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Selection and optimization of lignocellulosic substrate for laccase production from *Pleurotus* species

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The aim of the current study was to investigate the potential of *Pleurotus* species for laccase production on different lignocellulosic substrates and determine the optimal levels of physicochemical conditions required for the production. The study was conducted in National Agricultural Biotechnological Research Center, Holetta. The fruiting bodies of fungi were collected based on their morphology and inoculated on potato dextrose agar plate. Six different lignocellulosic substrates were collected and prepared for cultivation of *Pleurotus* species for laccase production. The highest enzyme production was obtained from bean straw compared to other substrates with an activity of 0.112 U/ml. The best three substrates; bean straw, *Eucalyptus* sawdust and wheat straw were selected, and a mixture of each of them on equal proportions was tested for laccase production potential. A mixture of *Eucalyptus* sawdust and bean straw on equal proportion was found to be the best, showing an activity of 0.137 U/ml and hence selected for different parameter optimization. Optimal laccase production (0.292 U/ml) was obtained on the 10th day of incubation period and the optimum temperature and pH were 28°C and 5.5, respectively. Soluble starch and peptone were found to be the most preferred carbon and nitrogen sources for laccase production, respectively. Asparagine and alanine induced more laccase production, with asparagine being the most potent inducer.

Key words: *Pleurotus species*, lignocellulosic substrates, bean straw, *Eucalyptus* sawdust, laccase.

INTRODUCTION

Laccase is copper-containing protein, which belongs to the large and diverse superfamily of multicopper oxidases (Arora and Sharma, 2010). It is widely distributed in higher plants and fungi (Kiiskinen et al., 2004) and also been found in insects and bacteria (Viswanath et al., 2014). Laccase of fungi is of particular interest with regard to potential industrial applications because of its capability to oxidize a wide range of industrially relevant

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substrates. Oxidation reaction is comprehensively used in industrial processes, for instance, in the textile, food, wood processing and pharmaceutical and chemical industries. Enzymatic oxidation is a potential substitute to chemical methods since enzymes are very specific and efficient catalysts, and are ecologically sustainable. Laccase is currently intensively used in large scale in the textile industry. Together with low molecular weight redox-mediator compounds, laccase can generate a desired worn appearance on denim by bleaching indigo dye.

Another potential environmental application for laccase is the bioremediation of contaminated soils as laccase is able to oxidize toxic organic pollutants, such as polycyclic aromatic hydrocarbons and chlorophenols. The most useful method for this application would probably be inoculating the soil with fungi that are efficient laccase producers because the use of isolated enzymes is not economically feasible for soil remediation in large scale. The current practical applications of the use of laccase have led to a search for source of the enzyme from white-rot fungi and the use of mediators, which promote or facilitate enzyme action (Viswanath et al., 2014). Owing to its vivid biotechnological applications, studies on laccase producing organisms have been intensified and the optimization of laccase production from different microorganisms is being carried out by several groups (Sivakumar et al., 2010). The ever-increasing demand for this enzyme requires the production process to be economical. Identifying inexpensive raw materials for enzyme production could be viewed as a solution to make the entire process cost effective.

Laccase production by fungi is influenced by culture conditions such as nature and concentration of carbon and nitrogen sources, media composition, pH, temperature, and other factors. In general, fungi were cultivated at temperatures between 25 and 30°C for optimal laccase production. The initial pH value of the medium before inoculation range between pH 4.5 and pH 6.0, but the pH is not generally controlled during cultivation (Arora and Gill, 2001). Carbon sources as well as its concentration in the medium play an important role in ligninolytic enzyme production. An excess of glucose or saccharose in the liquid medium eliminated the induction of laccase, where the constitutive production of laccase is maintained but the biosynthesis of induced laccase is repressed by both sugars (Bollag and Leonowicz, 1984). Hence, it has been a matter of concern to find environmentally sound and economically feasible media constituents for laccase production. The study was undertaken to isolate white rot fungi, *Pleurotus* species, from its natural habitat, that is the bark of old *Eucalyptus* trees, and detect laccase activity on potato dextrose agar (PDA) plate containing guaiacol, with the aim of using the isolate for laboratory scale production of laccase.

MATERIALS AND METHODS

Culture collection and maintenance

The study was conducted in the National Agricultural Biotechnological Research Center, in Holetta which is geographically located at 34 km west of Addis Ababa, Ethiopia. The fruiting bodies of fungi were collected based on their morphology following the methods of *Pleurotus* species identification guideline given by Consensus Document on the Biology of *Pleurotus* species (OECD, 2005). The major morphological traits considered during fruiting body collection were; occurrence, stem/stipe, odor, shape and color of fruiting body. The fungi grown in shelf-like clusters, nearly absent stem, smooth and thick flesh with whitish kidney shaped cap having anise odor, were considered. The fungi having the above-mentioned features were collected from different trees and were inoculated on PDA following standard microbiological procedures and incubated at 28°C. The colonies from the plates were observed for morphological difference and the colony having cylindrical to narrowly kidney shaped features was considered. The colonies with those morphologies were picked with a sterilized inoculating needle and inoculated on a freshly prepared PDA plates in order to obtain pure cultures. The pure *Pleurotus* species cultures were maintained in PDA slants according to Naraian et al. (2010).

Source and preparation of lignocellulosic substrates

The growth substrate like *Cupressus lusitanica* sawdust (CSD), *Eucalyptus* bark (EB) and *Eucalyptus* sawdust (ESD) were collected from local small wood processing firm. Bean straw (BS), Teff straw (TS) and Wheat straw (WS) were collected from Holetta Agricultural Research Center farm field. All lignocellulosic substrates were air dried and then oven dried in-order to free all substrates from moisture for ease of grinding. After that all substrates were ground gently by using a blender and stored at room temperature in capped glass bottles separately until used.

