Earthline Journal of Chemical Sciences
E-ISSN: 2581-9003; CODEN: EJCSB4
Volume 11, Number 2, 2024, Pages 249-265
https://doi.org/10.34198/ejcs.11224.249265

Degradation of textile polyazodyes by *Polyporus ciliatus* laccase produced in peanut shell solid medium: purification and characterization

Fatma Kallel*

Laboratory of Plant Improvement and Valorization of Agricultural Resources, National School of Engineering, Sfax University, P.O. Box 1173-3038, Tunisia
e-mail: f111fatma@yahoo.fr

Nadia Etiss

Laboratory of Plant Improvement and Valorization of Agricultural Resources, National School of Engineering, Sfax University, P.O. Box 1173-3038, Tunisia

Semia Ellouz Chaabouni

Laboratory of Plant Improvement and Valorization of Agricultural Resources, National School of Engineering, Sfax University, P.O. Box 1173-3038, Tunisia

Mohamed Neifar

Laboratory of Plant Improvement and Valorization of Agricultural Resources, National School of Engineering, Sfax University, P.O. Box 1173-3038, Tunisia

Common Services Bioreactor Unit Coupled with an Ultrafilter, National School of Engineering, Sfax University, P.O. Box 1173-3038, Tunisia

Abstract

This study describes decolorization of reactive sulfonated azoic dye Direct Blue 71, one of the recalcitrant and highly used industrial dyes, by *Polyporus ciliatus* derived laccase. Agro-industrial wastes are increasingly used as substrates for laccase production from white rot fungi through solid-state fermentation (SSF). Peanut shell (PS) is an attractive substrate for laccase production, and SSF is an adequate strategy for that purpose. It has been employed as a novel support-substrate for laccase production by Polyporus ciliatus under solid-state fermentation. The highest laccase yield of 1260 U/ml was attained using PS after 13 days of incubation at 30°C. The purified laccase (55 kDa) showed maximum activity at pH 3 and 60°C. The extracellular laccase enzyme was purified 67.6-fold to a

Received: March 19, 2024; Accepted: April 10, 2024; Published: April 14, 2024

Keywords and phrases: laccase; white-rot fungus *Polyporus ciliatus*; peanut shells solid medium; purification; dye decolorization.

*Corresponding author

specific activity of 1466 U/mg by a Mono-Q anion-exchange column. The purified enzyme Km value was found to be 24 µM, while the Vmax value was observed to be 0.96 µM s^{-1} with ABTS as a substrate. It had high thermotolerance and pH stability and was resistant to several metal ions including copper, cadmium, and iron. *P. ciliatus* pure laccase showed high potential for the decolorization and detoxification of the reactive sulfonated azoic dye Direct Blue 71, which suggested that this enzyme could be used for textile effluent treatment.

1. Introduction

Synthetic dyes are frequently used in the textile, food, pharmaceutical, paper and photographic, leather, cosmetic, and other industries. Massive amounts of dyes are discharged in the wastewater during dye manufacturing. These textile dyes persist as environmental pollutants since they are highly toxic, carcinogenic agents, and resistant to biodegradation [1, 2].

Physico-chemical processes such as coagulation, flocculation, filtration, sedimentation, oxidation, and flotation have been extensively applied for textile wastewater treatment. Although effective, these techniques are often insufficient to maintain performance over time. They also have certain drawbacks such as high cost and extra waste generation. Recalcitrant dye degradation by ligninolytic fungi has recently been shown to have great and promising potential to replace or complement current textile effluent treatment processes [3, 4]. Among ligninolytic enzymes, laccases (EC 1.10.3.2) are multicopper phenol oxidases, which catalyze the oxidation of a wide range of reducing aromatic phenolic substrates using dioxygen as a renewable electron acceptor. The substrate range of fungal laccases may be extended to nonphenolic and highly recalcitrant compounds by the inclusion of low-molecular-weight mediators like 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). Free and immobilized laccase reactors have been developed and applied for dye pollutant removal and wastewater treatment [5–9].

Laccases produced by white rot fungi (WRF) are widely studied at laboratory, pilot, and industrial scales. Conventional submerged fermentation has been the typical and the most commonly industrial process used for the production of microbial enzymes such as laccases. However, solid-state fermentation (SSF) represents an interesting alternative to the production of fungal enzymes at lower costs, since it simulates the natural growth conditions of WRFs. SSF well adapted for fungal growth has many advantages like being

a simpler fermentation process, having lower energy consumption, less pollution and water consumption as well as higher added-value product recovery [10]. Substrates used for laccase production by WRF under SSF conditions are typically agricultural and agro-industrial wastes such as fruit peels, cereal bran, straw, or oil cake [11, 12]. These agro-residues serve as an economic substrate, reducing the environmental pollution caused by their accumulation.

Polyporus species are white-rot basidiomycetes exhibiting high ligninolytic capabilities. [13–15]. Laccases from the *Polyporaceae* family generally have high redox potentials [16], making them interesting candidates for industrial applications, including textile wastewater treatment [17].

