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Optimization of Different Carbon Sources For The Production of Laccase Enzyme

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Abstract
Laccase is used industrially for pulp delignification, polycyclic aromatic hydrogen degradation, pesticide or insecticide degradation and organic synthesis. With microbial enzymes dominating world markets, more innovation and improvisation is needed to increase the efficiency of production at an economical rate. Laccases are multi-copper oxidases having wide substrate specificity mainly found in white-rot fungi, which are the only microorganisms able to degrade the whole wood components. As they are capable of degrading a wide variety of compounds they are commercially very significant. Present study aims at studying the production and optimization of laccase using different carbon sources. Agro-industrial residues, such as wheat bran, rice bran, glucose and glucose along with guiuacol were screened for laccase production, under aqueous-state fermentation conditions, by the white-rot fungus, *Pleurotus pulmonarius*. Glucose with guiuacol gave the highest activity, reaching about 5.0 U/ml within 10 days.

Keywords: laccase, aqueous-state fermentation, guiuacol, *Pleurotus pulmonarius*.

INTRODUCTION

Laccase (E.C. 1.10.3.2) is the most common lignin modifying enzyme produced by the white-rot fungi belonging to the family Polyporaceae *sensu lato* (Pelaez *et al.* 1995). Among them, *Trametes versicolor* has extensively been used as the main experimental organism for laccase production studies. However, another fungus belonging to the same genus, *Trametes hirsuta* has also been described as a very promising candidate for the production of laccase (Vares andHatakka., 1997). Moreover, laccase from *T. hirsuta* can efficiently degrade a wide variety of synthetic dyes (Abadulla *et al.* 2000 and Campos *et al.* 2001). This makes this biocatalyst very suitable for the treatment of waste water from the textile industry. Most studies dealing with ligninolytic enzyme production by white-rot fungi have been carried out using liquid culture conditions (Rosales *et al.*, 2007). Laccase is used industrially for pulp delignification, polycyclic aromatic hydrogen degradation, pesticide or insecticide degradation and organic synthesis. With microbial enzymes dominating world markets, more innovation and improvisation is needed to increase the efficiency of production at an economical rate. Laccases are multi-copper oxidases having wide substrate specificity mainly found in white-rot fungi, which are the only microorganisms able to degrade the whole wood components. As they are capable of degrading a wide variety of compounds they are commercially very significant. The selection of a substrate for aqueous-state fermentation processes depends upon several factors mainly related with cost and availability and thus may involve screening of agro-industrial residues. Wheat bran has commonly been used for the cultivation of microorganisms in aqueous-state fermentation processes. In the present work, in addition to wheat bran, some other agro-industrial residues such as rice bran, glucose and glucose along with guiuacol have been tested for laccase production by *Pleurotus pulmonarius* under aqueous-state conditions.

MATERIALS AND METHODS

Microorganism:

The white rot fungus, *Pleurotus pulmonarius* was isolated from soil sample. Pure culture was maintained on potato dextrose agar. *Pleurotus pulmonarius* was maintained and cultured in Potato Dextrose Agar (PDA) media and stored at 4°C.

Fungal Staining:

Lacto-phenol Cotton Blue was used for staining the fungal culture.

Confirmation of laccase production:

Laccases, which are extracellular secretion of white rot fungus, were able to oxidize different substrates such as guiuacol, syringoldazine, and non-phenolic compounds (Moorthi *et al.*, 2007). The oxidase enzyme system of *Pleurotus pulmonarius* was checked based on Trejo Hernandez *et al.* (2001). The laccase production media containing wheat bran flakes 4.5%, yeast extract1.5%, glucose 1%, NH_4Cl 0.25%, thiamine dichloride 0.05%, KH_2PO_4 0.2%, $MgSO_4.7H_2O$ 0.05%, $CaCl_2$0.01%, KCl 0.05% and pH 5.0 was prepared and the guiuacol (50 µg/100 ml) was added as a substrate for laccase screening. In

this study, a disc of 6 day-oldculture was placed at the centre of the plate. The plates were incubated at the dark place and observed for reddish brown zone in the medium which will be formed as a result of laccase oxidative polymerization with guiuacol (Moorthi *et al.*, 2007).

Carbohydrate Source:

The primary carbon source used was agricultural wastes which were used in place of Glucose and Guiuacol in a composite medium consisting of 0.1% KH_2PO_4, 0.05%$MgSO_4.7H_2O$, 0.02% NH_4NO_3, 0.001% $CaCl_2$,0.0001% $CuSO_4.5H_2O$, 0.0001% $FeSO_4.7H_2O$,0.0001% $MnSO_4$ and 0.01% yeast extract.

Primary Carbon Source:

- Wheat
- Rice

The laccase production using the two carbon sources was compared with composite medium containing 1% Glucose and 1% glucose along with guiuacol which acts as an inducer for laccase enzyme.

Culture conditions:

Four composite media containing four different carbohydrate sources namely, wheat bran, rice bran, 1%glucose and 1% glucose along with guiuacol were prepared based on Jhadav *et al.*, (2009). For test organism, 250-ml Erlenmeyer flask containing 100-ml of media were prepared in triplicate. The flasks were inoculated with 1g wet weight of mycelial spores of the organisms grown on PDA plates for 72 hours at 28±2°C. The flasks were rotated at 150 rpm at 28±2°C for 10 days. After 10 days the contents were filtered and centrifuged at 8000 rpm for 20 min.

