Brazilian Journal of Microbiology
https://doi.org/10.1007/s42770-024-01257-6

BACTERIAL, FUNGAL AND VIRUS MOLECULAR BIOLOGY - RESEARCH PAPER

Copper increases laccase gene transcription and extracellular laccase activity in *Pleurotus eryngii* KS004

Sara Sharghi[1] · Farajollah Shahriari Ahmadi[1] · Amin Mirshamsi Kakhki[1] · Mohammad Farsi[1]

Received: 30 September 2023 / Accepted: 9 January 2024

Abstract

The white-rot fungus *Pleurotus eryngii* secretes various laccases involved in the degradation of a wide range of chemical compounds. Since the laccase production is relatively low in fungi, many efforts have been focused on finding ways to increase it, so in this study, we investigated the effect of copper on the transcription of the *pel3* laccase gene and extracellular laccase activity. The results indicate that adding 0.5 to 2 mM copper to liquid cultures of *P. eryngii* KS004 increased both *pel3* gene transcription and extracellular laccase activity in a concentration-dependent manner. The most significant increase in enzyme activity occurred at 1 mM Cu^{2+}, where the peak activity was 4.6 times higher than in control flasks. Copper also induced the transcription of the laccase gene *pel3*. The addition of 1.5 and 2 mM Cu^{2+} to fungal culture media elevated *pel3* transcript levels to more than 13-fold, although the rate of induction slowed down at Cu^{2+} concentrations higher than 1.5 mM. Our findings suggest that copper acts as an inducer in the regulation of laccase gene expression in *P. eryngii* KS004. Despite its inhibitory effect on fungal growth, supplementing cultures with copper can lead to an increased extracellular laccase production in *P. eryngii*.

Keywords Induction · Laccase · *Pleurotus eryngii* · Transcriptional regulation · White-rot fungi

Introduction

Fungi serve as cell factories for producing various proteins, including enzymes. *Pleurotus eryngii*, the king oyster mushroom, is both edible and medicinal, producing ligninolytic enzymes like peroxidase, aryl-alcohol oxidase, and laccase [1]. Laccases are extracellular enzymes that catalyze the oxidation of diverse substrates such as phenolic compounds, aromatic, and aliphatic amines by safe and eco-friendly reactions dealing with reduction of oxygen to water. High-redox-potential laccases which are more desirable for biotechnological purposes are mostly of fungal origin, while low-redox-potential laccases are found in bacteria, higher plants, and animals. In fungi, the reactions catalyzed by laccase are involved in decomposition of lignocellulose, defense responses, pathogenesis, pigmentation, and sporulation [2, 3]. These copper-containing enzymes find applications in industries like pulp, paper and textiles, detoxification of recalcitrant pollutants, bioremediation, and more [4, 5]. However, realizing laccase's biotechnological potential faces challenges. A primary obstacle is the high production cost due to low enzyme yields in fungi. Consequently, efforts concentrate on determining molecular characteristics of fungal laccases, unraveling laccase gene regulation mechanisms, and enhancing expression or expanding enzyme properties for better production. One of the strategies practiced to produce larger quantities of laccase is using inducers such as aromatic compounds [6], amino acids [7], plant extracts [8], and metal ions [9–11]. In silico analysis of laccase gene promoters has revealed various responsive elements scattered across the promoter region. Correlation between specific cis-acting elements and laccase gene transcription exists, with potential mechanisms proposed. Differences in element copy number, location, or orientation contribute to the intricate landscape of laccase gene regulation [11, 12].

The fact that laccases are mostly coded by several genes within a given species is a usual phenomenon in eukaryotes [2]. In *P. eryngii*, four laccase genes, including *pel3*, have

Responsible Editor: Admir Giachini

✉ Farajollah Shahriari Ahmadi
shahriari@um.ac.ir

[1] Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

been isolated and characterized so far [13, 14]. Analysis of the *pel3* promoter region has highlighted diverse regulatory elements, including a copper-sensing sequence, a yeast copper-response element, and two putative metal response elements (MREs) [14]. In certain fungi, MREs can bind to metal ions like Cu^{2+}, Fe^{2+}, and Cd^{2+} in culture media, stimulating extracellular laccase production [4, 15].

