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Isolation and Molecular Characterization of Indigenous Yeast Strains from the Remote Satpura Forests in Narmadapuram District, Madhya Pradesh

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ABSTRACT: This study investigates the isolation and molecular identification of indigenous yeast strains from remote regions of Narmadapuram district of Madhya Pradesh, India, specifically from the bark and fruits of the Mahua tree (*Madhuca longifolia*). Employing classical microbiological techniques and molecular characterization, we aimed to uncover the biodiversity of yeast in this region and explore their potential applications in fermentation processes. Samples were collected from the Satpura forests and processed using a serial dilution method to isolate pure cultures of yeast. The isolated strains were cultivated on potato dextrose agar (PDA) and subsequently characterized using polymerase chain reaction (PCR) to amplify the internal transcribed spacer (ITS) region of their DNA. We successfully identified various yeast species, with a particular emphasis on *Saccharomyces cerevisiae*, known for its essential role in fermentation. The methodology included Sanger sequencing to confirm species identification, aligned with the NCBI database for accuracy. Our findings revealed a diverse range of yeast species, including *Hanseniaspora thailandica*, *Hanseniaspora opuntiae*, and *Pseudozyma antarctica*, each contributing unique characteristics valuable in fermentation processes. This study not only enhances our understanding of the microbial diversity in Madhya Pradesh but also opens avenues for harnessing indigenous strains in the production of high-quality fermented foods and beverages. Future research should focus on the specific fermentation properties of these strains to optimize traditional practices and promote sustainability within the fermentation industry.

Keywords: Indigenous yeast strains, fermentation, molecular identification, Madhya Pradesh, *Saccharomyces cerevisiae*, PCR.

INTRODUCTION

The quality of fermented foods and beverages is largely determined by the diverse types of microorganisms involved in their production. Fermentation, a complex ecological process, transforms grapes into wine with the help of various bacteria and microorganisms, particularly yeast (Liu *et al.*, 2019). The diversity of *Saccharomyces cerevisiae* strains significantly influences the chemical composition and sensory properties of the resulting alcohol. In pure fermentation, the ability of inoculated *S. cerevisiae* to suppress wild microflora is a crucial feature determining the starter culture's dominance in the process (Ciani *et al.*, 2016). Yeasts play a vital role in flavor development, shelf life, and the nutritional value of fermented products. To nurture and enhance the quality of these traditional fermented products, it is essential to obtain detailed knowledge about the microorganisms involved, their growth requirements, and their interactions. The presence of yeast in beer was first reported in 1680, although the genus Saccharomyces was not named until 1837. In 1876, Louis Pasteur confirmed the involvement of living organisms in fermentation, and in 1883, Emil C. Hansen isolated brewing yeast and successfully cultured it (Bello *et al.*, 2019). Saccharomyces yeasts can form symbiotic matrices with bacteria and are used to produce various fermented beverages, including Kombucha, Kefir, and Ginger Beer. For example, *S. fragilis* is used in Kefir culture and can grow on lactose contained in whey, a byproduct of cheese making, which can be used as animal fodder (Botstein *et al.*, 1997).

S. cerevisiae is a facultative anaerobe capable of growing on various fermentable and non-fermentable carbon sources. When grown on fermentable carbon sources like glucose, it primarily derives metabolic energy from glycolysis. The Pasteur Effect, which inhibits fermentation in the presence of high oxygen concentrations, is well-documented (Sebald *et al.*, 1995). Using molecular techniques, Šuranská *et al.* (2016) have isolated and identified various indigenous *S. cerevisiae* strains and screened them for selected

oenological properties. Modern genotyping techniques, such as PCR-fingerprinting and inter-delta PCR typing, were employed to differentiate among indigenous *S. cerevisiae* strains. Ultimately, some indigenous strains from organically treated grape berries were chosen for their promising oenological properties and will be used as starter cultures. The quality of fermented foods and beverages is significantly influenced by the microorganisms involved in their production. Among these, *Saccharomyces cerevisiae*, commonly known as baker's yeast, plays a crucial role in wine fermentation. The diversity of *S. cerevisiae* strains present during spontaneous fermentation contributes to the chemical composition and sensory qualities of the resulting wine.

