Evaluation of Laccase Production by Two White-rot Fungi Using Solid-state Fermentation with Different Agricultural and Forestry Residues

Qi An,[a,b] Cong-Sheng Li,[a] Jing Yang,[a] Si-Yu Chen,[a] Kai-Yue Ma,[a] Zhi-Yan Wu,[a,b,c] Lu-Sen Bian,[d] and Mei-Ling Han [a,b,c,*]

Pleurotus ostreatus and a newly isolated *Ganoderma lingzhi* strain were evaluated for their laccase secretion capacity by solid-state fermentation with different agricultural and forestry residues. There was a significant difference among fungi for biosynthetic potential. In principle, the laccase secretion capacity of *P. ostreatus* CY 568 was stronger than that from *G. lingzhi* Han 500. Different species of fungi had a preference for agricultural and forestry residues. The presence of cottonseed hull and *Populus beijingensis* were helpful for accelerating the rate of laccase enzyme production of *P. ostreatus* CY 568. Cottonseed hull and corncob were useful for improving the production of laccase from *G. lingzhi* Han 500. Continuous and stable laccase production was found on cottonseed hull by *P. ostreatus* CY 568 and *G. lingzhi* Han 500. Maximum laccase activity obtained from *P. ostreatus* CY 568 on *Toona sinensis*, *Sophora japonica*, *Salix babylonica*, *Populus beijingensis*, corncob, cottonseed hull, and straw of *Oryza sativa* was higher than that from *G. lingzhi* Han 500, and was nearly 1.16-fold, 1.59-fold, 3.32-fold, 1.39-fold, 1.08-fold, 1.08-fold, and 1.36-fold, respectively. These findings will be helpful for developing new productive strains and expanding more species for industrial application to obtain efficient and low-cost laccase.

Keywords: Pleurotus ostreatus; Ganoderma lingzhi; *Laccase production; Agricultural and forest residues; Solid-state fermentation*

Contact information: a: College of Life Science, Langfang Normal University, Langfang 065000, Hebei, China; b: Technical Innovation Center for Utilization of Edible and Medicinal Fungi in Hebei Province, Langfang 065000, Hebei, China; c: Edible and Medicinal Fungi Research and Development Center of Universities/Colleges in Hebei Province, Langfang 065000, Hebei, China; d: Experimental Centre of Forestry in North China, Chinese Academy of Forestry, Beijing 102300, China;
** Corresponding author: meilinghan309@163.com*

INTRODUCTION

White-rot fungi (WRF) are important eukaryotic microorganisms that grow on dead and fallen trees and degrade lignocellulose (Ko *et al*. 2001). Lignin degradation plays an important role in the carbon cycle and/or refractory degradation in nature. Formation of the fungi fruit body is also a process of producing lignocellulolytic enzymes using agricultural and forestry residues. Scientists have studied white-rot fungi for its ability to produce extracellular lignocellulotic enzymes, including ligninolytic enzymes that degrade lignin and hydrolytic enzymes that degrade cellulose and hemicellulose (An *et al.* 2016a,b; Han *et al.* 2020b; Lira-Perez *et al*. 2020). Ligninolytic enzymes of WRF mainly include oxidase (*e.g.*, laccase) and peroxidases (*e.g.*, lignin peroxidase, manganese peroxidase [versatile peroxidase]). Hydrolytic enzymes are comprised of cellulase and hemicellulase

corresponding to the role of degrading cellulose and hemicellulose. In addition, some genera of white-rot basidiomycetes, *e.g.*, *Trametes* and *Pleurotus*, are an efficient producer of extracellular enzymes, such as laccases (EC 1.10.3.2) (Castanera *et al*. 2015; An *et al*. 2018, 2020a,b; Wang *et al*. 2018; Singh *et al*. 2019).

