Isolation, Morphological Identification and Molecular Characterization of Cantharellus tropicalis and Evaluation of Its Antibacterial Activity

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Abstract

Cantharellus tropicalis is a tropical ectomycorrhizal chanterelle associated with *Dendrocalamus strictus* in central India that remains poorly characterized at the molecular level, largely because its polysaccharide-rich cell wall complicates genomic DNA extraction. In this study, a pure isolate of *C. tropicalis* (designated CtDIC#001) was procured from the Bio-Design Innovation Centre, Rani Durgavati University, Jabalpur, and cultured on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). Morphological identification was confirmed by macroscopic colony characters and by microscopic examination using the slide-culture technique with lactophenol cotton blue staining. Genomic DNA was extracted using the LETS (lithium chloride-EDTA-Tris-SDS) method followed by phenol:chloroform:isoamyl alcohol purification. UV-Vis spectrophotometry gave A_{260}/A_{280} and A_{260}/A_{230} ratios consistent with high-purity DNA (~1.8 and 2.0-2.2, respectively), and agarose gel electrophoresis confirmed intact, high-molecular-weight bands suitable for downstream molecular applications such as ITS amplification. The antibacterial activity of a methanolic extract of the fungus was evaluated against *Pseudomonas aeruginosa* by the disc/well diffusion method; the extract produced a 16 mm zone of inhibition compared with 25 mm for the standard antibiotic and no inhibition for the water control. These findings establish a validated, low-cost protocol for isolating and molecularly characterizing *C. tropicalis* and provide preliminary evidence of antibacterial activity in this species, warranting further phytochemical and sequencing-based investigation.

Keywords: *Cantharellus tropicalis*; ectomycorrhizal fungi; genomic DNA extraction; LETS method; antibacterial activity; disc diffusion assay

1. Introduction

The genus *Cantharellus* (family Cantharellaceae), commonly known as chanterelles, comprises more than 100 described species of ectomycorrhizal fungi distributed across temperate and tropical forests worldwide [6]. Members of the genus are recognized by their funnel- or trumpet-shaped, brightly coloured fruiting bodies and by a ridged hymenium ("false gills") rather than true lamellae, and they form mutualistic ectomycorrhizal associations with forest trees that assist nutrient and water uptake [4,6]. *Cantharellus tropicalis* Rahi, Rajak & Pandey is a chanterelle reported from tropical Central India, where it forms an ectomycorrhizal association with *Dendrocalamus strictus* (bamboo) [12]. Beyond its ecological role, the species has been reported to produce acid phosphatase under acidic culture conditions, a trait linked to phosphorus solubilization and nutrient cycling in forest soils [2,4].

Despite this ecological interest, molecular studies on *C. tropicalis* remain scarce. Basidiomycete cell walls, rich in chitin, glucans and other polysaccharides, are difficult to lyse [8], and co-extracted polysaccharides and phenolic secondary metabolites frequently inhibit downstream enzymatic reactions and reduce DNA yield and purity [10]. A range of extraction strategies for fungal and basidiomycete DNA have been developed and comparatively evaluated, with no single method proving universally optimal for every species [7,15]. The LETS (lithium chloride-EDTA-Tris-SDS) method offers a comparatively rapid, simple and low-cost alternative to reagent-intensive protocols such as CTAB-based lysis [14].

Chanterelles are also increasingly recognized as a source of bioactive secondary metabolites: related species such as *C. cibarius* contain phenolic compounds and carotenoids with reported antioxidant activity [3], and the close relationship of *C. tropicalis* to these medicinally relevant chanterelles suggests it may harbour comparable bioactive potential that has not yet been evaluated experimentally.

Given the limited molecular and pharmacological characterization of *C. tropicalis*, the present study was undertaken with the following objectives: (i) to survey, isolate and morphologically identify *C. tropicalis* from Rani Durgavati University, Jabalpur; (ii) to extract and characterize (quantitatively and qualitatively) genomic DNA from the pure isolate; and (iii) to evaluate the antibacterial activity of the fungus against a pathogenic bacterium.

2. Materials and Methods

2.1 Fungal culture and maintenance

A pure culture of *C. tropicalis* was procured, by courtesy of the Bio-Design Innovation Centre (DIC), Rani Durgavati University, Jabalpur (M.P.), and maintained at 4°C until use. All subsequent experimental work was carried out at the Fungal Biotechnology and Invertebrate Pathology Laboratory, Department of P.G. Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur.

2.2 Isolation and media preparation

Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) (compositions given in Table 1) were used as selective media, supplemented with chloramphenicol to suppress bacterial contamination. The isolate was streaked onto PDA and SDA plates using a sterile inoculating needle and incubated at 26 ± 2°C for 7 days. Colonies were sub-cultured onto antibiotic-free PDA for 5-7 days prior to identification. Pure cultures were preserved as slants: molten PDA/SDA was dispensed into test tubes, autoclaved at 121°C for 20 min, allowed to solidify at a 15° incline, and inoculated for maintenance at 25-30°C.

Table 1. Composition of culture media used in this study

Medium	Component	Amount (per litre)
PDA	Potato infusion	200 g
	Dextrose	20 g
	Agar	20 g

Medium	Component	Amount (per litre)
SDA	Peptone	10 g
	Dextrose	40 g
	Agar	20 g

2.3 Morphological and microscopic identification

Macroscopic colony characters (colour, texture and pigmentation) were recorded over the incubation period. Microscopic identification was performed by the slide-culture technique: a block of PDA was inoculated on all four sides with the fungus, covered with a sterile coverslip, and incubated in a moist chamber at 25-30°C for 2-5 days. The coverslip was then mounted in lactophenol cotton blue stain and examined under a compound microscope to visualize hyphae and other diagnostic structures.

