Effects of Different Induction Media as Inducers on Laccase Activities of *Pleurotus ostreatus* Strains in Submerged Fermentation

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Sequential submerged cultivation with different induction media as inducers of ligninolytic enzyme production by *Pleurotus ostreatus* strains was assessed by measuring laccase activities. An unconventional material, alkali lignin, was used for the first time as an inducer for different strains to enhance laccase activity. The *P. ostreatus* strains secreted similar but relatively high levels of laccase activity when the induction media contained alkali lignin with or without glucose. The laccase enzyme of different *P. ostreatus* strains in the different media exhibited large differences, and the wild strain YAASM 0568 exhibited enhanced production of laccase compared to other cultivated strains. The laccase activities of wild strain YAASM 0568 were nearly 3.4-, 3.3-, and 5.4-fold higher than that for cultivated strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336, respectively, when the induction media contained alkali lignin, inorganic salt, and vitamin B_1. In general, induction media containing alkali lignin with or without glucose were favorable for laccase secretion. The results revealed that the type of induction material and the nature of the fungus play important roles in the expression of ligninolytic enzymes. These findings would be helpful for selection of the appropriate type of strain and for optimization of integrated industrial ligninolytic enzyme production.

Keywords: Pleurotus ostreatus; Alkali lignin; Poplar wood; Induction medium; Laccase activity; Mycelial biomass

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INTRODUCTION

Laccases (*p*-diphenol: dioxygen oxidoreductase; EC 1.10.3.2) are glycoproteins that belong to a large and diverse family of blue multi-copper oxidases (Heinzkill *et al*. 1998; Tlecuitl-Beristain *et al*. 2008). These are also called blue enzymes, and they catalyze the oxidation of a wide range of phenolic compounds and aromatic amines using molecular oxygen as a terminal electron acceptor (Giardina *et al*. 2010; Pezzella *et al*. 2013). They are one of the oldest known classes of enzymes and were first described by Yoshida (1883). The enzymes are widely distributed in a variety of organisms such as plants, fungi, bacteria, and some insects (Mayer and Staples 2002; Giardina *et al*. 2010; Pardo *et al*. 2012). Moreover, laccase activity has also been demonstrated in *Basidiomycetes*, *Ascomycetes*, and *Deuteromycetes* (Bourbonnais *et al*. 1995; Gedikli *et al*. 2010). White rot fungi belonging to *Basidiomycetes* are the best-known producers of extracellular ligninolytic enzymes that are involved in lignin degradation, including manganese peroxidase (MNP), lignin peroxidase (LiP), and laccase (Pointing *et al*. 2000; Gomes *et al*. 2009). To date,

research on laccases has focused on fungi, especially white rot fungi. The typical white-rot fungus *Pleurotus ostreatus* (Jacq.) P. Kumm., a *Basidiomycete* fungus belonging to the genus *Pleurotus*, is one of the most important laccase producers worldwide. On account of their high non-specific oxidation capacity, laccases are used in numerous biotechnology applications, including pulp bleaching in the paper industry, dye decolorization, removal of phenolic compounds in food and beverages, biological detection, detoxification of environmental pollutants, wastewater treatment, and other miscellaneous applications (Arora and Sharma 2010; Piscitelli *et al*. 2010; Pezzella *et al*. 2013). Fungal laccases perform a variety of other key roles including lignin degradation, pigment biosynthesis, fruiting body formation, morphogenesis, and plant pathogenesis (Janusz *et al*. 2015; An *et al*. 2016a). Given this diverse applicability in industry, agriculture, and environmental and biological processes, laccases have been intensively studied since the 19th century. Because of the wide application base, large scale laccase production has become important from an economic standpoint despite the long standing technical difficulties in producing large amounts of laccase (Cardona *et al*. 2010).

Biotechnology processes typically depend on the use of a large number of low cost enzymes. In this context, the selection of appropriate induction media for fungal growth and enzyme production is one of the key factors in development of efficient processes (An *et al*. 2016a). Hence, research concerning the production of laccases is a topic of ongoing interest (Cohen *et al*. 2002; Giardina *et al*. 2010). On the other hand, the eco-friendly resources derived from lignocellulose has been paid much attention in recent years because the lignocellulose is abundant and renewable.

