Journal of Bioresources 11 (1): 84-91, January – March 2024

JOURNAL OF BIORESOURCES

journal webpage: https://jbr.rgu.ac.in

ISSN: 2394-4315 (Print)
ISSN: 2582-2276 (Online)

RESEARCH ARTICLE

Optimization of culture conditions for laccase production by *Ganoderma gibbosum* (Blume & T. Nees) Pat. under solid-state fermentation

Junmoni Das, Marjum Badak and Rajiv Kumar Singh*

Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh 791 112, Arunachal Pradesh, India

*Corresponding Author e-mail: rajiv.singh@rgu.ac.in
Article No.: JMDJBR80; Received: 21.01.2024; Peer-reviewed: 10.02.2024; Revised and Accepted: 09.03.2024; Published: 31.03.2024
Doi: https://doi.org/10.5281/zenodo.11318393

Abstract

Fungal laccases have gained significance in diverse industrial, biotechnological and environmental applications. Wood degrading White Rot Fungi (WRF) of the phylum Basidiomycota are an important source of laccase. Optimization of culture conditions for solid state fermentation is reported to enhance laccase production. In the present study, optimization of the physical and biochemical factors was carried out using one factor–at–time (OFAT) approach to maximize laccase production by *Ganoderma gibbosum* collected from nearby sub-tropical forest. Under unoptimized conditions, the fungus showed ~186 U/L laccase activity. Maltose/fructose, $NaNO_3$, and Polysorbate 60 were found to be the most effective carbon source, nitrogen source, and surfactant, respectively, enhancing the laccase yield. With the incorporation of 10 mM ethanol and 0.5 mM $CuSO_4$ to the growth medium, laccase yield increased approximately 4-fold and 8-fold, respectively. These findings may be utilized for further optimization of laccase production by *G. gibbosum*.

Keywords: White Rot Fungi; Lignolytic Enzyme; OFAT, Carbon and Nitrogen Source; Inducer; Surfactant

1. Introduction

Laccases (EC 1.10.3.2) are copper-containing oxidoreductase produced by many fungi. They are ligninolytic enzymes, owing to their higher redox potential and broad substrate specificity, they are able to transform or degrade a wide range of phenolic and non-phenolic compounds, as well as several recalcitrant pollutants, such as polycyclic aromatic hydrocarbons (PAHs), pesticides, and synthetic dyes (Gałązka et al., 2023), by using molecular oxygen as the electron acceptor (Weng et al., 2021).

By virtue of their versatility, laccases are of great importance in various industrial, biotechnological and environmental applications, including pulp and paper, food processing, textiles, cosmetics, medicines, diagnostics, and bioremediation (Chaudhary et al., 2022; López-Pérez et al., 2024).

Among fungi, laccases are prominently found in White Rot Fungi (WRF) belonging to the phylum Basidiomycota. They are wood-degrading fungi that selectively remove lignin from woods, leaving aside cellulosic components (Janusz et al., 2017; Dashora et al., 2023). In order to meet the expansive demand for laccases for various applications, several WRF have been explored in earlier decades for laccase production. It is reported that the amount of laccase produced varies in different species and strains, as well as on the production system and type of substrate used (Das et al., 2024). Additionally, several inducers (metal ions, alcohol, phenolics, and lignin-like substances) and surfactants (fatty acid derivatives) have been utilized for inducing high laccase production by WRF and its exogenous secretion in the growth medium, respectively (Chmelová et al., 2022).

Commonly, Solid-State Fermentation (SSF) is employed for laccase production from WRF as it is economical and eco-friendly, and mimics the natural growth environment desirable for fungal growth and metabolism and allows the usage of lignocellulosic agricultural wastes as substrates (Singhania et al., 2009; Wang et al., 2019).

Optimization of the fermentation conditions, both physical and nutritional, is another vital step for obtaining a maximum laccase production from a particular fungal species. Various workers have used a classical method, called one factor–at–time (OFAT) approach, which involves changing one independent factor while keeping the others constant, for the screening of significant factors for further optimization.

In the present study, a WRF, namely *Ganoderma gibbosum* was explored for laccase production under SSF on wheat bran by screening certain physical (incubation periods, pH, temperature and substrate to moisture ratio) and nutritional (carbon and nitrogen sources) parameters, inducers (copper sulphate, ethanol, ferulic acid and Veratryl alcohol) and surfactants (Polysorbate-20, 40, 60, 80 and Triton X-100).

2. Materials and methods

2.1. Chemicals and raw biomass material

All the chemicals were of analytical grade and purchased from Sigma-Aldrich Pvt. Ltd. (USA), HiMedia (India), and Merck (USA). The wheat bran was obtained from local market.

2.2. Test fungus

The test fungus *Ganoderma gibbosum* was earlier collected from a nearby subtropical forest, growing on a living plant, namely, *Callicarpa macrophylla* Vahl, and identified based on morphological characters. It was raised in pure culture on Potato Dextrose Agar (PDA) and stored at 4°C for further use. Subsequently, confirmation of correct identification was done by sequence matching of its 629 bp long PCR-amplified segment (NCBI Accession number: OP257154) covering a region between ITS1 and 28S rDNA.

©JBR2024 Published by Centre with Potential for Excellence in Biodiversity, Rajiv Gandhi University, Rono Hills, Doimukh-791112, Itanagar, Arunachal Pradesh, India. Visit: https://jbr.rgu.ac.in; Email: editor.jbr@rgu.ac.in

2.3. Qualitative Screening of test fungus for laccase

Primary screening for laccase production was performed by the plate assay method on a PDA plate containing 0.02% guaiacol (Kiiskinen et al., 2004). The formation of a reddish-brown zone around the fungal colony was considered laccase-positive.

2.4. Laccase production under un-optimized Solid-State Fermentation (SSF)

Wheat bran was used as the substrate for SSF due to its rich content in growth factors, vitamins, and proteins (Bagewadi et al., 2017). SSF was carried out in a 250 ml flask containing 5g of wheat bran. A mineral salt solution (MSS) containing 0.05% $(NH_4)_2SO_4$, KH_2PO_4, and $MgSO_4$ was prepared, and the pH was adjusted to 5.0 with 1 N HCl or 1M NaOH (Sharma et al., 2005). Then, 15 ml of MSS was added to moisten the wheat bran (1:3 substrate to moisture ratio) in each flask and autoclaved. The substrate was then inoculated with 4 mycelial discs (each 10 mm diameter), taken from a 10-day-old pure culture of the test fungus.

2.5. Extraction of enzyme

The enzyme produced in SSF was extracted by solid-liquid extraction using Citrate phosphate buffer (100 mM, pH 5.0) with a 1:10 ratio (w/v), followed by vortexing the mixture at 150 rpm for 45 minutes at 25 °C. The solids were separated by filtration using muslin cloth followed by centrifugation at 10,000 rpm for 12 min. at 4 °C. The supernatant obtained was taken as the enzyme source and stored at 4 °C for enzyme assay.