In this study, we focused on isolating and identifying indigenous *S. cerevisiae* strains from remote regions of Madhya Pradesh, India. These strains were obtained from berries and spontaneously fermented musts. Our goal was to screen these indigenous strains for specific oenological properties, including ethanol production capacity, sulfur dioxide tolerance, osmotic stress tolerance, flocculation intensity, and desirable enzymatic activities. Additionally, we examined their ability to produce and utilize acetic/malic acid and screened for undesirable properties such as H2S production.Modern genotyping techniques, such as PCR-fingerprinting and interdelta PCR typing, were employed to differentiate among the indigenous *S. cerevisiae* strains. This combination of methods provides a rapid and relatively simple approach for identifying yeast at the strain level. Ultimately, our findings will contribute to enhancing the regional character of wines by utilizing selected indigenous *S. cerevisiae* strains as starter cultures.

This study focuses on the isolation, identification, and molecular characterization of indigenous yeast strains from Satpura forests in Narmadapuram District of Madhya Pradesh. The isolation and identification of yeast strains will be accomplished using classical microbial techniques, with strain-level validation performed via Sanger sequencing methods. The identified strains will be analyzed for their role in the winemaking process, highlighting the commercial importance of this study.

MATERIALS AND METHODS

Sample Collection: Total 10 samples of Mahua (*Madhuca longifolia*) Tree bark and fruits samples were collected from various sites within the Satpura forest in the Narmadapuram District of Madhya Pradesh. The sampling sites were strategically selected to ensure a representative collection of indigenous fungal species, particularly yeast strains.

Sample Preparation and Inoculation: The collected bark and fruit samples of Mahua (*Madhuca longifolia*) were aseptically transferred to the laboratory in sterile containers and processed within 24 hours of collection. Each sample was surface sterilized using 70% ethanol followed by rinsing with sterile distilled water to minimize external microbial contaminants. Small portions of the sterilized material were then macerated in sterile phosphate-buffered saline, and aliquots of the resulting suspension were streaked onto Yeast Extract Peptone Dextrose (YEPD) agar plates supplemented with chloramphenicol to inhibit bacterial growth. The inoculated plates were incubated at 28 ± 2 °C for 48–72 hours under aerobic conditions to facilitate the selective growth of indigenous yeast colonies. Distinct yeast colonies emerging on the plates were subsequently sub-cultured onto fresh YEPD agar to obtain pure isolates for further morphological and molecular characterization.

DNA Extraction: Genomic DNA was isolated from the pure fungal cultures using the phenol-chloroform extraction method, a widely accepted protocol for nucleic acid extraction from fungal cells (Sambrook & Russell 2001). Briefly, fungal cells were lysed, and the cell debris was extracted with phenol-chloroform to obtain high-quality genomic DNA.

PCR Amplification_ The internal transcribed spacer (ITS) region of the fungal DNA was amplified using the polymerase chain reaction (PCR) with universal primers, ITS1 and ITS2 (White, 1990). The PCR conditions were optimized to ensure specific amplification, as follows: an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes.

DNA Sequencing: The PCR products were purified using a PCR purification kit and then subjected to DNA sequencing to ascertain the precise species identification. Sequencing was performed using an automated DNA sequencer, and the resulting sequences were aligned and compared against known sequences in the NCBI GenBank database using BLAST (Altschul *et al.*, 1990). These methods ensured the accurate isolation and molecular identification of indigenous *Saccharomyces cerevisiae* strains from the remote areas of Madhya Pradesh, facilitating a deeper understanding of the region's microbial diversity and its potential applications in fermentation industries.

Table 1: Universal Primer sequence used in present study are given below.

Primer	Sequence	PCR Product	Reference
ITS1 Fw	CTTGGTCATTTAGA GGAAGTAA	(~) 450 bp	Hite *et al.* (1990)
ITS2 Rv	GCTGCGTTCTTCATC GATGC		

The sequences of DNA were further analyzed using basic local alignment tool (BLAST) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for identification and Clustal W software was used to get genetic similarity.

