Isolation and identification of yeast strains for beverage production: methods, challenges, and industrial applications

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Abstract. The isolation and identification of yeast strains remain central to the development of diverse and high-quality beverages, ranging from beer and wine to emerging functional drinks. Accurate identification is vital not only for functional characterization but also for quality assurance, spoilage prevention, and strain traceability in commercial applications. Yeasts can be isolated by classical and modern methods from a wide range of natural and industrial environments, including fruits, tree bark, brewery waste, and spontaneous fermentations. Yeast identification through classical microbiological methods, though foundational, is limited in capturing the full diversity of yeasts. Modern molecular and proteomic tools—including ITS and D1/D2 rDNA sequencing, PCR-based genotyping, MALDI-TOF mass spectrometry, and next-generation sequencing—have significantly improved taxonomic resolution, strain discrimination, and community profiling. The review addresses industrial and regulatory considerations, highlighting safety, scalability, and consumer acceptance as critical factors.

1 Introduction

Alcoholic fermentation is a process in which microorganisms convert carbohydrates into ethanol, organic acids, carbon dioxide, and a range of secondary metabolites that contribute to the sensory, nutritional, and functional properties of the final product. Yeasts, particularly *Saccharomyces cerevisiae*, play a central role in alcoholic fermentation [1]. *S. cerevisiae* is the dominant fermenter in beer, wine, and spirits, valued for its efficient sugar conversion, ethanol production, and broad stress tolerance. *S. pastorianus* (a hybrid of *S. cerevisiae* and *S. eubayanus*) are central to lager beer fermentation [2]. Spontaneous fermentation, driven by the natural and often variable microbiota, can lead to fermented beverages with inconsistent quality, composition, and sensory properties, but it can contribute to the uniqueness and terroir of artisanal and craft beverages. Controlled use of selected starters ensures predictable fermentations and consistent product quality [3].

This review aims to provide a comprehensive overview of current methodologies used for the isolation and identification of yeast strains relevant to beverage production.

2 Importance of yeast strain diversity

Yeast diversity plays a fundamental role in shaping the biochemical and beverage fermentations sensory outcomes. While *S. cerevisiae* remains the foundational species for alcoholic beverage production due to its robust fermentation capacity and high ethanol tolerance, numerous non-*Saccharomyces* genera also contribute to flavour complexity, acidity modulation, and functional diversification. Yeasts of particular interest include *Brettanomyces* sp., which is often associated with spontaneous and mixed fermentations; *Pichia* sp., which is frequently found in kombucha and early stages of spontaneous fermentations; *Torulaspora*, which is often used in sequential or co-fermentations with *S. cerevisiae* in wine and cider production and *Hanseniaspora*, which dominate early stages of fermentation and produce high levels of acetate esters, enhancing fruity aromas [4, 5]. Wild or indigenous yeasts — isolated from vineyard soils, brewery environments, or natural substrates often exhibit diverse metabolic pathways and can generate rare or unexpected flavor compounds. However, their use may result in slower or incomplete fermentations and greater batch variability [6].

3 Sources of yeast strains

3.1 Natural habitats

Many yeast strains originate from diverse natural environments where they inhabit sugar-rich niches, offering an extensive reservoir of wild yeasts that can be harnessed for beverage innovation. Ripe fruits and floral nectars, for example, contain high concentrations of simple sugars and serve as primary habitats for *Saccharomyces*, *Hanseniaspora*, *Metschnikowia*, and

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Pichia species [7]. Tree bark, sap, and leaf surfaces—particularly in forest ecosystems—also provide a home for yeasts such as *S. paradoxus*, *Candida* sp., and *Torulaspora* sp. [8]. These strains are often adapted to variable moisture and nutrient availability and may carry unique traits for stress tolerance [9, 10]. Soil ecosystems harbor a remarkable diversity of non-*Saccharomyces* yeasts with promising biotechnological potential, while insects such as fruit flies and wasps serve as natural vectors, dispersing yeasts across habitats and occasionally maintaining symbiotic populations within their gut microbiota. These environments represent an extensive yet underexplored reservoir for isolating strains with unique metabolic traits capable of enhancing the aroma, flavor, and functional attributes of fermented beverages [11, 12].