2.6. Laccase assay

Laccase activity was assessed by preparing a 2 ml reaction mixture comprising 1.8 ml of 10 mM guaiacol in 100 mM citrate-phosphate buffer (pH 5.0) and 0.2 ml of the crude enzyme. The mixture was then incubated at room temperature for 20 minutes, and subsequently, absorbance was measured at 470 nm using a UV–Visible spectrophotometer (Eppendorf). Laccase activity was quantified as one unit (U/L), defined as the enzyme concentration needed to oxidize 1.0 µM of substrate per minute (Baltierra-Trejo et al., 2015).

$$\frac{U}{L} = \frac{\Delta A \times V_t \times D_f \times 10^6}{\varepsilon \times t \times d \times V_s}$$

Here, ΔA = absorbance, V_t = Total volume of the reaction (ml) D_f = Dilution factor, 10^6 = correction factor (µmoL mol^{-1}), ε = Molar extinction coefficient (26,600 M^{-1} cm^{-1})
V_s = Sample volume (ml), d = Optical path (1 cm), t = Reaction time (min.)

2.7. Optimization of physical parameters

2.7.1. Incubation period
The effect of the incubation period on laccase production was studied by incubating the culture flasks for 2–22 days and quantifying laccase activity at two-day intervals.

2.7.2. pH of the medium

The pH of MSS was adjusted between 3 and 7 using 1N HCl or 1N NaOH, and the laccase activity of the test fungus was measured at the end of the optimized incubation period.

2.7.3. Temperature of the medium

To determine the optimal temperature for maximum laccase production by the test fungus, fermentation was conducted with the optimized pH at different incubation temperatures ranging between 20 and 35 °C. Laccase activity was measured at the end of the optimized incubation period.

2.7.4. Substrate Moisture Ratio

The effect of substrate moisture level on laccase production was studied by varying the substrate-to-moisture ratio in the range of 1:2 to 1:6. Fermentation was carried out at the optimized pH and incubation temperature. Laccase activity was measured at the end of the optimized incubation period.

2.7.5. Optimization of biochemical parameters

All the experiments mentioned here were conducted at the optimized levels of the above-mentioned physical factors. Laccase activity was measured at the end of the optimized incubation period.
The effects of various carbon, nitrogen, surfactants, and inducers on laccase production were separately evaluated at different concentrations (Table 1).

Table 1. List of the tested nutritional and biochemical factors and their concentration

Factors with applied Concentration
Carbon Sources (Glucose, Sucrose, Fructose, Mannitol, Glycerol and Maltose) : 0.05 – 2.50 (%, w/w)
Nitrogen Source $(NH_4)_2SO_4$, $NaNO_3$, KNO_3, Peptone, Urea, Soyabean Meal): 0.05 – 2.50 (%, w/w)
Surfactants (Poly-20, Poly- 40, Poly- 60, Poly- 80, Triton X-100): 0.05 – 2.50 (%, v/v)
Ethanol: 0.2 – 20.0 mM
Ferulic Acid: 0.01–2.0 mM
Veratryl Alcohol: 0.5–5.0 %
Copper Sulphate : 0.05–5.0 mM

2.8. Statistical Analysis

All the experiments were performed in triplicate. One-way and two-way Analysis of Variance (ANOVA) was performed using IBM SPSS software (trial version), followed by multiple comparisons between groups using Fisher's LSD. OriginPro (trial version) was used for creating graphs.

3. Results

3.1. Identification of fungal isolate

The ITS region analysis was conducted using the BLAST tool on NCBI, revealing a 99% identity match to *Ganoderma gibbosum*. Phylogenetic analysis indicated close similarity to other *G. gibbosum* strains, confirming the identity of fungal isolate WRJ01.
The identification of *G. gibbosum* was carried out using ITS sequences and the phylogenetic tree was constructed with *Pleurotus ostreatus*, used as an outgroup (Figure 1). The obtained sequence was submitted to NCBI GenBank, Accession number: OP257154.

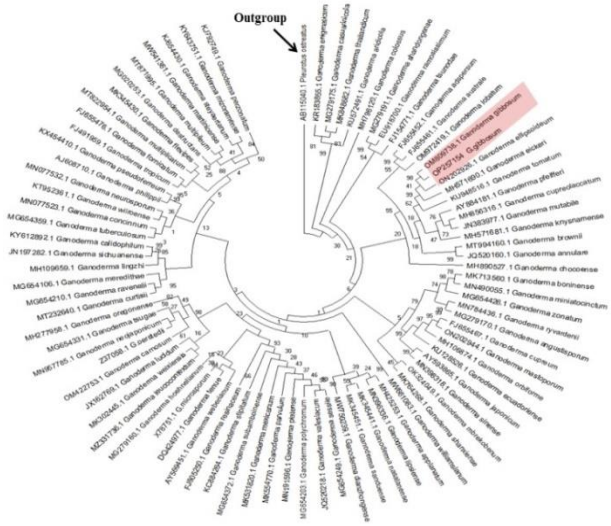

Figure 1. Phylogenetic tree of *G. gibbosum*

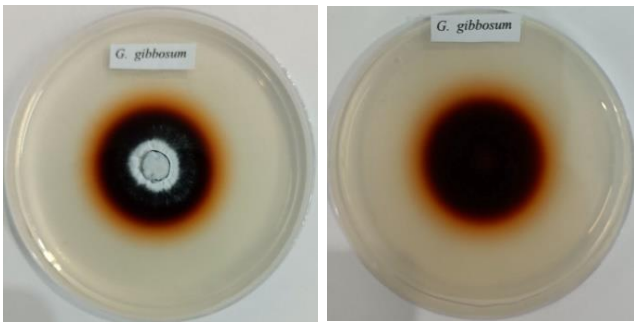

Figure 2. Laccase activity by *G. gibbosum* on guaiacol supplemented PDA

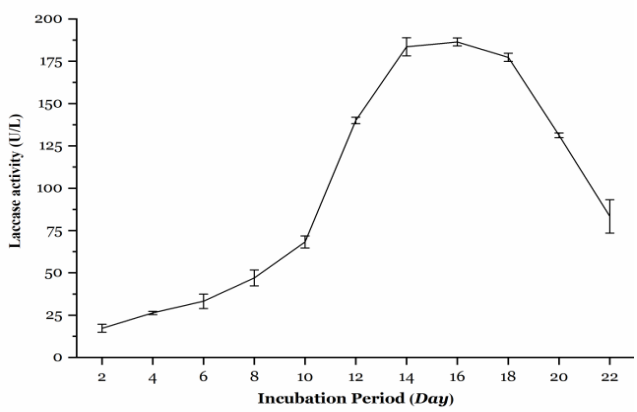

Figure 3. Effect of incubation time on laccase production (mean ± SD). SSF conditions: incubation temperature - 25°C, Moisture ratio - 1:3, pH- 5.0.

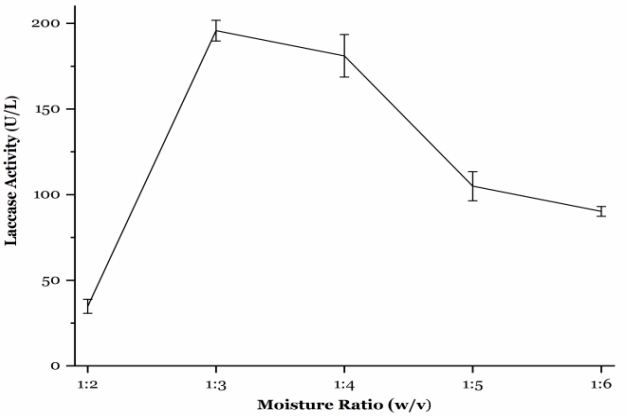

Figure 4. Effect of Moisture ratio on laccase production (mean ± SD). SSF conditions: incubation temperature - 25°C, pH- 5.0, Day-16.