Although *P. eryngii* has a great potential to be used as a valuable source of enzymes *due to its* short growth period and easy and cheap cultivation compared to other basidiomycetes, there is relatively little knowledge about laccase induction and transcriptional regulation in this fungus. Since the influence of copper on laccase synthesis and secretion is species and isolate dependent [12, 16], this study explores the effect of copper as an inducer on laccase activity and *pel3* gene transcription in *P. eryngii* KS004.

Materials and methods

Strains, medium, and culture conditions

The *Pleurotus eryngii* strain KS004 was provided by the Edible Mushrooms Research Center of Ferdowsi University of Mashhad, Iran, and maintained on potato dextrose agar (PDA) plates containing 200 g/L potato extract, 20 g/L dextrose, and 15 g/L agar at 26 °C. The potato dextrose broth (PDB) medium used for shaken cultures had the same ingredients, excluding agar, and included 5 g/L yeast extract as the best nitrogen source for laccase production [17]. The pH was adjusted to 6 before sterilization. Seven mycelial plugs (4 mm diameter) were taken from the periphery of PDA plates and inoculated into 100 mL of PDB medium in Erlenmeyer flasks. They were then grown for 8 days at 26 °C with agitation at 150 rpm. Cultures were supplemented with $CuSO_4.5H_2O$ to final concentrations of 0, 0.5, 1, 1.5, and 2 mM. Previous studies on other *Pleurotus* species showed that the best laccase production in *Pleurotus ostreatus* was induced by adding copper during the mid-logarithmic phase of cultivation (the 5th day) [17]. In our study, filter-sterilized copper sulfate was added to actively growing 5-day-old *P. eryngii* cultures, followed by 3 more days of growth. Samples were taken from 8-day-old cultures, with a reference culture without any inducer serving as the control. All experiments were conducted in triplicate.

Determination of the mycelial dry weight

To assess the impact of copper on fungal growth, culture media were augmented with varying concentrations of copper sulfate at the time of inoculation [15]. After 8 days of growth, the dry weights of the mycelium were measured. The quantification was performed using the method proposed by Manubens et al. [18]. Fungal mycelia were harvested from triplicate flasks of 8-day-old cultures and filtered through Whatman No.1 papers that were previously dried at 100 °C and weighed. The retained mycelium was then dried at 100 °C to a constant weight, and the mycelial dry weight was determined by calculating the difference in measured values.

To better illustrate the effect of copper on fungal growth, the *Pleurotus eryngii* KS004 mycelia were also cultured on PDA plates containing different Cu^{2+} concentrations.

Enzyme activity assay

Laccase activity was assessed at room temperature by monitoring the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS) as the substrate (SIGMA, Saint Louis, Missouri, USA). The reaction mixture contained 5 mM ABTS, 0.1 M sodium acetate buffer (pH = 5), and 100 µL of culture supernatant [14]. It was then incubated at room temperature for 3 min, after which the laccase activity was determined by the increase in absorbance at 420 nm ($\varepsilon = 36{,}000\ M^{-1}\ cm^{-1}$). One unit of enzyme activity was defined as the amount of enzyme needed to oxidize 1 µmol of ABTS per minute. Measurements were conducted in 10-mm quartz cuvettes using a UV-Vis spectrophotometer (Unico S-2100-UV, USA).

Extraction of RNA and cDNA synthesis

Upon harvest, 8-day-old cultures of *P. eryngii* mycelia were collected by filtration, washed twice with distilled water, and rapidly frozen in liquid nitrogen. The frozen mycelia were subsequently pulverized using a mortar and pestle. Total RNA extraction was performed using the DENAzist total RNA extraction kit (DENAzist Asia, Mashhad, Iran), following the manufacturer's instructions. All RNA samples were prepared in triplicate. RNA quality and quantity were assessed through gel electrophoresis and a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). Next, 625 ng of total RNA was reverse transcribed into cDNA in a 20 µL reaction volume using the Parstous cDNA synthesis kit (Parstous, Mashhad, Iran), as per manufacturer's protocol.

