Laccase Activity from *Pleurotus ostreatus* and *Flammulina velutipes* Strains Grown on Agro- and Forestry Residues by Solid-state Fermentation

Qi An,[a,b] Ze-Yang Liu,[a] Chun-Rui Wang,[a] Jing Yang,[a] Si-Yu Chen,[a] Xi Chen,[a] Yi-Jie Zhang,[a] Lu-Sen Bian,[d] and Mei-Ling Han [a,b,c,*]

Laccase activity from *Pleurotus ostreatus* and *Flammulina velutipes* strains was investigated with various agro- and forestry residues by solid-state fermentation. Different species or strains belonging to the same species had the unique capacity of secreting laccase on solid-state fermentation with various agro- and forestry residues. Overall, the capacity of secreting laccase for *P. ostreatus* strains was superior to *F. velutipes* strains due to the value of maximum activity on various agro- and forestry residues, except on the stalk of straw. Compared with *Populus beijingensis*, corncob, and stalk of straw, the presence of cottonseed hull was helpful to improve laccase activity for *P. ostreatus* strains because the maximum laccase activity from cottonseed hull was higher than that from the other three agro- and forestry residues. The presence of stalk of straw was more helpful to improve laccase activity for *F. velutipes* strains because of the maximum laccase activity from stalk of straw was higher that from *Populus beijingensis*, corncob, and cottonseed hull. These results indicated the importance of selecting suitable agro- and forestry residues for fungi producing laccase. These findings contributed to the selection of suitable strains to obtain an integrated application of low-cost laccase in the factory.

Keywords: Pleurotus ostreatus; *Flammulina velutipes*; *Laccase activity*; *Agro- and forestry 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

The development of agriculture and forestry has brought great economic benefits and contributed to environmental protection because of their ability in absorbing carbon dioxide and releasing oxygen. The role agriculture and forestry plays in soil and water conservation is also extremely important. However, the rapid development of agriculture and forestry also brings some environmental problems, mainly including the residues agro- and forestry harvesting practices. What is more serious is that these agro- and forestry residues will cause more air pollution if they were disposed of *via* burning. However, these agro- and forestry residues belonging to biomass resources have broad application value in various aspects including green energy fuels and industrial chemical products (Gaikwad and Meshram 2019). Agro- and forestry residues, *e.g.*, sugarcane bagasse, grasses, corncob,

cottonseed hull, bamboos, *etc.*, are composed of lignin, cellulose, and hemicellulose (Pinheiro *et al*. 2020). The production of enzymes *via* agro- and forestry residues has attracted widespread attention, especially laccase, due to the wide existence and low price of agro- and forestry residues (Lizardi-Jimenez *et al*. 2019; Palazzolo *et al*. 2019; Srinivasan *et al*. 2019; Thamvithayakorn *et al*. 2019; Agrawal and Verma 2020; Atilano-Camino *et al*. 2020; Pinheiro *et al*. 2020; Xu *et al*. 2020).

Laccase (EC 1.10.3.2), one of most important ligninolytic enzymes, alone or together with other ligninolytic enzymes, including lignin peroxidase (Lip) and manganese peroxidase (Mnp), belongs to a family of copper oxidases and is distributed in higher plants, cyanobacteria, bacteria, insects, and fungi (Yang *et al*. 2017; Srinivasan *et al*. 2019). Laccase has the ability of catalyzing a wide range of reactions, including organic and inorganic substrates (Sharma *et al*. 2007; Jaya Mary *et al*. 2018). High catalytic capacity and wide substrate adaptation of laccase greatly improves the wide application of enzyme in various fields, including bioremediation, biodegradation, biosensors, medicine, nanoscience, pulp and paper industry, baking industry, and beverage and beer industry (Bertrand *et al.* 2017; Kudanga *et al*. 2017; Mate and Alcalde 2017; Agrawal *et al*. 2018; Su *et al*. 2018; Yashas *et al*. 2018; Deska and Konczak 2019; Garlapati *et al*. 2019; Singh and Arya 2019; Wang *et al*. 2019; Zerva *et al*. 2020; Liu *et al*. 2020). The wide application of laccase to the above-mentioned biotechnological process requires large amounts of laccase with low production cost (Couto and Toca-Herrera 2007; An *et al*. 2018). Therefore, reducing the production costs of laccase by optimising the fermentation condition and selecting suitable strains to obtain high laccase production are the key research questions for industrial applications (Elisashvili *et al*. 2008; Mate and Alcalde 2017; Rodrigues *et al*. 2019; An *et al*. 2020a,b). At present, fungal laccase and bacterial laccase have been widely studied, especially fungal laccase. Many fungi have ability of secreting laccase, mainly basidiomycetes. White-rot fungi, belonging to basidiomycetes, are recognized as excellent laccases producers, and almost all species of white-rot fungi can secrete laccase to varying degrees (Couto and Toca-Herrera 2007; An *et al*. 2016a,b; Agrawal *et al*. 2018). Among the white-rot fungi, laccase production from the genus *Ganoderma*, *Trametes*, *Lentinus*, and *Pleurotus* are the most widely studied (Elissetche *et al.* 2007; Guo *et al.* 2017; Gupta and Jana 2018, 2019; Palazzolo *et al*. 2019; Sadeghian-Abadi *et al.* 2019; Atilano-Camino *et al*. 2020; Han *et al.* 2020).

The activity of laccase secreted by fungi is affected by many factors. Additionally, it mainly includes the following categories: 1) the concentration, proportion, and type of carbon or nitrogen sources, such as glucose, peptone, and lignocellulosic biomass (Songulashvili *et al*. 2008; An *et al*. 2015; Han *et al*. 2017; Palazzolo *et al*. 2019; Thamvithayakorn *et al*. 2019; Agrawal and Verma 2020; Atilano-Camino *et al*. 2020; Pinheiro *et al*. 2020); 2) various kinds or concentration of metal ions, *e.g.*, Ca^{2+}, Cu^{2+}, Cd^{2+}, Ag^{2+}, Fe^{2+}, Hg^{2+}, and Mn^{2+} (Wang *et al*. 2011; Pezzella *et al*. 2013; Divya *et al*. 2015; Suetomi *et al*. 2015; Zhuo *et al*. 2017; Xu *et al*. 2018; An *et al*. 2020a); 3) fungal secondary metabolites, such as veratrol and ferulic acid (Galhaup *et al*. 2002; Janusz *et al.* 2015); 4) temperature and pH (Diaz *et al*. 2013; Hu *et al*. 2014); 5) fermentation method, including submerged fermentation, solid-state fermentation and unconventional method (solid-state fermentation followed by liquid fermentation) (An *et al*. 2016b; Schalchli *et al*. 2017; Wasak *et al*. 2018; Gupta and Jana 2019; Wang *et al*. 2019); 6) different species or strains (Elisashvili and Kachlishvili 2009; An *et al*. 2016a, 2018; Zhang *et al*. 2020; Han *et al*. 2021).

