A Comparative Study on the Laccase Activity of Four Basidiomycete Fungi with Different Lignocellulosic Residues *via* Solid-state Fermentation

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The laccase producing abilities of four Basidiomycete fungi species were compared using solid-state fermentation using four different lignocellulosic residues. The biosynthetic potential of the Basidiomycetes was highly dependent on the type of fungi. In general, the laccase secreting ability of *Cerrena unicolor* Han 849 was greater than *Lenzites betulinus* Han 851, *Stropharia rugosoannulata* Han 1321, and *Auricularia heimuer* Han 1333. The maximum laccase production of *C. unicolor* Han 849 was approximately 11.25, 122.26, and 15.27 times higher than *L. betulinus* Han 851, *S. rugosoannulata* Han 1321 and *A. heimuer* Han 1333, respectively. Different species of fungi had a preference in lignocellulosic residues. The presence of *Firmiana platanifolia* was conducive to secreting laccase *via C. unicolor* Han 849 during solid-state fermentation. A continuous and stable laccase production *via C. unicolor* Han 849 was an obvious advantage of solid-state fermentation with any of the four lignocellulosic residues used. The maximum laccase production of *C. unicolor* Han 849 using *Firmiana platanifolia* was approximately 2.12, 1.68, and 6.13 times higher than *Populus beijingensis*, *Sorghum bicolor*, and *Oryza sativa*, respectively. These findings will be helpful for developing new productivity strains in industrial applications and selecting suitable lignocellulosic residues for laccase production.

Keywords: Basidiomycete Fungi; Laccase activity; Lignocellulosic residues; Solid-state fermentation

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INTRODUCTION

The application of enzymes in order to degrade pollutants has attracted a large amount of attention in the past few decades. Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are a group of blue multicopper oxidases that were first isolated from *Rhus vernicifera* (Yoshida 1883) and are widely present in plants, fungi, bacteria, and insects. Laccases can catalyze the oxidation of a wide variety of phenolic and nonphenolic lignin-related compounds, causing the reduction of molecular oxygen to water (Li *et al*. 2011; Yang *et al*. 2015; Zerva *et al*. 2019). Fungal laccases are best known for their role in lignin degradation, but they serve many other roles involving various aspects of multiple fields (An *et al*. 2018). Since they are energy-saving and environmentally friendly, laccases have potential applications in numerous industrial and biotechnological fields, *e.g.*, wine and

juice stabilization, fiber properties improvement, biosensor development, detoxification of environmental pollutants, dye decolorization, bio-synthesis, and bioremediation (Bilal *et al.* 2019; Deska and Kończak 2019; An *et al*. 2020a,b; Han *et al*. 2020). However, their application to biotechnological processes has been limited due to high production costs, which results in low enzyme activity and low yields. Increasing amounts of attention have been paid to studies demonstrating effective laccase production strategies associated with increased activity and reduced costs (Akpinar and Urek 2017; Chenthamarakshan *et al*. 2017; An *et al*. 2018; Singh and Arya 2019).

Among the various fungal phyla, basidiomycete fungi produce different kinds of extracellular oxidoreductases including laccases, peroxidases (manganese peroxidase and lignin peroxidase), and oxidases that generate H_2O_2 (Shrestha *et al*. 2016). Laccase production is highest in white rot fungi (Basidiomycetes) (Wang *et al*. 2019). Commonly used fermentation methods are submerged fermentation and solid-state fermentation. In the past, submerged fermentation (SF) was the most commonly used technology for the production of most enzymes, including laccase. The advantage of submerged fermentation is the homogeneous distribution of nutrients, which can result in full contact by the cultured microorganisms and therefore the full absorption of the nutrients. However, it also causes the dilution of the enzyme. There has been a trend during the past decade towards the increased use of solid-state fermentation (SSF) for the production of certain enzymes (Xin and Geng 2011; Nguyen *et al*. 2018). During solid-state fermentation, the microorganism is grown in the near or complete absence of free water with an natural substrate as solid support (Jaramillo *et al*. 2017). Therefore, SSF more closely simulates the natural environment of the microorganism. In addition, SSF has numerous advantages over SF, *e.g.*, lower energy consumption and higher product recovery (Jiang *et al*. 2017). However, disadvantages for SSF have been observed during laccase production, which included difficulties in scaling up and large batch-to-batch variation.