Primer design

The specific primer pairs used for the *pel3* gene in real-time PCR (qPCR) were designed by the Primer3 program (version 4.1.0). These primers were based on the genomic DNA sequence of *pel3* (accession no. AY686700) and amplified a 107 bp fragment in each reaction. The primer sequences were as follows: 5′-TCTTGCTATGGGCTTCGACT-3′ and

5′-GGCGTAGCACCTGACAGAAT-3′. The β-actin gene of *P. eryngii* was employed as an internal control. The primer pairs for β-actin were obtained from the literature [19]. The forward and reverse primers were 5′-CCCCTGAGCGAA AGTACTCC-3′ and 5′-AGGGCCTGACTCGTCGTATT-3′, respectively.

Quantitative real-time PCR

To quantify the transcription level of the *pel3* gene, quantitative real-time PCRs were conducted using a QIAGEN Rotor Gene 3000. SYBR green fluorescent dye was employed for product detection. Each reaction was prepared in a final volume of 20 µL, containing 10 µL of 2× master mix green (Ampliqon), 0.5 µM of both forward and reverse primers, and 1250 ng of cDNA, and the remaining volume was adjusted to 20 µL using nuclease-free water. The qRT-PCR was performed as follows: an initial step of 15 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 57 °C, and 30 s at 72 °C. Subsequently, a melting cycle ranging from 50 to 99 °C was performed to ensure amplification specificity. Each sample's amplification experiment was conducted in triplicate, with three biological replicates, each run in three technical replicates. All data were normalized to the internal control, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All measurements were conducted in three independent experiments and were analyzed using one-way analysis of variance (ANOVA). Post hoc Tukey HSD test was employed to compare the treatments. Statistical analysis was carried out using IBM SPSS Statistics 23, and the charts were generated using GraphPad Prism (version 9.5.1.733). The plotted results represent the mean values derived from the collected data. A *p*-value less than or equal to 0.05 has been interpreted statistically significant. Significant differences between treatments are denoted by letters on graph bars.

Results

The presence of multiple regulatory elements related to heavy metal response in the promoter region of *pel3* gene implied that laccase gene transcription in *P. eryngii* may be regulated by metal ions, specifically copper. To validate this hypothesis and also efficiently improve enzyme yield in this strain, here we examined the impact of varying concentrations of copper in the culture media on laccase activity and *pel3* laccase gene transcription in *P. eryngii* KS004.

In terms of enzyme activity, laccase activity remained relatively low in the absence of inducer. But when copper was added, the extracellular laccase activity clearly started to rise. With the addition of only 0.5 mM Cu^{2+}, laccase activity increased by more than 2-fold. The most significant increase was observed at 1 mM Cu^{2+}, reaching a peak activity of 381.1 U/L approximately 4.6 times higher than that in control flasks. As the amount of copper in the culture media was further elevated, the increasing trend was interrupted; as in 1.5 mM and 2 mM Cu^{2+}, the extracellular laccase activity began to slightly decline, reaching 371.85 U/L and 290.43 U/L, respectively. The changes in laccase activity are illustrated in Fig. 1.

To investigate the potential impact of copper as a heavy metal on the growth of *P. eryngii* KS004, the dry weight of fungal cultures exposed to escalating Cu^{2+} amounts was measured. As depicted in Fig. 2, the addition of copper to *P. eryngii* KS004 cultures resulted in slower mycelial growth. Eight days after the addition of metal, the average dry weight of the control group increased by 0.56 g per 100 mL of culture. While 0.5 mM Cu^{2+} did not significantly affect fungal biomass, mycelial growth started to decelerate with increasing copper concentration, reaching only 0.33 g and 0.23 g per 100 mL at 1.5 mM and 2 mM Cu^{2+}, respectively.

