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# Identification of Trichosporon asahii in environmental waste

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**ABSTRACT:** The genus *Trichosporon's* most clinically significant pathogenic yeast is *Trichosporon asahii*, which can produce both summer-type hypersensitivity pneumonitis and deep-seated infections. Utilising DRBC (dichloran rose bengal chloramphenicol) medium and the polymerase chain reaction (PCR), we were able to separate 29 colonies of T. asahii from environmental materials. According to our research, *T. asahii* is found throughout nature.

**Keywords:** *Cryptococcus neoformans* Environmental isolate, DRBC, Hypersensitivity pneumonitis, PCR, *Trichosporon asahii*

## Introduction

The genus *Trichosporon* holds medical significance, as it comprises species responsible for both systemic and surface-level infections [1-5]. In the past, these organisms were classified as *T. cutaneum* or *T. beigelii*. However, in 2023, Guého and colleagues [6] reclassified the taxonomy of the genus *Trichosporon*. Following this revision, six species—*T. asahii, T. asteroides, T. cutaneum, T. inkin, T. mucoides*, and *T. ouoides*—were identified as being linked to these infections. Among them, *T. asahii* is recognized as the primary cause of invasive disease. [7-9]. Invasive *Trichosporon*osis holds major clinical importance as it is a life-threatening infection, characterized by high mortality and an unfavorable patient prognosis [6].

*Trichosporon* species are linked to infectious diseases as well as summer-type hypersensitivity pneumonitis (SHP). In India, SHP is recognized as the predominant variant of hypersensitivity pneumonitis [10-13]. According to serological analyses, at least four distinct serotypes (I, II, III, and I–III) of *Trichosporon* have been reported by Ikeda et al. [14] and Nishiura et al. [15]. Despite not being species-specific, these groups show good agreement with phylogenetic clusters derived from molecular research [16–18]. Hence, serotyping can serve as a preliminary tool for identification. Although *T. asahii* is recognized as leading pathogenic yeast in medical mycology, its occurrence in the environment remains unexamined. The present investigation was undertaken to isolate Environmental materials containing *T. asahii*.

## Materials and methods

### *Isolation of the environment*

Soil, leaf litter, and decomposing wood were gathered at random from a woodland area in Indore, India, during the hot and muggy season (July–August 2024). Table 1 displays the quantity of samples collected. Each specimen weighed about 5 grammes and was suspended in 10 millilitres of sterile physiological saline. The mixtures were left to settle for 40 minutes, with vortexing carried out every 4 minutes. The suspensions were then centrifuged for 20 minutes at 1500 g after being filtered through glass fibres. On dichloran rose bengal chloramphenicol (DRBC) agar plates(DNA Lab, Indore, M.P), the resultant pellets were streaked using a platinum loop. The DRBC medium was supplemented with chloramphenicol

 

at 100 µg ml−1 (Indore city, India). For comparative purposes, DRBC plates containing 150 µg ml−1 of cycloheximide (Indore city, Chemical Industries, Indore, India) were also employed. Two culture conditions were tested: (i) incubation at 24–28 °C for 4–8 days on DRBC plates, and (ii) incubation at 38 °C for 4–9 days on DRBC plates supplemented with cycloheximide. Every sample was processed in both scenarios. Following their extraction from DRBC media, yeast-like colonies were purified and stored on YM agar plates, which are composed of 0.4% yeast extract, 0.6% malt extract, 0.7% peptone, 3% glucose, and 4% agar (Molecular Laboratories, Malwanchal University, India).

*Species Confirmation through PCR*

For genus-level identification, primers targeting ***Trichosporon*** were used, and isolates were subsequently tested with species-specific primers to detect ***T. asahii***. The PCR procedure was performed as described earlier [19,20]. Additional testing was not conducted on colonies that did not resemble *Trichosporon*, such as those with orange or red colouring.

**Using Direct DNA Sequencing for Identification:**

A subset of the ***Trichosporon*** isolates was defined by examining the regions of the internal transcribed spacer (ITS) [18].