3.2. Screening for laccase production

G. gibbosum was confirmed as laccase positive fungus due to formation of a reddish-brown ring after oxidation of guaiacol indicator around its colony in PDA plates (Figure 2).

3.3. Optimization of physical parameters

3.3.1. Incubation period: Laccase production by *G. gibbosum* increased gradually up to day 10, peaked thereafter reaching to its maximum on day 14–16th , (183–186 U/L), and declined sharply afterwards (Figure 3).

3.3.2. Moisture ratio: Among the five substrate to moisture ratio tested (1:2–1:6), 1:3 ratio provided the maximum laccase activity (~196 U/L) closely followed 1:4 ratio (~181 U/L). At both the substrate moisture ratios, the laccase production was many-fold higher in comparison to 1:2 ratio (~35 U/L) (Figure 4).

3.3.3. Incubation temperature: Laccase production jumped almost four fold when incubated at 25°C (~192 U/L) in comparison to 20°C (~46 U/L). On further increasing the incubation temperature to 30°C, its production showed almost 25% decline (~141 U/L) (Figure 5).

3.3.4. pH of growth medium: Laccase production increased sharply from pH 3 to pH 4 and again at pH 5 giving a maximum yield of 192 U/L. The production declined sharply while further increasing the pH of the growth medium (Figure 6).

3.4. Optimization of biochemical parameters

3.4.1. Carbon sources: Analysis of the results of various carbon sources (Mannitol, Glycerol, Glucose, Sucrose, Fructose, and Maltose) applied at different concentrations showed that on laccase activity is significantly influenced by the type of carbon source as well their concentration (Figure 7). *G. gibbosum* showed the highest laccase activity at 0.5% maltose or fructose (~330 U/L) followed sucrose under the given culture conditions. Further increase in concentration of these carbon sources showed a pronounced negative effect on laccase activity. Maltose was the the best source even at 0.05% (~290 U/L), followed by glucose (~257 U/L), fructose (~236 U/L) and sucrose (~215 U/L). Addition of Glycerol did not increase laccase activity at any of the applied concentration whereas mannitol appeared inhibitory.

3.4.2. Nitrogen sources: Substrate supplementation with additional nitrogen significantly influenced laccase production. Except KNO_3, other five nitrogen sources (organic sources: Urea, Soybean meal, peptone; inorganic sources $(NH_4)_2SO_4$, $NaNO_3$) promoted laccase activity, and with a further increase in their dose, the activity was generally higher (Figure 8).

The highest laccase activity was at 1.5% concentration of $NaNO_3$ (~ 655 U/L) followed by peptone (~ 418 U/L), $(NH_4)_2SO_4$ (~ 319 U/L) and soyabean meal (~ 283 U/L). Even at a lower concentration of 0.5%, $NaNO_3$ was superior in performance than others. On the other hand, the beneficial effect of urea gradually increased with concentration, and at 2.5% concentration its performance reaching to a maximum (~ 576U/L).

3.4.3. Surfactants: Effects of five different surfactants (Polysorbate-20, 40, 60, 80, and Triton X-100) were investigated on laccase activity under SSF condition (Figure 9). Among them, Polysorbate 60 at 1.5% concentration provided the highest laccase activity (~391 U/L). It was comparatively better than the other surfactants even at 0.5% concentration. Other surfactants exhibited a peak in laccase activity only at 0.05% concentration, and among them, the best performance was shown by Polysorbate 80 (~265 U/L).

3.4.4. Inducers: Among different inducers (Copper sulphate, Ethyl alcohol, Ferulic acid, and Veratryl alcohol) evaluated for their effect on laccase production, copper sulphate appeared to be the best providing a highest laccase activity of 1485 U/L at 0.5 mM (Figure 10a). Ethanol at 10mM provided 640 U/L laccase activity which remained statistically the same at 20mM (Figure 10b). For ferulic acid and veratryl alcohol, the optimal concentration for a maximum laccase activity was 0.01 mM (284 U/L) and 2.5mM (366 U/L) respectively, beyond which the activity declined sharply (Figure 10c, d).

4. Discussion

Laccase production by white rot fungi is affected by various physical and nutritional factors of the growth media for the metabolism and growth of the fungal mycelia (Rivera-Hoyos et al., 2013). In the present study, *G. gibbosum* showed approximately 190 U/L laccase activity on the 16th day after optimization of solid-state fermentation conditions on wheat through OFAT, with pH 5.0, temperature 25°C, and substrate moisture ratio of 1:3.

Under unoptimized conditions, laccase activity gradually increased from the 2nd day onwards, reaching its maximum level on days 14–16, and declining sharply afterward. The incubation period has been recognized to play a very important role in fungal growth, reproduction, and metabolism, and varies among fungal species, as well as according to the production conditions (Abd El Monssef et al., 2016; Hasan et al., 2023). Several researchers have reported

variations in time ranging from 5 to 17 days for the highest laccase activity (Umar and Ahmed, 2022; Han et al., 2022; Boran and Yesilada, 2022; Ibarra-Islas et al., 2023). A decline in laccase activity afterward can be attributed to the imposed physiological stresses on fungi due to the depletion of nutrients in the medium, leading to the inactivation of the secretory machinery of the enzymes (Chhaya and Modi, 2013; Sun et al., 2017).

A wide variation in substrate moisture ratio under SSF has been reported among fungal species. Optimum moisture content is essential as it facilitates substrate swelling, nutrient solubility, gas exchange, substrate utilization, microbial metabolism and enhanced enzyme production (Xin and Geng 2011; Dutt and Kumar, 2014). In this study, a substrate moisture ratio of 1:3 and 1:4 were far superior to any other ratios for laccase activity. Patel and Gupte. (2016) and Bhoyar et al. (2024) found similar results for *Tricholoma giganteum* AGHP and *Lentinus tigrinus* SSB_W2 respectively grown on wheat bran. Sharma et al. (2015) reported that maximum laccase activity by *Ganoderma* sp. rckk-02 was found on wheat bran moistened with mineral salt solution in substrate to moisture ratio of 1:2.5 supplemented with 4.5 mM copper and 2.0 % tryptophan. On the other hand, Ravenkar et al. (2006) and Boran and Yesilada (2022) have reported 1:0.7 as optimal substrate moisture ratio for wheat bran for high laccase production by *Ganoderma* spp.

Temperature serves as the primary determinant of both laccase activity and fungal growth, particularly during industrial scale-up processes. It is reported that the optimal temperature for laccase production varies significantly among fungi (Naz et al., 2022). We found the highest laccase activity by *G. gibbosum* at 25°C, with a sharp decline observed with further increases in temperature. This finding is in agreement with many other reports suggesting 25–30°C as the optimal temperature range for WRF (Thurston, 1994; Elsayed et al., 2012; Ergun and Urek, 2017; Patel et al., 2019). Umar and Ahmed (2022) have reported maximum laccase production (855 U/L) *by G. leucocontextum* at 40°C. Therefore, it appears that the optimum temperature for laccase production also varies in WRF.

