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Morphological and ITS-based identification of *Meripilus lineatus* from Keralam, India, with antibacterial, antioxidant and reactive orange decolourisation activities

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Abstract
The wood-decaying basidiomycete *Meripilus lineatus* was collected from a living *Delonix regia* tree on the campus of Government College for Women, Thiruvananthapuram, Keralam, India, and characterised using morphological and internal transcribed spacer (ITS) sequence data. The isolate produced a 504-bp ITS sequence (GenBank accession PZ575695.1), which showed 96.61% identity and 100% query coverage to reference sequences assigned to *M. lineatus*. Morphological observations and sequence comparisons supported its identification. The methanolic extract exhibited concentration-dependent antibacterial activity against *Bacillus cereus* and *Escherichia coli* across the reported 250–1000 µg test range. In the DPPH assay, radical-scavenging activity increased from 16.1% at 50 µg/mL to 69.8% at 250 µg/mL; the reported IC50 was approximately 185–190 µg/mL. The FRAP response increased from 0.162 to 0.210 absorbance units at 593 nm across 50–150 µg/mL. The isolate also visibly decolourised Reactive Orange dye on agar after 5–7 days. These findings identify *M. lineatus* as a candidate for further study of fungal bioactivity and dye removal. The reported activities are preliminary and require follow-up using validated quantitative assays and chemical characterisation.

Keywords: Macrofungi, taxonomy, antibacterial activity, DPPH, FRAP, reactive orange, mycoremediation

Introduction
Wood-decaying basidiomycetes contribute to lignocellulose decomposition and nutrient cycling and are investigated as sources of enzymes and secondary metabolites with potential biotechnological applications. White-rot fungi can transform complex aromatic compounds through extracellular oxidative systems, which has motivated research on their use in dye-containing wastewater treatment (Wesenberg *et al*., 2003; Gao *et al*., 2010; Rodríguez-Couto, 2017) [3, 5].
Accurate identification is essential when linking fungal taxa to biological activity. Macromorphological and microscopic characters provide an important taxonomic foundation, while DNA sequence data can help resolve morphologically similar taxa. The nuclear ribosomal ITS region is widely used as a fungal barcode because it combines broad amplification success with useful interspecific variation (White *et al*., 1990; Schoch *et al*., 2012) [4, 7]. Recent taxonomic studies have reassessed species boundaries and generic relationships within Meripilaceae, supporting the placement of *M. lineatus* in *Meripilus*, formerly treated as *Rigidoporus lineatus* (Westphalen *et al*., 2025) [6].
Although biological activities have been reported for several *Meripilus* species, the available information on *M. lineatus* remains limited. This study combined morphological examination and ITS-based identification of a collection from Thiruvananthapuram, Kerala, with preliminary assessment of antibacterial activity, antioxidant capacity and dye decolourisation.

Materials and Methods
Collection, isolation and morphology
The basidiocarp of *M. lineatus* were collected from wood at the basal region of a *Delonix regia* tree on the campus of Government College for Women, Thiruvananthapuram, Keralam, India.
The specimen was transported to the laboratory, surface-treated with 70% ethanol, and internal tissue was aseptically transferred to potato dextrose agar (PDA; HiMedia, India). Cultures were incubated at 28–30 °C and purified by repeated subculturing (Fig.1). Morphological observations were made on fresh and dried material. Hand-cut sections were stained with 1% Congo Red and mounted in 3% KOH for microscopic examination using an Olympus CX43 microscope. Twenty basidiospores were measured, and colour terminology followed Kornerup and Wanscher (1978) [2]. The specimens were deposited in the Mycological Herbarium of the Postgraduate and Research Department of Botany, Government College for Women, Thiruvananthapuram.