Laccase is generally produced at low concentrations by fungi grown on basal media (Songulashvili *et al*. 2011), but relatively higher concentrations are obtained by the addition of various supplements or altering other factors, *e.g.*, pH, temperature, and included aromatic compounds (Dominguez *et al*. 2007; Janusz *et al*. 2015; Metreveli *et al.* 2017; An *et al*. 2018; Filipe *et al.* 2019; An *et al*. 2020a,b; Atilano-Camino *et al*. 2020; Rajavat *et al.* 2020). Metal ions, *e.g.*, Cd^{2+}, Ag^{+}, Hg^{2+}, Mn^{2+}, and Cu^{2+}, are often used as additional supplements (Mäkela *et al.* 2013; An *et al.* 2016a, 2020a). Among the various metal ions, Cu^{2+} is considered to be an excellent inducer at certain concentrations. An *et al.* (2016) reported that in general, the presence of copper could increase the laccase activity obtained from strains belonging to the *Flammulina* genus. An *et al.* (2020a) reported that a final ion concentration of 2 mM could increase the laccase activity obtained from *Pleurotus ostreatus* CCEF 89. A combination of copper and manganese ions as the inducer for enhancing the laccase activity of *P. ostreatus* was shown to be superior to the use of single copper ions or manganese ions as inducers. The substrates used for SSF are typically lignocellulosic wastes needed for microorganism growth, enzyme production, and metabolite synthesis (Hatvani and Mécs 2001). Fungi, particularly white rot fungi, have a strong ability to degrade lignins and other cellulosic substances. Many research groups have attempted to improve laccase production by screening fungal strains based on the choice of lignocellulosic waste and optimization of the medium (Tišma *et al*. 2012; Soumya *et al*. 2016). Approximately 200 billion tons of agricultural wastes are generated each year all over the world (Ren *et al*. 2009). Lignocellulose, the major form of agricultural waste, is regarded as a low-cost nutrient substitute for laccase production in

SSF systems in comparison with other complex nutrient sources (Huang *et al*. 2017). Lignocellulosic biomass, *e.g.*, agro-industrial residues (straw, corncob, sugar cane bagasse, cottonseed hull, and corn stover) and forestry materials (leaves and sawdust), are principally made up of cellulose, hemicellulose, and lignins. A large majority of agricultural waste is used as livestock feed, fuel, and in paper production, or burned or left to rot, which contributes to environmental pollution and resource waste. Therefore, the efficient bioconversion of lignocellulose is an important goal in terms of agricultural waste resource utilization. It is a better method for producing laccase *via* culture fungus with lignocellulosic wastes. In addition, laccase activity is greatly dependent on the type of fungal species or strain (An *et al*. 2016, 2018; Han *et al*. 2018, 2020). The capacity of producing laccase from different fungi is different, so it is necessary to compare the laccase production of different species (Agrawal *et al*. 2018; An *et al*. 2018, 2020a,b).

Most of the previous studies in this area were focused on the laccase production secreted by the fungal genera *Pleurotus* and *Trametes*. However, the development of new productive strains is a persistent topic. Meanwhile, using inexpensive lignocellulosic wastes to ferment productive strains to produce laccase is a popular method. However, it is extremely necessary to evaluate the laccase activity of new productive strains using various lignocellulosic wastes. Under the circumstances, the present work deals with the evaluation of the laccase producing ability of four strains belonging to different species *via* solid-state fermentation using various lignocellulosic residues.

EXPERIMENTAL

Materials

Microorganisms

The four tested Basidiomycete strains (Han 849, Han 851, Han 1321, and Han 1333) were maintained on malt extract agar (MEA) medium (composed of 10 g/L of glucose, 20 g/L of malt extract, 3 g/L of KH_2PO_4, and 20 g/L of agar) at 4 °C at the College of Life Science, Langfang Normal University. Two wild strains (Han 849 and Han 851) were isolated from the Wulingshan National Nature Reserve, Xinglong county, Chengde city, Hebei province, China. One cultivated strain (Han 1321) was purchased from a market and isolated, and another cultivated strain (Han 1333) was donated by farmers from the Heilongjiang province, China. The fungus was reactivated in Petri dishes containing a complete yeast medium (CYM) (composed of 20 g/L of glucose, 2 g/L of peptone, 2 g/L of yeast extract, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 1 g/L of $K_2HPO_4 \cdot 3H_2O$, 0.46 g/L of KH_2PO_4, and 20 g/L of agar) at 26 °C for 9 d.

Lignocellulosic materials

Populus beijingensis was obtained from the Langfang Normal University (Hebei, China). *Firmiana platanifolia* was also obtained from Langfang. *Sorghum bicolor* and *Oryza sativa* were kindly provided by farmers in Chengde city, Hebei province, China. All of the lignocellulosic residue samples were chopped into small pieces before being air-dried and ground. The particle size of the lignocellulosic residues was between 20 mesh and 60 mesh.