The initial pH of the medium is also recognized as one of the most influential factors in fungal growth, enzyme production, and the transport of various components across the cell membrane. Further, a change in the pH of the growth medium may affect metabolic activity as well as enzyme activity (Kapoor et al., 2007; Adak et al., 2016). Usually, higher growth and laccase production are observed in acidic pH conditions (Thurston, 1994; Hamed et al., 2024), and in many studies, the initial pH of the medium between 4 and 6 has been reported as optimum (Nandal et al., 2013; Ding et al., 2014; Ghosh and Ghosh, 2017; Vantamuri et al., 2019). We found pH 5.0 to be optimum for laccase production by *G. gibbosum*. A similar result has been reported by Shrestha et al. (2016) for *G. lucidum*–CDBT1.

The carbon source in the medium plays a crucial role by promoting mycelial growth and inducing the transcription of the laccase gene (Teerapatsakul et al., 2007; Adamian et al., 2021). However, the effect can vary depending on the specific fungal strain. In the present study, out of the six carbon sources supplemented individually to the production medium, only maltose, fructose and sucrose enhanced laccase activity in *G. gibbosum*, whereas glucose, mannitol and glycerol appeared inhibitory. The former three carbon sources showed their best beneficial effect at 0.5% concentration, but their higher concentrations suppressed laccase activity. Gutiérrez-Antón et al. (2023) have also reported fructose and maltose as a suitable carbon source for enhanced laccase production by *Thielavia terrestris* Co3Bag1. On the other hand, Sharma and Murty (2021) reported fructose at 1% as the best carbon source compared to maltose, dextrose, sucrose and xylose for *Pleurotus sajor-caju*, providing the maximum laccase activity under SSF. Suppressed laccase production at higher concentrations of additional carbon has been reported for many fungal strains (Lee et al., 2004; Sharma and Murty, 2021).

Nitrogen sources also play a crucial role in fungal physiology and metabolism, impacting enzyme production (Reddy and Kanwal, 2022), however, nitrogen source may vary depending on the fungal species (Jaramillo et al. 2017). Out of the six nitrogen sources, except KNO_3, the other five sources used in the present study promoted laccase activity when increasing their dose up to a certain level.

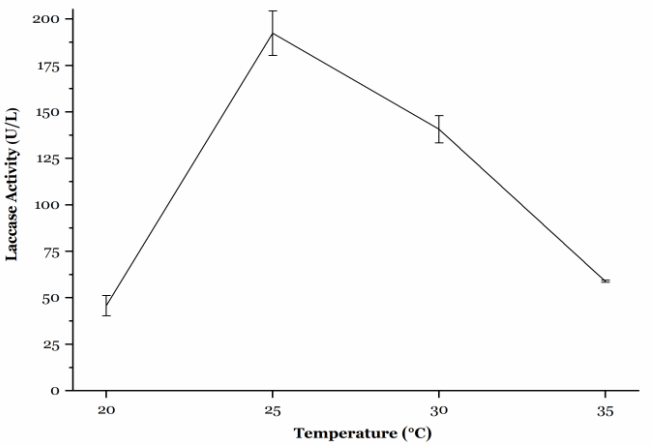

Figure 5. Effect of incubation temperature on laccase production (mean ± SD). SSF conditions: pH- 5.0, Moisture ratio - 1:3, Day-16.

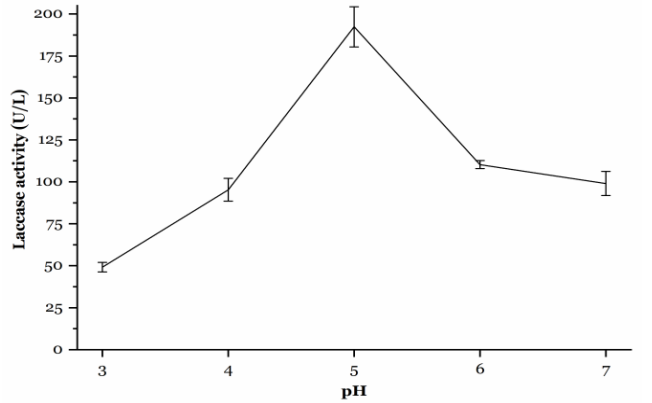

Figure 6. Effect of initial medium pH on laccase production (mean ± SD). SSF conditions: incubation temperature - 25°C, Moisture ratio - 1:3, Day-16.

Among these sources, $NaNO_3$ at a 1.5% concentration provided much higher laccase activity compared to the others. Gutiérrez-Antón et al. (2023) found 3.18-fold enhancement in the laccase activity by *Thielavia terrestris* Co3Bag1.

Production of laccase is significantly influenced by the presence of surfactants in the growth medium because they increase cell membrane permeability, thereby allowing a rapid release of enzymes. Additionally, they increase the solubility of substrate molecules present in the lignocellulosic structure, enhance enzyme stability, and reduce enzyme dosage during hydrolysis (Muñoz et al., 2022). We recorded maximum laccase activity in media supplemented with 1.5% Polysorbate 60. However, the specific effect of different surfactants on laccase production can vary depending on various factors, including the fungal strain, fermentation conditions, etc. (Singh and Singh, 2017; Geethanjali et al., 2020).

Various types of inducers (metal ions, phenolic and aromatic compounds) have been utilized for obtaining enhanced laccase production from fungi. Copper ion act as a cofactor, transcription activator, and promotes laccase synthesis and maturation (Sharestha et al., 2016; Wang et al., 2019; Sharghi et al., 2024). Phenolic and aromatic compounds induce secondary metabolism in fungi and to enhance laccase production (Tavares et al., 2005). We used four different inducers, viz. (copper sulphate, ethanol, veratryl alcohol and ferulic acid), and among them copper sulphate at 0.5 mM was the most effective in enhancing laccase activity by 8-fold in *G. gibbosum*. Next in effectiveness were ethanol, veratryl alcohol and ferulic acid providing 3.4-fold, 1.9-fold, and 1.5-fold more laccase activity respectively.

