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Prospecting laccase-producing wood-decaying fungi from the UPR peat swamp forest, Central Kalimantan

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ABSTRACT

Tropical peat forests of Kalimantan harbor diverse wood-decaying fungi with strong ligninolytic potential, yet their functional enzymatic characteristics remain poorly documented. This study prospectively examined the physiological expression of laccase production in native fungi isolated from the Universitas Palangka Raya peat swamp forest, Central Kalimantan. Three stable pure cultures were obtained: MIPA 2 (*Ganoderma* sp.), MIPA 4 (*Trametes* sp. 1), and MIPA 8 (*Trametes* sp. 3). All isolates produced extracellular laccase but showed clear differences in growth and enzyme productivity. Growth analysis revealed distinct radial expansion rates, with MIPA 2 exhibiting the fastest mycelial growth (13.64 mm/day). The faster radial growth of MIPA 2 suggests higher metabolic activity and biomass formation, which may contribute to its superior laccase production compared to the other isolates. Qualitative screening on syringaldazine-amended PDA also confirmed extracellular laccase activity in all isolates, with activity levels ranging from weak (+) to strong (+++). Quantitative assays in potato dextrose broth and modified glucose–peptone medium revealed strong nutrient-dependent regulation of laccase biosynthesis, with MIPA 2 reaching 93.71 U/L on day 21. The pronounced stimulation by organic nitrogen indicates ecological adaptation to acidic, nutrient-limited peat environments and distinguishes these isolates from many previously reported *Ganoderma* and *Trametes* strains that typically require external inducers for high activity. These findings demonstrate that pea t swamp ecosystems represent not only a geographic source but also a reservoir of fungi with distinct ligninolytic expression profiles and potential biotechnological relevance.

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1. Introduction

Indonesia's tropical forests, especially in Kalimantan, are internationally acknowledged for their remarkable biodiversity, encompassing a substantial and distinctive collection of microorganisms (Liu et al., 2020). Notwithstanding this abundance, the biological potential of forest microorganisms remains underexplored (Liu et al., 2020; Peraza-Jiménez et al., 2022). Among these microorganisms, fungi assume a vital ecological function in the breakdown of lignocellulosic biomass, thereby playing a significant role in nutrient cycling and carbon turnover within forest ecosystems (Peraza-Jiménez et al., 2022). Wood-decaying fungi, particularly white-rot fungi belonging to the Basidiomycota phylum, are distinguished by their remarkable capacity to decompose lignin via the secretion of extracellular ligninolytic enzymes (Dashtban et al., 2010; Kumar et al., 2024; Moreno et al., 2015). Essential enzymes in this process comprise laccase (EC 1.10.3.2), manganese peroxidase (MnP), and lignin peroxidase (LiP), which initiate oxidative depolymerization of lignin into smaller aromatic compounds that can subsequently enter downstream metabolic pathways for further transformation and eventual mineralization (Dashtban et al., 2010; Kumar et al., 2024; Martínez et al., 2005; Suryadi et al., 2022). However, the sustainability of these microbial resources is increasingly jeopardized by ecological disturbances, including land-use change, peatland drainage, forest fires, and overexploitation (Bandla et al., 2024). The stresses are especially intense in Kalimantan's peat ecosystems, which have seen repeated fires and habitat degradation in the past two decades (Schmidt et al., 2024). Thus, investigating and preserving native ligninolytic fungi is essential for harnessing their biotechnological potential, particularly for the discovery of new laccase-producing strains.

Laccase, a multicopper oxidase, has garnered significant scientific interest owing to its broad substrate specificity and ability to oxidize phenolic and other aromatic compounds using molecular oxygen as the terminal electron acceptor, producing water as the sole by-product (Arica et al., 2017; Dashtban et al., 2010). Its catalytic versatility facilitates applications in the bioremediation of textile colors and phenolic contaminants (Agnestisia et al., 2024, 2025; Aragaw et al., 2024; Mahdy and Suttinun, 2023; Paraschiv et al., 2022), wastewater treatment (Paraschiv et al., 2022), lignocellulosic biomass processing in the pulp and paper sector

(Moreno et al., 2020; Singh and Arya, 2019; Upadhyay et al., 2016), bioethanol production, biosensors, and pharmaceutical biocatalysis (Moreno et al., 2020; Upadhyay et al., 2016). Moreover, laccase is extensively found throughout various biological kingdoms, including fungi, bacteria, plants, and certain insects, although Basidiomycete fungi are the most abundant natural producers (Baldrian, 2006).