Previous study showed that solid-state fermentation is better than submerged

fermentation in that the enzymes are not diluted (Oostra *et al*. 2000). Moreover, solid-state fermentation is closer to the real living environment of fungi in nature. Lignocellulosic biomass is often used as the material for fungal growth by solid-state fermentation. Previous studies indicated that a difference among different fungal species or different strains belonging to the same species for biosynthetic potential was significant (Janusz *et al.* 2015; Vrsanska *et al*. 2016; Han *et al.* 2020). However, it is necessary to select the substrates suitable for each fungus to grow and secrete laccase because different fungi prefer different substrates. Meanwhile, laccase activity from *Pleurotus ostreatus* CCEF 89 or CCEF 99 on corncob, poplar wood and cottonseed hull had been detected in previous studies (Han *et al.* 2017, 2018). However, more strains and more kinds of agro- and forestry residues were necessary for obtaining suitable strain and fermentation condition. Based on this, in this study, laccase activity of strains belonging to *Pleurotus ostreatus* and *Flammulina velutipes* were investigated with various agro- and forestry residues on solid-state fermentation. The enzyme production capacity of strains from *Pleurotus ostreatus* and *Flammulina velutipes* was also compared. The results will provide a basis for the selection of suitable agro- and forestry residues for different strains to obtain low-cost laccase in the factory.

EXPERIMENTAL

Materials

Microorganisms

Three *Pleurotus ostreatus* strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 and three *Flammulina velutipes* strains CCMSSC 00114, CCMSSC 00118, and CCMSSC 05317 were used in this study. All strains were kindly provided by Institute of Microbiology, Beijing Forestry University (Beijing, China) and preserved on Malt Extract Agar (MEA) medium in the College of Life Science, Langfang Normal University (Langfang, China).

Collection and treatment of agro- and forestry residues

Populus beijingensis was collected from Langfang (China), while corncob, stalk of straw, and cottonseed hull were collected from farmland in Chengde (China). All collected agro- and forestry residues were air-dried and milled to a particle size of between 20- and 60-mesh.

Methods

Microbial culture and inoculum preparation

All microorganisms were reactivated on MEA medium with a stable culture temperature of 26 ℃. After 7 days of culture, round pieces with a diameter of 5 mm were punched out with a hole punch, forming the inoculants for subsequent using. Then, to prepare the seed fluid, 5 inoculants were cultured in 250-mL Erlenmeyer flasks with 100 mL of MEA liquid medium (without agar) at 26 ℃ in a shaking condition with a speed of 150 rpm. Seven days later, well grown seed fluid was homogenized by a laboratory blender for 2 min with a stable speed of 5000 rpm, and the well-stirred suspension was taken for the inoculum.

Laccase production from P. ostreatus and F. velutipes strains

A total of 3 g of dry *Populus beijingensis* was added to a 250-mL Erlenmeyer flask and moistened with 12 mL deionized water. All flasks were sterilized, cooled down to room temperature, and inoculated with 3 mL inoculum under sterile conditions. Other agro- and forestry residues, corncob, stalk of straw, and cottonseed hull, were followed the steps aforementioned. All flasks were incubated at 26 °C and underwent the step of solid-state fermentation. Every 24 h, the flasks were taken out and added into 100 mL acetate-sodium acetate buffer (50 mM, pH 5.5) to perform the extraction process. Extraction process was carried out in a shaker for 4 h (10 °C, 120 rpm) according to Han *et al*. (2020b). Then, fermentation liquor was filtered through Whatman No. 1 filter paper after removing the agro- and forestry residues. The obtained liquid was centrifuged for 20 min (4 °C, 12000 rpm). The supernatant after centrifugation was used for measurement of laccase activity.

Determination of Laccase activity

Laccase activity of *P. ostreatus* and *F. velutipes* strains was determined by the oxidation of 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) (1 mM), as described in a previous study (Han *et al*. 2020b). One activity unit was defined as the amount of laccase forming 1 μmol of $ABTS^+$ per minute ($\varepsilon_{420\ nm} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$) in the acetate-sodium acetate buffer (50 mM, pH 4.2) monitored by measuring the optical density (OD) at 420 nm using a Unico UV-4802 spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China). The assay mixture contained 100 μL of supernatant prepared and 1 mM ABTS in 50 mM acetate-sodium acetate buffer (pH 4.2). The specify duration of the reaction was 5 min with the model of kinetic measurement. The blanks were comprised of ABTS, acetate-sodium acetate buffer, and inactive supernatant enzyme solution.

Date analysis

Two-way analysis of variance was used to examine the effects of agro- and forestry residues and strains on laccase activity and performed by the method of Han *et al*. (2020b). The software used to complete two-way analysis of variance was SPSS software version 22.0 (PROC GLM, Armonk, NY, USA). All data figures were prepared using Origin 2016 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Results of Statistical analysis

The effect of the agro- and forestry residues on laccase activity of different strains belonging to *Pleurotus ostreatus* and *Flammulina velutipes* was significant ($P < 0.001$) during the 10 days of solid-state fermentation. The effect of the strains belonging to *P. ostreatus* and *F. velutipes* on laccase activity was significant ($P < 0.001$) during the 10 days of solid-state fermentation. Meanwhile, the interactions of strains and agro- and forestry residues on laccase activity were also significant ($P < 0.001$) at 10 days on solid-state fermentation (Table 1).

Laccase Activity of Strains Grown on Different Agro- and Forestry Residues

Agro- and forestry residues were considered as low-cost materials to produce various valuable chemical products including bioethanol and enzymes. Previous studies

indicated that lignocellulosic materials, belonging to a type of complex carbon and nitrogen sources, could be affected the laccase production secreted by fungi (Pinar *et al*. 2017; Leite *et al.* 2019; An *et al*. 2020b; Han *et al*. 2020; Rajavat *et al*. 2020). Widely used kinds of lignocellulosic materials were wood chips, olive pomace, wheat bran, bamboo, and coffee shells (Gaikwad and Meshram 2019; Leite *et al.* 2019; Xu *et al*. 2020).

Table 1. Effects of Strains, Agro- and Forestry Residues, and Strains ×Agro- and Forestry Residues Interactions on Laccase Activities of Different Strains (Two-way ANOVA)

Incubation Period (d)	Strains	Agro- and Forestry Residues	Strains × Agro- and Forestry Residues
1	940.907***	1194.372***	381.677***
2	4853.474***	11864.856***	2614.743***
3	915.119***	2465.461***	470.000***
4	1305.317***	3255.921***	742.631***
5	2034.876***	4814.965***	1087.556***
6	2642.866***	3173.666***	1175.300***
7	5661.829***	1641.142***	678.269***
8	2114.103***	710.668***	254.109***
9	2991.470***	400.593***	494.888***
10	1787.983***	225.465***	298.289***
Note: Degrees of freedom = 5, 3, 15; ***P < 0.001; The values were the F-value of Two-way ANOVA.			

The planting area of corn, cotton, *Populus beijingensis*, and *Oryza sativa* is large, so there are large amounts of corresponding residues. Moreover, *P. beijingensis*, *O. sativa*, corn, and cotton were also used for the growing of *Pleurotus ostreatus* and *Flammulina velutipes* to obtain a fruit body, which was a process of secreting lignocellulolytic enzymes (Han *et al*. 2017, 2020; An *et al*. 2020b). Thus, the investigation on the laccase activity from *P. ostreatus* and *F. velutipes* strains on different agro- and forestry residues was necessary to select suitable biomass for obtaining the low-cost laccase. A previous study had selected the actinobacteria strains to produce lignocellulolytic enzymes with olive pomace as substrate (Lamia *et al*. 2017). Therefore, this study investigated the laccase activity from *P. ostreatus* and *F. velutipes* strains on *Populus beijingensis*, stalk of straw, corncob, and cottonseed hull *via* solid-state fermentation.

