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Assessment of *Schizophyllum commune* and *Trametes hirsuta* as efficient laccase-producing white-rot fungi

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ABSTRACT: White-rot fungi are important microbial resources in industrial and environmental applications due to their ability to produce ligninolytic enzymes. Among these, laccases and extracellular blue copper oxidases are of particular interest because of their broad substrate specificity and lignin-degrading potential. In this study, two previously identified white-rot fungal isolates *Trametes hirsuta* (PSF7) and *Schizophyllum commune* (WRPF6), were evaluated for laccase production using guaiacol as substrate. Both isolates exhibited significant enzymatic activity indices of 1.3 ± 0.024 mm and 1.12 ± 0.022 mm, respectively. PSF7 showed the highest laccase activity, reaching 97.92 U/mL on the 12th day of incubation, followed by WRPF6 at 55.63 U/mL on the 15th day. Laccase oxidized guaiacol using molecular oxygen as the electron acceptor, highlighting the strong oxidative enzyme systems of these isolates. These findings suggest that PSF7 and WRPF6 are promising candidates for diverse industrial and biotechnological applications, including biofuel production from lignocellulosic biomass, textile dye degradation, and food or pharmaceutical processing.

Keywords: Guaiacol, *Laccase*, lignin, *Schizophyllum commune,Trametes hirsuta,*white rot fungus

Laccases possess high redox potential and catalyze oxidative transformation and ring cleavage of diverse aromatic compounds, a property attributed to their multicopper oxidase structure with interconnected cupredoxin-like domains folded into a compact globular conformation.Their wide range of substrate specificity, catalytic versatility, and ecological importance make them key enzymes in lignin degradation and aromatic compound transformation (Janusz *et al.,* 2020). Fungal laccases are generally monomeric glycoproteins, though dimeric and multimeric forms have also been reported across different species (Giardina *et al.,* 2010). Basidiomycete fungi are well known for their ability to produce a range of extracellular enzymes that contribute to lignin degradation. Key enzymes include laccases (benzenediol:oxygen oxidoreductases) and several peroxidases, such as MnP, LiP, and VP. LiP acts as a diarylpropane:hydrogen-peroxide oxidoreductase, catalyzing C–C bond cleavage, whereas MnP functions as a Mn(II):hydrogen-peroxide oxidoreductase. VP exhibits the combined catalytic capabilities of both LiP and MnP, enabling it to oxidize a broader spectrum of lignin-derived compounds (Pozdnyakova *et al.,* 2018). White rot basidiomycetes are viewed as the most efficient natural producers of laccase and other ligninolytic enzymes due to their superior ability to mineralize lignin compared to other microorganisms (Rabha *et al.,* 2023). Species such as *Trametes versicolor*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Ceriporiopis subvermispora*, and *Lentinula edodes* have been extensively studied for their strong ligninolytic capabilities and high laccase yields (Birhanli *et al.,* 2013). Laccase-producing fungi play a major role in the bioremediation of aromatic pollutants, dyes, xenobiotics, and various industrial contaminants owing to their oxidative versatility (Zhuo and Fan, 2021). Laccases also have wide industrial applications, including dye decolorization, pulp delignification, wastewater detoxification, and environmentally friendly processing in the textile, food, and paper industries (Arregui *et al.,* 2021). Their ability to catalyze oxidation under mild conditions makes them suitable for green and sustainable processing technologies (Mate and Alcalde, 2017). In lignocellulosic biomass pretreatment, biological methods utilizing white-rot fungi provide a sustainable alternative to conventional physicochemical processes that often require high energy and may generate inhibitory compounds (Kumar and Sharma, 2017). Recent studies have demonstrated that laccase from white-rot fungi, including *L. betulina* and *Trametes* spp., can effectively depolymerize lignin and enhance the digestibility and bioconversion efficiency of lignocellulosic materials (Kumar *et al.,* 2022; Cui *et al.,* 2021). Therefore, the present study aims to evaluate and quantify laccase production by selected white-rot fungal isolates to assess their potential applicability in the biodegradation of lignin from lignocellulosic biomass.