Tropical peat swamp ecosystems represent a distinct ecological niche characterized by acidic pH, persistent waterlogging, low oxygen availability, and nutrient limitation. Such environmental pressures may influence the regulation and expression of ligninolytic enzyme systems, potentially resulting in physiological traits that differ from those of fungi inhabiting typical wood-decaying environments. Despite numerous reports of laccase-producing fungi from Indonesia, primarily isolated from decaying wood, plantation forests, and agro-industrial residues, studies focusing specifically on tropical peat swamp fungi remain limited. To the best of our knowledge, no previous study has reported the isolation and physiological evaluation of laccase-producing fungi from the Universitas Palangka Raya peat swamp forest, highlighting the ecological and scientific relevance of the present work.

Nevertheless, systematic data on the diversity and physiological expression of laccase-producing fungi in the Universitas Palangka Raya (UPR) peat swamp forest remain limited. This gap prompts a specific scientific question: whether peat swamp fungi constitute a distinct and potentially promising source of laccases with adaptive production profiles. We therefore propose the hypothesis that fungi inhabiting acidic, nutrient-limited peat environments exhibit differentiated regulation of laccase expression compared with those from typical wood-decaying habitats. To address this question, the present study aims to (i) isolate native wood-decaying fungi from the UPR peat swamp forest, (ii) perform qualitative screening of extracellular laccase activity, and (iii) comparatively evaluate enzyme production under different culture conditions.

The scope of this work was confined to physiological screening and comparative production profiling of crude enzyme extracts; it does not extend to enzyme purification, kinetic characterization, or detailed biochemical analysis. This study is intended to provide baseline data to support future in-depth enzymatic and molecular investigations.

2. Materials and methods

2.1. Materials

The materials used in this study included fungal fruiting-body samples collected from the peat forest area of the University of Palangka Raya, along with culture media such as Potato Dextrose Agar (PDA; 39 g/L, Merck, ≥99% purity) and Potato Dextrose Broth (PDB; 24 g/L, Merck, ≥99% purity), as well as a modified glucose – peptone medium prepared using analytical-grade reagents. Sample sterilization employed absolute ethanol (Merck, ≥99.9% purity) and sterile distilled water. Laccase screening and activity assays utilized syringaldazine (Sigma-Aldrich, ≥98% purity) and 0.1 M sodium tartrate buffer (pH 5.3; analytical grade).

2.2. Methods

2.2.1. Fungal sampling, identification, and isolation

Fungal fruiting-body samples were obtained from the peat forest surrounding the University of Palangka Raya area by a purposeful sampling method. The fungal fruiting bodies were photographed using a digital camera and identified based on macroscopic characteristics with reference to established fungal identification literature and reputable online taxonomic resources. Subsequent to collection, the fungi were separated employing the surface sterilisation technique (Ilyas et al., 2006). The fungal fruiting bodies were initially cleansed using a sterile brush to eliminate debris, subsequently diced into small cubes. The surface sterilization process was performed by immersing the samples in 75% ethanol for 30 s, followed by treatment with 1% sodium hypochlorite solution for 2 minutes, and subsequently rinsing three times with sterile distilled water. The fungal fragments were desiccated on sterile paper towels to eliminate surplus moisture. Upon drying, the samples were placed onto PDA (39 g/L) media supplemented with 0.01% chloramphenicol and incubated at room temperature. The fungal growth rate was monitored during incubation by measuring radial colony expansion (mm) along two perpendicular axes, and the average value was recorded. Measurements were taken at 24-hour intervals. Upon sufficient colony development, actively growing mycelial tips were aseptically transferred onto slanted PDA medium to establish stable pure isolates.