Cultivation of *Pleurotus* species

The earlier described powdered substrates (CSD, ESD, BS, EB, TS, and WS) were transferred to flasks each in 10 g separately with 30 ml of distilled water added following the method of Demir et al. (2011). After autoclaved, all flasks were inoculated with 5 discs of the fungal culture (Patel et al., 2009) of 5-day old under aseptic condition, then incubated at 28°C for complete growth of mycelium.

Detection of laccase production

Guaiacol was used for qualitative enzyme assay. Agar discs of 5 day old cultures were placed on PDA petri-dishes containing 4 mM guaiacol and incubated at 28°C. The intense brown color development around the colony due to oxidation of guaiacol was considered as an indication for laccase activity. So, if the plate changes color to brown, it was concluded that there was laccase production by *Pleurotus* species (Desai et al., 2011).

Laccase extraction

After the 14th day of incubation, 30 ml of 100 mM acetate buffer (pH 5.0) was added to the flasks under aseptic condition (Kumari and Negi, 2014) and kept on shaker at 120 rpm at room

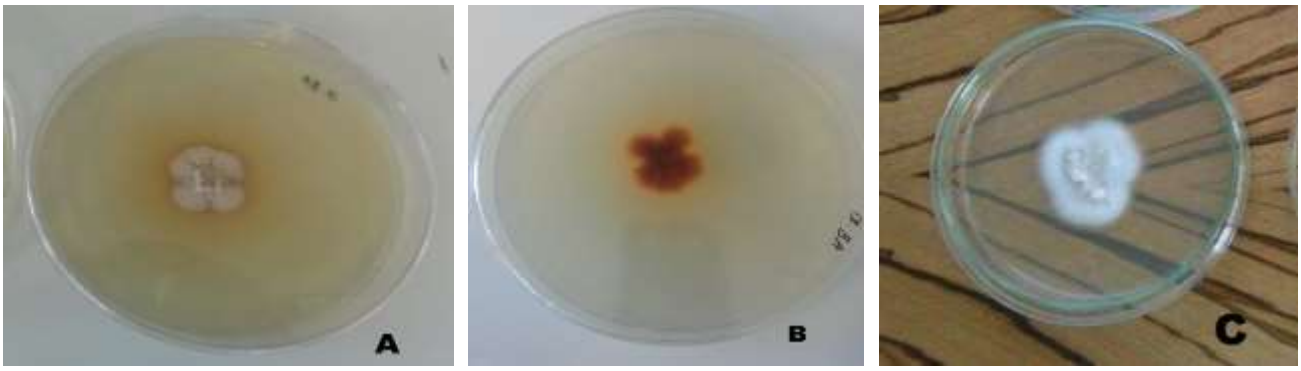

Figure 1. Detection of laccase production by *Pleurotus* species on PDA plate. A, PDA plate with guaiacol front view; B, PDA plate with guaiacol backside view; C- PDA plate no guaiacol).

temperature for 30 min to allow the buffer to extract the enzyme. Later, the contents were filtered with Whatman No.1 filter paper and the culture filtrate was centrifuged at 10,000 rpm for 15 min at 4°C to remove spores and insoluble. The clear supernatants (crude enzymes) were then collected in separate containers by decantation (Elsayed et al., 2012). This was used directly for the determination of laccase activity.

Laccase activity assay

For measuring laccase activity, 0.1 ml of culture filtrate (enzyme source) was added to 4.9 ml of 0.1 M sodium acetate buffer (pH 4.5) and 1 mM guaiacol as substrate (Demir et al., 2011). The reaction mixture prepared was incubated at 50°C for 15 min. Incubation without culture filtrate was used as control. Enzyme activity was measured by reading absorbance in the UV-Visible spectrophotometer adjusted to 465 nm. Enzyme activity was expressed as Enzyme units (U), where 1 U is defined as the amount of enzyme required to oxidize 1 micromole of guaiacol (substrate) per min and laccase activity in U/ml was calculated with the following formula (Ping et al., 2008; Kalra et al., 2013).

$$U/ml = (A \times V) / (\varepsilon \times t \times l \times v)$$

Where: U = Enzyme unit; t = time; A = Absorbance; *l* = sample thickness; V = Total reaction volume; v = Enzyme volume.

Selection of lignocellulosic substrate

Of all tested substrates, the best three were selected and mixed in equal proportion to determine the better substrate composition. Accordingly; BS, ESD and WS were the best three and selected for further reselection. Then, a mix of BS-ESD, BS-WS, and ESD-WS were prepared and inoculated with fungal plug. Enzyme activity was determined at the end of incubation period and BS-ESD mixture shows more activity and hence selected for further physicochemical parameter optimization.

Optimization of growth condition

A series of experiments were performed to optimize different physicochemical growth condition of *Pleurotus* species for maximal laccase production. Those include: incubation time (2 to 20 day), incubation temperature (20 to 35°C), initial pH (4.5 to 6.5), addition of carbon source (glucose, galactose, fructose, and mannitol, maltose, lactose and sucrose, cellulose and soluble starch), nitrogen source (sodium nitrate, peptone, beef extract, ammonium sulphate, and ammonium chloride), and metal ions (Mn^{2+}, Zn^{2+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, Cu^{2+}). The classical strategy was adopted; hence a single parameter was varied at a time and previously optimized parameter was kept at optimal level.

Data analysis

All the experiments were carried out in triplicates and the data presented is the mean value of the triplicates. The standard error was calculated using the mean values.

RESULTS AND DISCUSSION

Detection of laccase production by *Pleurotus* species

Five days old culture of *Pleurotus* species was placed on PDA petri-dishes containing 4 mM guaiacol and incubated at 28°C for 5 days. The culture was unable to grow quickly as expected compared to guaiacol free plate; although there was suppression effect from guaiacol together with mycelial growth initiation. After the 10th day, the culture was able to develop intense brown color around the colony (Figure 1) due to oxidation of guaiacol by laccase which can be correlated to its activity (Kumari and Negi, 2014). So it can be concluded that the fungus under investigation (*Pleurotus* species) was a laccase producer.