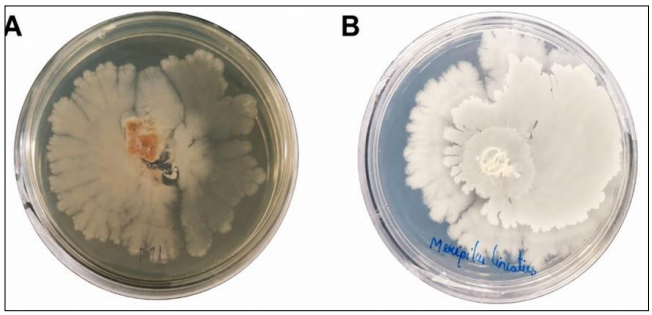


Fig 1: A- Surface sterilized fungal tissue inoculated on PDA medium and showing mycelial growth. B - Pure cultures of *M. lineatus* obtained by subculturing.

DNA extraction and ITS sequence analysis

Genomic DNA was extracted from seven-day-old mycelium using an SDS-based method. The ITS region was amplified using the universal primers ITS1 and ITS4 (White *et al*., 1990) [7], and the PCR products were verified by electrophoresis on a 2% (w/v) agarose gel. The amplified products were purified and subjected to Sanger sequencing. Sequence quality was assessed using Sequence Scanner software, and terminal regions were trimmed to obtain a 504-bp ITS sequence. The sequence was compared with reference sequences in NCBI GenBank using BLASTN. Sequence assembly and alignment were performed using BioEdit v7.0.5 and MAFFT v. 7, respectively. Phylogenetic analysis was conducted in MEGA12 using the neighbour-joining method with 1,000 bootstrap replicates. The ITS sequence was deposited in GenBank under accession number PZ575695.

Preparation of methanolic extract

Dried basidiocarp powder (10 g) was mixed with 100 mL methanol (1:10, w/v) and agitated on an orbital shaker for 24–48 h at room temperature. The suspension was filtered, and the solvent was evaporated. The crude extract obtained was 1.134 g, with a reported extraction yield of 11.34% (w/w). The extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution at a concentration of 20 mg/mL for antibacterial screening.

Antibacterial assay

The antibacterial activity of the methanolic extract of *M. lineatus* was evaluated by the agar-well diffusion method against *Bacillus cereus* and *Escherichia coli*. Freshly grown bacterial cultures were uniformly swabbed onto Mueller–Hinton agar plates, and wells of uniform diameter were aseptically prepared using a sterile cork borer. The extract stock solution (20 mg/mL in dimethyl sulfoxide) was added to the wells at loads of 250, 500, 750 and 1000 µg/well. Gentamicin (120 µg) served as the positive control. The plates were allowed to stand at room temperature for 15–30 min to facilitate diffusion and subsequently incubated at 37°C for 24 h. Antibacterial activity was assessed by measuring the diameters of the inhibition zones in millimetres. The assay was performed in triplicate, and results were expressed as mean inhibition-zone diameters with variation. Fig. 3

DPPH radical-scavenging assay

The antioxidant activity of the methanolic extract of *M. lineatus* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. The extract was dissolved in methanol to prepare a stock solution of 1 mg/mL, from which working concentrations of 50, 100, 150, 200 and 250 µg/mL were prepared. For each concentration, 3 mL of the extract solution was mixed with 1 mL of freshly prepared 0.1 mM methanolic DPPH solution. The reaction mixtures were shaken thoroughly and incubated in the dark at 27 °C for 20 min. A control containing methanol in place of the extract and a methanol blank without DPPH were used. After incubation, absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The percentage of DPPH radical inhibition was calculated using the formula: (%) Inhibition = [(Acontrol − Asample) / Acontrol] × 100, where Acontrol and Asample represent the absorbance of the control and extract-containing reaction mixture, respectively. The assay was performed in triplicate.

Ferric-reducing antioxidant power (FRAP)

The ferric-reducing antioxidant power of the methanolic extract of *M. lineatus* was evaluated using the FRAP assay. The assay was based on the reduction of the ferric (Fe^{3+})–TPTZ complex to the ferrous (Fe^{2+}) form in the presence of antioxidant compounds. The resulting colour change was measured spectrophotometrically at 593 nm. The extract was assessed at concentrations of 50, 100 and 150 µg/mL, with absorbance values of 0.162, 0.172 and 0.210, respectively. The assay was performed in triplicate. The increase in absorbance with increasing extract concentration indicated an enhanced ferric-reducing response.