2.2.2. Fungal laccase-producing screening

All fungal isolates were cultivated on PDA plates supplemented with 0.1% syringaldazine, an indicator substrate for laccase activity, in order to screen for laccase synthesis (Vaidyanathan et al., 2011). After six days of incubation at room temperature, plates displaying characteristic reddish-brown or purplish color changes surrounding the fungal colonies were deemed positive for laccase production and were chosen for subsequent investigations.

Table 1. Morphological and taxonomic characteristics of fungal samples

Sample code	Species name	Family	Class	Genus	Mode of Nutrition	Substrate	Color
MIPA 1	*Trametes* sp.1	Polyporaceae	Agaricomycetes	*Trametes*	Saprophytic	Wood trunk	Creamy white with yellowish tint
MIPA 2	*Ganoderma* sp.1	Ganodermataceae	Basidiomycetes	*Ganoderma*	Parasitic	Tree trunk	Dark brownish-purple
MIPA 3	*Ganoderma* sp.2	Ganodermataceae	Basidiomycetes	*Ganoderma*	Parasitic	Tree trunk	Reddish brown
MIPA 4	*Trametes* sp.2	Polyporaceae	Agaricomycetes	*Trametes*	Saprophytic	Tree trunk	Creamy white with light brown margin
MIPA 5	*Polyporus* sp.	Polyporaceae	Agaricomycetes	*Polyporus*	Saprophytic	Wood trunk	White to pale pink
MIPA 6	*Trametes* sp.3	Polyporaceae	Agaricomycetes	*Trametes*	Saprophytic	Tree trunk	Yellowish cream
MIPA 7	*Ganoderma* sp.1	Ganodermataceae	Basidiomycetes	*Ganoderma*	Saprophytic	Tree trunk	Bright orange
MIPA 8	*Trametes* sp.4	Polyporaceae	Agaricomycetes	*Trametes*	Saprophytic	Tree trunk	Light brown to creamy beige

2.2.3. Fungal culture for laccase production

Ten agar discs (5 mm diameter) of actively growing fungal mycelia from PDA plates were aseptically transferred into 250 mL Erlenmeyer flasks containing 100 mL of culture medium. Two media were used: potato dextrose broth (PDB, 24 g/L) and a modified glucose–peptone medium. The modified glucose–peptone medium (per liter) consisted of 10 g glucose, 3 g peptone, 0.4 g K_2HPO_4, 0.5 g $MgSO_4$, 0.05 g $MnSO_4$, 0.001 g $ZnSO_4$, 0.0005 g $FeSO_4$, and 100 mL of 0.1 M phosphate buffer (pH 5.5). The initial pH of the medium was adjusted to 5.5 prior to sterilization (Agnestisia et al., 2024). Cultures were incubated at 27 ± 2 °C under static conditions in the dark for 7, 14, 21, and 28 days. Static cultivation was selected to allow surface-associated mycelial growth and natural oxygen diffusion, conditions commonly associated with enhanced extracellular laccase production in filamentous fungi. The culture broth was then filtered through Miracloth to remove mycelial biomass, and the resulting supernatant was used as the crude enzyme extract for laccase activity assays.

2.2.4. Laccase activity assay

Laccase activity was assessed using a syringaldazine substrate (Leonowicz and Gzrywnowicz, 1981). The reaction was conducted within a 3 mL cuvette. The cuvette contained 0.1 M sodium tartrate buffer (750 µL) with a pH of 5.3, 0.5 mM syringaldazine (200 µL), and an enzyme (1.8 mL). The reaction was monitored through the increasing absorbance at 525 nm (ε^{525} = 65,000 M_{-1}•cm^{-1}) over a duration of 2 minutes. Enzyme activity was expressed as U/mL of laccase, with one unit (U) denoting the amount of enzyme required to catalyze the oxidation of 1 µmol of syringaldazine per minute under the specified assay conditions.