Laccase production on different lignocellulosic substrates

The cultivation of *Pleurotus* species on different lignocellulosic substrates; bean straw (BS), *Cupressus lusitanica* sawdust (CSD), *Eucalyptus* bark (EB),

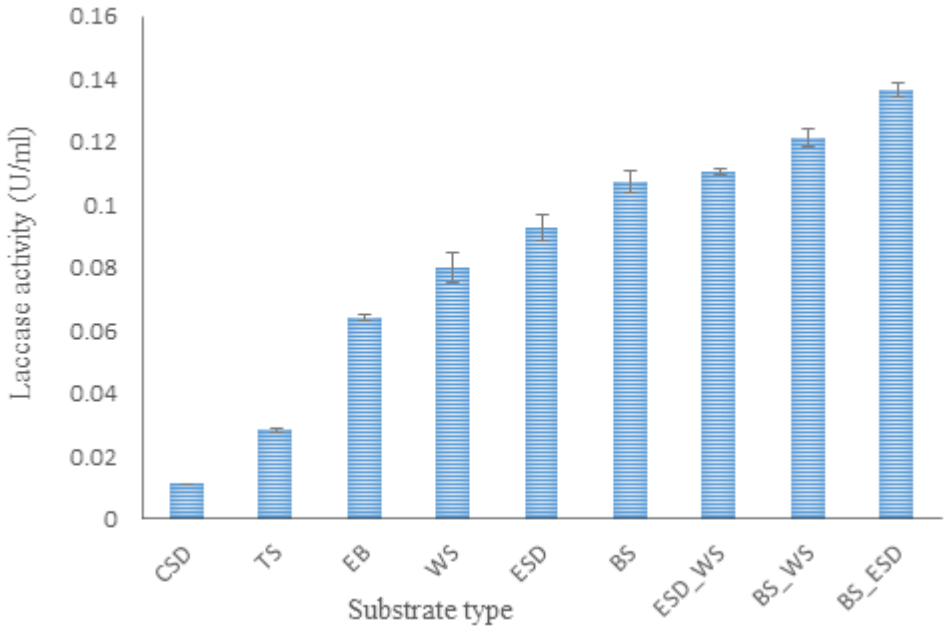

Figure 2. Laccase production by *Pleurotus* species grown on different lignocellulosic substrates.

Eucalyptus sawdust (ESD), teff straw (TS), and wheat straw (WS) was followed for 14 days. At the end, laccase was extracted from each substrate separately and assayed to check the potential of each substrate for laccase production. All substrates show a good mycelium growth except CSD, which are very poor and suppress fungal growth. BS, ESD, and WS, shows a good growth while TS and EB had a moderate growth. EB is the natural substrate for the study organism, but the study showed that it had moderate mycelial growth probably because of the poor nutrient content available in bark. The highest enzyme production was obtained from bean straw compared to other substrates with an activity of 0.112 U/ml as shown in Figure 2.

The best three substrates were selected and each mixed in equal proportion to optimize the best composition. Accordingly, BS, ESD and WS were selected and a mixture of them on equal proportions was tested for laccase production potential. A mix of ESD and BS on equal proportion was found to be the best, showing an activity of 0.137 U/ml and was selected for further physicochemical parameter optimization. There has been an increasing trend towards the utilization of organic waste such as residues from the agricultural, forestry and agro-based industries as raw materials to produce value added products by SSF technique.

Present investigation confirms and evaluates the use of ESD and BS on equal proportion as an inexpensive and easily available substrate for production of laccase. The laccase production ability of *Pleurotus* species can be enhanced further by supplementing the media with various inducers. Optimization studies have thus led to increase the laccase production. The substrates and inducers are cheap, safe and can be suggested for the higher laccase production.

Optimal incubation period

Many of the wood degrading white rot fungi were found to be excellent producers of laccase. But variations prevailed in relation to optimum expression period of this enzyme among the organisms (Sivakumar et al., 2010). In the present study, the time course of laccase production by white rot fungi *Pleurotus* species was evaluated by harvesting enzyme every other day for 20 days. The results in Figure 3 show that the optimum incubation time was the 10th day, at which laccase activity reached 0.142 U/ml. There were increments in laccase production from the second day up to the sixth day of incubation. After the sixth day laccase production was increased slightly and reached its maximum on the 10th day. After the tenth day, activity decreased slowly up to the 14th day, and then sharply dropped up to the 20th day. So, the maximal incubation period of the study organism for laccase production was the 10th day.

Nearly similar results were found by Kumar et al. (2011) who reported that maximal laccase activity in *P.*

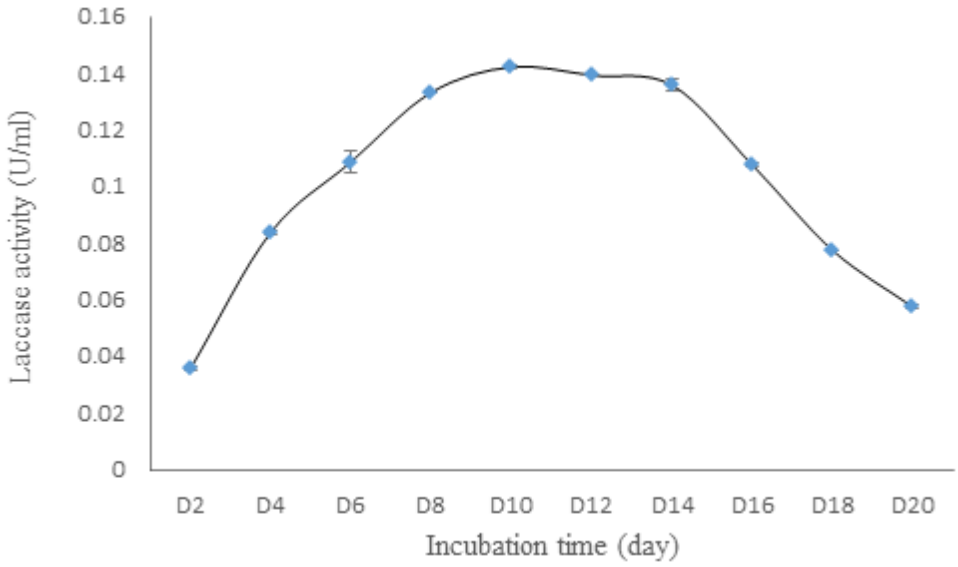

Figure 3. Effect of incubation period on laccase production by *Pleurotus* species.

