Evaluation of the Capacity of Laccase Secretion of Four Novel Isolated White-rot Fungal Strains in Submerged Fermentation with Lignocellulosic Biomass

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The capacity of novel isolated white-rot fungi secreting laccase was evaluated for various kinds of lignocellulosic biomass in submerged fermentation. The laccase secreted by *Neofomitella fumosipora* Han 386 and *Pleurotus pulmonarius* Han 527 was significantly faster than that by *Coriolopsis trogii* Han 751 and *Coriolopsis sanguinaria* An 282. Maximum laccase from *N. fumosipora* Han 386 on the four kinds of lignocellulosic biomass tested appeared on the first day. This phenomenon indicated that *N. fumosipora* Han 386 secreted laccase rapidly compared with other tested strains in this study and showed the superiority in the rate of secreting laccase. Based on the maximum laccase activity, the ability of secreting laccase of *C. sanguinaria* An 282 was superior to other tested novel isolated strains. On the whole, *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 preferred *Toona sinensis* to produce laccase, *C. trogii* Han 751 preferred to produce laccase on *Populus beijingensis*, and *C. sanguinaria* An 282 grown on Sorghum straw was more suitable for secreting laccase. The results will be helpful for developing bioprocesses using various kinds of lignocellulosic biomass for lignocellulolytic enzyme production and enlarging the number of laccase producing strains for industrial application.

Keywords: Capacity of laccase secretion; Novel isolated strains; White-rot fungi; Lignocellulosic biomass; Submerged fermentation

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INTRODUCTION

Enzymes enable a process of biological catalysis that has a rapid rate of conversion in biochemical reactions. Therefore, enzymes usually play an important role in various aspects of biotechnology and industry. Using enzymes has many advantages compared with the pure chemical method, *i.e.*, rapid and high catalytic efficiency, user-friendly control, high substrate specificity, extensive substrate range, energy saving, mild reaction process, and no toxicity (Senthivelan *et al*. 2016). Generally, enzymes are protein in nature, and they have the ability to degrade multiple types of complex substances into other intermediate metabolites. Therefore, enzymes are beneficial catalysts for green and ecological industrial applications, which can effectively achieve cleaner production. In recent years, basidiomycetes have been extensively studied by researchers to isolate new

organisms that can secrete lignocellulosic decomposing enzymes with superior properties for industrial application (Senthivelan *et al*. 2016; An *et al*. 2018, 2020a,b; Han *et al*. 2020b). The major enzymes related to lignin degradation ability of white-rot fungi are lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase. Laccases (EC 1.10.3.2, p-diphenol: dioxygen oxido-reductases), an interesting oxidase among various enzymes, are biologically important enzymes that belong to the group of copper oxidases and are useful as green enzymes for cleaner industrial application to reduce the environmental pollution (Ba and Kumar 2017; Agrawal *et al*. 2018; Bilal *et al*. 2019). Laccase can oxidize a variety of substrates, containing aromatic and non-aromatic compounds, by degrading them into smaller components that help to reduce molecular oxygen to water (Yang *et al*. 2015; Zerva *et al*. 2019). Because laccase has numerous applications in various fields, such as biosensors, improving fiber properties, DNA (deoxyribonucleic acid) labeling, immunochemical assay, fuels, biodegradation of wastes, synthesis of organic molecules, pulp bleaching in the paper industry, baking industry, stabilization of wine, and nanobiotechnology, it has attracted attention from researchers worldwide for further intensive studies (Arora and Sharma 2010; Strong and Claus 2011; Senthivelan *et al*. 2016; Mate and Alcalde 2017; Agrawal *et al*. 2018; Su *et al*. 2018; Becker and Wittmann 2019; Singh and Arya 2019; Unuofin *et al*. 2019a; Zerva *et al*. 2019).

Usually, laccase activity secreted by fungi could be affected by many factors, *e.g.*, carbon/nitrogen source, metal ion, and species (Metreveli *et al.* 2017; Filipe *et al.* 2019; Rajavat *et al.* 2020). Because the efficient biotechnological applications require large amounts of low-cost enzymes, the appropriate approaches for this purpose are to develop new strains with high production of laccase and utilize the potential of lignocellulosic biomass (Rodrigues *et al*. 2019; An *et al*. 2020a). Meanwhile, the selection of suitable lignocellulosic biomass for fungal growth and the synthesis of specified enzymes is an important link in the development of biotechnology.

Lignocellulosic biomass is the most abundant renewable carbon source on earth, and it has attracted considerable interest for the production of second-generation biofuels and value-added chemicals (Guerriero *et al*. 2016; Haldar *et al*. 2016; Kumar *et al*. 2016). Agricultural and forestry residues, industrial and municipal wastes, and energy crops, usually considered as lignocellulosic materials, have great potential to help reduce the production cost of biofuels and value-added chemicals (Agrawal *et al.* 2018; Sharma *et al*. 2019). Producing enzymes with lignocellulosic biomass is an effective, environmentally friendly, attractive, and economical method (Medouni-Haroune *et al*. 2017). Various types of agricultural and forestry residues, such as corn stover, corncob, cottonseed hull, leaves, sawdust, straw, and sugar cane bagasse, are widely used in research of laccase. It is valuable to find more low-cost lignocellulosic biomass for the production of laccase by fungi. The main methods used for fermentation with various kinds of lignocellulosic biomass are conventional solid-state fermentation (SSF), conventional submerged fermentation (SmF), and solid pre-fermentation followed by submerged fermentation (An *et al*. 2016b). The advantage of submerged fermentation is that it is easy to manipulate and expand the scale of production. In addition, the research on laccase production by white-rot fungi has focused on several genera, such as *Ganoderma*, *Trametes*, *Pleurotus*, *Lentinus*, *Flammulina*, and *Lentinula* (An *et al*. 2016a,b; Guo *et al.* 2017; Gupta and Jana 2018; Sadeghian-Abadi *et al.* 2019; Han *et al*. 2020b; Xu *et al*. 2020). Finding more new strains with the ability of secreting laccase is necessary.