Addition of copper in low concentrations to the culture medium in reported to enhance laccase production in several cases and its optimum concentrations may vary depending on the fungal species

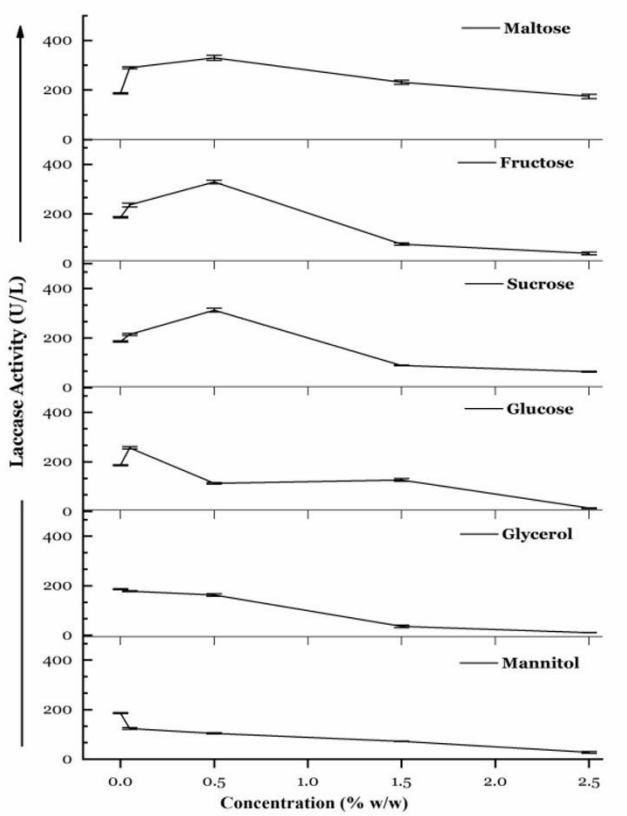

Figure 7. Effect of different carbon sources on the laccase production (mean ± SD). SSF conditions: pH-5.0, temperature - 25°C, Moisture ratio - 1:3, Day-16.

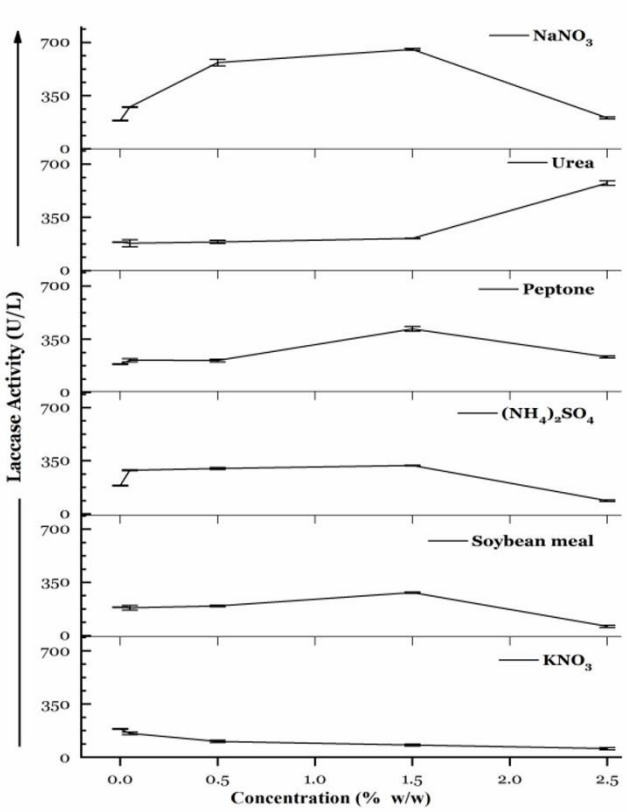

Figure 8. Effect of different nitrogen sources on the laccase production (mean ± SD). SSF conditions: pH-5.0, temperature - 25°C, Moisture ratio - 1:3, Day-16.

and even specific strains. (Palmeiri et al., 2000; Wang et al., 2019; Durán-Sequeda et al., 2022). Manavalan et al. (2013) and Fonseca et al. (2010) have also reported 0.4 mM and 0.5 mM copper sulphate as optimum for stimulating laccase production in *G. lucidum* and *G. applanatum* respectively. Similar findings for ethanol, veratryl alcohol and ferulic acid as effective inducers have been reported for various WRF (Arora and Gill, 2001; Lomascolo et al., 2003; Elisashvili et al. 2010; Kocyigit et al., 2012; Sharma et al. 2014). These inducers activate oxidative stress within the fungal cells which may indirectly induce laccase production (Lee et al., 1999; Karp et al., 2012; Chhaya and Gupte, 2013; Swatek and Staszczak, 2020).

5. Conclusion

Fungal laccases are excellent biocatalysts, with a great demand for various industrial, biotechnological, and environmental applications. This study reports *Ganoderma gibbosum* as a potential laccase-producing White rot fungus. Optimization of certain physical and nutritional factors under Solid-State Fermentation enhanced laccase production, which further increased manyfold in the presence of inducers and surfactants. Optimization of fermentation conditions through statistical designs for ideal levels of various factors, and their interactions, may further enhance laccase yield.

Acknowledgement

The 1st and 2nd authors thank Rajiv Gandhi University (RGU), Arunachal Pradesh and Council of Scientific and Industrial Research (CSIR), New Delhi India, respectively for providing financial support and infrastructure to conduct the experiments. Additionally, authors also acknowledge Department of Biotechnology, Govt. of India for the financial support under the grant BT/PR25530/NER/95/1239/2017.

Author(s) contribution

Junmoni Das: Conceptualization, Methodology, Experimentation, Formal Analysis, Writing – Original draft, review & editing; Marjum Badak: Writing – Validation, review & editing; Rajiv Kumar Singh: Conceptualization, Supervision, Validation, Writing – review & editing.

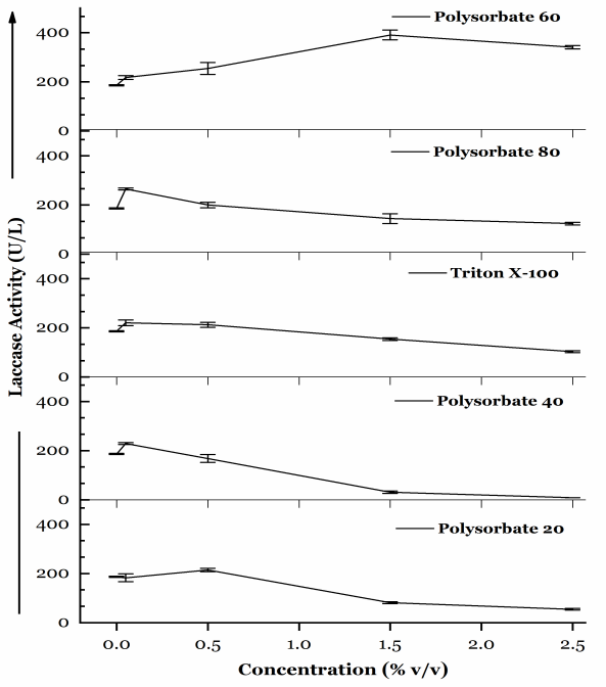

Figure 9. Effect of different surfactants sources on the laccase production (mean ± SD). SSF conditions: pH-5.0, temperature - 25°C, Moisture ratio - 1:3, Day-16.

Conflict of interest

All authors declare that there is no conflict of interest.

References

Abd El Monssef RA, Hassan EA and Ramadan EM. 2016. Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment. Annals of Agricultural Sciences 61: 145-154. doi: 10.1016/j.aoas.2015.11.007

Adak A, Tiwari R, Singh S., Sharma S and Nain L. 2016. Laccase production by a novel white-rot fungus Pseudolagarobasidium acaciicola LA 1 through solid-state fermentation of *Parthenium* biomass and its application in dyes decolorization. Waste and Biomass Valorization 7: 1427-1435. doi: 10.1007/s12649-016-9550-0

Adamian Y, Lonappan L, Alokpa K, Agathos SN and Cabana H. 2021. Recent developments in the immobilization of laccase on carbonaceous supports for environmental applications-a critical review. Frontiers in Bioengineering and Biotechnology. 9: 778239. doi: 10.3389/fbioe.2021.778239.