In basidiomycete fungi, extracellular laccases are produced in small amounts (Bollag and Leonowicz 1984). However, their production can be considerably stimulated by the presence of a wide variety of inducing substances, mainly aromatic or phenolic compounds related to lignin or lignin derivatives, such as ferulic acid, 2,5-xylidine, p-anisidine, and veratryl alcohol (Gianfreda *et al*. 1999). In addition, the expression of lignin-modifying laccases is affected by carbon and/or nitrogen sources and the concentrations, the concentration ratio, and the level of complexity of the media (Leatham and Kirk 1983; Asgher *et al*. 2012; Sulej *et al*. 2013; An *et al*. 2016b). Often, higher nitrogen levels are required to increase laccase formation (Gianfreda *et al*. 1999), but for certain organisms such as *Pycnoporus cinnabarinus* or *P. sanguineus* nitrogen-limiting culture conditions enhance the production of this enzyme (Eggert *et al*. 1996; Pointing *et al*. 2000). Whereas the increased production of laccase activity by fungi in response to aromatic and phenolic substances is well documented, the regulation of laccase expression by metals has only been reported recently. Among the various metals (Cu^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}), copper is one of the best known inducers of laccase activity (Karahanian *et al*. 1998; Baldrian and Gabriel 2002; Galhaup *et al*. 2002; Soden and Dobson 2001, 2003; An *et al*. 2016a). Laccase activity is not only affected by the type of metal ion, but is also affected by the concentration of the metal ion. In addition, the optimal metal ion concentrations for different fungi are different. For *Trametes pubescens*, the optimal Cu^{2+} concentration for laccase production was determined to be 1.5 mmol/L to 2.0 mmol/L (Galhaup and Haltrich 2001). The optimal Cu^{2+} concentrations for *T. multicolor* and *T. orientalis* were 0.5 mmol/L to 1 mmol/L and 2 mmol/L, respectively (Si *et al*. 2011). Besides the above factors, the strain is also a key consideration in laccase production (Boran and Yeşilada 2011; An *et al*. 2016b).

Previous studies have demonstrated that simple carbon and/or nitrogen sources, and even complex lignocellulosic biomass, could affect the production of laccase and hypha

biomass by the action of fungi. However, there have been no reports on the effects of alkali lignin as inducer on laccase activity and hypha biomass of fungi, particularly in white rot fungi. The aim of the present work was to study the effect of alkali lignin on the production of laccase and hypha biomass by different *P. ostreatus* strain cultures in a submerged fermentation (SF) method. Moreover, the differences between laccase production obtained from different *P. ostreatus* strains induced by alkali lignin or by simple carbon/nitrogen sources were investigated. Insights into laccase production derived from complex lignocellulose biomass as an inducer are also given.

EXPERIMENTAL

Materials

Microorganisms

Three white-rot basidiomycete *P. ostreatus* strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 were obtained from the China Center for Mushroom Spawn Standards and Control (CCMSSC) of the Chinese Academy of Agricultural Sciences (Beijing, China). One wild *P. ostreatus* strain YAASM 0568 was obtained from Yunnan Academy of Agricultural Sciences (Yunnan, China). All organisms were maintained in 2% (w/v) malt-extract agar (MEA) plates at 4 °C at the Institute of Microbiology, Beijing Forestry University.

Raw materials

Fresh poplar wood (*Populus beijingensis* W. Y. Hsu) obtained from the Forest Production Station of Beijing Forestry University (Beijing, China) was chopped into small pieces and air-dried. The samples were ground, and the particles of size between 20- and 80-mesh were prepared for subsequent use.

Induction medium

The induction medium used in the submerged fermentation method was divided into six categories, named IM 1, IM 2, IM 3, IM 4, IM 5, IM 6, and IM 7. The formulations were as follows: IM 1: contained alkali lignin 3 g/L, KH_2PO_4 3 g/L, vitamin B_1 0.03 g/L; IM 2: contained glucose 10 g/L, alkali lignin 3 g/L, KH_2PO_4 3 g/L, vitamin B_1 0.03 g/L; IM 3: contained peptone 2 g/L, alkali lignin 3 g/L, KH_2PO_4 3 g/L, vitamin B_1 0.03 g/L; IM 4: contained peptone 2 g/L, alkali lignin 3 g/L, KH_2PO_4 3 g/L; IM 5: contained glucose 10 g/L, peptone 2 g/L, alkali lignin 3 g/L, KH_2PO_4 3 g/L, vitamin B_1 0.03 g/L; IM 6: contained glucose 10 g/L, peptone 2 g/L, KH_2PO_4 3 g/L, vitamin B_1 0.03 g/L; and IM 7: contained poplar wood 3 g/L, KH_2PO_4 3 g/L, vitamin B_1 0.03 g/L.