The goal of the present study was to evaluate the capacity of four novel isolated white-rot fungi secreting laccase on various kinds of lignocellulosic biomass in submerged

fermentation. Among the four white-rot fungi, Han 386 and Han 751 showed discoloration on guaiacol selective medium and the ratio of colony diameters (d_1) to photochramic laps (d_2) is less than 1 ($d_1/d_2 < 1$) (data not shown). The other fungi, Han 527 and An 282, were of the genera *Pleurotus* and *Coriolopsis*, which are considered as excellent laccase producers (Agrawal and Shahi 2017). Hence, the results from this study will be helpful for developing bioprocesses using various kinds of lignocellulosic biomass for lignocellulolytic enzyme production and enlarging the number of laccase producing strains for industrial application. At the same, the selection of suitable lignocellulosic biomass for novel isolated species secreting laccase has also been investigated.

EXPERIMENTAL

Materials

Fungus strain

Four newly isolated fungal strains, Han 386, Han 527, Han 751, and An 282, were used to compare the capacity of laccase secretion in this study. Han 386 was isolated from Tianluhu Forest Park, Guangzhou City, Guangdong Province, China. Han 527 was isolated from Tuoliang National Nature Reserve, Shijiazhuang City, Hebei Province, China. Han 751 was isolated from Maojingba National Nature Reserve, Chengde City, Hebei Province, China. An 282 was isolated from Daweishan Nature Reserve, Pingbian County, Honghe Prefecture, Yunnan Province, China. These strains were isolated on malt extract agar (MEA) medium (g/L, glucose 10, malt extract 20, KH_2PO_4 3, and agar 20) and purified on complete yeast medium (CYM) (g/L, glucose 20, peptone 2, yeast extract 2, $MgSO_4 \cdot 7H_2O$ 0.5, $K_2HPO_4 \cdot 3H_2O$ 1, KH_2PO_4 0.46, and agar 15). They were maintained by subculturing them on CYM by incubating the cultures at 26 °C for 7 days. Then, these strains were kept at 4 °C in the College of Life Science, Langfang Normal University, Langfang City, Hebei Province, China. These strains were used in this study due to their ability to grow on wood in nature.

Lignocellulosic biomass collection and treatment

Populus beijingensis and *Toona sinensis* were obtained from Langfang City (Hebei Province, China), while *Salix babylonica* and Sorghum straw were obtained from Chengde City (Hebei Province, China). All lignocellulosic biomass were milled. The particle passed through the 20-mesh screen and were retained on the 60-mesh screen, then air-dried.

Methods

Identification of the fungus

Mycelia of Han 386, Han 527, Han 751, and An 282 for DNA extraction were grown on CYM. After 7 days, mycelium was scraped using a clean surgical blade from the surface of the Petri plates cultivated microorganisms. The cetyltrimethylammonium bromide (CTAB) rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used for extracting genomic DNA, and the extraction process was followed the instruction with some modifications (Han *et al*. 2016, 2020a). Internally transcribing spacer (ITS) regions of ribosomal DNA (rDNA) from Han 386, Han 527, and Han 751 were amplified in this study with primer pairs ITS5 and ITS4 (Han *et al*. 2016), while the primers used for amplifying the ITS of An 282 were ITS1 and ITS4.

The polymerase chain reaction (PCR) schedule was carried according to Han *et al*. (2020a,b). Then, the PCR products were purified and sequenced by Beijing Genomics Institute (Beijing, China). Finally, the ITS sequences of above four strains were deposited to GenBank. Phylogenetic analysis followed Han *et al.* (2016). PAUP* version 4.0b10 (Swofford 2002) was used to perform maximum parsimony analysis. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985).

Inoculum preparation

All tested white-rot fungi were incubated at 26 °C on CYM agar medium. After 8 days, inoculants with a diameter of 5 mm were made using a hole punch. Then, 5 inoculants were added into 250-mL flasks containing 100 mL of CYM without agar and cultured on a rotary shaker with a speed of 150 rpm at 26 °C. After 7 days, cultures were gently homogenized (2 min, 5000 rpm) with a laboratory blender under sterile conditions. These homogenized suspensions were used as an inoculum.

Submerged fermentation

Erlenmeyer flasks (250 mL) containing 1 g *Populus beijingensis* were soaked with 100 mL of solution (g/L, $MgSO_4 \cdot 7H_2O$ 0.5, $K_2HPO_4 \cdot 3H_2O$ 1, KH_2PO_4 0.46) and autoclaved at 121 °C for 30 min. After autoclaving, the Erlenmeyer flasks were inoculated with 3 mL of the inoculum mentioned above and incubated at 26 °C under rotary conditions with a speed of 150 rpm for various time periods.

