Effect of Different Nutritional Materials on Laccase Activity and Biomass Accumulation of the *Auricularia cornea* var. Li Strain

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The laccase activity changes of the *Auricularia cornea* var. Li strain were studied over a continuous 9-day period using different carbon sources, nitrogen sources, and lignocellulose as liquid fermentation inducers. The results showed that the addition of carbon sources, nitrogen sources, and alkaline lignin all stimulated *Auricularia cornea* var. Li to secrete laccase and promoted the accumulation of mycelial biomass. Both carbon and nitrogen deficiencies could stimulate *Auricularia cornea* var. Li to produce more laccase, but they were detrimental to the accumulation of mycelial biomass. Maltose and peptone should be prioritized as materials for cultivating high-laccase-producing *Auricularia cornea* var. Li strains through liquid fermentation. Lignocellulosic biomass could significantly enhance laccase activity in *Auricularia cornea* var. Li. During the cultivation of *Auricularia cornea* var. Li., wheat bran and cottonseed hulls that produced high levels of laccase should be recommended. This study partially revealed the laccase production characteristics of *Auricularia cornea* var. Li and identified culture substances that were beneficial for laccase secretion during different growth stages of the mushroom. The results provide a foundation for improving the yield and quality of *Auricularia cornea* var. Li.

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Keywords: Auricularia cornea var. Li; Laccase activity; Carbon sources; Nitrogen sources; Mycelial biomass; Lignocellulose

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INTRODUCTION

Edible fungi have garnered significant attention due to their fruiting bodies being rich in nutritional, medicinal, and economic value (Jacinto-Azevedo *et al*. 2021; Zhang *et al*. 2021; Dimopoulou *et al*. 2022). Various edible fungi have been isolated from nature and cultivated on a large scale for market supply (Jacinto-Azevedo *et al*. 2021; Zhang *et al*. 2021; Dimopoulou *et al*. 2022; Juárez-Hernández *et al*. 2023). During the process of artificial domestication and cultivation, different carbon and nitrogen sources often have been used to explore the characteristics of edible mushroom strains (Li *et al*. 2021b). Various lignocellulosic biomasses are commonly used in the large-scale cultivation of edible fungi to provide nutrients for mycelial growth and fruiting body development (Luo and Chen 2010; Kumla *et al*. 2020; Viriato *et al*. 2022). Lignocellulosic biomass is primarily composed of long-chain cellulose and hemicellulose, which are interconnected by heterogeneous polymerization of lignin units (Nunes and Kunamneni 2018; Govil *et al*.

2022; Jindal *et al*. 2023). Edible fungi can obtain sufficient nutrients from lignocellulose, due to their strong secretion capacity of lignin-degrading enzymes (Kumla *et al*. 2020; Su *et al*. 2023). Lignin-degrading enzymes can break down lignin, thereby disrupting the microfibrillar structure and allowing cellulose to be efficiently hydrolyzed into sugars (Nunes and Kunamneni 2018; Govil *et al*. 2022; Jindal *et al*. 2023). Some studies have suggested a correlation among the yield of edible fungi, the production of lignin-degrading enzymes, and the degradation of lignin (Fang *et al.* 2018; Colla *et al*. 2023; Su *et al*. 2023).

Auricularia cornea var. Li is a novel white variety from *Auricularia cornea* Ehrenb (Wang *et al*. 2018; Lin *et al*. 2024). This variant is rich in protein, dietary fiber, polysaccharides, unsaturated fatty acids, and various trace elements, offering significance in both nutritional and medical contexts (Wang *et al.* 2018; Lin *et al.* 2024). Laccase is a representative enzyme of lignin-degrading enzymes, capable of completely degrading lignin into carbon dioxide and water (Janusz *et al*. 2013). Laccase is the most ideal green catalyst for biological degradation (Janusz *et al*. 2013). The mycelial growth and fruiting body development of edible fungi are closely related to their laccase activity (Sun *et al*. 2011; Fang *et al*. 2018; Cesur *et al*. 2022). Fungal strains with high laccase activity often exhibit faster mycelial growth and shorter growth cycles (Sun *et al*. 2011). *Volvariella brumalis* strains could grow rapidly when cultivated on lignocellulosic substrates that promote high laccase production (Xiong *et al*. 2024). The *Auricularia cornea* var. Li strains with the highest laccase activity also yielded the highest fruiting body production (Fang *et al*. 2018). Laccase expression is influenced by multiple factors, including the developmental stage and cultivation environment (Rivera-Hoyos *et al*. 2013; Reddy and Kanwal 2022). Factors, such as cultivation methods, complexity and concentration ratios of carbon and nitrogen sources, metal ions, secondary metabolites, temperature, and pH levels, can impact laccase activity (Rivera-Hoyos *et al*. 2013; Durán-Sequeda *et al*. 2021; Razavi *et al*. 2021; Reddy and Kanwal 2022; Colla *et al*. 2023; González-González *et al*. 2023). Carbon sources, nitrogen sources, and other inducers often play the major role in influencing laccase activity (Rivera-Hoyos *et al*. 2013; Reddy and Kanwal 2022). Laccase production can be triggered by the depletion of carbon or nitrogen sources and is influenced by the type and concentration of these sources (Janusz *et al*. 2013, 2015). The inducers typically refer to water-soluble lignocellulosic materials and small molecular aromatic compounds with structures similar to lignin, or metabolites derived from lignin degradation, such as alkaline lignin, ferulic acid, and lignin-derived phenols (An *et al*. 2018; Swatek and Staszczak 2020; Reddy and Kanwal 2022). These substances promote laccase secretion (An *et al*. 2018; Swatek and Staszczak 2020; Reddy and Kanwal 2022). Various lignocellulosic biomasses not only act as inducers for laccase production (Atilano-Camino *et al*. 2020; Li *et al*. 2021a; Xiong *et al*. 2021, 2024; Colla *et al*. 2023), but also serve as the primary nutrient source for edible fungi during the cultivation process (Luo and Chen 2010; Kumla *et al*. 2020; Viriato *et al*. 2022).