Arora DS and Gill PK. 2001. Effects of various media and supplements on laccase production by some white rot fungi. Bioresource Technology 77: 89-91. doi: 10.1016/s0960-8524(00)00114-0

Bagewadi ZK, Mulla SI and Ninnekar HZ. 2017. Optimization of laccase production and its application in delignification of biomass. International Journal of Recycling of Organic Waste in Agriculture 6: 351-365. doi: 10.1007/s40093-017-0184-4

Battan B, Sharma J, Dhiman SS and Kuhad RC. 2007. Enhanced production of cellulase-free thermostable xylanase by *Bacillus pumilus* ASH and its potential application in the paper industry. Enzyme and Microbial Technology 41: 733-739. doi: 10.1016/j.enzmictec.2007.06.006

Bhoyar SS, Chaudhari AU, Desai MA, Latpate RV, Sartale SD and Kodam KM. 2024. Wheat bran as an efficient agro-process waste for enhanced yellow laccase production by *Lentinus tigrinus* SSB_W2 and its application in anthraquinone dye degradation. 3 Biotech 14: 33. doi: 10.1007/s13205-023-03881-9

Boran F and Yeşilada Ö. 2022. Laccase production by newly isolated *Ganoderma lucidum* with solid state fermentation conditions and its using for dye decolorization. Adıyaman Üniversitesi Mühendislik Bilimleri Dergisi 9: 458-470. doi: 10.54365/adyumbd.1107682

Chaudhary S, Singh A, Varma A and Porwal S. 2022. Recent advancements in biotechnological applications of laccase as a multifunctional enzyme. Journal of Pure and Applied Microbiology 16: 1479-1491. doi.org/10.22207/JPAM.16.3.72

Chmelová D, Legerská B, Kunstová J, Ondrejovič M and Miertuš S. 2022. The production of laccases by white-rot fungi under solid-state fermentation conditions. World Journal of Microbiology and Biotechnology 38: 21. doi: 10.1007/s11274-021-03207-y

Chhaya U and Gupte A. 2013. Effect of different cultivation conditions and inducers on the production of laccase by the litter-dwelling fungal isolates *Fusarium incarnatum* LD-3 under solid substrate fermentation. Annals of Microbiology 63: 215–223. doi: 10.1007/s13213-012-0464-1

Das J, Badak M. and Singh RK. In Microbiology-2.0 Update for a Sustainable Future, Singapore: Springer Nature Singapore. 2024. Sustainable Innovations and Production Strategies of White Rot Fungi-Derived Laccase. 285– 06. doi: 10.1007/978-981-99-9617-9_13

Dashora K, Gattupalli M, Tripathi GD, Javed Z Singh S, Tuohy M, Sarangi PK, Diwan D, Singh HB and Gupta VK. 2023. Fungal Assisted Valorisation of Polymeric Lignin: Mechanism, Enzymes and Perspectives. Catalysts 13: 149. doi: 10.3390/catal13010149

Ding Z, Chen Y, Xu Z. Peng L, Xu G, Gu Z. Zhang L, Shi G and Zhang K. 2014. Production and characterization of laccase from *Pleurotus ferulae* in submerged fermentation. Annals of Microbiology 64: 121-129. doi: 10.1007/s13213-013-0640-y

Durán-Sequeda D, Suspes D, Maestre E, Alfaro M, Perez G, Ramírez L, Pisabarro AG, Sierra R. 2022. Effect of Nutritional Factors and Copper on the Regulation of Laccase Enzyme Production in *Pleurotus ostreatus*. Journal of Fungi 8: 7. doi: 10.3390/jof8010007

Dutt D and Kumar A. 2014. Optimization of cellulase production under solid-state fermentation by Aspergillus flavus (AT-2) and *Aspergillus niger* (AT-3) and its impact on stickies and ink particle size of sorted office paper. Cellulose Chemistry and Technology 48: 285-298.

Elisashvili V, Kachlishvili E, Khardziani T and Agathos SN. 2010. Effect of aromatic compounds on the production of laccase and manganese peroxidase by white-rot basidiomycetes. Journal of Industrial Microbiology and Biotechnology 37: 1091–1096. doi: 10.1007/s10295-010-0757-y

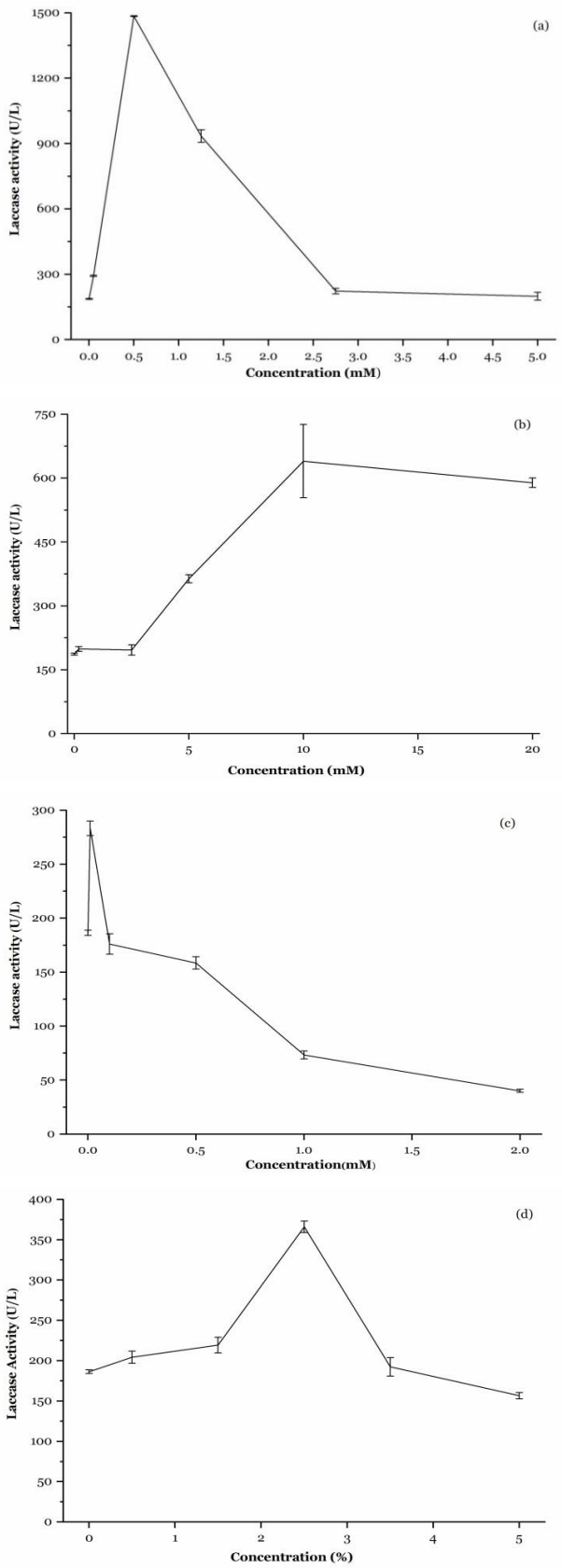

Figure 10. Effect of inducers on laccase production (mean ± SD). (a) Copper sulphate, (b) Ethanol, (c) Ferulic acid, (d) Veratryl alcohol. SSF conditions: pH-5.0, temperature - 25°C, Moisture ratio - 1:3, Day-16.

