Comparative Study on Laccase Activity of White Rot Fungi under Submerged Fermentation with Different Lignocellulosic Wastes

Qi An,[a,b] Jie Qiao,[a] Lu-Sen Bian,[d] Mei-Ling Han,[a,c,*] Xun-You Yan,[a] Zi-Zheng Liu,[a] and Chun-Yan Xie [e]

Different *Pleurotus ostreatus* and *Flammulina velutipes* species were compared relative to their ability to produce laccase in submerged fermentation of various lignocellulosic wastes. Fungi cultivation in identical culture conditions revealed wide differences among both species and strains of the same species. The laccase secretion ability of *P. ostreatus* strains was superior to *F. velutipes* strains. Maximum laccase production on cottonseed hull, corncob, and poplar wood was secreted by *P. ostreatus* CY 568, *P. ostreatus* CCEF 89, and *P. ostreatus* CY 568, respectively. The nature of lignocellulosic materials played an important role in determining the expression of laccase potential of fungi. The presence of cottonseed hull improved laccase activity and accelerated the rate of enzyme production. Maximum laccase production on cottonseed hull was nearly 1.29-fold and 1.53-fold higher than that on corncob and poplar wood, respectively. Laccase activity was detected in almost all tested strains on cottonseed hull on the first day, while only a few strains on poplar wood and corncob were detected on the first day. These findings will be helpful for selecting the appropriate strain in industrial applications and for optimization of integrated industrial laccase production.

Keywords: White rot fungi; Laccase production; Lignocellulosic wastes; Submerged 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; e: The Key Laboratory Study on Microbial Fermentation of Langfang city, Langfang 065000, Hebei, China;
** Corresponding author: meilinghan309@163.com*

INTRODUCTION

Extensive research on fungi, especially white rot fungi (basidiomycetes), has been conducted to gain new productivity strains with great potential for lignocellulolytic enzymes secretion and better properties for application in industry (Elisashvili *et al.* 2008; An *et al.* 2018; Han *et al.* 2020). Among these lignocellulolytic enzymes, laccase (EC 1.10.3.2), a blue multi-copper oxidase, is one of the oldest known and important enzymes (Zerva *et al.* 2019). Laccase was first isolated from *Rhus vernicifera* (Yoshida 1883), and these enzymes are widely distributed in plants, some insects, bacteria, and fungi. Laccase has potential applications in numerous biotechnology, including delignification of pulp and paper, production of biofuels, drug analysis, removal of phenolic compounds in food and beverages, bioremediation, cosmetics, paints, furniture, agricultural and forestry waste disposal, nanobiotechnology, and biomedicine (Madhavi and Lele 2009; Kudanga and Le

Roes-Hill 2014; Bertrand *et al.* 2017; Mate and Alcalde 2017; Singh and Arya 2019; Zerva *et al.* 2019; Han *et al.* 2020). Bioremediation mainly involves trichlorophenol, alkenes, industrial wastes, decolorisation of dyes, polluted soils, and herbicide degradation (Mayer and Staples 2002; Strong and Claus 2011; Ba and Kumar 2017). Furthermore, laccase plays an important role in lignin degradation, pigment biosynthesis, fruiting body formation, morphogenesis, and plant pathogenesis (Janusz *et al*. 2015; An *et al.* 2016a, 2018). In view of the rich effect in industry, agriculture, and environmental and biological processes, laccase has attracted much attention. However, all these effective applications are based on large-scale laccase production. Large-scale laccase production has become important from an economic standpoint despite the long standing technical difficulties in producing large amounts of laccase (Cardona *et al*. 2010; Agrawal *et al.* 2018; An *et al.* 2018). The capacity of producing laccase from different fungi is different, so it is necessary to compare the laccase production of different strains belonging to different species (Agrawal *et al.* 2018; An *et al.* 2018).