3.1.1 Traditional beverages and spontaneous fermentations

These systems often show uncontrolled microbial successions of yeasts and bacteria. Such microbial consortia can be found in uncontrolled fermented **wines** (*Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Pichia membranifaciens*), **kombucha** (*Brettanomyces bruxellensis*, *Zygosaccharomyces bailii*, *S. cerevisiae*.), **fermented sap beverages** (*S. cerevisiae*, *Pichia kudriavzevii*, *Candida tropicalis*, *Debaryomyces hansenii*), **chicha and cauim** (*S. cerevisiae*, *Candida parapsilosis*, *Torulaspora delbrueckii*, *Issatchenkia orientalis*), **tej** (*Saccharomyces sp.*, *Dekkera anomala*, *Kluyveromyces marxianus*, *Candida lusitaniae*), **boza** (*S. cerevisiae*, *Candida krusei* (*Issatchenkia orientalis*), *Kazachstania exigua*), **ogi and kunu** (*S. cerevisiae*, *P. kudriavzevii*, *Candida stellata*, *Wickerhamomyces anomalus*); **Cheonggukjang and Makgeolli** (*S. cerevisiae*, *Saccharomycopsis fibuligera*, *Pichia anomala*); **Brem** (*S. cerevisiae*, *Candida glabrata*, *Endomycopsis fibuligera*) [4, 13, 14]. Traditional fermented beverages are valuable sources of wild yeasts with traits such as ethanol tolerance, aroma-forming capacity, stress resistance, and useful enzymatic activities [15, 16].

3.1.2 Industrial environments

Industrial fermentation environments—such as breweries and wineries —serve as reservoirs of both commercial and indigenous yeast strains [17, 18]. Wild yeasts such as *Brettanomyces bruxellensis*, *Torulaspora delbrueckii*, and *Pichia* spp. are frequently detected in breweries, particularly in barrel-aging facilities, where they can persist and enhance the complexity of mixed-culture beers. Wineries often develop their own "house microbiota," that may include both inoculated *S. cerevisiae* strains and naturally occurring yeasts like *Hanseniaspora uvarum* and *Candida zemplinina* [19].

4 Isolation techniques

Effective isolation of yeast strains is crucial for characterizing microbial diversity and identifying functionally relevant strains for beverage fermentation. Isolation strategies should be tailored to the source material and desired yeast traits, with particular attention to recovering non-*Saccharomyces* species that may be underrepresented in competitive environments. Selective and differential media are employed to isolate yeasts from complex microbial communities, especially when bacteria or filamentous fungi are present [15, 20, 21].

5 Identification and characterization

Accurate identification and functional characterization of yeast strains are essential for their application in beverage fermentation. While traditional phenotypic methods provide preliminary insights, molecular techniques such as MALDI-TOF MS offer high-throughput, cost-effective species identification. Functional characterization, including stress tolerance and metabolic profiling, is crucial for selecting strains with desirable traits for fermentation processes [22].

5.1 Focus on molecular-genetic methods

5.1.1 DNA barcoding

DNA barcoding is widely used for yeast species identification, based on conserved and variable regions in ribosomal DNA. **Internal Transcribed Spacer (ITS)** - The ITS1-5.8S-ITS2 region is the official fungal barcode and provides good resolution at the species level. ITS sequencing is a reliable method for rapid identification and phylogenetic placement of yeasts. **D1/D2 Domain of 26S rRNA** is the sequencing of the D1/D2 domain of the large subunit (LSU) rRNA gene is also commonly used for yeast taxonomy [23, 24].

5.1.2 PCR-based techniques

PCR fingerprinting techniques provide species or strain-level discrimination based on DNA polymorphisms. **Random Amplified Polymorphic DNA (RAPD)** and **Amplified Fragment Length Polymorphism (AFLP)** are used for genotyping yeasts from the same species or for population studies. **Restriction Fragment Length Polymorphism (RFLP)** of ITS or rDNA regions provides a rapid and cost-effective method to distinguish between common yeast species without sequencing. **Interdelta PCR** is a technique that targets interdelta sequences specific to *S. cerevisiae* and is used for strain differentiation, particularly in industrial or ecological studies [25, 26].

5.2 Quantitative PCR (qPCR) and Real-Time Detection

qPCR allows specific and sensitive detection of yeast species or strains using fluorescent probes or intercalating dyes. Its applications include monitoring population dynamics during fermentation, rapid

detection of spoilage yeasts (e.g., *Brettanomyces*) and strain tracking in inoculated fermentations [27, 28].