Methods

Inoculum preparation

The different microorganisms were placed in 250 mL flasks containing 100 mL basic medium (10 g/L glucose, 20 g/L yeast extract, 3 g/L KH_2PO_4) and cultured on a rotary shaker at 26 °C at a speed of 150 rpm. After 6 days, the mycelial pellets were harvested and homogenized with a laboratory blender for 30 s at 5000 rpm. The suspension was used as an inoculum.

Culture conditions

The submerged fermentation (SF) was performed using a rotary shaker at 150 rpm and 26 °C in 250 mL flasks containing 100 mL of one of the above-mentioned induction media. All flasks containing induction media were autoclaved at 121 °C for 30 min. Three mL of homogenized mycelium were used to inoculate the flasks containing induction media.

To determine the dynamics of laccase activities under different induction medium conditions in SF, *P. ostreatus* culture liquid from the 1st day to the 10th day was obtained by filtering the culture through Whatman No. 1 filter paper, and the filtrate was then centrifuged (4 °C, 12000 rpm, 15 min). The supernatant was used for measurement of enzyme activity.

Enzyme and biomass assays

Enzyme activity was measured in the culture broth from each inoculated flask, at different growth times after removing the mycelia. All measurements were derived from three independent experiments and the mean values were taken. The standard deviations for the experiments were less than ± 10%.

Laccase (EC 1.10.3.2) activity was determined by changes in the absorbance at 420 nm related to the rate of oxidation of 1 mM 2,2'-azinobis-[3-ethyltiazoline-6-sulfonate] (ABTS) in 50 mM sodium acetate buffer (pH 4.2). The assay mixture was measured in a 3 mL cuvette with 50 μL of adequately diluted culture liquid (Woolfenden and Wilson 1982). One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute ($\varepsilon_{420} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$).

To determine the biomass dynamics under different induction medium conditions in SF, *P. ostreatus* cultures from the 1st day to the 10th day were filtered by Whatman No. 1 filter paper, washed 3 times with distilled water, and oven-dried at 60 °C for 24 h.

Statistical analysis

To examine the effects of treatments and strains on laccase activities, 2-way analysis of variance followed by the Tukey post hoc test was applied to these variables, with treatments and strains being fixed factors (PROC GLM, IBM SPSS software version 22.0; Armonk, NY, USA). All statistical figures were generated using the program Origin Pro 8.0 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

Effect of Induction Medium on Production of Laccase Enzymes and Mycelium Biomass

As an inducible oxidoreductase, laccase is necessary for lignin degradation by white-rot fungi such as *Ganoderma lucidum*, *G. applanatum*, *Fomes fomentarius*, and *Pleurotus ostreatus* (Tien and Kirk 1983; Badalyan and Sakeyan 2004; An *et al*. 2016a). Among these fungi, *P. ostreatus* is a white-rot basidiomycete that has a ligninolytic system that consists of laccase and manganese peroxidase (Baldrian and Gabriel 2002). Many studies have been performed on the effect of nutrient medium on enzyme production (Buswell *et al.* 1995; Stajić *et al.* 2006; Elisashvili *et al.* 2008a,b; Membrillo *et al.* 2008; Isikhuemhen and Mikiashvilli 2009). These studies indicated that fungal culture conditions, including carbon/nitrogen (C/N) sources and heavy metals, affect fungal physiology and

ligninolytic enzyme expression (Janusz *et al*. 2015; An *et al*. 2016b). Most studies have focused on substituting the nitrogen or carbon source, including even the complex lignocellulosic substrate. Fewer articles have considered the effect of pure lignin as a substrate inducer with existing C/N sources or even lacking a simple C/N source. Also, there are no articles on the differences in laccase activity obtained from alkaline lignin or renewable lignocellulose biomass as the inducer. Thus, in this research a comparative study of laccase production by *Pleurotus* strains in lignocellulose biomass and various carbon/nitrogen sources with alkali lignin was conducted. While monitoring the extracellular laccase activities of *Pleurotus* strains during the SF of induction medium containing alkali lignin, most strains showed high laccase expression after 1 day of incubation, except for one, the *P. ostreatus* strain CCMSSC 00406. This suggested the presence of alkali lignin was helpful for improving laccase activity and for accelerating the rate of enzyme production (Fig. 1).

