Comparative Analysis of the Laccase Secretion Ability of Five White-rot Fungi in Submerged Fermentation with Lignocellulosic Biomass

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Lignocellulosic biomass is widely used in the field of laccase production because it has the advantages of low price and easy availability. Thus, a comparative analysis was performed of the laccase secretion ability of five white-rot fungi in submerged fermentation using single or mixtures of lignocellulosic biomass. Maximum laccase activity of *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 was 55.83 ± 0.28 U/L on the mixture of corncob and cottonseed hull, 77.96 ± 1.60 U/L on corncob, 443.33 ± 15.49 U/L on corncob, 686.57 ± 16.49 U/L on corncob, and 162.04 ± 11.33 U/L on cottonseed hull. The mixed lignocellulosic material effectively improved the laccase activity of *T. gibbosa* An 360 compared with other fungal strains. However, the presence of corncob contributed to the secretion of laccase activity for *V. fraxinea* An 369, *P. pyricola* Han 202, and *C. trogii* Han 474. Meanwhile, cottonseed hull was conducive to the secretion of laccase of *T. versicolor* Han 1504. Laccase activity of *P. pyricola* Han 202 was very stable throughout most of the fermentation time. The results from this study provide new methods and increase fungal strains to improve laccase activity.

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INTRODUCTION

Lignocellulosic biomass, the most abundant renewable resource on earth, is usually treated as a cheap source of raw materials for renewable energy, chemicals, and other valuable products (Xiao *et al*. 2013). Lignocellulosic biomass has three main polymeric constituents: lignin, cellulose, and hemicellulose (Sánchez 2009). Lignin is dispersed between cellulose fibers, and hemicellulose is present and connected between lignin and cellulose fibers, thus forming a strong cellulose-hemicellulose-lignin structure that is similar to the "reinforced concrete structure" (Mosier *et al*. 2005). This "reinforced concrete structure" limits the contact between hydrolase and cellulose, and the insolubility of lignin and the complexity of chemical structure are the main factors leading to the difficulty of lignocellulosic biomass degradation (Zhen *et al*. 2017; Choi *et al*. 2019).

Although physical or chemical methods can be used to effectively degrade lignocellulosic biomass, they usually require harsh and extreme conditions (Singh *et al*. 2015; Ma *et al*. 2020; Singh and Gupta 2020). The microorganisms can ferment and degrade lignocellulosic biomass by secreting extracellular lignocellulolytic enzymes (Sánchez 2009), which has the advantages of mild reaction conditions and lack of secondary pollution to the environment. It is of great significance to isolate and screen efficient lignocellulosic biomass-degrading microorganisms. Therefore, it is necessary to screen the microorganisms that can effectively break down lignin structure.

There are many kinds of microorganisms in nature, and white-rot fungi are recognized as a large group of microorganisms that can effectively degrade lignin (Dinis *et al*. 2009; Muthuvelu *et al*. 2020). Traditional lignin-degrading enzymes primarily include laccase, manganese peroxidase, and lignin peroxidase, among which laccase plays an important role in the process (Arora and Gill 2001). Laccase (EC 1.10.3.2), belongs to a family of blue copper oxidases; it has the capacity of oxidizing phenols and aromatic amines by reducing molecular oxygen to water (Sharma *et al*. 2019; An *et al*. 2021a,b). Laccases are widespread among higher plants, fungi, insects, and bacteria (Yadav *et al*. 2018; Zhao *et al*. 2018; Nuskern *et al*. 2021). Since laccase was discovered by Yoshida in 1883, it has become a hot research topic because of its potential application value (Pandi *et al*. 2019; Song *et al*. 2020). Today, laccase can be applied in various aspects of the industrial, biotechnological, and environmental fields, such as energy exploitation, bio-detection, bio-degradation, bio-synthesis, nanoscience, and the beverage and food industry for the removal of phenolic compounds, and other miscellaneous applications (Baldrian 2006; Kudanga and Le Roes-Hill 2014; Bertrand *et al*. 2017; Yashas *et al*. 2018; Bilal *et al*. 2019; Singh and Arya 2019; Atilano-Camino *et al*. 2020; Liu *et al*. 2020; Perez-Montiel *et al*. 2021; Khatami *et al*. 2022). However, the production of large amounts of low cost and high activity laccases is the basis for the wide application of laccases (Couto and Sanroman 2005; Couto and Toca-Herrera 2007). Thus, it is necessary to promote the effective production of laccase by white-rot fungi.

Previous studies indicated that there are many factors affecting the laccase activity of fungi, such as carbon/nitrogen sources, lignocellulose materials, exogenous metal ions (Na^+, Ca^{2+}, Cu^{2+}, Fe^{2+}), pH, fungal species, and so on (Atila *et al*. 2017; Zhou *et al*. 2017; Zhuo *et al*. 2017; Kostadinova *et al*. 2018; Xu *et al*. 2018; Lallawmsanga *et al*. 2019; Jasinska *et al*. 2019; Rodriguez *et al*. 2019; Sun *et al*. 2021). Lignocellulosic materials are good substrates for fungi-producing enzymes because of their low price. Thus, selecting suitable cheap lignocellulosic biomass for microorganisms to efficiently produce laccase is an important and lasting work (Mate and Alcalde 2017; Rodrigues *et al*. 2019; Habimana *et al*. 2021; Han *et al*. 2022a). Furthermore, submerged fermentation is generally recognized as the most economical way for the production of extracellular enzymes because of their significant advantages, such as low water requirements and no dilution of the enzymes (Kapich *et al*. 2004). There are few studies on the activity of mixed lignocellulosic materials on fungal laccase activity. Xu *et al*. (2020) investigated the effects of mixed lignocellulosic biomass (corn crop waste) on lignin-degrading enzyme activities, growth, and quality of *Lentinula edodes*. Han *et al*. (2021) analyzed the effect of the mixture of *Pinus tabuliformis* and *Firmiana platanifolia* on laccase activity secreted by three fungi. Therefore, five newly isolated fungal species were used for detecting the laccase activity. The comparative analysis of the laccase secretion ability of these fungi in submerged fermentation with lignocellulosic biomass was investigated. Meanwhile, the effect of using a mixture of lignocellulosic biomass on laccase activity was investigated

for five tested fungi at the same time. These results contribute to new methods and increase the available fungal strains to improve laccase activity.

