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# Rapid Identification of *through* Real time PCR form Indigenous Yeast Strains from Satpura Forests of Madhya Pradesh

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**ABSTRACT: This study investigates the rapid identification and characterization of indigenous yeast strains, with a particular focus on *Saccharomyces cerevisiae*, sourced from the Satpura forests of Madhya Pradesh, India. A total of 60 samples were collected from the bark and fruits of the Jamun (*Syzygium cumini*), Tendu (*Diospyros melanoxylon*), and Mahua (*Madhuca longifolia*) across diverse sampling sites to ensure representative coverage of indigenous fungal species. Employing a combination of classical microbiological techniques and modern molecular methods, including real-time PCR, this research aimed to isolate and accurately identify yeast species within this unique ecological niche. The yeast colonies were cultivated on potato dextrose agar, which provided optimal conditions for growth, while Lactophenol cotton blue staining facilitated detailed morphological examination of the isolates. Through quantitative PCR (qPCR) analysis, we confirmed the presence of S. cerevisiae, noting consistent melting points of 73.5°C and 78.0°C across the samples, which validated the identity of the yeast. This study underscores the significant microbial diversity inherent in the Satpura forests and highlights the potential applications of these indigenous yeast strains in various fermentation processes. The findings not only contribute to the understanding of local yeast biodiversity but also pave the way for further investigations into sustainable practices in the food and beverage industry, with the potential to enhance the flavor profiles of traditional fermented products.**

**Keywords:** Fermentation, molecular identification, Madhya Pradesh, *Saccharomyces cerevisiae*, Real Time PCR.

## INTRODUCTION

The identification and characterization of indigenous yeast strains have garnered significant attention in recent years due to their potential applications in various biotechnological processes. Yeasts, particularly the species *Saccharomyces cerevisiae*, play a crucial role in the food and beverage industry, widely utilized in the production of bread, beer, and wine (Gupta *et al.*, 2015; Wang *et al.*, 2007). Recognized for its fermentation abilities and reliability as a starter culture, *S. cerevisiae* serves not only as an essential ingredient in food production but also as a model organism for scientific research. This underscores the importance of understanding the genetic and ecological diversity of yeast strains found in unique environments. The Satpura forests of Madhya Pradesh, India, represent a distinctive and underexplored ecological niche (Sarkhel, 2023). They encompass a variety of habitats that support diverse microbial communities, including indigenous yeast strains that may possess unique fermentation properties or resilience to environmental stresses. The rapid identification and characterization of these strains are crucial for their effective utilization in industrial applications and to enhance our understanding of the microbial diversity within this biodiverse region.

In responding to the need for faster and more reliable identification methods, real-time PCR (Polymerase Chain Reaction) has emerged as a powerful tool for the rapid and accurate identification of microbial species, including yeast. This technique amplifies specific DNA sequences, allowing for the effective detection of target organisms with high sensitivity and specificity (Sabaté *et al.*, 2000). Real-time PCR has proven particularly useful in distinguishing *Saccharomyces cerevisiae* from closely related species, such as Saccharomyces bayanus, Saccharomyces pastorianus, and Saccharomyces paradoxus (Torriani *et al.*, 2004). The ability to quickly and accurately identify these species

is essential, especially in the context of the fermentation industry, where different yeast strains can dramatically influence the outcomes of fermentation processes. The present study aims to explore and characterize the indigenous yeast strains from the Satpura forests of Madhya Pradesh, focusing on developing a rapid identification method for *Saccharomyces cerevisiae* using real-time PCR. This research not only seeks to enhance our understanding of microbial diversity in this unique ecological niche but also aspires to provide valuable insights into the potential applications of these strains in various biotechnological processes. Furthermore, the introduction of a rapid identification technique will streamline the screening and utilization of indigenous yeast strains in various industrial applications, ultimately contributing to the development of more sustainable and efficient fermentation practices. Previous research has effectively demonstrated the efficacy of real-time PCR in the identification and quantification of *Saccharomyces cerevisiae* in diverse substrates, including wines and other fermented products (Querol *et al.,* 2005). However, the application of this advanced technique to indigenous yeast strains from the Satpura forests remains largely unexplored. By bridging this gap, this study aims to employ real-time PCR for the identification and characterization of *Saccharomyces cerevisiae* strains from this unique ecological setting. The rapid identification of *Saccharomyces cerevisiae* through real-time PCR from indigenous yeast strains in the Satpura forests of Madhya Pradesh holds significant promise for biotechnological applications. The findings from this study will contribute to the growing body of knowledge on microbial diversity and provide a foundation for future research into the industrial utilization of indigenous yeast strains, possibly enriching the flavor profiles of traditional fermented products and fostering the sustainability of local fermentation practices.