Table 1. Effects of Strains, Media, and Strains × Media Interactions on Laccase Activities of *Pleurotus ostreatus* (Two-Way ANOVA)

Incubation Period (d)	Laccase Activity (U/L)		
	Strain	Media	Strain × media
1	1215.1***	1288.7***	255.6***
2	69.8***	934.8***	203.4***
3	98.8***	847.1***	397.1***
4	255.1***	593.7***	339.6***
5	640.9***	486.2***	273.2***
6	2002.1***	1117.7***	615.7***
7	1683.9***	871.9***	517.5***
8	2473.3***	1312.9***	1145.9***
9	4004.5***	2002.5***	2077.4***
10	1039.2***	645.3***	674.3***

Note: df = 3, 6, 18; *P < 0.05, **P < 0.01, ***P < 0.001

Table 2. Effects of Strains, Media, and Strains × Media Interactions on Mycelium Biomass of *Pleurotus ostreatus* (Two-Way ANOVA)

Incubation Period (d)	Mycelium Biomass (g)		
	Strain	Media	Strain × media
1	188.466***	595.2***	63.5***
2	64***	846.4***	53***
3	30.2***	909.9***	43.7***
4	39.8***	854.2***	46.9***
5	55.6***	966.5***	47.6***
6	90.5***	1001.2***	58.4***
7	125.6***	1173.8***	69***
8	77.3***	774.5***	50.2***
9	134.7***	826.4***	51.4***
10	126.7***	587.3***	37.6***

Note: df = 3, 6, 18; *P < 0.05, **P < 0.01, ***P < 0.001

Table 3. Maximum Laccase Activities, Culture Condition, Strains, and Time of Different *Pleurotus ostreatus* Strains

Maximum Laccase Activity （U/L）	Culture Condition	Strain	Time (day)
49.1 ± 3.2	IM 1	CCMSSC 00322	1 th
51.3 ± 0.4	IM 1	CCMSSC 00406	1 th
168.8 ± 10.9	IM 1	YAASM 0568	6 th
31.1 ± 1.9	IM 1	CCMSSC 00336	3 th
63.6 ± 5.1	IM 2	CCMSSC 00322	1 th
54.7 ± 4.6	IM 2	CCMSSC 00406	2 th
105.3 ± 4.5	IM 2	YAASM 0568	8 th
49.8 ± 3.8	IM 2	CCMSSC 00336	2 th
15.4 ± 0.5	IM 3	CCMSSC 00322	3 th
-	IM 3	CCMSSC 00406	-
10.1 ± 0.3	IM 3	YAASM 0568	6 th
32.7 ± 1.6	IM 3	CCMSSC 00336	6 th
25.8 ± 2.0	IM 4	CCMSSC 00322	2 th
-	IM 4	CCMSSC 00406	-
6.0 ± 0.4	IM 4	YAASM 0568	6 th
12.9 ± 0.7	IM 4	CCMSSC 00336	3 th
29.5 ± 1.5	IM 5	CCMSSC 00322	1 th
-	IM 5	CCMSSC 00406	-
20.3 ± 2	IM 5	YAASM 0568	6 th
26.7 ± 2.2	IM 5	CCMSSC 00336	4 th
12.5 ± 0.5	IM 6	CCMSSC 00322	8 th
-	IM 6	CCMSSC 00406	-
-	IM 6	YAASM 0568	-
-	IM 6	CCMSSC 00336	-
82.3 ± 1.8	IM 7	CCMSSC 00322	7 th
58.2 ± 2.5	IM 7	CCMSSC 00406	4 th
139.1 ± 3.4	IM 7	YAASM 0568	6 th
41.2 ± 0.4	IM 7	CCMSSC 00336	6 th

Data are presented as mean ± standard deviation for triplicates and are expressed as U/L. IM 1 = Induction Medium 1; IM 2 = Induction Medium 2; IM 3 = Induction Medium 3; IM 4 = Induction Medium 4; IM 5 = Induction Medium 5; IM 6 = Induction Medium 6; IM 7 = Induction Medium 7

As shown in Tables 1 and 2, the effect of the induction medium on laccase activities and mycelium biomass of *P. ostreatus* was very significant. When induction medium 1 (IM 1) was used as the growth medium, *P. ostreatus* secreted almost the same relatively high levels of laccase activity compared with the laccase activity from other induction media. The maximum laccase activities were up to 49.1 ± 3.2 U/L, 51.3 ± 0.4 U/L, 168.8 ± 10.9 U/L, and 31.1 ± 1.9 U/L for CCMSSC 00322, CCMSSC 00406, YAASM 0568, and CCMSSC 00336, respectively (Table 3). The maximum laccase activity occurred after 1 d, 1 d, 6 d, and 3 d for CCMSSC 00322, CCMSSC 00406, YAASM 0568, and CCMSSC 00336, respectively (Table 3). Clearly, the laccase activity observed for strain YAASM 0568 was much greater than that observed for strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336, being nearly 3.4-fold, 3.3-fold, and 5.4-fold greater, respectively. When IM 2 was used as the growth medium, three cultivated *P. ostreatus* strains secreted almost the same high levels of laccase activity compared with the laccase activity from other conditions, including even that of IM 1. The trend of laccase activity obtained from strain YAASM 0568 was run in the opposite direction comparing with the trend of laccase