ostreatus was on the 9th day (570 U/L). Nadeem et al. (2014) reported that, there was a sharp increase in laccase production by *P. ostreatus* from 2 to 6th day and after 10th day its activity decreased slightly. Laccase produced in late phase of *P. ostreatus* that is on 6th and 8th day, reached its maximum value with activity of 5.53 ± 0.11 and 5.68 ± 0.08 U/ml, respectively and after that decreased subsequently. The laccase production on day 8 was 61.7 and 41.4-fold more than the 2nd and 16th day of incubation, respectively. In addition to this Sivakumar et al. (2010) also found optimum laccase production by *Ganoderma sp.* after 10th day of incubation.

On the other hand, Abdulah (2008) found maximum laccase production on the 5th day of incubation with activity of 0.127 U/ml. The report also indicated that after 5 days there was a sharp decline in enzyme activity observed and reached (0.043 U/ml) after 15 days. Maximum level of laccase activity (0.395 U/ml) was observed after 15 days of incubation and there was no activity observed even after 25 days of incubation as reported by Hashim (2012). Time course study of laccase showed that maximum production was 75 IU/ml in 8 days of SSF of banana stalk by *Schizophyllum commune* IBL-06 as reported by Irshad and Asgher (2011).

Optimal incubation temperature

Temperature is considered as a crucial and effective factor in the growth of microorganisms and their metabolism that is temperature significantly influences the production of mycelial biomass, protein and laccase. The results of the present study indicate that an incubation temperature 25, 28 and 30°C was good for laccase production with 28°C being optimal with activity of 0.142 U/ml (Figure 4). But at 35 and 20°C, the fungus growth decreased and consequently laccase's units dropped, because high temperature causes cell membrane composition alteration, stimulation of protein catabolism, and lower temperature results in suppression of nutrient transportation which was in line with the report given by Nadeem et al. (2014). At 35°C, laccase's activity dropped to 0.062 U/ml which is nearly 47% of its maximum activity at 28°C. Generally, temperature more than 30°C and less than 25°C does not favor the growth of the study organism and subsequently lesser laccase is produced. This indicates that cultivating *Pleurotus* species under optimum temperature is important for more laccase production.

The above result is in line with Abdulah (2008), who reported that the highest laccase production was obtained at 28˚C; with an enzyme activity of 0.132 U/ml. The same result was also reported by Patel et al. (2009) who showed that the best temperature for laccase production by *P. ostreatus* was 28˚C, while Snajdr and Baldrain (2007) indicated that the highest laccase production was obtained at 25 to 30°C with the same organism. The optimum temperature for maximum laccase production by *P. sajor-caju* was found to be 30°C on day 9 with an activity of 0.2844 U/ml. Very little ligninolytic activities were observed at temperatures above 30°C probably due to the fact that increasing the temperature could have inhibited the fungal growth and hence, decreased enzyme activities (Patrick et al., 2011).

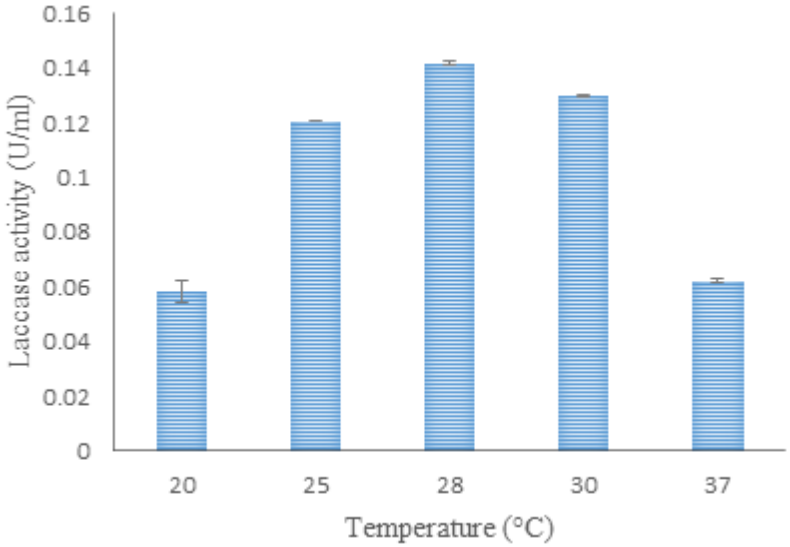

Figure 4. Effect of incubation temperature on laccase production by *Pleurotus species.*

Sivakami et al. (2012) also reported that the optimal temperature for laccase production by *P. ostreatus* was 30°C. On the other hand, Patrick et al. (2009) indicated that the optimum temperature for maximum production of ligninolytic enzymes was determined to be 25°C for laccase on day 6 with an activity of 0.36 U/ml.