To explore the impact of copper on laccase gene transcription, 5-day-old *P. eryngii* cultures were exposed to various Cu^{2+} concentrations. After a 3-day incubation period, the transcription levels of the laccase gene *pel3* were assessed through qRT-PCR. As depicted in Fig. 3, copper significantly stimulated the transcription of the *pel3* gene in *P. eryngii* KS004. While the transcription level of

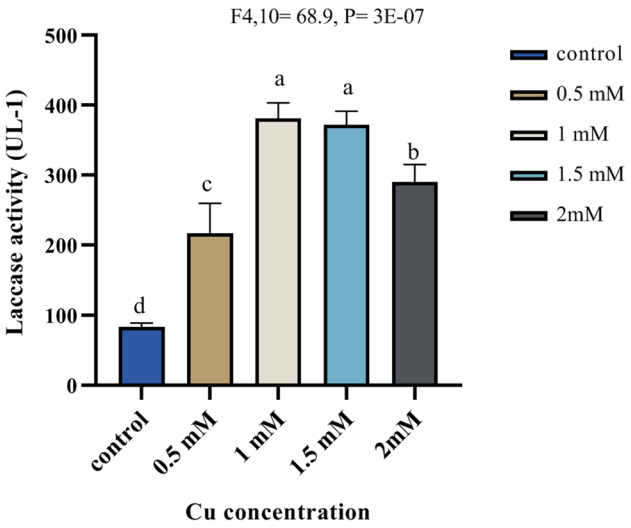

Fig. 1 Influence of various Cu concentrations on extracellular laccase activity (enzyme activity units per liter of culture). Vertical bars represent the standard deviation of three independent replicates