## MATERIALS AND METHODS

**Sample Collection:** A total of 60 samples, consisting of tree bark and fruit, were collected from three tree species: Jamun (*Syzygium cumini*), Tendu (*Diospyros melanoxylon*), and Mahua (*Madhuca longifolia*) from various strategically selected sites within the Satpura Forests of Narmadapuram District, Madhya Pradesh. These trees were particularly recognized for their succulent flowers, which create a natural sugary substrate conducive to yeast proliferation. Swabs were taken from the surfaces of the tree bark and flowers and subsequently transferred to vials containing yeast extract peptone dextrose (YPD) broth to maintain the nutrient integrity of the samples. These locations were carefully chosen to ensure a representative collection of indigenous fungal species, with a specific focus on yeast strains.

Sample Preparation and Inoculation: The collected samples underwent a serial dilution method (Wang *et al.,* 2007) to effectively reduce the microbial load and isolate pure fungal cultures. Following the dilutions, the samples were inoculated onto potato dextrose agar (PDA) media, which is a standard medium for fungal cultivation (Pitt & Hocking 2009).

**Culturing:** After inoculation, the plates were incubated at 30°C for 3 to 5 days, allowing sufficient time for fungal colonies to develop. Pure cultures were established by repeatedly streaking individual colonies onto fresh PDA plates until achieving uniform colony morphology, indicating clonality.

Staining of yeast Lactophenol cotton blue staining was performed to visualize yeast cells for morphological examination. First, a small amount of yeast culture was taken using a sterile loop and placed onto a clean glass slide. A drop of lactophenol cotton blue stain was added to the yeast sample and mixed gently to ensure even distribution of the dye. The slide was then covered with a coverslip to avoid bubbles and to ensure uniform contact with the dye. The preparation was heated gently over a flame for a few seconds to facilitate the penetration of the dye into the cells, which enhances the staining quality. After cooling, the slide was examined under a light microscope at various magnifications. The lactophenol cotton blue stain effectively highlights the morphology of yeast cells, allowing for the observation of their shape, size, and arrangement, thus providing valuable information for identification and characterization. The stained samples were documented, capturing their structural details for further analysis

**DNA Extraction:** Genomic DNA was extracted from the pure fungal cultures using a phenol-chloroform extraction method, a widely accepted technique for isolating nucleic acids from fungal cells (Sambrook & Russell 2001). This process involved lysing the fungal cells and extracting cell debris with phenol-chloroform to yield high-quality genomic DNA.

**Real-Time PCR Analysis:** For the real-time PCR analysis, primers specific to *S. cerevisiae* were utilized. The forward primer (SC1d) was 5′-ACA TAT GAA GTA TGT TTC TAT ATA ACG GGT G-3′, and the reverse primer (SC1r) was 5′-TGG TGC TGG TGC GGA TCT A-3′. These primers, as described by Martorell *et al.* (2005), were designed to amplify an approximate 300-bp region of the *S. cerevisiae* genome, ensuring specificity for this yeast species.

For quantification using the Areamx management system, qPCR was performed in 25-µL reaction volumes comprising 5 µL of the extracted DNA, 0.06 µM of each primer, and 240 nM of ROX reference dye (Roche) incorporated within a hot-start reaction mix containing 1× FastStart SYBR Green Master (ROX) from Roche to facilitate the qPCR process.