The presence of laccase, alone or together with lignin peroxidase (Lip) and manganese peroxidase (Mnp), has been demonstrated in a wide variety of white rot fungi (Mayer and Staples 2002), such as the genus *Trametes*, *Coriolus*, *Pleurotus*, *Lentinus*, *Flammulina* and *Ganoderma* (Galhaup *et al.* 2002; Jang *et al.* 2006; Elissetche *et al.* 2007; Arockiasamy *et al.* 2008; An *et al.* 2016a, b; Guo *et al.* 2017; Gupta and Jana 2018; Sadeghian-Abadi *et al.* 2019; Han *et al.* 2020). Some *Trametes* and *Pleurotus* species are efficient producers of laccase (Lorenzo *et al.* 2002; Castanera *et al.* 2015), such as *T. versicolor*, *T. pubescens*, and *P. ostreatus* (Galhaup *et al.* 2002; Dominguez *et al.* 2007; Park *et al.* 2015). Laccase activity depends on the type of fungal species or strains (Elisashvili *et al.* 2008; Janusz *et al.* 2015; An *et al.* 2016a, 2018; Han *et al.* 2017, 2018, 2020). The secretion of laccase from fungi is affected by many factors, including the concentration, ratio and complexity of carbon and nitrogen sources, fermentation method, the type and concentration of metal ions, pH, temperature, co-cultivation of different fungi, and aromatic compounds (Stajić *et al.* 2006; Elisashvili and Kachlishvili 2009; Janusz *et al.* 2015; Metreveli *et al.* 2017; Filipe *et al.* 2019; Rajavat *et al.* 2020).

Research on the complexity of carbon and nitrogen sources has mainly focused on lignocellulosic biomass. Lignocellulosic biomass, the most abundant and renewable eco-friendly resources on earth, is attractive because it can be used to make valuable industrial products (Pinar *et al*. 2017; Han *et al.* 2020). The area of agricultural and forestry crops is vast in China, and the types and quantities of agricultural and forestry wastes are huge. However, the majority of biomass resources are wasted. White rot fungi can utilize a variety of lignocellulosic residues by producing several extracellular secreted enzymes to grow fruiting bodies for human consumption or play an important role in the process of conversion of lignocellulosic biomass to bioethanol. Furthermore, using lignocellulosic residues to produce enzymes is an environmentally friendly and economical method (Lamia *et al*. 2017). The common fermentation methods are solid-state fermentation (SSF) and submerged fermentation (SmF) (An *et al.* 2016b). The advantage of solid-state fermentation is that it avoids dilution of enzyme products due to the high content of water in submerged fermentation (Oostra *et al.* 2000). However, it is easier to regulate the fermentation conditions of submerged fermentation compared with solid-state fermentation. Submerged fermentation is the main method used in industry.

Previous studies investigated the laccase production secreted by strains belonging to the genus *Pleurotus* with wood, tree leaves, corncob, and wheat straw (Elisashvili *et al*. 2008; Han *et al*. 2017). Laccase production of strains belonging to the genus *Flammulina*

have been discussed in the context of the effect of metal ions and aromatic compounds (Janusz *et al.* 2015; An *et al.* 2016a). However, studies on laccase secretion of the genus *Pleurotus* and *Flammulina* strains cultured by lignocellulosic waste with different lignin content have not been reported. The present work compared the laccase activity of different *P. ostreatus* and *F. velutipes* strains by lignocellulosic wastes with different lignin content in submerged fermentation. The strains and lignocellulosic wastes that contribute to the high yield of laccase were screened to provide a basis for expanding the number of strains used in industrial application and for obtaining low-cost laccase.

EXPERIMENTAL

Materials

Microorganisms

Four white-rot basidiomycete *Pleurotus ostreatus* strains CCEF 89, CY 568, CCMSSC 00322, and CCMSSC 00406, and four white-rot basidiomycete *Flammulina velutipes* CCMSSC 00114, CCMSSC 00118, CCMSSC 05317, and CCMSSC 05331 were obtained from Institute of Microbiology, Beijing Forestry University (Beijing, China). All organisms were maintained on Complete Yeast Medium (CYM) agar medium (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 20 g/L) at 4 °C in the College of Life Science, Langfang Normal University.

Lignocellulosic materials

Poplar wood was obtained from Langfang Normal University (Hebei, China). Corncob and cottonseed hull were kindly provided by farmers in Chengde city, Hebei province, China. All lignocellulosic wastes were chopped into small pieces, air-dried, and ground. The particles size of lignocellulosic wastes was between 20- and 60-mesh.