Sampling of crude enzyme

For obtaining the crude enzyme liquid, fermentation liquor in each inoculated flask at different growth times was filtered using Whatman No. 1 filter paper to remove the mycelia and lignocellulosic biomass, then centrifuged at 4 °C and 12000 rpm for 20 min. The supernatant was used for the laccase assay.

Determination of enzyme activity

Laccase activity was determined with an iMark™ Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA) by monitoring the increase in absorbance at 415 nm according to the methods of An *et al*. (2020b). One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μmol of 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) per min at pH 4.2.

Data analysis

Results were the mean values and standard deviations of three independent experiments. Two-way analysis was applied to determine the significant differences and a p-value less than the level of 0.05 indicated that the results were statistically significant. The analysis of statistical tests used the software SPSS 22.0 (IBM Corp., Armonk, NY, USA). The software Origin 2016 (OriginLab Corporation, Northampton, MA, USA) was used for generating the colorful figures.

RESULTS AND DISCUSSION

Identification of Novel Isolated Strains

Four novel isolated white-rot fungi strains used to produce laccase were classified and identified based on their nucleotide sequences. The ITS sequence number submitted to GenBank of Han 386, Han 527, Han 751, and An 282 strains was MW547890, MW547891, MW547892, and MW547893, respectively. According to the phylogenetic tree (Fig. 1) inferred from ITS sequences, tested strains Han 386, Han 527, Han 751, and An 282 belonged to 3 genera and 4 species, and identified as *Neofomitella fumosipora* (Corner) Y.C. Dai, Hai J. Li & Vlasák, *Pleurotus pulmonarius* (Fr.) Quél., *Coriolopsis trogii* (Berk.) Domański, and *Coriolopsis sanguinaria* (Klotzsch) Teng, respectively.

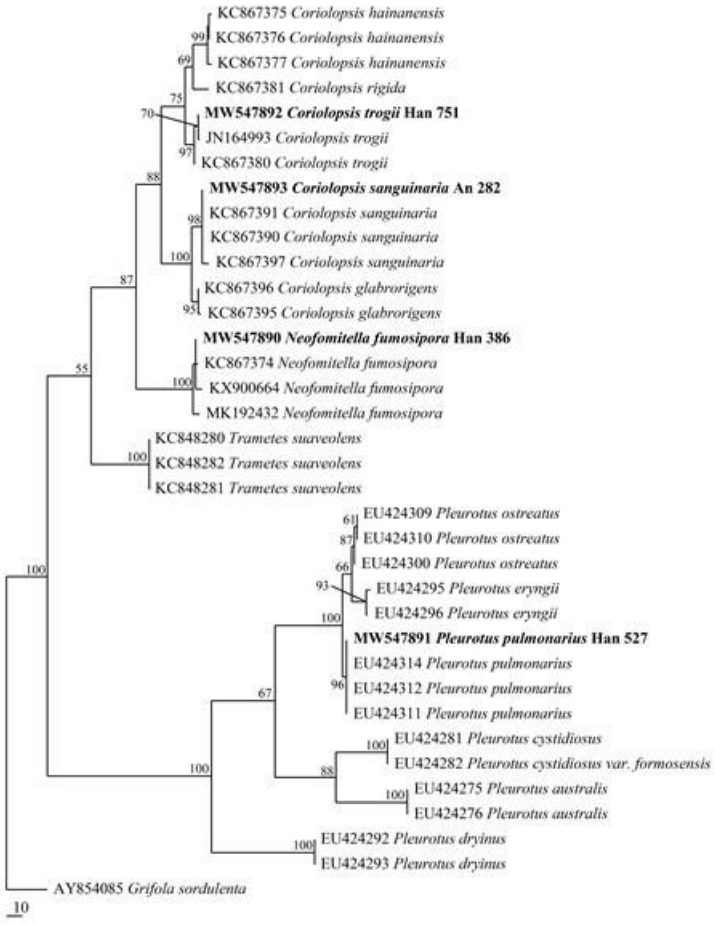

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of four novel isolated white-rot fungal strains based on ITS sequences. Branches are labeled with parsimony bootstrap proportions ⩾ 50%.

Results of Statistical Analysis

As shown in Table 1, species and lignocellulosic biomass significantly affected the laccase production throughout the fermentation phase ($P < 0.001$). Meanwhile, the effect of species with lignocellulosic biomass interactions on laccase production was also significant ($P < 0.001$).

Evaluation the Capacity of Laccase Secretion of Four Novel Isolated Fungal Strains

Laccase activity could be affected by many factors, including pH, metal ions, organic and inorganic compounds, temperature, fermentation method, and co-culture (Songulashvili *et al*. 2011; Sun *et al*. 2012; Kuhar and Papinutti 2014; Qin *et al*. 2019; Sharma *et al*. 2019; Lira-Perez *et al*. 2020; Han *et al*. 2021). Meanwhile, different species or strains belonging to the same species significantly affected laccase activity (Elisashvili *et al*. 2008; Janusz *et al*. 2015; An *et al*. 2016a; Han *et al*. 2017, 2018; Ikubar *et al*. 2018; An *et al*. 2020a, 2020b; Han *et al*. 2020b). There are many kinds of wild white-rot fungi in nature, and most of them grow on tree stumps of rotting or fallen trees, which causes the white decay of the trees. In the process of growth, white-rot fungi can secrete extracellular enzymes to degrade macromolecules and complex lignocellulose into small molecules for their own growth and reproduction (An *et al*. 2016b; Pinar *et al*. 2017; Filipe *et al*. 2019; Leite *et al*. 2019; Rajavat *et al*. 2020). Therefore, it is necessary to collect, isolate, and purify new laccase producing strains in their natural habitat. However, the study on laccase of the novel isolated white-rot fungal strains used in this article had hardly been reported previously. Therefore, it is important to evaluate the laccase secretion capacity of these strains, not only to develop new laccase producing strains, but also to lay a foundation for the production of high-quality laccase.