MATERIALS AND METHODS

Collection and maintenance of ligninolytic fungal cultures

Two previously identified ligninolytic fungal cultures, *viz*., *Schizophyllum commune* (WRPF6) and *Trametes hirsuta* (PSF7), were obtained from the Department of Microbiology, College of Basic Science and Humanities, GBPUA&T Pantnagar, Uttarakhand.The fungal cultures were reactivated using the spot inoculation method on PDA plates. After inoculation, the plates were maintained at 25–27°C for seven days.

Qualitative screening of cultures for *laccase* production

The fungal cultures were screened qualitatively for laccase activity using PDA medium supplemented with 0.01% (v/v) guaiacol (Pandey *et al.,* 2018; Kiiskinen *et al.,* 2004). Each culture was centrally inoculated onto Petri plates and incubated at 28 ± 1 °C for 5–10 days under static conditions. The appearance of a dark-red to reddish-brown zone surrounding the fungal mycelium due to oxidation of guaiacol indicated laccase activity. Plates without fungal inoculation served as negative controls. The level of extracellular laccase production was quantified by calculating the relative enzyme activity index using the formula:

$$I_{laccase} = \frac{\text{Diameter of the colored halozone (in mm)}}{\text{Diameter of colony (in mm)}}$$

Quantitative estimation of laccase enzyme

Fungal cultures were cultivated for laccase production following the method described by Kalra *et al.* (2013). Based on the relative laccase activity observed in the qualitative plate assay, the isolates were grown in a laccase-inducing liquid medium composed of the following components (g/L^{-1} w/v): KH_2PO_4 50, glucose 3.0, $MgSO_4.7H_2O$ 1.0, NH_4NO_3 12.5 mM, KCl 0.5, Tween 20 0.2, veratryl alcohol 1 mM, $FeSO_4$ 0.001, trace metal solution 0.1%, and pH 5.0 at 28±1^{0}C for 24 days. After incubation, the crude enzyme was extracted by centrifugation at 10,000 rpm at 4^{0}C for 10 minutes. The obtained supernatant was used as the crude laccase enzyme.The reaction mixture included 1 mL of diluted crude enzyme and 3 mL of sodium acetate buffer (10 mM), pH 5.5. After that, 1.0 mL of guaiacol (2mM) was added as substrate. Mixed the solution and incubated at 30^{0}C for 15 minutes. After incubation, laccase activity was indicated by the development of a brown colour. Afterwards, the absorbance was measured at 450 nm against a blank, which contained 1.0 mL of distilled water instead of crude enzyme in the reaction mixture. 1 unit of laccase enzyme activity is defined as the amount of enzyme needed to oxidize one µmol of guaiacol/ minute. The activity (Unit /mL) was determined according to the following formula:

$$\text{Enzyme activity } (Unit/ml) = \frac{A * V}{t * e * v}$$

Where A (Absorbance), V (Total volume of the reaction mixture), t (Incubation time), e (Extinction coefficient) and v denotes thevolume of crude enzyme solution (mL)

Statistical Analysis

The experiment was performed three times, and the resulting data were analyzed using the Statistical Package for the Social Sciences (SPSS) software. Enzyme activity measurements were subjected to one-way analysis of variance (ANOVA). Before ANOVA, the dataset was subjected to a square root transformation to achieve normality and stabilize variance. Statistical significance was considered at $p < 0.05$, and mean comparisons were done using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Qualitative screening of selected isolates based on I_{lac} plate assay

Qualitative screening for laccase production by the selected microbial isolates was performed on guaiacol-amended agar medium**.** Guaiacol serves as the primary carbon source and chromogenic substrate for laccase, and its enzymatic oxidation leads to the formation of a distinct dark brick-red to brown halo surrounding the colonies. The microbial isolate PSF7 exhibited the maximum I_{lac} value of 1.30±0.024 mm (Fig. 1A), followed by WRPF6 followed with an I_{lac} value of 1.12±0.022 mm (Fig. 1B).The absence of a dark halo confirms that the medium components and guaiacol substrate do not spontaneously oxidize or change colour in the absence of the laccase enzyme (Fig. 1C). The results of the qualitative screening for laccase production using the guaiacol-amended agar medium are presented in Table1. The data quantifies the laccase activity using the relative enzyme activity index (I_{lac}), which is calculated as the ratio of the diameter of the coloured halo zone to the diameter of the colony (in mm). Among them, PSF7 exhibited the maximum I_{lac}value (1.30 mm), followed by WRPF6 (1.12mm)**.** The critical difference at 5% level was provided for each measurement, 0.049 for PSF7 and 0.042 for WRPF6, showing thatthe difference between means was statistically significant.