EXPERIMENTAL

Materials

Microorganisms

Five fungal species, *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 were isolated from Zizhen Park in Xiangyang city of Hubei Province (China), Yanghu Mountain in Xiangyang city of Hubei Province, Laoxiying Village in Chengde city of Hebei Province (China), Nature Park in Langfang city of Hebei Province, and Wuling Mountain in Chengde city of Hebei Province, respectively. The strains were purified on complete yeast medium (CYM) (g/L: glucose 20, peptone 2, yeast extract 2, $MgSO_4 \cdot 7H_2O$ 0.5, $K_2HPO_4 \cdot 3H_2O$ 1, KH_2PO_4 0.46, and agar 15), maintained on malt extract agar (MEA) medium (g/L: glucose 10, malt extract 20, KH_2PO_4 3, and agar 20), and stored in College of Life Science, Langfang Normal University (Langfang, China).

Lignocellulosic biomass

Corncob and cottonseed hull were obtained from Chengde city, Hebei province (China). All of these air-dried lignocellulosic materials were ground to the practical size between 20- and 60-mesh.

Methods

Microbial culture and inoculum preparation

All fungal strains, *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504, were cultured on CYM at 26 ℃ to perform the process of activation. After 6 days, five inoculants with a diameter of 1.0 cm were obtained and transferred into 250-mL triangular flask containing 100 mL liquid CYM. All flasks were cultured in shaking incubators at 26 ℃ with the speed of 150 rpm. After 7 days, the mycelium pellets obtained were homogenized using a blender at 8000 rpm for 2 min to prepare the homogenized inoculum.

Submerged fermentation for laccase activity

Triangular flasks (250 mL) containing 100 mL liquid (KH_2PO_4 1.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, and deionized water 1 L) and 2 g lignocellulosic biomass were autoclaved at 121 ℃ for 30 min. Each component of the experimental group is described in Table 1. Then, 3 mL homogenized inoculum was added into each flask. All flasks were cultured into a rotary shaker with the speed of 150 rpm at 26 ℃ for submerged fermentation.

Assay of laccase activity

The crude enzyme solution was obtained by filtering the fermentation liquid through a filter paper and centrifuging it at 12000 rpm (4 ℃) for 20 min. Laccase activity of the crude enzyme solution was detected by the UV-4802 spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China) with the optical density (OD) at 420 nm. The substrate used for laccase assay was 2,2’-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS).

The UV-determination process was performed according to the method of Han *et al*. (2021b, 2022a). One activity unit was defined as the amount of enzyme required to oxidize 1.0 μmol ABTS per minute (ε_{420} nm = 3.6×10^4 M^{-1} cm^{-1}).

Table 1. Description of Each Component of the Experimental Group

Species	Lignocellulosic Biomass (g)	Liquid (mL)	Homogenized Inoculum (mL)
Han 474	Corncob 2	100	3
Han 474	Cottonseed hull 2	100	3
Han 474	Corncob 1 and Cottonseed hull 1	100	3
Han 202	Corncob 2	100	3
Han 202	Cottonseed hull 2	100	3
Han 202	Corncob 1 and Cottonseed hull 1	100	3
An 360	Corncob 2	100	3
An 360	Cottonseed hull 2	100	3
An 360	Corncob 1 and Cottonseed hull 1	100	3
Han 1504	Corncob 2	100	3
Han 1504	Cottonseed hull 2	100	3
Han 1504	Corncob 1 and Cottonseed hull 1	100	3
An 369	Corncob 2	100	3
An 369	Cottonseed hull 2	100	3
An 369	Corncob 1 and Cottonseed hull 1	100	3

Data analysis

Two-way analysis of variance (ANOVA) was applied to analyze the effects of lignocellulosic biomass and fungal species on laccase activity by following Han *et al*. (2021b) using and using SPSS 22.0 (PROC GLM, Armonk, NY, USA). All colorful figures were generated by the software of Origin 2016 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Statistical Analysis Result

Previous studies indicated that fungal species and agricultural and forest residues can significantly affect the laccase activity (Elisashvili *et al*. 2008; Janusz *et al*. 2015; An *et al*. 2016, 2021a,b; Han *et al*. 2021, 2022a). Similarly, as shown in Table 2, fungal species and lignocellulosic biomass could affect the laccase activities significantly ($P < 0.001$) through the whole submerged fermentation stage. Further, the interactions of fungal species and lignocellulosic biomass could affect the laccase activity significantly ($P < 0.001$).

Effect of Various Fungal Species on Laccase Activity

Previous studies evaluated the laccase secretion ability and found that it varies from different *Basidiomycetous* white-rot fungus or different strains belonging to same species (Janusz *et al*. 2015; An *et al*. 2016; Han *et al*. 2021, 2022a). Thus, the assessment of capacity of laccase production from new strains isolated from their natural ecological environment is an important task for obtaining strains with high production capacity. The

levels of extracellular laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 were compared.

Table 2. Two-way ANOVA to Analyze the Effects of Fungal Species, Lignocellulosic Biomass, and the Interactions between Fungal Species and Lignocellulosic Biomass on Laccase Activities

Incubation Period (d)	Fungal Species	Lignocellulosic Biomass	Fungal Species × Lignocellulosic Biomass
1	1394.026***	169.469***	222.485***
2	1989.127***	165.302***	446.185***
3	1815.177***	361.831***	707.617***
4	989.416***	49.343***	283.889***
5	921.434***	32.932***	301.983***
6	825.474***	70.958***	95.143***
7	1102.169***	4.123***	62.616***
8	3226.846***	391.073***	409.607***
9	9312.340***	641.941***	657.984***
10	2216.403***	55.879***	99.642***
Note: df = 4, 2, 8; ***P < 0.001; The values were the F-value of Two-way ANOVA			