Table 1. Two-way ANOVA to Examine the Effects of Species, Lignocellulosic Biomass, and Species × Lignocellulosic Biomass Interactions on Laccase Activity

Incubation Period (d)	Species	Lignocellulosic Biomass	Species × Lignocellulosic Biomass
1	12016.379***	2346.957***	338.811***
2	2762.028***	214.220***	117.142***
3	2377.716***	95.151***	132.659***
4	1817.221***	620.308***	310.157***
5	1549.573***	144.775***	118.186***
6	1623.408***	152.727***	141.817***
7	9842.421***	772.763***	518.241***
8	6602.810***	362.888***	150.522***
9	2214.949***	207.709***	384.153***
10	6973.398***	656.951***	2007.677***
*Note: df = 3, 3, 9; *P < 0.05, **P < 0.01, ***P < 0.001			

Generally speaking, the ability of secreting laccase from four novel isolated strains *Neofomitella fumosipora* Han 386, *Pleurotus pulmonarius* Han 527, *Coriolopsis trogii* Han 751, and *Coriolopsis sanguinaria* An 282 on different lignocellulosic biomass was different (Figs. 2 through 5). Moreover, the trend of laccase activity of *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 was similar, and the trend of laccase activity of *C. trogii* Han 751 and *C. sanguinaria* An 282 was similar. Laccase activity of *N. fumosipora* Han 386 on Sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* was 68.72 ± 0.30 U/L, 73.34 ± 0.63 U/L, 115.43 ± 1.59 U/L, and 48.42 ± 0.92 U/L on the 1st day, respectively (Fig. 2). Laccase activity of *P. pulmonarius* Han 527 on Sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* was 14.57 ± 1.40 U/L, 21.00 ± 1.06 U/L, 53.35 ± 2.35 U/L, and 18.28 ± 1.71 U/L on the 1st day (Fig. 3).

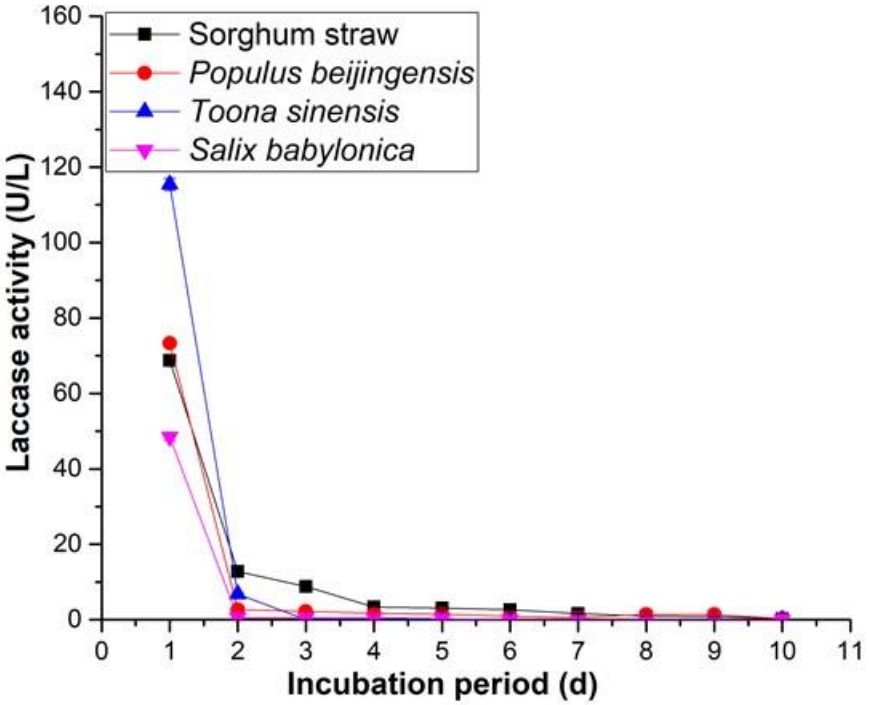

Fig. 2. Laccase activity from *Neofomitella fumosipora* Han 386 grown on *Populus beijingensis*, *Toona sinensis*, *Salix babylonica*, and Sorghum straw in submerged fermentation. Average values were taken through three individual parallel measurements.