Quantification of extracellular laccase enzyme activity

The two isolates selected from the preliminary screening were subjected to quantitative laccase activity analysis. The cultures were incubated in a laccase-inducing liquid medium for 24 days, during which samples were collected at regular intervals. The culture broth was centrifuged to obtain the supernatant containing the crude enzyme, and quantitative assays were performed at 3-day intervals. The evaluation revealed distinct strain-specific differences in laccase production (Fig .2), which visually confirms the superiority of PSF7 as a laccase producer in the tested conditions, as it achieved a higher enzyme titer and reached its peak production earlier compared to WRPF6. This difference underscores the importance of selecting high-performing fungal strains for biotechnological applications.The initial activity of the isolate PSF7 started at a low (0.96± 0.004 U/mL) on day 3, and increased moderately up to day 9 (20.02 ±0.511 U/mL). The highest activity was exhibited, reaching its maximum production of 97.92 ± 2.395 U/mL on the 12th day of incubation. The activity dropped sharply to 33.38 ±0.069 U/mL on day 15 and

Table 1: Relative activity indices of laccase enzymes of two fungal cultures as determined by plate assay

S. No.	Fungal cultures	Relative enzyme activity indices (mm)	CD (5%)
		Laccase (I_{lac})	
1	*Trametes hirsuta* (PSF7)	1.30±0.024	0.049
2	*Schizophyllum commune* (WRPF6)	1.12±0.022	0.042

CD, critical difference

Table 2: *In-vitro* laccase enzyme activities (U/mL) in culture extract of selected fungal cultures

Fungal cultures	Extracellular Laccase enzyme activities (U/mL) at different time intervals (d)							
	3	6	9	12	15	18	21	24
Trametes hirsuta (PSF7)	0.96±0.004	2.44±0.005	20.02±0.511	97.92±2.395	33.38±0.069	14.68±0.099	7.04±0.059	4.82±0.015
Schizophyl lum commune (WRPF6)	1.04±0.026	4.08±0.096	9.79±0.194	19.28±0.249	55.63±0.573	12.75±0.046	7.86±0.094	2.59±0.043
SEm±: a (cultures) = 0.184; b (time period) = 0.300; a*b (interaction) = 0.519								
CD (5%): a (cultures) = 0.524; b (time period) = 0.855; a*b (interaction) = 1.482								

CD, critical difference; SEm, standard error of mean; d, days

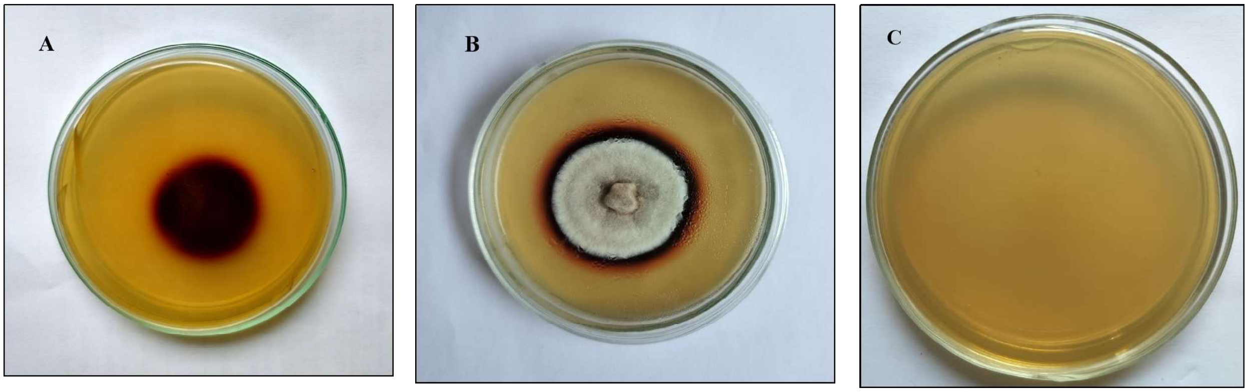

Fig. 1: Qualitative detection of laccase enzyme activity by (A) *Trametes hirsuta* (PSF7); (B) *Schizophyllum commune* (WRPF6); (C) Control (untreated) plate assay of laccase