Laccases are distributed among plants, fungi, bacteria, and some insects (Shrestha *et al*. 2016). Laccase is a multi-copper oxidase that catalyzes the oxidation of various phenolic and non-phenolic substrates related to lignin structure, while reducing oxygen to water (Sharma *et al*. 2019). Laccase exhibits a wide and extraordinary range of natural substrates. For this reason, it is used in industrial and biotechnology applications in several areas, such as improving fiber properties, degradation of antibiotics and other pharmaceutical products, fuels, detoxification of environmental pollutants, stabilization lignin by providing precursors to chemicals, and pulp bleaching in the paper industry (Bertrand *et al*. 2017; Mate and Alcalde 2017; Agrawal *et al*. 2018; Su *et al*. 2018; Bilal *et al.* 2019; Singh and Arya 2019; Zerva *et al*. 2019). In addition, it is also used in healthcare products, nanobiotechnology, the pharmaceutical industry (Fperrer-Miralles *et al*. 2009). In addition, it is used in the removal of phenolic compounds in wine and as a biosensor (Ba and Kumar 2017; Yang *et al*. 2017; Becker and Wittmann 2019; Unuofin *et al*. 2019; Zerva *et al.* 2019).

A wide range of applications in biotechnological, industrial, and environmental protection require large amounts of enzymes. Unfortunately, laccases secreted by wild or cultivated fungi in simple fermentation conditions are not suitable for commercial purposes due to the disadvantages of low yields, weak activity, and low economic efficiency (Mate and Alcalde 2017; Rodrigues *et al*. 2019; An *et al*. 2020a). Developing new productive strains and selecting low-cost lignocellulosic materials for fermentation are helpful for opening new commercial and industrial opportunities for their uses in economic development and environmental protection (Janusz *et al*. 2015; Huang *et al*. 2019; An *et al*. 2020b).

Currently, enzymes are mainly produced by conventional fermentation methods (Christopher *et al*. 2005). However, in recent years, more attention has been paid to the production of enzymes by solid-state fermentation (SSF), utilizing agro-industrial residues (Singhania *et al*. 2009; Sharma *et al*. 2019). The advantages of conventional submerged fermentation are that it is convenient to control fermentation conditions and suitable for large-scale fermentation in industry. However, the main advantages of solid-state fermentation, compared to submerged fermentation, have been higher product yield and higher cost-effectiveness, as well as ease in controlling conditions and using the method (*e.g.*, less spraying and vibration) (Szendefy *et al*. 2006; Osma *et al*. 2011; Sharma *et al*. 2019). Furthermore, solid-state fermentation, which uses cultivated types of fungi to produce enzymes, is particularly beneficial because it mimics the fungi natural habitat, thus providing higher enzyme yields (Steudler and Bley 2015). In recent years, the SSF method has emerged as an attractive strategy for producing lignocellulolytic enzymes (Soccol *et al*. 2017). The application of enzymes obtained from SSF will make the enzymatic production process less cumbersome to reduce the overall cost and improve its feasibility and economic viability on a commercial scale (Sharma *et al*. 2019). Meanwhile, lignocellulosic biomass is the most commonly used material for solid-state fermentation. Lignocellulosic biomass, mainly including agro-industrial residues (*e.g.*, straw, corncob, and hazelnut husk) and forest residue (*e.g.*, leaves, branches), are principally made up of three major components: cellulose, hemicellulose, and lignin (Sitarz *et al.* 2013; Han *et al.* 2020b). The types and quantities of agricultural and forestry wastes, such as cottonseed hull, corn straw,

and leaves, and wheat straw are huge in China. Rational utilization of these lignocellulosic residues will become an important aspect to solve environmental problems. The use of various types of lignocellulosic residues to produce enzymes is convenient, efficient, environmentally friendly, and economical (Lamia *et al*. 2017).