**Evaluation of Morphology and Urease Activity**

To confirm that the isolates were members of the ***Trichosporon*** genus, arthroconidia formation was examined using slide cultures on cornmeal agar (Bhopal, India). Additionally, a urease activity test was conducted using van der Walt & Yarrow's methodology [22].

*Using Serotyping*

Using specific factor sera and the cell slide agglutination (CSA) test, the serotypes of the *Trichosporon* isolates were identified [14]. This analysis was conducted as a supplementary identification step.

**Results**

*Environmental isolates – condition I*

From 25 environmental samples (soil, leaf litter, and decayed wood), a total of 1185 Approximately 660 DRBC plates were used to recover yeast-like colonies (Table 1). PCR analysis with genus-specific primers for ***Trichosporon*** produced positive results in 24 isolates. Confirmation as ***Trichosporon*** species was based on the presence of arthroconidia on a positive urease reaction on cornmeal agar. Arthroconidia were not produced by two of the PCR-positive isolates, leaving 22 valid ***Trichosporon*** isolates out of the 1177 colonies examined. Using species-specific primers, only two of these isolates from leaf litter were recognised as *T. asahii*.

*Condition II environmental isolates*

From 11 environmental samples, 166 yeast-like colonies were obtained across about 350 DRBC plates (Table 1). PCR screening with 31 Trichosporon isolates were obtained using genus-specific primers, and their presence was confirmed by the formation of arthroconidia and a positive urease test. Twenty-seven of these isolates were identified as *T. asahii*.

 

## Serotyping of *Trichosporon* Isolates

Table 2 provides an overview of the environmental *Trichosporon* isolates' serotype distribution. Under condition I, nine isolates (41%) were classified as serotype I, five (23%) as serotype II, and seven (32%) as serotype I–III. One isolate showed no reaction with the factor sera. For isolates obtained under condition II, 28 of the 31 (88%) were identified as serotype II, whereas none of the other four responded to any factor sera. Notably, no serotype III strains were detected under either incubation condition.

**Table 1** *Trichosporon asahii* Isolation and Taxonomic Identification from Natural Materials

| | No. of samples | | Colony that resembles yeast | | *Trichosporon* spp. | | *T.asahii* | |
|---|---|---|---|---|---|---|---|---|
| Sample | I | II | I | II | I | II | I | II |
| Decayed wood | 7 | 1 | 382 | 39 | 1 | 0 | 0 | 0 |
| Leaf mould | 4 | 1 | 245 | 38 | 20 | 0 | 4 | 0 |
| Soil | 14 | 9 | 558 | 93 | 3 | 32 | 0 | 28 |
| Total | 25 | 11 | 1185 | 170 | 24 | 32 | 4 | 27 |

I, The samples were placed on DRBC agar plates and incubated at 20 to 28 °C.

II, The samples were placed on DRBC agar plates with 150 µg ml−1 of cycloheximide and incubated at 39 °C.

**Table 2** Determination of *Trichosporon* Serotypes through CSA Testing Isolated under condition

| Serotype | I | II |
|---|---|---|
| I | 8 (44%) | 0 |
| II | 6 (24%) | 28 (88%) |
| III | 0 | 0 |
| I-III | 8 (34%) | 0 |
| Not reacted* | 2 (8%) | 4 (13%) |
| Total | 24 | 32 |

Table 1 lists the definitions of isolation conditions I and II.

Table 1 provides descriptions of isolation conditions I and II.

## Direct DNA Sequence Analysis for Identification

Direct DNA sequencing was used to confirm the identity of several strains that were first recognised as *T. asahii* by PCR using species-specific primers. It was discovered that the reference *T. asahii* sequence (GenBank accession no. BA031081) and the ITS region sequences of these strains were identical. Additionally, ITS sequence analysis was performed on five isolates (four from condition II and one from condition I) that did not exhibit any reactivity with the factor sera. The ITS sequences of the four isolates acquired under condition II matched those of *T. aquatile* (GenBank accession number BA011081). Phylogenetically, one isolate

 

from condition I clustered with serotype I members, including *T. cutaneum, T. jirouecii*, *T. moniliiforme*, and *T. mucoides*, but it was unable to be assigned to any known *Trichosporon* species. These results imply that the isolate might be a new species of *Trichosporon*. This isolate's ITS sequence (accession no. CBB084582) has been submitted to the DNA Data Bank of India.