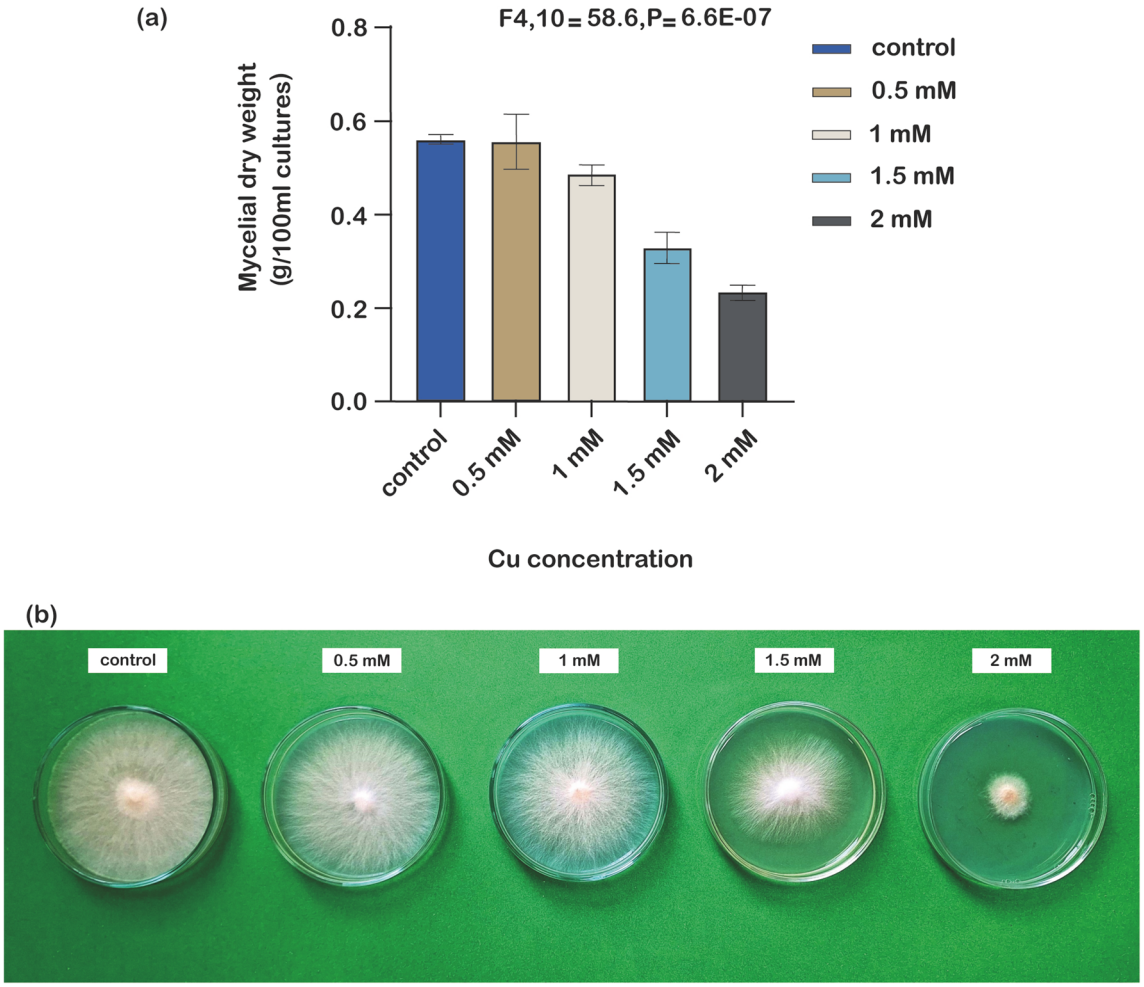

Fig. 2 Impact of copper sulfate on mycelial growth of *P. eryngii* KS004. **a** Changes in dry weight of mycelia cultured on PDB medium. Vertical bars represent the standard deviation of three independent replicates. **b** Mycelial growth of *P. eryngii* KS004 on PDA culture medium with different concentrations of Cu^{2+}

this laccase gene was notably low in cultures without any inducer, the transcription levels of the *pel3* gene subjected to 0.5 mM Cu^{2+} began to gradually increase. The *pel3* gene transcription continued to rise consistently with escalating Cu^{2+} amounts, reaching 6.6-fold at 1 mM and over 13-fold in 1.5 and 2 mM $CuSO_4$ treatments. It is worth noting that, despite the continuous increase in *pel3* gene transcription, the rate of induction slowed down at Cu^{2+} concentrations higher than 1.5 mM.

This study examined the impact of Cu^{2+} as a potential inducer on total production of extracellular laccase and how this heavy metal affects *pel3* gene expression on transcriptional level in liquid cultures of *P. eryngii* KS004. The results indicated that the addition of copper to actively growing *P. eryngii* cultures could lead to a significant increase in transcription of *pel3* gene, with the effect of copper being concentration-dependent. Moreover, it was demonstrated that the cultures supplemented with copper experienced an enhancement in extracellular laccase activity; however, higher Cu^{2+} concentrations curtailed mycelial growth. Consequently, while *pel3* laccase gene transcription displayed a notable increase at higher Cu^{2+} concentrations, the increase in extracellular laccase activity followed a different pattern, reaching its peak at 1 mM Cu^{2+} and declining at higher copper concentrations.

Discussion

Extracellular laccases are typically produced in limited quantities during secondary metabolism in fungi. Amplifying laccase production becomes imperative for the enzyme's industrial applications. Laccase transcription has been observed to be regulated by metal ions [20], aromatic compounds [6], and nitrogen and carbon sources [21] in culture media. The induction of laccase by copper has been