RESULTS AND DISCUSSION

In this study, we embarked on the exciting journey of isolating and identifying indigenous yeast strains from the remote areas of Madhya Pradesh, specifically from

the tree bark and fruits of the Mahua tree, scientifically known as *Madhuca longifolia*. Our process began with strategically collecting samples from various locations within the Satpura forest. To ensure we captured pure cultures of yeast strains, we employed a serial dilution technique that effectively minimized the microbial load present in the samples. Upon processing the collected samples, we yielded a variety of yeast colonies cultivated on potato dextrose agar (PDA) media (Fig. 1).

Following an incubation period of 3 to 5 days at 30°C, we observed distinct fungal colonies emerging. Molecular characterization of these yeast strains was accomplished with extraction of genome of pure cultures (Fig. 2) amplifying the internal transcribed spacer (ITS) region of the fungal DNA using universal primers, ITS1 and ITS2. The PCR amplification successfully generated a targeted fragment of approximately 450 bp (Fig. 3). After amplifying the DNA, we proceeded with Sanger sequencing of the PCR products. This method allowed us to accurately identify the yeast strains by comparing the sequences obtained with those available in the NCBI database through the BLAST tool.

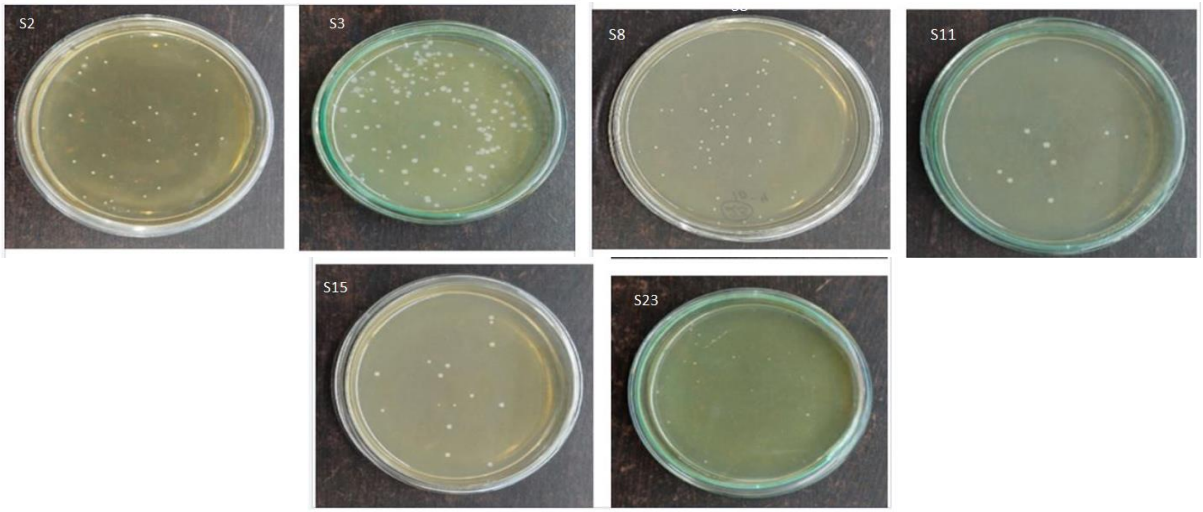

Fig. 1A. The figure showing selected images of isolated yeast species in present study.

