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ORIGINAL PAPER

A newly isolated *Paecilomyces* sp. WSH-L07 for laccase production: isolation, identification, and production enhancement by complex inducement

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Abstract Laccase can catalyze the oxidation of a wide range of organic and inorganic substrates. In this study, an easily detectable method was employed for screening laccase-producing microorganisms by using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as laccase-secretion indicator. A novel laccase-producing strain was isolated and identified as *Paecilomyces* sp. WSH-L07 according to the morphological characteristics and the comparison of internal transcribed spacer (ITS) ribosomal DNA (rDNA) gene sequences. In further investigation, the production of laccase by *Paecilomyces* sp. WSH-L07 was greatly enhanced by the nontoxic inducers of copper sulphate and methylene blue. Under the induction of 50 μM copper sulphate and 20 μM methylene blue, the maximum laccase production was obtained. When these inducers were added into cultivation medium at 24 h and 12 h, respectively, an increment of about 100 times of laccase activity compared with that of in inducer-free medium and about two times of that of in single copper-supplemented medium was observed. Compared with other *Paecilomyces* species, *Paecilomyces* sp. WSH-L07 exhibit the better laccase-producing characteristics with an activity of 1,650 U/l on the eighth day, suggesting its potential ability for industrial application.

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Introduction

Laccases (*p*-diphenol:dioxygen oxidoreductase; EC 1.10.3.2) belong to the group of multicopper oxidases containing four copper ions per molecule distributed in three different copper-binding sites [25]. Laccases use molecular oxygen as an electron acceptor to catalyze the oxidation of a wide range of organic and inorganic substrates, including *o,p*-diphenols, aminophenols, polyphenols, polyamines, lignin, some inorganic ions, aryl diamines, and methoxyphenol acid [25]. Due to their broad substrate spectrum, laccases have attracted increasing attention in industrial and environmental fields, such as pulp delignification, dye decolorization, environmental pollutant detoxification, biopolymer modification, and biotransformation [8, 13, 17, 20, 25].

Laccases were first detected in the juice of the lacquer tree, *Rhus vernicifera*, and were sequentially found in fungi, insects, and bacteria, whereas the most popular laccase producers were white-rot fungi [1, 6, 7, 9, 16]. In recent years, some other potential laccase producers were found, including different genera of basidiomycetes, ascomycetes, and some deuteromycetes. A newly isolated deuteromycete fungus. *Pestalotiopsis* sp., gave the maximum laccase activity of 32.7 U/ml in glucose–ammonium tartrate medium supplemented with 2.0 mM Cu, which was comparable with that of many white-rot fungi [8]. *Paecilomyces* species have also proven to be laccase producers. Two *Paecilomyces inflatus* strains were first reported by Kluczek-Turpeinen et al. [11], giving about 30 U/l laccase activity after 4 weeks of cultivation in the compost extract.

Later, Liang et al. [14] reported a strain of *Paecilomyces biformis* that exhibited a colored zone on potato dextrose agar (PDA) medium containing *o*-methoxyphenol, indicating laccase secretion.

Although most laccases are constitutively produced in a small amount, the production of laccases could be significantly enhanced by a wide variety of substances, including metal ions, aromatic compounds, and dyes [3, 7, 15, 23]. Tavares et al. [22] reported a cooperative effect between the inducers on laccase production, and nearly 104 times enhancement of laccase activity (759.8 U/l per day) was obtained using complex-inducer-supplemented (copper, xylidine, and phenolic mixture) medium. However, most potent laccase inducers, such as aromatic compounds, are volatile, toxic, and expensive. Furthermore, laccase production generally requires long fermentation time, which is still not appreciated for industrial applications. A *Trametes versicolor* laccase was reported in efficient decolorization of synthetic dyes, but it required a fermentation time as long as 15–20 days, with laccase activity of 90–100 U/l [24]. Thus, it is urgent to exploit novel potential laccase producers with short fermentation time and improved laccase production, as well as nontoxic compounds as potent inducers.