Laccase production by fungi can be affected by a variety of factors, including kinds of lignocellulosic residues, pH, metal ions, temperature, and aromatic compounds (Metreveli *et al.* 2017; Filipe *et al.* 2019; An *et al*. 2020a,b; Rajavat *et al.* 2020). Moreover, type of fungal species or strains is an important factor affecting laccase production. Laccase activity of different strains from the genera, *Pleurotus* and *Flammulina* on substrate of wood, tree leaves, corncob, and wheat straw have been investigated (An *et al*. 2020b; Han *et al*. 2017, 2020b). Laccase production of *Ganoderma lucidum* has been discussed in the effect of rice and sunflower agro-residues, phenolic, and metallic inducers (Kuhar and Papinutti 2014; You *et al*. 2014; Postemsky *et al*. 2017; Palazzolo *et al*. 2019). However, comparative study of strain belonging to genera, *Ganoderma* and *Pleurotus* has not been reported. Furthermore, laccase production of genus *Ganoderma* and *Pleurotus* cultured by numerous agricultural and forestry residues, *e.g.* straw of *Oryza sativa*, *Salix babylonica*, and *Sophora japonica*, has not been reported. However, in order to reduce the production cost of laccase, it is very necessary to screen low-cost lignocellulosic materials suitable for laccase production by fungi. In this context, the objective of the present work was to evaluate the presence of different agricultural and forestry residues on the production of laccase by *P. ostreatus* and one newly isolated white-rot fungus *Ganoderma* sp. on solid-state fermentation. The aim was to select suitable agricultural and forestry residues to provide a basis for: 1.) developing new productive strains and 2.) expanding more species for industrial application to obtain efficient and low-cost laccase.

EXPERIMENTAL

Materials

Fungi

The microorganisms used in this study were *Pleurotus ostreatus* CY 568 and *Ganoderma* sp. Han 500. Pure cultures of *P. ostreatus* CY 568 preserved at 4 °C were kindly provided by the Institute of Microbiology, Beijing Forestry University (Beijing, China). Another selected strain, *Ganoderma* sp. Han 500 was collected from Mount Tai Scenic Spot in Taian city, Shandong Province, China. The strain was isolated and purified on Complete Yeast Medium (CYM) (glucose 20 g/L, peptone 2 g/L, yeast extract 2 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $K_2HPO_4 \cdot 3H_2O$ 1 g/L, KH_2PO_4 0.46 g/L, and agar 15 g/L). They were maintained by subculturing them in Malt Extract Agar (MEA) medium (glucose 10 g/L, malt extract 20 g/L, KH_2PO_4 3 g/L, and agar 20 g/L) by incubating the cultures at 26 °C for 6 days. Then, these strains were kept at 4 °C in the College of Life Science, Langfang Normal University (Langfang, China).

Agricultural and forestry residues

Seven kinds of agricultural and forestry residues were used in this study. Among them, *Populus beijingensis* (POB), *Toona sinensis* (TOS), and *Sophora japonica* (SOJ) were obtained from Langfang city (Hebei province, China). Corncob (COC), cottonseed hull (COH), *Salix babylonica* (SAB), and straw of *Oryza sativa* (STOS) were obtained from Chengde city (Hebei province, China). All agricultural and forestry residues were air-

dried and ground. Particle diameter of all agricultural and forestry residues were between 20- and 60-mesh.

Methods

Organism and inoculum preparation

The microorganisms were incubated on CYM at 26 °C. After 11 days, 5 inoculants as cut by use of a hole punch with diameter of 5 mm were placed in 250-mL flasks containing 100 mL of CYM without agar. The medium containing microorganism was cultured under oscillating conditions (120 rpm) for 7 days at 26 °C. Then, microorganisms were homogenized using a laboratory blender (2 min, 5000 rpm) and used as an inoculum.

Identification of the fungus

Mycelia of *Ganoderma* sp. Han 500 used for DNA extraction were grown on CYM agar medium for 9 days, and then scraped from the surfaces of CYM agar medium. The genomic DNA of the fungus was extracted by a cetyl trimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) according to the manufacturer's instructions with some modifications (Han *et al.* 2016, 2020a). The ITS regions of ribosomal DNA (rDNA) were amplified by polymerase chain reaction (PCR) using primer pairs ITS1 and ITS4. The PCR reaction schedule for ITS was referred to the method of Han *et al*. (2016, 2020a). The PCR products were purified and sequenced at Beijing Genomics Institute (Beijing, China). The newly generated sequence was deposited at GenBank.