Minimum laccase activity from *P. ostreatus* strain CCMSSC 00322 grown on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 2.35 ± 0.11, 2.59 ± 0.19, 9.26 ± 0.19, and 28.21 ± 1.55 U/L, respectively, and the time of minimum laccase activity all occurred on the 1st day (Figs. 1, 2, 3, and 4, respectively). Thus, minimum laccase activity from strain CCMSSC 00322 on cottonseed hull was nearly 12.00-fold, 10.89-fold, and 3.05-fold higher than that on stalk of straw, *P. beijingensis*, and corncob, respectively. Therefore, the presence of cottonseed hull was advantageous for *P. ostreatus* strain CCMSSC 00322 to rapidly secrete laccase. Maximum laccase activity from *P. ostreatus* strain CCMSSC 00322 grown on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 312.04 ± 5.52, 303.52 ± 5.41, 321.98 ± 6.55, and 594.58 ± 4.15 U/L, respectively, and the corresponding occurrence time was on the 7th day, 7th day, 7th day, and 6th day (Table 2). Clearly, maximum laccase activity secreted by strain CCMSSC 00322 on stalk

of straw, *P. beijingensis*, and corncob maintained an equal level, and was lower than that on cottonseed hull. Previous study indicated that cottonseed hull was helpful for *P. ostreatus* strains to obtain higher laccase production compared with corncob or poplar wood (Han *et al*. 2017, 2018, 2020), and the advantages of cottonseed hull in improving laccase activity emerged in this study were similar to previous studies. At the end of fermentation (on 10th day), laccase activity from strain CCMSSC 00322 on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 23.89 ± 1.79, 103.89 ± 4.51, 129.07 ± 1.65, and 177.47 ± 11.88 U/L, respectively (Figs. 1 through 4). To some extent, *P. beijingensis*, corncob, and cottonseed hull were all helpful for strain CCMSSC 00322 to secrete stable laccase during whole process of fermentation (Figs. 1, 2, and 4).

Minimum laccase activity from *P. ostreatus* strain CCMSSC 00406 on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 2.16 ± 0.11 U/L (on 1st day), 1.05 ± 0.11 U/L (on 1st day), 2.90 ± 0.28 U/L (on 1st day), and 4.07 ± 0.32 U/L (on 1st day), respectively (Figs. 1, 2, 3 and 4). Maximum laccase activity for *P. ostreatus* strain CCMSSC 00406 from cottonseed hull was 417.72 ± 17.99 U/L on 5th day, which was higher than that from stalk of straw (253.95 ± 5.86 U/L, 8th day), *Populus beijingensis* (265.86 ± 13.28 U/L, 7th day), and corncob (148.15 ± 7.38 U/L, 6th day), by 1.64-fold, 1.57-fold, and 2.82-fold, respectively (Table 2). Laccase activity from strain CCMSSC 00406 at 10th day on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 32.35 ± 2.39, 67.84 ± 1.83, 16.23 ± 1.50, and 49.20 ± 3.18 U/L, respectively. The maximum activity of laccase on *P. beijingensis* was lower than that on cottonseed hull, but laccase activity on *P. beijingensis* was higher than that on cottonseed hull at 10th day (Figs. 1 and 4).

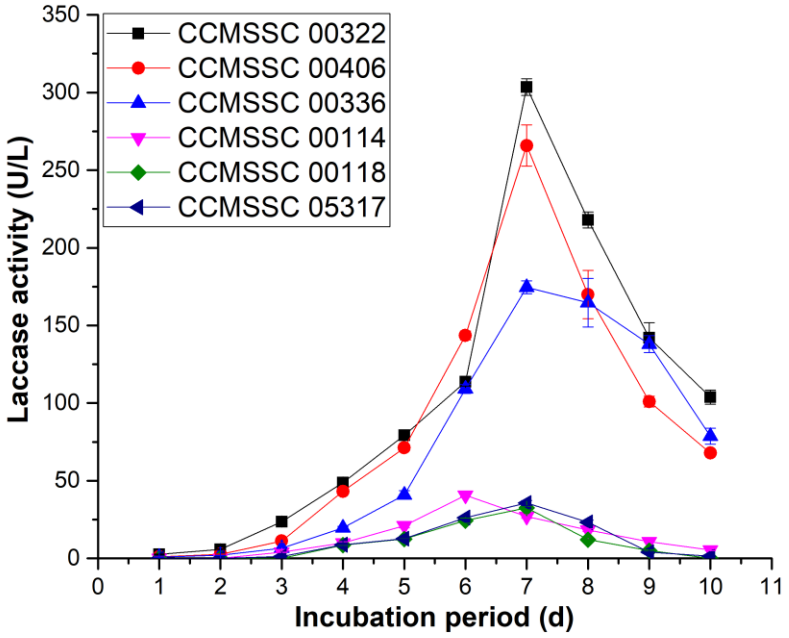

Fig. 1. Laccase activity from *P. ostreatus* strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 and *F. velutipes* strains CCMSSC 00114, CCMSSC 00118, and CCMSSC 05317 on *P. beijingensis*. Mean value was calculated using three independent parallel values.

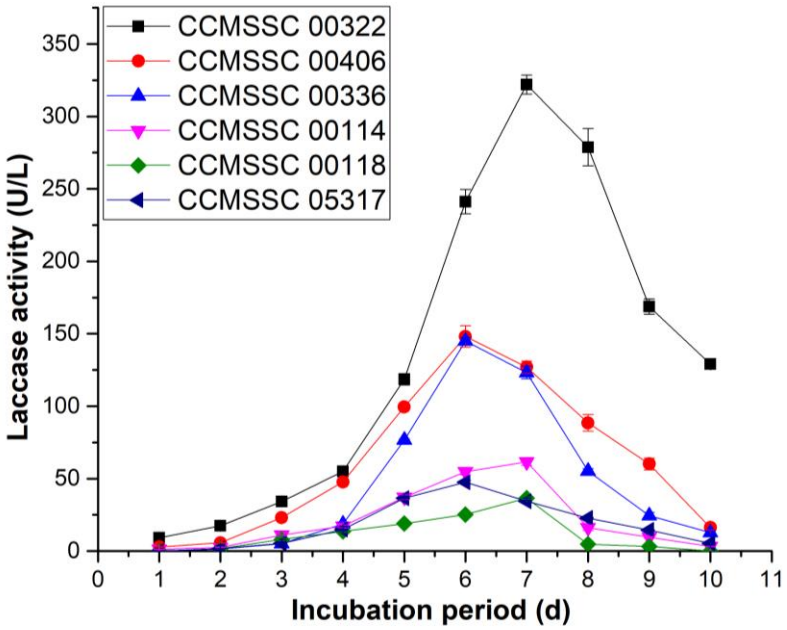

Fig. 2. Laccase activity from *P. ostreatus* strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 and *F. velutipes* strains CCMSSC 00114, CCMSSC 00118, and CCMSSC 05317 on corncob. Mean value was calculated using three independent parallel values.