In this study, a deuteromycete *Paecilomyces* sp. WSH-L07 was isolated from lignin-containing samples by the selective medium supplemented with common laccase substrate 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as indicators. The time course of cultivation was studied to analyze the properties of laccase secretion by *Paecilomyces* sp. WSH-L07 and put forward possible strategies to improve laccase production in future studies. In addition, the inductive effects of potential inducers as well as the cooperative effect with copper sulphate on laccase production were investigated, and the positive cooperative induction effect by copper sulphate and methylene blue was found. Optimization of complex inducers (copper sulphate and methylene blue) concentrations and addition time was also performed.

Materials and methods

Microorganism, media, and culture conditions

Microorganisms were isolated from various lignin-containing sources, such as soil samples, rotten wood, and decaying wheat straw, and maintained on PDA slants (potato extract 200 g/l, glucose 20 g/l, agar 20 g/l) at 30°C. After 6 days of incubation, spores were washed with sterilized physiological sodium chloride solution and diluted to a suspension with 1×10^6–10^7 spores/ml. The prepared spore suspensions (2 ml) were used as the standard inoculums for laccase production in submerged cultivation. The modified PDA medium (PDA–ABTS) used for preliminary screening of laccase-active microorganisms was supplemented with 0.5 g/l ABTS (Sigma Chemical Co., USA) into PDA medium before autoclaving. The yeast extract peptone dextrose (YPD) medium, containing glucose 20 g/l, peptone 5 g/l, and yeast extract 2 g/l, was supplemented with 100 μM copper sulphate as the basal medium (YPD–Cu) for laccase production. The YPD–ABTS medium was employed to monitor the start of laccase secretion in submerged cultivation, in which 0.5 g/l ABTS was added into YPD–Cu medium before autoclaving. The pH of media above was adjusted to 6.5 before autoclaving. Flask experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml YPD–Cu or YPD–ABTS medium cultivated at 30°C and 150 rpm on a rotary shaker.

Screening for laccase-producing stains

Preliminary screening of laccase-producing strains was performed based on the intensity of the dark-green dye according to Danneel et al. [4]. The lignin-containing samples from field soil, rotten wood, and decaying wheat straw were sieved (2-mm mesh) and homogenized, and 5 g of samples were added into 250-ml flasks containing 20 ml sterilized physiological sodium chloride solution and glass beads and kept on a rotary shaker for 30 min for full distribution. The prepared suspensions were spread onto PDA–ABTS plates and incubated at 30°C. Laccase secreted into PDA–ABTS medium by positive strains could oxidize ABTS to a green-colored compound. Colonies with obvious dye zones were inoculated onto PDA slants and incubated at 30°C for 6 days. Secondary screening was then developed by incubating the above-mentioned selected strains into YPD–ABTS and YPD–Cu media in duplicate, respectively. YPD–ABTS medium was used to visualize the start of laccase secretion, and the supernatant of YPD–Cu medium cultured for 5–11 days was assayed for laccase activity.

Characteristics and identification of strain S2

The isolated strain was inoculated onto PDA plates and cultured at temperatures in the range of 25−55°C to check its optimal growth temperature and thermal tolerance. The morphological characteristics of the isolated strain on agar plates were observed, and the sporulation of the isolated strain was determined by the change of colony color and optical microscopic observation. In addition, comparison of the internal transcribed spacer (ITS) ribosomal DNA (rDNA) gene sequence was employed. Genomic DNA of the isolated strain was extracted with Universal Genomic DNA Extraction Kit. The gene encoding ITS regions was amplified by Fungi Identification polymerase chain reaction

(PCR) kit. After purification of the amplified products by agarose gel DNA purification kit, ITS rDNA gene was sequenced in both directions with ITS forward primer and ITS reverse primer. The resulting sequence was compared with others in the GenBank database and analyzed by Clustalx 2.0 and Phylip 3.68 software. All kits and primers were purchased from Takara Biotechnology (Dalian, China).