5.2.1 Next-Generation Sequencing (NGS) and Metagenomics

NGS technologies have revolutionized the study of microbial communities in spontaneous and mixed-culture fermentations. **Amplicon-Based Sequencing** uses high-throughput sequencing of ITS or 26S rDNA amplicons enables profiling of yeast diversity in complex matrices without the need for culturing [29]. **Shotgun Metagenomics** is an approach that sequences all genetic material in a sample and allows functional analysis of metabolic pathways and interactions between yeast and other microbes [30]. **Strain-Level Resolution**- new bioinformatics tools such as StrainPhlAn and MetaPhlAn3 can now provide strain-level profiling from metagenomic data [31].

5.3 Focus on biochemical and proteomic tools

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a proteomic tool increasingly used in yeast identification. This technique profiles the peptide mass fingerprint of ribosomal proteins extracted from intact yeast cells. It allows rapid, cost-effective species-level identification within minutes and is widely adopted in clinical and food microbiology. Other biochemical techniques, including **Fourier-transform infrared spectroscopy (FTIR)** and flow cytometry, are also being explored for high-throughput yeast phenotyping and viability assessment in fermentation systems [32, 33].

6 Functional characterization for beverage applications

6.1 Fermentation efficiency and kinetics

One of the primary screening criteria for yeasts used in beverage production is their ability to efficiently convert sugars into ethanol and secondary metabolites under production conditions. Key performance indicators include fermentation rate, sugar utilization profile, ethanol yield, and degree of attenuation. While *S. cerevisiae* strains completely and rapidly utilize substrate sugars, non-*Saccharomyces* species often exhibit slower fermentation kinetics but can contribute beneficially in co-cultures by modulating early-stage fermentation dynamics. High-throughput micro-fermentation assays and automated fermentation analyzers (e.g., Bioscreen C, FermLab) are frequently employed to compare strain performance under controlled conditions [34, 35].

6.2 Flavor and aroma compound production

Yeasts contribute significantly beverages sensory profile by producing volatile compounds such as esters, higher alcohols, organic acids, and sulfur-containing compounds. Although *Saccharomyces* yeast are commonly used for beverage fermentation, some non-*Saccharomyces* yeasts can produce volatile compounds that contribute to fruity, floral or spicy aromas and to diversify beverage flavor profile. Analytical techniques like gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O) are commonly used to quantify and characterize aroma profiles in fermentation samples. Gene expression studies focusing on flavor-associated genes, such as ATF1 and BAT1, are being explored as biomarkers for yeast flavor potential using transcriptomics [36, 37].

6.3 Stress tolerance (alcohol, pH, temperature)

A strain's ability to thrive under fermentation stressors—such as high ethanol concentrations, low pH, osmotic pressure, and temperature extremes—is critical for industrial scalability and process robustness. *S. cerevisiae* shows high ethanol tolerance (up to 16–18% v/v), but many non-*Saccharomyces* strains are inhibited at lower concentrations. Thermotolerance is relevant for fermentations in warm climates or when using non-refrigerated conditions. Strains that maintain membrane integrity and redox balance under acidic pH are desirable [13]. Stress assays using synthetic media, spot tests, and viability staining (e.g., propidium iodide, methylene blue) allow comparative screening [17, 38].

6.4 Antimicrobial activity or spoilage potential

While most yeasts are non-pathogenic, certain species may produce metabolites that inhibit spoilage microorganisms or conversely act as spoilers themselves. Some yeasts, notably *Metschnikowia* and *Candida* species, produce pulcherrimin or killer toxins with antimicrobial activity, which can be advantageous in natural fermentations. Conversely, spoilage yeasts like *Brettanomyces* sp., *Zygosaccharomyces* sp, and *Pichia* sp. can cause undesirable turbidity, gas production, or off-flavors in packaged beverages. Killer toxin activity can be tested using overlay assays or co-culture inhibition experiments, while spoilage potential is evaluated by challenge testing in model beverage systems [39, 40].

6.5 Co-culturing potential

Mixed fermentations involving *Saccharomyces* and non-*Saccharomyces* yeasts are gaining attention for their ability to enhance complexity, reduce ethanol levels, and modulate mouthfeel. Sequential or co-inoculation strategies are employed to harness the early metabolic activity of non-*Saccharomyces* yeasts, followed by domination or stabilization by *S. cerevisiae*. Successful co-culturing depends on strain compatibility, nutrient competition, oxygen tolerance, and killer activity [41]. Monitoring population dynamics in co-cultures often involves qPCR with species-specific primers or flow cytometry with fluorescent labeling [42].