Time course of laccase production by P. ostreatus CY 568 and Ganoderma sp. Han 500

Production of laccase under solid-state fermentation was performed individually in 250-mL Erlenmeyer flasks, containing dry POB (3.0 g) moistened with 12 mL deionized water. The operation method of other agricultural and forestry residues, such as TOS (3.0 g), SOJ (3.0 g), COC (3.0 g), COH (3.0 g), SAB (3.0 g), and STOS (3.0 g), was the same as the POB. All flasks were sterilized and inoculated with 3 mL prepared inoculum and incubated at 26 °C. The flasks with fermentation agricultural or forestry residue was taken out from the incubator every 24 h and suspended in 100 mL acetate-sodium acetate buffer (50 mM, pH 5.5). The process of extraction was performed on a rotary shaker at 10 °C with a speed of 120 rpm for 4 h (Han *et al*. 2020b). The contents of the flask were filtered through Whatman No. 1 filter paper, then centrifuged through a centrifuge at 4 °C (12,000 rpm, 20 min). The supernatant after centrifugation was used to analyze laccase activity.

Laccase assay

Laccase activity was measured using 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) 1 mM as substrate in sodium acetate buffer 50 mM, pH 4.2. The reaction mixture and assay method were referred to the method of Han *et al*. (2020b). Absorbance of the product was detected by an iMark^{TM} Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA) and recorded at 415 nm. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute ($\varepsilon_{415} = 3.16 \times 10^4$ M^{-1} cm^{-1}).

Data analysis

All values reported are the mean values of triplicate experiments. Two-way analysis of variance followed by the Tukey *post hoc* test was applied to examine the effects of agricultural and forestry residues and species on laccase activities according to An *et al*.

(2020a,b) using SPSS software version 22.0 (PROC GLM, IBM SPSS software version 22.0, Armonk, NY, USA). Statistical figures were generated by the software of Origin 2016 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Identification of *Ganoderma* sp. Han 500

The test strain of *Ganoderma* sp. Han 500 was identified by molecular biology as *Ganoderma lingzhi* and GenBank no. MW504827.

Two-way Analysis Results

The effect of different fungi on laccase production was significant ($P < 0.001$) during whole process of fermentation, except on the 8th day ($P > 0.05$). Different agricultural and forestry residues significantly affected the laccase production ($P < 0.001$) throughout the fermentation stage. Moreover, the interaction of different fungi with agricultural and forestry residues on laccase production was significant ($P < 0.001$) (Table 1).

Table 1. Two-way Analysis of Variance to Examine the Effects of Different Fungi, Agricultural and Forestry Residues, and Different Fungi × Agricultural and Forestry Residues Interactions on Laccase Production

Incubation Period (d)	Different Fungi	Agricultural and Forestry Residues	Different Fungi × Agricultural and Forestry Residues
1	2765.257***	2788.678***	205.041***
2	52.783***	38.403***	7.049***
3	1247.785***	866.749***	77.795***
4	1050.193***	1039.914***	81.347***
5	644.688***	1132.814***	56.715***
6	288.239***	1425.959***	28.687***
7	270.973***	2025.066***	52.851***
8	0.347	1260.403***	111.124***
9	440.071***	863.389***	88.849***
10	244.813***	684.067***	149.581***
*Note: df = 1, 6, 6; *P < 0.05, **P < 0.01, ***P < 0.001			

Production of Laccase under Different Agricultural and Forestry Residues

Important factors affecting the laccase activity of fungi are agricultural and forestry residues belonging to complex carbon and nitrogen sources (Han *et al*. 2020b). Fungal growth on lignocellulosic biomass is a cheap process of enzyme production. Previous studies used less variety or quantity of lignocellulose material, such as wood chips, tree leaves, wheat bran, peanut powder or coffee shells, cultivated the fungus for growth to produce enzymes (Elisashvili *et al.* 2008; Fang *et al*. 2015). Because of the difference of laccase activity on different lignocellulosic biomass by fungi, it is important to study the laccase activity among different agricultural and forestry residues. Previous studies on

Pleurotus ostreatus and *Ganoderma lingzhi* focused on laccase production induced by metals, temperature, and grown on two or three substrates (Elissetche *et al*. 2007; Wang *et al*. 2015; Postemsky *et al*. 2017; An *et al*. 2020b; Han *et al*. 2020b). Hence, this paper presents evaluation of laccase production by *P. ostreatus* CY 568 and *G. lucidum* Han 500 in various agricultural and forestry residues.