Methods

Inoculum preparation

Inoculants with a diameter of 5 mm were made with a hole punch from reactivated fungus in Petri dishes. Then, 5 inoculants were placed in 250 mL flasks containing 100 mL of CYM medium (composed of 20 g/L of glucose, 2 g/L of peptone, 2 g/L of yeast extract, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 1 g/L of $K_2HPO_4 \cdot 3H_2O$, and 0.46 g/L of KH_2PO_4) and cultured on a rotary shaker at temperature of 26 °C and 150 rpm. After 8 d, the 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 solid-state fermentation (SSF) processes of the *Populus beijingensis*, *Firmiana platanifolia*, *Sorghum bicolor*, and *Oryza sativa* wastes were performed individually at a temperature of 26 °C in 250 mL flasks containing 3 g of the substrate with 12 mL of deionized water. The Erlenmeyer flasks containing the substrate and deionized water were autoclaved at 121 °C for 30 min. When it cooled to room temperature, 3 mL of inoculum was added to inoculate each flask. All flasks were incubated at 26 °C.

Extracellular laccase was extracted with 100 mL of 50 mM acetate-sodium acetate buffer (a pH 5.5). The extractions were performed on a rotary shaker at a temperature of 10 °C and a speed of 150 rpm for 4 h (Han *et al*. 2020). The extracted liquid was filtered with Whatman No. 1 filter paper and then centrifuged at a temperature of 4 °C with a speed of 12000 rpm for 20 min. The supernatant was the crude enzyme liquid used for measuring the enzyme activity.

Enzyme activities assays

Laccase activity was assayed *via* the changes in the absorbance at 415 nm, which is related to the rate of oxidation of 1 mM 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS). The reaction mixture was referred to in Han *et al*. (2020) and measured using an iMark™ microplate absorbance reader (Bio-Rad, Hercules, CA). One unit of enzyme activity was defined as the amount of enzyme forming 1 μmol of $ABTS^+$ per min (ε_{415} = 3.16×10^4 M^{-1} cm^{-1}).

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

Statistical analysis

Two-way analysis of variance between the lignocellulosic residues and the different species was performed according to Han *et al*. (2020), using SPSS software (PROC GLM, version 22.0, IBM, Armonk, NY). All statistical figures were generated using the program Origin 2016 (OriginLab Corporation, Northampton, MA).

Genomic DNA extraction, polymerase chain reaction, and sequencing

Mycelia for the DNA extraction were grown on CYM for 9 d, and the samples were obtained from the surfaces of the CYM *via* scraping. The total genomic DNA of the mycelia was extracted *via* a cetyltrimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) according to the instructions provided by the manufacturer with some modifications (An *et al.* 2016; Han *et al.* 2016; An *et al.* 2020a). The method of amplifying the ITS regions and polymerase chain reaction (PCR) cycling schedule for ITS was in accordance with a study by Han *et*

al. (2016). The PCR products were purified and sequenced at Beijing Genomics Institute (Beijing, China). The newly generated sequence was deposited in GenBank.

RESULTS AND DISCUSSION

Molecular Biological Results

The four tested basidiomycete strains (Han 849, Han 851, Han 1321, and Han 1333) were identified *via* molecular biology as *Cerrena unicolor*, *Lenzites betulinus*, *Stropharia rugosoannulata*, and *Auricularia heimuer*. The corresponding GenBank numbers were MW467890, MW467891, MW467892, and MW467893, respectively.

Laccase Activity from Different Lignocellulosic Residues

Recent studies have shown that lignocellulosic residues stimulate enzyme production in basidiomycetes (Pinar *et al*. 2017; Gupta and Jana 2018; Huang *et al*. 2019; Leite *et al*. 2019; Sadeghian-Abadi *et al*. 2019; Atilano-Camino *et al*. 2020; Han *et al*. 2020). Moreover, lignocellulosic residues are suitable for solid-state fermentation and submerged fermentation. Previous studies on the laccase production of fungi grown on lignocellulosic residues have primarily used common lignocellulosic materials, *e.g.*, poplar wood, cottonseed hull, tree leaves, corncob, and coffee shells (An *et al*. 2020b; Han *et al*. 2020). However, there is a large variety of lignocellulose residues. Finding new suitable lignocellulosic residues for the growth of fungi in order to produce laccase is also important for solid-state fermentation. In this study, *Populus beijingensis*, *Firmiana platanifolia*, *Sorghum bicolor*, and *Oryza sativa* were used to stimulate the tested fungi grown into secreting laccase. As shown in Table 1, the effect of the lignocellulosic residues on the laccase production of four Basidiomycete fungi strains was significant (*p*-value was less than 0.001) throughout the fermentation stage.