Optimal initial pH

The extracellular laccase production in microorganisms is influenced by pH since it affects the characteristics of the medium including nutrient solubility and transportation, and thus it affects the nutrient availability to the growing microorganism. Secondly it also affects the enzyme ionizable group and thus affects the stability of an enzyme (Abdulah, 2008). In the present study, after determination of optimum incubation time and temperature which was 10th day and 28°C, respectively, the fungi were cultivated at a series of initial pH values ranging from 4.5 to 6.5, with 0.5 interval. During the course of growth of fungal cultures, pH of culture medium was not regulated as it is barely possible. The highest laccase activity was obtained when the initial pH of the medium was adjusted to 5.0 and 6.0; with 5.5 being an optimal at an activity of 0.159 U/ml (Figure 5). It was also observed that laccase activity was raised with increased pH from 4.5 up to 5.5; later on it showed a declining trend in activity as the pH approached neutral.

The results obtained in the present study are in agreement with Nadeem et al. (2014) who reported that most of the fungal cultures preferred a slightly acidic pH of medium for growth and enzyme production. Prasad et al. (2005), Adejoye and Fasidi (2009) and Sivakami et al. (2012) also showed that in *Schizophyllum commune* and *P. ostreatus* the optimal pH for fungi mycelia biomass yield and laccase activities was 5.5. Selim et al. (2013) reported that enzyme production was found to be increasing gradually with the increase in initial pH, reaching the maximum at 7 (0.472 U/ml) and then decreasing at higher pH values such as pH 9 (0.005 U/ml). The culture pH strongly affects many enzymatic processes and transport of various components across the cell membrane.

The most preferred carbon source

In order to determine the most preferred carbon source by *Pleurotus* species for laccase production, different supplementary carbon sources at 2% concentration were used in production media. The highest laccase activity was obtained when soluble starch was used as additional carbon source and laccase activity reached up to 0.205 U/ml, while lactose resulted in low laccase level (0.122 U/ml). Sucrose and glucose were also another good supplementary carbon source for the study organism with an activity of 0.181 and 0.178 U/ml, respectively (Figure 6).

In agreement with the current study Elsayed et al. (2012) reported that soluble starch was found to be the best carbon source for laccase production. However, galactose, fructose and maltose significantly repressed laccase formation by *P. ostreatus* which was in line with

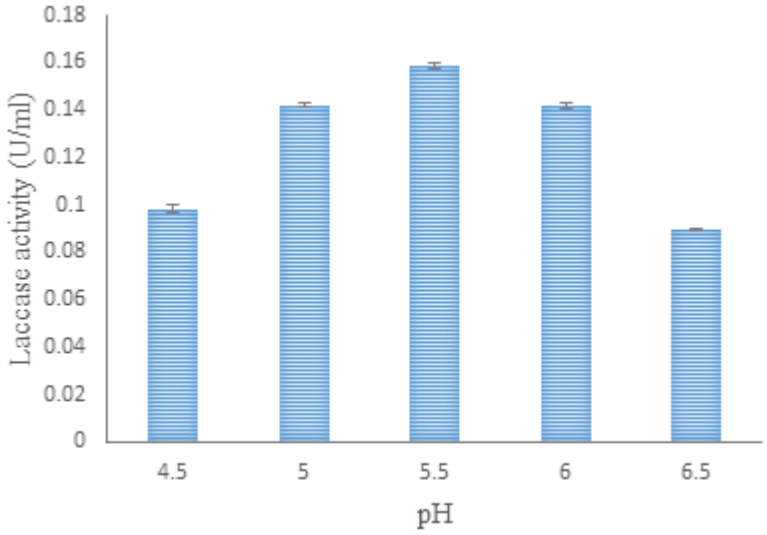

Figure 5. Effect of initial pH on laccase production by *Pleurotus species.*

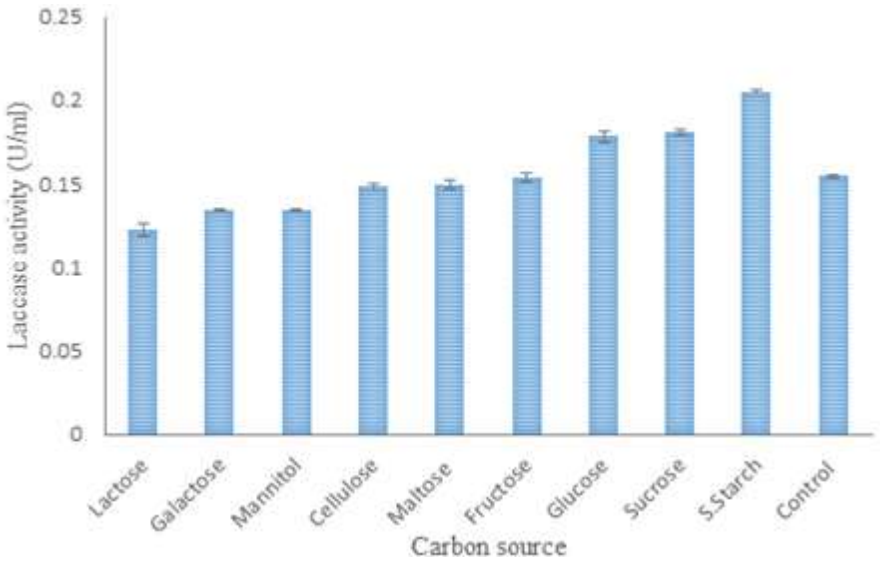

Figure 6. Effect of supplementary carbon source on laccase production by *Pleurotus* species.

the current finding. Additionally, Sivakumar et al. *(*2010) confirmed that among six different carbon sources such as mannitol, maltose, sucrose, glucose, lactose and starch tested at 2% for laccase production in *Ganoderma* species, starch supported a maximum laccase activity of 0.18 U/ml. Abdulah (2008) also reported that the highest laccase activity was obtained when glucose was used as carbon source, but cellulose resulted in low laccase levels (0.01 U/ml). On the other hand, Ticlo et al. (2006) described that among several of the carbon sources tested maximum laccase activity was detected when fructose was used as the carbon source.