3. Results and discussion

3.1. Fungal identification and isolation

Eight fungal fruiting-body samples were successfully collected during this study, all of which were found on wood-derived surfaces, including living trees, decaying trunks, and partially degraded woody debris inside the peat forest area of UPR. The sampling location was situated at 2.219614° S, 113.887265° E. The distribution of these specimens in different microhabitats demonstrates the ecological adaptability of wood-decaying fungi in peatland environments, where high moisture, acidity, and limited nutrients create selective pressures that favor versatile decomposer species. The occurrence of fruiting bodies on both living and deceased woody substrates signifies diversity in fungal lifestyles, encompassing parasitic or opportunistic taxa as well as exclusively saprophytic species engaged in lignocellulosic breakdown. Fig. 1 illustrates the macroscopic properties of a representative fruiting body and its corresponding substrate, offering an overview of the morphological parameters utilized for first field identification. These discoveries collectively emphasize the functional diversity of fungi in tropical peat forests and their potential as sources of ligninolytic enzymes, including laccases, for biotechnological applications.

The eight fruiting-body specimens obtained from the peat forest of the UPR area were subsequently analyzed for morphological identification (Table 1).

The findings indicate that the fungal samples were predominantly composed of members from the Polyporaceae (*Trametes*, *Polyporus*) and Ganodermataceae (*Ganoderma*), a taxonomic arrangement frequently encountered in the woody substrates of tropical and temperate forests (Mafia et al., 2020; Panjaitan et al., 2023).

These taxa are recognized as primary wood degraders within Basidiomycota and encompass groupings that contain numerous white-rot species proficient in substantial lignin breakdown (Li et al., 2022; Zhao et al., 2024). Furthermore, the majority of *Trametes* isolates (MIPA 1, MIPA 4, MIPA 6, MIPA 8) were documented from dead or rotting wood, referred to as "wood trunk" or "tree trunk" in Table 1, and were classified as saprophytic in their nutritional mode. This assignment aligns with the ecology of *Trametes* spp., recognized as saprotrophic white-rot fungus that inhabit decaying hardwood and play a significant role in lignocellulose decomposition and nutrient cycling (Frasconi et al., 2010; Galhaup and Haltrich, 2001; Suboh et al., 2022). *Trametes* sp.1 has been identified as an effective laccase producer in laboratory settings, underscoring its involvement in lignin degradation and rendering it a common focus in research on extracellular oxidases (Galhaup and Haltrich, 2001; Gonzalez et al., 2013).

Fig.1. Macroscopic characteristics of the fungal fruiting body samples

The *Ganoderma* isolates (MIPA 2, MIPA 3 and MIPA 7) display a more variable ecological strategy: while many *Ganoderma* species are parasitic pathogens of living trees, several species or strains also act as saprotrophs on dead wood or as opportunistic saprotrophs following host decline (Mafia et al., 2020). Thus, labeling *Ganoderma* sp.2 vas parasitic/opportunistic and *Ganoderma* sp.3 as saprophytic (Table 1) agrees with contemporary mycological accounts that emphasize facultative parasitism and trophic plasticity within the genus. The presence of *Ganoderma* fruiting bodies on standing trunks in our survey therefore plausibly reflects either active parasitism or colonisation of physiologically weakened host tissues. *Polyporus* sp. (MIPA 5) was observed on dead wood and displayed the orange-to-cream color transition typical of this species; the species is broadly documented as causing white rot on dead hardwoods and is therefore correctly assigned as saprophytic in Table 1. Its thin, tough context and characteristic pore morphology further support the field identification. The color descriptions recorded in the table serve as supportive morphological characters for preliminary field identification. Nevertheless, these traits must be interpreted with caution because coloration can vary with age, weathering, and substrate conditions. In comprehensive taxonomic practice, such color notes are typically evaluated alongside macroscopic features such as pileus architecture, context thickness, and pore surface, and ideally complemented by microscopic or molecular data such as ITS sequencing to achieve species-level resolution. In the present study, identification is limited to field-based morphological observations, while detailed laboratory identification through microscopic examination and DNA barcoding will be conducted in a subsequent research phase and is not included within the scope of the current work.