In terms of *Cerrena unicolor* Han 849, laccase activity was detected in all lignocellulosic residues on day 1 (as shown in Figs. 1 through 4). The laccase activity of *C. unicolor* Han 849 with *Oryza sativa*, *Sorghum bicolor*, *Firmiana platanifolia* and *Populus beijingensis* residues was 35.56 U/L ± 1.81 U/L, 26.92 U/L ± 0.87 U/L, 121.86 U/L ± 3.48 U/L, and 45.11 U/L ± 1.96 U/L, respectively, on day 1. Compared to the other three lignocellulosic residues, *C. unicolor* Han 849 secreted higher laccase activity with *Firmiana platanifolia* from day 1 (as shown in Fig. 3). The laccase activity of *C. unicolor* Han 849 with *Oryza sativa*, *Sorghum bicolor*, *Firmiana platanifolia*, and *Populus beijingensis* residues ranged from 25.12 U/L ± 0.97 U/L to 102.17 U/L ± 3.55 U/L, 26.92 U/L ± 0.87 U/L to 371.71 U/L ± 5.69 U/L, 121.86 U/L ± 3.48 U/L to 625.98 U/L ± 24.08 U/L, and 45.11 U/L ± 1.96 U/L to 295.96 U/L ± 4.85 U/L, respectively (as shown in Figs. 1 through 4). The laccase activity of *C. unicolor* Han 849 with *Firmiana platanifolia* was maintained at a high level during the entire fermentation stage (as shown in Fig. 3). The maximum laccase activity using *Firmiana platanifolia* was higher than *Oryza sativa*, *Sorghum bicolor*, and *Populus beijingensis*, approximately 6.13, 1.68, and 2.12 times as much (as shown in Table 2). The time to reach maximum laccase activity for *C. unicolor* Han 849 with *Oryza sativa*, *Sorghum bicolor*, *Firmiana platanifolia*, and *Populus beijingensis* was 6 d, 6 d, 7 d, and 10 d, respectively (as shown in Table 2). The time to reach maximum laccase activity for soft lignocellulosic residues (*Oryza sativa* and *Sorghum bicolor*) was slightly sooner than for hard wood residues (*Firmiana platanifolia* and *Populus beijingensis*). Han *et al*. (2020) reported that the time to reach maximum

laccase activity using *Pleurotus ostreatus* CY 568 with poplar sawdust was earlier than when using corncob, while the time to reach maximum laccase activity using *P. ostreatus* CCEF 99 with corncob was earlier than when using poplar sawdust. An *et al*. (2020b) reported that the time to reach and the value of the maximum laccase activity from some of *P. ostreatus* and *F. velutipes* strains using corncob were earlier and higher, respectively, than those strains using poplar wood. Therefore, different species or different strains of the same species have different preferences for lignocellulosic residues. Based on *C. unicolor* Han 849, generally speaking, *Firmiana platanifolia* was more suitable for *C. unicolor* Han 849 in terms of producing laccase. For *Lenzites betulinus* Han 851, no laccase activity was detected when using *Sorghum bicolor* on the 1st day (as shown in Figs. 1 through 4). The laccase activity of *L. betulinus* Han 851 using *Oryza sativa*, *Sorghum bicolor*, *Firmiana platanifolia*, and *Populus beijingensis* was 5.22 U/L ± 0.17 U/L, 0 U/L ± 0 U/L, 3.32 ± 0.30 U/L, and 5.83 U/L ± 0.17 U/L, respectively, on day 1. Furthermore, laccase activity was first detected in *Sorghum bicolor* on the day 5 and was very low (as shown in Fig. 2). The maximum laccase activity for *L. betulinus* Han 851 using *Oryza sativa* (55.66 U/L ± 3.60 U/L on day 5) was higher than *Sorghum bicolor* (3.62 U/L ± 0.30 U/L on day 7), *Firmiana platanifolia* (6.73 U/L ± 0.35 U/L on day 10), and *Populus beijingensis* (21.20 U/L ± 1.42 U/L on day 8), 15.38, 8.27, and 2.63 times greater, respectively (as shown in Table 2). In general, *Oryza sativa* was more suitable for *L. betulinus* Han 851 in terms of producing laccase according to the value and the time to reach maximum laccase activity. For *Stropharia rugosoannulata* Han 1321, the laccase activity was barely detected when using *Populus beijingensis* on day 1 (as shown in Figs. 1 through 4). The laccase activity of *S. rugosoannulata* Han 1321 when using *Sorghum bicolor* was undetected during the entire fermentation stage (as shown in Fig. 2). The maximum laccase activity for *S. rugosoannulata* Han 1321 using *Populus beijingensis*, *Firmiana platanifolia*, and *Oryza sativa* was 3.11 U/L ± 0.17 U/L, 1.61 U/L ± 0.17 U/L, and 5.12 U/L ± 0.30 U/L, respectively (as shown in Table 2). Relatively speaking, *Oryza sativa* was more suitable for *S. rugosoannulata* Han 1321 in term of producing laccase. For *Auricularia heimuer* Han 1333, the laccase activity when using *Oryza sativa*, *Firmiana platanifolia*, and *Populus beijingensis* ranged from 7.13 U/L ± 0.46 U/L to 40.99 U/L ± 2.09 U/L, 1.00 U/L ± 0.17 U/L to 15.07 U/L ± 0.80 U/L, and 0.30 U/L ± 0 U/L to 11.05 U/L ± 0.17 U/L, respectively (as shown in Figs. 1, 3, and 4). No laccase activity was detected when using *Sorghum bicolor* (as shown in Fig. 2). Therefore, *Oryza sativa* was more suitable for *A. heimuer* Han 1333 in terms of secreting laccase.