Therefore, alkaline lignin was used as an inducer, while different carbon sources, nitrogen sources, or lignocelluloses were utilized for the liquid fermentation of *Auricularia cornea* var. Li strains. The changes in laccase activity under each induction medium were continuously monitored for 10 days, while the biomass of mycelium accumulated under carbon-limiting or nitrogen-limiting conditions was also measured. The aim was to study the secretion patterns of laccase and the accumulation of biomass in *A. cornea* var. Li under conditions of limited carbon or nitrogen sources. Results showed that the laccase expression characteristics of *A. cornea* var. Li under liquid fermentation with different nutrient sources. The cultivating materials for high laccase production in *A. cornea* var. Li

are recommended, based on this work. These conclusions may lay a theoretical foundation for accelerating the growth of *A. cornea* var. Li mycelium and improving the yield and quality of fruiting bodies.

EXPERIMENTAL

Materials

The tested *Auricularia cornea* var. Li strain was provided by the Edible Fungi Engineering Center of Guizhou Province, and the DNA testing of the strain was conducted in the Guizhou Institute of Biology, China (Li *et al*. 2021b).

Induction media

The Malt Extract Agar (MEA) Media used was comprised of the following: 10 g/L glucose, 20 g/L malt extract, 3 g/L potassium dihydrogen phosphate, and 20 g/L agar. All biological reagents were dissolved in deionized water, and the volume was adjusted to 1 L. The MEA media were sterilized in the autoclave at 121 °C for 20 min in an autoclave.

Liquid-state fermentation media was comprised of the following: 20 g/L glucose, 2 g/L peptone, 2 g/L yeast extract, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L $K_2HPO_4 \cdot 3H_2O$, and 0.46 g/L KH_2PO_4. All biological reagents were dissolved in deionized water, and the volume was adjusted to 1 L. The media were sterilized in the autoclave at 121 °C for 20 min in an autoclave.

Based on the description of fungal culture media for the genus *Auricularia* in the book *Encyclopedia of Mushroom Industry in China* (Luo and Chen 2010), several lignocellulosic materials were selected to cultivate *Auricularia* fungi to induce *Auricularia cornea* var. Li.

Table 1. The Formulations of Different Induction Media

Induction Media	Formulations
X1	10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X2	3 g/L lignin alkali, 10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X3	3 g/L lignin alkali, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X4	3 g/L lignin alkali, 10 g/L glucose, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X5	3 g/L lignin alkali, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X6	3 g/L lignin alkali, 10 g/L sucrose, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X7	3 g/L lignin alkali, 10 g/L maltose, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X8	3 g/L lignin alkali, 2 g/L yeast extract, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X9	3 g/L lignin alkali, 2 g/L beef extract, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X10	3 g/L cottonseed hulls, 10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X11	3 g/L *Quercus acutissima* sawdust, 10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X12	3 g/L corn cobs, 10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X13	3 g/L wheat bran, 10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1
X14	3 g/L fermented soybean meal powder, 10 g/L glucose, 2 g/L peptone, 1 g/L KH_2PO_4, 0.03 g/L Vb1

The materials included corn cobs, *Quercus acutissima* sawdust, cottonseed hulls, wheat bran, and fermented soybean meal powder. Fermented soybean meal powder and biological reagents, lignin alkali, were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The other lignocellulosic materials were collected from Guiyang City, Guizhou Province, China. The substrates were chopped into small fragments, sun-dried, and then oven-dried at 60 °C. Some fragments were pulverized to a mesh size ranging between 40-mesh and 60-mesh using an electric grinder. All reagents and materials were dried to constant weight before using. Different induction media were prepared according to the compositions listed in Table 1. Then, the 250-mL Erlenmeyer flasks containing 100 mL of different induction media were sterilized at 121 °C for 30 min in an autoclave.