Dye decolourisation

The dye decolourisation potential of *M. lineatus* was evaluated using the synthetic azo dyes Reactive Orange into potato dextrose agar (PDA). The medium was sterilised by autoclaving at 121 °C for 15 min and cooled to 45–50 °C before adding 0.0009 g of Reactive Orange to the media. The dye-amended media was mixed thoroughly, poured into sterile Petri plates and allowed to solidify. A 5 mm diameter mycelial disc from the actively growing margin of a fresh fungal culture was inoculated at the centre of each plate. The plates were incubated aerobically at 28 ± 2 °C for 5–7 days, and the experiment was performed in triplicate. Dye-containing uninoculated plates served as controls for changes in colour unrelated to fungal growth, while inoculated PDA plates without dye were maintained to assess normal fungal growth. The plates were examined periodically for reductions in colour intensity and the formation of decolourised zones around the growing mycelium. Changes were documented photographically and compared with the corresponding uninoculated controls. Decolourisation was assessed qualitatively.

Results

Isolation and morphological identification

A pure culture of the fungal isolate was successfully established from the inner tissue of the basidiocarp following surface sterilisation and repeated subculturing on potato dextrose agar (PDA). Based on the macroscopic and microscopic characteristics, the specimen was identified as *M. lineatus* (Pers.) Westph. & Rajchenb. The basidiomata were annual, pileate and sessile, occurring singly or in imbricate clusters, with dimidiate pilei measuring up to 3 × 2 cm and 10 mm in thickness. The pilear surface was concentrically zonate, pale orange (5A3) when fresh and greyish orange (5B5) upon drying. The pore surface was pale orange (5A3) when fresh, becoming brownish orange (5C5) on drying, with round to angular pores at a density of 6–7 per mm. The context was homogeneous, pale yellow (4A3) and up to 2 mm thick, while the yellowish-white (4A2) pore tubes reached 3 mm in depth. The margins were acute and up to 5 mm thick. Microscopic examination revealed a monomitic hyphal system with hyaline, simple-septate, moderately branched generative hyphae, up to 8 µm wide. Cystidia were rare, club-shaped, thick-walled and coarsely encrusted, measuring 35.4–42.8 × 7.8–12.6 µm. The basidia were clavate, four-sterigmate and lacked basal clamps, measuring 11.5–12.6 × 4.1–4.6 µm. The basidiospores were smooth, thin-walled, hyaline and

cylindrical, measuring (4.1–) 4.3–4.6 (–5) × (2.3–) 2.5–2.9 (–3.4) µm. The combination of these macroscopic and microscopic characteristics supported the identification of the collection as *M. lineatus*. The principal morphological features are presented in Figure 2.



Fig 2: *Meripilus lineatus.* A-C Habit *in situ*. D – Generative hyphae

ITS sequence analyses

The molecular identification of the isolate was supported by analysis of the internal transcribed spacer (ITS) region of ribosomal DNA. Following sequence quality assessment and trimming of the terminal regions, a 504-bp ITS sequence was obtained and deposited in the NCBI GenBank database under accession number PZ575695.1. BLASTN analysis revealed 100% query coverage and 96.61% sequence identity with the reference *M. lineatus* sequences MT309474 and OR636131. The E-value of 0.0 indicated that the sequence matches were highly significant. These molecular findings, considered alongside the observed macroscopic and microscopic characteristics, support the identification of the isolate as *M. lineatus*.

Antibacterial activity

The methanolic extract of *M. lineatus* exhibited concentration-dependent antibacterial activity against Bacillus cereus and Escherichia coli, as determined by the agar well diffusion assay. The diameter of the inhibition zone increased with increasing extract concentration in both bacterial species, indicating an enhanced inhibitory response at higher extract loads. Against B. cereus, the inhibition zone increased from 20.0 ± 0.5 mm at 250 µg/well to 24.0 ± 0.5 mm at 1000 µg/well. Similarly, E. coli exhibited an increase in the inhibition zone from 18.0 ± 0.6 mm to 24.0 ± 0.6 mm across the same concentration range. At the lowest tested concentration, B. cereus showed a larger inhibition zone than E. coli, suggesting greater susceptibility under the experimental conditions (Fig. 3). However, at 1000 µg/well, both organisms exhibited an inhibition-zone diameter of 24.0 mm, although the reported variation differed slightly between them. These findings demonstrate that the methanolic extract inhibited both Gram-positive and Gram-negative bacteria, with the magnitude of inhibition increasing with extract concentration. Gentamicin (120 µg), used as the positive control, produced inhibition zones against the tested bacterial strains