7 Applications in specific beverage types

Yeasts play diverse and critical roles across the spectrum of fermented beverages, influencing not only ethanol production but also the development of complex flavor and aroma profiles. Increasing interest in non-*Saccharomyces* yeasts, strain diversification, and region-specific microbial communities has broadened the functional landscape of yeasts in beverages, from alcoholic drinks such as beer, wine, and spirits to non-alcoholic fermented products.

7.1 Beer: Use of non-Saccharomyces for flavor diversity and low-alcohol beer

In beer production, *S. cerevisiae* and *S. pastorianus* have traditionally dominated, selected for their efficient maltose/maltotriose utilization and robust ethanol production. However, the craft brewing industry has embraced non-*Saccharomyces* yeasts to introduce novel flavor profiles, reduce alcohol content, or produce sour and mixed-fermentation styles. Species such as *Torulaspora delbrueckii*, *Hanseniaspora uvarum*, and *Lachancea thermotolerans* contribute fruity, floral, or lactic notes Additionally, yeasts like *Saccharomycodes ludwigii* and *Metschnikowia pulcherrima*, which cannot metabolize maltose, have been applied in the production of naturally low- or non-alcoholic beers [35, 41, 43].

7.2 Wine: Spontaneous fermentation and terroir expression

The expression of regional terroir—defined as the microbial signature of a vineyard—is increasingly linked to native yeast diversity [44]. Spontaneous fermentations begin with a diversity of non-*Saccharomyces* species (e.g., *Hanseniaspora* sp., *Metschnikowia* sp., *Candida* sp., *Pichia* sp.), eventually dominated by *S. cerevisiae* as alcohol levels rise [45]. These early yeasts shape wine complexity by producing volatile compounds and modulating fermentation dynamics. For example, *Lachancea thermotolerans* produces lactic acid, contributing to freshness and acidity, while *Torulaspora delbrueckii* enhances aroma and reduces volatile acidity [46, 47]. Commercialization of selected non-*Saccharomyces* starter cultures has allowed winemakers to manage spontaneous-like fermentations while reducing risk [48].

7.3 Spirits: Influence on pre-distillation fermentation

In the production of distilled beverages (e.g., rum, whisky, tequila), yeasts primarily influence flavor development during the pre-distillation fermentation phase. Although *S. cerevisiae* is the main fermentative species due to its ethanol tolerance and sugar metabolism, the presence of non-*Saccharomyces* species has been noted in traditional and artisanal fermentations [49]. Studies in mezcal and rum fermentations have identified *Kluyveromyces marxianus*, *Pichia kudriavzevii*, and *Hanseniaspora* sp. as key contributors to ester and higher alcohol production [50].

7.4 Non-alcoholic beverages: kombucha, kefir, and fermented juices

In the expanding sector of functional and fermented non-alcoholic beverages, yeasts contribute to carbonation, flavor complexity, and symbiotic interactions with bacteria [51]. Kombucha, a fermented tea, involves a complex community of acetic acid bacteria and yeasts such as *Brettanomyces*, *Zygosaccharomyces*, and *Pichia*. These yeasts convert sugars into ethanol, which is subsequently oxidized to acetic acid by bacteria, resulting in a tangy, effervescent beverage [52]. Water kefir and milk kefir grains contain mixed microbial consortia, including *Saccharomyces*, *Kluyveromyces*, and *Candida* species, which contribute to lactic acid, CO_2, and aroma formation [53]. Fermented fruit juices also rely on wild or selected yeast strains for controlled sugar reduction, improved shelf life, and enhancement of bioactive compounds [54].

8 Current challenges and future directions

8.1 Safety and GRAS status of isolated strains

Before a newly isolated yeast strain can be used to develop starter cultures for fermented beverages, it must undergo comprehensive taxonomic identification, molecular analyses, and functional characterization to evaluate its technological suitability, safety, and contribution to desirable fermentation attributes. Only after meeting these criteria the strain can be confidently incorporated into starter culture formulations [55]. In the U.S., the Generally Recognized as Safe (GRAS) designation by the Food and Drug Administration (FDA) is a critical regulatory milestone. A microorganism may be granted GRAS status either through scientific procedures or based on a long history of safe use in food [56].