Effect of inducers on laccase production

Guaiacol, *p*-hydroxybenzoic acid, *p*-aminobenzoic acid, dimethylaniline, *o*-phenylene diamine, phloroglucinol, phenol red, bromothymol blue, and indigo were dissolved in ethanol; catechol, orcinol, gallic acid, *p*-anisidine, dimethyl phthalate, methyl orange, methylene blue, safranine T, eriochrome black T, rhodamine B, fuchsin basic, chromotropic acid, and gallocyanine were dissolved in deionized water. All reagents were of analytical−reagent grade and obtained from Sinopharm Chemical Reagent (Shanghai, China). The compounds were added into YPD medium and YPD–Cu medium at the beginning of incubation to investigate the inductive effect of different inducers and the cooperative effect with copper sulphate (used as complex inducers) on the production of laccase by the isolated strain, respectively. The concentrations of compounds were 100 μM for aromatic compounds and 10 μM for dye compounds. The sample cultured in YPD medium was used as control. The strategy, which changed one parameter and kept the rest of the parameters at the optimal levels, was employed to optimize the concentration and addition time of complex inducers (cooper sulphate and methylene blue). The concentrations of complex inducers were employed from 0 μM to 200 μM for copper sulphate and 5 μM to 35 μM for methylene blue. The addition time of complex inducers was determined by detecting laccase activity in the YPD medium supplemented with copper sulphate and methylene blue at the different stages of cultivation, respectively. All flask experiments were performed in duplicate, and the results were the average values from triplicate assays.

Analytical methods

Enzyme assay

Culture medium (including YPD, YPD–Cu or inducer-supplemented medium) was centrifuged at 8,000× g for 10 min, and the supernatant was used for enzyme assay. Laccase activity was assayed spectrophotometrically by measuring the oxidation of ABTS at 420 nm (ε_{420} = 36 $mmol^{-1}$ cm^{-1}) at 30°C, as described by Bourbonnais and Paice [2] with some modifications. The assay mixture in a total volume of 1 ml contained 0.1 ml cell-free supernatants at various dilutions and 1 mM ABTS in 100 mM citrate buffer (pH 3.4). One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol ABTS per minute.

Residual glucose, pH and biomass assay

Samples were taken every 24 h from each flask to investigate glucose consumption, cell growth, and pH changes during cultivation in YPD–Cu medium. Glucose concentration was assayed by colorimetric quantification at 535 nm using 3,5-dinitrosalicylic acid (DNS) [19]. Cell concentrations were estimated as dry cell weight (DCW). YPD–Cu medium was filtered through qualitative filter papers, and the wet mycelia were then dried at 70°C to constant weight, and filter-paper weight was subtracted to evaluate DCW. The pH of YPD–Cu medium was measured using a pH meter.

Results and discussion

Screening for laccase-producing strains

To screen the laccase-producing strain, various lignin-containing samples were cultured in PDA–ABTS medium. As shown in Fig. 1, colonies with dark-green dye zones on PDA–ABTS plates indicated laccase active cultures. After preliminary screening, more than 100 target colonies were obtained from various lignin-containing samples (data not shown).

These pure cultures from preliminary screening were inoculated into YPD–ABTS and YPD–Cu media for secondary screening as described in "Materials and methods". The highest laccase production in YPD–Cu medium was found by strain S2 isolated from crop soil with laccase activity of 820 U/l on the eighth day. Besides, PDA–ABTS and YPD–ABTS media inoculated with strain S2 exhibited a green color at the second day, which was earlier than that of other strains, indicating quick secretion of laccase by strain S2 both in agar and submerged cultivations. With the better characteristics of enzyme production, strain S2 was selected for further investigation.