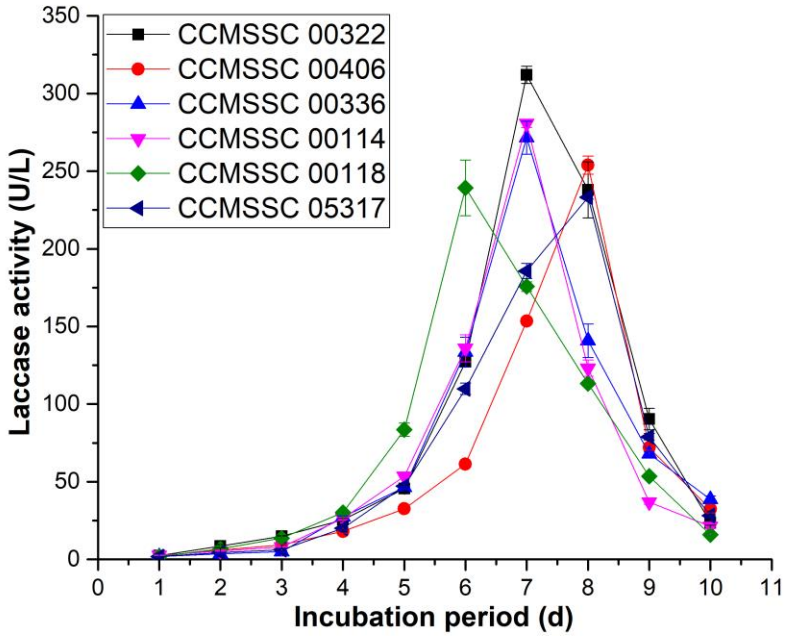

Fig. 3. Laccase activity from *P. ostreatus* strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 and *F. velutipes* strains CCMSSC 00114, CCMSSC 00118, and CCMSSC 05317 on stalk of straw. Mean value was calculated using three independent parallel values.

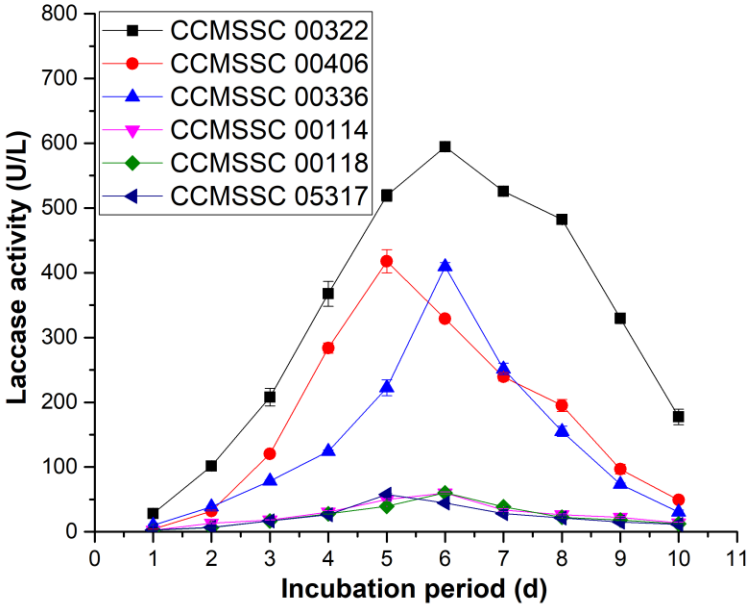

Fig. 4. Laccase activity from *P. ostreatus* strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 and *F. velutipes* strains CCMSSC 00114, CCMSSC 00118, and CCMSSC 05317 on cottonseed hull. Mean value was calculated using three independent parallel values.

Minimum laccase activity for *P. ostreatus* strain CCMSSC 00336 from agro- and forestry residues with stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 1.91 ± 0.11, 0.86 ± 0.11, 0 ± 0, and 10.19 ± 1.13 U/L, respectively, and all appeared on 1st day. Minimum laccase activity for strain CCMSSC 00336 on cottonseed hull was nearly 5.34-fold and 11.85-fold higher than that on stalk of straw and *P. beijingensis*, respectively, while no laccase activity was detected on corncob at the same time. The maximum laccase activity from strain CCMSSC 00336 on cottonseed hull (409.44 ± 6.49 U/L, 6th day) was higher than that on stalk of straw (271.54 ± 10.58 U/L, 7th day), *Populus beijingensis* (174.51 ± 4.18 U/L, 7th day), and corncob (145.00 ± 2.59 U/L, 6th day), by 1.51-fold, 2.35-fold, and 2.82-fold, respectively (Table 2). Thus, cottonseed hull and stalk of straw improved laccase activity for strain CCMSSC 00336. At the 10th day, laccase activity from strain CCMSSC 00336 grown on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 38.64 ± 2.29, 78.64 ± 5.18, 12.84 ± 1.12, and 30.62 ± 2.93 U/L (Figs. 1 through 4, respectively).

Minimum laccase activity from *F. velutipes* strain CCMSSC 00114 on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 2.59 ± 0.19 U/L, 0 ± 0 U/L, 1.23 ± 0.11 U/L, and 2.47 ± 0.11 U/L (Figs. 1, 2, 3, and 4, respectively). Maximum laccase activity from strain CCMSSC 00114 on stalk of straw was 280.80 ± 2.75 U/L, higher than that on *P. beijingensis* (40.62 ± 0.95 U/L), corncob (61.67 ± 2.49 U/L), and cottonseed hull (59.88 ± 1.95 U/L), by nearly 6.91-fold, 4.55-fold, and 4.69-fold (Table 2), respectively.

Additionally, laccase activity on stalk of straw was higher that on *P. beijingensis*, corncob, and cottonseed hull, by nearly 3.88-fold, 6.76-fold, and 1.54-fold, respectively. Clearly, stalk of straw contributed to *F. velutipes* strain CCMSSC 00114 to secrete laccase rapidly and improve laccase activity. At the beginning of the fermentation (1st day), laccase activity from *F. velutipes* strain CCMSSC 00118 on stalk of straw and cottonseed hull was

1.91 ± 0.11 and 2.16 ± 0.11 U/L, respectively, while no laccase activity was detected on *P. beijingensis* and corncob (Figs. 1, 2, 3, and 4, respectively). Maximum laccase activity from strain CCMSSC 00118 on stalk of straw (239.26 ± 17.89 U/L, 6th day) was higher than that on *P. beijingensis* (32.47 ± 1.12 U/L, 7th day), corncob (36.60 ± 0.70 U/L, 7th day), and cottonseed hull (60.19 ± 2.80 U/L, 6th day), by 7.37-fold, 6.54-fold, and 3.98-fold, respectively (Table 2).

At the end stage of fermentation (10th day), similarly to the beginning stage of fermentation, laccase activity on stalk of straw, and cottonseed hull was 15.99 ± 1.30 and 12.35 ± 0.60 U/L, and laccase activity was undetected on *P. beijingensis* and corncob. Overall, the presence of stalk of straw was helpful for strain CCMSSC 00118 to accelerate laccase secretion and increase laccase activity. Thus, comparing with other three agro- and forestry residues, stalk of straw was suitable for strain CCMSSC 00118 secreting laccase. On the 1st day, laccase activity for *F. velutipes* strain CCMSSC 05317 was 1.79 ± 0.11 U/L on stalk of straw and 2.65 ± 0.11 U/L on cottonseed hull. No laccase activity was detected on *P. beijingensis* and corncob (Figs. 1, 2, 3 and 4, respectively). Maximum laccase activity for strain CCMSSC 05317 was 233.21 ± 1.13 U/L on stalk of straw, 35.62 ± 1.62 U/L on *P. beijingensis*, 47.53 ± 1.23 U/L on corncob, and 57.78 ± 1.30 U/L on cottonseed hull (Table 2), respectively. Furthermore, on the 10th day, laccase activity for strain CCMSSC 05317 on stalk of straw, *P. beijingensis*, corncob, and cottonseed hull was 28.21 ± 0.60, 1.36 ± 0.11, 5.43 ± 0.43, and 11.42 ± 0.11 U/L, respectively. Clearly, stalk of straw was extremely helpful for strain CCMSSC 05317 to secrete laccase during whole process of solid-state fermentation.