Methods

Organism and inoculum preparation

The microorganism was transferred to new CYM agar medium plates and incubated at 26 °C for 7 days. Inoculants with a diameter of 5 mm were made on the new flat plate covered with mycelium by a hole punch. Under aseptic conditions, 5 inoculants were placed in 250 mL flasks containing 100 mL of CYM medium (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, and KH_2PO_4 0.46 g/L). The medium was cultured on a rotary shaker at 26 °C and 150 rpm. After 7 days, mycelial pellets were harvested and homogenized with a laboratory blender for 2 min at 5000 rpm. The resulting suspension was used as an inoculum.

Culture conditions

The submerged fermentation (SmF) of the poplar sawdust, corncob, and cottonseed hull was performed individually at 26 °C in 250 mL flasks containing 3 g of the substrate with 100 mL of deionized water. All Erlenmeyer flasks were autoclaved at 121 °C for 30 min; after cooling, 3 mL of homogenized mycelium were used to inoculate each flask. All flasks were incubated at 26 °C.

To determine the laccase dynamics under different lignocellulosic wastes and strains conditions in SmF, laccase activities were tested from fungi growth on the 1st day

to the 10th day.

Enzyme activity was measured in the culture broth from each inoculated flask, at different growth times after removing the mycelia and lignocellulosic substrate. Fermentation liquor was filtered by Whatman No. 1 filter paper and then centrifuged at 4 °C and 12000 rpm for 20 min. The supernatant was the crude enzyme liquid, which was used for measurement of enzyme activity.

Enzyme activities assays

All measurements were derived from three independent experiments, and the mean values were taken. The standard deviations for the experiments were less than ± 10%.

Laccase activity was assayed by examining the oxidation of 1 mM 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) to its cation radical $ABTS^+$ at 420 nm ($\varepsilon_{420} = 3.6 \times 10^4$ M^{-1} cm^{-1}). The reaction mixture contained 1 mM ABTS in 50 mM sodium acetate buffer, pH 4.2, and 50 μL of adequately diluted culture liquid (Woolfenden and Wilson 1982; An *et al.* 2018). One unit of enzyme activity was defined as the amount of enzyme forming 1 μmol of $ABTS^+$ per minute.

Statistical analysis

To examine the effects of lignocellulosic materials and strains on laccase activities, two-way analysis of variance followed by the Tukey post hoc test was applied to these variables, with lignocellulosic materials and strains as fixed factors (PROC GLM, IBM SPSS software version 22.0; Armonk, NY, USA). All statistical figures were generated using the program Origin 2016 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Effects of Lignocellulosic Materials on Production of Laccase Enzymes

Many studies have indicated the effect of nutrient medium on laccase activity (Isikhuemhen and Mikiashvilli 2009), including the kinds, concentration, and complexity of carbon/nitrogen sources. The complex carbon and nitrogen sources used are mainly lignocellulose materials. The selection of lignocellulosic materials is one of the important factors affecting the secretion of laccase by fungi. Fungi growing on different lignocellulosic materials show different laccase activities (Han *et al.* 2017, 2018, 2020; Leite *et al.* 2019). Previous studies have used a single lignocellulose material, such as wood chips, tree leaves or coffee shells, as the substrate for fungal growth. The comparative studies of different lignocellulosic materials on laccase secretion by white-rot fungi are relatively few (Elisashvili *et al.* 2008). Thus, this paper presents a comparative study of laccase production by *P. ostreatus* and *F. velutipes* strains in different lignocellulose materials. Poplar wood, corncob, and cottonseed hull are commonly used as lignocellulosic materials in the cultivation of *P. ostreatus* and *F. velutipes* (Han *et al*. 2017). Their lignin contents differ greatly. The lignin content of corncobs, hardwood stems, and cottonseed hull is 15%, 18 to 25%, and 31.7%, respectively (Sánchez 2009; Tian *et al*. 2013). While monitoring the extracellular laccase activities of *P. ostreatus* and *F. velutipes* strains during the submerged fermentation of cottonseed hull, most strains showed laccase activity after 1 day of incubation, except for one, the *P. ostreatus* CCMSSC 00406. This suggested that the presence of cottonseed hull improved laccase activity and accelerated the rate of enzyme production (Fig. 1). As shown in Table 1, the effect of the lignocellulosic material

on laccase activities of tested *P. ostreatus* and *F. velutipes* strains was significant ($P < 0.001$) throughout the fermentation stage.