Characteristics and identification of strain S2

Strain S2 can grow at temperatures in the range of 25–45°C, and the higher the temperature, the more spores formed according to colony color and optical microscope observation. When cultured at 30°C on a PDA plate for 6 days, the colonies of strain S2 were large, flat, rounded, with short and velvety vegetative hyphae, gray color on the surface and black on the back, which indicates the formation of

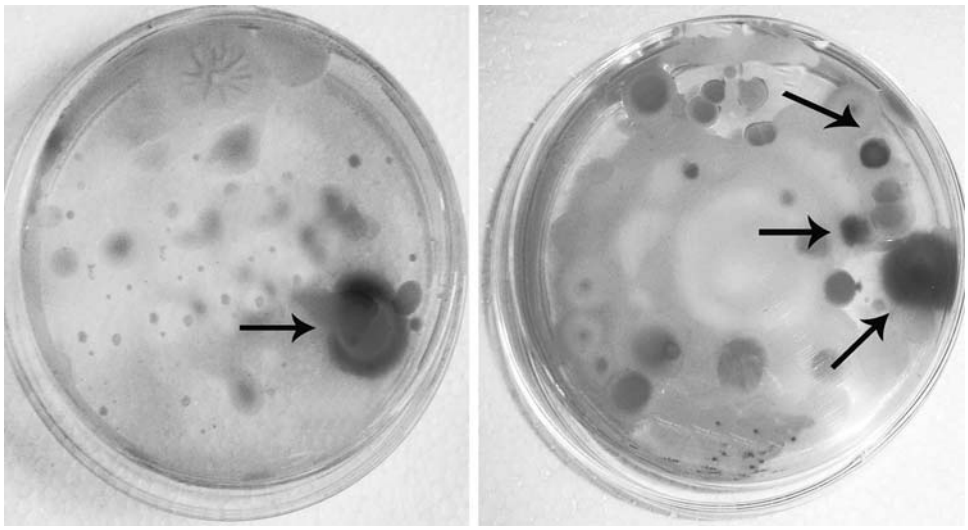

Fig. 1 Laccase active cultures with green halos (indicated by *arrows*) on potato dextrose agar−2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonate (PDA–ABTS) plates incubated at 30°C

spores and melanin. The ITS region of strain S2 was amplified, sequenced, and submitted to GenBank (Accession no. EU375749). The obtained sequence was compared with those in the National Center for Biotechnology Information Nucleotide Sequence Database by using the Basic Local Alignment Search Tool (BLAST) algorithm. A comparative analysis by Clustalx and Phylip software demonstrated that ITS rDNA gene sequence from strain S2 had a significant identity to a number of *Paecilomyces* sp. (Fig. 2). According to the analysis of ITS rDNA gene sequence, together with its morphological characteristics, strain S2 was identified as *Paecilomyces* sp. and designated as *Paecilomyces* sp. WSH-L07.

The time course of extracellular laccase production

To investigate the time course of laccase production, medium pH, residual sugar, cell growth, and laccase activity were monitored in flask experiments (Fig. 3). In the first 4 days, pH of YPD–Cu medium decreased continuously, indicating the formation of organic acid by primary metabolism of *Paecilomyces* sp. WSH-L07. At the same time, the concentration of glucose in YPD–Cu medium decreased rapidly and was almost completely utilized on the fourth day of cultivation (2 g/l), coinciding with the maximum amount of biomass formed.

Under lower environmental pH and depleted glucose, the biomass stopped increasing, the pH began to increase, and a sharp increment of laccase secretion was observed, indicating an alteration to secondary metabolism. This is in accordance with reports by Galhaup et al. [6]. The secretion of laccase was comparatively low in the glucose-rich environment (the first 4 days) with the production of 48.5 U/l per day, whereas an enhanced laccase secretion was detected on the following days. The maximum laccase activity of 819 U/l was obtained on the eighth day and declined afterward. Such a phenomenon may be explained by the turnoff of a large number of genes in the presence of glucose, resulting in the repression of laccase-secretion-