Elsayed MA, Hassan MM, Elshafei AM, Haroun, BM and Othman AM. 2012. Optimization of cultural and nutritional parameters for the production of laccase by *Pleurotus ostreatus* ARC280. British Biotechnology Journal 2: 115–132. doi: 10.9734/BBJ/2012/1305

Ergun OS and Urek RO. 2017. Production of ligninolytic enzymes by solid state fermentation using *Pleurotus ostreatus*. Annals of Agricultural Sciences 15: 73–277. doi: 10.1016/j.aasci.2017.04.003

Fonseca MI, Shimizu E, Zapata PD and Villalba LL. 2010. Copper inducing effect on laccase production of white rot fungi native from Misiones (Argentina). Enzyme and Microbial Technology 46: 534-539. doi: 10.1016/j.enzmictec.2009.12.017

Gałązka A, Jankiewicz U and Szczepkowski A. 2023. Biochemical characteristics of laccases and their practical application in the removal of xenobiotics from water. Applied Sciences 13: 4394. doi: 10.3390/app13074394

Geethanjali PA, Gowtham HG and Jayashankar M. 2020. Optimization of culture conditions for hyper-production of laccase from an indigenous litter dwelling fungus *Mucor circinelloides* GL1. Environmental Sustainability 3: 481-495. doi: 10.1007/s42398-020-00137-7

Ghosh P and Ghosh U. 2017. Statistical optimization of laccase production by *Aspergillus flavus* PUF5 through submerged fermentation using agro-waste as cheap substrate. Acta Biologica Szegediensis 61: 25-33.

Gutiérrez-Antón M, Santiago-Hernández A, Rodríguez-Mendoza J, Cano-Ramírez C. Bustos-Jaimes I, Aguilar-Osorio G, Campos JE and Hidalgo-Lara M E. 2023. Improvement of Laccase Production by *Thielavia terrestris* Co3Bag1. Enhancing the Bio-Catalytic Performance of the Native Thermophilic TtLacA via Immobilization in Copper Alginate Gel Beads. Journal of fungi 9: 308. doi: 10.3390/jof9030308

Hamed AA, Abd-Elaziz AM, Ghanem MM, ElAwady ME and Abdel-Aziz MS. 2024. Production of laccase enzyme from *Curvularia lunata* MY3: purification and characterization. Folia Microbiologica 69: 221-234. doi: 10.1007/s12223-023-01088-2

Han ML, Li XQ, Zhang CD, Li MX, Zhang MH, An M, Dou XY, Zhang TX, Yan XY, Bian LS. and An Q. 2022. Effect of Different Lignocellulosic Biomasses on Laccase Production by *Pleurotus* Species. BioResources 18: 584-598. doi: 10.15376/biores.18.1.584-598

Hasan S, Anwar Z, Khalid W, Afzal F, Zafar M, Ali U, Refai MY., Afifi M, AL-Farga A. and Aljobair MO. 2023. Laccase production from local biomass using solid state fermentation. Fermentation 9: 179. doi: 10.3390/fermentation9020179

Ibarra-Islas A. Hernández JEM, Armenta S, López JE, López PMG, León SH and Arce-Cervantes O. 2023. Use of nutshells wastes in the production of lignocellulolytic enzymes by white-rot fungi. Brazilian Archives of Biology and Technology 66: e23210654. doi: 10.1590/1678-4324-2023210654

Jaramillo AC, Cobas M, Hormaza A and Sanromán MÁ. 2017. Degradation of adsorbed azo dye by solid-state fermentation: Improvement of culture conditions, a kinetic study, and rotating drum bioreactor performance. Water, Air, & Soil Pollution 228: 1-14. doi: 10.1007/s11270-017-3389-2

Janusz G, Pawlik A, Sulej J, Świderska-Burek U, Jarosz-Wilkołazka A and Paszczyński A. 2017. Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiology Reviews 4: 941-962. doi: 10.1093/femsre/fux049

Kapoor M, Kapoor RK and Kuhad RC. 2007. Differential and synergistic effects of xylanase and laccase mediator system (LMS) in bleaching of soda and waste pulps. Journal of Applied Microbiology 103: 305-317. doi: 10.1111/j.1365-2672.2006.03251.x

Karp SG, Faraco V, Amore A, Birolo L, Giangrande C, Soccol VT, Pandey A and Soccol CR. 2012. Characterization of laccase isoforms produced by *Pleurotus ostreatus* in solid state fermentation of sugarcane bagasse. Bioresource Technology 114: 735–739. doi: 10.1016/j.biortech.2012.03.058.

Kiiskinen LL, Rättö M and Kruus K, 2004. Screening for novel laccase-producing microbes. Journal of Applied Microbiology 97: 640-646. doi: 10.1111/j.1365-2672.2004.02348.x

Kocyigit A. Pazarbasi MB, Yasa I, Ozdemir G and Karaboz I. 2012. Production of laccase from *Trametes trogii* TEM H2: a newly isolated white-rot fungus by air sampling. Journal of Basic Microbiology 52: 661-669. doi: 10.1002/jobm.201100341

Lee IY, Jung KH, Lee CH and Park YH. 1999. Enhanced production of laccase in *Trametes vesicolor* by the addition of ethanol. Biotechnology Letters 21: 965-968.

Lee KH, Wi SG, Singh AP and Kim YS. 2004. Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus *Coniophora puteana*. Journal of Wood Science 50: 281–284. doi: 10.1007/s10086-003-0558-2

Lomascolo A, Record E, Herpoël-Gimbert I, Delattre M, Robert JL, Georis J. Dauvrin T. Sigoillot JC and Asther M. 2003. Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer. Journal of Applied Microbiology 94: 618-624. doi: 10.1046/j.1365 2672.2003.01879.x

López-Pérez M, Aguirre-Garrido JF, Herrera-Zúñiga L and García-Arellano H. 2024. Structure, expression regulation, and applications of fungal laccases, an interesting prospective in biotechnology. Studies in Natural Products Chemistry 80: 227-267. doi: 10.1016/B978-0-443-15589-5.00008-6

Manavalan T, Manavalan A, Thangavelu KP and Heese K. 2013. Characterization of optimized production, purification and application of laccase from *Ganoderma lucidum*. Biochemical Engineering Journal 70: 106–114. doi: 10.1016/j.bej.2012.10.007

Muñoz SS, Balbino TR, Alba EM, Barbosa FG, de Pier FT, de Almeida ALM, Zilla AHB, Antunes FAF, Hilares RT. Balagurusamy N. et al. 2022. Surfactants in biorefineries: Role, challenges & perspectives. Bioresource Technology 345: 126477. doi: 10.1016/j.biortech.2021.126477