The aims of this study were to assess the potential of selected agro-residues for laccase production by the WRF *Polyporus ciliatus* since the use of such substrates would mean a significant decrease in production costs. In the other hand, this aims to purify and characterize the enzyme produced under SSF conditions and to study the enzyme decolorization of Direct Blue 71 as a model polyazo. To the best of our knowledge, this is the first report on the characterization of *P. ciliatus* laccase produced by SSF.

2. Materials and Methods

2.1. Chemicals

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and 2,6-dimethoxyphenol (DMP) were from Sigma–Aldrich. Q-Sepharose was from Pharmacia LKB Biotechnology, and Biogel P100 was from Bio-Rad. All other chemicals used were of analytical grade.

2.2. Fungal strain

Polyporus ciliatus was provided by the Belgian Coordinated Collections of Microorganisms/Mycothèque of the Catholic University of Leuven (BCCM/MUCL). The fungus was maintained on malt extract agar (MEA, 2%) plates grown at 30 °C and stored at 4 °C.

2.3. Fermentation and enzyme extraction

Agro-industrial wastes used as economical support-substrates to produce laccase by

P. ciliatus were: pea pomace (PP), pomegranate husk (PH), peanut shells (PS), cactus-pear peel (CPP), and acacia leaves (AL). The physiochemical composition of these by-products is shown in Table 1. Laccase production was performed in Erlenmeyer flasks (500 ml) containing 5 g of selected agro-residue, 30 ml of sodium acetate buffer 20 mM pH 5.0, 11.25 mg $CuSO_4$, and 0.75 mg $CaCl_2$. The medium was autoclaved then inoculated with six disks (6 mm in diameter) taken from the active borders of MEA medium, followed by incubation at 30 °C under dark.

After cultivation, the enzyme extraction was conducted with tartrate buffer (pH 5, 10 ml buffer/g substrate) by stirring for 1 h at 150 rpm and the suspension was filtered on Whatman paper [12].

Table 1. Chemical composition of the different lignocellulosic agro-residues employed.

Agro-residue	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Reference
Pea pomace	22.12	62.3	8.2	[18]
Pomegranate husk	4.5	11.4	3.4	[19]
Peanut shells	26.4	40.5	14.7	[20]
Cactus-pear peel	3.06	13.18	7.64	[21]
Acacia leaves	14.13	14.12	5.76	[22]

2.4. Enzyme and protein assays

Laccase activity was assayed, as described by Neifar et al. [3] using 5 mM DMP in sodium tartrate buffer (100 mM; pH 5) (ε469 = 27, 500 M^{-1} cm^{-1}, referred to DMP concentration). One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 µmol of DMP per minute. During the enzyme purification process, protein concentration and absorbance at 280 nm were measured to monitor and quantify protein concentration, using Bovine Serum Albumin (BSA) as a standard.

2.5. Laccase purification

Laccase purification was carried out at 4 °C in three steps as described by Neifar et al. [23]. The culture cell-free was first saturated with ammonium sulfate (approximately at 80%), followed by centrifugation (30 min at 10,000 × g) to separate the precipitated proteins from the solution. The resulting pellet was resuspended in citrate-phosphate buffer (20 mM; pH 6). The enzyme solution was then applied to a size-exclusion

chromatography column (Biogel P100, 2.5 × 90 cm). The column was equilibrated with the same buffer (20 mM citrate-phosphate buffer, pH 6.0), and the flow rate was maintained at 20 ml h^{-1}. Fractions containing laccase activity were pooled and applied to a Mono-Q anion-exchange column (Q-Sepharose Cl 6B, 1.6 × 11 cm). The column was equilibrated with 20 mM citrate-phosphate buffer (pH 6.0), and the elution was performed using a non-linear NaCl gradient ranging from 0 to 500 mM. The laccase peak fractions are pooled and stored at 4 °C or at -20 °C in glycerol to preserve their activity.

2.6. Gel electrophoresis and zymograms

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was carried out according to the method of Laemmli [24]. Proteins separated on the gel are visualized by staining with Coomassie Brilliant Blue G-250. Zymogram was obtained using 10 mM 2,6-dimethoxyphenol (DMP) in 0.1 M sodium tartrate buffer at pH 4. The gel was washed for 10 minutes with the same buffer and then incubated with DMP [25]**.**

2.7. Enzyme characterization

The temperature profile of the purified laccase was determined by incubating the enzyme with DMP at different temperatures ranging from 25 to 70 °C. The thermal stability of the enzyme was determined by assaying for residual enzyme activity after incubation for 4 hours at different temperatures (25, 50, 60, and 70°C). The optimal pH of the purified enzyme was examined at pH 2–9 under standard assay conditions using 50 mM of citrate buffer (pH 2 to 6) and Tris–HCl buffer (pH 7 to 9). For pH stability, the purified laccase was pre-incubated at room temperature in buffer systems in the range of pH 2–9 for 1 and 24 hours. The remaining enzyme activity was then assayed under optimal conditions.

The effect of metal ions on the purified laccase was determined by using different metal salts, including $Co(NO_3)_2$, $CdCl_2$, $MnSO_4$, $HgCl_2$, $FeSO_4$, $MgSO_4$, $ZnSO_4$, and $CuSO_4$, at a final concentration of 10 mM separately. The effect of other potential inhibitors (ethylenediaminetetraacetic acid [EDTA], sodium azide [NaN_3], sodium dodecyl sulfate [SDS], and L-cysteine) on laccase activity was also determined.