Laccase activity was detected in almost all tested *P. ostreatus* and *F. velutipes* strains on the substrate of cottonseed hull on the 1st day, except *P. ostreatus* CCMSSC 00406 (Fig. 1). Half of the tested strains, as *P. ostreatus* CCEF 89, CY568 and *F. velutipes* CCMSSC 00114, CCMSSC 00118, exhibited laccase activity on 1st day on the substrate of corncob (Fig. 2). Only two strains, *P. ostreatus* CCEF 89 and CY568, demonstrated laccase activity on 1st day on poplar wood substrate (Fig. 3). Many strains showed detectable laccase activity after being cultivated for 3 days on poplar wood substrate, for example, *F. velutipes* CCMSSC 05317 and CCMSSC 00118. None of these strains showed this phenomenon on corncob or cottonseed hull substrate.

Table 1. Effects of Strains, Lignocellulosic Materials, and Strains ×Lignocellulosic Materials Interactions on Laccase Activities of tested *P. ostreatus* and *F. velutipes* strains (Two-Way ANOVA)

Incubation Period (d)	Strain	Lignocellulosic Material	Strain × lignocellulosic material
1	1498.275***	822.921***	162.644***
2	2082.150***	2342.089***	256.738***
3	1594.021***	2116.185***	284.262***
4	2824.763***	5220.347***	842.133***
5	1989.862***	3191.318***	435.906***
6	4486.930***	5409.257***	637.548***
7	5621.516***	1230.118***	248.862***
8	2828.872***	119.089***	139.807***
9	2994.666***	848.695***	121.429***
10	796.043***	318.850***	76.209***
*Note: df = 7, 2, 14; *P < 0.05, **P < 0.01, ***P < 0.001			

In terms of laccase production and maximum laccase activity, the tested strains showed variations on cottonseed hull, corncob, and poplar wood. Laccase production of *P. ostreatus* CCEF 89 in cottonseed hull, corncob, and poplar wood 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, respectively (Figs. 1, 2, and 3). Maximum laccase activity for strain CCEF 89 obtained from cottonseed hull was higher than that obtained from corncob and poplar wood, by 1.07-fold and 1.47-fold, respectively (Table 2). The time of maximum laccase activity for strain CCEF 89 from cottonseed hull, corncob, and poplar wood was the 6th day, 8th day, and 7th day, respectively (Table 2). Han *et al.* (2017) reported that laccase activity in synthetic medium containing cottonseed hull was higher than that in synthetic medium containing sawdust or corncob, indicating that the cottonseed hull could be considered as a better inducer for enhancing the laccase activities of *P. ostreatus* CCEF 89 and CCEF 99. There was a similar result in the present study. The maximum laccase activity for *P. ostreatus* strain CY 568 obtained from cottonseed hull was 902.92 ± 25.42 U/L on 7th day, which was higher than that obtained from corncob (611.71 ± 24.21 U/L, 7th day) and poplar wood (590.72 ± 14.98 U/L, 7th day), by 1.48-fold and 1.53-fold, respectively (Table 2).