Overall, laccase activity from *P. ostreatus* CY 568 or *G. lingzhi* Han 500 on TOS, SOJ, SAB, POB, COC, COH, and STOS exhibited large variation in terms of maximum or minimum laccase activity, occurrence time of maximum or minimum laccase activity, and trends in enzyme production (Figs. 1 and 2). Minimum laccase activity from *P. ostreatus* CY 568 occurred on day 1 on all agricultural and forestry residues, except on SAB (Fig. 1). While time of minimum laccase activity from *G. lingzhi* Han 500 was on day 1 from all agricultural and forestry residues (Fig. 2). In terms of time and value of maximum laccase activity, *P. ostreatus* CY 568 or *G. lingzhi* Han 500 secreted on different agricultural and forestry residues showed larger differences (Figs. 1 and 2). Elisashvili *et al*. (2008) reported that extracellular laccase activity from different *P. ostreatus* strains exhibited large variation on tree leaves, wheat straw, mandarin peels, apple, and banana peels. A previous study showed that the activity of laccase from *P. ostreatus* and *F. velutipes* strains was different on different lignocellulosic materials (An *et al*. 2020b). Similarly, the range of laccase enzyme production was larger on different agricultural and forestry residues in this study. Laccase activity from *P. ostreatus* CY 568 on COH was 82.88 ± 1.98 U/L on day 1, nearly 5.85-fold, 2.71-fold, 2.24-fold, 2.70-fold, 15.29-fold, and 12.13-fold on TOS, SOJ, SAB, POB, COC, and STOS, respectively. Laccase activity from *G. lingzhi* Han 500 on COH was 61.28 ± 1.66 U/L on day 1, nearly 10.00-fold, 11.52-fold, 12.71-fold, 17.41-fold, and 21.81-fold on TOS, SAB, POB, COC, and STOS, respectively. Moreover, laccase activity from *G. lingzhi* Han 500 on SOJ was undetected on day 1. Thus, it can be seen that COH has a greater advantage in accelerating laccase enzyme production. Additionally, it has been previously mentioned that the presence of cottonseed hull was useful for producing laccase production quickly (An *et al*. 2020b).

Maximum laccase activity of *P. ostreatus* CY 568 on COH was 849.61 ± 22.74 U/L on day 7, which was higher than that from TOS (375.33 ± 7.96 U/L, day 8), SOJ (412.80 ± 6.74 U/L, day 9), SAB (122.16 ± 3.03 U/L, day 4), POB (316.25 ± 4.10 U/L, 4th day), COC (345.79 ± 2.42 U/L, day 6), and STOS (367.69 ± 11.58 U/L, day 7), by 2.26-fold, 2.06-fold, 6.95-fold, 2.69-fold, 2.46-fold, and 2.31-fold, respectively (Table 2). In terms of the value of maximum laccase activity, COH was beneficial to the accumulation of laccase activity for *P. ostreatus* CY 568. Based on the earliest occurrence time of maximum laccase activity (day 4), SAB (122.16 ± 3.03 U/L) and POB (316.25 ± 4.10 U/L) were beneficial for *P. ostreatus* CY 568. At the same time, laccase activity from *P. ostreatus* CY 568 on TOS, SOJ, COC, COH, and STOS was 213.68 ± 12.58 U/L, 174.90 ± 5.14 U/L, 270.14 ± 24.49 U/L, 437.11 ± 13.68 U/L, and 41.39 ± 1.71 U/L, respectively (Fig. 1). Therefore, the presence of COH and POB were helpful for accelerating the rate of laccase enzyme production of *P. ostreatus* CY 568. Maximum laccase activity of *G. lingzhi* Han 500 on COH was 788.73 ± 6.12 U/L on day 8, which was higher than that from TOS (324.49 ± 15.83 U/L, day 8), SOJ (259.49 ± 20.92 U/L, day 9), SAB (36.77 ± 1.68 U/L, day 6), POB (228.05 ± 10.10 U/L, day 7), COC (319.07 ± 19.64 U/L, day 7), and STOS (271.15 ± 14.81 U/L, day 7), by 2.43-fold, 3.04-fold, 21.45-fold, 3.46-fold, 2.47-fold, and 2.91-fold, respectively (Table 2). In terms of the value of maximum laccase activity, COH was beneficial to the accumulation of laccase activity for *G. lingzhi* Han 500. Based on the earliest occurrence time of maximum laccase activity (day 6), SAB (36.77 ± 1.68 U/L) was