In terms of corncob, the laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 at the first day of the submerged fermentation stage was 0 ± 0 U/L, 9.54 ± 0.70 U/L, 38.06 ± 3.06 U/L, 2.87 ± 0.16 U/L, and 10.65 ± 0.16 U/L (Fig. 1), respectively. However, *P. pyricola* Han 202 needs a shorter adaptation time to grow on corncob and produced laccase faster than other fungal species. Han *et al*. (2022b) analyzed the extracellular laccase activity among *Ganoderma* and *Coriolopsis* species on different lignocellulosic wastes and found that the laccase activity of *Coriolopsis trogii* Han 1211, *Ganoderma lingzhi* Han 1345, *Coriolopsis strumosa* Han 1356, and *Ganoderma applanatum* Han 1578 fermentation on corncob at the 1st day was 1.61 ± 0.17 U/L, 0.30 ± 0 U/L, 0 ± 0 U/L, and 0 ± 0 U/L, respectively. Thus, the laccase activity of *V. fraxinea* An 369 and *P. pyricola* Han 202 fermented on corncob at the 1st day was higher than that from some strains belonging to genus of *Coriolopsis* and *Ganoderma*. Maximum laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 was 12.13 ± 1.12 U/L, 77.96 ± 1.60 U/L, 443.33 ± 15.49 U/L, 686.57 ± 16.49 U/L, and 49.07 ± 4.57 U/L, and the corresponding time was 2nd day, 2nd day, 3rd day, 8th day, and 3rd day (Fig. 1, Table 3), respectively. Based on this, maximum laccase activity of *C. trogii* Han 474 was nearly 56.60-fold, 8.81-fold, 1.55-fold, and 13.99-fold higher than that from *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, and *T. versicolor* Han 1504, relatively. Maximum laccase activity of *C. trogii* Han 1211, *G. lingzhi* Han 1345, *C. strumosa* Han 1356, and *G. applanatum* Han 1578 grown on corncob was 151.50 ± 3.32 U/L at 3rd day, 333.74 ± 11.41 U/L at 8th day, 116.94 ± 2.09 U/L at 6th day, and 35.16 ± 2.93 U/L at 6th day (Han *et al*. 2022b). Maximum laccase activity of *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751 fermentation on corncob was 1.51 ± 0 U/L, 19.89 ± 1.31 U/L, and 90.92 ± 4.01 U/L, respectively (Liu *et al*. 2022). Clearly, maximum laccase activity of *P. pyricola* Han 202 and *C. trogii* Han 474 was higher when compared with the laccase activity from other strains in previous studies (Han *et al*. 2022b; Liu *et al*. 2022).

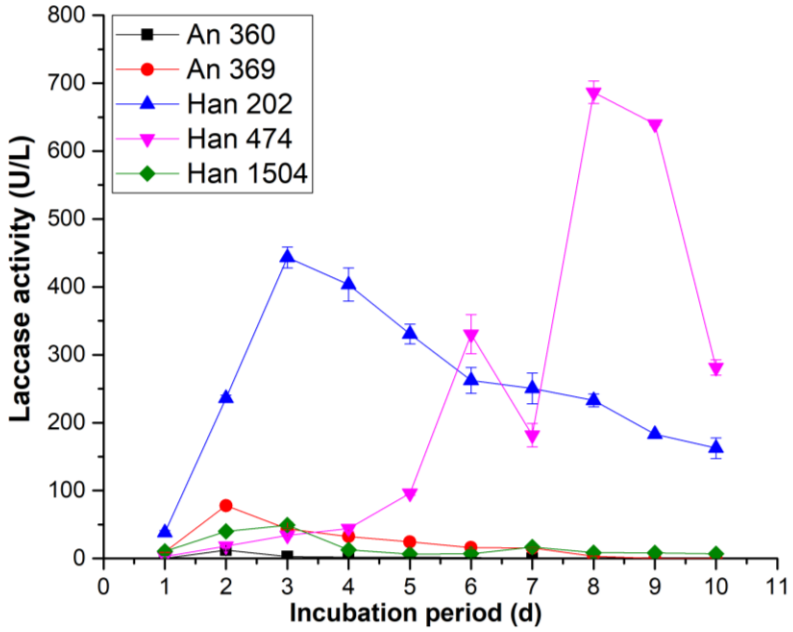

Fig. 1. Laccase activity from *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 in submerged fermentation with corncob

With cottonseed hull, the laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 was 7.22 ± 0.48 U/L, 11.76 ± 0.42 U/L, 105.83 ± 7.52 U/L, 9.26 ± 0.16 U/L, and 0 ± 0 U/L for the 1st day of the submerged fermentation (Fig. 2). Thus, the laccase activity from *P. pyricola* Han 202 at the 1st day was 14.66-fold, 9.00-fold, and 11.43-fold higher than that from *T. gibbosa* An 360, *V. fraxinea* An 369, and *C. trogii* Han 474, respectively. The laccase activity of *Coriolopsis trogii* Han 1211, *Ganoderma lingzhi* Han 1345, *Coriolopsis strumosa* Han 1356, and *Ganoderma applanatum* Han 1578 fermentation on cottonseed hull at the 1st day was 28.03 ± 2.09 U/L, 7.03 ± 0.63 U/L, 0 ± 0 U/L, and 19.09 ± 0.76 U/L, respectively (Han *et al*. 2022b). The values of laccase activity from *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751 fermented on cottonseed hull were 1.51 ± 0 U/L, 1.21 ± 0 U/L, and 1.21 ± 0 U/L on the 1st day, respectively (Liu *et al*. 2022). Therefore, *P. pyricola* Han 202 showed higher laccase activity on day 1 than the strains previously used and exhibited excellent ability to secrete laccase. Maximum laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 was 29.54 ± 1.12 U/L (3rd day), 65.46 ± 0.32 U/L (4th day), 159.26 ± 3.31 U/L (7th day), 288.80 ± 14.49 U/L (9th day), and 162.04 ± 11.33 (2nd day), respectively (Table 3). Maximum laccase activity of *C. trogii* Han 1211, *G. lingzhi* Han 1345, *C. strumosa* Han 1356, *G. applanatum* Han 1578, *P. acerina* Han 618, *T. hirsuta* Han 726, and *C. trogii* Han 751 grown on cottonseed hull was 223.03 ± 11.51 U/L at 8th day, 84.09 ± 7.69 U/L at 5th day, 203.64 ± 5.89 U/L at 5th day, 41.09 ± 3.60 U/L at 2nd day, 28.03 ± 1.88 U/L at 9th day, 27.43 ± 1.59 U/L at 5th day, and 16.58 ± 0 U/L at 8th day, respectively (Han *et al*. 2022b; Liu *et al*. 2022).