Many species from the Basidiomycetes genus are excellent laccase producers, *e.g.*, *Trametes trogii* and *Pleurotus ostreatus*. Bacteria and actinomycetes also have ability to secrete laccase, but the activity and the yield of the secreted laccase are lower. The activity of laccase secreted by Actinobacteria strains using *Olive pomace* as substrate is between 5.63 x 10^{-3} U/mL and 2.15 x 10^{-3} U/mL (Medouni-Haroune *et al*. 2017). Therefore, most of the studies related to laccase have focused on Basidiomycetes. Lakhtar *et al*. (2010) reported a laccase activity of 0.2 U/mL *via* the cultivation of *Lentinula edodes* with olive mill wastewater. The maximum laccase activities obtained from SSF cultures of *Trametes trogii* and *T. versicolor* using *Corylus maxima*, *Zea mays* and *Triticum sativum* were 384 U/L and 68 U/L, 198 U/L and 387 U/L, and 244 U/L and 215 U/L, respectively (Birhanli and Yesilada 2013). The maximum laccase activity for *Flammulina velutipes* CCMSSC 05331 using cottonseed hull, corncob, and poplar wood was 68.11 U/L ± 1.09 U/L, 50.13 U/L ± 3.68 U/L, and 39.98 U/L ± 0.17 U/L, respectively (An *et al*. 2020b). In this study, the maximum laccase activity of *C. unicolor* Han 849 using *Firmiana platanifolia* was

625.98 U/L ± 24.08 U/L (as shown in Fig. 3); therefore, *C. unicolor* Han 849 showed a strong laccase secretion ability with *Firmiana platanifolia* compared to the other lignocellulosic residues. The maximum laccase activity of *Coriolopsis trogii* or *T. versicolor* using walnut shells was higher than using wheat straw (Birhanli and Yeşilada 2013). The time to reach of maximum laccase activity for strains in genus *Pleurotus* and *Flammulina* when using cottonseed hull was earlier than using corncob or poplar wood, except for *P. ostreatus* CY 568 and CCMSSC 00406 (An *et al*. 2020b).

Table 1. Effects of Different Fungi, Lignocellulosic Residues, and Different Fungi and Lignocellulosic Residues Interactions in Terms of Laccase Activity (Two-Way ANOVA)

Incubation Period (d)	Different Fungi	Lignocellulosic Residues	Different Fungi and Lignocellulosic Residue Interactions
1	6866.645***	1226.357***	1072.840***
2	1205.870***	431.322***	468.957***
3	11201.673***	1511.725***	1763.314***
4	960.380***	127.401***	171.851***
5	8285.991***	778.426***	1348.293***
6	9255.133***	809.694***	1116.479***
7	3527.157***	739.195***	870.547***
8	2527.963***	209.090***	300.075***
9	3417.071***	542.353***	644.346***
10	3729.877***	558.370***	601.512***
*Note: df = 3, 3, 9; *P < 0.05, **P < 0.01, ***P < 0.001			

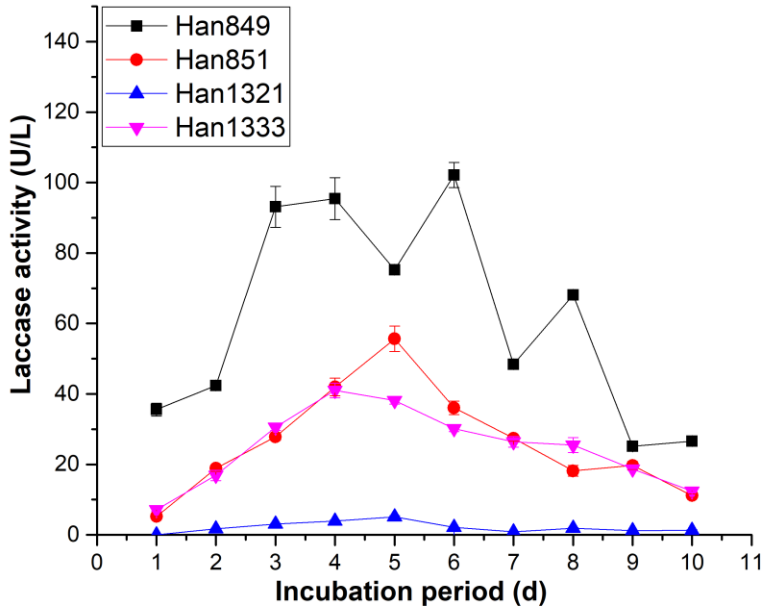

Fig. 1. The laccase activity from Han 849, Han 851, Han 1321, and Han 1333 grown on *Oryza sativa*. The average values were calculated from individual measurements from three parallel cultures of the corresponding strains.