beneficial for *G. lingzhi* Han 500. At the same time, laccase activity from *G. lingzhi* Han 500 on TOS, SOJ, POB, COC, COH, and STOS was 247.04 ± 5.79 U/L, 25.02 ± 1.21 U/L, 197.51 ± 12.31 U/L, 300.38 ± 5.36 U/L, 670.28 ± 36.28 U/L, and 154.61 ± 5.36 U/L, respectively (Fig. 2). Therefore, the presence of COH and COC were helpful for accelerating the rate of laccase enzyme production of *G. lingzhi* Han 500.

In terms of total laccase activity throughout the fermentation stage, *P. ostreatus* CY 568 and *G. lingzhi* Han 500 can always bring better enzyme production on COH due to the stable and high laccase activity. Han *et al.* (2020b) showed that laccase activity of tested *P. ostreatus* strains on sawdust was not always higher than that on corncob under solid-state fermentation. Similarly, maximum laccase activity from *Coriolopsis trogii* or *Trametes versicolor* on powdered walnut shell is higher than that on powdered wheat straw in submerged fermentation (Birhanli and Yeşilada 2013). Thus, these studies indicate that the lignin content is not directly related to laccase production secreted by fungi. This study showed a similar result. Han *et al*. (2020b) indicated that fungi grown on poplar sawdust could gain continuous and stable laccase production through solid-state fermentation. However, fungi induced by poplar sawdust could not gain stable and continuous laccase activity in submerged fermentation (An *et al*. 2020b). Grown on cottonseed hull moistened with deionized water, laccase production of fungi was higher than that grown on poplar wood or corncob moistened with deionized water (An *et al*. 2020b). In this study, continuous and stable laccase production was found on COH by *P. ostreatus* CY 568 and *G. lingzhi* Han 500.

In general, COH was beneficial to the accumulation of laccase activity and accelerated the rate of laccase enzyme production for *P. ostreatus* CY 568 and *G. lingzhi* Han 500. The STOS was disadvantageous for *P. ostreatus* CY 568 and *G. lingzhi* Han 500 producing laccase enzyme.

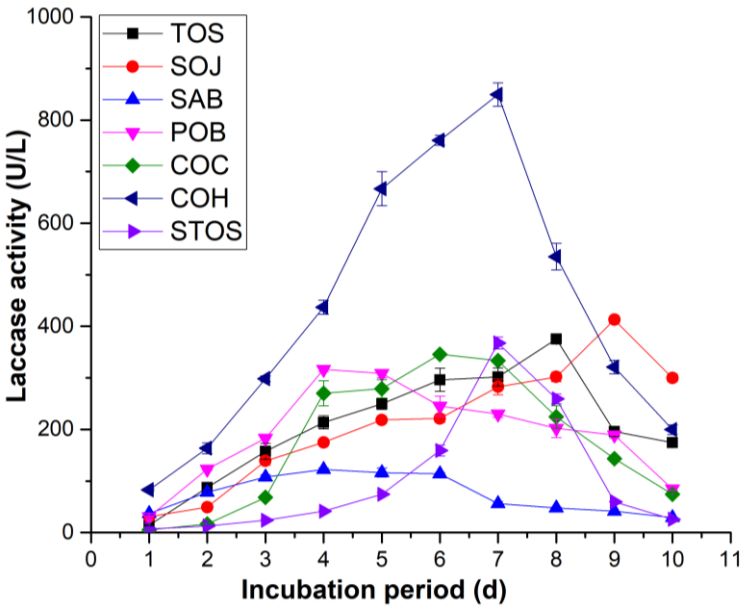

Fig. 1. Laccase production obtained from *Pleurotus ostreatus* CY 568 on TOS, SOJ, SAB, POB, COC, COH, and STOS by solid-state fermentation. TOS indicates *Toona sinensis*; SOJ indicates *Sophora japonica*; SAB indicates *Salix babylonica*; POB indicates *Populus beijingensis*; COC indicates corncob; COH indicates cottonseed hull; STOS indicates straw of *Oryza sativa*

