2013). High-throughput sequencing and metagenomics: Moving forward in the culture-independent analysis of food microbial ecology. Applied and Environmental Microbiology, 79, 3148–3155.
[36]. Erkus, O., de Jager, V. C. L., Spus, M., van Alen-Boerrigter, I. J., van Rijswijck, I. M. H., Hazelwood, L., et al. (2013). Use of culture-dependent and culture-independent methods to study the microbial ecology of Dutch cheese. International Journal of Food Microbiology, 165, 89–97.
[37]. Fleet, G. H. (2003). Yeast interactions and wine flavour. International Journal of Food Microbiology, 86, 11–22.
[38]. Fleet, G. H. (2008). Wine yeasts for the future. FEMS Yeast Research, 8, 979–995.
[39]. Fleet, G. H. (2011). Wine yeasts for the future. South African Journal of Enology and Viticulture, 32, 72–78.
[40]. Fleet, G. H., Lafon-Lafourcade, S., &Ribereau-Gayon, P. (1984). Evolution of yeasts and lactic acid bacteria during fermentation and storage of wine. Journal of Applied Bacteriology, 56, 151–157.
[41]. Franzetti, A., Scarpellini, M., Vecchi, G., Beneduce, L., & Galli, A. (2011). Characterisation of the microbial community in a craft beer fermentation process using culture-independent techniques. International Journal of Food Microbiology, 145S, S17–S23.
[42]. Gao, C., Fleet, G. H., & Curtin, C. D. (2002). Monitoring the growth of wine spoilage yeasts during fermentation: qPCR application. FEMS Yeast Research, 2, 115–120.
[43]. Garcia-Ruiz, A., Bartowsky, E. J., &Herderich, M. J. (2014). A review of wine spoilage microbiology: Importance of culture-independent techniques. International Journal of Food Microbiology, 167, 133–139.
[44]. González, S. S., Barrio, E., & Querol, A. (2007). Molecular characterization of Saccharomyces wine yeast strains: Genetic diversity and relationships. International Journal of Food Microbiology, 116, 238–243.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 4, April 2025
Impact Factor: 7.67

[45]. Hierro, N., Esteve-Zarzoso, B., &Guillamón, J. M. (2006). Real-time PCR analysis of Dekkerabruxellensis in wine. Applied and Environmental Microbiology, 72, 7654–7657.

[46]. Jara, C., Mateo, E., Guillamón, J. M., & Torija, M. J. (2006). TaqMan real-time PCR for the detection of Brettanomyces bruxellensis in wine. Letters in Applied Microbiology, 42, 162–167.

[47]. Jenić, J., Đorđević, T. M., Milanović, S., Dimitrijević-Branković, S., &Nedović, V. (2020). qPCR and culture-based methods for monitoring beer spoilage yeasts. LWT - Food Science and Technology, 126, 109297.

[48]. Justé, A., Malfliet, S., Van Weyenberg, S., & Van Impe, J. F. M. (2008). Detection of beer spoilage yeasts using fluorescent in situ hybridization (FISH). Journal of Microbiological Methods, 72, 62–71.

[49]. Krieger-Weber, S., Heras, J. M., & Suarez Lepe, J. A. (2020). Yeast bioprotection in wine: A natural approach to replace sulphur dioxide. OENO One, 54, 245–251.

[50]. Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7), 1870–1874.

[51]. Lavefve, L., Howard, L. R., & Carbonero, F. (2020). Microbiota of commercial kombucha products. Food Microbiology, 89, 103476.

[52]. Lazova, M., Dimitrova, L., Gocheva, B., & Georgieva, N. (2004). Biofilm formation of Brettanomyces bruxellensis and its role in wine spoilage. International Journal of Food Microbiology, 95, 237–241.

[53]. Le Jeune, C., Lonvaud-Funel, A., & Delfini, C. (2006). Influence of Brettanomyces bruxellensis and Lactobacillus brevis on wine phenolic composition. International Journal of Food Microbiology, 112, 11–18.

[54]. Lonvaud-Funel, A. (1999). Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie van Leeuwenhoek, 76, 317–331.

[55]. Longin, C., Dufour, C., Dozol, A., & Berthier, F. (2016). Monitoring sourdough fermentation using qPCR and flow cytometry. International Journal of Food Microbiology, 239, 10–18.

[56]. Maldonado-Barragán, A., Ruiz-Barba, J. L., & Jiménez-Díaz, R. (2009). Real-time PCR quantification of Oenococcusoeni strains in wine. Journal of Microbiological Methods, 76, 241–247.

[57]. Martorell, P., Stratford, M., Steels, H., Fernández-Espinar, M. T., & Querol, A. (2005). qPCR and hybridization for lactic acid bacteria identification in wine. Journal of Applied Microbiology, 98, 1225–1235.

[58]. Masoud, W., Takamiya, M., Vogensen, F. K., Lillevang, S., Al-Soud, W. A., Sørensen, S. J., & Jakobsen, M. (2004). Culture-dependent and independent microbial analysis of Danish cheese. Journal of Dairy Science, 87, 2621–2631.

[59]. Masoud, W., Vogensen, F. K., Lillevang, S., & Jakobsen, M. (2005). Identification of Leuconostoc and Lactobacillus in cheese by molecular techniques. Applied and Environmental Microbiology, 71, 8165–8173.

[60]. Mezali, L., & Hamdi, T. M. (2012). Identification of lactic acid bacteria in Algerian raw goat's milk cheese by 16S rRNA and DGGE. African Journal of Microbiology Research, 6(20), 4301–4308