Laccase Activities from Three White-rot Fungal Species Isolated from Their Native Habitat in North China Using Solid-State Fermentation with Lignocellulosic Biomass

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Laccase has huge potential application in all aspects of biotechnology due to the ability of laccase to oxidize a wide range of phenolic and non-phenolic compounds. The future success of such applications requires large amounts of laccase with low costs. White-rot fungi are important groups of laccase production. In this study, three white-rot fungi, *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751, isolated from a native North China habitat, were identified by the method of molecular biology and preliminary screening of the ability of laccase-production by guaiacol selection medium. Then they were fermented on different lignocellulosic biomass. Three species showed consistency in preference of lignocellulosic biomass, and the presence of stalk of *Sorghum bicolor* was more suitable for secreting laccase. The capacity of laccase secretion from different species was significantly different. The capacity of secreting laccase of *C. trogii* Han 751 was superior to that of *P. acerina* Han 618 and *T. hirsuta* Han 726. The discovery of a new strain with superior capacity of secreting laccase and suitable lignocellulosic biomass were helpful for laying a foundation for the optimization of the fermentation conditions for the highest laccase production, the isolation and purification of laccase, and the industrial application of laccase.

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INTRODUCTION

With the rapid economic development and the rapid increase in population, the human energy demand and energy consumption is increasing (Li *et al*. 2021; Yousef *et al*. 2021a, 2021b). Fossil energy is irreplaceable and finite, and the pollutants released during the combustion process of fossil energy cause serious pollution to the environment (Moreira *et al*. 2016; Darwesh *et al*. 2020). Lignocellulosic biomass, as the most abundant and cheapest renewable eco-friendly resource on Earth, is usually disposed of in a simple

and crude way, such as burning it or piling it up to rot, causing serious air pollution. Meanwhile, lignocellulosic biomass has attracted increasing attention due to its ability to be converted into other types of products, such as bioenergy and other chemicals (An *et al.* 2020, 2021; Atilano-Camino *et al.* 2020; Gaikwad and Meshram 2020; Han *et al.* 2021a). Producing enzymes with lignocellulosic biomass through the method of solid-state or submerged fermentation has been widely accepted by researchers. Among the enzymes, laccase is one of the oldest enzymes and has been studied extensively by researchers all over the world.

Laccase (EC 1.10.3.2), belonging to blue multi-copper oxidase, was first found in *Rhus vernicifera* and is widely distributed in various higher plants, some insects, fungi, and bacteria (Zhang *et al.* 2020; Han *et al.* 2021c). Based on its ability to oxidize various kinds of phenolic and non-phenolic compounds, laccase has huge potential application in all aspects of biotechnology involving materials science, biodegradation, bioremediation, drug analysis, biosensor, nanobiotechnology, the paper and pulp industry, food chemistry, and biofuels (Unuofin *et al.* 2019; Zerva *et al.* 2019; Huang *et al.* 2020; Coria-Oriundo *et al.* 2021; Shokri *et al.* 2021; Sun *et al.* 2021a, 2021b; Zhou *et al.* 2021). Furthermore, laccase plays an important role in lignin degradation, fruiting body formation, and plant pathogenesis (Janusz *et al.* 2015; An *et al.* 2018). Laccase is secreted mainly by bacteria and fungi. Among all types of fungi, white-rot fungi are recognized as excellent laccase producers. Meanwhile, the prerequisites of the above wide application of laccase are the availability of a large number of low-cost laccases. Unfortunately, laccase secreted by common wild or cultivated fungi under simple fermentation conditions has the characteristics of low yield, weak activity, and low economic benefit, so it is not suitable for commercial, large-scale application (Rodrigues *et al.* 2019; An *et al.* 2021). On this basis, the development of new laccase producing strains and the selection of low-cost lignocellulosic biomass used for fermentation could be helpful for opening new opportunities in aspects of commercial and industrial applications (Huang *et al.* 2019; An *et al.* 2020).

Laccase secreted by fungi could be affected by many factors, including pH, temperature, secondary metabolites and category, co-culture of fungi, concentration, and proportion of carbon and nitrogen sources, metal ions (An *et al.* 2016; Ottoni *et al.* 2016; Bettin *et al.* 2019; Yin *et al.* 2019; Han *et al.* 2021a; Hu *et al.* 2021). Similarly, fermentation method is also an important factor to fungi secreting laccase (Sharma *et al.* 2019; An *et al.* 2021). The main advantage of submerged fermentation (SmF) is that it is easy to control the fermentation conditions; thus it is more suitable for industry producing laccase. But there is no denying that the enzymes are diluted during the process of submerged fermentation, and solid-state fermentation (SSF) avoids the dilution of the enzyme. Furthermore, the process of solid-state fermentation is more similar to the environment in which fungi are living in in their natural habitat (Steudler and Bley 2015). In recent years, the solid-state fermentation method has attracted a great deal of attention in producing enzymes, especially laccase (Soccol *et al.* 2017). Lignocellulosic biomass is the main material used in solid-state fermentation for fungi grown and the types of lignocellulosic biomass used in previous studies were mainly tree leaves, sugar cane bagasse, coffee shell, and corncob. Previous studies had indicated that the difference in the production of laccase secreted by fungi was noticeable (An *et al.* 2020, 2021). Thus, developing new producing laccase fungi species is very meaningful and essential work.