Table 3. Maximum Value of Laccase Activity and Occurrence Time of *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 in Submerged Fermentation with Lignocellulosic Biomass

Maximum Laccase Activity (U/L)	Lignocellulosic Biomass	Species	Time (Day)
12.13 ± 1.12	Corncob	*Trametes gibbosa* An 360	2nd
29.54 ± 0.32	Cottonseed hull	*Trametes gibbosa* An 360	3rd
55.83 ± 0.28	Corncob and Cottonseed hull	*Trametes gibbosa* An 360	4th
77.96 ± 1.60	Corncob	*Vanderbylia fraxinea* An 369	2nd
65.46 ± 0.32	Cottonseed hull	*Vanderbylia fraxinea* An 369	4th
42.5 ± 0.96	Corncob and Cottonseed hull	*Vanderbylia fraxinea* An 369	2nd
443.33 ± 15.49	Corncob	*Perenniporia pyricola* Han 202	3rd
159.26 ± 3.31	Cottonseed hull	*Perenniporia pyricola* Han 202	7th
172.50 ± 15.00	Corncob and Cottonseed hull	*Perenniporia pyricola* Han 202	7th
686.57 ± 16.49	Corncob	*Coriolopsis trogii* Han 474	8th
288.80 ± 14.49	Cottonseed hull	*Coriolopsis trogii* Han 474	9th
379.54 ± 22.46	Corncob and Cottonseed hull	*Coriolopsis trogii* Han 474	8th
49.07 ± 4.57	Corncob	*Trametes versicolor* Han 1504	3rd
162.04 ± 11.33	Cottonseed hull	*Trametes versicolor* Han 1504	2nd
75.19 ± 4.05	Corncob and Cottonseed hull	*Trametes versicolor* Han 1504	3rd
Maximum values of laccase activity are presented as mean value ± standard deviation for biological triplicates			

It was clear that the laccase secretion capacity of *C. trogii* Han 474 was superior to other strains used in the previous study (Han *et al*. 2022b; Liu *et al*. 2022) due to their maximum laccase activities. In contrast, although the maximum laccase activity of *P. pyricola* Han 202 was not the largest among the five fungi, its laccase activity was very stable and remained above 100 U/L through most of the fermentation time (Fig. 2).

When a mixture of corncob and cottonseed hull was used, the laccase activity of *P. pyricola* Han 202 on the 1st day was 57.69 ± 1.89 U/L, which is nearly 12.46-fold and 1.52-fold higher than that from *C. trogii* Han 474 and *T. versicolor* Han 1504, respectively (Fig. 3). Meanwhile, no laccase activity was detected from *T. gibbosa* An 360 and *V. fraxinea* An 369 on 1st day. Thus, *P. pyricola* Han 202 could produce higher laccase activity in a short time compared with other fungal species.

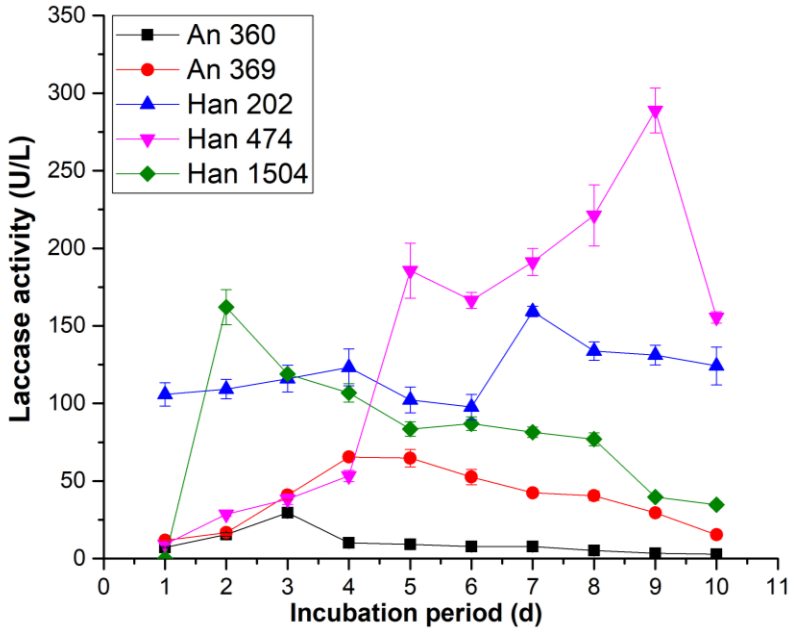

Fig. 2. Laccase activity from *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 in submerged fermentation with cottonseed hull

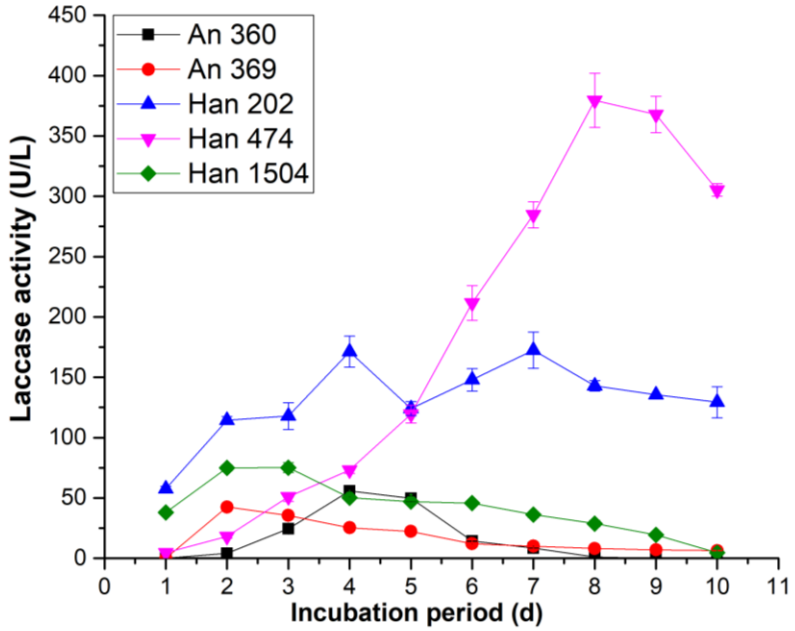

Fig. 3. Laccase activity from *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, *Coriolopsis trogii* Han 474, and *Trametes versicolor* Han 1504 in submerged fermentation with the mixture of corncob and cottonseed hull

Maximum laccase activity of *C. trogii* Han 474 was 379.54 ± 22.46 U/L at the 8th day, nearly 6.80-fold, 8.93-fold, 2.20-fold, and 5.05-fold higher than that from *T. gibbosa* An 360 (55.83 ± 0.28 U/L, 4th day), *V. fraxinea* An 369 (42.5 ± 0.96 U/L, 2nd day), *P. pyricola* Han 202 (172.50 ± 15.00 U/L, 7th day), and *T. versicolor* Han 1504 (75.19 ± 4.05 U/L, 3rd day), respectively (Table 3).