The Lineweaver-Burk plot method was used to determine kinetic constants such as the Michaelis-Menten constant (K_m) and the maximum velocity (V_{max}) of an enzyme-

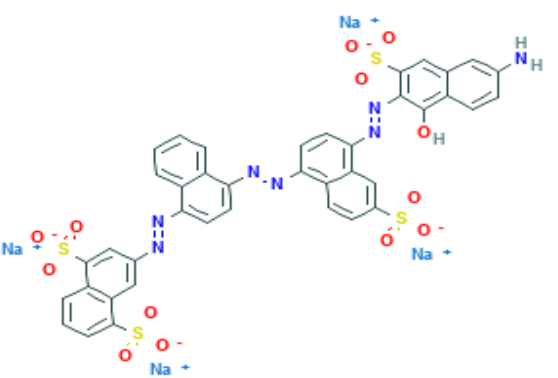
catalyzed reaction by using DMP as a substrate at their optimal temperature and pH values.

2.8. Enzymatic dye decolorization and toxicity assays

The dye decolorization ability of the purified enzyme was assessed using the recalcitrant dye DB71 (Table 2). The reaction mixture involved the dye DB71 at a concentration of 150 mg L^{-1}, purified laccase enzyme at a concentration of 0.1 U mL^{-1}, and a 0.1 M tartrate buffer at pH 4.0.

The DB71 decolorization (as absorbance at 518 nm) was measured using a Shimadzu UV-VIS Scanning spectrophotometer (UV-mini-1240; Shimadzu, Kyoto, Japan) and expressed in terms of percentage. The control test contains the same amount of denatured laccase. In order to determine the effect of ABTS as a laccase mediator, experiments were also conducted with the addition of 1 mM ABTS. Phytotoxicity assays were performed as described by Daâssi et al. [26] using laccase-treated and untreated dye.

Table 2. Characteristics of the dye employed in this study.

Property	**Direct Blue 71**
λ_{max} (nm)	594
CA	4399-55-7
C.I.	34140
Chemical formula	$C_{40}H_{23}N_7Na_4O_{13}S_4$
Molecular weight (g mol^{-1})	1029.9
Molecular structure	

2.9. Statistical analysis

All experiments and analytical measurements were conducted in triplicate. Duncan's multiple range tests were employed for the statistical analysis of the data. Differences between treatments were considered significant at the 5% level ($p< 0.05$).

3. Results and Discussion

3.1. Laccase production

Fungal laccases are enzymes that have gained attention for their potential applications in treating dye wastewaters (WW). Therefore, for practical and economical implementation of fungal laccases in WW treatment, enzyme production by SSF must be efficient and cost-effective. An interesting strategy for this challenge is the use of easily available low-cost substrates, such as agricultural wastes, for fungal growth and enzyme production [12]. In this study, *P. ciliatus* could able to grow extensively on all substrates tested. Figure 1 illustrates the profile of laccase productivity during degradation of various by-products by *P. ciliatus*. There was a rapid increase in laccase productivity, reaching its maximum on day 13 of fermentation. This suggests that the fungal strain *P. ciliatus* was capable of producing laccase efficiently during SSF. Then laccase productivity increased during the last days of SSF. Peanut shells were determined as the most suitable substrate for enzyme production by *P. ciliatus* with a laccase activity of 1420 U/L, as shown in Figure 1. Polyphenols, which are often present in peanut shells, can serve as inducers for laccase synthesis. These compounds may act as signaling molecules or substrates that enhance the expression of laccase genes in *P. ciliates* [27]. Pea pomace, pomegranate husk, and acacia leaves can be also considered as promising and potential inducers of laccase activities (910, 770, and 759 U/L, respectively), while the production in cactus-pear peel solid medium was low (325 U/L). These findings show the potential of using some abundant agricultural residues in Tunisia for laccase production without any addition of enzyme inducers. In fact, these substrates had not been previously used as support-substrates for enzyme production, which opens up new avenues for research and innovation in the field of laccase production from agricultural residues.

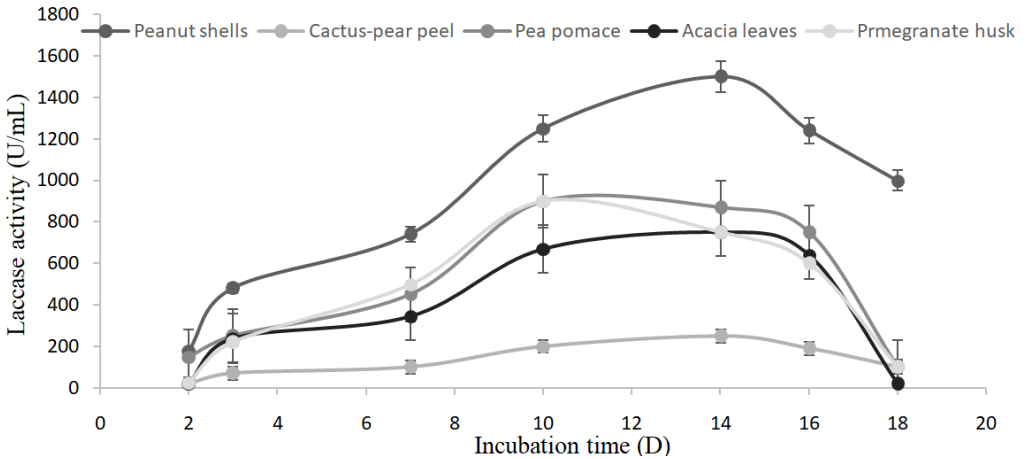


Figure 1. Laccase production by *P. ciliatus* grown on different natural supports under SSF conditions.