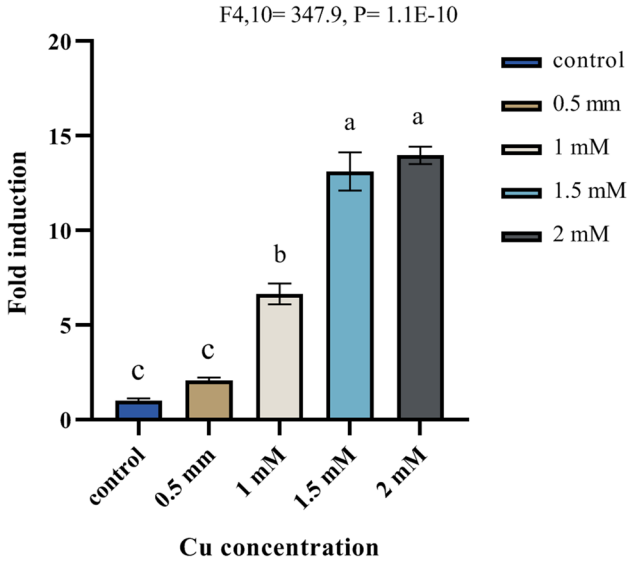

Fig. 3 Relative induction effect of copper on *pel3* laccase gene transcription

documented in various fungi [12, 15, 17]. Copper's influence on transcript levels has been reported in *Trametes versicolor* [22], *Ceriporiopsis subvermispora* [23], *Pleurotus ostreatus* [24], *Pleurotus sajor-caju* [21], *Trametes pubescens* [20], and *Aspergillus flavus* [25], although the degree of induction in transcription varies among different species .This study marks the first report of copper-induced laccase gene transcription for the *pel3* gene of *P. eryngii*. Our findings suggest that copper regulates *pel3* gene expression at the transcriptional level, with the impact of Cu^{2+} being dose-dependent. Two putative metal-responsive elements (MREs), along with the copper-sensing sequence and yeast copper-response element found in the promoter region of the *pel3* gene [14], might contribute to the induction of *pel3* gene transcription by Cu^{2+}. Our findings align with similar findings in other fungi, particularly those within the *Pleurotus* genus.

This study also delved into the effect of copper on laccase activity. *P. eryngii* KS004 exhibited laccase activity in both the absence and presence of Cu^{2+}, but the addition of copper to the culture media significantly augmented laccase activity, reaching its peak at 1 mM Cu^{2+} concentration. Baldrian and Gabriel (2002) reported analogous results for copper induction in *Pleurotus ostreatus* within a nitrogen-limited liquid medium. Their findings proposed that the increased laccase activity upon $CuSO_4$ addition stems from both heightened laccase production through improved expression of laccase genes and the stabilization of the enzyme in the extracellular environment [26]. Results from Palmieri et al. also indicated that the inclusion of 1 mM Cu^{2+} led to a decrease in extracellular proteolytic activity, thereby hindering laccase degradation [24].

Copper concentration is also variable in studies, as exemplified by Zhu et al. [9] and Baldrian and Gabriel [26], who explored $CuSO_4$ effects ranging from 1 to 5 mM. Gomaa and Momtaz [25] employed 1, 5, and 10 mM for *Aspergillus flavus*, and Yang et al. [15] assessed 0.5 and 1 mM for copper induction in *Trametes velutina*. To have an accurate picture of the effect of copper on laccase induction in *P. eryngii* KS004, we examined four distinct copper concentrations: 0.5, 1, 1.5, and 2 mM. Previous investigations have shown that Cu can hinder mycelial growth in select *Pleurotus* species [17, 27], aligning with our findings. It has been suggested that concentrations beyond the fungal tolerance inhibit fungal growth as a result of oxidative stress that is extremely toxic to fungal cells [16, 28, 29]. Consequently, the inhibitory impact of copper on growth should be factored in when interpreting extracellular laccase activity results.

It has been suggested that fungi produce laccases to scavenge reactive oxygen species (ROS) in order to protect themselves from oxidative stress caused by copper [16, 30] and as a defense mechanism against this heavy metal [5]. Considering the results obtained in the present study, the elevated gene expression and enzyme activity despite the lowered growth, facing stress conditions caused by copper seems to be the case for *P. eryngii* KS004.