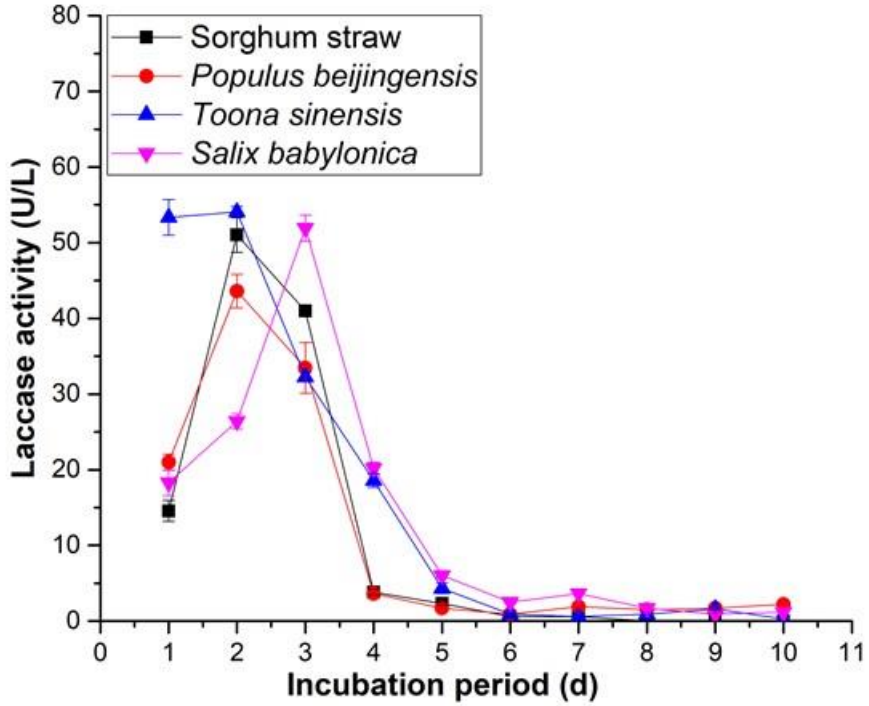

Fig. 3. Laccase activity from *Pleurotus pulmonarius* Han 527 grown on *Populus beijingensis*, *Toona sinensis*, *Salix babylonica*, and Sorghum straw in submerged fermentation. Average values were taken through three individual parallel measurements.

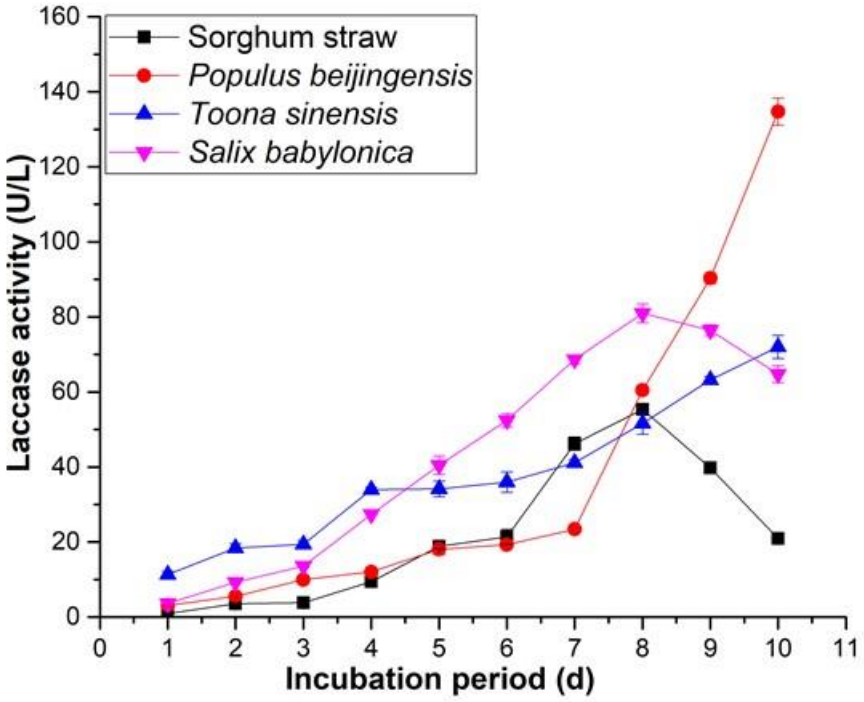

Fig. 4. Laccase activity from *Coriolopsis trogii* Han 751 grown on *Populus beijingensis*, *Toona sinensis*, *Salix babylonica*, and Sorghum straw in submerged fermentation. Average values were taken through three individual parallel measurements.