All collected fungal fruiting bodies was the isolated using a surface sterilization method. This procedure is essential for obtaining pure cultures, as wood inhabiting fungi naturally coexist with diverse microbial communities, including bacteria and other contaminating fungi that may interfere with accurate identification

and culture development (Ilyas et al., 2006). Sterilization was carried out aseptically using 75% alcohol followed by sterile distilled water to remove external contaminants while preserving internal mycelial tissues. The disinfected fragments were then transferred onto PDA, a carbohydrate rich medium suitable for fungal growth. Chloramphenicol (0.01%) was added to suppress bacterial proliferation during incubation without affecting fungal development. Incubation was carried out at room temperature for ten days until visible mycelial development appeared. Of the eight fruiting body samples processed, only three produced stable and consistent colonies displaying uniform radial growth and good adaptability to PDA, indicating their suitability for further laboratory analysis (Fig. 2). In contrast, the remaining five samples failed to establish viable cultures, likely due to factors such as suboptimal freshness of the fruiting bodies, an initial dominance of contaminating microorganisms, or physiological incompatibility of the fungi with the provided media and environmental conditions.

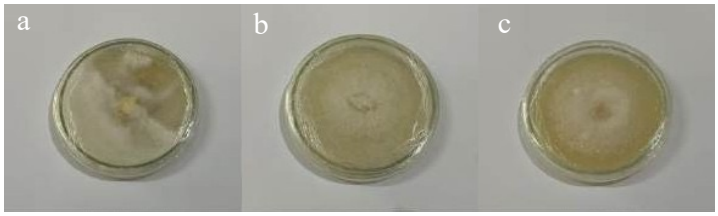

Fig. 2. Fungal isolates grown on PDA medium. Note: (a) MIPA 2, (b) MIPA 4, (c) MIPA 8

The three successfully isolated fungi also demonstrated distinct variations mycelial growth rates throughout the ten-day incubation period (Fig. 3). Growth was monitored daily by measuring colony diameter as the primary indicator. The observed differences in growth patterns and expansion rates suggest species specific metabolic capacities and differential physiological adaptation to the in vitro culture medium.

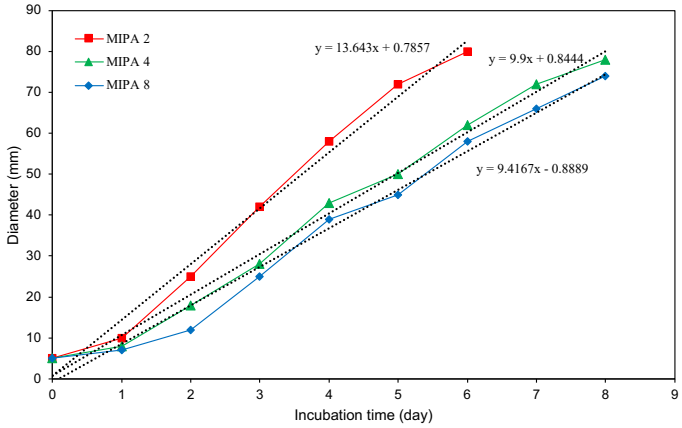

Fig. 3. The growth curve of the fungal isolates in the radial direction

The radial growth rate (Kr) of each isolate was determined from the slope of the linear portion of the mycelial growth curve. Radial growth was measured as colony diameter (mm) along two perpendicular axes, and the average value was recorded at 24-hour intervals. The obtained Kr values showed clear variation among isolates, with MIPA 2 exhibiting the fastest growth (13.64 mm/day), followed by MIPA 4 (9.90 mm/day) and MIPA 8 (9.42 mm/day). The growth rate of MIPA 2 was faster to that reported for the wood-decaying basidiomycete *Perenniporia* sp., which demonstrated a Kr of 9.28 mm/day under similar culture conditions (Abudarin et al., 2025). These values fall within the general range reported for white-rot and other wood-inhabiting fungi. For instance, *Pleurotus ostreatus* typically grows between 8-15 mm/day on PDA, depending on strain and temperature (Krupodorova et al., 2024). However, radial growth on PDA primarily reflects vegetative expansion under nutrient-rich laboratory conditions and does not directly measure ligninolytic efficiency or wood degradation capacity, as PDA lacks lignocellulosic substrates. Therefore, the Kr values presented here should be interpreted strictly as comparative indicators of in vitro growth performance.