3.2. Laccase purification and biochemical characterization

A laccase was successfully purified from *P. ciliatus* culture filtrate in two chromatographic steps (Figure 2A and Figure 2B). The summary of purification results is given in Table 3. A total of 68-fold purification of crude enzyme extract was achieved, and the purification overall yield was about 30% (Table 3). This result is better than that obtained by Kumar et al. [28], who reported a 4.24-fold purification of *Aspergillus flavus* laccase after filtration, ammonium sulfate precipitation (ASP), and dialysis steps. Laccase from the basidiomycete fungus *Stereum Ostrea* was successfully purified by ASP followed by Sephadex G-100 column chromatography, leading to a purification fold of 70 [29].

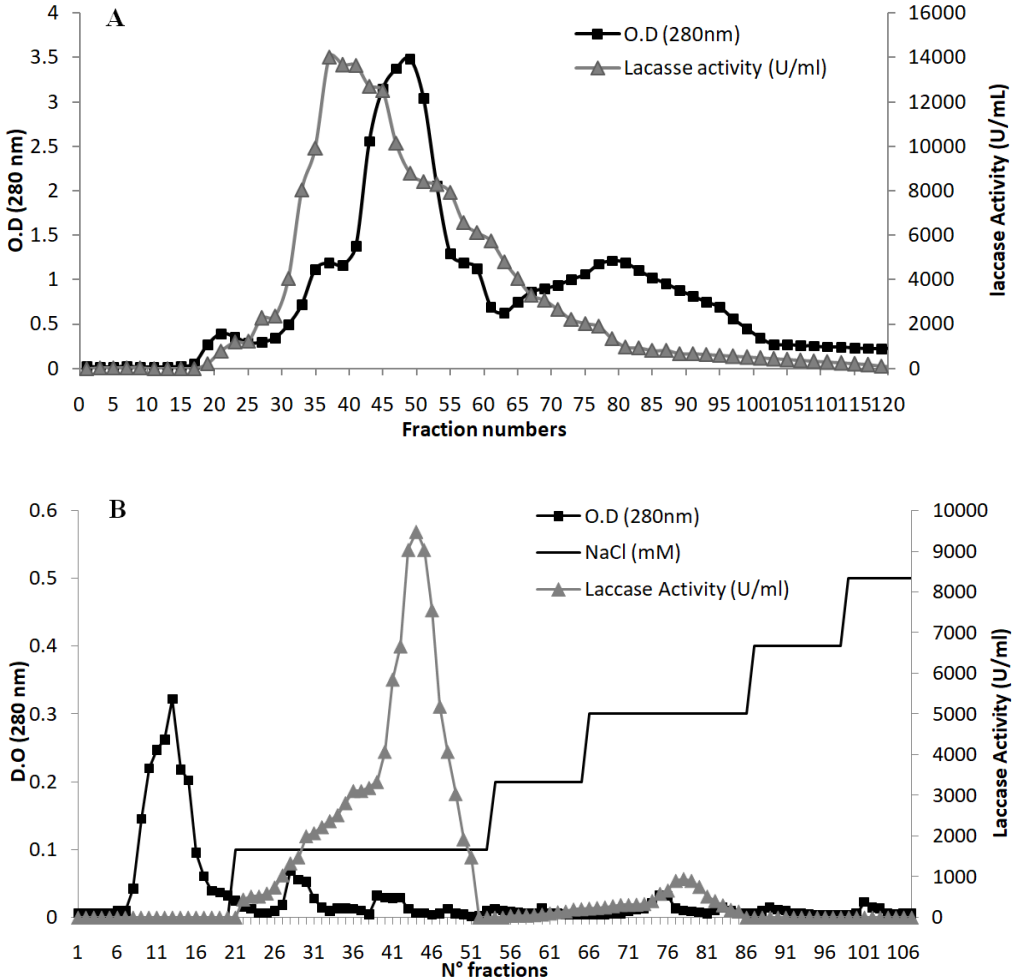


Figure 2. Purification of *P. ciliatus* laccase. Subsequent chromatography steps on Biogel P100 (**A**) and Mono-Q (**B**) columns.