2.4 Genomic DNA extraction (LETS method)

Genomic DNA was extracted following the LETS protocol [14]. Briefly, wet mycelium was washed 3-4 times with sterile distilled water and ground in a pestle and mortar with LETS buffer (0.1 M lithium chloride, 10 mM EDTA, 10 mM Tris-base, 0.5% SDS). The homogenate was vortexed and centrifuged at 10,000 rpm for 10 min, and the supernatant was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). Following a second centrifugation, the aqueous phase was precipitated with chilled 70% ethanol, incubated on dry ice for 15 min, and centrifuged again; the resulting DNA pellet was air-dried and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

2.5 DNA quantification and quality assessment

DNA purity and concentration were assessed by UV-Vis spectrophotometry, using absorbance at 260 nm to estimate DNA concentration and the A_{260}/A_{280} and A_{260}/A_{230} ratios as indicators of protein and polysaccharide/phenolic contamination, respectively. DNA integrity was further verified by electrophoresis on a 1% agarose gel prepared in TAE buffer, stained with ethidium bromide (0.5 µg/mL), run at 80 V, and visualized on a gel documentation system against a 1 kb DNA ladder.

2.6 Antibacterial activity assay

The antibacterial activity of a methanolic extract of *C. tropicalis* was evaluated against the pathogenic bacterium *Pseudomonas aeruginosa* by the disc/well diffusion method. Sterile Nutrient Agar plates were seeded with the test bacterium, wells were cut with a sterile cork borer, and the fungal extract was loaded into the wells alongside a standard antibiotic disc (positive control), sterile water and methanol (solvent control). Plates were incubated at 37°C for 24 h, and the diameter of the zone of inhibition around each well was measured.

3. Results and Discussion

3.1 Morphological and cultural characteristics

The isolate, designated **CtDIC#001** (Table 2), grew slowly on PDA and SDA (radial growth of 3-4 cm/week), consistent with previously reported growth rates for *C. tropicalis* [12]. The optimum pH for growth was found to be pH 4. Colonies were initially white, turning pale yellow with maturity, particularly following sporulation.

Table 2. Fungal strain used in this study

S. No.	Fungal strain	Source	Strain code
1	*Cantharellus tropicalis*	Bio-Design Innovation Centre, RDVV, Jabalpur (M.P.)	CtDIC#001

3.2 Microscopic identification

Slide-culture preparations stained with lactophenol cotton blue revealed hyaline, septate hyphae, supporting the identification of the isolate as *C. tropicalis*, a species rarely documented from Indian tropical forests.

3.3 Genomic DNA extraction and quality assessment

The LETS method yielded genomic DNA suitable for downstream molecular work. UV-Vis spectrophotometry gave A_{260}/A_{280} ratios close to the widely accepted value of 1.8, indicating negligible protein contamination, and A_{260}/A_{230} ratios within the 2.0-2.2 range considered indicative of high-purity DNA, suggesting effective removal of co-extracted polysaccharides and phenolic compounds that commonly interfere with fungal DNA preparations [10]. Agarose gel electrophoresis confirmed clear, high-molecular-weight bands with no visible smearing, indicating minimal shearing or degradation. Together, these results indicate that the LETS protocol, despite its simplicity relative to CTAB- or bead-beating-based methods commonly evaluated for fungal DNA [7,15], was effective for a species known to possess abundant extracellular polysaccharides, and that the resulting DNA is suitable for applications such as ITS amplification and phylogenetic analysis.

3.4 Antibacterial activity

The methanolic extract of *C. tropicalis* produced a 16 mm zone of inhibition against *P. aeruginosa*, compared with 25 mm for the standard antibiotic and no inhibition for the water control (Table 3). The activity of the methanolic extract relative to the aqueous control suggests that the responsible bioactive compounds are more soluble in organic solvents, a pattern commonly reported for phenolic and terpenoid constituents of related chanterelles such as *C. cibarius* [3]. While this study did not chemically isolate or identify these compounds, the observed activity provides a basis for future phytochemical screening and compound purification.

Table 3. Antibacterial activity of *C. tropicalis* against *P. aeruginosa* (disc/well diffusion method)

Sample	Zone of inhibition (mm)
Standard antibiotic (positive control)	25
C. tropicalis methanolic extract	16
Methanol (solvent control)	3
Water (negative control)	0

3.5 Broader significance

As an ectomycorrhizal fungus, *C. tropicalis* forms mutualistic associations with forest trees that can enhance nutrient uptake and contribute to plant health [4]. Its tolerance of acidic conditions (optimum pH 4) suggests an ability to persist in nutrient-poor soils. Although sequencing was beyond the scope of the present study, the validated DNA extraction protocol described here lays the groundwork for future multilocus phylogenetic and barcoding studies, which have proven valuable for resolving diversity within tropical *Cantharellus* [6,11].

4. Conclusion

Cantharellus tropicalis presents itself as a promising fungal species from both a taxonomic and a biotechnological perspective. This study establishes a reliable, low-cost workflow, comprising morphological identification, LETS-based genomic DNA extraction, and UV-Vis/gel-based quality assessment, for characterizing *C. tropicalis*, and provides preliminary evidence that the fungus possesses antibacterial activity against *P. aeruginosa*. Given the limited data available on tropical *Cantharellus* species from India, these findings help fill an important gap and open avenues for further research involving molecular barcoding, genome sequencing, secondary-metabolite profiling and bioprospecting.

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Conflict of interest

The authors declare no conflict of interest.

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