Laccase activities in tree leaves and mandarin peels in submerged fermentation from *Lentinus edodes* IBB 123, *L. edodes* IBB 363, *Pleurotus dryinus* IBB 903, *P. ostreatus* IBB 8, *P. ostreatus* 2175, *P. ostreatus* 2191, and *P. tuberregium* IBB 624 were respectively 39 ± 2.8 and 4 ± 0.3 U/flask, 89 ± 6.5 and 6 ± 0.6 U/flask, 6 ± 0.5 and 205 ± 2.1 U/flask, 11 ± 0.9 and 24 ± 2.0 U/flask, 5 ± 0.5 and 17 ± 1.7 U/flask, 4 ± 0.5 and 17 ± 1.4 U/flask, and 7 ± 0.6 and 19 ± 1.3 U/flask (Elisashvili *et al*. 2008). It is easy to find that various fungal species and different strains of the same species have great differences in the secretion of laccase.

Effect of Various Lignocellulosic Biomass on Laccase Activity

Solid lignocellulosic biomass used for cultivating fungi has the potential not only to reduce the pollution of solid agricultural and forestry waste, but also it could create economic value, such as the enzyme products (Gupta and Jana 2019; Leite *et al*. 2019). There are many kinds of lignocellulose biomass used in producing chemical products, and poplar, sugarcane bagasse, tree leaves, corncob, and straw were commonly used in previous studies (Gaikwad and Meshram 2019; Atilano-Camino *et al*. 2020; Malhotra and Suman 2021; Liu *et al*. 2022). Cottonseed hull and corncob used in the present study are by-products of cotton and corn, which are of great yield and low price in China. Thus, evaluation of the cheap and readily available lignocellulosic biomass materials for fungi to produce enzymes is of interest for the production of low-cost enzyme products.

Laccase activity of *T. gibbosa* An 360 on the 1st day was only detected on cotton hull (7.22 ± 0.48 U/L) (Figs. 1 to 3). However, the maximum value of laccase production from *T. gibbosa* An 360 was obtained with a lignocellulosic mixture of cottonseed hull and corncob, 55.83 ± 0.28 U/L, which was 4.62-fold and 1.89-fold higher than that from corncob and cottonseed hull used alone (Table 3). It was clear that the mixed lignocellulosic material effectively improved the laccase activity of *T. gibbosa* An 360. At the 1st day, laccase activity from *V. fraxinea* An 369 on corncob, cottonseed hull, and the mixture of corncob and cottonseed hull was 9.54 ± 0.70 U/L, 11.76 ± 0.42 U/L, and 0 ± 0 U/L, respectively. Maximum laccase activity from *V. fraxinea* An 369 on corncob (77.96 ± 1.60 U/L) and cottonseed hull (65.46 ± 0.32 U/L) was higher than that on the mixture of corncob and cottonseed hull (35.56 ± 2.37 U/L). The value of laccase activity from *P. pyricola* Han 202 for the 1st day was 38.06 ± 3.06 U/L on corncob, 105.83 ± 7.52 U/L on cottonseed hull, and 57.69 ± 1.89 U/L on the mixture of corncob and cottonseed hull. During the test with various lignocellulosic biomass, maximum laccase activity of *P. pyricola* Han 202 was 443.33 ± 15.49 U/L, which occurred on corncob for the 3rd day. Similar to the phenomenon from *V. fraxinea* An 369, mixture of corncob and cottonseed hull was not useful for improving laccase activity to *P. pyricola* Han 202. However, it is worth noting that the laccase activity of *P. pyricola* Han 202 on each material remained relatively stable. For example, the laccase activity on corncob was greater than 200 U/L for most of the time. Similarly, maximum laccase activity of *C. trogii* Han 474 and *T. versicolor* Han 1504 appeared on corncob (686.57 ± 16.49 U/L) and cottonseed hull (162.04 ± 11.33 U/L), respectively. Highest laccase activity from *Pseudolagarobasidium* sp. PP17-33 fermented on oil palm decanter cake was 5.841 U/g, which was obtained *via* Plackett–Burman design (Thamvithayakorn *et al*. 2019). The optimal conditions for *Trametes versicolor* to produce laccase used wheat bran as submerged fermentation substrate at 35 ℃ and 5 g/L, approximately 200 U/mL on 11 days (Atilano-Camino *et al*. 2020). Most studies have focused on the effects of single lignocellulosic materials on laccase production by fungi, whereas few studies have investigated the effect of mixed

lignocellulosic materials on fungal laccase production (Han *et al*. 2021). Han *et al*. (2021) found that the mixture of *Pinus tabuliformis* and *Firmiana platanifolia* was beneficial for improving the laccase activity by *Cerrena unicolor* Han 849. Similarly, the mixture lignocellulosic biomass with cottonseed hull and corncob was useful for enhancing the laccase activity of *T. gibbosa* An 360. Thus, it also can be seen that laccase activity of fungal strains fermented on different lignocellulosic biomass was existent.

CONCLUSIONS

1. Overall, the capacity of secreting laccase by *Coriolopsis trogii* Han 474 on all tested lignocellulosic biomass materials was superior to other four fungal strains, *Trametes gibbosa* An 360, *Vanderbylia fraxinea* An 369, *Perenniporia pyricola* Han 202, and *Trametes versicolor* Han 1504.
2. It was demonstrated that the mixed lignocellulosic material effectively improved the laccase activity of *T. gibbosa* An 360 compared with other fungal strains. However, the presence of corncob contributed to the secretion of laccase from *V. fraxinea* An 369, *P. pyricola* Han 202, and *C. trogii* Han 474. Meanwhile, cottonseed hull was conducive to the secretion of laccase of *T. versicolor* Han 1504.
3. Although the maximum laccase activity of *P. pyricola* Han 202 was not the largest among the five fungi, its laccase activity was very stable throughout most of the fermentation time.
4. Maximum laccase activity of *T. gibbosa* An 360, *V. fraxinea* An 369, *P. pyricola* Han 202, *C. trogii* Han 474, and *T. versicolor* Han 1504 were 55.83 ± 0.28 U/L on the mixture of corncob and cottonseed hull, 77.96 ± 1.60 U/L on corncob, 443.33 ± 15.49 U/L on corncob, 686.57 ± 16.49 U/L on corncob, and 162.04 ± 11.33 U/L on cottonseed hull.