Presently, related studies on laccase are mainly focused on a few genera, such as *Pleurotus*, *Flammulina*, *Ganoderma*, *Trametes*, *Lentinus*, and *Phanerochaete* (Elisashvili

et al. 2008; Huang *et al.* 2019; An *et al.* 2020, 2021; Atilano-Camino *et al.* 2020). Of course, new studies have begun to develop new productive strains and evaluate their capacity of secreting laccase (Han *et al.* 2021a, 2021c). Very few studies have considered using more numbers of lignocellulosic biomass for grown fungi to produce laccase. However, it is important to develop new laccase producing fungi and screen suitable lignocellulosic biomass to produce laccase. Under the circumstances, three white-rot fungal species, Han 618, Han 726, and Han 751, isolated from their native habitat in North China, were used to evaluate their capacities of secreting laccase under solid-state fermentation with six lignocellulosic biomasses (stalk of *Helianthus annuus*, stalk of *Sorghum bicolor*, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob). The results were helpful for screening a new strain and suitable lignocellulosic biomass, and also laid a foundation for the optimization of the fermentation conditions for laccase production, the isolation and purification of laccase, and the industrial application of laccase.

EXPERIMENTAL

Materials

Microorganisms and chemicals

Three white-rot fungi, Han 618, Han 726, and Han 751, used in the present study were collected from Maojingba National Nature Reserve (Chengde City, Hebei Province, China) and were isolated on malt extract agar (MEA) medium (glucose 10 g, malt extract 20 g, KH_2PO_4 3 g, agar 18 g, and deionized water 1 L) and purified on CYM medium (glucose 20 g, peptone 2 g, yeast extract 2 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $K_2HPO_4 \cdot 3H_2O$ 1 g, KH_2PO_4 0.46 g, agar 15 g, and deionized water 1 L). All microorganisms were maintained at the College of Life Science of Langfang Normal University.

All basal chemicals were purchased from Tianjin Zhiyuan Chemical Reagent Co. Ltd. (Tianjin, China). Malt extract, yeast extract, and peptone were purchased from Beijing Aobo Star Biotechnology Co. Ltd. (Beijing, China). Agar and 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) were purchased from Beijing BioDee Bio. Tech. Co. Ltd. (Beijing, China) and Sigma Aldrich Trading Co., Ltd. (Shanghai, China), respectively.

Lignocellulosic biomass

The lignocellulosic biomasses used were the stalk of *Helianthus annuus* (SOHA), stalk of *Sorghum bicolor* (SOSB), *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob. Among them, *H. annuus*, stalk of *S. bicolor*, *P. tabuliformis*, cottonseed hull, and corncob were kindly provided by farmers from Chengde City (Hebei Province, China). *Populus beijingensis* was obtained from Langfang City (Hebei Province, China). All lignocellulosic biomasses were air-dried and milled with the particle size between 20- and 60-mesh.

Methods

Fungal culture and screening for laccase-producing fungi

Three white-rot fungi were grown on CYM medium for 8 days at 26 °C to perform the activation. Each fungal isolate (5 mm agar disc) was punched by a hole punch on the activated Petri dishes and transferred to guaiacol selection medium (guaiacol 1 g, ammonium tartrate 0.1 g, peptone 2.6 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 1 g, Na_2HPO_4 0.2 g, agar 18 g, and deionized water 1 L) for 10 days at 26 °C.

Inoculum preparation

Five fungal isolate (5 mm agar disc) were added into 250-mL flasks containing 100 mL CYM liquid medium and cultured under oscillating conditions with 150 rpm at 26 °C. After 8 days, the mycelium pellets were homogenized with a modular homogenizer S10 (Ningbo Xinzhi Biotechnology Co., Ltd., Zhejiang, China) at 8,000 rpm for 2 min and the homogenized liquid was used as an inoculum.

Laccase-producing by fungi on solid-state fermentation

Erlenmeyer flasks (250 ml) containing 2 g lignocellulosic biomass were moistened with 8 mL of basal solution and autoclaved at 121 °C for 30 min. The components of the basal solution were as follows: $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $K_2HPO_4 \cdot 3H_2O$ 1 g/L, and KH_2PO_4 0.46 g/L. After sterilizing, each Erlenmeyer flasks was added to 3 mL of homogenized inoculum. Then, all flasks were transferred to a constant temperature incubator (26 °C), and the whole fermentation process was performed.

Preparation of crude enzyme

To obtain the crude enzyme, the flasks with fermentation lignocellulosic biomass were suspended in a 100 mL acetate-sodium acetate buffer (50 mM, pH 5.5), extracted on a rotary shaker at 10 °C with a speed of 120 rpm for 4 h (An *et al.* 2021), and filtered through a filter paper. The filtrate was centrifuged at 4 °C (12,000 rpm, 20 min), and the supernatant was used for the determination of laccase activity.

Determination of laccase activity

Laccase activity was assayed by the method of ABTS (2,2'-azinobis-[3-ethyltiazoline-6-sulfonate]) (Han *et al.* 2021a,c). The ABTS was used as substrate, and the reaction system was detected using an iMark^{TM} microplate absorbance reader (Bio-Rad, Hercules, CA, USA). The reaction mixture contained 190 μL acetate-sodium acetate buffer (50 mM, pH 4.2), 100 μL ABTS, and 10 μL crude enzyme. One unit of laccase activity was defined as the amount of crude enzyme required to oxidize 1 μmol of ABTS per min ($\varepsilon_{415} = 3.16 \times 10^4$ M^{-1} cm^{-1}).

Statistical analysis

All data were expressed as the mean value ± standard deviation (SD) ($n = 3$). The obvious differences were calculated by ANOVA (analysis of variance) using SPSS software version 22.0 (PROC GLM, IBM SPSS software version 22.0, Armonk, NY, USA). Statistical figures were generated by the Origin software version 2016 (OriginLab Corporation, Northampton, MA, USA).