activity from strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336. The maximum laccase activities were up to 63.6 ± 5.1 U/L, 54.7 ± 4.6 U/L, 105.3 ± 4.5 U/L, and 49.8 ± 3.8 U/L for CCMSSC 00322, CCMSSC 00406, YAASM 0568, and CCMSSC 00336, respectively (Table 3), the maximum values occurring after 1 d, 2 d, 8 d, and 2 d, respectively.

The laccase activities observed from IM 3, IM 4, IM 5, and IM 6 were clearly lower than those for IM 1, IM 2, and IM 7 (Fig. 1). In addition, no laccase activity was detected in the case of CM 6 for strains, CCMSSC 00406, YAASM 0568, and CCMSSC 00336 (Table 3). At the same time, the laccase activity from strain CCMSSC 00322 was also very low (Table 3). The maximum laccase activities were up to 82.3 ± 1.8 U/L, 58.2 ± 2.5 U/L, 139.1 ± 3.4 U/L, and 41.2 ± 0.4 U/L for CCMSSC 00322, CCMSSC 00406, YAASM 0568, and CCMSSC 00336, respectively (Table 3). The maximum values occurred after 1 d, 2 d, 8 d, and 2 d, respectively. This result showed that poplar wood affected the secretion of laccase. Similar results have been found in previous studies. A previous study showed that the laccase activity of the *P. ostreatus* strain CCEF 89 was probably less than 100 U/L when poplar wood, corncob, or cottonseed were used as the inducer in SF (An *et al*. 2016a). However, laccase production for the *P. ostreatus* strains CCEF 89 and CCEF 99 led to a slow accumulation process when poplar wood, corncob, or cottonseed with complete yeast media as an inducer in SF was used (Han *et al.* 2017). However, in the present study, the maximum laccase activity of strain YAASM 0568 was up to 168.8 ± 10.9 U/L in a relatively short period of time when alkali lignin was used as the inducer. Although the alkali lignin had been used as an induction material for the growth of *G. lucidum*, *Polyporus brumalis*, *Polyporus ciliates* and *Trametes versicolor*, the laccase activity was not measured on media supplemented with alkali lignin (Sitarz *et al.* 2013). Besides, scanning electron microscope (SEM) images of the alkali lignin before and after treatment with laccase (5%) and methyl syringate (MS) mediator in aerobic environment had been reported, but the laccase activity affected by alkali lignin was still not studied (Yao *et al.* 2017). Hence, the present study is the first study which has reported promising results on basidiomycetes when using alkali lignin as an induction material.

Clearly, the appearance times for maximum laccase activity by strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 from IM 1-5 were earlier than that for the group IM 6. Furthermore, alkaline lignin was one of the ingredients of IM 1-5, hence suggesting that alkaline lignin is beneficial for *P. ostreatus* secreting laccase enzymes.

Out of the four basidiomycetes fungi tested, *G. lucidum*, *Polyporus brumalis*, *P. ciliates*, and *T. versicolor*, only *G. lucidum* exhibited significant growth on malt extract medium or minimal medium with alkali lignin (Sitarz *et al.* 2013). And the hyphae biomass could be detected in all induction medium with alkali lignin in the present study. The hyphae biomass of IM 1 and 2 followed a trend similar to laccase activity (Figs. 1, 2). The hyphae biomass for IM 2 was greater than that for IM 1. The only difference between the two induction media was that IM 1 contained glucose. One plausible explanation for this result might be that the presence of glucose could promote the growth of mycelium (An *et al.* 2016b). Although simple carbon sources such as glucose are often found to repress genes which are used in the metabolism of alternative carbon sources to induce an energy-saving response (Galhaup *et al.* 2002; An *et al.* 2016b), laccase synthesis was repressed only when glucose above a certain concentration was used with the fungus (Galhaup *et al.* 2002; Mikiashvili *et al*. 2004). As long as the glucose concentration in the medium never exceeded a certain low and critical value, glucose repression could be avoided, and the production of laccase was almost doubled compared with batch cultivation (Galhaup *et al.*

2002). This finding indicates that the presence of glucose in the medium is advantageous to the mycelial biomass accumulation to some degree, which in turn is beneficial to the secretion of laccase by *P. ostreatus*.