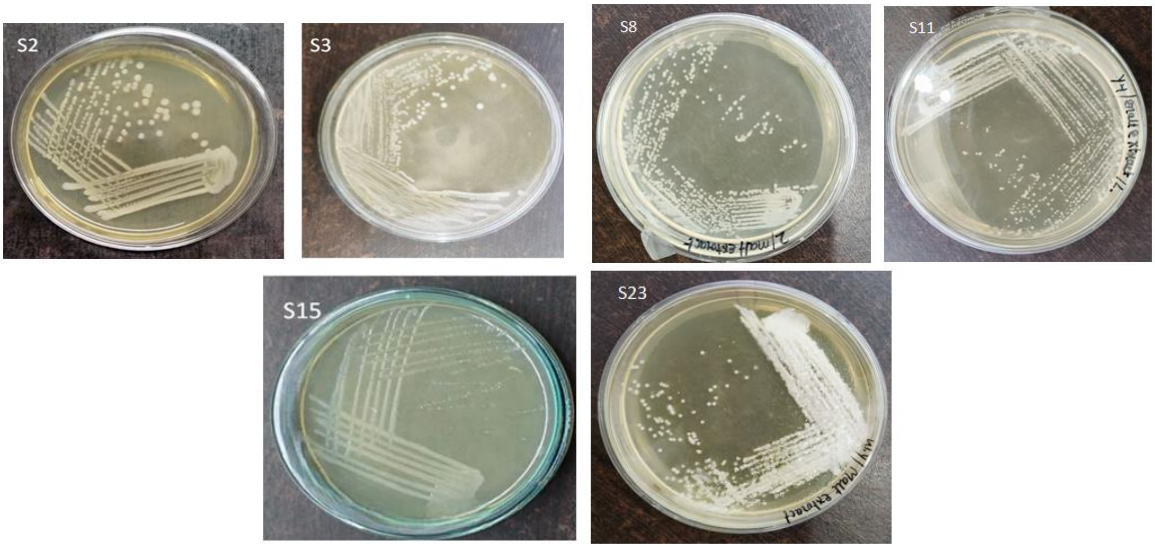

Fig. 1B. The figure showing the pure cultures of isolated yeast species in present study.

Our molecular analysis revealed a rich diversity of yeast species, which we summarized in a Table 2. Among the strains we successfully isolated, *Saccharomyces cerevisiae* emerged as a particularly important find. This yeast species is commonly known for its widespread use on both tree bark and fruit surfaces (Rajasekhar *et al.*, 2022). It is well known for its efficient fermentation capabilities, making it a crucial organism for the baking and brewing industries (Parapouli *et al.*, 2020). Its significance extends beyond practical application; *S. cerevisiae* also serves as a significant model organism in various scientific studies (Botstein *et al.*, 1997). We also identified other notable species such as *Hanseniaspora thailandica*, which is associated with fermentation processes and contributes to the flavor and aroma profiles of beverages (Niyomvong *et al.*, 2023).

Table 2: Characteristics of Yeast Species Identified in the Current Study.

Sr. No.	Species	Characteristics
1.	*Saccharomyces cerevisiae*	Found on surfaces of fruits and tree bark. Highly efficient fermenter, widely used in baking and brewing; a key model organism in scientific research
2.	*Hanseniaspora thailandica*	Associated with fermentation; contributes to flavor and aroma in beverages
3.	*Hanseniaspora opuntiae*	Found on surfaces of fruits and tree bark; important in fermentation processes, particularly in arid environments
4.	*Pseudozyma antarctica*	Known for producing cold-adapted enzymes, particularly lipases, useful in biotechnological applications
5.	Hanseniasporameyeri	Involved in fermentation for wines and other alcoholic beverages, contributing to flavor complexity

Another strain, *Hanseniaspora opuntiae*, was found on fruit surfaces and is especially relevant in fermentation processes occurring in arid environments, demonstrating its adaptability. Additionally, we isolated *Pseudozyma antarctica*, recognized for its production of cold-adapted enzymes like lipases, which hold promise for various biotechnological applications. Lastly, Hanseniasporameyeri is involved in the fermentation of wines, enhancing the flavor complexity of alcoholic beverages (Martin *et al.*, 2022). The successful molecular identification of these yeast strains reveals the significant biodiversity that exists within Madhya Pradesh. Moreover, it underscores the ecological importance of these microorganisms, not just in fermentation but also regarding their potential applications in food and beverage production. The findings of this research extend our understanding of microbial diversity in Madhya Pradesh. They highlight the potential of these indigenous yeast strains for various applications in the fermentation industry. The identification of strains like *S. cerevisiae* suggests they can be utilized to enhance traditional fermentation processes, potentially improving the quality and flavor of products. For instance, leveraging unique properties of various Hanseniaspora species might yield distinctive flavors that reflect the local environment, thereby enriching the cultural and economic significance of traditional drinks (Coutinho *et al.*, 2018).