Nandal P, Ravella SR and Kuhad RC. 2013. Laccase production by *Coriolopsis caperata* RCK2011: Optimization under solid state fermentation by Taguchi DOE methodology. Scientific Reports 3: 1386. doi: 10.1038/srep01386

Naz S, Gupta S and Chatterjee T. 2022. A review: optimization of fungal laccase production and their potential application in effluents treatment. Journal of Scientific Research 66: 86-102. doi: 10.37398/JSR.2022.660110

Palmieri G, Giardina P, Bianco C, Fontanella B and Sannia G. 2000. Copper Induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Applied and Environmental Microbiology 66: 920–924. doi: 10.1128/AEM.66.3.920-924.2000

Patel H and Gupte A. 2016. Optimization of different culture conditions for enhanced laccase production and its purification from *Tricholoma giganteum* AGHP. Bioresources and Bioprocessing 3: 1-10. doi: 10.1186/s40643-016-0088-6

Patel HK, Sharma PJ and Kalaria RK. 2019. Bioprospecting and molecular characterization of laccase-producing bacteria from industrial contaminated sites. Global Journal of Environmental Science and Management 5: 203–212. doi: 10.22034/gjesm.2019.02.06

Reddy MS and Kanwal HK. 2022. Influence of carbon, nitrogen sources, inducers, and substrates on lignocellulolytic enzyme activities of *Morchella spongiola*. Journal of Agriculture and Food Research 7: 100271. doi: 10.1016/j.jafr.2022.100271

Revankar MS and Lele SS. 2006. Increased production of extracellular laccase by the white rot fungus *Coriolus versicolor* MTCC 138. World Journal of Microbiology and Biotechnology 22: 921-926. doi: 10.1007/s11274-006-9136-2

Rivera-Hoyos CM, Morales-Alvarez ED, Poutou-Pinales RA, Pedroza-Rodriguez AM, Rodriguez-Vazquez R, Delgado-Boada JM. 2013. Fungal laccases. Fungal Biology Reviews. 27:67–82. doi: 10.1016/j.fbr.2013.07.001

Sharghi S, Ahmadi FS, Kakhki AM and Farsi M. 2024 Copper increases laccase gene transcription and extracellular laccase activity in *Pleurotus eryngii* KS004. Brazilian Journal of Microbiology. 55: 111–116. doi: 10.1007/s42770-024-01257-6

Sharma A, Thakur VV, Shrivastava A, Jain RK, Mathur RM, Gupta R. and Kuhad RC. 2014. Xylanase and laccase-based enzymatic kraft pulp bleaching reduces adsorbable organic halogen (AOX) in bleach effluents: a pilot scale study. Bioresource Technology 169: 96–102. doi: 10.1016/j.biortech.2014.06.066

Sharma A, Shrivastava B and Kuhad RC. 2015. Reduced toxicity of malachite green decolorized by laccase produced from *Ganoderma* sp. rckk-02 under solid-state fermentation. 3 Biotech 5: 621-631. doi: 10.1007/s13205-014-0258-1

Sharma KK, Kapoor M and Kuhad RC. 2005. In vivo enzymatic digestion, *in vitro* xylanase digestion, metabolic analogues, surfactants and polyethylene glycol ameliorate laccase production from *Ganoderma* sp. kk-02. Letters in Applied Microbiology 41: 24-31. doi: 10.1111/j.1472-765X.2005.01721.x.

Sharma S and Murty DS. 2021. Enhancement of Laccase Production by Optimizing the Cultural Conditions for *Pleurotus sajor-caju* in Solid-State Fermentation. Journal of Pure and Applied Microbiology 15: 958-967. doi: 10.22207/JPAM.15.2.54

Shrestha P, Joshi B, Joshi J, Malla R and Sreerama L. 2016. Isolat physicochemical characterization of laccase from *Ganoderma lucidum* isolated from its native habitat in Nepal. BioMed Research International 2016: 3238909. doi: 10.1155/2016/3238909

Singh A and Singh RK. 2017. Effect of surfactants on lignin modifying enzyme activity of *Ganoderma* species KX879638 and *Lentinus* species KY006984 under solid state fermentation. African Journal of Microbiology Research 11: 1520-1527. doi: 10.5897/AJMR2017.8589

Singhania RR, Patel AK, Soccol CR and Pandey A. 2009. Recent advances in solid-state fermentation. Biochemical Engineering Journal 44: 13–18. doi: 10.1016/j.bej.2008.10.019

90

Sun J, Zheng M, Lu Z, Lu F and Zhang C. 2017. Heterologous production of a temperature and pH-stable laccase from *Bacillus vallismortis* fmb-103 in Escherichia coli and its application. Process Biochemistry 55: 77-84. doi: 10.1016/j.procbio.2017.01.030

Swatek A and Staszczak M. 2020. Effect of Ferulic Acid, a Phenolic Inducer of Fungal Laccase, on 26S Proteasome Activities *In Vitro*. International Journal of Molecular Sciences 21: 2463. doi: 10.3390/ijms21072463

Tavares APM, Coelho MAZ, Coutinho JAP and Xavier AMRB. 2005. Laccase improvement in submerged cultivation, induced production and kinetic modeling. Journal of Chemical Technology and Biotechnology 80: 669–676. doi: 10.1002/jctb.1246

Teerapatsakul C, Parra R, Bucke C and Chitradon L, 2007. Improvement of laccase production from Ganoderma sp. KU-Alk4 by medium engineering. World Journal of Microbiology and Biotechnology 23: 1519-1527. doi: 10.1007/s11274-007-9396-5

Thurston CF. 1994. The structure and function of fungal laccases. Microbiology 1: 19–26. doi: 10.1099/13500872-140-1-19

Umar A and Ahmed S. 2022. Optimization, purification, and characterization of laccase from *Ganoderma leucocontextum* along with its phylogenetic relationship. Scientific Reports 12: 2416. doi.org/10.1038/s41598-022-06111-z

Vantamuri AB, Manawadi SI, Guruvin SK, Holeyannavar VM and Shettar DS. 2019. Production of laccase by *Ganoderma* sp. in submerged fermentation. Journal of Advanced Scientific Research 10: 67-71. https://sciensage.info/index.php/JASR/article/view/331

Wang F, Xu L, Zhao L, Ding Z, Ma H and Terr Y. 2019. Fungal laccase production from lignocellulosic agricultural wastes by solid-state fermentation: A review. Microorganisms 7: 665. doi: 10.3390/microorganisms7120665

Weng C, Peng X, and Han Y. 2021. Depolymerization and conversion of lignin to value-added bioproducts by microbial and enzymatic catalysis. Biotechnology for biofuels 1: 84. doi: 10.1186/s13068-021-01934-w

Xin F and Geng A. 2011. Utilization of horticultural waste for laccase production by *Trametes versicolor* under solid-state fermentation. Applied Biochemistry and Biotechnology 163: 235–246. doi: 10.1007/s12010-010-9033-x

©JBR2024 Published by Centre with Potential for Excellence in Biodiversity, Rajiv Gandhi University, Rono Hills, Doimukh-791112, Itanagar, Arunachal Pradesh, India. Visit: https://jbr.rgu.ac.in; Email: editor.jbr@rgu.ac.in