Laccase activity:

Extracellular laccase activity was assayed spectrophotometically as described by Jhadav *et al.*, (2009) with Guiuacol as substrate. The reaction mixture contains 10 mM Guiuacol with 1 ml of culture filtrate (enzyme). The oxidation reaction was monitored by measuring the change in A_{530} for 10 min. One unit enzyme activity was defined as 1 µ mole of Guiuacol oxidized per minute at 25°C. The activities were expressed in U/ml.

RESULTS AND DISCUSSION

The potential of agro-industrial residues, such as wheat bran and rice bran, as substrates for laccase production by *Pleurotus pulmonarius* under aqueous-state conditions, was investigated. These materials were selected due to their availability and low cost.

Screening of fungus for extracellular enzyme production:

White rot fungi are capable of producing extracellular enzymes such as lignin peroxidase, manganese peroxidase and laccase. Laccase is mainly responsible for the decolorization of aromatic compounds. It is able to oxidize substrates such as ABTS (2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid)), guiuacol. In this test dark reddish brown zones appeared on the culture plate. In *Pleurotus pulmonarius* inoculated plate, the appearance of dark reddish brown colour takes place within 24 hrs and the complete color change was observed in 2nd day suggesting that the *Pleurotus pulmonarius*, a test organism secretes extracellular enzyme which oxidatively polymerized guiuacol. The similar finding was observed by Moorthi *et al.*, (2007) during which they observed dark reddish brown zones appeared on culture plate on 4th day for *Trametes hirsuta*.

Laccase production optimization with different carbon sources:

Laccase production and activity was measured byculturing the fungi in medium containing different carbon sources. Laccase production time was standardized using composite medium containing glucose and guiuacol. Standard time for production of laccase was found to be on the 10th day. Hence 10 days was taken as the standard incubation for production of laccase in medium containing different carbon sources. We observed that there is a slight increase in the activity of laccase when the culture is grown in rice bran containing medium (Laccase activity 2.25 U/ml) as compared to wheat bran (Laccase activity 2.90 U/ml) and glucose containing medium (Laccase activity 3.90U/ml). Activity of laccase when the culture is grown in rice bran, wheat bran and glucose containing medium was found to be slightly different suggesting that wheat bran and glucose are the best alternative for enzyme production. We also observed that medium containing glucose in presence of guiuacol gave higher laccase activity (5.0 U/ml) as compared to other medium containing wheat bran, rice bran and glucose as carbon source (Figure-1). The similar observation regarding the laccase production in aqueous-state condition by the white rot fungus *Phanerochaete chrysosporium* has been observed by Jhadav *et al.*, (2009) in which they reported the activity of laccase, 0.15 U/ml, 0.24 U/ml, 0.21U/ml, and 0.44 U/ml with wheat bran, rice bran, glucose and glucose with guiuacol containing medium respectively. Laccase is a very unique enzyme capable of degrading multiple substrates. Hence

it is no surprise that such an enzyme will be of considerable market value. However, as this enzyme is secreted in very low amounts by the organism a lot of work is needs to be carried out in increasing the production output and optimization. The main objective of present study was to determine if the production efficiency of the enzyme would increase with an alternate carbon source other than glucose. We found increase in the activity when the culture was grown in presence of wheat bran as a carbon source similar to the glucose. We also observed in the process that presence of an inducer like guiuacol is required to boost the production. Though we used the inducer in a glucose containing medium we can safely assume that use of inducer in a medium containing alternate carbon source like wheat or rice bran would further increase the production of the enzyme.

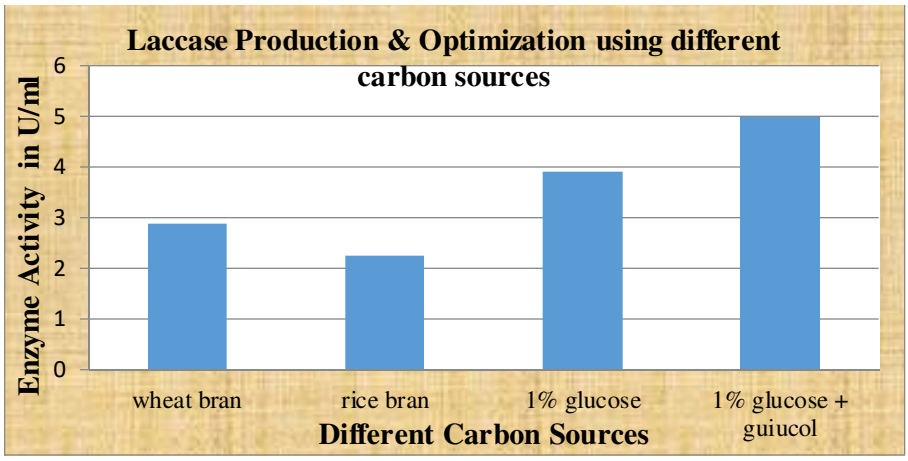

Figure-1. Laccase production and optimization using different carbon sources.

CONCLUSION:

According to the results obtained in the present work, agro-industrial residues, especially wheat bran and rice bran, have an enormous potential as substrate for laccase production by *Pleurotus pulmonarius* in aqueous-state conditions. Furthermore, these substrates make the process more economical due to their availability and low cost, also they provide some nutrients for the microorganism. Guiuacol could be used as an inducer as well as substrate for laccase production using white rot fungi.

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