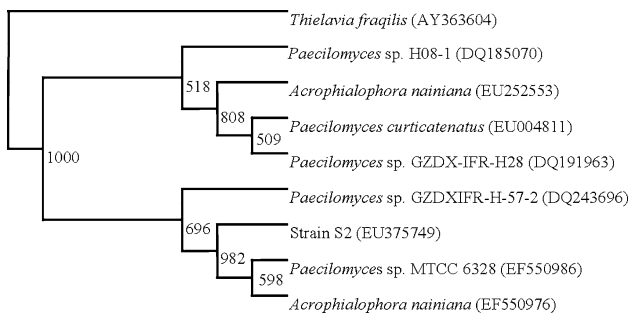

Fig. 2 The phylogenetic tree for strain S2 and relative strains based on the internal transcribed spacer (ITS) ribosomal DNA (rDNA) gene sequence. Bootstrap values >500 are shown based on 1,000 iterations

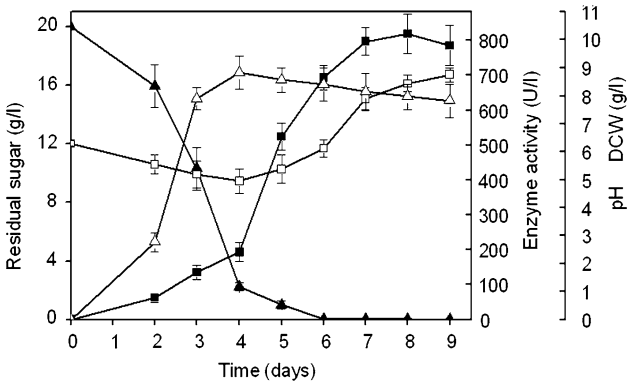

Fig. 3 The time course of extracellular laccase production by *Paecilomyces* sp. WSH-L07 in yeast extract peptone dextrose−copper sulphate (YPD–Cu) medium cultured at 30°C and 150 rpm on a rotary shaker. Laccase activity (*filled square*), pH (*open square*), residual sugar (*filled triangle*), and dry cell weight (DCW) (*open triangle*). *Error bars* correspond to the standard deviation of duplicate experiments

related enzyme synthesis [21]. The repression of glucose could be reduced by continuously feeding with a low, non-repressing amount of glucose to the cultures to improve laccase production [6]. As shown in Fig. 3, laccase from *Paecilomyces* sp. WSH-L07 is not a growth-associated product and could be accumulated rapidly during the stationary phase, which may be correlated with the physiological function of fungi laccase in sporulation, pigmentation, and morphogenesis [18]. To our knowledge, the highest laccase production by *Paecilomyces* sp. strains was up to 30 U/l, with fermentation time as long as 4 weeks [11, 14], whereas in this report, *Paecilomyces* sp. WSH-L07 exhibited laccase production of 819 U/l, incubated in a simple composition of YPD–Cu medium for 8 days, indicating *Paecilomyces* sp. WSH-L07 as a potential laccase producer.

Effect of inducers on laccase production

Most laccases are extracellular inducible enzymes, and inducers play an important role in the enhancement of laccase production. Thus, experiments of potential inducers, including various aromatic and dye compounds on laccase production, were carried out. According to the results (Table 1), laccase activity could be readily detected only in copper-supplemented medium. It is demonstrated that copper is the most efficient inducer for laccase production among all tested inducers, which is in agreement with Gnanamani et al.'s report [7]. As laccase could catalyze the oxidation of aromatic compounds as well as decolorization of dyes, complex inducers of copper sulphate and aromatic or dye compounds were employed for further enhancement of the cooperative induction effect. Although single aromatic and dye inducers gave poor laccase activity in YPD medium, a cooperative effect of copper sulphate with tested aromatic and dye compounds was observed, especially for complex inducers of copper sulphate with methylene blue (>890 U/l laccase activity). However, an antagonist effect of other inducers on copper sulphate by reducing the production of laccase was observed. The reason is not clear, but it may be due to the poison of these inducers for cell growth as well as laccase production under selected concentrations, which requires more investigation in future studies.