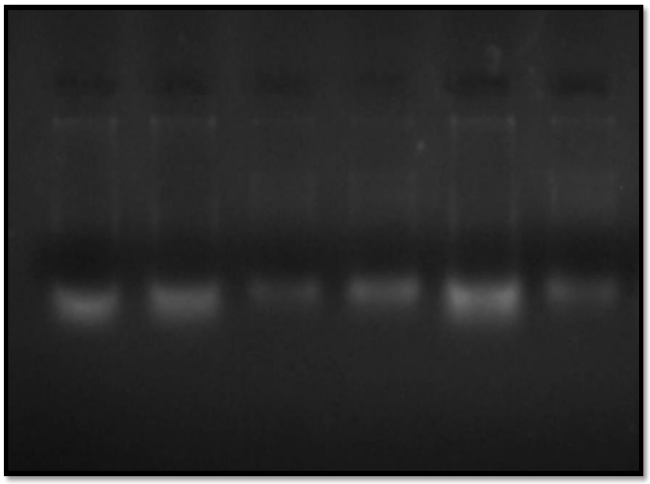

Fig. 2. Figure showing genome of extracted genomes of yeast species.

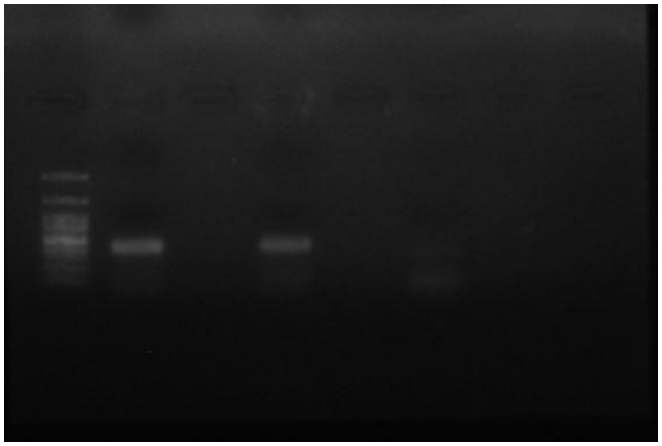

Fig. 3. Displays selected results demonstrating the PCR amplification of the ITS gene, highlighting the amplification of the ITS region (~450 bp) of yeast.

Looking ahead, further research is essential to explore how these strains contribute to fermentation processes. Future studies could assess the fermentation efficiency of these yeast strains, examine their ability to produce desirable flavors and aromas, and determine how they impact the nutritional value of fermented products. Additionally, investigating the interactions of these strains with lactic acid bacteria during fermentation could offer insights into optimizing traditional practices, ultimately fostering sustainability and innovation within the fermentation industry. Fig. 2 further illustrates our molecular findings, with depicting the genomic analysis of the isolated bacteria and fungi. Fig. 3 shows the successful PCR amplification of the ITS gene, indicating the robustness of our molecular methods in identifying the fungal populations. The diversity of yeast strains isolated from the remote areas of Madhya Pradesh highlights the ecological significance of these microorganisms in natural fermentation processes. Their isolation not only enriches our understanding of regional microbial biodiversity but also holds potential for applications in food and beverage fermentation, aligning with the growing interest in traditional and indigenous fermentation practices (Mudoor *et al.*, 2023). The successful identification and characterization of these yeast species underline their importance in fermentation industries and traditional practices. Future research should explore their specific roles in flavor development, fermentation efficiency, and potential health benefits, contributing to sustainable agricultural practices and enhancing regional food production.

CONCLUSIONS

This study successfully isolated and identified diverse indigenous yeast strains from the Satpura forests in Madhya Pradesh, with a particular focus on *Saccharomyces cerevisiae*, which is integral to fermentation processes. These findings highlight the potential for utilizing local yeast strains to enhance traditional fermentation practices, fostering improved quality and flavor in food and beverages.

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Conflict of Interest. None.

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