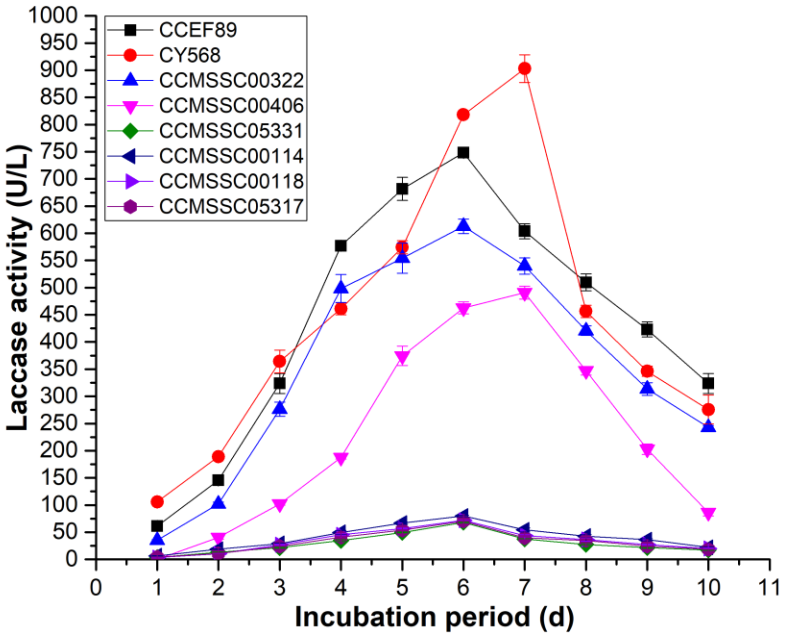

Fig. 1. Effects of cottonseed hull on laccase production by tested *P. ostreatus* and *F. velutipes* strains. Average values were calculated from individual measurements for each of three parallel cultures of eight strains.

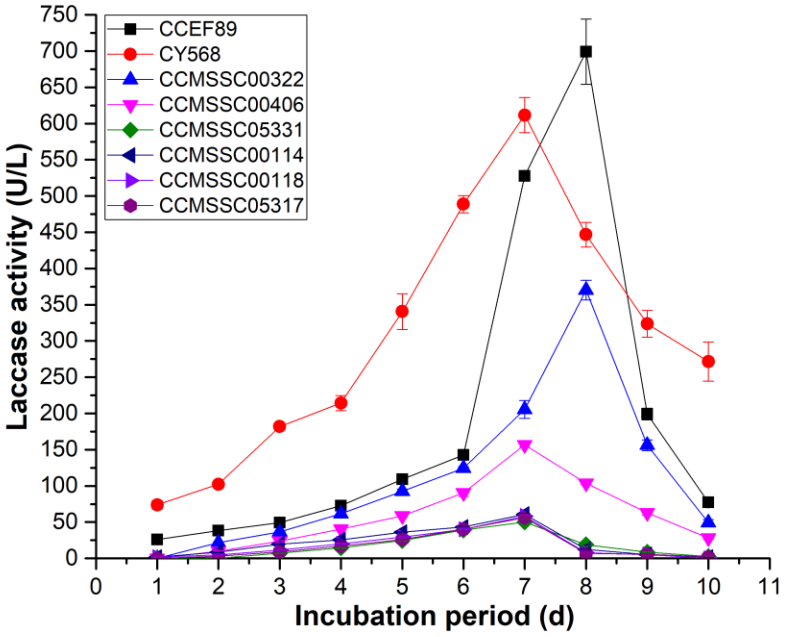

Fig. 2. Effects of corncob on laccase production by tested *P. ostreatus* and *F. velutipes* strains. Average values were calculated from individual measurements for each of three parallel cultures of eight strains.

The maximum laccase activity for *P. ostreatus* strain CCMSSC 00322 from cottonseed hull (612.82 ± 13.16 U/L, 6th day) was higher than that from corncob (370.20 ± 13.51 U/L, 8th day) and poplar wood (371.81 ± 7.01 U/L, 8th day), by 1.66-fold and 1.65-fold, respectively (Table 2). Similarly, maximum laccase activity for *P. ostreatus* strain CCMSSC 00406 on cottonseed hull was 490.66 ± 11.87 U/L on 7th day, which was higher than that from corncob (156.62 ± 3.17 U/L, 7th day) and poplar wood (270.44 ± 5.98 U/L, 8th day), by 1.48-fold and 1.53-fold, respectively (Table 2). It was easy to see that laccase activity of four *P. ostreatus* strains on cottonseed hull was higher than that of the substrate of poplar wood and corncob. Meanwhile, the laccase activity of *P. ostreatus* CCEF 89 and CY 568 obtained from corncob was higher than that from poplar wood. The laccase activity of *P. ostreatus* CCMSSC 00322 and CCMSSC 00406 from corncob was lower than that from poplar wood.