Previous studies concerning lignocellulosic materials using in laccase production secreted by *F. velutipes* were mainly ramie stalk, corncob, and poplar wood (Xie *et al*. 2017; An *et al*. 2020b). Han *et al.* (2017) reported that laccase production from *P. ostreatus* CCEF 89 and CCEF 99 in synthetic medium containing cottonseed hull was higher than that in poplar wood or corncob with the same synthetic medium.

In the present study, a similar phenomenon was found, because the occurrence time of maximum laccase activity on cottonseed hull was earlier that than on stalk of straw, *P. beijingensis*, and corncob. Maximum laccase activity from *F. velutipes* CCMSSC 05317 in submerged fermentation with cottonseed hull was higher than that with corncob and poplar wood (An *et al*. 2020b). Meanwhile, maximum laccase activities obtained from *F. velutipes* CCMSSC 00118 grown on cottonseed hull, corncob, and poplar wood were 71.83 ± 0.35, 58.27 ± 1.14, and 42.50 ± 0.80 U/L (An *et al*. 2020b). The presence of cottonseed hull was certainly helpful to improve laccase activity for *P. ostreatus* strains in this study, while, the presence of stalk of straw was more helpful to improve laccase activity for *F. velutipes* strains than cottonseed hull. Additionally, this result confirmed that it was important to select the suitable agro- and forestry residues for different fungi to produce laccase *via* fermentation.

Table 2. Maximum Laccase Activity, Agro- and Forestry Residues, and Occurrence Time of Tested *Pleurotus ostreatus* and *Flammulina velutipes* Strains

Maximum Laccase Activity (U/L)	Agro- and Forestry Residues	Tested Strains	Time (Day)
312.04 ± 5.52	Stalk of straw	*P. ostreatus* CCMSSC 00322	7th
303.52 ± 5.41	*Populus beijingensis*	*P. ostreatus* CCMSSC 00322	7th
321.98 ± 6.55	Corncob	*P. ostreatus* CCMSSC 00322	7th
594.58 ± 4.15	Cottonseed hull	*P. ostreatus* CCMSSC 00322	6th
253.95 ± 5.86	Stalk of straw	*P. ostreatus* CCMSSC 00406	8th
265.86 ± 13.28	*Populus beijingensis*	*P. ostreatus* CCMSSC 00406	7th
148.15 ± 7.38	Corncob	*P. ostreatus* CCMSSC 00406	6th
417.72 ± 17.99	Cottonseed hull	*P. ostreatus* CCMSSC 00406	5th
271.54 ± 10.58	Stalk of straw	*P. ostreatus* CCMSSC 00336	7th
174.51 ± 4.18	*Populus beijingensis*	*P. ostreatus* CCMSSC 00336	7th
145.00 ± 2.59	Corncob	*P. ostreatus* CCMSSC 00336	6th
409.44 ± 6.49	Cottonseed hull	*P. ostreatus* CCMSSC 00336	6th
280.80 ± 2.75	Stalk of straw	*F. velutipes* CCMSSC 00114	7th
40.62 ± 0.95	*Populus beijingensis*	*F. velutipes* CCMSSC 00114	6th
61.67 ± 2.49	Corncob	*F. velutipes* CCMSSC 00114	7th
59.88 ± 1.95	Cottonseed hull	*F. velutipes* CCMSSC 00114	6th
239.26 ± 17.89	Stalk of straw	*F. velutipes* CCMSSC 00118	6th
32.47 ± 1.12	*Populus beijingensis*	*F. velutipes* CCMSSC 00118	7th
36.60 ± 0.70	Corncob	*F. velutipes* CCMSSC 00118	7th
60.19 ± 2.80	Cottonseed hull	*F. velutipes* CCMSSC 00118	6th
233.21 ± 1.13	Stalk of straw	*F. velutipes* CCMSSC 05317	8th
35.62 ± 1.62	*Populus beijingensis*	*F. velutipes* CCMSSC 05317	7th
47.53 ± 1.23	Corncob	*F. velutipes* CCMSSC 05317	6th
57.78 ± 1.30	Cottonseed hull	*F. velutipes* CCMSSC 05317	5th
Data are presented as mean value ± standard deviation for biological triplicates and are expressed as U/L.			

Laccase Activity from Different *Pleurotus ostreatus* and *Flammulina velutipes* Strains

Pleurotus ostreatus and *Flammulina velutipes* have the capacity of secreting laccase (An *et al*. 2016a, 2018). Previous studies indicated that metal ions have ability of inducing laccase secreted by fungi. Among different metal ions, the presence of Cu^{2+} or Mn^{2+} contributed to improving laccase production from *F. velutipes* and *P. ostreatus* strains, and Fe^{2+} was disadvantageous for *F. velutipes* and *P. ostreatus* strains secreting laccase (Janusz *et al*. 2015; An *et al*. 2016a, 2020a). The effects of lignocellulosic materials, such as cottonseed hull and poplar wood, on the activity of laccase in *P. ostreatus* and *F. velutipes* strains *via* submerged fermentation were also studied (Elisashvili *et al*. 2008; An *et al*. 2015, 2020b; Han *et al*. 2017, 2018, 2020). However, enlarging the screening range of strains and suitable agro- and forestry residues is helpful for obtaining high-produced laccase strains used in industrial application. Thus, in present study, the capacity of secreting laccase from three *P. ostreatus* strains and three *F. velutipes* strains on agro- and forestry residues was considered.