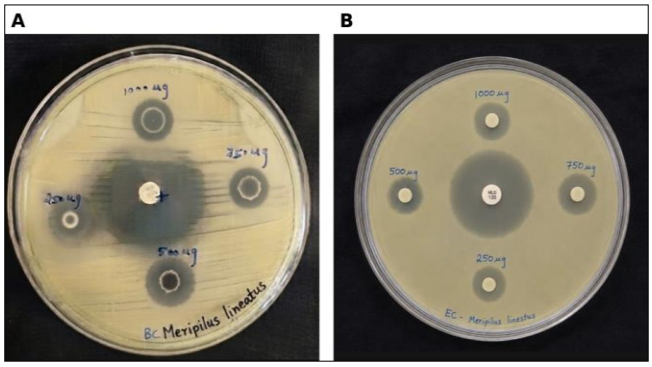


Fig 3: Antimicrobial plates of different organisms showing inhibition zone. A-*Bacillus cereus*, B- *E. coli*

Table 1: Positive antibacterial responses reported for the methanolic extract of *M. lineatus.*

Test organism	Inhibition zone at 250 µg (mm)	Inhibition zone at 1000 µg (mm)
Bacillus cereus	20.0 ± 0.5	24.0 ± 0.5
Escherichia coli	18.0 ± 0.6	24.0 ± 0.6

DPPH radical-scavenging activity

The methanolic extract of *M. lineatus* exhibited concentration-dependent DPPH radical-scavenging activity across the tested concentration range of 50–250 µg/mL (Table 2). The percentage of radical inhibition increased progressively from 16.1 ± 0.4% at 50 µg/mL to 26.2 ± 0.5%, 39.6 ± 0.6%, and 56.4 ± 0.7% at 100, 150, and 200 µg/mL, respectively, reaching a maximum of 69.8 ± 0.8% at 250 µg/mL. This progressive increase in inhibition indicates an enhanced capacity of the extract to scavenge DPPH radicals at higher concentrations.

Ascorbic acid, used as the reference antioxidant, exhibited greater radical-scavenging activity than the fungal extract at all tested concentrations. Its inhibition increased from 29 ± 0.6% at 50 µg/mL to 49 ± 0.7%, 65 ± 0.8%, and 80 ± 0.7% at 100, 150, and 200 µg/mL, respectively, reaching 90 ± 0.9% at 250 µg/mL. Although the extract showed lower activity than the standard, its steadily increasing inhibition demonstrates appreciable concentration-dependent antioxidant activity under the experimental conditions.

The IC_{50} of the methanolic extract was reported to be approximately 185–190 µg/mL, representing the concentration required to achieve 50% DPPH radical inhibition. This value is consistent with the observed increase in scavenging activity between 150 and 200 µg/mL. Overall, the findings indicate that the methanolic extract of M. lineatus possesses measurable free-radical-scavenging potential, although its activity was lower than that of ascorbic acid at the corresponding concentrations. (Fig. 4)