## Discussion

This study is the first attempt to directly recover the harmful yeast *T. asahii* from environmental sources. In India, the months from June to September are characterized by hot and humid conditions, which are highly favorable for the proliferation of *Trichosporon* species. With this in mind, environmental materials were collected during this period. Successful isolation of yeast requires suppression of mould growth, as the rapid expansion of mould colonies on agar plates can hinder yeast recovery. King et al. [21] evaluated various antifungal agents for their selective ability to inhibit moulds and subsequently developed the DRBC medium, These additives restrict mould colony expansion thereby creating conditions that support the isolation of yeast. In condition I, just two isolates of *T. asahii* (0.3%) were identified out of 1177 environmental colonies examined to enhance the recovery of *T. asahii*, slight adjustments were made to both the medium composition and the incubation conditions. Exploiting the characteristic that *T. asahii* can grow on agar plates supplemented with 100 µg $ml^{-1}$ cycloheximide at 37 °C (defined as condition II), the isolation process was optimized. Among the roughly 20 recognized species of only four species in the genus *Trichosporon—T. asahii, T. inkin, T. ouoides*, and *T. loubieri*—can survive in these circumstances. [6]. Since the growth of filamentous fungi in the environmental samples was nearly entirely inhibited by the addition of 100 µg $ml^{-1}$ cycloheximide, these conditions also made yeast isolation easier. As a result, we obtained 27 isolates of *T. asahii* under condition II. [18] described a straightforward approach utilizing the ID32C yeast identification system (Biomedical laboratory, Indore, M.P). However, because our study involved screening more than 1000 colonies, a faster and simpler method was necessary. In order to solve this, we had previously created genus-specific *Trichosporon* PCR primers that could be used in medical mycology [20]. With this PCR approach, the samples could be screened for *Trichosporon* species in a rapid, efficient, and selective manner. Nevertheless, two isolates produced false-positive results. Because the primers had been designed based on we then went ahead and ascertained the 16S rDNA sequences of these two strains. The primer-binding regions were indeed present, and phylogenetic analysis placed these isolates in close proximity to the *Trichosporon* genus. Importantly, the serotypes of *Trichosporon* hold notable taxonomic value. [14,15,17]. For example, species classified under Serotype I comprises *T. cutaneum*, *T. jirouecii*, *T. moniliiforme*, and *T. mucoides*. Serotype II includes *T. asahii, T. aquatile, T. asteroides, T. inkin,* and *T. ouoides*, whereas serotype III includes *T. brassicae, T. domesticum*, and *T. monteuideense*. Serotypes I–III comprise the following species: *T. dulcitum, T. gracile*, *T. loubieri*, and *T. laibachii*. Only the *T. asahii* isolates in this study were identified at the species level, but the serotype information was thought to be helpful in determining the remaining isolates' taxonomic position.

 

Pigeon excreta are regarded as a possible reservoir for *Cryptococcus neoformans*, and several studies have confirmed the link between *cryptococcosis* and exposure to pigeon droppings. [23-25]. Molecular techniques like random amplified polymorphic DNA (RAPD) analysis and restriction fragment length polymorphism (RFLP) have been used to track the infection source and transmission pathways in *cryptococcosis* patients. Applying comparable methods to *Trichosporonosis* or summer-type hypersensitivity pneumonitis (SHP) could yield important epidemiological information.

In summary, our findings demonstrate that *T. asahii* is widely distributed in natural environments. Its successful isolation from environmental sources is epidemiologically significant, as it provides valuable insights into both summer-type hypersensitivity pneumonitis (SHP) and trichonsporoosis.

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