The most preferred nitrogen source

Ligninolytic enzyme production by the wood-rotting basidiomycetes has been influenced by the nature of nitrogen sources and they are the most important factors for regulation of ligninolytic enzyme production

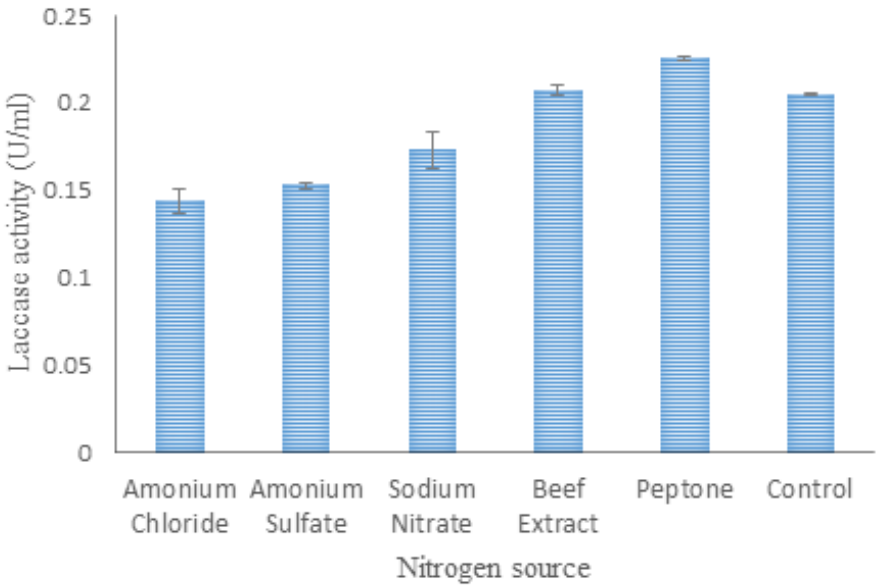

Figure 7. Effect of supplementary nitrogen source on laccase production by *Pleurotus* species.

(Bakkiyaraj et al., 2013). The results depicted in Figure 7 indicate that nitrogen source like peptone and beef extract gave better laccase activities than ammonium chloride, ammonium sulfate and sodium nitrate. Maximum laccase activity was recorded with peptone which was 0.225 U/ml. When compared to control, laccase obtained with inorganic nitrogen source showed less activity, while results from organic nitrogen showed more laccase activity. Inorganic nitrogen sources like ammonium chloride, ammonium sulfate and sodium nitrate have failed to produce high laccase when used as supplementary nitrogen source compared to organic ones, that is peptone and beef extract. The current result is in line with the reports of Abdel-Azeem and Salem (2012), Kenkebashvili et al. (2012), Bakkiyaraj et al. (2013) and Junyao et al. (2014) which showed that organic nitrogen sources such as peptone were more suitable than inorganic sources for different fungi.

The most preferred divalent metallic ions

In order to select the best salt solution for laccase production by *Pleurotus* species, 5 mM of each salt (metal ions) was used namely: manganese chloride, zinc chloride, iron sulfate, magnesium sulfate, calcium chloride, and copper sulfate. The results showed that all compounds gave good fungal growth other than copper sulfate and iron sulfate. Maximum laccase activity was observed in medium with calcium chloride which was 0.248 U/ml and the minimum was 0.07 U/ml with iron sulfate medium. Copper sulfate and iron sulfate inhibited the growth of the fungus in the production media. There were only very little mycelia on the media, which were brought along with the PDA plugs used as inocula. In general, other than calcium chloride medium, all salt solutions (metal ions) reduced laccase production compared to control unit without any salt solutions (Figure 8).

Metal ions can significantly affect the growth and extracellular enzymes production capabilities of white rot fungi (Baldrian, 2004). Selim et al. (2013) reported similar results by showing that laccase production reached the maximum value with supplementation of Ca^{2+} (0.730 U/ml) and decreased with Fe^{+2} (0.49 U/ml). Mongkolthanaruk et al. (2012) also indicated that Ca^{2+} activated more laccase production. The requirement for specific metal ions depends on the organisms, that is different organism prefers different metal ions and thus the product varies. Ions such as iron may interrupt the electron transport system of laccase and substrate conversion and thus results in little or no activity (Kim and Nicell, 2006).

The most potent inducers

Different inducers were investigated for their potential of laccase induction, namely: biotin, alanine, asparagine, cystien, leucine, lysine, phenylalanine, and glycine. The production media were amended with the aforementioned inducers at a concentration of 0.2% (w/v) amino acids.

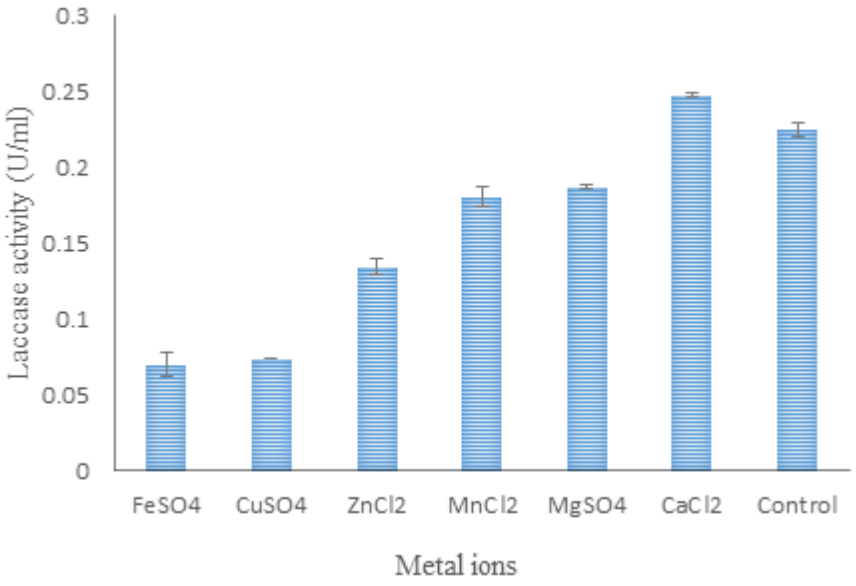

Figure 8. Effect of addition of metal ions on laccase production by *Pleurotus* species.