The molecular mass of the purified laccase from *P. ciliatus* is 55 kDa as determined by SDS-PAGE (Fig. 3). It was similar to most WRF laccases (50-80 kDa) [30]. The UV-visible spectrum analysis indicates that *P. ciliatus* laccase exhibits a typical electronic structure of blue laccases, with characteristic absorption peaks corresponding to type 1 and type 3 copper sites with intensive peaks at 600 and 330 nm, respectively [31]. The K_m and V_{max} values of *P. ciliatus* laccase were 24 µM and 0.96 µM s^{-1}, respectively, using ABTS as a substrate. The K_m value of the purified laccase is very close to that of

laccase from *Trametes sp. strain* AH28-2 (25 µM) [32]. Most of the reported fungal laccases, specifically in terms of their activity toward ABTS, exhibit a range of Km values of approximately 30-80 µM. This result indicates a higher substrate affinity, and the purified laccase is effective at lower substrate concentrations, which can be important for industrial processes [33].

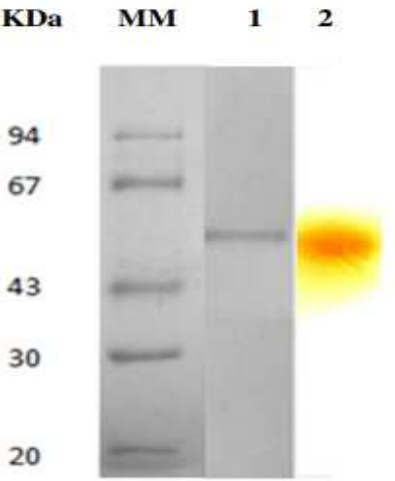


Figure 3. Electrophoretic analysis of the purified *P. ciliates* laccase. (MM: Molecular mass markers. Lane 1: purified laccase, Lane 2: Laccase activity band detected by zymogram with DMP as a substrate).

Table 3. Purification of *Polyporus ciliatus* laccase from the peanut shells solid culture.

Purification step	**Total activity (IU)**	**Total protein (mg)**	**Specific activity (IU/mg)**	**Yield (%)**	**Purification factor (X-fold)**
Culture supernatant	3600	166	21.7	100	1
AS precipitation (80%)	3200	115	27.8	88.9	1.3
Biogel P100	1203	15	80.2	33.4	3.7
Mono-Q	1100	0.75	1466	30.5	67.6

P. ciliatus laccase required a temperature of 60°C for optimal activity (Figure 4A), similar to that of other fungal laccases [34], but higher than those of others such as *Tricholoma mongolicum* [35] and *P. ostreatus* [36] (optimal temperatures at 30 and 35°C, respectively). Laccase was active over a wide temperature range since approximately 65% of activity was detected at 20°C and was maintained at temperatures as high as 80°C (Figure 4A). The purified laccase was relatively thermostable, presenting an advantage for industrial applications. Indeed, half-lives at 60 and 70°C were estimated at 220 and 20 min, respectively (Figure 4B). The pH optimum of the purified enzyme was 3.0 using DMP as a substrate (Figure 4C). Enzyme stability at acidic pH values was remarkably lower than under more neutral conditions (Figure 4D), as already observed for other fungal laccases [23].

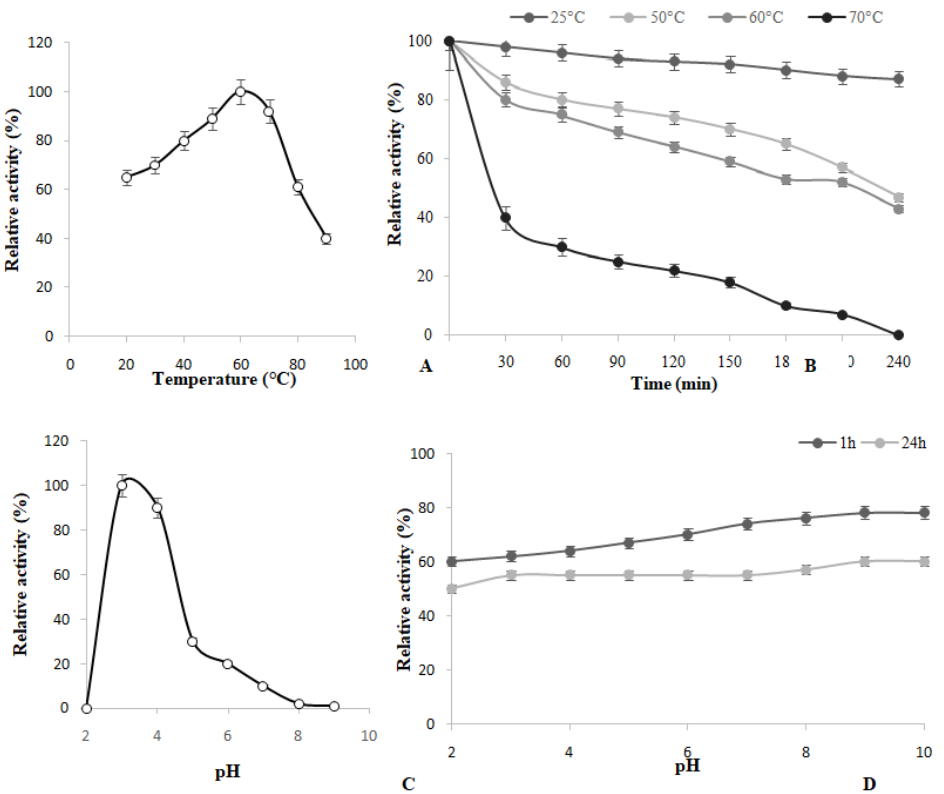


Figure 4. Effect of pH and temperature on activity and stability of purified laccase from *P. ciliatus*: (**A**) optimum temperature using DMP as substrate. (**B**) thermal stability at 25° C, 50 °C, 60 °C and 70 °C. (**C**) optimum pH using DMP as substrate. (**D**) pH stability after 1 h and 24 h incubation at 25°C.