Identification of the selected fungal strain

The potential laccase-producing fungal strain was identified based on the sequence of internal transcribed spacer (ITS). The strain was grown on CYM medium for 8 days, and the cultivated microorganism was scraped by a clean surgical blade from the surface of the Petri dishes. The total genomic DNA was extracted using the cetyltrimethyl-ammonium bromide (CTAB) rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) (Han *et al.* 2016, 2020a, 2021b). The ITS (internal transcribed spacer) region of white-rot fungi was used by general sequence ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGAT-ATGC-3') (White *et al.* 1990). After amplification, the PCR products were purified and

sequenced at Beijing Genomics Institute (Beijing, China), and the newly generated sequences were deposited at GenBank. Maximum parsimony phylogenetic analysis was performed in PAUP* version 4.0b10 (Swofford 2002) and followed the operational process described by Han *et al.* (2016). Branch support was determined by 1000 bootstrap replicates (Felsenstein 1985).

RESULTS AND DISCUSSION

Screening the Laccase-Producing Fungi Isolate

Research on laccase from white-rot fungi has mainly been focused on a few genera, such as *Pleurotus*, *Flammulina*, *Ganoderma*, *Trametes*, *Lentinus*, and *Phanerochaete* (Elisashvili *et al*. 2008; Huang *et al*. 2019; An *et al*. 2020, 2021; Atilano-Camino *et al*. 2020). However, the development of new strains that have the capacity of producing laccase is extremely important. On the other hand, the difference in degradation efficiency of lignin, the type and activity of the enzyme, and requirements for environmental conditions was huge due to different strains belonging to different species or different genus. Therefore, it is of great practical significance to isolate and obtain more abundant white-rot fungi strains from the native habitat and screen for laccase-producing fungi. Thus, three white-rot fungi were isolated from their native habitat in North China for the aim to evaluate the capacity of secreting laccase. Three white-rot fungi, *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751, showed discoloration by screening with guaiacol selective medium. As shown in Table 1, three white-rot fungi showed discoloration on guaiacol selective medium and the ratio of colony diameters (d1) to photochramic laps (d2) was less than 1 (d1/d2 < 1). The ratio of colony diameters to photochramic laps of Han 618, Han 726, and Han 751 was 0.83, 0.21, and 0.40 (Table 1). Microorganisms with discoloration on guaiacol selective medium indicate that the microorganisms have the ability to degrade lignin (Nishida *et al*. 1988). Thus, all tested white-rot fungi were shown to have the ability to degrade lignin.

Table 1. Discoloration Results of Three White-Rot Fungi on Selected Medium

White-rot Fungi	Colony Diameters d1 (cm)	Photochramic Laps d2 (cm)	d1/d2
Han 618	4.4	5.3	0.83
Han 726	0.8	3.8	0.21
Han 751	1.9	4.8	0.40

Identification of the Selected Laccase-Producing Fungal Strain

The MW547892, MZ413707, and MZ413708 are GenBank numbers of newly generated ITS sequences for the three laccase-producing fungal strains Han 751, Han 726, and Han 618, respectively. In the ITS phylogenetic tree, the strain Han 751 was grouped with two samples of *Coriolopsis trogii* (Berk.) Domański, the strain Han 726 was grouped with three samples of *Trametes hirsuta* (Wulfen) Lloyd, and the strain Han 618 was clustered with three samples of *Phlebia acerina* Peck (Fig. 1). Thus, the strains Han 751, Han 726, and Han 618 were identified as *Coriolopsis trogii*, *Trametes hirsute*, and *Phlebia acerina*, respectively.