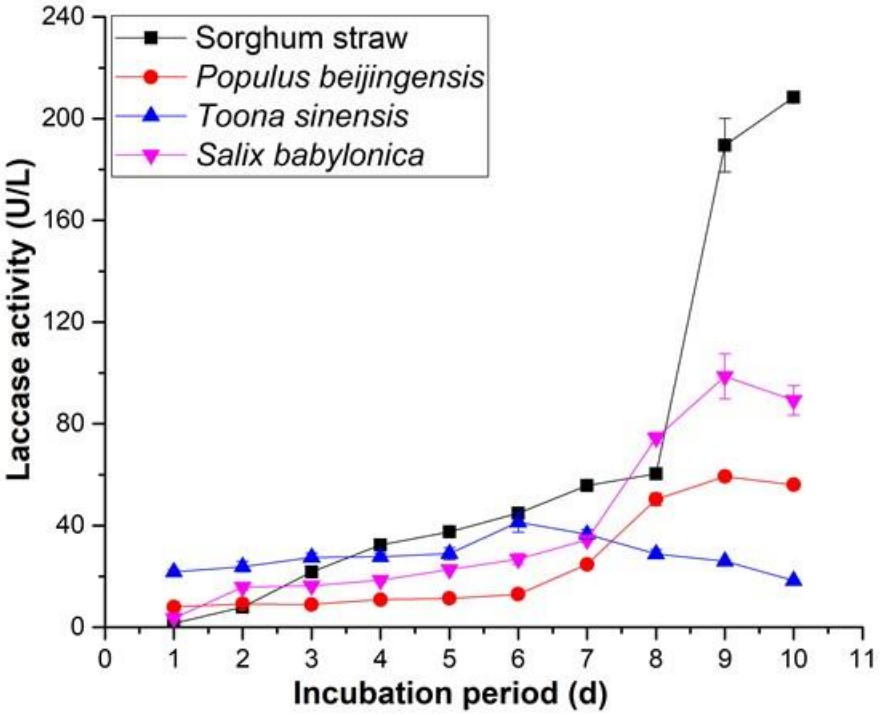

Fig. 5. Laccase activity from *Coriolopsis sanguinaria* An 282 grown on *Populus beijingensis*, *Toona sinensis*, *Salix babylonica*, and Sorghum straw in submerged fermentation. Average values were taken through three individual parallel measurements.

Laccase activity of *C. trogii* Han 751 on Sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* was 0.90 ± 0.00 U/L, 3.11 ± 0.17 U/L, 11.35 ± 0.92 U/L, and 3.62 ± 0.30 U/L on the 1st day, respectively (Fig. 4). Laccase activity of *C. sanguinaria* An 282 on Sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* was 1.51 ± 0.00 U/L, 8.04 ± 0.17 U/L, 21.80 ± 0.97 U/L, and 3.62 ± 0.30 U/L on the 1st day (Fig. 5). Obviously, the laccase secreted by *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 was significantly faster than that by *C. trogii* Han 751 and *C. sanguinaria* An 282 (Figs. 2 through 5). Meanwhile, the time of maximum laccase from *N. fumosipora* Han 386 on four kinds of lignocellulosic biomass tested occurred on the 1st day. To some extent, this phenomenon also indicated that *N. fumosipora* Han 386 secreted laccase rapidly compared to other tested strains in this study and showed the superiority in the rate of secreting laccase. In other previous studies of laccase from white rot fungi, it was rare to see a case of maximum laccase activity on the 1st day; most occurred after the 4th day *via* optimized treatment (Elissetche *et al*. 2007; Elisashvili *et al*. 2008; Songulashvili *et al*. 2008; Elisashvili and Kachlishvili 2009; Hilden *et al*. 2013; Moiseenko *et al*. 2018; Rodrigues *et al*. 2019; Sharma *et al*. 2019; An *et al*. 2020b; Han *et al*. 2020b). Based on this, the *N. fumosipora* Han 386 strain secreted laccase rapidly and showed great application prospects in various fields. Furthermore, the ability of *N. fumosipora* to secrete laccase was the not reported until now. Maximum laccase activity from *P. pulmonarius* Han 527 on Sorghum straw, *Populus beijingensis*, and *Toona sinensis* appeared on the 2nd day, while, on *Salix babylonica* it appeared on the third day (Fig. 3). Obviously, *P. pulmonarius* Han 527 also secreted laccase more rapidly than *C. trogii* Han 751 and *C. sanguinaria* An 282 strain (Figs. 3 through 5). A previous study indicated that maximum laccase activity of *P. ostreatus* CY 568 and CCEF 99 on poplar sawdust appeared on the 5th day and the 9th day (Han *et al*. 2020b). The time of maximum laccase activity from CCEF 89, CY 568, CCMSSC 00322, and CCMSSC 00406 on cottonseed hull was on the 6th day, 7th day, 6th day, and 7th day, respectively (An *et al*. 2020b). Thus, *P. pulmonarius* Han 527 could reach the peak of laccase activity in a short time compared to the *P. ostreatus* strains. Maximum laccase activity of another two strains, *C. trogii* Han 751 and *C. sanguinaria* An 282, belonging to the same genus of *Coriolopsis*, appeared after the 6th day (Figs. 4 and 5). The average 7-day activity of laccase from *Trichoderma asperellum* MR1, *T. virens* UKM1, *T. viride* MMS3, *Aspergillus niger* EFB1, *A. awamori* MMS4, and *A. fumigatus* SK1 on oil palm frond petiole was 3.08 ± 0.75 IU/g, 8.30 ± 0.92 IU/g, 2.30 ± 0.66 IU/g, 0.97 ± 0.90 IU/g, 1.03 ± 0.92 IU/g, and 3.13 ± 0.68 IU/g, respectively (Ikubar *et al*. 2018). Therefore, different strains belonging to the same species have various abilities of secreting laccase. The highest laccase from novel isolated strain *Pseudolagarobasidium* sp. PP17-33 was 5.841 U/g observed from oil palm wastes on solid-state fermentation (Thamvithayakorn *et al*. 2019). The maximum laccase activity from novel isolated white-rot fungi *N. fumosipora* Han 386, *P. pulmonarius* Han 527, *C. trogii* Han 751, and *C. sanguinaria* An 282 was 115.43 ± 1.59 U/L, 54.05 ± 0.76 U/L, 134.72 ± 3.55 U/L, and 208.36 ± 1.49 U/L (Table 2). Thus, the results were good for a certain strain based on the laccase activity of the tested novel strains under simple conditions.