Conclusion

The outcomes presented in this study show that *Pleurotus eryngii* KS004 laccase is not only constitutive but also inducible and establish a link between laccase enzyme production in this fungus and the presence of Cu^{2+} ions in culture media. This underscores that introducing this metal at low concentrations can significantly induce laccase gene transcription. Considering the toxic and inhibitory effect of copper on fungal growth and increased *pel3* gene transcription, it is suggested that *pel3* is a stress-regulated gene in *P. eryngii* KS004, although further investigations into this are required. Our result seen as a whole aids in comprehending laccase gene regulation within *Pleurotus eryngii* KS004 through a variety of cis-acting elements and offers a promising approach to enhance native laccase enzyme production for biotechnological applications.

Funding This work was partly supported by the research council of Ferdowsi University of Mashhad (Project number 3/47998).

Declarations

Conflict of interest The authors declare that they have no competing interests.

References

1. Villani A, Galli E, Paciolla C, Stea G, Logrieco AF, Siniscalco C, Mulè G, Susca A (2015) Molecular characterization of Pleurotus eryngii varieties occurring in Italy. Sydowia 67:37. https://doi.org/10.12905/0380.sydowia67-2015-0033
2. Janusz G, Pawlik A, Świderska-Burek U, Polak J, Sulej J, Jarosz-Wilkołazka A, Paszczyński A (2020) Laccase properties, physiological functions, and evolution. Int J Mol Sci 21(3):966. https://doi.org/10.3390/ijms21030966
3. Mate DM, Alcalde M (2017) Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. Microb Biotechnol 10(6):1457–1467
4. Brugnari T, Braga DM, dos Santos CS, Torres BH, Modkovski TA, Haminiuk CW, Maciel GM (2021) Laccases as green and versatile biocatalysts: from lab to enzyme market—an overview. Bioresour Bioprocess 8(1):1–29. https://doi.org/10.1186/s40643-021-00484-1
5. Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G (2014) Fungal laccases and their applications in bioremediation. Enzyme Research 2014. https://doi.org/10.1155/2014/163242
6. Terrón MC, González T, Carbajo JM, Yagüe S, Arana-Cuenca A, Téllez A, Dobson AD, González AE (2004) Structural close-related aromatic compounds have different effects on laccase activity and on lcc gene expression in the ligninolytic fungus Trametes sp. I-62. Fungal Genet Biol 41(10):954–962. https://doi.org/10.1016/j.fgb.2004.07.002
7. Dhawan S, Kuhad RC (2002) Effect of amino acids and vitamins on laccase production by the bird's nest fungus Cyathus bulleri. Bioresour Technol 84(1):35–38
8. Ardon O, Kerem Z, Hadar Y (1996) Enhancement of laccase activity in liquid cultures of the ligninolytic fungus Pleurotus ostreatus by cotton stalk extract. J Biotechnol 51(3):201–207
9. Lettera V, Del Vecchio C, Piscitelli A, Sannia G (2011) Low impact strategies to improve ligninolytic enzyme production in filamentous fungi: the case of laccase in Pleurotus ostreatus. Comptes Rendus Biologies 334(11):781–788
10. Hernández-Monjaraz WS, Caudillo-Pérez C, Salazar-Sánchez PU, Macías-Sánchez KL (2018) Influence of iron and copper on the activity of laccases in Fusarium oxysporum f. sp. lycopersici. Braz J Microbiol 49:269–275
11. Durán-Sequeda D, Suspes D, Maestre E, Alfaro M, Perez G, Ramírez L, Pisabarro AG, Sierra R (2021) Effect of nutritional factors and copper on the regulation of laccase enzyme production in *Pleurotus ostreatus*. J Fungi 8(1):7
12. Piscitelli A, Giardina P, Lettera V, Pezzella C, Sannia G, Faraco V (2011) Induction and transcriptional regulation of laccases in fungi. Curr Genom 12(2):104–112