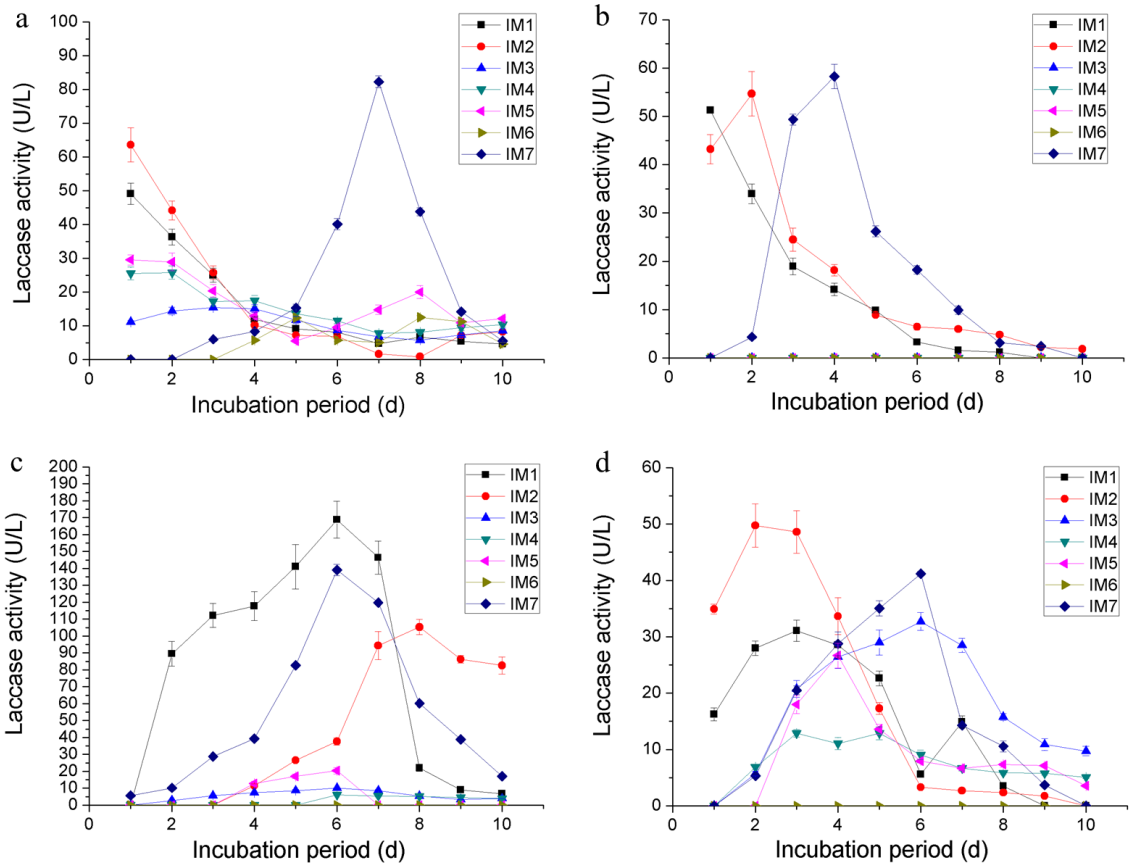

Fig. 1. Effect of different induction media on laccase production by four *Pleurotus ostreatus* strains grown over a period of 10 days (a strain: *P. ostreatus* CCMSSC 00322; b strain: *P. ostreatus* CCMSSC 00406; c strain: *P. ostreatus* YAASM 0568; d strain: *P. ostreatus* CCMSSC 00336). Average values were calculated from individual measurements for each of three parallel cultures of all strains. IM1 = induction medium 1; IM2 = induction medium 2; IM3 = induction medium 3; IM4 = induction medium 4; IM5 = induction medium 5; IM6 = induction medium 6; IM7 = induction medium 7.