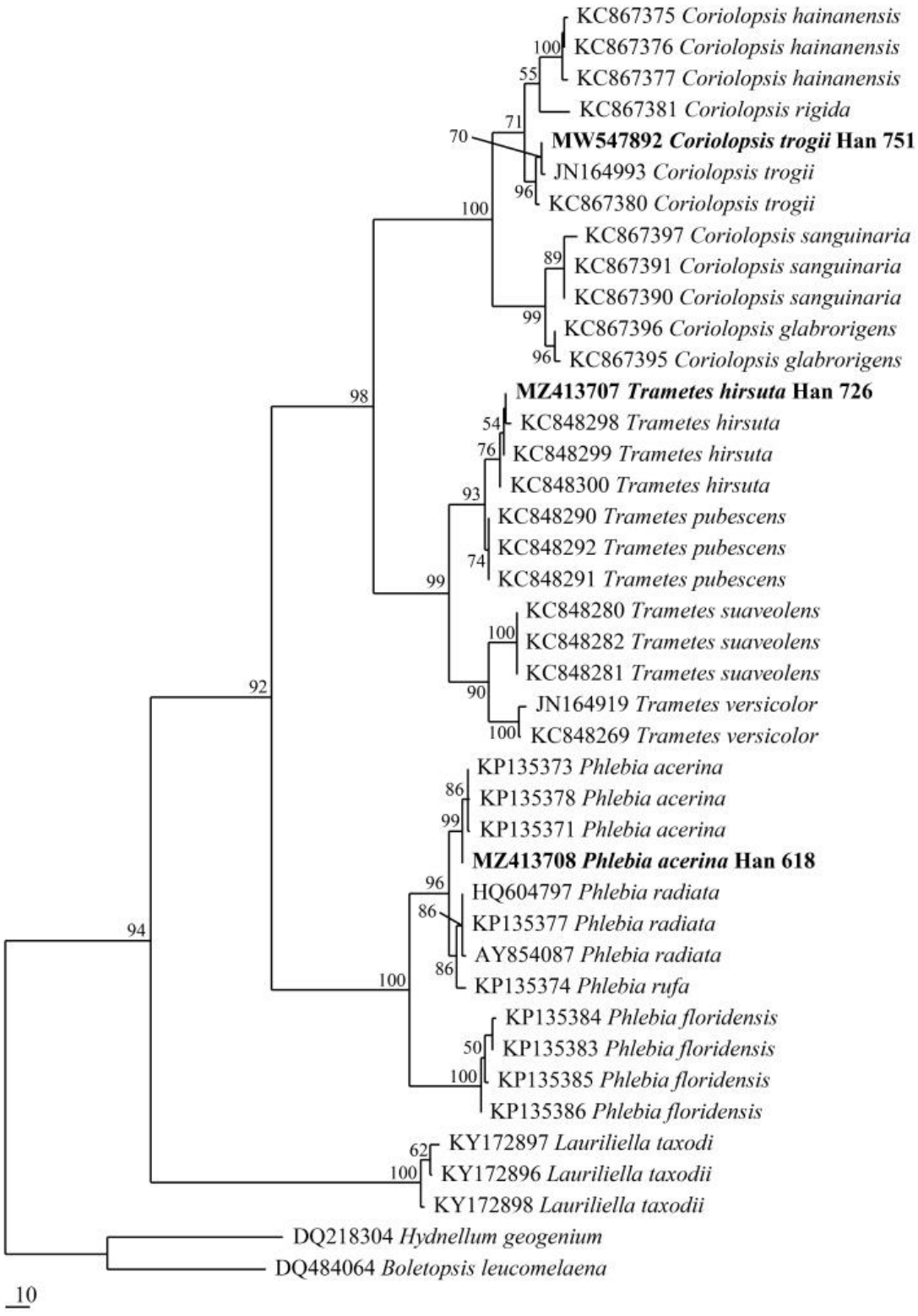

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of three white-rot fungal strains based on ITS sequences. Branches are labeled with parsimony bootstrap proportions ≥ 50%.

Statistical Analysis Results

The effects of species and lignocellulosic biomass on laccase activity were significant ($P < 0.001$) at different fermentation times (Table 2). Similarly, the interaction of species and lignocellulosic biomass on laccase activity was significant at different fermentation times ($P < 0.001$) (Table 2).

Table 2. Effects of Species, Lignocellulosic Biomass, and the Interactions of Species and Lignocellulosic Biomass on Laccase Activity (Two-way ANOVA)

Incubation Period (d)	Species	Lignocellulosic Biomass	Species × Lignocellulosic Biomass
1	580.063***	504.009***	648.398***
2	735.058***	303.308***	342.138***
3	1423.847***	308.847***	720.647***
4	418.315***	394.285***	401.622***
5	1693.379***	2398.408***	1839.419***
6	292.894***	760.048***	330.429***
7	1148.417***	2311.367***	1035.541***
8	380.293***	968.723***	198.423***
9	1329.299***	1238.833***	958.366***
10	1113.058***	2879.468***	876.494***
*Note: df = 2, 5, 10; ***P < 0.001			

Laccase Activity from *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751 on Different Lignocellulosic Biomass

Recent studies have indicated that the presence of lignocellulosic biomass could stimulate laccase production by basidiomycetes (Birhanli and Yeşilada 2013; Zhou *et al*. 2014; Palazzolo *et al*. 2019; An *et al*. 2020, 2021; Han *et al*. 2021a, 2021c). Meanwhile, the selection of appropriate lignocellulosic biomass for fungus growth and enzyme production plays an important role in the development of efficient biotechnology (Elisashvili *et al*. 2008; Han *et al*. 2021a). There have been few studies on laccase related to the genus *Phlebia*, and previous studies were mainly focused on the effect of chemical and metallic compounds on laccase or the heterologous expression of a laccase gene (Kaneko *et al*. 2009; Fonseca *et al*. 2014, 2018; Janusz *et al*. 2016). However, the effect of lignocellulosic biomass on laccase activity from the genus *Phlebia* has not been reported. The laccase of genus *Trametes* has been extensively studied, and *Trametes hirsuta* Han 726 used in this study also belongs to one species of genus *Trametes* (Kołodziejczak-Radzimska *et al*. 2020; Sun *et al*. 2020; Bilal *et al*. 2021; Mejía-Otálvaro *et al*. 2021; Navada and Kulal 2021; Wulandari *et al*. 2021; Zhang *et al*. 2021). However, the laccase of genus *Coriolopsis* has been less studied, and previous studies were mainly focused on recombinant expression, heterologous expression, and the selection of high production of laccase (Songulashvili *et al*. 2016; Xu *et al*. 2016; Avelar *et al*. 2017; Pinar *et al*. 2017; Glazunova *et al*. 2018). Additionally, lignocellulosic biomass used in previous studies merely related to laccase from genus *Coriolopsis* were chestnut shell and barley bran. According to the above mentioned, there are few studies on the effects of lignocellulosic biomass on genus *Phlebia*, *Trametes*, and *Coriolopsis*.

