EFFECT OF DIFFERENT AGRO-WASTES ON LACCASE PRODUCTION BY PLEUROTUS OSTREATUS IMI 395544

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Abstract: This study was carried out to utilize the agro-wastes like rice bran, wheat bran, and rice straw, and saw dust for the production of commercially important enzyme laccase. The effect of various agro-wastes on the production of laccase by white rot fungi Pleurotus ostreatus IMI 395544 was tested under stationery culture. Agro-waste (10 g) and nitrogen source (0.5 g) in 100 ml medium were found to be optimized concentration of carbon and nitrogen sources respectively. Among the carbon source, wheat bran showed the maximum activity of laccase (0.31 U/ml) and the same carbon source along with malt extract showed 0.36 U/ml activity. The influence of different inducers and metals on the production of laccase also assayed. Laccase production was further increased by adding aromatic inducer like 2, 5 xylidine to 0.47 U/ml. The effect of copper was greatest among the tested metal ions, which gives 0.41 U/ml laccase activity. The results suggest that P. ostreatus IMI 395544 is a high potential producer of the industrially important enzyme laccase with suitable medium components.

Keywords: Agro-waste, Inducers, Laccase activity, Pleurotus ostreatus, wheat-bran

1. INTRODUCTION
Large quantity of agricultural residues accumulates in agro-industries. Disposing of agro-wastes is one of the serious problems for industries. Some of these can be recycled and upgraded for the use as animal feed or for preparation of other products (Cohen et al., 2002) generally these agro-wastes contain large amount of lignin and cellulose which is a good substrate for the growth of fungus. Currently, these agro-residues are either allowed to decay naturally in the fields or burnt. These residues are, however, potential substrates for microbial conversion via solid substrate fermentation into value added products such as enzymes (Pandey et al., 2000). Solid state culture (SSC) is defined as a cultivation, in which microorganism grows on a moist water-insoluble solid material in the absence or near absence of free water (Mooyoung et al., 1983; Gervais and Molin, 2003). It mimics the natural environment of the white-rot fungi and holds tremendous potential for the production of enzymes, including laccase and Manganese peroxidase (MnP) (Pandey et al., 1999). Laccase (benzinediol: oxygen oxidoreductases; E.C. 1.10.3.2) copper containing enzymes, which catalyze the oxidation of a variety of phenolic and non-phenolic compounds with the concurrent reduction of $O_2$ to $H_2O$.

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The broad specificity for the reducing substrates, laccases from white-rot fungi are receiving increasing attention as potential industrial enzymes in various applications, such as pulp delignification, wood fiber modification, dye or stain bleaching, chemical or medicinal synthesis, and contaminated water or soil remediation (Couto et al., 2001). The main limitation for the extensive industrial application of microbial enzymes is their high cost. Therefore, a good strategy to increase the productivity of the fermentation processes would expand the spectrum of laccase-producing organisms and enhance their enzyme production. SSF offers many advantages over submerged fermentation, which include higher product titers, lower wastewater output, reduced energy requirements, simpler fermentation media, etc. (Pandey et al., 2001).

The aim of this study were to assess the potential of selected agro-residues for laccase production via SSC using Pleurotus ostreatus IMI 595544 and to find out the effect of various nitrogen sources, inducer and metal ions for the production of laccase in stationary culture.

2. MATERIALS AND METHODS

The strain, Pleurotus ostreatus IMI 395544 was obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The strain was acknowledged and certified by Commonwealth Agricultural Bureaux International (CABI) London, United Kingdom. It was cultured on malt extract agar slants for two weeks at 28°C. The stock culture was stored at 4°C and subcultured every month. Chemicals such as guaiacol (Sigma, Mumbi), yeast extract, malt extract, peptone, soya peptone, beef extract and meat extract were purchased from Himedia (Chennai). All the other chemicals used for study were of analytical grade. Rice bran, wheat bran, saw dust; rice straw was purchased from local market. Static cultivation was carried out at 25°C in 100 ml Erlenmeyer flasks with 50 ml of medium. 10 g of sterilized agro wastes were supplemented with nutrient solution containing (w/v): 0.2 % KH₂PO₄, 0.05 % MgSO₄·7H₂O and 0.002 % MnSO₄. The flasks were inoculated with two malt extract agar plugs (10 mm diameter) cut from an actively growing part of a colony on Petri plate and incubated at 27°C for 20 days. Three culture flasks of each substrate were randomly sampled for laccase assay. Laccase enzyme activity was assayed by the method of Arora and Sandhu (1985) with minor modifications. One unit of enzyme activity was defined as the amount catalyzing the production of one μmol of substrate per ml per min. Enzyme activity was expressed in U/ml.