The sensitivity of the purified enzyme to metal ions and chemical reagents was highlighted in Table 4. *P. ciliatus* laccase activity seems unaffected by the presence of Mg^{2+}, Cd^{2+}, and Mn^{2+} ions. The enzyme was highly sensitive to Hg^{2+}, a common inhibitor of metalloenzymes, indicating the presence of thiol groups essential for its activity. It was also inhibited by Co^{2+}, Zn^{2+}, and Fe^{2+}. In contrast, the laccase activity was improved by Cu^{2+} at 10 mM (Table 4). The enzyme activation by Cu^{2+} ions may be related to the fact that Cu^{2+} is an active center of fungal laccases [37]. *P. ciliatus* laccase was significantly inhibited by sodium azide, L-cysteine, and SDS. These results showed that P. ciliatus laccase was inhibited by typical inhibitors of metal-containing oxidases [38].

Table 4. Effect of various metals and potential inhibitors on oxidation of DMP by purified *P. ciliatus* laccase.

Compound	**Concentration (mM)**	**Residual activity (%)**
Cu^{2+}	10	116.3
Fe^{2+}	10	90.5
Cd^{2+}	10	96.4
Zn^{2+}	10	72.7
Co^{2+}	10	40.9
Hg^{2+}	10	4.4
Mg^{2+}	10	102.3
Mn^{2+}	10	97.1
L-Cys	1	8.8
NaN_3	0.01	0.0
SDS	1	0.0
EDTA	1	81.8

Synthetic dyes are widely used in textile industries thanks to their vibrant colors and versatility in dyeing processes [39]. In this study, the ability of P. ciliatus laccase to decolorize and detoxify the reactive polyazoic dye Direct Blue 71 (Table 2) has been investigated. The phytotoxicity of untreated and laccase-treated dye DB71 on Solanum lycopersicum, Lepidium sativum, and Medicago sativa seeds was performed to evaluate the variation of the degree of phytotoxicity. As shown in Table 5, a highly significant inhibition of all seed germination by untreated DB71 dye was recorded. In fact, treated dye of laccase of *P. ciliatus* exerted a positive effect on the germination. The index of germination of DB71 dye under Solanum lycopersicum, Medicago sativa, and Lepidium

sativum was 76.4%, 67.7%, and 51.7%, respectively. Furthermore, DB71 treated with laccase and mediated by ABTS contributed to the amelioration of the germination of the different tested seeds.

Table 5. Germination index of untreated and treated DB 71 dye by *P. ciliatus* laccase.

Germination Index (%)	*Solanum lycopersicum*	*Medicago sativa*	*Lepidium sativum*
Untreated DB 71	0	0	12
Treated DB 71	76.4	67.7	51.7
Treated DB 71 + ABTS	86.3	41	98

4. Conclusions

The results obtained in this work illustrated the potential advantage of employing cheap agro-industrial wastes such as peanut shells as a support-substrate for laccase production by the White Rot Fungi *P. ciliatus* under SSF conditions. The enzyme produced on peanut shells in a solid medium was purified to homogeneity using only two chromatographic steps. The apparent molecular mass of this enzyme was 55 kDa, and the optimum pH value of the purified laccase was 3 with DMP as substrate. The optimum temperature of the purified laccase was 60°C. The purified enzyme K_m value was 24 µM, while the V_{max} value was found to be 0.96 µM s^{-1} with ABTS as a substrate. The laccase activity was enhanced by Cu^{2+} at a concentration of 10 mM. The purified laccase could be effectively used as a promising alternative for the development of efficient and eco-friendly industrial processes for dye decolorization, such as Direct Blue 71.

Declarations

Competing interests

The authors have declared no conflict of interest

Authors' contributions

We, all authors, certified that we have contributed to the work described in the manuscript. By common consent, we agree to submit it for publication in your esteemed journal. We also state, on our honor, that the content of this manuscript has not been published nor has it been submitted elsewhere.

Funding

This work was supported financially by the Ministry of Higher Education and Scientific Research of Tunisia through a grant to "Laboratory of Plant Improvement and Valorization of Agricultural Resources-ENIS."

References

[1] Drumond Chequer, F. M., De Oliveira, G. A. R., Anastacio Ferraz, E. R., et al. (2013). Textile dyes: dyeing process and environmental impact. In M. Gunay (Ed.), *Eco-Friendly Textile Dyeing and Finishing*. InTech.