The value of laccase activity from *Phlebia acerina* Han 618 on SOHA, SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob was 0 U/L, 0 U/L, 0 U/L, 1.51 ± 0 U/L, 1.51 ± 0 U/L, and 0 U/L on the 1st day, respectively. The first time laccase activity was detected on stalk of *Helianthus annuus* (SOHA) was on the 6th day, and the value of laccase activity was 0.60 ± 0 U/L. The maximum laccase activity from SOHA was 18.98 ± 1.81 U/L on 7th day (Table 3, Fig. 2). The trend of laccase activity on SOSB was similar to the trend from SOHA, and laccase activity (6.63 ± 0.60 U/L) was detected on the 5th day for the first time (Fig. 2). The maximum laccase activity from SOSB was 110.21 ± 1.82 U/L on 10th day (Table 3, Fig. 2). Laccase activity from *Pinus tabuliformis* and corncob was detected only on one day (the 6th day and 5th day) and was very low (4.62 ± 0.46 U/L and 1.51 ± 0 U/L), respectively. The trend of laccase activity on *Populus beijingensis* was different from that of other lignocellulosic biomass in that the laccase activity reached its maximum values (7.43 ± 0.35 U/L) on the 3rd day but remained low throughout the whole stage of fermentation (Fig. 2). The laccase activity on cottonseed hull showed a small peak (15.47 ± 1.55 U/L) on the 4th day and reached the maximum value (28.03 ± 1.88 U/L) on the 9th day (Table 3). Obviously, the maximum laccase activity from *P. acerina* Han 618 was 110.21 ± 1.82 U/L on SOSB, nearly 5.81-fold, 23.85-fold, 14.83-fold, 3.93-fold, and 72.99-fold, higher than that on SOHA, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob, respectively. Additionally, SOSB was more suitable for *P. acerina* Han 618 with respect to secreting laccase.

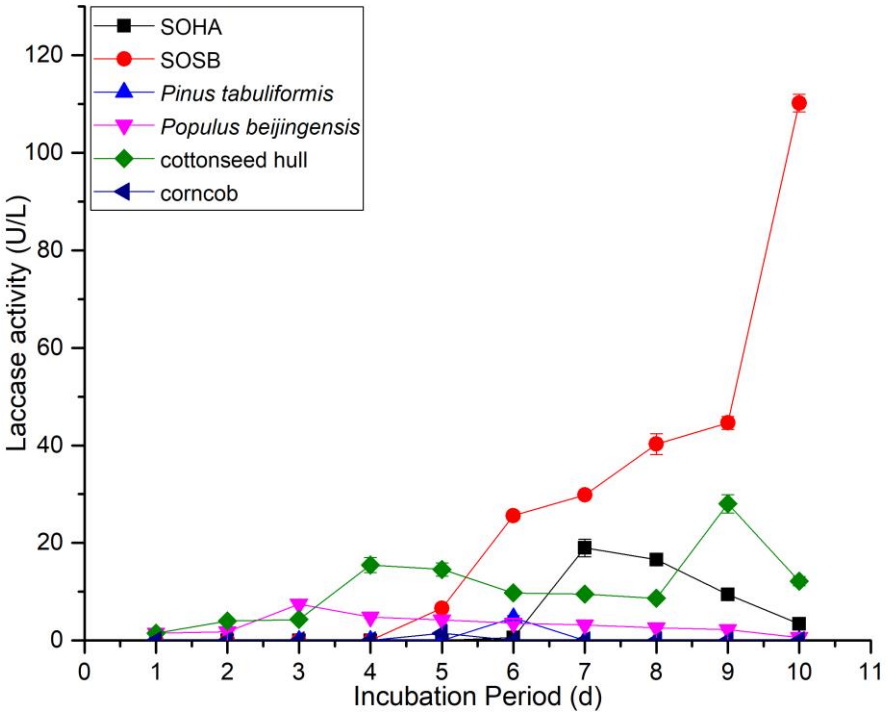

Fig. 2. Laccase activity of *Phlebia acerina* Han 618 from the SOHA, SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob on solid-state fermentation. SOHA indicates stalk of *Helianthus annuus*; SOSB indicates stalk of *Sorghum bicolor*

Table 3. Maximum Laccase Activity, Lignocellulosic Biomass, and Occurrence Time of Tested Three White-Rot Fungi

Maximum Laccase Activity (U/L)	Lignocellulosic	Species	Time (Day)
18.98 ± 1.81	SOHA	*Phlebia acerina* Han 618	7th
110.21 ± 1.82	SOSB	*Phlebia acerina* Han 618	10th
4.62 ± 0.46	*Pinus tabuliformis*	*Phlebia acerina* Han 618	6th
7.43 ± 0.35	*Populus beijingensis*	*Phlebia acerina* Han 618	3rd
28.03 ± 1.88	Cottonseed hull	*Phlebia acerina* Han 618	9th
1.51 ± 0	Corncob	*Phlebia acerina* Han 618	5th
82.68 ± 4.39	SOHA	*Trametes hirsuta* Han 726	10th
183.75 ± 16.63	SOSB	*Trametes hirsuta* Han 726	8th
4.62 ± 0.17	*Pinus tabuliformis*	*Trametes hirsuta* Han 726	7th
12.66 ± 0.52	*Populus beijingensis*	*Trametes hirsuta* Han 726	7th
27.43 ± 1.59	Cottonseed hull	*Trametes hirsuta* Han 726	5th
19.89 ± 1.31	Corncob	*Trametes hirsuta* Han 726	6th
122.26 ± 4.57	SOHA	*Coriolopsis trogii* Han 751	9th
799.03 ± 40.89	SOSB	*Coriolopsis trogii* Han 751	9th
13.96 ± 0.46	*Pinus tabuliformis*	*Coriolopsis trogii* Han 751	1st
18.59 ± 0.92	*Populus beijingensis*	*Coriolopsis trogii* Han 751	8th
16.58 ± 0	Cottonseed hull	*Coriolopsis trogii* Han 751	8th
90.92 ± 4.01	Corncob	*Coriolopsis trogii* Han 751	8th
Data are presented as mean ± standard deviation for biological triplicates and are expressed as U/L.			