3. RESULTS AND DISCUSSION

Agro-residues contain high amount of cellulose, which supports good growth of fungus and laccase productivity. The first signs of growth were seen after two to three days after inoculation. As the culture grew older, mycelium spreads all over the media within 25 days of fermentation. The profile of laccase productivity during degradation of various agro-residues by Pleurotus ostreatus IMI 565454 is given in Fig 1. Generally, substrates that were utilized efficiently and rapidly by the organism produce high level of laccase. Wheat bran gave the highest laccase production of 0.31 U/ml. The reason may be the wheat brawn is ligninocellulose source, which provide both carbon and nitrogen source and cause an effect called carbon repression which supports better production of laccase (Krishna Prasad et al., 2005). Next to the wheat bran, banana leaf waste shows 0.27 U/ml of laccase production, but the growth of the fungus was very slow when compared with wheat bran. Both saw dust and rice bran were poorly utilized.
for the growth by the fungus, resulted in the low laccase levels 0.18 U/ml and 0.11 U/ml respectively.

Fig. 1: Effect of Different Agrowastes on Laccase Production by Pleurotus Ostreatus IMI 395544

Another essential factor for efficient laccase production by fungi is the nitrogen source used for cultivation. The effect of different nitrogen sources on laccase production by Pleurotus ostreatus IMI 595545, when grown on 10 g/L, wheat brawn in basal medium was assayed was shown in Fig. 2. The effect of peptone, soya peptone, yeast extract, malt extract, beef extract and meat extract of (5 g/L) on laccase production was determined. The highest enzyme production (0.36 U/ml) was achieved when using malt extract as the N-source. There was a slight reduction in the production of laccase (0.34 U/ml) was obtained when malt extract was replaced by yeast extract. Less than 0.20 U/ml of laccase activity were obtained when using beef extract and meat extract as N-sources. Earlier reports in the production of laccase support the malt extract is one of the best nitrogen sources, when compared with other organic sources (Arora and Gill, 2005).

Figure 2: Effect of Different Nitrogen Source on Laccase Production by Pleurotus Ostreatus IMI 395544
Extracellular laccases are constitutively produced in small amounts. However, their production can be greatly stimulated by presence of wide variety of inducing substances. Different inducers such as vanillic acid, gallic acid, 2, 5 xylidine, 1-hydroxybenzotriazole, guaiacol and p-anisidine were added at 1 mM concentration after 8 days of incubation. The effect of inducers in the production of laccase was shown in the Fig 3. Substantial increase in laccase activity was observed using hydroxylbenzotriazole (HBT) (0.37 U/ml), (0.34 U/ml) as compared to control (0.32 U/ml). 2, 5 xylidine was found to be the best inducer, which gave 0.47 U/ml. This data is well agreed with earlier reports in Pleurotus spp. (Leonowicz et al., 2001; Krishna Prasad et al., 2005).

Interestingly, the addition of some of the metal ions resulted in markedly enhanced the formation of extra cellular laccase activity was shown in Fig. 4. Different metal ions were selected to study the stimulatory effect in the production medium; the final concentration of the metal ion in the medium is 1 mM. A control was maintained to determine the effect of added metal ions contains only wheat bran (10 g/dL and yeast extract (0.5 g/dL). The effect of copper was very much noticeable when compared with other metal ions. The highest laccase activity was observed in the flask added with copper ions (0.41 U/ml). Copper is an essential micronutrient for most of living organisms (Cervantes and Gutierrez-Corona, 1994). Copper requirement by microorganism are usually satisfied in very low concentration, ranging between 1 to 10 M. copper present in higher concentrations in its free, cupric form, is extremely toxic to microbial cells (Labbe and Thiele, 1999). In contract, metal ions like Hg and Cd reduce the laccase production when compared with control (0.35 U/ml), 0.13 U/ml and 0.20 U/ml respectively. The obtained data shows, these elements are highly toxic for the fungus in the selected concentration.
4. CONCLUSION

The study has explored how to utilize the agrowaste for the production of commercially important enzyme laccase. Wheat bran is the one of the best carbon source among the other agro-wastes for the production of enzyme. We can minimize the production time in solid state fermentation by using yeast extract and increase the yield by adding inducer like 2,5 xylidine. As a metal ion copper has certain stimulatory effect, which should be extensively studied along with inducer for achieving maximum production of industrial applications enzyme laccase with less cost.

References