[2] Slama, H. B., Chenari Bouket, A., Pourhassan, Z., et al. (2021). Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. *Applied Sciences*, *11*, 6255. https://doi.org/10.3390/app11146255

[3] Neifar, M., Jaouani, A., Kamoun, A., et al. (2011). Decolorization of Solophenyl Red 3BL Polyazo dye by Laccase-Mediator system: Optimization through response surface methodology. *Enzyme Research*, *2011*, e179050. https://doi.org/10.4061/2011/179050

[4] Singh, A., Pal, D. B., Mohammad, A., et al. (2022). Biological remediation technologies for dyes and heavy metals in wastewater treatment: new insight. *Bioresource Technology*, *343*, 126154. https://doi.org/10.1016/j.biortech.2021.126154

[5] Kucharzyk, K. H., Janusz, G., Karczmarczyk, I., & Rogalski, J. (2012). Chemical modifications of laccase from white-rot basidiomycete cerrena unicolor. *Applied Biochemistry and Biotechnology*, *168*, 1989-2003. https://doi.org/10.1007/s12010-012-9912-4

[6] Latif, W., Ciniglia, C., Iovinella, M., et al. (2023). Role of white rot fungi in industrial wastewater treatment: a review. *Applied Sciences*, *13*, 8318. https://doi.org/10.3390/app13148318

[7] Pavko, A. (2011). Fungal decolourization and degradation of synthetic dyes some chemical engineering aspects. In *Waste Water - Treatment and Reutilization*. IntechOpen.

[8] Viswanath, B., Rajesh, B., Janardhan, A., et al. (2014). Fungal laccases and their applications in bioremediation. *Enzyme Research*, *2014*, 163242. https://doi.org/10.1155/2014/163242

[9] Wang, W., Zhang, Z., Ni, H., et al. (2012). Decolorization of industrial synthetic dyes using engineered Pseudomonas putida cells with surface-immobilized bacterial laccase. *Microbial Cell Factories*, *11*, 75. https://doi.org/10.1186/1475-2859-11-75

[10] Costa, J. A. V., Treichel, H., Kumar, V., & Pandey, A. (2018). Chapter 1 - Advances in

solid-state fermentation. In A. Pandey, C. Larroche, & C. R. Soccol (Eds.), *Current Developments in Biotechnology and Bioengineering* (pp. 1-17). Elsevier.

[11] López-Gómez, J. P., Manan, M. A., & Webb, C. (2020). Chapter 7 - Solid-state fermentation of food industry wastes. In M. R. Kosseva & C. Webb (Eds.), *Food Industry Wastes* (Second Edition) (pp. 135-161). Academic Press.

[12] Neifar, M., Jaouani, A., Ellouze-Ghorbel, R., et al. (2009). Effect of culturing processes and copper addition on laccase production by the white-rot fungus Fomes fomentarius MUCL 35117. *Letters in Applied Microbiology*, 49, 73-78. https://doi.org/10.1111/j.1472-765X.2009.02621.x

[13] Imran, M., Asad, M. J., Hadri, S. H., & Mehmood, S. (2012). Production and industrial applications of laccase enzyme. *Journal of Cell and Molecular Biology 10*, 1-11.

[14] Kumar, A., & Chandra, R. (2020). Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. *Heliyon*, *6*, e03170. https://doi.org/10.1016/j.heliyon.2020.e03170

[15] Lundell, T. K., Mäkelä, M. R., de Vries, R. P., & Hildén, K. S. (2014). Chapter Eleven - Genomics, lifestyles and future prospects of wood-decay and litter-decomposing basidiomycota. In F. M. Martin (Ed.), *Advances in Botanical Research* (pp. 329-370). Academic Press.

[16] Zimbardi, A. L. R. L., Camargo, P. F., Carli, S., et al. (2016). A high redox potential laccase from *Pycnoporus sanguineus* RP15: Potential application for dye decolorization. *International Journal of Molecular Sciences*, *17*, 672. https://doi.org/10.3390/ijms17050672

[17] Nakade, K., Nakagawa, Y., Yano, A., et al. (2010). Characterization of an extracellular laccase, PbLac1, purified from *Polyporus brumalis*. *Fungal Biology*, *114*, 609-618. https://doi.org/10.1016/j.funbio.2010.05.002

[18] Verma, N., Bansal, M. C., & Kumar, V. (2011). Pea peel waste: A lignocellulosic waste and its utility in cellulase production by *Tricoderma reesei* under solid state cultivation. *BioResources*, *6*, 1505-1519. https://doi.org/10.15376/biores.6.2.1505-1519

[19] Shabtay, A., Eitam, H., Tadmor, Y., et al. (2008). Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed. *Journal of Agricultural and Food Chemistry*, *56*, 10063-10070. https://doi.org/10.1021/jf8016095

[20] Rivilli, P., Alarcón, R., Isasmendi, G., & Pérez, J. (2011). Stepwise isothermal fast pyrolysis (SIFP). Part II. SIFP of peanut shells - Antifungal properties of phenolic fractions. *Bioresources*, *7*. https://doi.org/10.15376/biores.7.1.0112-0117

[21] Tegegne, F., Kijora, C., & Peters, K. J. (2005). Study on the effects of incorporating various levels of cactus pear (*Opuntia ficus-indica*) on the performance of sheep. Conference on International Agricultural Research for Development, Stuttgart-Hohenheim, October 11-13, 2005.