When sorghum straw was used as the growth lignocellulosic biomass, the maximum laccase activity of *C. sanguinaria* An 282 was 208.36 ± 1.49 U/L, which was nearly 3.03-fold, 4.08-fold, and 3.76-fold more than that of *N. fumosipora* Han 386, *P. pulmonarius* Han 527, and *C. trogii* Han 751. Maximum laccase activity from *C. trogii* Han 751 grown on *Populus beijingensis* was 134.72 ± 3.55 U/L, which was higher than that from *N. fumosipora* Han 386, *P. pulmonarius* Han 527, and *C. sanguinaria* An 282,

by nearly 1.84-fold, 3.09-fold, and 2.27-fold, respectively. When *Toona sinensis* was used as the growth lignocellulosic biomass, maximum laccase activity of *N. fumosipora* Han 386 was 115.43 ± 1.59 U/L, nearly 2.14-fold, 1.60-fold, and 2.79-fold more than that of *P. pulmonarius* Han 527, *C. trogii* Han 751, and *C. sanguinaria* An 282. Maximum laccase activity from *C. sanguinaria* An 282 with *Salix babylonica* as the growth lignocellulosic biomass was 98.65 ± 8.80 U/L, which was higher than that of *N. fumosipora* Han 386, *P. pulmonarius* Han 527, and *C. trogii* Han 751, by nearly 2.04-fold, 1.90-fold, and 1.22-fold, respectively. This phenomenon indicated that each white-rot fungus was different in the degree of adaptation to the lignocellulosic biomass. Certainly, similar phenomenon was confirmed by previous studies (Metreveli *et al*. 2017; Huang *et al*. 2019).

Overall, four novel isolated strains showed their unique capacity of secreting laccase. Among them, *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 could reach their peak of laccase activity in shortened time. While, based on the maximum laccase activity, the ability of secreting laccase of *C. sanguinaria* An 282 was superior to the other tested strains. Every white-rot fungus was different in the degree of adaptation to the lignocellulosic biomass.

Table 2. Maximum Laccase Production, Lignocellulosic Biomass, and Time of Four Tested Fungi

Maximum Laccase Production (U/L)	Lignocellulosic Biomass	Fungi	Time (Day)
68.72 ± 0.30	Sorghum straw	*Neofomitella fumosipora* Han 386	1st
73.34 ± 0.63	*Populus beijingensis*	*Neofomitella fumosipora* Han 386	1st
115.43 ± 1.59	*Toona sinensis*	*Neofomitella fumosipora* Han 386	1st
48.42 ± 0.92	*Salix babylonica*	*Neofomitella fumosipora* Han 386	1st
51.03 ± 2.30	Sorghum straw	*Pleurotus pulmonarius* Han 527	2nd
43.60 ± 2.22	*Populus beijingensis*	*Pleurotus pulmonarius* Han 527	2nd
54.05 ± 0.76	*Toona sinensis*	*Pleurotus pulmonarius* Han 527	2nd
51.94 ± 1.71	*Salix babylonica*	*Pleurotus pulmonarius* Han 527	3rd
55.35 ± 0.76	Sorghum straw	*Coriolopsis trogii* Han 751	8th
134.72 ± 3.55	*Populus beijingensis*	*Coriolopsis trogii* Han 751	10th
72.03 ± 3.06	*Toona sinensis*	*Coriolopsis trogii* Han 751	10th
80.97 ± 2.53	*Salix babylonica*	*Coriolopsis trogii* Han 751	8th
208.36 ± 1.49	Sorghum straw	*Coriolopsis sanguinaria* An 282	10th
59.27 ± 0.35	*Populus beijingensis*	*Coriolopsis sanguinaria* An 282	9th
41.39 ± 4.01	*Toona sinensis*	*Coriolopsis sanguinaria* An 282	6th
98.65 ± 8.80	*Salix babylonica*	*Coriolopsis sanguinaria* An 282	9th
Data are presented as mean ± standard deviation for triplicates and are expressed as U/L.			

Evaluation of the Effect of Lignocellulosic Biomass on Laccase Activity

Agroindustrial residues provide substantial, economic, and renewable natural biological resources for large-scale, profitable biofuel production and types of chemical products including industrial biocatalysts (Birhanli and Yesilada 2013; Ikubar *et al*. 2018; Unuofin *et al*. 2019b; Agrawal and Verma 2020). Due to these advantages, more studies have focused on the effects of various lignocellulosic materials on laccase secretion of fungi or bacteria, especially white-rot fungi, in recent years (Simonic *et al*. 2010; Singh *et al*. 2010; Srinivasan *et al*. 2019; Thamvithayakorn *et al*. 2019; Unuofin *et al*. 2019b; Atilano-Camino *et al*. 2020; Shanmugam *et al*. 2020). Thus, sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* were used to grow white-rot fungi producing laccase.