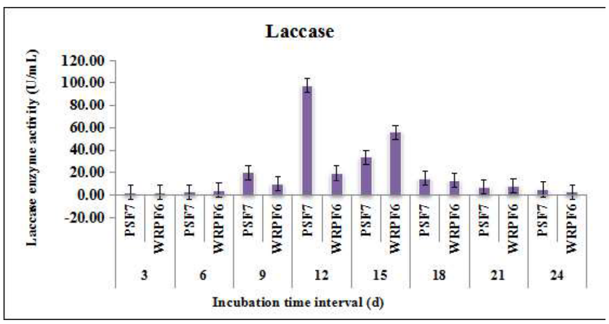

Fig.2: Laccase activity in the crude enzyme extracts of the selected fungal cultures, *Trametes hirsuta* (PSF7) and *Schizophyllum commune* (WRPF6), measured at different incubation intervals over 24 days

continued to decrease until the final measurement on day 24 (4.82 ± 0.015 U/mL). Similarly, the initial activity of WRPF6 was slightly higher compared to PSF7 on day 3 (1.04 ± 0.026 U/mL) and reached its peak activity later than PSF7, achieving 55.63 ± 0.573 U/mL on the 15 days of incubation. After that, the activity decreased significantly, reaching 2.59 ± 0.043 U/mL by day 24, demonstrating significant enzymatic potential under the same fermentation conditions (Table 2).

The findings from the qualitative and quantitative laccase assays confirmed that the high enzyme activity of the isolates, particularly PSF7, was consistent with literature on wood-rotting fungi. These observations are consistent with recent studies reporting that wood-rotting fungi and other ligninolytic microorganisms often display higher qualitative laccase activity on guaiacol plates due to their robust oxidative enzyme systems and multiple laccase isoenzymes (Umar et al., 2023; Sharma *et al.,* 2023; Pandey *et al*., 2018).Recent literature further emphasizes that isolates demonstrating higher I_{lac} values frequently possess enhanced extracellular enzyme secretion capabilities, making them potential candidates for downstream biotechnological applications. For instance, higher guaiacol-oxidizing laccase activity has been strongly associated with improved performance in dye decolorization, bioremediation, and lignocellulosic biomass conversion processes (Alhomaidi *et al.,* 2023). Similarly, kinetic characterization of guaiacol-oxidizing fungal laccases suggests that strains with strong guaiacol reactivity often exhibit favourable catalytic parameters such as lower Km and higher Vmax, strengthening their utility in industrial enzyme production (Saad Abd El-latif *et al.,* 2024).The appearance of a colored zone is a widely accepted indicator of extracellular laccase production and continues to be routinely used due to its reliability, rapid detection, and cost-effectiveness (Elsaba *et al.,* 2023). These findings highlight the biotechnological promise of potent isolates such as PSF7 and WRPF6, which may serve as efficient candidates for large-scale laccase production and downstream industrial or environmental applications.

Several studies indicate that laccase productivity in *Trametes hirsuta* varies by strain and culture conditions, with higher enzyme yields recorded under optimized incubation (Li *et al.,* 2016; Wang *et al.,* 2024). Bagewadi *et al.* (2017) reported that *Trichoderma harzianum* strain HZN10 produced laccase activities of 63 U/g, 55 U/g, and 53 U/g when grown on rice straw and sugarcane bagasse substrates. The present results highlight clear variation in laccase synthesis between the two fungal isolates, with PSF7 exhibiting markedly higher enzymatic activity. The isolates, particularly PSF7, are efficient candidates for large-scale laccase production and various downstream industrial or environmental applications.This difference underscores the importance of selecting high-performing fungal strains for biotechnological applications that rely on strong oxidative enzyme systems, including lignocellulose degradation, dye decolorization, and environmental bioremediation.

CONCLUSION

The present study demonstrates that fungal cultures PSF7 and WRPF6 possess significant potential for ligninolytic enzyme production, particularly laccase. Qualitative and quantitative screening confirmed their efficiency as laccase producers. These fungi represent valuable resources for sustainable industrial and environmental applications. Future work may focus on optimizing culture conditions, including substrate, pH, temperature, and inducer supplementation, as well as exploring enzyme stabilization or immobilization to enhance laccase yield and industrial applicability.

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