[22] Reddy, D. V., & Elanchezhian, N. (2008). Evaluation of tropical tree leaves as ruminant feedstuff based on cell contents, cell wall fractions and polyphenolic compounds. *Livestock Research for Rural Development*, *20*.

[23] Neifar, M., Jaouani, A., Ellouze-Ghorbel, R., & Ellouze-Chaabouni, S. (2010). Purification, characterization and decolourization ability of Fomes fomentarius laccase produced in solid medium. *Journal of Molecular Catalysis B: Enzymatic*, *64*, 68-74. https://doi.org/10.1016/j.molcatb.2010.02.004

[24] Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680-685. https://doi.org/10.1038/227680a0

[25] Zouari-Mechichi, H., Mechichi, T., Dhouib, A., et al. (2006). Laccase purification and characterization from *Trametes trogii* isolated in Tunisia: decolorization of textile dyes by the purified enzyme. *Enzyme and Microbial Technology*, *39*, 141-148. https://doi.org/10.1016/j.enzmictec.2005.11.027

[26] Daâssi, D., Mechichi, T., Nasri, M., & Rodriguez-Couto, S. (2013). Decolorization of the metal textile dye Lanaset Grey G by immobilized white-rot fungi. *Journal of Environmental Management*, *129*, 324-332. https://doi.org/10.1016/j.jenvman.2013.07.026

[27] Mahmoud, M., Rifaat, H. M., Sayed, O. H., et al. (2013). Effect of inducers and process parameters on laccase production by locally isolated marine *Streptomyces lydicus* from Red Sea, Egypt. *International Journal of ChemTech Research*, *5*, 15-23.

[28] Kumar, R., Kaur, J., Jain, S., & Kumar, A. (2016). Optimization of laccase production from *Aspergillus flavus* by design of experiment technique: Partial purification and characterization. *Journal of Genetic Engineering and Biotechnology*, *14*, 125-131. https://doi.org/10.1016/j.jgeb.2016.05.006

[29] Viswanath, B., Chandra, M. S., Kumar, K. P., & Reddy, B. R. (2008). Production and purification of laccase from *Stereum ostrea* and its ability to decolorize textile dyes. *DBPBMB*, 2, 19-25.

[30] Baldrian, P. (2006). Fungal laccases - occurrence and properties. *FEMS Microbiology Reviews*, *30*, 215-242. https://doi.org/10.1111/j.1574-4976.2005.00010.x

[31] Palmieri, G., Giardina, P., Bianco, C., et al. (1997). A novel white laccase from *Pleurotus ostreatus*. *Journal of Biological Chemistry*, *272*, 31301-31307. https://doi.org/10.1074/jbc.272.50.31301

[32] Xiao, Y. Z., Tu, X. M., Wang, J., et al. (2003). Purification, molecular characterization and reactivity with aromatic compounds of a laccase from basidiomycete *Trametes sp.* strain AH28-2. *Applied Microbiology and Biotechnology*, *60*, 700-707. https://doi.org/10.1007/s00253-002-1169-3

[33] Zhang, G.-Q., Tian, T., Liu, Y.-P., et al. (2011). A laccase with anti-proliferative activity against tumor cells from a white root fungus *Abortiporus biennis*. *Process Biochemistry*, 46, 2336-2340. https://doi.org/10.1016/j.procbio.2011.09.020

[34] Wang, H. X., & Ng, T. B. (2006). A laccase from the medicinal mushroom *Ganoderma lucidum*. *Applied Microbiology and Biotechnology*, *72*, 508-513. https://doi.org/10.1007/s00253-006-0314-9

[35] Li, M., Zhang, G., Wang, H., & Ng, T. (2010). Purification and characterization of a laccase from the edible wild mushroom *Tricholoma mongolicum*. *Journal of Microbiology and Biotechnology*, *20*, 1069-1076. https://doi.org/10.4014/jmb.0912.12033

[36] Palmieri, G., Cennamo, G., Faraco, V., et al. (2003). An atypical laccase isozyme from copper supplemented *Pleurotus ostreatus* cultures. *Enzyme and Microbial Technology*, 220-230. https://doi.org/10.1016/S0141-0229(03)00117-0

[37] Sulistyaningdyah, W. T., Ogawa, J., Tanaka, H., et al. (2004). Characterization of alkaliphilic laccase activity in the culture supernatant of *Myrothecium verrucaria* 24G-4 in comparison with bilirubin oxidase. *FEMS Microbiology Letters*, *230*, 209-214. https://doi.org/10.1016/S0378-1097(03)00892-9

[38] Zhao, D., Zhang, X., Cui, D., & Zhao, M. (2012). Characterisation of a novel white laccase from the deuteromycete fungus *Myrothecium verrucaria* NF-05 and its decolourisation of dyes. *PLOS ONE*, *7*, e38817. https://doi.org/10.1371/journal.pone.0038817

[39] Novotný, Č., Rawal, B., Bhatt, M., et al. (2001). Capacity of *Irpex lacteus* and *Pleurotus ostreatus* for decolorization of chemically different dyes. *Journal of Biotechnology*, 89, 113-122. https://doi.org/10.1016/S0168-1656(01)00321-2