In terms of *Populus beijingensis*, laccase activity from three *P. ostreatus* strains was detected on the 1st day, while no laccase activity was detected from three *F. velutipes* strains (Fig. 1). Maximum laccase activity for *P. ostreatus* CCMSSC 00322 was 303.52 ± 5.41 U/L, nearly 1.14-fold, 1.74-fold, 7.47-fold, 9.35-fold, and 8.52-fold than that for *P. ostreatus* CCMSSC 00406, *P. ostreatus* CCMSSC 00336, *F. velutipes* CCMSSC 00114, *F. velutipes* CCMSSC 00118, and *F. velutipes* CCMSSC 05317, respectively (Table 2). Clearly, *P. ostreatus* CCMSSC 00322 was the best producer with the capacity of secreting laccase on *P. beijingensis*. The level of secreting laccase from three *F. velutipes* strains was roughly equivalent and was lower than that from three *P. ostreatus* strains (Fig. 1). Laccase activity obtained from *P. ostreatus* CCMSSC 00322, *P. ostreatus* CCMSSC 00406, and *F. velutipes* CCMSSC 00114 on corncob was measured on the 1st day, while the other three strains were not measured this day. Similarly, to the phenomenon on the *Populus beijingensis*, maximum laccase activity for *P. ostreatus* CCMSSC 00322 was 321.98 ± 6.55 U/L, which was nearly 2.17-fold, 2.22-fold, 5.22-fold, 8.80-fold, and 6.77-fold higher than that for *P. ostreatus* CCMSSC 00406, *P. ostreatus* CCMSSC 00336, *F. velutipes* CCMSSC 00114, *F. velutipes* CCMSSC 00118, and *F. velutipes* CCMSSC 05317, respectively (Table 2). Thus, *P. ostreatus* CCMSSC 00322 was the best producer with the capacity of secreting laccase on corncob and other two *P. ostreatus* strains had similar laccase secretion capacity (Fig. 2). Maximum laccase activity from *F. velutipes* CCMSSC 00114 on corncob was 61.67 ± 2.49 U/L, nearly 1.68-fold and 1.30-fold higher than that from *F. velutipes* CCMSSC 00118 and *F. velutipes* CCMSSC 05317. Maximum laccase activity from these *F. velutipes* strains indicated that the specificity of enzyme production in different strains belonging to the same species was significant. Furthermore, it was also demonstrated once again that the enzyme production capacity of *P. ostreatus* CCMSSC 00322 was much stronger than that of other strains, and each strain had a certain specificity of its own enzyme production through the laccase activity of the six strains grown on corncob on the 10th day. In terms of stalk of straw, laccase activity was detected in these six strains on the 1st day (Fig. 3). Maximum laccase activity from *P. ostreatus* CCMSSC 00322, CCMSSC 00406, CCMSSC 00336, *F. velutipes* CCMSSC 00114, CCMSSC 00118, and CCMSSC 05317 on stalk of straw was 312.04 ± 5.52, 253.95 ± 5.86, 271.54 ± 10.58, 280.80 ± 2.75, 239.26 ± 17.89, and 233.21 ± 1.13 U/L (Table 2), respectively. Among the tested six strains, *P. ostreatus* CCMSSC 00322 was still the best producer in secreting laccase on stalk of straw. Meanwhile, three *F. velutipes* strains exhibited superior capacity

of secreting laccase due to the high level of laccase activity, especially *F. velutipes* CCMSSC 00114 (Fig. 3). This is the first report about the laccase activity secreted by *P. ostreatus* and *F. velutipes* strains grown on stalk of straw. The report found that stalk of straw was more suitable for *F. velutipes* strains to secrete laccase. Maximum laccase activity from *P. ostreatus* CCMSSC 00322 on cottonseed hull was 594.58 ± 4.15 U/L, which was nearly 1.42-fold, 1.45-fold, 9.93-fold, 9.88-fold, and 10.29-fold than that for *P. ostreatus* CCMSSC 00406, *P. ostreatus* CCMSSC 00336, *F. velutipes* CCMSSC 00114, *F. velutipes* CCMSSC 00118, and *F. velutipes* CCMSSC 05317.

Previous studies indicated that species or different strains belonging to the same species have their own unique characteristics of enzyme production (Janusz *et al.* 2015; Huang *et al.* 2019; An *et al*. 2020a,b; Han *et al.* 2020). The value of laccase activity from four *P. ostreatus* strains 2175, IBB8, IBB108, and 2191, on tree leaves was no different by solid-state fermentation (Elisashvili *et al.* 2008). Additionally, the present study indicated that there were differences and showed differentiated specificity in the capacity of secreting laccase from different *P. ostreatus* and *F. velutipes* strains on various agro- and forestry residues. An *et al*. (2020b) reported that the capacity of secreting laccase from *P. ostreatus* strains was superior to that from *F. velutipes* strains. In this study, similar phenomenon appeared most times, except fermentation on stalk of straw. In terms of overall laccase production capacity, *P. ostreatus* was indeed better than *F. velutipes* due to the value of maximum laccase activity and duration of enzyme production.

CONCLUSIONS

1. The capacity of secreting laccase was varied from different species or strains belonging to the same species on solid-state fermentation with various agro- and forestry residues.
2. Overall, the capacity of secreting laccase for *P. ostreatus* strains was superior to *F. velutipes* strains due to the value of maximum activity on various agro- and forestry residues, except on stalk of straw.
3. Comparing with *Populus beijingensis*, corncob and stalk of straw, the presence of cottonseed hull was certainly helpful to improve laccase activity for *P. ostreatus* strains in this study, while, the presence of stalk of straw was more helpful to improve laccase activity for *F. velutipes* strains.

ACKNOWLEDGMENTS

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

REFERENCES CITED

Agrawal, K., Chaturvedi, V., and Verma, P. (2018). "Fungal laccase discovered but yet undiscovered," *Bioresources and Bioprocessing* 5, article no. 4. DOI: 10.1186/s40643-018-0190-z

Agrawal, K., and Verma, P. (2020). "Production optimization of yellow laccase from *Stropharia* sp. ITCC 8422 and enzyme-mediated depolymerization and hydrolysis of lignocellulosic biomass for biorefinery application," *Biomass Conversion and Biorefinery* (Early Access). DOI: 10.1007/s13399-020-00869-w

An, Q., Han, M. L., Bian, L. S., Han, Z. C., Han, N., Xiao, Y. F., and Zhang, F. B. (2020a). "Enhanced laccase activity of white rot fungi induced by different metal ions under submerged fermentation," *BioResources* 15(4), 8369-8383. DOI: 10.15376/biores.15.4.8369-8383

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

An, Q., Ma, H. F., Han, M. L., Si, J., and Dai, Y. C. (2018). "Effects of different induction media as inducers on laccase activities of *Pleurotus ostreatus* strains in submerged fermentation," *BioResources* 13(1), 1143-1156. DOI: 10.15376/biores.13.1.1143-1156

An, Q., Qiao, J., Bian, L. S., Han, M. L., Yan, X. Y., Liu, Z. Z., and Xie, C. Y. (2020b). "Comparative study on laccase activity of white rot fungi under submerged fermentation with different lignocellulosic wastes," *BioResources* 15(4), 9166-9179. DOI: 10.15376/biores.15.4.9166-9179

An, Q., Wu, X. J., Han, M. L., Cui, B. K., He, S. H., Dai, Y. C., and Si, J. (2016b). "Sequential solid-state and submerged cultivation of white rot fungus *Pleurotus ostreatus* on lignocellulosic biomass for the activity of lignocellulolytic enzymes," *BioResources* 11(4), 8791-8805. DOI: 10.15376/biores.11.4.8791-8805

An, Q., Wu, X. J., Wu, B., and Dai, Y. C. (2015). "Effects of carbon and nitrogen sources on lignocellulose decomposition enzyme activities in *Flammulina velutipes*," *Mycosystema* 34(4), 761-771. DOI: 10.13346/j.mycosystema.150060

Atilano-Camino, M. M., Alvarez-Valencia, L. H., Garcia-Gonzalez, A., and Garcia-Reyes, R. B. (2020). "Improving laccase production from *Trametes versicolor* using lignocellulosic residues as cosubstrates and evaluation of enzymes for blue wastewater biodegradation," *Journal of Environmental Management* 275, article no. 111231. DOI: 10.1016/j.jenvman.2020.111231

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

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

Deska, M., and Konczak, B. (2019). "Immobilized fungal laccase as "green catalyst" for the decolourization process - state of the art," *Process Biochemistry* 84, 112-123. DOI: 10.1016/j.procbio.2019.05.024

Diaz, R., Tellez-Tellez, M., Sanchez, C., Bibbins-Martinez, M. D., Diaz-Godinez, G., and