A previous study showed that the inducement ability of wood chips and corncob to laccase production was different in different strains of *P. ostreatus* (Han *et al.* 2017). The maximum laccase activity for *F. velutipes* CCMSSC 00114 from cottonseed hull, corncob, and poplar wood was 79.67 ± 1.22 U/L on 6th day, 60.98 ± 1.49 U/L on 7th day and 45.61 ± 0.97 U/L on 7th day. The maximum laccase activities were up to 71.83 ± 0.35 U/L, 58.27 ± 1.14 U/L, and 42.50 ± 0.80 U/L for *F. velutipes* CCMSSC 00118 from substrates of cottonseed hull, corncob, and poplar wood, respectively (Table 2), the maximum values occurring after 6 d, 7 d, and 7 d, respectively. A previous study found that the laccase activity of *F. velutipes* CCMSSC 00118 was 10.1 U/L in the glucose-free CYM medium with 5% cottonseed hull (An *et al.* 2015). The laccase activity was lower than in this study. The maximum laccase activity for *F. velutipes* CCMSSC 05317 obtained from cottonseed hull was 70.42 ± 0.35 U/L, which was higher than that obtained from corncob (55.96 ± 1.22 U/L) and poplar wood (40.18 ± 0.46 U/L), by 1.26-fold and 1.75-fold, respectively (Table 2).

The time of maximum laccase activity for strain CCMSSC 05317 from cottonseed hull, corncob, and poplar wood was 6th day, 7th day and 7th day (Table 2). Maximum laccase activity for *F. velutipes* CCMSSC 05331 from cottonseed hull, corncob, and poplar wood was 68.11 ± 1.09 U/L, 50.13 ± 3.68 U/L, and 39.98 ± 0.17 U/L, respectively (Table 2). The time of maximum laccase activity was 6th day, 7th day and 7th day, respectively (Table 2). Furthermore, the appearance times for maximum laccase activity of tested strains on cottonseed hull was earlier than that on corncob or poplar wood, except *P. ostreatus* CY 568 and CCMSSC 00406.

Elisashvili *et al.* (2008) reported that the laccase activity in tree leaves solid-state fermentation or in wheat straw solid-state fermentation by *P. ostreatus* 2175, *P. ostreatus* IBB8, *P. ostreatus* IBB108, and *P. ostreatus* 2191 has almost no difference. The maximum laccase activity with a powdered walnut shell as the inducer by *F. trogii* or *Trametes versicolor* is higher than with powdered wheat straw as the inducer in submerged fermentation (Birhanli and Yeşilada 2013). The laccase activity of *P. ostreatus* CCEF 99 and CY 568 on sawdust substrate was sometimes higher than that on corncob substrate, but sometimes lower than that on corncob substrate on solid-state fermentation (Han *et al.* 2020). All the above studies indicate that the lignin content is not directly proportional to the laccase production secreted of fungi induced by these lignocellulosic materials. A roughly similar phenomenon was found in this study.

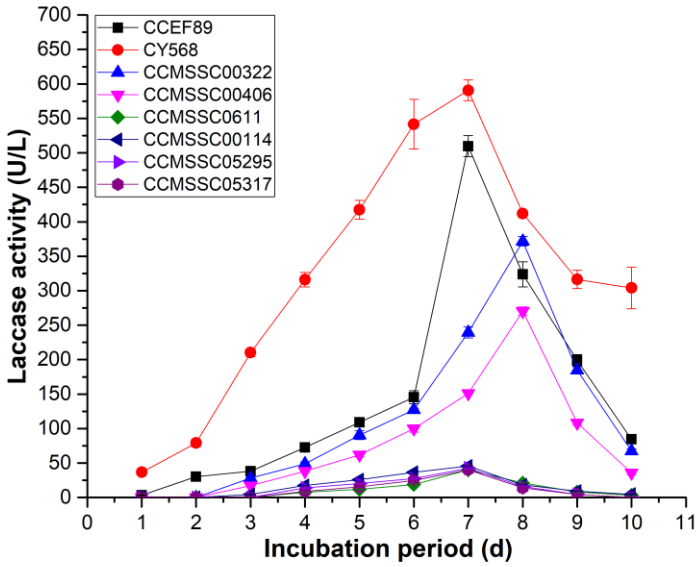

Fig. 3. Effects of poplar wood on laccase production by tested *P. ostreatus* and *F. velutipes* strains. Average values were calculated from individual measurements for each of three parallel cultures of eight strains.