It should be noted that the measurements were conducted on single plates for each isolate, and no biological replication was performed in this preliminary study. Consequently, no statistical analysis was applied. This represents a limitation of the present work, as the reported values reflect observational screening rather than statistically validated growth parameters. The primary objective at this stage was comparative evaluation among isolates to identify promising candidates for further investigation. Future studies will incorporate biological replicates and appropriate statistical analysis to strengthen the quantitative assessment of growth performance.

The observed variation among isolates likely reflects intrinsic physiological differences and differential adaptation to artificial culture conditions. Although rapid radial growth does not necessarily indicate superior wood-degrading ability, isolates with faster vegetative expansion may offer practical advantages for biotechnological applications, such as shorter inoculum preparation time, faster biomass accumulation, and more efficient colonization of production media. Nevertheless, further evaluation under lignocellulosic conditions and enzyme-based assays is required to determine their true degradation potential.

3.2. Laccase production screening of fungal isolates

All three fungal isolates tested exhibited positive qualitative responses for extracellular laccase production when grown on PDA supplemented with 0.1% syringaldazine. After six days of incubation, each isolate developed a distinct reddish-brown to purplish halo around the colony, indicating successful oxidation of syringaldazine, a chromogenic substrate specific for fungal laccases. The results of the screening are summarized in Table 2. Although the intensity of pigmentation varied among isolates, the consistent appearance of the characteristic color change confirms that all three isolates possess active laccase-secreting capabilities under the tested culture conditions. Syringaldazine oxidation is widely used as a reliable indicator of laccase activity because it is not significantly oxidized by other oxidoreductases, making it a robust qualitative assay for preliminary enzyme screening (Vaidyanathan et al., 2011). However, no positive or negative control strains were included in this preliminary screening study. The primary objective was to compare laccase-producing potential among newly isolated peat swamp fungi rather than to benchmark their activity against a well-characterized reference strain. This is acknowledged as a limitation of the present study. Future work will incorporate established laccase-producing and non-producing control strains to strengthen comparative validation and improve methodological robustness.

Table 2. Qualitative screening results for extracellular laccase activity in fungal isolates

Sample Code	Species Name	Screening Result
MIPA 2	*Ganoderma* sp.1	+++
MIPA 4	*Trametes* sp.2	+
MIPA 8	*Trametes* sp.4	++

Note: +++, strong laccase activity; ++, moderate laccase activity; +, weak laccase activity

These findings are consistent with previous reports indicating that wood-decaying Basidiomycota, particularly species of *Trametes* and *Ganoderma*, often exhibit constitutive laccase expression even in the absence of specific inducers (Na et al., 2025). The observed

variation in halo intensity among isolates likely reflects differences in physiological adaptation, enzyme secretion efficiency, or metabolic activity on PDA medium. Importantly, the detection of laccase activity across all isolates underscores their ecological function as ligninolytic wood-rot fungi and emphasizes their suitability for further enzymatic characterization and potential biotechnological applications.

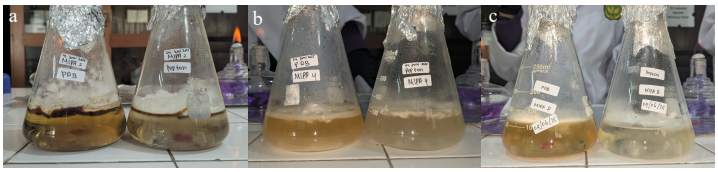

Fig. 4. Culture conditions of fungal isolates in potato dextrose broth (PBD) and modified glucose–peptone medium (mGP) after 28 days of incubation direction