The DNA amplification from the extracted samples was executed using the following thermal cycling program:
Initial Denaturation: 10 minutes at 95°C to ensure complete denaturation of the DNA.
**Amplification Cycles:** Followed by 45 cycles consisting of:
15 seconds at 95°C (to denature the DNA)
60 seconds at 60°C (to allow primer annealing)
90 seconds at 72°C (for extension and synthesis of new DNA strands)
**Final Extension:** A concluding step of 10 minutes at 72°C to ensure the completion of the reaction.
It is important to note that these cycling parameters reflected a longer amplification time than typically observed with standard PCR sequences using the AreaMxqPCR, having been selected based on prior optimization with established PCR instrumentation.
Post-quantitative PCR (qPCR) runs, the resulting data were analyzed to determine the threshold cycle (Ct) values, which indicate the cycle number at which the fluorescence signal surpassed the background level, correlating directly with the initial quantity of *S. cerevisiae* present in the samples. By comparing these Ct values against a standard curve generated from known concentrations of S. cerevisiae, accurate quantification of the yeast populations in the collected samples was realized.
This meticulous approach to qPCR amplification ensured precise quantification of the presence and abundance of *Saccharomyces cerevisiae* in the samples, providing essential information for subsequent analyses and applications.

## RESULTS AND DISCUSSION

In this study, we focused on isolating and identifying indigenous yeast strains from the remote areas of Madhya Pradesh, specifically from the bark and fruits of three tree species: Jamun (*Syzygium cumini*), Tendu (*Diospyros melanoxylon*), and Mahua (*Madhuca longifolia*). A total of 60 samples were collected from various strategically selected sites within the Satpura Forests of Narmadapuram District, Madhya Pradesh. The sampling locations were chosen to ensure a representative collection of indigenous fungal species.
To facilitate the growth of yeast populations, swabs were taken from both the tree bark and flowers, which are known to provide a natural sugary substrate that promotes yeast proliferation. The samples were transferred to vials containing yeast extract peptone dextrose (YPD) broth, effectively preserving the nutrient content essential for yeast growth. To ensure the successful isolation of pure yeast cultures, a serial dilution technique was employed. This method minimized the microbial load present in the samples, allowing for the selective growth of yeast strains. The approach proved effective in isolating diverse yeast cultures from the collected samples, which will be further characterized for their potential applications in fermentation and other biotechnological processes. Upon processing the collected samples, we yielded a variety of yeast colonies cultivated on potato dextrose agar (PDA) media and stained the cells with Lactophenol cotton blue (Fig. 1).