Table 2. Maximum Laccase Activities, Lignocellulosic Material, Strains, and Time of tested *P. ostreatus* and *F. velutipes* Strains

Maximum Laccase Activity (U/L)	Lignocellulosic Material	Strain	Time (day)
509.74 ± 15.43	Poplar wood	CCEF 89	7 th
590.72 ± 14.98	Poplar wood	CY 568	7 th
371.81 ± 7.01	Poplar wood	CCMSSC 00322	8 th
270.44 ± 5.98	Poplar wood	CCMSSC 00406	8 th
45.61 ± 0.97	Poplar wood	CCMSSC 00114	7 th
42.50 ± 0.80	Poplar wood	CCMSSC 00118	7 th
40.18 ± 0.46	Poplar wood	CCMSSC 05317	7 th
39.98 ± 0.17	Poplar wood	CCMSSC 05331	7 th
699.12 ± 44.91	Corncob	CCEF 89	8 th
611.71 ± 24.21	Corncob	CY 568	7 th
370.20 ± 13.51	Corncob	CCMSSC 00322	8 th
156.62 ± 3.17	Corncob	CCMSSC 00406	7 th
60.98 ± 1.49	Corncob	CCMSSC 00114	7 th
58.27 ± 1.14	Corncob	CCMSSC 00118	7 th
55.96 ± 1.22	Corncob	CCMSSC 05317	7 th
50.13 ± 3.68	Corncob	CCMSSC 05331	7 th
748.24 ± 9.53	Cottonseed hull	CCEF 89	6 th
902.92 ± 25.42	Cottonseed hull	CY 568	7 th
612.82 ± 13.16	Cottonseed hull	CCMSSC 00322	6 th
490.66 ± 11.87	Cottonseed hull	CCMSSC 00406	7 th
79.67 ± 1.22	Cottonseed hull	CCMSSC 00114	6 th
71.83 ± 0.35	Cottonseed hull	CCMSSC 00118	6 th
70.42 ± 0.35	Cottonseed hull	CCMSSC 05317	6 th
68.11 ± 1.09	Cottonseed hull	CCMSSC 05331	6 th
Data are presented as mean ± standard deviation for triplicates and are expressed as U/L.			

Effects of Strains on Production of Laccase Enzymes

Laccase is necessary for lignin degradation by white-rot fungi such as *Ganoderma lucidum, Flammulina velutipes, Fomes fomentarius,* and *Pleurotus ostreatus* (Badalyan and Sakeyan 2004; An *et al*. 2016a; Han *et al.* 2020). Laccase production is significantly affected by different species or different strains of the same species (Janusz *et al.* 2015; An *et al.* 2016a, 2018; Huang *et al.* 2019; Han *et al.* 2020). Analysis the capacity of laccase production from different species or strains is helpful to obtain strains with high yield of laccase and provide more valuable strains for industrial production. In this study, the effect of the strains on laccase activities of tested *P. ostreatus* and *F. velutipes* strains was significant ($P < 0.001$) throughout the fermentation stage (Table 1). Eight strains belonging to *P. ostreatus* and *F. velutipes* showed their unique ability of secreting laccase (Figs. 1, 2 and 3). On the whole, the laccase secretion ability of *P. ostreatus* strains was stronger than that of *F. velutipes* strains in this study.