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REFERENCES CITED

An, Q., Han, M. L., Wu, X. J., Si, J., Cui, B. K., Dai, Y. C., and Wu, B. (2016). "Laccase production among medicinal mushrooms from the Genus *Flammulina* (Agaricomycetes) under different treatments in submerged fermentation," *International Journal of Medicinal Mushrooms* 18(11), 1049-1059. DOI: 10.1615/IntJMedMushrooms.v18.i11.90

An, Q., Li, C. S., Yang, J., Chen, S. Y., Ma, K. Y., Wu, Z. Y., Bian, L. S., and Han, M. L. (2021a). "Evaluation of laccase production by two white-rot fungi using solid-state fermentation with different agricultural and forestry residues," *BioResources* 16(3), 5287-5300. DOI: 10.15376/biores.16.3.5287-5300

An, Q., Shi, W. Y., He, Y. X., Hao, W. Y., Ma, K. Y., Chen, X., Yan, X. Y., Bian, L. S., Li, C. S., and Han, M. L. (2021b). "Evaluation of the capacity of laccase secretion of four novel isolated white-rot fungal strains in submerged fermentation with lignocellulosic biomass," *BioResources* 16(4), 6706-6722. DOI: 10.15376/biores.16.4. 6706-6722

Arora, D. S., and Gill, P. K. (2001). "Effects of various media and supplements on laccase production by some white rot fungi," *Bioresource Technology* 77(1), 89-91. DOI: 10.1016/S0960-8524(00)00114-0

Atila, F., Tuzel, Y., Cano, A. F., and Fernandez, J. A. (2017). "Effect of different lignocellulosic wastes on *Hericium americanum* yield and nutritional characteristics," *Journal of the Science of Food and Agriculture* 97(2), 606-612. DOI: 10.1002/jsfa.7772

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 no. 111231. DOI: 10.1016/j.jenvman.2020.111231

Baldrian, P. (2006). "Fungal laccases – Occurrence and properties," *FEMS Microbiology Reviews* 30(2), 215-242. DOI: 10.1111/j.1574-4976.2005.00010.x

Bertrand, B., Martinez-Morales, F., and Trejo-Hernandez, M. R. (2017). "Upgrading laccase production and biochemical properties: Strategies and challenges," *Biotechnology Progress* 33(4), 1015-1034. DOI: 10.1002/btpr.2482

Bilal, M., Rasheed, T., Nabeel, F., Iqbal, H. M. N., and Zhao, Y. P. (2019). "Hazardous contaminants in the environment and their laccase-assisted degradation – A review," *Journal of Environmental Management* 234, 253-264. DOI: 10.1016/j.jenvman.2019.01.001

Choi, J. H., Park, S. Y., Kim, J. H., Cho, S. M., Fang, S. K., Hong, C., and Choi, I. G. (2019). "Selective deconstruction of hemicellulose and lignin with producing derivatives by sequential pretreatment process for biorefining concept," *Bioresource Technology* 291, article ID 121913. DOI: 10.1016/j.biortech.2019.121913

Couto, S. R., and Sanroman, M. A. (2005). "Coconut flesh: a novel raw material for laccase production by *Trametes hirsuta* under solid-state conditions. Application to Lissamine Green B decolourization," *Journal of Food Engineering* 71(2), 208-213. DOI: 10.1016/j.jfoodeng.2004.10.035

Couto, S. R., and Toca-Herrera, J. L. (2007). "Laccase production at reactor scale by filamentous fungi," *Biotechnology Advances* 25(6), 558-569. DOI: 10.1016/j.biotechadv.2007.07.002

Dinis, M. J., Bezerra, R. M. F., Nunes, F., Dias, A. A., Guedes, C. V., Ferreira, L. M. M., Cone, J. W., Marques, G. S. M., Barros, A. R. N., and Rodrigues, M. A. M. (2009). "Modification of wheat straw lignin by solid state fermentation with white-rot fungi," *Bioresource Technology* 100(20), 4829-4835. DOI: 10.1016/j.biortech.2009.04.036

Elisashvili, V., Penninckx, M., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Kharziani, T., and Kvesitadze, G. (2008). "*Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of

lignocellulosic wastes of different composition," *Bioresource Technology* 99(3), 457-462. DOI: 10.1016/j.biortech.2007.01.011

Gaikwad, A., and Meshram, A. (2019). "Effect of particle size and mixing on the laccase-mediated pretreatment of lignocellulosic biomass for enhanced saccharification of cellulose," *Chemical Engineering Communications* 207(12), 1696-1706. DOI: 10.1080/00986445.2019.1680364

Gupta, A., and Jana, A. K. (2019). "Production of laccase by repeated batch semi-solid fermentation using wheat straw as substrate and support for fungal growth," *Bioprocess and Biosystems Engineering* 42(3), 499-512. DOI: 10.1007/s00449-018-2053-6

Habimana, P., Gao, J., Mwizerwa, J. P., Ndayambaje, J. B., Liu, H. R., Luan, P. Q., Ma, L., and Jiang, Y. J. (2021). "Improvement of laccase activity *via* covalent immobilization over mesoporous silica coated magnetic multiwalled carbon nanotubes for the discoloration of synthetic dyes," *ACS Omega* 6(4), 2777-2789. DOI: 10.1021/acsomega.0c05081

Han, M. L., Yang, J., Liu, Z. Y., Wang, C. R., Chen, S. Y., Han, N., Hao, W. Y., An, Q., and Dai, Y. C. (2021). "Evaluation of laccase activities by three newly isolated fungal species in submerged fermentation with single or mixed lignocellulosic wastes,"

Han, M. L., Li, X. Q., Zhang, C. D., Li, M. X., Zhang, M. H., An, M., Dou, X. Y., Zhang, T. X., Yan, X. Y., Bian, L. S., and An, Q. (2022a). "Effect of different lignocellulosic biomasses on laccase production by *Pleurotus* species," *BioResources* 17(3), 4921-4936. DOI: 10.15376/biores.17.3.4921-4936