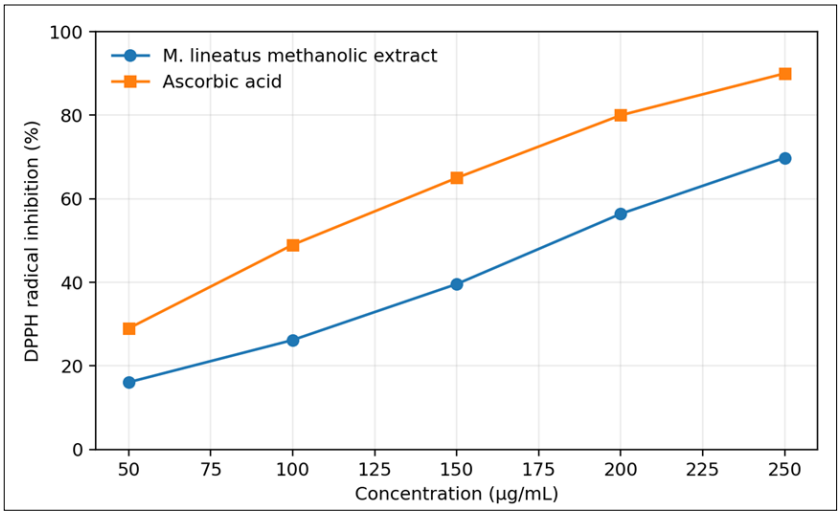


Fig 4: DPPH radical-scavenging activity of the methanolic extract compared with ascorbic acid

Table 2: DPPH radical-scavenging activity reported in the dissertation (mean ± reported variation; n = 3)

Concentration (µg/mL)	Extract inhibition (%)	Ascorbic acid inhibition (%)
50	16.1 ± 0.4	29 ± 0.6
100	26.2 ± 0.5	49 ± 0.7
150	39.6 ± 0.6	65 ± 0.8
200	56.4 ± 0.7	80 ± 0.7
250	69.8 ± 0.8	90 ± 0.9

Ferric-reducing antioxidant power (FRAP) activity

The ferric-reducing antioxidant power of the methanolic extract of *M. lineatus* was evaluated by measuring absorbance at 593 nm. The extract exhibited an increase in absorbance with increasing concentration, rising from 0.162 ± 0.003 at 50 µg/mL to 0.172 ± 0.004 at 100 µg/mL and 0.210 ± 0.005 at 150 µg/mL (Table 3). The highest absorbance was recorded at 150 µg/mL among the three extract concentrations for which results are clearly documented.

The increase in absorbance indicates an enhanced ferric-reducing response at higher extract concentrations. In the FRAP assay, antioxidant constituents reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, resulting in the formation of a coloured Fe^{2+}–TPTZ complex. The intensity of this colour, measured spectrophotometrically, reflects the reducing capacity of the sample. Accordingly, the observed increase in absorbance suggests that the extract contains constituents capable of electron donation and ferric-ion reduction.

These findings, considered alongside the DPPH radical-scavenging results, provide complementary evidence of the *in vitro* antioxidant potential of M. lineatus (Fig. 5).

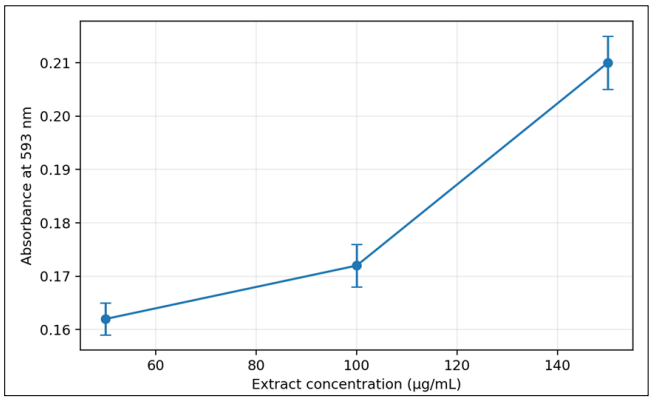


Fig 5: FRAP absorbance of the methanolic extract at 593 nm

Reactive Orange decolourisation

The isolate of *M. lineatus* exhibited visible decolourisation of Reactive Orange dye incorporated into potato dextrose agar (PDA). A reduction in colour intensity was observed around the actively growing mycelium after 5–7 days of incubation at 28 ± 2°C under aerobic conditions. The uninoculated dye-containing control plates showed no visible change in colour, supporting the association of the observed decolourisation with fungal growth. The assay was performed in triplicate. (Fig. 6)