Laccase activity from *Trametes hirsuta* Han 726 on SOHA, SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob was 22.70 ± 1.66 U/L, 0.90 ± 0 U/L, 0.60 ± 0 U/L, 0.60 ± 0 U/L, 1.21 ± 0 U/L, and 0.90 ± 0 U/L on the 1st day, respectively. Obviously, laccase activity on SOHA was nearly 25.22-fold, 37.83-fold, 37.83-fold, 18.76-fold, and 25.22-fold higher than that on SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob. The activity of laccase on SOHA decreased from the 1st day, increased from the 4th day, and reached a maximum value of 82.68 ± 4.39 U/L on the 10th day (Fig. 3, Table 3). Maximum laccase activity on SOSB was 183.75 ± 16.63 U/L on the 8th day (Table 3). The level of laccase activity on *Pinus tabuliformis* was low throughout the whole fermentation process and the maximum laccase activity was merely 4.62 ± 0.17 U/L on the 7th day. Maximum laccase activity on *Populus beijingensis*, cottonseed hull, and corncob was 12.66 ± 0.52 U/L, 27.43 ± 1.59 U/L, and 19.89 ± 1.31 U/L, and its corresponding time was 7th day, 6th day, and 5th day (Table 3), respectively. Briefly, the maximum laccase activity from *T. hirsuta* Han 726 appeared on SOSB, nearly 2.22-fold, 39.77-fold, 14.51-fold, 6.70-fold, and 9.24-fold higher than that

on SOHA, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob, respectively. In other words, the SOSB was more suitable for *T. hirsuta* Han 726 secreting laccase.

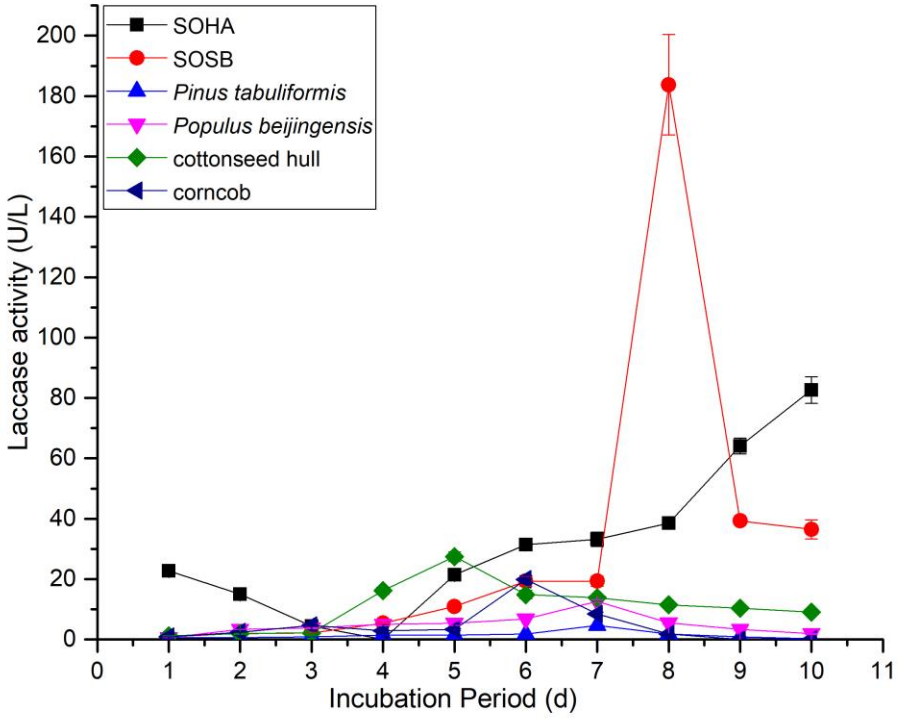

Fig. 3. Laccase activity of *Trametes hirsuta* Han 726 from the SOHA, SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob on solid-state fermentation. SOHA indicates stalk of *Helianthus annuus*; SOSB indicates stalk of *Sorghum bicolor*

On the 1st day, laccase activity from *Coriolopsis trogii* Han 751 on SOHA, SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob was 4.12 ± 0.17 U/L, 9.64 ± 0.60 U/L, 13.96 ± 0.46 U/L, 0 U/L, 1.21 ± 0 U/L, and 0.90 ± 0 U/L, respectively (Fig. 4). The trend of laccase activity on SOHA, SOSB, *Populus beijingensis*, cottonseed hull, and corncob was similar, and the trend of laccase activity increased first and then decreased after reaching the maximum laccase activity (Fig. 4). Maximum laccase activity on SOHA, SOSB, *Populus beijingensis*, cottonseed hull, and corncob was 122.26 ± 4.57 U/L, 799.03 ± 40.89 U/L, 18.59 ± 0.92 U/L, 16.58 ± 0 U/L, and 90.92 ± 4.01 U/L, and its corresponding time was 9th day, 9th day, 8th day, 8th day, and 8th day, respectively (Table 3). Different from the trend of laccase activity on other lignocellulosic biomasses, the laccase activity on *Pinus tabuliformis* reached its maximum on the 1st day, which was only 13.96 ± 0.46 U/L. Then the laccase activity began to decline, and no laccase activity could be detected after the 7th day (Table 3, Fig. 4). In conclusion, maximum laccase activity from *C. trogii* Han 751 appeared on SOSB, nearly 6.54-fold, 57.24-fold, 42.98-fold, 48.19-fold, and 8.79-fold higher than that on SOHA, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob, respectively. Thus, the SOSB was more suitable for *C. trogii* Han 751 to secret laccase.