Han, M. L., Yang, J., Ma, J. J., Wang, C. R., Chen, S. Y., Xu, M. X., Yang, Q. Y., Bian, L. S., Yan, X. Y., and An, Q. (2022b). "Extracellular laccase activity among *Ganoderma* and *Coriolopsis* species grown on lignocellulosic wastes," *BioResources* 17(3), 5049-5064. DOI: 10.15376/biores.17.3.5049-5064

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(1), 121-133. DOI: 10.1007/s11274-014-1769-y

Jasinska, A., Goralczyk-Binkowska, A., Sobon, A., and Dlugonski, J. (2019). "Lignocellulose resources for the *Myrothecium roridum* laccase production and their integrated application for dyes removal," *International Journal of Environmental Science and Technology* 16(8), 4811-4822. DOI: 10.1007/s13762-019-02290-x

Kapich, A. N., Prior, B. A., Botha, A., Galkin, S., Lundell, T., and Hatakka, A. (2004). "Effect of lignocellulose-containing substrates on production of ligninolytic peroxidases in submerged cultures of *Phanerochaete chrysosporium* ME-446," *Enzyme and Microbial Technology* 34(2), 187-195. DOI: 10.1016/j.enzmictec.2003.10.004

Khatami, S. H., Vakili, O., Movahedpour, A., Ghesmati, Z., Ghasemi, H., and Taheri-Anganeh, M. (2022). "Laccase: Various types and applications," *Biotechnology and Applied Biochemistry* (In press). DOI: 10.1002/bab.2313

Kostadinova, N., Krumova, E., Boeva, R., Abrashev, R., Miteva-Staleva, J., Spassova, B., and Angelova, M. (2018). "Effect of copper ions on the ligninolytic enzyme complex and the antioxidant enzyme activity in the white-rot fungus *Trametes trogii* 4_6," *Plant Biosystems* 152(5), 1128-1133. DOI: 10.1080/11263504.2017.1418450

Kudanga, T., and Le Roes-Hill, M. (2014). "Laccase applications in biofuels production: Current status and future prospects," *Applied Microbiology and Biotechnology*

98(15), 6525-6542. DOI: 10.1007/s00253-014-5810-8

Lallawmsanga, Leo, V. V., Passari, A. K., Muniraj, I. K., Uthandi, S., Hashem, A., Abd Allah, E. F., Alqarawi, A. A., and Singh, B. P. (2019). "Elevated levels of laccase synthesis by *Pleurotus pulmonarius* BPSM10 and its potential as a dye decolorizing agent," *Saudi Journal of Biological Sciences* 26(3), 464-468. DOI: 10.1016/j.sjbs.2018.10.006

Leite, P., Silva, C., Salgado, J. M., and Belo, I. (2019). "Simultaneous production of lignocellulolytic enzymes and extraction of antioxidant compounds by solid-state fermentation of agro-industrial wastes," *Industrial Crops and Products* 137, 315-322. DOI: 10.1016/j.indcrop.2019.04.044

Liu, W. X., Zhao, M. Y., Li, M. X., Li, X. Q., Zhang, T. X., Chen, X., Yan, X. Y., Bian, L. S., An, Q., Li, W. J., *et al.* (2022). "Laccase activities from three white-rot fungal species isolated from their native habitat in north China using solid-state fermentation with lignocellulosic biomass," *BioResources* 17(1), 1533-1550. DOI: 10.15376/biores.17.1.1533-1550

Liu, Y., Luo, G., Ngo, H. H., Guo, W. S., and Zhang, S. C. (2020). "Advances in thermostable laccase and its current application in lignin-first biorefinery: A review," *Bioresource Technology* 298, article ID 122511. DOI: 10.1016/j.biortech.2019.122511

Ma, L. L., Lu, Y. Y., Yan, H., Wang, X., Yi, Y. L., Shan, Y. Y., Liu, B. F., Zhou, Y., and Lu, X. (2020). "Screening of cellulolytic bacteria from rotten wood of Qinling (China) for biomass degradation and cloning of cellulases from *Bacillus methylotrophicus*," *BMC Biotechnology* 20(6), article no. 2. DOI: 10.1186/s12896-019-0593-8

Malhotra, M., and Suman, S. K. (2021). "Laccase-mediated delignification and detoxification of lignocellulosic biomass: Removing obstacles in energy generation," *Environmental Science and Pollution Research* 28(42), 58929-58944. DOI: 10.1007/s11356-021-13283-0

Mate, D. M., and Alcalde, M. (2017). "Laccase: A multi-purpose biocatalyst at the forefront of biotechnology," *Microbial Biotechnology* 10(6), 1457-1467. DOI: 10.1111/1751-7915.12422

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresource Technology*, 96(6), 673-686. DOI: 10.1016/j.biortech.2004.06.025

Muthuvelu, K. S., Rajarathinam, R., Selvaraj, R. N., and Rajendren, V. B. (2020). "A novel method for improving laccase activity by immobilization onto copper ferrite nanoparticles for lignin degradation," *International Journal of Biological Macromolecules* 152, 1098-1107. DOI: 10.1016/j.ijbiomac.2019.10.198

Nuskern, L., Tkalec, M., Srezovic, B., Jezic, M., Gacar, M., and Curkovic-Perica, M. (2021). "Laccase activity in fungus *Cryphonectria parasitica* is affected by growth conditions and fungal-viral genotypic interactions," *Journal of Fungi* 7(11), article no. 958. DOI: 10.3390/jof7110958

Pandi, A., Kuppuswami, G. M., Ramudu, K. N., and Palanivel, S. (2019). "A sustainable approach for degradation of leather dyes by a new fungal laccase," *Journal of Cleaner Production* 211, 590-597. DOI: 10.1016/j.jclepro.2018.11.048

Perez-Montiel, G., Torres-Garcia, J. L., Juarez-Santacruz, L., Cortes-Espinosa, D. V., Rubio-Pina, J., and Ahuactzin-Perez, M. (2021). "*Pleurotus ostreatus* growth and

laccase enzymes production during the degradation process of bisphenol A," *Biotecnia* 23(2), 39-46. DOI: 10.18633/biotecnia.v23i2.1357