Besides the distinct effect of different inducers, the concentrations of inducers also affect laccase production. Therefore, the concentrations of copper sulphate in YPD–Cu medium supplemented with 10 μM methylene blue were employed in the range of 0–200 μM before sterilization. Figure 4a indicates that copper was essential for laccase production and enhanced laccase activity significantly compared with the copper-free cultures. The highest laccase activity (982 U/l) was detected in the medium supplemented with 50 μM copper sulphate, whereas further increment of copper concentration in medium resulted in an obvious decrease of laccase activity. As reported by Labbe and Thiele [12], copper requirements by microorganisms were usually satisfied by very low concentrations but toxic to fungal metabolism at higher concentrations. Although the obvious effect of copper sulphate concentration on laccase production, there were no significant differences between different concentrations of methylene blue (Fig. 4b). The highest laccase production, 1,139 U/l, was obtained by complex inducers of copper sulphate and methylene blue with optimal concentrations of 50 μM and 20 μM, respectively.

To investigate the effect of addition time of complex inducers on laccase production, methylene blue (20 μM) and copper sulphate (50 μM) were added into YPD medium at different times of the flask cultivations (Fig. 5), and optimal feeding time of methylene blue and copper were

Table 1 Effect of inducers on laccase production by *Paecilomyces* sp. WSH-L07

Inducer	Enzyme activity (U/l)	
	Inductive effect of different inducers	Cooperative effect with copper sulphate[a]
Aromatic compound (100 μM)		
Guaiacol	11.85	191.85
p-Hydroxybenzoic acid	7.19	342.25
p-Aminobenzoic acid	7.82	766.05
Dimethylaniline	9.68	413.66
o-Phenylene diamine	6.93	256.11
Phloroglucinol	4.55	298.42
Catechol	13.28	389.89
Orcinol	8.44	326.53
Gallic acid	4.94	455.08
p-Anisidine	10.50	723.68
Dimethyl phthalate	19.22	448.39
Dye compound (10 μM)		
Methyl orange	6.65	673.28
Phenol red	3.33	274.00
Methylene blue	7.80	898.64
Bromothymol blue	3.94	422.72
Safranine T	4.92	426.14
Eriochrome black T	6.11	657.14
Rhodamine B	3.33	677.36
Fuchsin basic	3.25	466.78
Indigo	6.80	346.33
Chromotropic acid	2.22	328.53
Gallocyanine	6.30	415.67

[a] Supplemented with different aromatic or dye compounds into yeast extract peptone dextrose−copper sulphate (YPD–Cu) medium

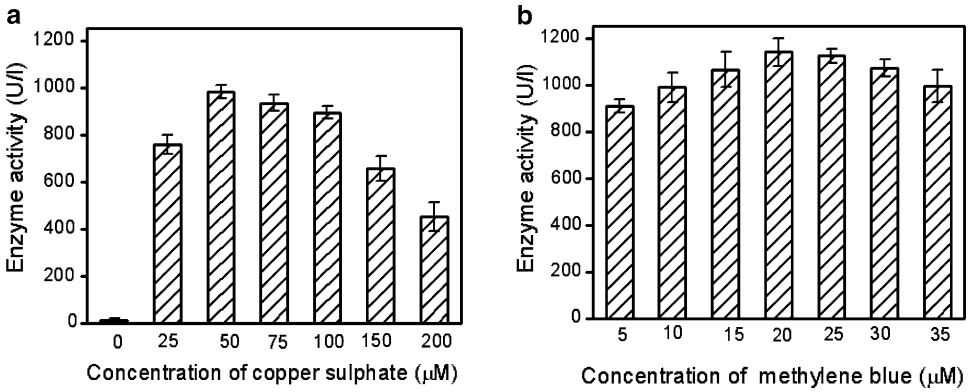

Fig. 4 Effect of copper sulphate (**a**) and methylene blue (**b**) concentrations on laccase production by *Paecilomyces* sp. WSH-L07; **a** was supplemented with different concentrations of copper sulphate and 10 μM methylene blue; **b** was supplemented with 50 μM copper sulphate and different concentrations of methylene blue. The yeast extract peptone dextrose (YPD) medium was used as basal medium, and both copper sulphate and methylene blue were added into YPD medium at the beginning of cultivation. *Error bars* correspond to the standard deviation of duplicate experiments