Depending on the fungus and the induction material, supplementation of additional nitrogen sources in the medium affects the yield of lignocellulolytic enzymes such as laccase, manganese peroxidase, and it also affects filter paper activity (Elisashvili *et al*. 2008a,b; Elisashvili *et al*. 2015). As a result, high nitrogen levels were required to obtain large amounts of laccase; this is consistent with the importance of the nitrogen source and the need for high nitrogen levels for laccase formation (Galhaup *et al.* 2002). Interestingly, although the mycelium biomass values obtained from IM 3 by the four strains of *P. ostreatus* were more than those obtained from IM 1 and 2, the trend for laccase activity differed from the trend of mycelium biomass for IM 3. That is to say, the laccase activity for IM 3 by the four strains of *P. ostreatus* was lower than that for IM 1 and 2, and laccase activity was not even detected in the case of strain CCMSSC 00406. The same conclusion also applied for the other induction medium compared to IM 1 and 2 in this study. This

suggested that the presence of exogenous peptone sources in the induction media can promote the mycelial biomass accumulation of *P. ostreatus*, but cannot enhance laccase activity. Besides, the mycelial biomass for IM 5 was much more than that for IM 6, the difference in these two media being the presence/absence of alkaline lignin, respectively. Similarly, the existence of alkaline lignin was conducive to the accumulation of mycelial biomass.

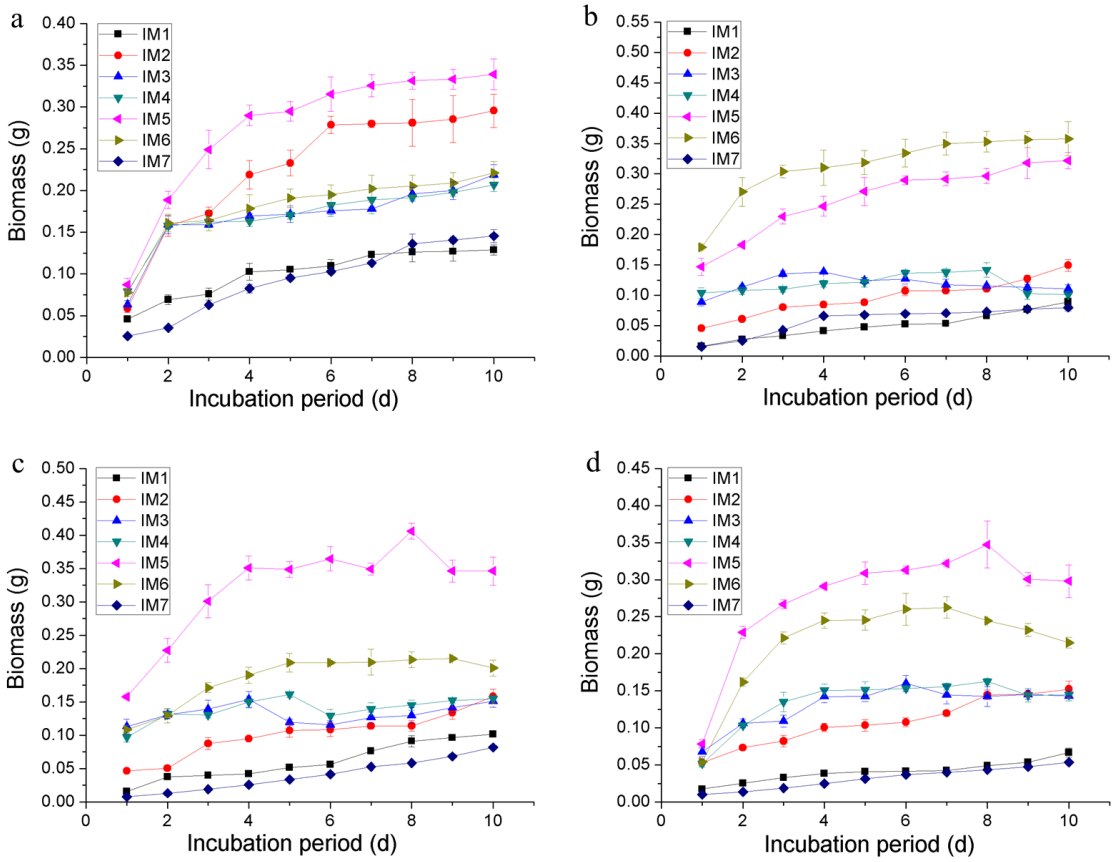

Fig. 2. Effect of different induction media on mycelium biomass by four *Pleurotus ostreatus* strains grown over a period of 10 days (a strain: *P. ostreatus* CCMSSC 00322; b strain: *P. ostreatus* CCMSSC 00406; c strain: *P. ostreatus* YAASM 0568; d strain: *P. ostreatus* CCMSSC 00336). Average values were calculated from individual measurements for each of three parallel cultures of all strains. IM1 = induction medium 1; IM2 = induction medium 2; IM3 = induction medium 3; IM4 = induction medium 4; IM5 = induction medium 5; IM6 = induction medium 6; IM7 = induction medium 7.