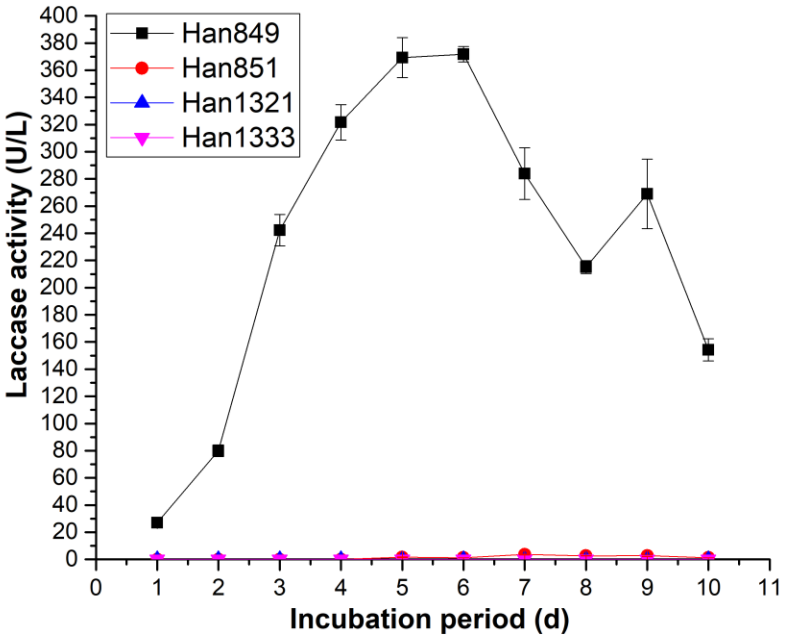

Fig. 2. The laccase activity from Han 849, Han 851, Han 1321, and Han 1333 grown on *Sorghum bicolor*. The average values were calculated from individual measurements from three parallel cultures of the corresponding strains.

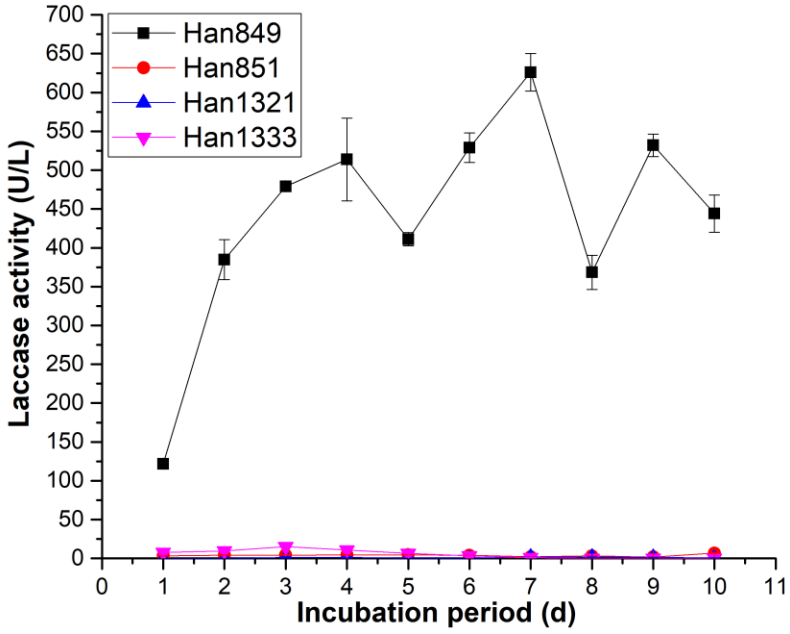

Fig. 3. The laccase activity from Han 849, Han 851, Han 1321, and Han 1333 grown on *Firmiana platanifolia*. The average values were calculated from individual measurements from three parallel cultures of the corresponding strains.

The laccase activity obtained from *P. ostreatus* CCEF 99 and CY 568 using sawdust as a substrate was sometimes higher than using a corncob substrate, but sometimes lower than using a corncob substrate in a solid-state fermentation process (Han *et al*. 2020). The results of this study were similar to previous studies, which indicated that there was no complete correlation between lignin content and laccase activity. Of course, the difference of lignocellulosic residues and the adaptability of fungi to different lignocellulosic residues will lead to great differences in laccase production time and yield.