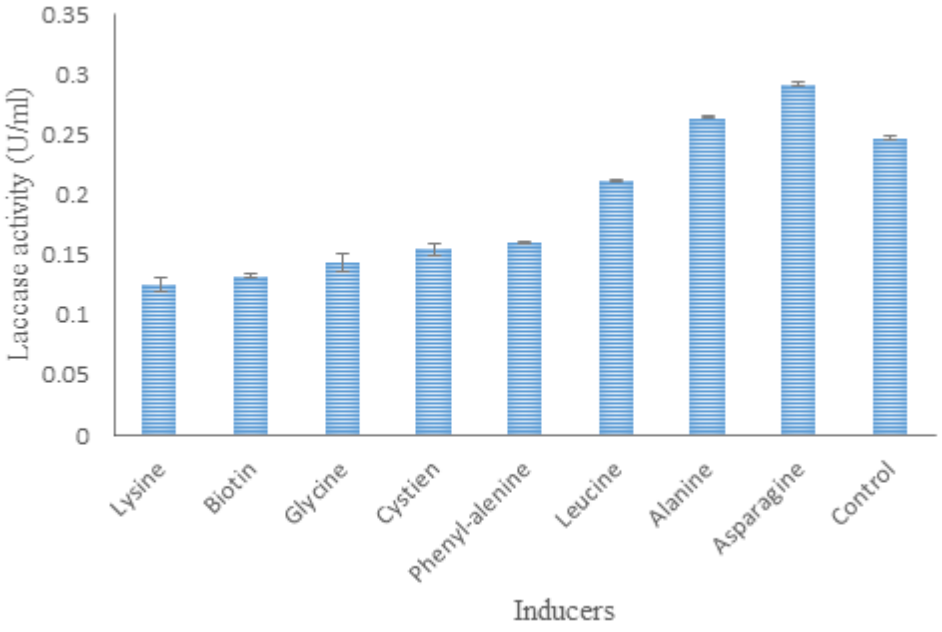

Figure 9. Effect of addition of metal ions on laccase production by Pleurotus species.

Inducer compounds have been widely employed to enhance laccase production by different organisms and the nature of the compound that induces laccase activity differs greatly with the species (Junyao et al., 2014). After full growth period, the recorded activity showed that asparagine and alanine induce more laccase production with an activity of 0.292 and 0.264 U/ml, respectively. All other remaining compounds resulted in decreased laccase production with lysine being the least inducer resulting in an activity of 0.125 U/ml (Figure 9). So, asparagine and alanine were concluded to be potent inducers, with asparagine being a better for study organism.

Addition of various amino acids in the medium

stimulates the ligninolytic enzyme production. The higher laccase activity (57.25 U/ml) was recorded with alanine at the concentration of 0.01% in the medium, and moderate to good level of enzyme activities were obtained with asparagine (Johnsy et al., 2014).

Conclusion

In view of the results obtained, it can be concluded that: Laccase production by *Pleurotus* species has been shown to depend markedly on the composition of the culture medium like: Type of lignocellulosic substrate, carbon and nitrogen source, metal ions and inducer compounds and also governed by parameters such as pH of the production medium, incubation period and temperature. In future, we are interested to study the properties of laccase and test the ability of this enzyme to degrade the various dyes as an important enzyme for various industrial applications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abdel-Azeem AM, Salem FM (2012). Biodiversity of laccase producing fungi in Egypt. Mycosphere 36:900-920.

Abdulah AR (2008). The use of partially purified laccase produced by some fungal isolates in Aflatoxin B1 degradation. M.Sc. Thesis in Biotechnology, University of Baghdad College of Science Department of Biotechnology.

Adejoye OD, Fasidi IO (2009). Effect of cultural conditions on biomass and laccase production in submerged medium by *Schizophylllum commune* (FR.). A Nigerian edible mushrooms. Electronic J. Environ. Agric. Food Chem..8(11):1186-1193.

Arora DS, Gill PK (2001). Effects of various media and supplements on laccase production by some white rot fungi. Biol. Resour. Technol. 77:89-91.

Arora DS, Sharma RK (2010). Ligninolytic fungal laccase and their biotechnological applications. Appl. Biochem. Biotechnol. 160:1760-1788.

Bakkiyaraj S, Aravindan R, Arrivukkarasan S, Viruthagiri T (2013). Enhanced laccase production by *Trametes hirusta* using wheat bran under submerged fermentation. Int. J. Chem. Technol. Res. 5(3):1224-1238.

Baldrian P (2004). Purification and characterization of laccase from the white rot fungus *Daedalea quercina* and decolorization of synthetic dyes by the enzyme. Appl. Microbiol. Biotechnol. 63:560-563.

Bollag JM, Leonowicz A (1984). Comparative studies of extracellular fungal laccase. Appl. Environ. Microbiol. 48:849-854.

Demir A, Aytar P, Gedikli S, Çabuk A, Arısoy M (2011). Laccase production with submerged and solid-state fermentation: Benefit and cost analysis. J. Biol. Chem. 39(3):305-313.

Elsayed MA, Hassan MM, Elshafei AM, Haroun BM, Othman AM (2012). Optimization of cultural and nutritional parameters for the production of laccase by *Pleurotus ostreatus* ARC280. Br. Biotechnol. J. 2:115-132.