Effect of Strains on Production of Laccase Enzymes and Mycelium Biomass

Various studies have examined the correlations between the capacity of lignocellulose degradation and fungal cultivation conditions (Elisashvili *et al*. 2008a,b; Zhang *et al*. 2010; Janusz *et al*. 2015; An *et al*. 2016b). However, there is limited knowledge on the effects of different strains on fungal lignocellulose-degrading capacities or fungal laccase-secreting capacities. In this study, the effect of different strains of *P. ostreatus* on laccase activities and mycelium biomass was very significant, as shown in Tables 1 and 2. The cultivated strains CCMSSC 00322, CCMSSC 00406, CCMSSC 00336, and the wild strain YAASM 0568 showed their unique capacities for secreting laccase and

accumulating hyphae (Figs. 1 and 2). However, the laccase activities for the cultivated strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336 were consistently low relative to that of the wild strain YAASM 0568 (Fig. 1). The maximum laccase activities for the three cultivated strains ranged from about 31.1 ± 1.9 U/L to 63.6 ± 5.1 U/L for IM 1 or 2 after 1 d to 3 d. The maximum laccase activity of the wild strain ranged from about 105.3 ± 4.5 U/L to 168.8 ± 10.9 for the same induction media after 6 d to 8 d (Table 3). Clearly, all these strains have their own individual characteristics. Although the maximum laccase activities of the cultivated strains were lower than that of the wild strain, the times to attain the maximum laccase activities for the cultivated strains were shorter than that of the wild strain. In other words, the laccase activity of the wild strain YAASM 0568 was a relatively slow accumulation process in contrast to that of the cultivated strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336. In terms of accelerating the laccase accumulation process, this suggested that alkaline lignin is less effective in inducing the wild strain YAASM 0568 to secreting laccase in comparison to that for the cultivated strains. The maximum laccase activity of the cultivated strains rose in going from IM 1 to IM 2, while the opposite trend occurred for the wild strain. However, with respect to biomass production, in comparing results for IM 1 with IM 2, no differences were detected for the four strains. When IM 3, 4, 5, and 6 were used to culture strain CCMSSC 00406, no laccase activity was detected from 1 d to 10 d, while the mycelial biomass maintained a stable growth trend throughout this period. One possible explanation for this phenomenon was the existence of peptones which inhibited or restricted laccase secretion by this strain. However, in the case of laccase activity from the wild strain YAASM 0568, this was lower than that from the three cultivated strains when the induction medium contained an exogenous nitrogen source, *e.g.*, a peptone. This clearly shows the differences in sensitivity for nitrogen sources by these strains. The laccase activity of YAASM 0568 from IM 7 was higher than that from other three strains, but the mycelium biomass from IM 7 was also consistently low compared with other three strains. The maximum laccase activities of strain CCMSSC 00322 and CCMSSC 00406 from IM 7 were slightly higher than that from other induction medium, but the occurrence time of maximum laccase activities from IM 7 was later than that from other induction medium (Fig. 1). However, the maximum laccase activities of strain YAASM 0568 and CCMSSC 00336 from IM 7 were lower than that from other induction medium.

CONCLUSIONS

1. *Pleurotus ostreatus* strains showed exceptional potential for laccase synthesis through conventional SF with alkaline lignin as the inducer. Both the nature of the fungus and the type of the induction material are critical for control of the expression of ligninolytic enzymes by fungi.
2. The combination of alkaline lignin and glucose as an additional carbon source for enhancing laccase activity was shown to be superior to that of alkaline lignin and peptone as an additional nitrogen source.
3. The existence of poplar wood was conducive to secreting laccase by *P. ostreatus* strains, but that is a slow process of accumulating laccase. While the growth of *P. ostreatus* strains in an induction medium containing alkaline lignin with/without additional

glucose as a carbon source resulted in enhanced enzyme activity within a short period of time. This phenomenon was clearly evident in the case of the cultivated strains.

4. The maximum laccase activity of wild strain YAASM 0568 was nearly 3.4-fold, 3.3-fold, and 5.4-fold higher than that for the domestic cultivated strains CCMSSC 00322, CCMSSC 00406, and CCMSSC 00336, respectively, when the induction media contained alkali lignin, inorganic salt, and vitamin B1.

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