Maximum laccase activity of *Neofomitella fumosipora* Han 386 on sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* was 68.72 ± 0.30 U/L, 73.34 ± 0.63 U/L, 115.43 ± 1.59 U/L, and 48.42 ± 0.92 U/L, respectively, and the time that the maximum was observed was on the 1st day (Fig. 2). Therefore, the maximum laccase activity on *Toona sinensis* was higher than that on sorghum straw, *Populus beijingensis*, and *Salix babylonica*, by nearly 1.68-fold, 1.57-fold, and 2.38-fold. The trend of laccase activity from *N. fumosipora* Han 386 on these lignocellulosic biomasses was similar and maintained low levels after the 3rd day (Fig. 2). In brief, four types of lignocellulosic biomass could promote the rapid secretion of laccase by *N. fumosipora* Han 386, especially the lignocellulosic biomass of *Toona sinensis*. Similar to *N. fumosipora* Han 386, *Pleurotus pulmonarius* Han 527 could rapidly secrete laccase at the beginning of the whole fermentation stage (Fig. 3). Maximum laccase activity of *P. pulmonarius* Han 527 on *Toona sinensis* was 54.05 ± 0.76 U/L, which was nearly 1.06-fold, 1.24-fold, and 1.04-fold higher than that on sorghum straw, *Populus beijingensis*, and *Salix babylonica* (Table 2). The maximum laccase activity on *Toona sinensis*, sorghum straw, *Populus beijingensis*, and *Salix babylonica* appeared on the 2nd, 2nd, 2nd, and 3rd day, respectively. Thus, these tested lignocellulosic biomasses were helpful to accelerate the production of laccase by *P. pulmonarius* Han 527, especially *Toona sinensis*. Different from *N. fumosipora* Han 386 and *P. pulmonarius* Han 527, the enzyme production of *Coriolopsis trogii* Han 751 mainly concentrated in the middle and late period, after approximately 5 days (Fig. 4). Maximum laccase activity of *C. trogii* Han 751 on Sorghum straw, *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* was 55.35 ± 0.76 U/L (on the 8th day), 134.72 ± 3.55 U/L (on the 10th day), 72.03 ± 3.06 U/L (on the 10th day), and 80.97 ± 2.53 U/L (on the 8th day), respectively. Thus, *C. trogii* Han 751 preferred to produce laccase on *Populus beijingensis* compared with the other three tested lignocellulosic biomass. The enzyme production trend of *Coriolopsis sanguinaria* An 282 was similar on the four kinds of lignocellulosic biomass (Fig. 5). Maximum laccase activity of *C. sanguinaria* An 282 on sorghum straw was 208.36 ± 1.49 U/L, which was higher than that on *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica* by nearly 3.52-fold, 5.03-fold, and 2.11-fold, respectively (Table 2). It can be seen that *C. sanguinaria* An 282 preferred to produce laccase by fermentation on sorghum straw compared with *Populus beijingensis*, *Toona sinensis*, and *Salix babylonica*. Elisashvili *et al*. (2008) reported that laccase activity of *P. tuber-regium* IBB 624 and *Lentinus edodes* IBB 123 grown on tree leaves was higher than that on wheat straw. Laccase activity of *F. trogii* using olive oil mill wastewater as an inducer was higher than cheese factory wastewater used as an inducer (Boran and Yesilada 2011). Compared with growing on powdered wheat straw, a powdered walnut shell was more suitable for *Funalia trogii* or *Trametes versicolor* secreting laccase (Birhanli and Yeşilada 2013). A previous study indicated that *P. ostreatus* and *Flammulina velutipes* strains fermented on cottonseed hull produced laccase better than on corncob or poplar wood (An *et al*. 2020b). Maximum laccase activity of *P. ostreatus* CCEF 99 grown on poplar sawdust was lower than that on corncob, and maximum laccase activity of *P. ostreatus* CY 568 grown on poplar sawdust was higher than that on corncob (Han *et al.* 2020b). All these studies also showed that different fungi preferred different substrates for laccase enzyme production. Meanwhile, these studies also indicate that different contents of lignin could affect laccase activity, but there is no clear linear relationship between lignin content and laccase activity. And, present study showed a similar result.

Overall, *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 preferred *Toona sinensis* to produce laccase, *C. trogii* Han 751 preferred to produce laccase on *Populus*

beijingensis, and *C. sanguinaria* An 282 grown on sorghum straw was more suitable for secreting laccase.

CONCLUSIONS

1. The biosynthetic potential in secreting enzymes of white-rot fungi was highly correlated with different species. The laccase secreted by *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 was significantly faster than that by *C. trogii* Han 751 and *C. sanguinaria* An 282.
2. The ability of *N. fumosipora* to secrete laccase was not reported until now. Maximum laccase from *N. fumosipora* Han 386 on the tested four kinds of lignocellulosic biomass appeared on the 1st day. To some extent, this phenomenon indicated that *N. fumosipora* Han 386 secreted laccase rapidly comparing with other tested strains in this study and showed the superiority in the rate of secreting laccase.
3. Based on the maximum laccase activity, the ability of secreting laccase of *C. sanguinaria* An 282 was superior to the other tested novel isolated strains.
4. Different fungi preferred different lignocellulosic biomass for laccase enzyme production. Overall, *N. fumosipora* Han 386 and *P. pulmonarius* Han 527 preferred *Toona sinensis* to produce laccase, *C. trogii* Han 751 preferred to produce laccase on *Populus beijingensis*, and *C. sanguinaria* An 282 grown on sorghum straw was more suitable for secreting laccase.

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