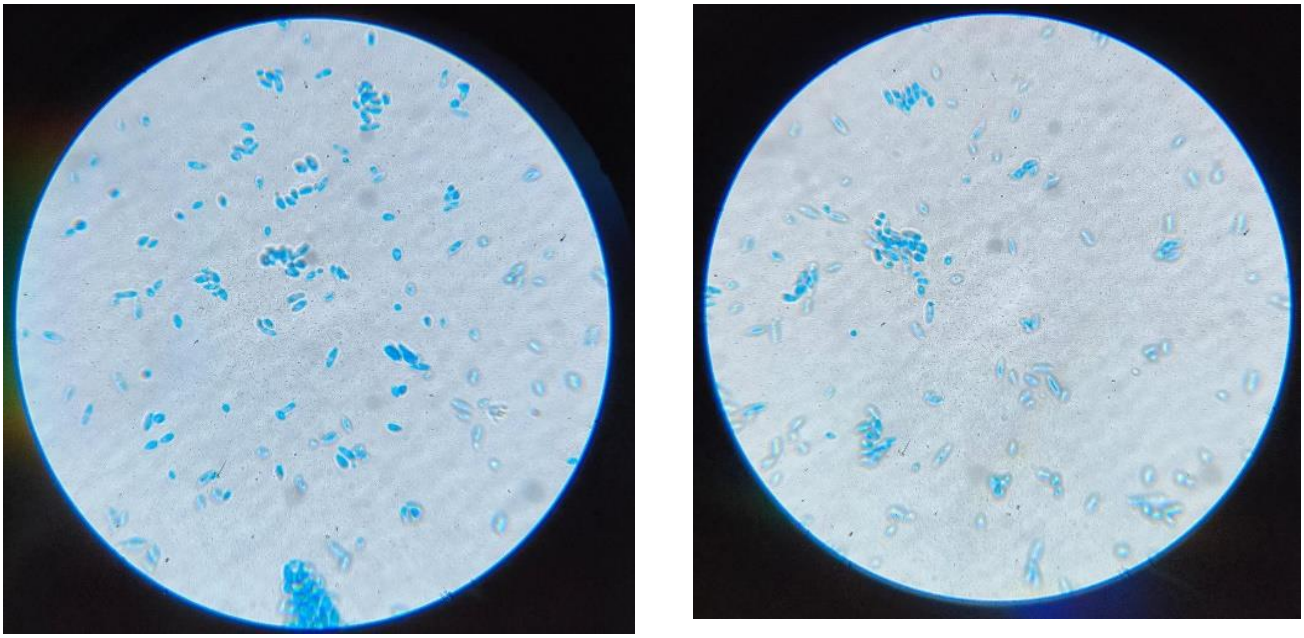

**Fig. 1.** The figure showing selected image (100X) of isolated stained the cells of yeast with Lactophenol cotton blue during present study.

The identification of *S. cerevisiae* was confirmed through real-time PCR, a reliable method allowing for the rapid detection of target DNA sequences. Using specifically designed primers, the amplification and quantification of the *S. cerevisiae* genome were achieved, yielding distinct melting points at 73.5°C and 78.5°C, which corroborated the identity of the yeast. Consistently observed melting points between the samples validated the presence of *S. cerevisiae* in the tested cultures, as indicated by previous studies (Powell *et al.*, 2010).

Among the 48 yeast species tested, three were confirmed to be *S. cerevisiae* through qPCR analysis (Table 1). The reliability of the real-time PCR technique not only enhances the efficiency of yeast identification but also represents a significant advancement in monitoring yeast populations in fermentation contexts. This study aligns with previous research indicating the practicality of real-time PCR for differentiating *S. cerevisiae* from related species (Torriani *et al.*, 2004; Martorell *et al.*, 2005). The findings underscore the rich microbial diversity present in the Satpura forests and highlight the potential of local yeast strains in biotechnological applications. Indigenous strains like *S. cerevisiae* can contribute significantly to the fermentation industry due to their unique fermentation abilities and potential resilience to environmental pressures. Furthermore, understanding the genetic diversity of these yeast species may pave the way for optimizing fermentation processes, thus enhancing the flavor profiles of traditional fermented products.

**Table 1: Summary of Yeast Species Tested and Detection of Saccharomyces cerevisiae through qPCR.**

| Sr. No. | Number of yeast species tested | *Saccharomyces cerevisiae* reported through qPCR |
|---|---|---|
| 1. | 48 | 06 |