Methods

Culture method

The *Auricularia cornea* var. Li strain was activated using MEA media and incubated at 25 °C for 8 days.

The best-growing colonies were selected for inoculum preparation. Five 5-mm diameter mycelial discs were excised from the activated agar plates and transferred into a 250-mL Erlenmeyer flask, which was filled with 100 mL of liquid-state fermentation media. All Erlenmeyer flasks were incubated at 25 °C and shaken at 150 rpm for 10 days. After incubation, the mycelial pellets in the Erlenmeyer flask were homogenized using a modular homogenizer HFJ-10 (Tianjin HengAo Technology Co., Ltd., China) at 5,000 rpm for 180 s. The mycelial pellets were used as inoculums. Then, 3 mL of well-mixed *Auricularia cornea* var. Li homogenate were added to each of the 250-mL Erlenmeyer flasks containing 100 mL of different induction media. All flasks were incubated in the dark at 25 °C and 150 r/min for 10 days on a shaking incubator, with three replicates for each treatment.

Assay of laccase activity

Starting from day 2 of cultivation, 2 mL of crude enzyme extract was collected daily from each treatment. The samples were centrifuged at a temperature of 4 ℃ with a rotational speed of 12,000 rpm for 20 min. Following centrifugation, the supernatants were stored at a temperature of -80 ℃ for subsequent analysis of the laccase activity.

The laccase activity was assayed with 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate) (ABTS) (Xiong *et al.* 2021). The amount of enzyme required to convert 1 μmol of ABTS per minute was defined as one unit of enzyme activity. The molar extinction coefficient of ABTS at 420 nm is 3.6×10^4L/(mol·cm).

Mycelial biomass measurement

Starting from day 2 of cultivation, mycelial biomass was measured every two days, with three replicates for each treatment. After filtering the mycelial mats through double-layer filter paper, they were rinsed multiple times with distilled water, dried at 70 °C to a constant weight, and the dry weight recorded.

Data statistics

Analyses of variance (ANOVA) between the test groups were performed with the PASW Statistics 18.0 (International Business Machines Corporation, version 18.0, New York, NY, USA). The figures were created by WPS Office (Kingsoft, V12.1.0.16388, Beijing, China).

RESULTS AND DISCUSSION

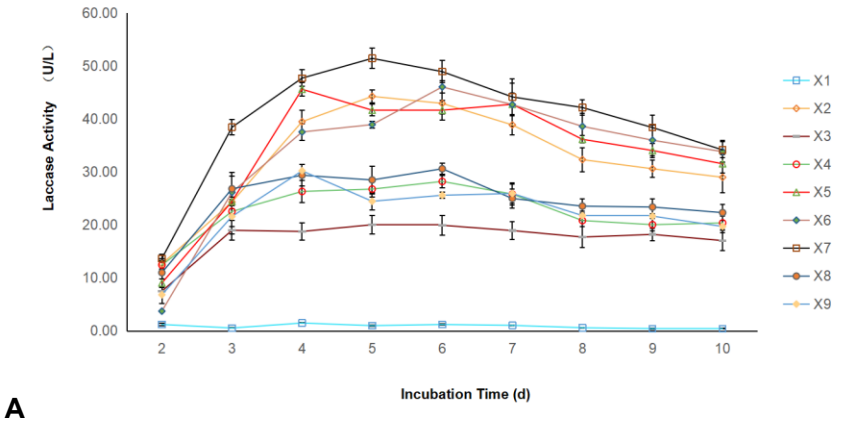
Effects of Different Carbon and Nitrogen Sources on Laccase Activity and Mycelial Biomass Production in Liquid Fermentation of *Auricularia cornea* var. Li

As shown in Table 1, different carbon and nitrogen sources had a highly significant effect on laccase activity during the liquid fermentation of *Auricularia cornea* var. Li throughout the entire cultivation period ($P < 0.001$). On day 5 of cultivation, different carbon and nitrogen sources had the most pronounced effect on laccase activity (Table 1). Except for day 2 after cultivation, different carbon and nitrogen sources significantly affected the accumulation of mycelial biomass in *A. cornea* var. Li ($P < 0.001$). After 8 days of cultivation, the rate of increase in mycelial biomass of *A. cornea* var. Li began to slow down (Table 1).