Soriano-Santos, J. (2013). "Influence of initial pH of the growing medium on the activity, production and genes expression profiles of laccase of *Pleurotus ostreatus* in submerged fermentations," *Electronic Journal of Biotechnology* 16(4), article no. 6. DOI: 10.2225/vol16-issue4-fulltext-6

Divya, L. M., Prasanth, G. K., and Sadasivan, C. (2015). "Assessing the tolerance of immobilized laccase from a salt-tolerant strain of *Trichoderma viride* Pers NFCCI-2745 to heavy metal ions, detergents and copper chelating agents," *International Journal of Environmental Science and Technology* 12(10), 3225-3234. DOI: 10.1007/s13762-014-0697-6

Elisashvili, V., and Kachlishvili, E. (2009). "Physiological regulation of laccase and manganese peroxidase production by white-rot Basidiomycetes," *Journal of Biotechnology* 144(1), 37-42. DOI: 10.1016/j.jbiotec.2009.06.020

Elisashvili, V., Penninckx, M., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Kharziani, T., and Kvesitadze, G. (2008). "*Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition," *Bioresource Technology* 99(3), 457-462. DOI: 10.1016/j.biortech.2007.01.011

Elissetche, J., Ferraz, A., Freer, J., and Rodríguez, J. (2007). "Enzymes produced by *Ganoderma australe* growing on wood and in submerged cultures," *World Journal of Microbiology and Biotechnology* 23, 429. DOI: 10.1007/s11274-006-9243-0

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

Galhaup, C., Wagner, H., Hinterstoisser, B., and Haltrich, D. (2002). "Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*," *Enzyme and Microbial Technology* 30(4), 529-536. DOI: 10.1016/S0141-0229(01)00522-1

Garlapati, D., Chandrasekaran, M., Devanesan, A., Mathimani, T., and Pugazhendhi, A. (2019). "Role of cyanobacteria in agricultural and industrial sectors: An outlook on economically important byproducts," *Applied Microbiology and Biotechnology* 103(12), 4709-4721. DOI: 10.1007/s00253-019-09811-1

Guo, C. L., Zhao, L. T., Wang, F., Lu, J., Ding, Z. Y., and Shi, G. Y. (2017). "Beta-carotene from yeasts enhances laccase production of *Pleurotus eryngii* var. ferulae in co-culture," *Frontiers in Microbiology* 8, article no. 1101. DOI: 10.3389/fmicb.2017.01101

Gupta, A., and Jana, A. K. (2018). "Effects of wheat straw solid contents in fermentation media on utilization of soluble/insoluble nutrient, fungal growth and laccase production," *3 Biotech* 8, article no. 35. DOI: 10.1007/s13205-017-1054-5

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

Han, M. L., An, Q., He, S. F., Zhang, X. L., Zhang, M. H., Gao, X. H., Wu, Q., and Bian, L. S. (2020). "Solid-state fermentation on poplar sawdust and corncob wastes for lignocellulolytic enzymes by different *Pleurotus ostreatus* strains," *BioResources* 15(3), 4982-4995. DOI: 10.15376/biores.15.3.4982-4995

Han, M. L., An, Q., Wu, X. J., Zheng, F., and Si, J. (2017). "Effects of different

lignocellulose as inducers on laccase activities of *Pleurotus ostreatus* in submerged fermentation," *Mycosystema* 36(3), 349-357. DOI: 10.13346/j.mycosystema.160055

Han, M. L., Du, J., An, Q., and Li, C. S. (2018). "Effects of different culture substrate on laccase activities of *Pleurotus ostreatus* under different fermentation conditions," *Mycosystema* 37(8), 1100-1108. DOI: 10.13346/j.mycosystema.180064

Han, M. L., Yang, J., Liu, Z. Y., Wang, C. R., Chen, S. Y., Han, N., Hao, W. Y., An, Q., and Dai, Y. C. (2021). "Evaluation of laccase activities by three newly isolated fungal species in submerged fermentation with single or mixed lignocellulosic wastes," *Frontiers in Microbiology* 12, article no. 682679. DOI: 10.3389/fmicb.2021.682679

Huang, L., Sun, N., Ban, L., Wang, Y., and Yang, H. P. (2019). "Ability of different edible fungi to degrade crop straw," *AMB Express* 9, article no. 4. DOI: 10.1186/s13568-018-0731-z

Hu, X., Wang, C. Y., Wang, L., Zhang, R. R., and Chen, H. (2014). "Influence of temperature, pH and metal ions on guaiacol oxidation of purified laccase from *Leptographium qinlingensis*," *World Journal of Microbiology and Biotechnology* 30(4), 1285-1290. DOI: 10.1007/s11274-013-1554-3

Janusz, G., Czuryło, A., Frąc, M., Rola, B., Sulej, J., Pawlik, A., Siwulski, M., and Rogalski, J. (2015). "Laccase production and metabolic diversity among *Flammulina velutipes* strains," *World Journal of Microbiology and Biotechnology* 31(1), 121-133. DOI: 10.1007/s11274-014-1769-y

Jaya Mary, J., Karthik, C., Smita, G. R., Kumar, S., Prabakar, D., Kadirvelu, K., and Pugazhendhi, A. (2018). "Biological approaches to tackle heavy metal pollution: A survey of literature," *Journal of Environmental Management* 217, 56-70. DOI: 10.1016/j.jenvman.2018.03.077

Kudanga, T., Nemadziva, B., and Le Roes-Hill, M. (2017). "Laccase catalysis for the synthesis of bioactive compounds," *Applied Microbiology and Biotechnology* 101(1), 13-33. DOI: 10.1007/s00253-016-7987-5

Lamia, M. H., Farid, Z., Sonia, M. A., Sevastianos, R., Samia, A., Véronique, D., and Mouloud, K. (2017). "Selective isolation and screening of actinobacteria strains producing lignocellulolytic enzymes using olive pomace as substrate," *Iranian Journal of Biotechnology* 15(1), 74-77. DOI: 10.15171/ijb.1278

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

Lizardi-Jimenez, M. A., Ricardo-Diaz, J., Quinones-Munoz, T. A., Hernandez-Rosas, F., and Hernandez-Martinez, R. (2019). "Fungal strain selection for protease production by solid-state fermentation using agro-industrial waste as substrates," *Chemical Papers* 73(10), 2603-2610. DOI: 10.1007/s11696-019-00814-w

Liu, Y., Luo, G., Ngo, H. H., Guo, W. S., and Zhang, S. C. (2020). "Advances in thermostable laccase and its current application in lignin-first biorefinery: A review," *Bioresource Technology* 298, article no. 122511. DOI: 10.1016/j.biortech.2019.122511

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

Oostra, J., Tramper, J., and Rinzema, A. (2000). "Model-based bioreactor selection for large-scale solid-state cultivation of *Coniothyrium minitans* spores on oats," *Enzyme*

and Microbial Technology 27(9), 652-663. DOI: 10.1016/S0141-0229(00)00261-1

Palazzolo, M. A., Postemsky, P. D., and Kurina-Sanz, M. (2019). "From agro-waste to tool: Biotechnological characterization and application of *Ganoderma lucidum* E47 laccase in dye decolorization," *3 Biotech* 9(6), article no. 213. DOI: 10.1007/s13205-019-1744-2

Pezzella, C., Lettera, V., Piscitelli, A., Giardina, P., and Sannia, G. (2013). "Transcriptional analysis of *Pleurotus ostreatus* laccase genes," *Applied Microbiology and Biotechnology* 97(2), 705-717. DOI: 10.1007/s00253-012-3980-9