Rodrigues, E. M., Karp, S. G., Malucelli, L. C., Helm, C. V., and Alvarez, T. M. (2019). "Evaluation of laccase production by *Ganoderma lucidum* in submerged and solid-state fermentation using different inducers," *Journal of Basic Microbiology* 59(8), 784-791. DOI: 10.1002/jobm.201900084

Rodriguez, R. D., Heredia, G., Siles, J. A., Jurado, M., Saparrat, M. C. N., Garcia-Romera, I., and Sampedro, I. (2019). "Enhancing laccase production by white-rot fungus *Funalia floccosa* LPSC 232 in co-culture with *Penicillium commune* GHAIE86," *Folia Microbiologica* 64(1), 91-99. DOI: 10.1007/s12223-018-0635-y

Sánchez, C. (2009). "Lignocellulosic residues: Biodegradation and bioconversion by fungi," *Biotechnology Advances* 27(2), 185-194. DOI: 10.1016/j.biotechadv.2008.11.001

Sharma, A., Jain, K. K., Srivastava, A., Shrivastava, B., Thakur, V. V., Jain, R. K., and Kuhad, R. C. (2019). "Potential of *in situ* SSF laccase produced from *Ganoderma lucidum* RCK 2011 in biobleaching of paper pulp," *Bioprocess and Biosystems Engineering* 42(3), 367-377. DOI: 10.1007/s00449-018-2041-x

Singh, D., and Gupta, N. (2020). "Microbial laccase: A robust enzyme and its industrial applications," *Biologia* 75(8), 1183-1193. DOI: 10.2478/s11756-019-00414-9

Singh, G., and Arya, S. K. (2019). "Utility of laccase in pulp and paper industry: A progressive step towards the green technology," *International Journal of Biological Macromolecules* 134, 1070-1084. DOI: 10.1016/j.ijbiomac.2019.05.168

Singh, J., Suhag, M., and Dhaka, A. (2015). "Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: A review," *Carbohydrate Polymer*s 117, 624-631. DOI: 10.1016/j.carbpol.2014.10.012

Song, Q., Deng, X., and Song, R. Q. (2020). "Expression of *Pleurotus ostreatus* laccase gene in *Pichia pastoris* and its degradation of corn stover lignin," *Microorganisms* 8(4), article no. 601. DOI: 10.3390/microorganisms8040601

Sun, Y., Liu, Z. L., Hu, B. Y., Chen, Q. J., Yang, A. Z., Wang, Q. Y., Li, X. F., Zhang, J. Y., Zhang, G. Q., and Zhao, Y. C. (2021). "Purification and characterization of a thermo- and pH-stable laccase from the litter-decomposing fungus *Gymnopus luxurians* and laccase mediator systems for dye decolorization," *Frontiers in Microbiology* 12, article ID 672620. DOI: 10.3389/fmicb.2021.672620

Thamvithayakorn, P., Phosri, C., Pisutpaisal, N., Krajangsang, S., Whalley, A. J. S., and Suwannasai, N. (2019). "Utilization of oil palm decanter cake for valuable laccase and manganese peroxidase enzyme production from a novel white rot fungus, *Pseudolagarobasidium* sp. PP17–33," *3 Biotech* 9, article no. 417. DOI: 10.1007/s13205-019-1945-8

Xiao, L. P., Shi, Z. J., Bai, Y. Y., Wang, W., Zhang, X. M., and Sun, R. C. (2013). "Biodegradation of lignocellulose by white-rot fungi: Structural characterization of water-soluble hemicelluloses," *Bioenergy Research* 6(4), 1154-1164. DOI: 10.1007/s12155-013-9302-y

Xu, S., Wang, F., Fu, Y. P., Li, D., Sun, X. Z., Li, C. T., Song, B., and Li, Y. (2020). "Effects of mixed agro-residues (corn crop waste) on lignin-degrading enzyme activities, growth, and quality of *Lentinula edodes*," *RSC Advances* 10(17), 9798-9807. DOI: 10.1039/c9ra10405d

Xu, X. Q., Huang, X. H., Liu, D., Lin, J., Ye, X. Y., and Yang, J. (2018). "Inhibition of metal ions on *Cerrena* sp laccase: Kinetic, decolorization and fluorescence studies,"

Journal of the Taiwan Institute of Chemical Engineers 84, 1-10. DOI: 10.1016/j.jtice.2017.12.028

Yadav, D., Ranjan, B., Mchunu, N., Roes-Hill, M. L., and Kudanga, T. (2018). "Secretory expression of recombinant small laccase from *Streptomyces coelicolor* A3(2) in *Pichia pastoris*," *International Journal of Biological Macromolecules* 108, 642-649. DOI: 10.1016/j.ijbiomac.2017.11.169

Yashas, S. R., Shivakumara, B. P., Udayashankara, T. H., and Krishna, B. M. (2018). "Laccase biosensor: Green technique for quantification of phenols in wastewater (a review)," *Oriental Journal of Chemistry* 34(2), 631-637. DOI: 10.13005/ojc/340204

Zhao, J., Zeng, S., Xia, Y., and Xia, L. (2018). "Expression of a thermotolerant laccase from *Pycnoporus sanguineus* in *Trichoderma reesei* and its application in the degradation of bisphenol," *Journal of Bioscience and Bioengineering* 125(4), 371-376. DOI: 10.1016/j.jbiosc.2017.11.010

Zhen, J., Li, G. J., Li, W., Du, Z. M., Wang, J. W., Yin, W. B., and Chen, G. C. (2017). "Fermentation condition optimization of laccase producing strain *Trametes hirsuta* XYG422 and its activity on corn straw degradation," *Mycosystema* 36(6), 718-729. DOI: 10.13346/j.mycosystema.160163 (in Chinese)

Zhou, C. X., Dong, A. X., Wang, Q., Yu, Y. Y., Fan, X. R., Cao, Y. L., and Li, T. J. (2017). "Effect of common metal ions and anions on laccase catalysis of guaiacol and lignocellulosic fiber," *BioResources* 12(3), 5102-5117. DOI: 10.15376/biores.12.3.5102-5117

Zhuo, R., Yuan, P., Yang, Y., Zhang, S., Ma, F. Y., and Zhang, X. Y. (2017). "Induction of laccase by metal ions and aromatic compounds in *Pleurotus ostreatus* HAUCC 162 and decolorization of different synthetic dyes by the extracellular laccase," *Biochemical Engineering Journal* 117, 62-72. DOI: 10.1016/j.bej.2016.09.016

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