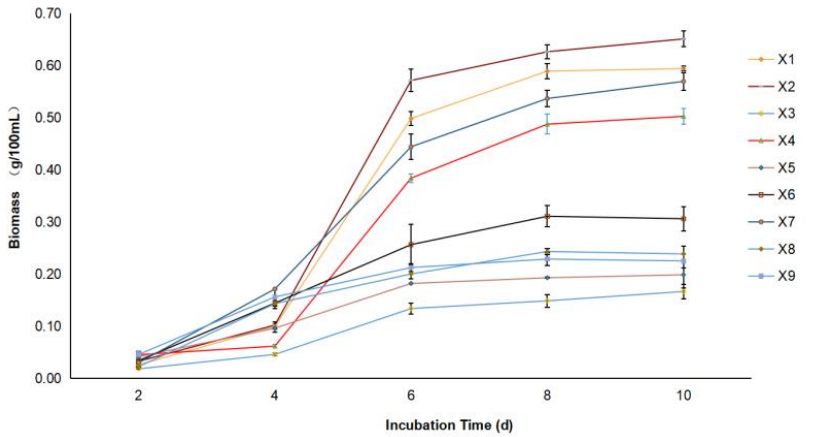
Laccase activities of *A. cornea* var. Li in the induction media X1 to X9 showed a trend of initially increasing and then decreasing with extended cultivation time (Fig. 1A), while the mycelial biomass exhibited an upward trend (Fig. 1B). Compared to the induction medium X1, the addition of alkaline lignin (X2) was beneficial for *A. cornea* var. Li to secrete laccase and accumulate mycelial biomass (Fig. 1). Alkaline lignin consistently demonstrated a notable induction capacity for laccase production in previous fungal laccase induction experiments (An *et al*. 2018; Xiong *et al*. 2024). Compared to the induction media X3, the increasing of carbon sources (X4, X6, X7), nitrogen sources (X5, X8, X9), or the combination of carbon and nitrogen sources (X2) could stimulate *A. cornea* var. Li to produce more laccase and accumulate greater biomass (Fig. 1). Compared to the induction of media X2, both nitrogen deficiency (X4, X6, X7) and carbon deficiency (X5, X8, X9) hindered the accumulation of mycelial biomass in *A. cornea* var. Li. The carbon deficiency had a greater impact on the mycelial biomass of *A. cornea* var. Li than the nitrogen deficiency (Fig. 1B). However, compared to the induction of medium X2, the laccase activity of *A. cornea* var. Li under carbon deficiency (X5) was higher than that under nitrogen deficiency (X4). This was likely related to the inhibitory effect of glucose on laccase expression, which had also been observed in other edible mushroom strains (Galhaup *et al*. 2002; Mikiashvili *et al*. 2004; Rivera-Hoyos *et al*. 2013; An *et al*. 2018). Researchers have stated that laccase expression can be influenced by adjusting glucose concentration (Galhaup *et al*. 2002; Mikiashvili *et al*. 2004; Rivera-Hoyos *et al*. 2013; An *et al*. 2018). At certain concentrations, glucose could also promote the secretion of laccase in fungi (An *et al*. 2018). At least under nitrogen deficiency, the addition of glucose promotes both laccase activity expression and biomass accumulation in *A. cornea* var. Li (Fig. 1). Both carbon and nitrogen limitation could stimulate *A. cornea* var. Li to produce more laccase (Fig. 1A). The specific activity of laccase also changed depending on the choice of carbon or nitrogen sources (Rivera-Hoyos *et al*. 2013). Compared to sucrose (X6) or glucose (X4), the carbon source maltose (X7) showed greater advantages in both laccase induction and mycelial biomass accumulation (Fig. 1). The nitrogen source peptone (X5) significantly stimulated laccase production in *A. cornea* var. Li more than yeast extract (X8) and beef extract (X9) (Fig. 1A), while its effect on mycelial biomass accumulation was second only to yeast extract (Fig. 1B). This suggested that the nitrogen source most favorable for *A. cornea* var. Li mycelial biomass accumulation was not necessarily the best laccase inducer. Therefore, to obtain the high-yielding laccase-producing liquid strains of *A. cornea* var. Li, maltose as the carbon source and peptone as the nitrogen source should be recommended.

Table 2. Effects of Different Carbon and Nitrogen Sources on Laccase Activity and Mycelial Biomass Production in Liquid Fermentation of *Auricularia cornea* var. Li by One-way ANOVA

Incubation Time (d)	Laccase Activity（U/L）	Biomass(g)
2	11.984***	3.459**
3	19.365***	-
4	78.525***	31.616***
5	98.916***	-
6	33.25***	90.621***
7	36.399***	-
8	70.145***	188.193***
9	53.426***	-
10	37.529***	175.509***
Note: df = 3;*P < 0.05,**P < 0.01,***P < 0.001		



A



B

Fig. 1. The effects of different carbon and nitrogen sources on Laccase activity and mycelial biomass production in liquid fermentation of *Auricularia cornea* var. Li; A: Laccase activity, B: Mycelial biomass

Effect of Different Cultivation Substrates on Laccase Activity in Liquid Fermentation of *Auricularia cornea* var. Li