3.3. Laccase activity of fungal isolates

The three laccase-positive isolates (MIPA 2, MIPA 4, and MIPA 8) were further cultivated in liquid media to evaluate their ability to synthesize extracellular laccase over an extended incubation period (Fig. 4). Each isolate was grown in both modified glucose–peptone broth and potato dextrose broth (PDB), two media widely used for stimulating oxidative enzyme secretion in basidiomycetes. These media were selected because nutrient composition is one of the most critical factors affecting extracellular laccase production. Glucose–peptone broth provides an easily assimilable carbon source (glucose) together with organic nitrogen from peptone, which is known to enhance biomass formation and promote the transcription and secretion of ligninolytic enzymes, including laccases (Vaidyanathan et al., 2011). In contrast, PDB is a carbohydrate-rich but relatively low-nitrogen medium derived from potato extract and dextrose, typically supporting constitutive baseline enzyme production rather than maximal induction (Drężek and Możejko-Ciesielska, 2025). By cultivating the isolates in these two nutritionally distinct media, the study aimed to determine how variations in carbon and nitrogen availability influence laccase biosynthesis and to identify the most favorable nutrient environment for enzyme production.

Ten actively growing agar plugs were inoculated into 250 mL flasks containing 100 mL of each medium and incubated statically at room temperature for 7, 14, 21, and 28 days. At each interval, the culture supernatant was collected and subjected to quantitative laccase analysis using syringaldazine as the chromogenic substrate. This comparative approach provides insights into strain-specific metabolic responses, enzyme secretion potential, and the suitability of each isolate for future biotechnological or scale-up applications.

The laccase activity profiles of the three fungal isolates (MIPA 2, MIPA 4, and MIPA 8) demonstrated clear differences influenced by medium composition and incubation time (Fig. 5). Among all isolates, MIPA 2, which was identified as *Ganoderma* sp., exhibited the highest laccase productivity in both media. In PBD medium, its activity increased from 33.20 U/L on day 7 to a maximum of 68.40 U/L on day 21, followed by a decline at day 28. When cultivated in glucose peptone medium, MIPA 2 produced markedly higher activities, reaching 93.71 U/L on day 21. Although this value represents the highest activity observed in the present study, it can be considered moderate compared to laccase titers reported for optimized or induced *Ganoderma* strains under controlled fermentation conditions. Under the non-induced conditions applied in this study, however, the activity level indicates that MIPA 2 has promising baseline laccase-producing capability. This enhanced performance is consistent with previous findings that organic nitrogen sources such as peptone stimulate laccase synthesis in basidiomycetes (Bettin et al., 2009; Tinoco et al., 2011)

Nevertheless, without direct benchmarking under comparable cultivation conditions, the designation of MIPA 2 as “superior” should be interpreted as relative only to the isolates examined in this study. Furthermore, while *Ganoderma* species are widely reported to possess multiple laccase isoenzymes and efficient secretion systems, the present study did not include gene-level or proteomic analyses; therefore, the mechanistic basis of the observed activity cannot be conclusively determined.

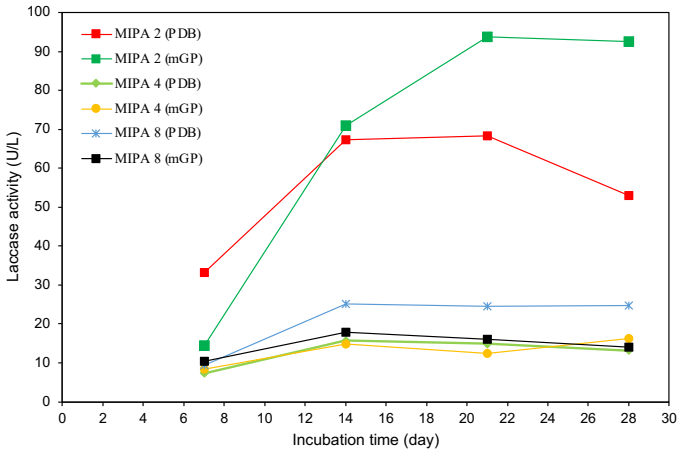

Fig. 5. Profiles of fungal isolates during 28 days of incubation in two liquid media. Note: PDB, potato dextrose broth; mGP., modified glucose-peptone