8.2 Limitations in culture-dependent methods

Traditional culture-dependent approaches remain the cornerstone of yeast isolation, but they suffer from critical drawbacks. These methods are inherently biased toward fast-growing and easily culturable species, often underrepresenting the true microbial diversity in natural or fermented ecosystems [57]. Many non-*Saccharomyces* species are either slow-growing, have specific nutritional requirements, or are outcompeted by dominant *Saccharomyces* strains under standard culture conditions [1]. Distinguishing morphologically similar strains often requires labor-intensive physiological assays or molecular tools, complicating the screening of large environmental samples [58].

8.3 Need for high-throughput screening

The functional characterization of isolated yeasts—particularly for traits such as flavor compound production, stress tolerance, and fermentation kinetics—remains a bottleneck. High-throughput screening systems that combine miniaturized fermentation platforms with analytical tools (e.g., GC-MS, LC-MS, biosensors) are urgently needed to accelerate strain selection for beverage applications [59]. Advances in lab automation, microfluidics, and machine learning are making it increasingly feasible to screen hundreds or thousands of yeast isolates under varying fermentation conditions. These tools can help link genotype to phenotype more efficiently and enable rapid identification of high-performing or niche-specific strains [60].

8.4 Synthetic biology and metabolic engineering of yeasts

The application of synthetic biology and metabolic engineering is transforming the way yeast strains are designed for beverage production. Genetically modified yeasts have been developed to reduce the formation of undesirable metabolites (e.g., hydrogen sulfide, acetaldehyde), to overproduce flavor-enhancing compounds, and to ferment alternative or complex sugars more efficiently—an important trait in the production of low-alcohol or functional beverages [61]. Furthermore, some engineered strains are designed to express heterologous enzymes or biosynthetic pathways that are not naturally present in yeasts, enabling the synthesis of novel compounds or the fortification of beverages with health-promoting molecules as vitamins, antioxidants, or terpenoids [62]. CRISPR/Cas9-based genome editing tools allow for precise modifications in industrial strains, while synthetic promoter libraries and modular pathway engineering enable the fine-tuning of metabolic flux [63].

8.5 Microbiome-based design of fermentation consortia

A promising direction for future research is the microbiome-informed design of mixed fermentation consortia. Increasing evidence suggests that microbial interactions such as mutualism, commensalism, and competitive exclusion play a crucial role in shaping fermentation outcomes, particularly in spontaneously fermented beverages like kombucha, lambic beer, and natural wines [44, 48, 64]. Integrating culture-independent methods such as metagenomics, metatranscriptomics, and metabolomics can help unravel the dynamics and functional roles of different yeast and bacterial species within these communities. This knowledge can guide the rational assembly of defined consortia with improved sensory profiles, fermentation robustness, and safety [64].

8.6 Scaling up from lab to industrial fermentation

Scaling yeast fermentation from laboratory to industrial scale presents several challenges, including maintaining consistent fermentation performance, product quality, and microbial stability. Critical parameters such as oxygenation, temperature control, mixing, and nutrient availability must be optimized for large-scale performance. Additionally, strain robustness—tolerance to ethanol, acid, and osmotic stress—must be validated under industrial conditions to ensure consistent yields and sensory attributes. Starter culture propagation methods must also be adapted for commercial operations, ensuring cell viability and vitality over time [65].

9 Conclusion

Yeasts are central to beverage fermentation, shaping both the biochemical and sensory qualities of alcoholic and non-alcoholic products. Beyond classical *S. cerevisiae*, diverse non-*Saccharomyces* genera offer traits such as ethanol tolerance, aroma production, stress resilience, and sugar utilization. Effective exploitation of this diversity relies on robust isolation and identification methods. Advanced molecular and proteomic approaches, together with functional screening and high-throughput fermentation assays, allow precise selection of strains for specific applications. Microbial biodiversity provides a rich source of strains that enhance flavor, functionality, and sustainability, including low-energy fermentations and valorization of alternative substrates. Integrating microbial ecology, synthetic biology, and systems-level analytics opens opportunities for next-generation yeast strains and consortia tailored to evolving beverage industry needs, while ensuring safety, regulatory compliance, and scalability.

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