The carbon-to-nitrogen ratio of different lignocellulosic materials varies greatly, significantly affecting the mycelial growth, mushroom yield, and quality of edible fungi (Hoa *et al*. 2015; Kumla *et al*. 2020; Colla *et al*. 2023). In the history of edible mushroom cultivation, low-nitrogen substrates have often been chosen as the primary materials, with organic or inorganic nitrogen added as supplementary materials (Luo and Chen 2010; Kumla *et al*. 2020). The nitrogen content in the cultivation supplements is often higher than that in the primary substrate; however, the primary substrate provides the main nutrients for the fungi (Luo and Chen 2010; Kumla *et al*. 2020). Based on the characteristics of the cultivation materials, this study employed different primary substrates (corn cobs, *Quercus acutissima* sawdust, and cottonseed hulls) and supplementary materials (wheat bran and fermented soybean meal powder) to induce laccase secretion in *Auricularia cornea* var. Li (Luo and Chen 2010; Kumla *et al*. 2020). Throughout the entire cultivation period, different substrates had a highly significant impact on the laccase activity produced by *A. cornea* var. Li in liquid fermentation ($P < 0.001$) (Table 3). On the 6th day of cultivation, different substrates had the most significant effect on laccase activity of *A. cornea* var. Li (Table 3, Fig. 2). The enzyme activity detected under different substrates was consistently higher than that observed under nitrogen-limited or carbon-limited conditions, showing a more significant and sustained effect on laccase activity (Tables 2 and 3, Figs. 1 and 2).

As shown in Fig. 3, the laccase activity of *A. cornea* var. Li induced by different substrates initially increased and then decreased over the cultivation period. The cultivation auxiliary material wheat bran stimulated the secretion of laccase in *A. cornea* var. Li more effectively than fermented soybean meal powder (Fig. 2). In induction culture experiments with various edible fungi, wheat bran was considered a cultivation auxiliary material that yielded high laccase production (Atilano-Camino *et al*. 2020; Kumla *et al*. 2020; Li *et al*. 2021; Xiong *et al*. 2021, 2024). Compared to *Q. acutissima* sawdust and corn cobs, cottonseed hulls more effectively stimulated the secretion of laccase by *A. cornea* var. Li (Fig. 2). Moreover, all tested cultivation additives demonstrated a stronger ability to stimulate laccase production in *A. cornea* var. Li compared to the tested primary substrates (Fig. 2). High nitrogen environments could stimulate fungi to produce laccase (D'Agostini *et al*. 2011; Colla *et al*. 2023; Pradeep Kumar *et al*. 2023). This indicated that both carbon or nitrogen limitation and excess could cause variations in laccase activity of the same fungal strain (Figs. 1 and 2). Both the addition of alkaline lignin and lignocellulosic biomass could significantly stimulate the production of more laccase by *Auricularia cornea* var. Li (Figs. 1, 2). This phenomenon was also observed on the other edible fungi (Xiong *et al*. 2024). However, the ability of single lignin addition to induce laccase secretion in the *A. cornea* var. Li strain was lower than that of complex lignocellulosic biomass (Fig. 2). Additionally, due to its single nutrient composition and high cost, pure lignin is not considered in edible mushroom cultivation. Some scholars believe that strains of *A. cornea* var. Li with high laccase activity tend to have higher fruiting body yields (Fang *et al*. 2018). Substrate formulations that yield high laccase production could promote the growth of fungal mycelia (Xiong *et al*. 2024). Therefore, the laccase activity of *A. cornea* var. Li could be enhanced by adjusting the substrate formulation, which could lead to increased yield. Therefore, the tested lignocellulosic biomass could be used to cultivate *A. cornea* var. Li, and cottonseed hulls and wheat bran should be prioritized (Luo and Chen 2010).

Table 3. Effect of Different Cultivation Substrates on Laccase Activity in Liquid Fermentation of *Auricularia cornea* var. Li by One-way ANOVA

Incubation Period (d)	Laccase Activity (U/L)
2	78.809***
3	71.999***
4	164.271***
5	272.531***
6	444.551***
7	430.946***
8	163.130***
9	272.967***
10	139.298***
Note: df = 3;*P < 0.05,**P < 0.01,***P < 0.001	

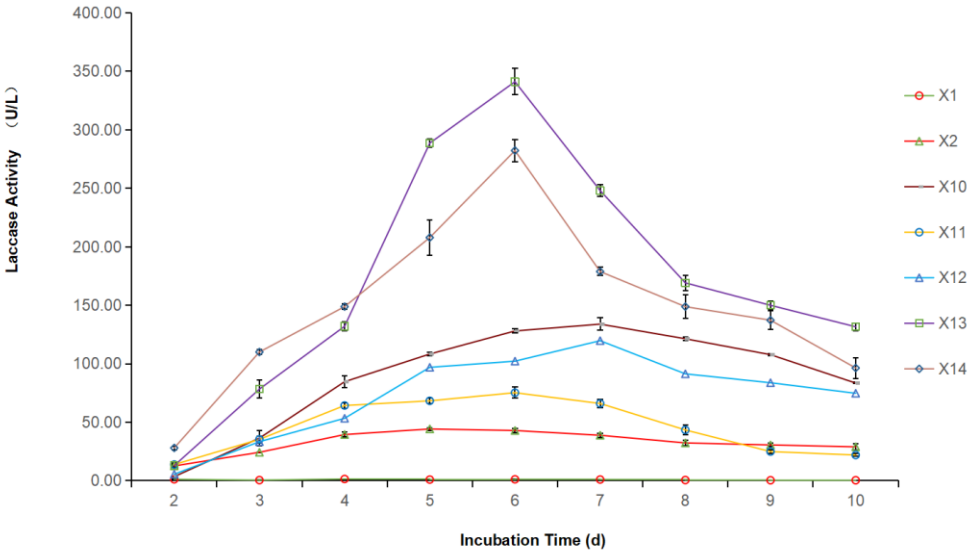


Fig. 2. The effect of different cultivation substrates on laccase activity in liquid fermentation of *Auricularia cornea* var. Li