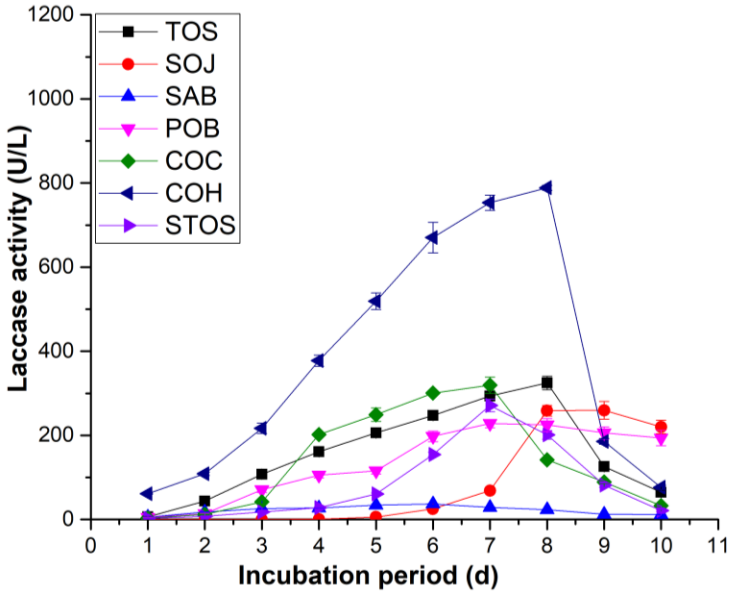

Fig. 2. Laccase production obtained from *Ganoderma lingzhi* Han 500 on TOS, SOJ, SAB, POB, COC, COH, and STOS by solid-state fermentation. TOS indicates *Toona sinensis*; SOJ indicates *Sophora japonica*; SAB indicates *Salix babylonica*; POB indicates *Populus beijingensis*; COC indicates corncob; COH indicates cottonseed hull; STOS indicates straw of *Oryza sativa*

Table 2. Maximum Laccase Production, Agricultural and Forestry Residues, and Time of *Pleurotus ostreatus* CY 568 and *Ganoderma lingzhi* Han 500

Maximum Laccase Production (U/L)	Agricultural and Forestry Residues	Fungi	Time (day)
375.33 ± 7.96	TOS	CY 568	8
412.80 ± 6.74	SOJ	CY 568	9
122.16 ± 3.03	SAB	CY 568	4
316.25 ± 4.10	POB	CY 568	4
345.79 ± 2.42	COC	CY 568	6
849.61 ± 22.74	COH	CY 568	7
367.69 ± 11.58	STOS	CY 568	7
324.49 ± 15.83	TOS	Han 500	8
259.49 ± 20.92	SOJ	Han 500	9
36.77 ± 1.68	SAB	Han 500	6
228.05 ± 10.10	POB	Han 500	7
319.07 ± 19.64	COC	Han 500	7
788.73 ± 6.12	COH	Han 500	8
271.15 ± 14.81	STOS	Han 500	7
Data are presented as mean ± standard deviation for triplicates and are expressed as U/L.			

Production of Laccase from Different Fungi

Different extracellular enzyme activities of fungi can cause different decomposition and utilization of lignin, cellulose, and hemicellulose in plant cell wall (An *et al*. 2015). Laccase is a ligninolytic enzyme and is necessary for lignin degradation by white-rot fungi, such as *Lentinus edodes*, *Pleurotus ostreatus*, *Ganoderma lucidum*, *Trametes versicolor*, and *L. crinitus* (Elisashvili *et al*. 2008; Kuhar *et al*. 2015; Han *et al*. 2020b; Serbent *et al*. 2020; Xu *et al*. 2020). The laccase secretion capacity of different white-rot fungi was significantly different. Thus, evaluation and analysis of the capacity of different species

secreting laccase is helpful to obtain new laccase productive species for industrial production.