Laccase activity was detected in almost all tested *P. ostreatus* and *F. velutipes* strains on cottonseed hull on the 1st day, except *P. ostreatus* CCMSSC 00406 (Fig. 1). Laccase secreted by *P. ostreatus* strains exceeded 35 U/L on the first day, except for strain CCMSSC 00406 (Fig. 1), while production of laccase secreted by *F. velutipes* strains were not exceeding 7 U/L on the first day. The laccase activity of four *F. velutipes* strains was not high throughout the fermentation stage, and the trend of laccase expression was similar (Fig. 1). The maximum laccase activity for *P. ostreatus* CY 568 on cottonseed hull was nearly 1.21-fold, 1.47-fold, 1.84-fold, 11.33-fold, 12.57-fold, 12.82-fold, and 13.26-fold than that for *P. ostreatus* CCEF 89, *P. ostreatus* CCMSSC 00322, *P. ostreatus* CCMSSC 00406, *F. velutipes* CCMSSC 00114, *F. velutipes* CCMSSC 00118, *F. velutipes* CCMSSC 05317 and *F. velutipes* CCMSSC 05331, respectively. Different from the substrate of cottonseed hull, only four strains on corncob demonstrated laccase activity on the first day, and the activity was lower than that under the condition of cottonseed hull (Fig. 2). The maximum laccase activity for *P. ostreatus* CCEF 89 on corncob was nearly 1.14-fold, 1.89-fold, 4.46-fold, 11.46-fold, 12.00-fold, 12.49-fold, and 13.95-fold than that for *P. ostreatus* CY 568, *P. ostreatus* CCMSSC 00322, *P. ostreatus* CCMSSC 00406, *F. velutipes* CCMSSC 00114, *F. velutipes* CCMSSC 00118, *F. velutipes* CCMSSC 05317 and *F. velutipes* CCMSSC 05331, respectively. The trend of laccase expression for four *F. velutipes* strains was similar (Fig. 2). Different from the substrate of cottonseed hull and corncob, only two strains showed laccase activity on poplar wood on the first day, and the activity was lower than that under the condition of cottonseed hull and corncob. In particular, some strains, such as *F. velutipes* CCMSSC 05331 and 05317, did not exhibit laccase activity until day 4 (Fig. 3). The maximum laccase activity for *P. ostreatus* CY 568 on poplar wood was nearly 1.16-fold, 1.59-fold, 2.18-fold, 12.95-fold, 13.90-fold, 14.70-fold, and 14.78-fold that for *P. ostreatus* CCEF 89, *P. ostreatus* CCMSSC 00322, *P. ostreatus* CCMSSC 00406, *F. velutipes* CCMSSC 00114, *F. velutipes* CCMSSC 00118, *F. velutipes* CCMSSC 05317, and *F. velutipes* CCMSSC 05331, respectively. The trend of laccase expression for four *F. velutipes* strains was similar (Fig. 3). Previous studies about *F. velutipes* were mainly focused on the effects of metal ions and aromatic compounds on laccase (Janusz *et al*. 2015; An *et al*. 2016a). Previous studies reported the laccase activity of *F. velutipes* under lignocellulosic materials with simple carbon and nitrogen sources (An *et al*. 2015). The lignocellulosic material used for studying laccase production by *F. velutipes* was ramie stalk (Xie *et al*. 2017). This study was the first to compare the laccase activity of different strains of *F. velutipes* in submerged fermentation of lignocellulosic wastes of different composition. A previous study indicated that continuous and stable

laccase production was an extremely important advantage of solid-state fermentation with poplar sawdust (Han *et al*. 2020). Unfortunately, no stable and continuous laccase activity was found on substrate of poplar sawdust under submerged fermentation. A previous study showed that cottonseed hull combined with simple carbon and nitrogen sources were more effective than corncobs and wood chips combined with simple carbon and nitrogen sources (Han *et al*. 2017). The result of this study also confirmed that laccase production of fungi cultured by cottonseed hull with deionized water was better than that by poplar wood or corncob with deionized water.

CONCLUSIONS

1. This study indicated that the biosynthetic potential of basidiomycetes was highly dependent on the type of fungi and the lignocellulosic substrate. Different species of fungi or different strains of the same fungus had a preference of lignocellulosic materials to some extent.
2. On the whole, the laccase secretion ability of *P. ostreatus* strains was superior to *F. velutipes* strains. The maximum laccase production of four *P. ostreatus* strains on cottonseed hull, corncob, and poplar wood was much higher than that of four *F. velutipes* strains on the same lignocellulosic waste.
3. The presence of cottonseed hull was conducive to secreting laccase by *P. ostreatus* and *F. velutipes* strains in submerged fermentation. Maximum laccase production of tested *P. ostreatus* and *F. velutipes* strains on cottonseed hull was nearly 1.29-fold and 1.53-fold higher than that on corncob and poplar wood, respectively.
4. The presence of cottonseed hull was helpful for accelerating the rate of enzyme production. Laccase activity was detected in almost all tested *P. ostreatus* and *F. velutipes* strains on cottonseed hull on the first day, while only a few strains on poplar wood and corncob could be detected on the first day.

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