Hashim AJ (2012). Determination of optimal conditions for laccase production by *Pleurotus ostreatus* using sawdust as solid medium and its use in phenol degradation. Baghdad Sci. J. 9(3):491-499.

Irshad M, Asgher M (2011). Production and optimization of ligninolytic enzymes by white rot fungus *Schizophyllum commune* IBL-06 in solid state medium banana stalks. Afr. J. Biotechnol. 10(79):18234-18242.

Johnsy G, Kaviyarasan V (2014). Effect of physico-chemical parameters on ligninolytic enzyme production of an indigenous isolate of *Neolentinus kauffmanii* -under submerged culture condition. World J. Pharmaceut. Sci. 2(5):464-468.

Junyao H, Ye X, Ling Q, Dong L (2014). Enhanced production of an acid-tolerant laccase by cultivation of *Armillariel latabescens*. J. Chem. Pharmaceut. Res. 6(1):240-245.

Kalra K, Chauhan R, Shavez M, Sachdeva S (2013). Isolation of laccase producing *Trichoderma* Spp. and effect of pH and temperature on its activity. Int. J. Chem. Technol. Res. 5(5):2229-2235.

Kenkebashvili N, Elisashvili V, Wasser SP (2012). Effect of carbon, nitrogen sources, and copper concentration on the ligninolytic enzyme production by *Coriolopsis gallica*. J. Waste Convers. Bioproducts Biotechnol. 1(2):22-27.

Kiiskinen LL, Palonen H, Linder M, Viikari L, Kruus K (2004). Laccase from *Melanocarpus albomyces* binds effectively to cellulose. FEBS Lett. 576:251-255.

Kim Y, Nicell JA (2006). Impact of reaction conditions on the laccase catalyzed conversion of bisphenol A. Bioresour. Technol. 97:1431-1442.

Kumar VV, Kirupha SD, Periyaraman P, Sivanesan S (2011). Screening and induction of laccase activity in fungal species and its application in dye decolorization. Afr. J. Microbiol. Res. 5:1261-1267.

Kumari J, Negi S (2014). Development of bioprocess for the production of laccase by *Pleurotus ostreatus* MTCC 1802 using evolutionary optimization technique. Indian J. Exp. Biol. 52:1106-1111.

Mongkolthanaruk W, Tongbopit S, Bhoonobtong A (2012). Independent behavior of bacterial laccases to inducers and metal ions during production and activity. Afr. J. Biotechnol. 11(39):9391-9398.

Nadeem A, Baig S, Sheikh N (2014). Mycotechnological production of laccase by *Pleurotus ostreatus* P1 and its inhibition study. J. Anim. Plant Sci. 24(2):492-502.

Naraian R, Singh D, Verma A, Garg SK (2010). Studies on *in vitro* degradability of mixed crude enzyme extracts produced from *Pleurotus* spp. J. Environ. Biol. 31(6):945-951.

Organisation for Economic Co-operation and Development (OECD) (2005). Consensus Document on the Biology of *Pleurotus* spp. (Oyster Mushroom). Environment, Health and Safety Publications Series on Harmonisation of Regulatory Oversight in Biotechnology. André-Pascal Press, Paris France 34:10-15.

Patel H, Gupte A, Gupte S (2009). Effect of different culture condition and inducer on production of laccae by basidiomycetes fungal isolate *Pleurotus ostreatus* HP-1 under solid state fermentation. Bio-resource 4(1):268-284.

Patrick F, Mtui G, Mshandete AM, Johansson G, Kivaisi A (2009). Purification and characterization of a laccase from the basidiomycete Funaliatrogii (Berk.) isolated in Tanzania. Afr. J. Biochem. Res. 3(5):250-258.

Patrick F, Mtui G, Mshandete AM, Kivaisi A (2011). Optimization of laccase and manganese peroxidase production in submerged culture of *Pleurotus sajor-caju*. Afr. J. Biotechnol. 10(50):10166-10177.

Ping W, Xuerong F, Li C, Qiang W, Aihui Z (2008). Decolorization of reactive dyes by laccase immobilized in alginate/gelatin blent with PEG. J. Environ. Sci. 20:1519-1522.

Prasad KK, Venkata Mohan S, Vijaya Bhaskar Y, Ramanaiah SV, Lalit

Babu V, Pati BR, Sarma PN (2005). Laccase Production using *Pleurotus ostreatus* 1804 immobilized on PUF cubes in batch and packed bed reactors: Influence of culture conditions. J. Microbiol. 43(3):301-307.

Selim MS, Mahmoud MG, Rifaat HM, El Sayed OH, El Beih FM (2013). Effect of inducers and process parameters on laccase production by locally isolated marine *Streptomyces lydicus* from Red Sea. Egypt. Int. J. Chem. Technol. Res. 5(1):15-23.

Sivakami V, Ramachandran B, Srivathsan J, Kesavaperumal G, Smily B, Kumar JM (2012). Production and optimization of laccase and lignin peroxidase by newly isolated *Pleurotus ostreatus* LIG 19. J. Microbiol. Biotechnol. Res. 2(6):875-881.

Sivakumar R, Rajendran R, Balakumar C, Tamilvendan M (2010). Isolation, screening and optimization of production medium for thermostable laccase production from *Ganoderma* sp. Int. J. Eng. Sci. Technol. 2(12):7133-7141.

Snajdr J, Baldrain P (2007). Temperature effect the production, activity and stability of ligninolytic enzymes in *Pleurotus ostreatus* and *Trametes versicolor*. Folia Microbial. 52(5):498-502.

Ticlo DD, Verma AK, Mathew M, Raghukumar C (2006). Effect of nutrient nitrogen on laccase production, its isozyme pattern and effluent decolorization by the fungus NIOCC #2a, isolated from mangrove wood. Indian J. Mar. Sci. 35(4):364-372.

Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G (2014). fungal laccase and their applications in bioremediation. Enzyme Res. 1-21.