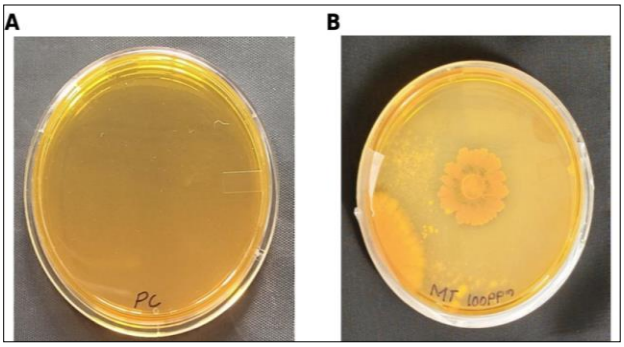


Fig 6: Positive controls of Reactive Orange (A) and Reactive orange supplemented plate showing dye degradation to fungal growth (B)

The observed reduction in colour intensity indicates the potential of *M. lineatus* for further investigation in fungal dye-decolourisation and bioremediation studies. However, the decolourisation was assessed qualitatively, as quantitative measurements of decolourised-zone diameter and spectrophotometric estimates of dye removal were not available. In addition, chemical analyses confirming dye transformation were not performed. Therefore, the results demonstrate visible Reactive Orange decolourisation under the experimental conditions but do not establish the extent of dye removal or confirm complete chemical degradation.

Discussion

The combined macroscopic and microscopic characteristics, together with ITS sequence analysis, support the identification of the Keralam collection as *M. lineatus*. The ITS sequence deposited in GenBank under accession number PZ575695.1 provides a traceable molecular record of the isolate.

The methanolic extract exhibited antibacterial activity against Bacillus cereus and Escherichia coli, with larger inhibition zones at the highest tested extract load than at the lowest. These findings indicate concentration-dependent antibacterial activity under the experimental conditions. However, agar well diffusion is a preliminary screening method, and inhibition-zone diameters are influenced by compound diffusion and assay conditions. The results therefore do not establish the minimum inhibitory concentration (MIC) or identify the compounds responsible for the observed activity. Further studies using standardised susceptibility-testing methods, appropriate solvent controls and independently verified replicate-level data are required to characterise the antibacterial potential of the extract.

The DPPH assay demonstrated a concentration-dependent increase in radical-scavenging activity, reaching 69.8% inhibition at 250 µg/mL. The reported IC_{50} of approximately 185–190 µg/mL is consistent with the observed response trend, although it should be verified by recalculation from the original replicate-level data. Similarly, the FRAP assay showed increasing absorbance across the clearly documented extract concentrations, indicating an enhanced ferric-reducing response. As DPPH and FRAP assess different aspects of antioxidant activity, their results provide complementary evidence of the *in vitro* antioxidant potential of the extract. Nevertheless, these findings alone do not establish biological or clinical antioxidant efficacy.

The qualitative decolourisation of Reactive Orange around the growing mycelium suggests that M. lineatus may have potential for further investigation in dye-removal and bioremediation studies. However, visible colour reduction alone cannot distinguish enzymatic degradation from biosorption or other processes. Further investigations involving quantitative absorbance measurements, appropriate abiotic and biomass controls, and chemical characterisation of the treated dye are necessary to determine the extent of dye removal and establish whether chemical transformation has occurred. Accordingly, the present findings provide a preliminary basis for further investigation but do not establish detoxification or the effectiveness of the fungus in full-scale wastewater treatment.

Conclusion

The Kerala isolate reported as *M. lineatu*s was characterised using morphology and ITS sequence data. Its methanolic extract showed positive antibacterial activity against *B. cereus and E. coli*, concentration-dependent DPPH radical-scavenging activity and an increasing FRAP response. The isolate also visibly decolourised Reactive Orange on agar. These findings provide preliminary evidence for further taxonomic, chemical and biotechnological investigation. Validation using original raw data, standardised assays, chemical characterisation and quantitative dye-removal analysis is needed before practical applications can be proposed.

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Declarations

Funding: No funding statement was identified in the supplied dissertation text.

Conflicts of interest: The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to the conceptualization, methodology, investigation, data analysis, interpretation of results, and preparation of the manuscript. All authors reviewed and approved the final version of the manuscript.

Data availability: The ITS sequence is reported in the dissertation as GenBank accession PZ575695.1. Other data are presented in the article and its figures/tables.

Ethics approval: Ethical approval was not required for this study, as it did not involve human participants or experimental procedures involving animals.

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