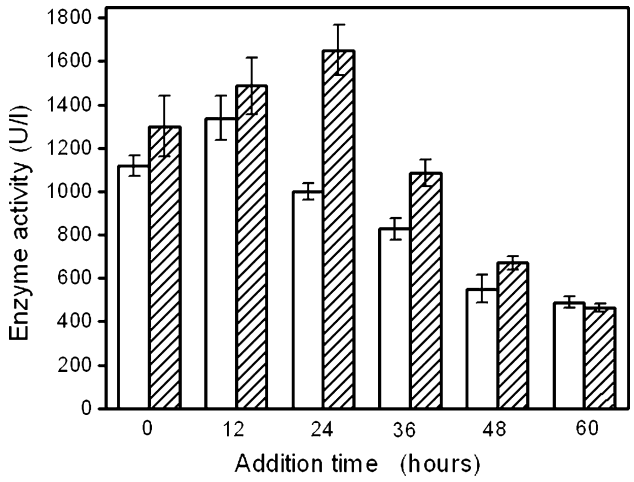

Fig. 5 Effect of addition time of methylene blue (*open bar*) and copper sulphate (*striped bar*) on laccase production by *Paecilomyces* sp. WSH-L07. The yeast extract peptone dextrose (YPD) medium was used as basal medium, and 20 μM methylene blue and 50 μM copper sulphate was employed. *Error bars* correspond to the standard deviation of duplicate experiments

obtained at 12 h and 24 h of cultivation, respectively, with the maximum laccase activity of 1,650 U/l.

According to the results, laccase from *Paecilomyces* sp. WSH-L07 is regarded as a typical copper-inducible enzyme, and the production of laccase could be significantly enhanced in complex-inducer-supplemented medium with >100% increment of laccase activity compared with simple YPD medium. The secretion of laccase induced by copper has been demonstrated by several researchers [7, 15]. It was suggested that the effect of copper on laccase production might be related to transcriptional regulation of laccase genes, as well as positive adjustment of laccase activity and stability [5, 10, 26].

Conclusion

In summary, a newly isolated *Paecilomyces* sp. with the highest laccase activity (820 U/l) in YPD–Cu medium was isolated from lignin-containing samples by PDA–ABTS plates and was designated as *Paecilomyces* sp. WSH-L07. Detectable laccase was only found in copper-supplemented medium, suggesting copper was essential for laccase production by *Paecilomyces* sp. WSH-L07. Further enhanced laccase production was achieved by complex inducers, especially with 20 μM methylene blue and 50 μM copper sulphate. The addition time of copper and methylene blue was at 24 h and 12 h of cultivation, respectively, resulting in the maximum laccase production with an activity of 1,650 U/l. Although laccase production was improved several times by complex inducers in this work, the enzyme activity is still relatively low for industrial application. More efforts should concentrate on improving laccase production with reduced cost.

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References

1. Alexandre G, Zhulin IB (2000) Laccases are widespread in bacteria. Trends Biotechnol 18:41–42
2. Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates: an expanded role for laccase in lignin biodegradation. FEBS Lett 267:99–102
3. Chen Q, Hang J, Shi YY, Xiao YZ, Wu J, Hong YZ, Wang YP (2004) Selective induction, purification and characterization of a laccase isozyme from the basidiomycete *Trametes* sp. AH28–2. Mycologia 96:26–35