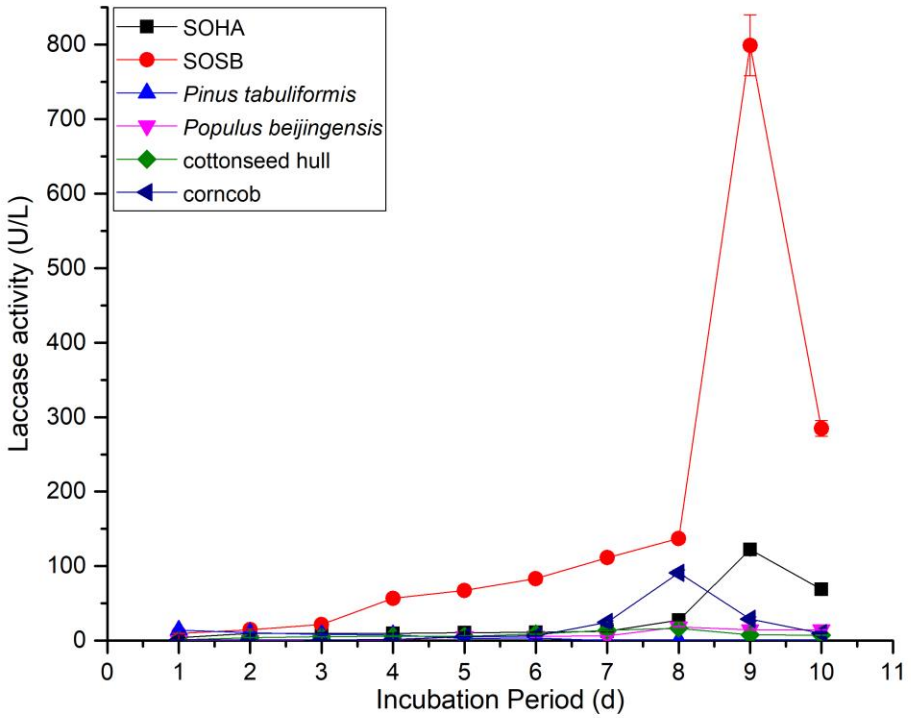

Fig. 4. Laccase activity of *Coriolopsis trogii* Han 751 from the SOHA, SOSB, *Pinus tabuliformis*, *Populus beijingensis*, cottonseed hull, and corncob on solid-state fermentation. SOHA indicates stalk of *Helianthus annuus*; SOSB indicates stalk of *Sorghum bicolor*

Comparison of Laccase Activity from Tested Three White-Rot Fungi

In terms of laccase activity on SOHA, the laccase activity on the 1st day was not detected by *Phlebia acerina* Han 618, and the value from *Trametes hirsuta* Han 726 was higher than that from *Coriolopsis trogii* Han 751. That seemed to indicate that SOHA was suitable for the rapid secretion of laccase by *T. hirsuta* Han 726. However, the maximum laccase activity from *C. trogii* Han 751 was nearly 6.44-fold and 1.48-fold higher than that from *P. acerina* Han 618 and *T. hirsuta* Han 726 (Table 3), respectively. In other words, SOHA was suitable for *C. trogii* Han 751 secreting laccase based on the maximum laccase activity. Laccase activity from *P. acerina* Han 618, *T. hirsuta* Han 726, and *C. trogii* Han 751 on SOSB on the 1st day expressed at low level and was 0 U/L, 0.90 ± 0 U/L, and 9.64 ± 0.60 U/L, respectively. But the maximum laccase activity on SOSB was higher than that on other lignocellulosic biomasses (Figs. 2 through 4), and the value was 110.21 ± 1.82 U/L from *P. acerina* Han 618, 183.75 ± 16.63 U/L from *T. hirsuta* Han 726, and 799.03 ± 40.89 U/L from *C. trogii* Han 751 (Table 3). Thus, SOSB was suitable for the three tested white-rot fungi to secret laccase. In terms of laccase activity on *Pinus tabuliformis* and *Populus beijingensis*, *P. acerina* Han 618, *T. hirsuta* Han 726, and *C. trogii* Han 751 showed a low level of laccase activity, and the maximum values were 4.62 ± 0.46 U/L and 7.43 ± 0.35 U/L, 4.62 ± 0.17 U/L and 12.66 ± 0.52 U/L, and 13.96 ± 0.46 U/L and 18.59 ± 0.92 U/L. Obviously, none of the three tested strains were suitable for secreting laccase on *Pinus tabuliformis* and *Populus beijingensis*. Similarly, cottonseed hull was not suitable

for testing white-rot fungi secreting laccase due to the low level of laccase activity (Figs. 2 through 4). For corncob, *P. acerina* Han 618, *T. hirsuta* Han 726, and *C. trogii* Han 751 showed different trends of laccase activity. *Phlebia acerina* Han 618 laccase activity could only be detected on the 5th day, *T. hirsuta* Han 726 laccase activity could be detected before 8 days, and the maximum value of laccase activity was 19.89 ± 1.31 U/L, while *C. trogii* Han 751 laccase activity could be detected during the whole fermentation stage and the maximum value of laccase activity was 90.92 ± 4.01 U/L. Interestingly, laccase activity from *C. trogii* Han 751 on different lignocellulosic biomasses was higher than that from *P. acerina* Han 618 or *T. hirsuta* Han 726. Thus, the capacity of *C. trogii* Han 751 secreting laccase was superior to that of *P. acerina* Han 618 and *T. hirsuta* Han 726.