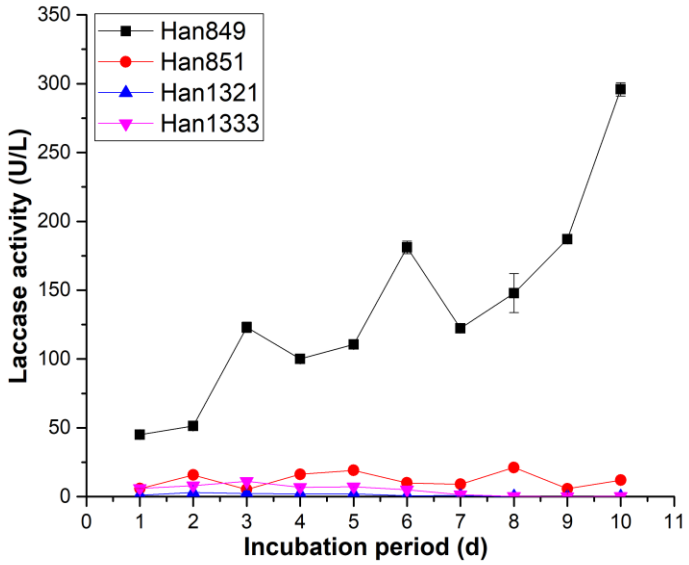

Fig. 4. The laccase activity from Han 849, Han 851, Han 1321 and Han 1333 grown on *Populus beijingensis*. The average values were calculated from individual measurements from three parallel cultures of the corresponding strains.

Table 2. Maximum Laccase Activities, Lignocellulosic Residues, and the Time Maximum Laccase Activity was Detected for Different Fungi

Maximum Laccase Activity (U/L)	Lignocellulosic Residues	Fungi	Time (d)
295.96 ± 4.85	*Populus beijingensis*	Han 849	10
625.98 ± 24.08	*Firmiana platanifolia*	Han 849	7
371.71 ± 5.69	*Sorghum bicolor*	Han 849	6
102.17 ± 3.55	*Oryza sativa*	Han 849	6
21.20 ± 1.42	*Populus beijingensis*	Han 851	8
6.73 ± 0.35	*Firmiana platanifolia*	Han 851	10
3.62 ± 0.30	*Sorghum bicolor*	Han 851	7
55.66 ± 3.60	*Oryza sativa*	Han 851	5
3.11 ± 0.17	*Populus beijingensis*	Han 1321	2
1.61 ± 0.17	*Firmiana platanifolia*	Han 1321	7
0	*Sorghum bicolor*	Han 1321	-
5.12 ± 0.30	*Oryza sativa*	Han 1321	5
11.05 ± 0.17	*Populus beijingensis*	Han 1333	3
15.07 ± 0.80	*Firmiana platanifolia*	Han 1333	3
0	*Sorghum bicolor*	Han 1333	-
40.99 ± 2.09	*Oryza sativa*	Han 1333	4
Note: Data is presented as the mean ± standard deviation in triplicate			

Laccase Activity in Different Fungi

Previously studies on improving laccase activity have primarily focused on the effects of different physico-chemical parameters, *e.g.*, substrate ratio, inoculum age, inoculum size, pH, and temperature (Elissetche *et al*. 2007; Vibha and Negi 2018; Gupta and Jana 2019; Unuofin *et al*. 2019a,b; Han *et al*. 2020). However, different species or different strains of the same species are an important factor affecting laccase activity (Lamia *et al*. 2017; Huang *et al.* 2019; An *et al*. 2020a,b; Han *et al.* 2020). It is important to analyze the capacity of laccase production from fungi among different genera for the development of new productive strains as well as providing more valuable strains for industrial production. In this study, using four different Basidiomycete fungi strains had a significant (*p*-value is less than 0.001) effect on laccase activities throughout the fermentation stage (as shown in Table 1). *Cerrena unicolor* Han 849, *Lenzites betulinus* Han 851, *Stropharia rugosoannulata* Han 1321, and *Auricularia heimuer* Han 1333 displayed their unique ability of secreting laccase (Figs. 1 through 4).