CONCLUSIONS

1. The addition of alkaline lignin was beneficial for *Auricularia cornea* var. Li to secrete laccase and accumulate mycelial biomass. Based on the inducer alkaline lignin, the addition of carbon sources, nitrogen sources, or a combination of both could stimulate *Auricularia cornea* var. Li to produce more laccase and accumulate greater biomass. Both carbon and nitrogen deficiencies could stimulate *Auricularia cornea* var. Li to produce more laccase, but they were detrimental to the accumulation of mycelial biomass. Carbon deficiency had a more significant impact on the biomass accumulation of *Auricularia cornea* var. Li than nitrogen deficiency.
2. Compared to sucrose and glucose, the carbon source maltose demonstrated greater advantages in both inducing laccase production and accumulating mycelial biomass

of *Auricularia cornea* var. Li. The nitrogen source peptone significantly stimulated laccase production in *Auricularia cornea* var. Li outperforming yeast extract and beef extract, while being second only to yeast extract in promoting mycelial biomass accumulation. Therefore, maltose and peptone should be used with priority for cultivating high laccase-producing *Auricularia cornea* var. Li strains through liquid fermentation.

3. The cultivation supplement wheat bran was more effective than fermented soybean meal in stimulating *Auricularia cornea* var. Li to secrete laccase. The cultivation substrate cottonseed hulls were more effective than *Q. acutissima* sawdust and corn cob in stimulating *Auricularia cornea* var. Li to secrete laccase. The addition of single lignin (alkaline lignin) induced lower laccase secretion in *Auricularia cornea* var. Li strains compared to complex lignocellulosic biomass. Wheat bran and cottonseed hulls should be recommended in cultivation to develop an optimal substrate formulation for high laccase production, thereby enhancing the growth rate and yield of *Auricularia cornea* var. Li.

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REFERENCES CITED

An, Q., Ma, H. F., Han, M. L., Si, J., and Dai, Y. C. (2018). "Effects of different induction media as inducers on laccase activities of *Pleurotus ostreatus* strains in submerged fermentation," *BioResources* 13(1), 1143-1156. DOI: 10.15376/biores.13.1.1143-1156

Atilano-Camino, M. M., Alvarez-Valencia, L. H., Garcia-Gonzalez, A., and Garcia-Reyes, R. B. (2020). "Improving laccase production from *Trametes versicolor* using lignocellulosic residues as cosubstrates and evaluation of enzymes for blue wastewater biodegradation," *Journal of Environmental Management* 275, article ID 111231. DOI: 10.1016/j.jenvman.2020.111231

Cesur, A., Yamamoto, R., Asada, Y., and Watanabe, A. (2022). "Relationship between fruiting body development and extracellular laccase production in the edible mushroom *Flammulina velutipes*," *Biochemistry and Biophysics Reports* 29, article 101204. DOI: 10.1016/j.bbrep.2022.101204

Colla, I. M., de O. Filho, O. B. Q., Bertéli, M. B. D., De Freitas, J. D. S., Avelino, K. V., Ruiz, S. P., Valle, J. S. do, Linde, G. A., and Colauto, N. B. (2023). "Carbon-to-nitrogen ratios on laccase and mushroom production of *Lentinus crinitus*," *International Journal of Environmental Science and Technology* 20(4), 3941-3952. DOI: 10.1007/s13762-022-04233-5

D'Agostini, É. C., Mantovani, T. R. D. A., Valle, J. S. D., Paccola-Meirelles, L. D., Colauto, N. B., and Linde, G. A. (2011). "Low carbon/nitrogen ratio increases laccase production from basidiomycetes in solid substrate cultivation," *Scientia Agricola* 68, 295-300. DOI: 10.1590/S0103-90162011000300004

Dimopoulou, M., Kolonas, A., Mourtakos, S., Androutsos, O., and Gortzi, O. (2022). "Nutritional composition and biological properties of sixteen edible mushroom species," *Applied Sciences* 12(16), article 8074. DOI: 10.3390/app12168074

Durán-Sequeda, D., Suspes, D., Maestre, E., Alfaro, M., Perez, G., Ramírez, L., Pisabarro, A. G., and Sierra, R. (2021). "Effect of nutritional factors and copper on the regulation of laccase enzyme production in *Pleurotus ostreatus*," *Journal of Fungi* 8(1), article 7. DOI: 10.3390/jof8010007