In contrast, the *Trametes* isolates MIPA 4 and MIPA 8 produced substantially lower laccase activities. MIPA 4 showed the weakest performance, with maximum activity in PBD reaching only 15.71 U/L on day 14, and in glucose peptone medium reaching only 16.36 U/L on day 28. MIPA 8 displayed moderate activity, reaching 25.24 U/L in PBD on day 14 and 17.89 U/L in glucose peptone medium before gradually declining. Although *Trametes* is widely recognized as a laccase producing genus, laccase output varies greatly across strains, with many isolates requiring specific inducers such as copper or aromatic phenolics to trigger high enzyme levels (Couto et al., 2002; Lee et al., 1999). Without such inducers, low or moderate activity is common. The lower productivity of MIPA 4 and MIPA 8 therefore likely reflects strain specific physiological limitations, including weaker regulatory response to nutrients, limited secretion capacity, or fewer active laccase isoenzymes compared with *Ganoderma*.

Overall, glucose peptone medium was more effective than PBD in enhancing laccase activity across all isolates, which supports previous observations that readily metabolizable carbon sources combined with organic nitrogen can upregulate oxidative enzyme pathways in ligninolytic fungi (Baldrian, 2006). Peak activity typically occurred between days 14 and 21, which corresponds to secondary metabolism phases characteristic of basidiomycetes. The decline at day 28 may be associated with nutrient depletion, shifts in pH, or the accumulation of metabolic inhibitors. These results clearly identify MIPA 2 as the most promising isolate for further development because of its substantially higher laccase activity and strong responsiveness to nutrient rich conditions.

4. Conclusion

This study successfully isolated and characterized wood-decaying fungi from the peat forest of the UPR area, revealing a and preliminarily evaluated wood-decaying fungi from the peat forest of the UPR area. The isolates were morphologically identified at the genus level and screened for extracellular laccase production, followed by quantification of crude enzyme activity in liquid culture. The recovered fungi were primarily affiliated with the genera *Trametes*, *Polyporus*, and *Ganoderma*, which are commonly

associated with ligninolytic activity in woody substrates. Although eight fruiting bodies were collected, only three isolates (MIPA 2, MIPA 4, and MIPA 8) established stable cultures and demonstrated active growth in vitro. These isolates exhibited distinct growth rates and laccase production profiles, reflecting species specific physiological traits and ecological adaptations. Qualitative screening using syringaldazine confirmed that all three isolates possessed extracellular laccase activity, with MIPA 2 showing the strongest response, followed by MIPA 8 and MIPA 4. Quantitative analysis further demonstrated that medium composition played a critical role in regulating enzyme synthesis. Glucose peptone medium consistently supported higher laccase activity compared with PDB, emphasizing the importance of organic nitrogen in promoting oxidative enzyme pathways. Among the isolates, MIPA 2, identified as *Ganoderma* sp., exhibited the highest laccase productivity, reaching 93.71 U/L on day 21. In contrast, the *Trametes* isolates MIPA 4 and MIPA 8 produced lower to moderate enzyme levels under the tested conditions. This difference may be associated with strain-specific physiological variation; however, detailed regulatory mechanisms and secretion capacity were not investigated in this study and therefore cannot be conclusively determined. All findings highlight the peat forest as a valuable reservoir of ligninolytic fungi. Among the isolates screened, *Ganoderma* sp.1 (MIPA 2) exhibited the highest laccase activity under the tested conditions and therefore represents the most promising isolate within this study. However, given that the evaluation was limited to crude enzyme activity without optimization, kinetic characterization, or stability assessment, its industrial applicability cannot yet be established. Further molecular identification, inducer optimization, and detailed enzymatic characterization are necessary to more comprehensively assess the biotechnological potential of these isolates.

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Conflict of interest

The authors declare no conflict of interest in this research.

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