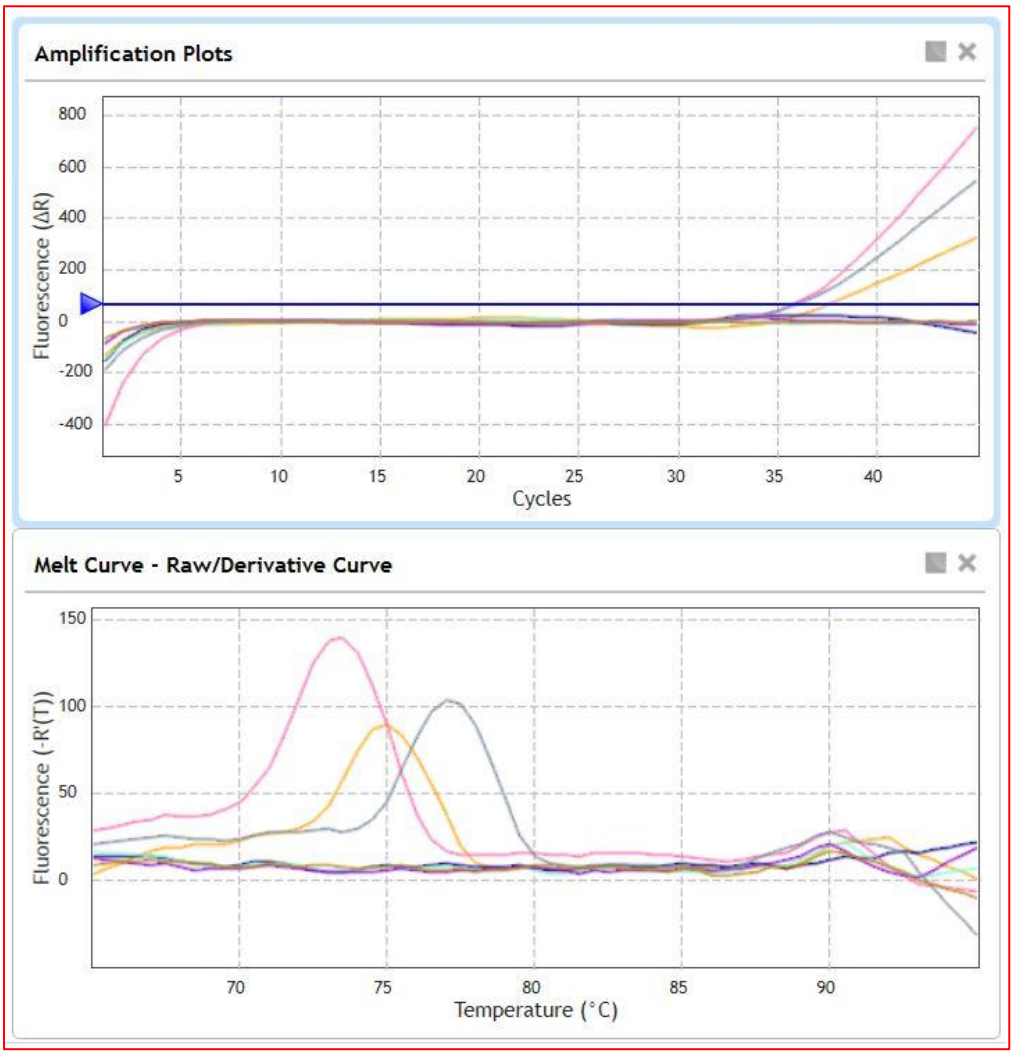


**Fig. 2.** Displays consistent melting points (73.5°C and 78.0°C) across samples, confirming the presence of *Saccharomyces cerevisiae* in the tested yeast cultures.

## CONCLUSIONS

This study effectively identified and characterized indigenous yeast strains from the Satpura forests of Madhya Pradesh, with a special emphasis on *Saccharomyces cerevisiae*. By combining traditional microbiological techniques with modern molecular methods like real-time PCR, we confirmed the presence of this important yeast species and highlighted the diverse microbial life in this unique ecosystem. These findings not only deepen our understanding of local yeast biodiversity but also suggest exciting possibilities for using these strains in sustainable food and beverage production, enhancing the quality of traditional fermentation processes.

## FUTURE SCOPE

Future research should concentrate on examining the fermentation efficiencies and metabolic characteristics of these indigenous yeast strains. Such insights will contribute to sustainable practices in the fermentation industry, leveraging local microbial resources to enrich traditional food and beverage production in Madhya Pradesh. The successful application of real-time PCR in this study establishes a foundation for further exploration into the utilization of indigenous yeast strains, encouraging the integration of microbial diversity into biotechnological innovations. The findings presented here not only contribute to the existing body of knowledge regarding yeast biodiversity but also open avenues for future research aimed at harnessing the potential of local strains in various industrial applications.

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**Conflict of Interest.** None.

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