Fang, H. Y., Ren, Z. M., Meng, X. X., Dai, J. J., Li, S. J., Li, Z. W., and Li, X. (2018). "The relationship between extracellular enzymes changes and agronomic traits in *Auricularia cornea* var Li.," *Molecular Plant Breeding* 16(13) ,4431-4435. DOI: 10.13271/j.mpb.016.004431

Galhaup, C., Wagner, H., Hinterstoisser, B., and Haltrich, D. (2002). "Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*," *Enzyme and Microbial Technology* 30(4), 529-536. DOI: 10.1016/S0141-0229(01)00522-1

González-González, P., Gómez-Manzo, S., Tomasini, A., Martínez y Pérez, J. L., García Nieto, E., Anaya-Hernández, A., Ortiz Ortiz, E., Castillo Rodríguez, R. A., Marcial-Quino, J., and Montiel-González, A. M. (2023). "Laccase production from *Agrocybe pediades*: Purification and functional characterization of a consistent laccase isoenzyme in liquid culture," *Microorganisms* 11(3), article 568. DOI: 10.3390/microorganisms11030568

Govil, T., Narayanan, A. S., Salem, D. R., and Sani, R. K. (2022). "Progress in consolidated bioprocessing of lignocellulosic biomass for biofuels and biochemicals," in: *Microbial Biotechnology for Renewable and Sustainable Energy*, Springer Nature Singapore, Singapore, pp. 35-54. DOI: 10.1007/978-981-16-3852-7_2

Hassan, A., and Muhammad, L. (2023). "Isolation and identification of edible mushroom *Pleurotus* spp. and evaluate its efficiency in the production of pleuran," *IOP Conference Series: Earth and Environmental Science* 1225(1), article ID 012080. DOI: 10.1088/1755-1315/1225/1/012080

Hoa, H. T., Wang, C. L., and Wang, C. H. (2015). "The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*)," *Mycobiology* 43(4), 423-434. DOI: 10.5941/MYCO.2015.43.4.423

Jacinto-Azevedo, B., Valderrama, N., Henríquez, K., Aranda, M., and Aqueveque, P. (2021). "Nutritional value and biological properties of Chilean wild and commercial edible mushrooms," *Food Chemistry* 356, article 129651. DOI: 10.1016/j.foodchem.2021.129651

Janusz, G., Czuryło, A., Frąc, M., Rola, B., Sulej, J., Pawlik, A., Siwulski, M., and Rogalski, J. (2015). "Laccase production and metabolic diversity among *Flammulina velutipes* strains," *World Journal of Microbiology and Biotechnology* 31, 121-133. DOI: 10.1007/s11274-014-1769-y

Janusz, G., Kucharzyk, K. H., Pawlik, A., Staszczak, M., and Paszczynski, A. J. (2013). "Fungal laccase, manganese peroxidase and lignin peroxidase: Gene expression and

regulation," *Enzyme and Microbial Technology* 52(1), 1-12. DOI: 10.1016/j.enzmictec.2012.10.003

Jindal, M., Uniyal, P., and Thallada, B. (2023). "Reductive catalytic fractionation as a novel pretreatment/lignin-first approach for lignocellulosic biomass valorization: A review," *Bioresource Technology* 385, article 129396. DOI: 10.1016/j.biortech.2023.129396

Juárez-Hernández, E., Pérez-Zavala, M., Román-Reyes, M., Barboza-Corona, J., and Macías-Sánchez, K. (2023). "Overview of *Pleurotus* spp., edible fungi with various functional properties," *International Food Research Journal* 30(5), 1074-1092. DOI: 10.47836/ifrj.30.5.01

Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K., Vadthanarat, S., and Lumyong, S. (2020). "Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste," *Molecules* 25(12), article 2811. DOI: 10.3390/molecules25122811

Li, P., Xiang, Z., Xiong, X., Yang, L., He, Y. W., and Zhang, X. J. (2021a). "Effects of different cultivation substrate inductions on laccase activity of *Auricularia polytricha*," *Northern Horticulture* 2021(24), 129-135.

Li, P., Zhang, L., Xiong, X., Xiang, Z., Yang, Y. H., Zhao, X. P., and Wan, C. (2021b). "Identification of *Auricularia cornea* var. Li. and research on optimum growth conditions," *Seed* 2021(05), 119-125+2. DOI:10.16590/j.cnki.1001-4705.2021.05.119

Lin, N., Li, M., Han, Z., Liu, X., Qu, J., Wang, X., Duan, X., and He, C. (2024). "Polysaccharides from *Auricularia cornea* var. Li.: Structural alterations during *in vitro* digestion and its potent immunomodulatory properties on macrophages," *Food Bioscience* 59, article ID 104014. DOI: 10.1016/j.fbio.2024.104014

Luo, X. C., and Chen, S. Y. (2010). *Encyclopedia of Mushroom Industry in China*, Tsinghua University Press, Beijing, China.