Laccase activity from *Pleurotus ostreatus* CY 568 was detected in all agricultural and forestry residues on day 1 (Fig. 1). Laccase activity from *Ganoderma lingzhi* Han 500 on different agricultural and forestry residues was detected, except on SOJ (Fig. 2). Laccase activity from *P. ostreatus* CY 568 on TOS, SAB, POB, COC, COH, and STOS on day 1 was higher than that from *G. lingzhi* Han 500 by nearly 2.33-fold, 6.95-fold, 6.38-fold, 1.54-fold, 1.35-fold, and 2.43-fold, respectively (Figs. 1, 2). Except for *P. ostreatus* CCMSSC 00406 strain, the laccase production of other tested *P. ostreatus* strains was more than 35 U/L on the first day, and the laccase production of *Flammulina velutipes* strains were less than 7 U/L on the first day (An *et al*. 2020b). From this view, the capacity of secreting laccase by *P. ostreatus* CY 568 and *G. lingzhi* Han 500 was superior to that by *F. velutipes*. The maximum laccase activity for *P. ostreatus* CY 568 on TOS, SOJ, SAB, POB, COC, COH, and STOS was higher than that from *G. lingzhi* Han 500 by nearly 1.16-fold, 1.59-fold, 3.32-fold, 1.39-fold, 1.08-fold, 1.08-fold, and 1.36-fold, respectively (Figs. 1, 2). Meanwhile, the time of maximum laccase activity for *P. ostreatus* CY 568 was earlier than that from *G. lingzhi* Han 500. In general, the capacity of *P. ostreatus* CY 568 secreting laccase was stronger than that of *G. lingzhi* Han 500 in this study. *G. australe* A464 grown on wood secreted low amount of laccase (1 to 2 IU/L) under submerged fermentation (Elissetche *et al*. 2007). The highest enzyme activity of *G. lucidum* extract cultured with ferulic acid was 49 U/L and 44 U/L on days 7 and 8, respectively (Rodrigues *et al*. 2019). Under the Plackett-Burman design, the highest laccase activity was observed in experiment 1 (707 U/L on day 6) and experiment 8 (785 U/L on day 7 and 607 U/L on day 8) (Rodrigues *et al*. 2019). In this study, maximum laccase of *G. lingzhi* Han 500 on TOS, SOJ, POB, COC, COH, and STOS was 324.49 ± 15.83 U/L, 259.49 ± 20.92 U/L, 228.05 ± 10.10 U/L, 319.07 ± 19.64 U/L, 788.73 ± 6.12 U/L, and 271.15 ± 14.81 U/L, respectively (Table 2). Thus, the laccase secretion ability of *G. lingzhi* Han 500 was good in comparison to results of previous studies.

CONCLUSIONS

1. There was a significant difference among fungi for biosynthetic potential. In general, the capacity of *P. ostreatus* CY 568 secreting laccase was stronger than that of *G. lingzhi* Han 500 in this study.
2. Different species of fungi had a preference for agricultural and forestry residues. The presence of cotton seed hull (COH) and *Populus beijingensis* (POB) were useful for accelerating the rate of laccase production of *P. ostreatus* CY 568. The presence of COH and corncob (COC) were useful for accelerating the rate of laccase production of *G. lingzhi* Han 500.
3. Continuous and stable laccase production was found on COH by *P. ostreatus* CY 568 and *G. lingzhi* Han 500.
4. The laccase secretion ability of newly isolated *G. lingzhi* Han 500 strain was high compared to the results about strains from genus *Ganoderma* in previous studies.

ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (31900009), the Fundamental Research Funds for the Universities in Hebei Province (JQ201905), the Fundamental Research Funds for the Universities in Hebei Province (JYQ201901), the Top-notch Youth Project of Langfang City, and the Top-notch Youth Project of Colleges and Universities in Hebei Province (BJ2019007).

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Article submitted: January 23, 2021; Peer review completed: April 17, 2021; Revised version received and accepted: May 28, 2021; Published: June 4, 2021.
DOI: 10.15376/biores.16.3.5287-5300