The laccase activity from *Cerrena unicolor* Han 849 with *Oryza sativa* was approximately 6.81 and 4.99 times greater than the activity from *Lenzites betulinus* Han 851 and *Auricularia heimuer* Han 1333, respectively, on day 1, while the laccase activity of *Stropharia rugosoannulata* Han 1321 with *Oryza sativa* was undetected on day 1 (as shown in Figs. 1 through 4). In addition, the maximum laccase activity of *C. unicolor* Han 849 on *Oryza sativa* was 102.17 U/L ± 3.55 U/L, which is approximately 1.84, 19.96, and 2.49 times greater than *L. betulinus* Han 851, *S. rugosoannulata* Han 1321, and *A. heimuer* Han 1333, respectively (as shown in Table 2). The laccase activity from *C. unicolor* Han 849 with *Oryza sativa* was higher than *L. betulinus* Han 851, *S. rugosoannulata* Han 1321, and *A. heimuer* Han 1333 during the entire fermentation process (as shown in Fig. 1). The laccase activity of *L. betulinus* Han 851 and *A. heimuer* Han 1333 was roughly equivalent, and the laccase activity from *S. rugosoannulata* Han 1321 was extremely low. Different from *Oryza sativa*, *C. unicolor* Han 849 showed a high laccase activity when using *Sorghum bicolor*, while the other three species showed extremely low or even undetectable laccase activity levels during the entire fermentation process (as shown in Fig. 2). The maximum laccase activity of *C. unicolor* Han 849 using *Sorghum bicolor* was 371.71 U/L ± 5.69 U/L, which was approximately 102.68 times greater than *L. betulinus* Han 851 (as shown in Table 2). Both *S. rugosoannulata* Han 1321 and *A. heimuer* Han 1333 had undetected laccase activity. The laccase activity trends of the four species using *Firmiana platanifolia* were similar to the trends of *Sorghum bicolor* (as shown in Fig. 3). As such, *C. unicolor* Han 849 showed a superior laccase secreting capacity with *Firmiana platanifolia* and a maximum laccase activity of 625.98 U/L ± 24.08 U/L (as shown in Table 2). The maximum laccase activity using *Firmiana platanifolia* was approximately 93.01, 388.81, and 41.54 times greater than *L. betulinus* Han 851, *S. rugosoannulata* Han 1321, and *A. heimuer* Han 1333, respectively. The laccase activity of the four species using *Populus beijingensis* was similar to the trends of the other three lignocellulosic residues. (as shown in Fig. 4). The maximum laccase activity of *C. unicolor* Han 849 using *Populus beijingensis* was 295.96 U/L ± 4.85 U/L, which was approximately 13.96, 95.16, and 26.78 times greater than *L. betulinus* Han 851, *S. rugosoannulata* Han 1321, and *A. heimuer* Han 1333, respectively. Only *C. unicolor* Han 849 maintained a high level of laccase activity; the other species all maintained a low level of laccase activity (as shown in Fig. 4). An *et al*. (2020b) reported that the maximum laccase activity for *P. ostreatus* CY 568 grown on cottonseed hull was approximately 1.21, 1.47, 1.84, 11.33, 12.57, 12.82, and 13.26 times greater than *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 maximum laccase activity of *P. ostreatus* CCEF 89 with corncob was approximately 1.14, 1.89, 4.46, 11.46, 12.00, 12.49, and 13.95 times greater than *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 (An *et al*. 2020b). Therefore, it was found that different strains have substrate bias and their laccase secretion capacities are different. In this study, *L. betulinus* Han 851 and *A. heimuer* Han 1333 showed a more obvious preference for *Oryza sativa* (as shown in Fig. 1). However, *C. unicolor* Han 849 preferred *Firmiana platanifolia*. Han *et al*. (2020) reported that a continuous and stable laccase production from *P. ostreatus* was an extremely important advantage when using solid-state fermentation with poplar sawdust. Similarly, a continuous and stable laccase production from *C. unicolor* Han 849 was obvious advantage when using solid-state fermentation with any of the four lignocellulosic residues used in this study (as shown in Figs. 1 through 4). In general, the laccase secreting ability of *C. unicolor* Han 849 was stronger than the other three tested species, and the laccase secreting ability of *S. rugosoannulata* Han 1321 was extremely poor in this study.

CONCLUSIONS

1. The biosynthetic potential of Basidiomycetes was highly dependent on the species of fungi. In general, the laccase secreting ability of *C. unicolor* Han 849 was stronger than *L. betulinus* Han 851, *S. rugosoannulata* Han 1321, and *A. heimuer* Han 1333. In addition, the laccase secreting ability of *S. rugosoannulata* Han 1321 was extremely poor.
2. The biosynthetic potential of Basidiomycetes was highly dependent on the lignocellulosic residues.
3. Different species of fungi had a preference in lignocellulosic residues. *Cerrena unicolor* Han 849 preferred *Firmiana platanifolia*, while *L. betulinus* Han 851 and *A. heimuer* Han 1333 showed a more obvious preference for *Oryza sativa*.
4. The continuous and stable laccase production from *C. unicolor* Han 849 was an obvious advantage of solid-state fermentation when using any of the four lignocellulosic residues tested in this study.
5. The presence of *Firmiana platanifolia* was conducive to *C. unicolor* Han 849 secreting laccase during solid-state fermentation. The maximum laccase production of *C. unicolor* Han 849 grown on *Firmiana platanifolia* was approximately 2.12, 1.68, and 6.13 times greater than *Populus beijingensis*, *Sorghum bicolor*, and *Oryza sativa*, respectively.

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