13. Munoz C, Guillen F, Martinez AT, Martinez MJ (1997) Induction and characterization of laccase in the ligninolytic fungus *Pleurotus eryngii*. Curr Microbiol 34:1–5
14. Rodríguez E, Ruiz-Dueñas FJ, Kooistra R, Ram A, Martínez ÁT, Martínez MJ (2008) Isolation of two laccase genes from the white-rot fungus *Pleurotus eryngii* and heterologous expression of the *pel3* encoded protein. J Biotechnol 134(1-2):9–19
15. Yang Y, Wei F, Zhuo R, Fan F, Liu H, Zhang C, Ma L, Jiang M, Zhang X (2013) Enhancing the laccase production and laccase gene expression in the white-rot fungus *Trametes velutina* 5930 with great potential for biotechnological applications by different metal ions and aromatic compounds. PLoS One 8(11):e79307. https://doi.org/10.1371/journal.pone.0079307
16. Buddhika UV, Savocchia S, Steel CC (2021) Copper induces transcription of BcLCC2 laccase gene in phytopathogenic fungus, *Botrytis cinerea*. Mycology 12(1):48–57
17. Zhu C, Bao G, Huang S (2016) Optimization of laccase production in the white-rot fungus *Pleurotus ostreatus* (ACCC 52857) induced through yeast extract and copper. Biotechnol Biotechnol Equip 30(2):270–276
18. Manubens A, Canessa P, Folch C, Avila M, Salas L et al (2007) Manganese affects the production of laccase in the basidiomycete *Ceriporiopsis subvermispora*. FEMS Microbiol Lett 275:139–145
19. Kim YH, Lee HS, Kwon HJ, Patnaik BB, Nam KW, Han YS, Bang IS, Han MD (2014) Effects of different selenium levels on growth and regulation of laccase and versatile peroxidase in white-rot fungus, *Pleurotus eryngii*. World J Microbiol Biotechnol 30:2101–2109
20. Galhaup C, Wagner H, Hinterstoisser B, Haltrich D (2002) Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*. Enzym Microb Technol 30(4):529–536
21. Soden DM, Dobson AD (2001) Differential regulation of laccase gene expression in *Pleurotus sajor-caju*. Microbiology 147(7):1755–1763
22. Collins PJ, Dobson A (1997) Regulation of laccase gene transcription in *Trametes versicolor*. Appl Environ Microbiol 63(9):3444–3450
23. Álvarez JM, Canessa P, Mancilla RA, Polanco R, Santibáñez PA, Vicuña R (2009) Expression of genes encoding laccase and manganese-dependent peroxidase in the fungus *Ceriporiopsis subvermispora* is mediated by an ACE1-like copper-fist transcription factor. Fungal Genet Biol 46(1):104–111
24. Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Appl Environ Microbiol 66(3):920–924. https://doi.org/10.1128/AEM.66.3.920-924.2000
25. Gomaa OM, Momtaz OA (2015) Copper induction and differential expression of laccase in *Aspergillus flavus*. Braz J Microbiol 46:285–292. https://doi.org/10.1590/S1517-838246120120118
26. Baldrian P, Gabriel J (2002) Copper and cadmium increase laccase activity in *Pleurotus ostreatus*. FEMS Microbiol Lett 206(1):69–74
27. Mohamadhasani F, Rahimi M (2022) Growth response and mycoremediation of heavy metals by fungus Pleurotus sp. Sci Rep 12(1):19947
28. Manzl C, Enrich J, Ebner H, Dallinger R, Krumschnabel G (2004) Copper-induced formation of reactive oxygen species causes cell death and disruption of calcium homeostasis in trout hepatocytes. Toxicology 196(1-2):57–64
29. Kannaiyan R, Mahinpey N, Mani T, Martinuzzi RJ, Kostenko V (2012) Enhancement of Dichomitus squalens tolerance to copper and copper-associated laccase activity by carbon and nitrogen sources. Biochem Eng J 67:140–147
30. Schouten A, Wagemakers L, Stefanato FL, Kaaij RM, Kan JA (2002) Resveratrol acts as a natural profungicide and induces self-intoxication by a specific laccase. Mol Microbiol 43(4):883–894