Pinar, O., Karaosmanoğlu, K., Sayar, N. A., Kula, C., Kazan, D., and Sayar, A. A. (2017). "Assessment of hazelnut husk as a lignocellulosic feedstock for the production of fermentable sugars and lignocellulolytic enzymes," *3 Biotech* 7, article no. 367. DOI: 10.1007/s13205-017-1002-4

Pinheiro, V. E., Michelin, M., Vici, A. C., de Almeida, P. Z., and Polizeli, M. D. T. D. (2020). "*Trametes versicolor* laccase production using agricultural wastes: A comparative study in Erlenmeyer flasks, bioreactor and tray," *Bioprocess and Biosystems Engineering* 43(3), 507-514. DOI: 10.1007/s00449-019-02245-z

Rajavat, A. S., Rai, S., Pandiyan, K., Kushwaha, P., Choudhary, P., Kumar, M., Chakdar, H., Singh, A., Karthikeyan, N., Bagul, S. Y., *et al.* (2020). "Sustainable use of the spent mushroom substrate of *Pleurotus florida* for production of lignocellulolytic enzymes," *Journal of Basic Microbiology* 60(2), 173-184. DOI: 10.1002/jobm.201900382

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

Sadeghian-Abadi, S., Rezaei, S., Yousefi-Mokri, M., and Faramarzi, M. A. (2019). "Enhanced production, one-step affinity purification, and characterization of laccase from solid-state culture of *Lentinus tigrinus* and delignification of pistachio shell by free and immobilized enzyme," *Journal of Environmental Management* 244, 235-246. DOI: 10.1016/j.jenvman.2019.05.058

Schalchli, H., Hormazabal, E., Rubilar, O., Briceno, G., Mutis, A., Zocolo, G. J., and Diez, M. C. (2017). "Production of ligninolytic enzymes and some diffusible antifungal compounds by white-rot fungi using potato solid wastes as the sole nutrient source," *Journal of Applied Microbiology* 123(4), 886-895. DOI: 10.1111/jam.13542

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

Sharma, N., Kalra, K. L., Oberoi, H. S., and Bansal, S. (2007). "Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation," *Indian Journal of Microbiology* 47, 310-316. DOI: 10.1007/s12088-007-0057-z

Songulashvili, G. G., Elisashvili, V., Wasser, S. P., Hadar, Y., and Nevo, E. (2008). "Effect of the carbon source and inoculum preparation method on laccase and manganese peroxidase production in submerged cultivation by the medicinal mushroom *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Aphyllophoromycetideae)," *International Journal of Medicinal Mushrooms* 10(1), 79-86. DOI: 10.1615/IntJMedMushr.v10.i1.100

Srinivasan, P., Selvankumar, T., Kamala-Kannan, S., Mythili, R., Sengottaiyan, A.,

Govarthanan, M., Senthilkumar, B., and Selvam, K. (2019). "Production and purification of laccase by *Bacillus* sp. using millet husks and its pesticide degradation application," *3 Biotech* 9(11), article no. 396. DOI: 10.1007/s13205-019-1900-8

Suetomi, T., Sakamoto, T., Tokunaga, Y., Kameyama, T., Honda, Y., Kamitsuji, H., Kameshita, I., Izumitsu, K., Suzuki, K., and Irie, T. (2015). "Effects of calmodulin on expression of lignin-modifying enzymes in *Pleurotus ostreatus*," *Current Genetics* 61(2), 127-140. DOI: 10.1007/s00294-014-0460-z

Su, J., Fu, J. J., Wang, Q., Silva, C., and Cavaco-Paulo, A. (2018). "Laccase: A green catalyst for the biosynthesis of poly-phenols," *Critical Reviews in Biotechnology* 38(2), 294-307. DOI: 10.1080/07388551.2017.1354353

Thamvithayakorn, P., Phosri, C., Pisutpaisal, N., Krajangsang, S., Whalley, A. J. S., and Suwannasai, N. (2019). "Utilization of oil palm decanter cake for valuable laccase and manganese peroxidase enzyme production from a novel white-rot fungus, *Pseudolagarobasidium* sp. PP17-33," *3 Biotech* 9(11), article no. 417. DOI: 10.1007/s13205-019-1945-8

Vrsanska, M., Voberkova, S., Langer, V., Palovcikova, D., Moulick, A., Adam, V., and Kopel, P. (2016). "Induction of laccase, lignin peroxidase and manganese peroxidase activities in white-rot fungi using copper complexes," *Molecules* 21(11), article no. 1553. DOI: 10.3390/molecules21111553

Wang, F., Xu, L., Zhao, L. T., Ding, Z. Y., Ma, H. L., and Terry, N. (2019). "Fungal laccase production from lignocellulosic agricultural wastes by solid-state fermentation: A review," *Microorganisms* 7(12), article no. 665. DOI: 10.3390/microorganisms7120665

Wang, X. W., Hu, J., Liang, Y., and Zhan, H. Y. (2011). "Effects of metal ions on laccase activity," *Asian Journal of Chemistry* 23(12), 5422-5424.

Wasak, A., Drozd, R., Grygorcewicz, B., Jankowiak, D., and Rakoczy, R. (2018). "Purification and recovery of laccase produced by submerged cultures of *Trametes versicolor* by three-phase partitioning as a simple and highly efficient technique," *Polish Journal of Chemical Technology* 20(4), 88-95. DOI: 10.2478/pjct-2018-0059

Xie, C. L., Gong, W. B., Yan, L., Zhu, Z. H., Hu, Z. X., and Peng, Y. D. (2017). "Biodegradation of ramie stalk by *Flammulina velutipes*: Mushroom production and substrate utilization," *AMB Express* 7, article no. 171. DOI: 10.1186/s13568-017-0480-4

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

Xu, X. Q., Huang, X. H., Liu, D., Lin, J., Ye, X. Y., and Yang, J. (2018). "Inhibition of metal ions on *Cerrena* sp. laccase: Kinetic, decolorization and fluorescence studies," *Journal of the Taiwan Institute of Chemical Engineers* 84, 1-10. DOI: 10.1016/j.jtice.2017.12.028

Yang, J., Li, W. J., Ng, T. B., Deng, X. Z., Lin, J., and Ye, X. Y. (2017). "Laccases: Production, expression regulation, and applications in pharmaceutical biodegradation," *Frontiers in Microbiology* 8, article no. 832. DOI: 10.3389/fmicb.2017.00832

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

Zerva, A., Simic, S., Topakas, E., and Nikodinovic-Runic, J. (2019). "Applications of microbial laccases: Patent review of the past decade (2009-2019)," *Catalysts* 9(12), article no. 1023. DOI: 10.3390/catal9121023

Zhang, Q., Zhao, L. T., Li, Y. R., Wang, F., Li, S., Shi, G. Y., and Ding, Z. Y. (2020). "Comparative transcriptomics and transcriptional regulation analysis of enhanced laccase production induced by co-culture of *Pleurotus eryngii* var. ferulae with *Rhodotorula mucilaginosa*," *Applied Microbiology and Biotechnology* 104(1), 241-255. DOI: 10.1007/s00253-019-10228-z

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

Article submitted: February 24, 2021; Peer review completed: April 17, 2021; Revised version received: September 8, 2021; Accepted: September 11, 2021; Published: September 14, 2021.
DOI: 10.15376/biores.16.4.7337-7354