4. Danneel HJ, Ullrich M, Giffhorn F (1992) Goal-oriented screening method for carbohydrate oxidases produced by filamentous fungi. Enzyme Microb Technol 14:898–903
5. Galhaup C, Goller S, Peterbauer CK, Strauss J, Haltrich D (2002) Characterization of the major laccase isoenzyme from *Trametes pubescens* and regulation of its synthesis by metal ions. Microbiology 148:2159–2169
6. Galhaup C, Wagner H, Hinterstoisser B, Haltrich D (2002) Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*. Enzyme Microb Technol 30:529–536
7. Gnanamani A, Jayaprakashvel M, Arulmani M, Sadulla S (2006) Effect of inducers and culturing processes on laccase synthesis in *Phanerochaete chrysosporium* NCIM 1197 and the constitutive expression of laccase isozymes. Enzyme Microb Technol 38:1017–1021
8. Hao JJ, Song FQ, Huang F, Yang CL, Zhang ZJ, Zheng Y, Tian XJ (2007) Production of laccase by a newly isolated deuteromycete fungus *Pestalotiopsis* sp. and its decolorization of azo dye. J Ind Microbiol Biotechnol 34:233–240
9. Hattori M, Konishi H, Tamura Y, Konno K, Sogawa K (2005) Laccase-type phenoloxidase in salivary glands and watery saliva of the green rice leafhopper, *Nephotettix cincticeps*. J Insect Physiol 51:1359–1365
10. Hoshida H, Fujita T, Murata K, Kubo K, Akada R (2005) Copper-dependent production of a *Pycnoporus coccineus* extracellular laccase in *Aspergillus oryzae* and *Saccharomyces cerevisiae*. Biosci Biotechnol Biochem 69:1090–1097
11. Kluczek-Turpeinen B, Tuomela M, Hatakka A (2003) Lignin degradation in a compost environment by the deuteromycete *Paecilomyces inflatus*. Appl Microbiol Biotechnol 61:374–379
12. Labbe S, Thiele DJ (1999) Copper ion inducible and repressible promoter systems in yeast. Method Enzymol 306:144–153
13. Leonowicz A, Cho NS, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J (2001) Fungal laccase: properties and activity on lignin. J Basic Microbiol 41:185–227
14. Liang ZQ, Han YF, Chu HL (2007) A new thermotolerant *Paecilomyces* species which produces laccase and a biform sporogenous structure. Fungal Divers 27:95–102
15. Malhotra K, Sharma P, Capalash N (2004) Copper and dyes enhance laccase production in γ-proteobacterium JB. Biotechnol Lett 26:1047–1050
16. Malmstrom BG, Reinhammar B, Vanngard T (1970) The state of copper in stellacyanin and laccase from the lacquer tree *Rhus vernicifera*. Biochim Biophys Acta 205:48–57
17. Marjasvaara A, Torvinen M, Vainiotalo P (2004) Laccase-catalyzed mediated oxidation of benzyl alcohol: the role of TEMPO and formation of products including benzonitrile studied by nano-electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. J Mass Spectrom 39:1139–1146
18. Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60:551–565
19. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428
20. Mohandass C, Knutson K, Ragauskas AJ (2008) Laccase treatment of recycled blue dyed paper: physical properties and fiber charge. J Ind Microbiol Biotechnol 35:1103–1108
21. Ronne H (1995) Glucose repression in fungi. Trends Genet 11:12–17
22. Tavares APM, Coelho MAZ, Coutinho JAP, Xavier A (2005) Laccase improvement in submerged cultivation: induced production and kinetic modelling. J Chem Technol Biotechnol 80:669–676
23. Vasconcelos AFD, Barbosa AM, Dekker RFH, Scarminio IS, Rezende MI (2000) Optimization of laccase production by *Botryosphaeria* sp. in the presence of veratryl alcohol by the response-surface method. Process Biochem 35:1131–1138
24. Wong YX, Yu J (1999) Laccase-catalyzed decolorization of synthetic dyes. Water Res 33:3512–3520
25. Yaropolov AI, Skorobogatko OV, Vartanov SS, Varfolomeyev SD (1994) Laccase: properties, catalytic mechanism, and applicability. Appl Biochem Biotechnol 49:257–280
26. Zhao J, Kwan HS (1999) Characterization, molecular cloning, and differential expression analysis of laccase genes from the edible mushroom *Lentinula edodes*. Appl Environ Microb 65:4908–4913