The maximum value of laccase from *Coriolopsis rigida* on barley bran was around 3×10^5 nkat/L, and the result showed that the capacity of *Coriolopsis rigida* secreting laccase by was excellent (Gómez *et al*. 2005; Alcántara *et al*. 2007). Similarly, the maximum laccase activity from *C. trogii* Han 751 on SOSB in the present study was 799.03 ± 40.89 U/L, and the result also showed a strong ability of laccase secretion for *C. trogii* Han 751. The laccase activity of *Pleurotus ostreatus* IBB 8, *P. ostreatus* 2175, *P. tuberregium* IBB 624, *Lentinus edodes* IBB 123, *L. edodes* IBB 363, and *L. edodes* IBB 369 fermentation on tree leaves or wheat straw through conventional solid-state fermentation was 7 ± 0.7 or 7 ± 0.8 U/flask, 15 ± 1.4 or 12 ± 1.2 U/flask, 20 ± 1.8 or 10 ± 1.0 U/flask, 57 ± 4.7 or 20 ± 1.5 U/flask, 52 ± 4.9 or 55 ± 5.1 U/flask, and 7 ± 0.7 or 38 ± 4.0 U/flask, respectively (Elisashvili *et al*. 2008). The values of the highest laccase activity were 386 U/L for *T. trogii* incubated in a medium containing pulverized apricot seed shell in submerged fermentation (Birhanli and Yeşilada 2013).

The laccase activity of *P. ostreatus* CY 568 wild strain on sawdust and corncob *via* solid-state fermentation ranged from 36.77 ± 2.17 U/L to 353.83 ± 11.94 U/L and 9.64 ± 0.52 U/L to 440.73 ± 8.36 U/L, and the laccase activity of *P. ostreatus* CCEF 99 cultivated strain on sawdust and corncob *via* solid-state fermentation ranged from 67.21 ± 3.67 U/L to 548.72 ± 19.59 U/L and 16.88 ± 1.51 U/L to 286.12 ± 25.80 U/L, respectively (Han *et al*. 2020b). The laccase production of *P. ostreatus* CCEF 89 in cottonseed hull, corncob, and poplar wood under submerged fermentation ranged from 61.38 ± 4.09 U/L to 748.24 ± 9.53 U/L, 26.12 ± 2.28 U/L to 699.12 ± 44.91 U/L, and 3.32 ± 0.30 U/L to 509.75 ± 15.43 U/L, and the maximum laccase activity for *P. ostreatus* strain CY 568 obtained from cottonseed hull, corncob, and poplar wood was 902.92 ± 25.42 U/L, 611.71 ± 24.21 U/L, and 590.72 ± 14.98 U/L, respectively (An *et al*. 2020). Overall, the presence of cottonseed hull was conducive to secreting laccase by *P. ostreatus* strains in submerged fermentation or on solid-state fermentation. But the presence of cottonseed hull was disadvantageous for secreting laccase from *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751 (Figs. 2 through 4). Laccase activity of four basidiomycete fungi fermentation on *Populus beijingensis*, *Firmiana platanifolia*, straw of *Sorghum bicolor*, and straw of *Oryza sativa* was investigated, and the results showed that different species of fungi had a preference in lignocellulosic residues (Han *et al*. 2021a). Among these, *Cerrena unicolor* Han 849 preferred *Firmiana platanifolia*, while *Lenzites betulinus* Han 851 and *Auricularia heimuer* Han 1333 showed a more obvious preference for straw of *Oryza sativa* (Han *et al*. 2021a). The presence of cottonseed hull and *Populus beijingensis* were useful for accelerating the rate of laccase production of *P. ostreatus* CY 568, while the presence of *Toona sinensis*, cottonseed hull, and corncob were useful for accelerating the rate of laccase production for *Ganoderma lingzhi* Han 500 (An *et al*. 2021). In the present study, three species, *P. acerina* Han 618, *T. hirsuta* Han 726, and *C. trogii*

Han 751 showed consistency in preference of lignocellulosic biomass, and the presence of SOSB was more suitable for their secreting laccase. The laccase secreting ability of *C. unicolor* Han 849 was stronger than those of *L. betulinus* Han 851, *Stropharia rugosoannulata* Han 1321, and *A. heimuer* Han 1333 based on analyses of their laccase activities on different lignocellulosic residues (Han *et al*. 2021a). In the present study, the capacity of secreting laccase of *C. trogii* Han 751 was superior to that of *P. acerina* Han 618 and *T. hirsuta* Han 726. Based on this, the optimization of fermentation conditions and analysis of enzymatic properties of *C. trogii* Han 751 will be the next research work of the current authors.

CONCLUSIONS

1. Three white-rot fungi, *Phlebia acerina* Han 618, *Trametes hirsuta* Han 726, and *Coriolopsis trogii* Han 751, isolated from their native habitat in North China, were identified based on the sequence of internal transcribed spacer (ITS) and were preliminarily screened for the ability of laccase-producing by guaiacol selection medium.
2. Three species, *P. acerina* Han 618, *T. hirsuta* Han 726, and *C. trogii* Han 751 showed consistency in preference of lignocellulosic biomass, and the presence of SOSB was more suitable for their secreting laccase.
3. The capacity of laccase secretion from different species used in the present study was significantly different. The capacity of secreting laccase of *C. trogii* Han 751 was superior to that of *P. acerina* Han 618 and *T. hirsuta* Han 726.

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