Mikiashvili, N. A., Chichua, D., and Elisashvili, V. I. (2004). "Lignocellulolytic enzyme activities of medicinally important basidiomycetes from different ecological niches," *International Journal of Medicinal Mushrooms* 6(1), article 70. DOI: 10.1615/IntJMedMushr.v6.i1.70

Nunes, C. S., and Kunamneni, A. (2018). "Laccases—Properties and applications," in: *Enzymes in Human and Animal Nutrition*, Academic Press, Cambridge, MA, USA, pp. 133-161. DOI: 10.1016/B978-0-12-805419-2.00007-1

Pradeep Kumar, V., Sridhar, M., Ashis Kumar, S., and Bhatta, R. (2023). "Elucidating the role of media nitrogen in augmenting the production of lignin-depolymerizing enzymes by white-rot fungi," *Microbiology Spectrum* 11(5), article e01419-23. DOI: 10.1128/spectrum.01419-23

Razavi, S. E., Sanei, S. J., and Taheri, A. (2021). "Optimization of nutritional factors and copper on laccase production by *Pleurotus florida*," *Microbiology, Metabolites and Biotechnology* 4(1), 39-48. DOI: 10.22104/ARMMT.2022.5881.1077

Reddy, M. S., and Kanwal, H. K. (2022). "Influence of carbon, nitrogen sources, inducers, and substrates on lignocellulolytic enzyme activities of *Morchella spongiola*," *Journal of Agriculture and Food Research* 7, article 100271. DOI: 10.1016/j.jafr.2022.100271

Rivera-Hoyos, C. M., Morales-Álvarez, E. D., Poutou-Piñales, R. A., Pedroza-Rodríguez, A. M., RodrÍguez-Vázquez, R., and Delgado-Boada, J. M. (2013). "Fungal laccases," *Fungal Biology Reviews* 27(3-4), 67-82. DOI: 10.1016/j.fbr.2013.07.001

Su, S., Li, J., Xu, Y. N., Tian, F., Xu, W., and Li, W. (2023). "Advances in decomposition and utilization of fungus-growing materials by edible fungi mycelia," *Journal of Yunnan University: Natural Sciences Edition* 45(3), 760-767. DOI: 10.7540/j.ynu.20220051

Su, W. Y., Tan, Y., L., Liu, X. M., Yang, H. C., Qin, Y. Y., Li, X., and Ren, L. K. (2021). "Cloning and prokaryotic expression analysis of laccase gene Aclac from *Auricularia cornea*," *Journal of Southern Agriculture* 52(8), 2158-2164. DOI: 10.3969/j.issn.2095-1191.2021.08.014

Sun, S. J., Liu, J. Z., Hu, K. H., and Zhun, H. X. (2011). "The level of secreted laccase activity in the edible fungi and their growing cycles are closely related," *Current Microbiology* 62, 871-875. DOI: 10.1007/s00284-010-9794-z

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(7), article 2463. DOI: 10.3390/ijms21072463

Viriato, V., Mäkelä, M. R., Kowalczyk, J. E., Ballarin, C. S., Loiola, P. P., and Andrade, M. C. (2022). "Organic residues from agricultural and forest companies in Brazil as useful substrates for cultivation of the edible mushroom *Pleurotus ostreatus*," *Letters in Applied Microbiology* 74(1), 44-52. DOI:10.1111/lam.13580

Wang, X., Lan, Y., Zhu, Y., Li, S., Liu, M., Song, X., Zhao H., Liu W., Zhang J, and Wang S., *et al.* (2018). "Hepatoprotective effects of *Auricularia cornea* var. Li. polysaccharides against the alcoholic liver diseases through different metabolic pathways," *Scientific Reports* 8(1), article 7574. DOI: 10.1038/s41598-018-25830-w

Xiong, X., Li, P., Xiang, Z., Huang, J., and Wang, J. (2024). "Influence of different lignocellulosic materials on laccase activity in liquid fermentation of Chinese low-temperature straw mushroom (*Volvariella brumalis*)," *BioResources* 19(4), 8608-8620. DOI: 10.15376/biores.19.4.8608-8620

Xiong, X., Li, P., Zhang, G. H., Xiang, Z., Tao, W. G., Zhou, G. Y., and He, Y. W. (2021). "Effects of different cultivation substrates on laccase activity in liquid fermentation of *Lentinula edodes* during liquid fermentation," *Biotechnology Bulletin* 37(12), 50-59. DOI: 10.13560/j.cnki.biotech.bull.1985.2021-0844

Zhang, Y., Wang, D., Chen, Y., Liu, T., Zhang, S., Fan, H., Liu, H., and Li, Y. (2021). "Healthy function and high valued utilization of edible fungi," *Food Science and Human Wellness* 10(4), 408-420. DOI: 10.1016/j.fshw.2021.04.003

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