ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIO CHEMISTRY

CHARACTERIZATION AND DECOLORIZATION OF DYE AND TEXTILE EFFLUENT BY LACCASE FROM PLEUROTUS FLORIDA- A WHITE-ROT FUNGI

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ABSTRACT

The textile industries by for the most passionate user of synthetic dyes are in need of ecoefficient solutions for its coloured effluents. The decolorization and detoxification potential of white rot fungi can be harnessed. Laccase has been utilized in biological treatment of effluents containing chemical dyes. The present study is planned to study the screening and chrecterization of laccase from *pleurotus florida*. Dye decolorization study in invitro condition. Screening of the *pleurotus florida* for the production of laccase enzyme on potato dextrose agar plates. Estimation of laccase production by laccase assay method. Determination of optimum pH, Temperature, substrate concentration, enzyme concentration. Synthetic dye decolorization by using the crude laccase (1:10) and dye decolorization on agar. plateKeywords: white rot fungi- pleurotus florida- synthetic dye decolorization.

ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

KEYWORDS

white rot fungi, pleurotus florida, substrate concentration, enyme concentration, synthetic dye decolorization.

INTRODUCTION

Worldwide over 10,000 different dyes and pigments are used in dyeing and printing industries. The total world colorant production is estimated to be 8, 00,000 tons per year and at least 10% of the used dyestuff enters the environment through wastes[1]. White rot fungi are the most efficient ligninolytic organisms capable of degrading various types of dyes such as azo, heterocyclic, reactive and polymeric dyes. This capability is due to extracellular non-specific and non stereo selective enzyme systems composed of laccases (EC 1.10.3.2), lignin peroxidases (EC 1.11.10.14) and manganese peroxidase (EC1.11.1.13)[2]. Laccase enzyme offers an attractive solution because of its potential to degrade wide spectrum dyes having diverse chemical structure[3].

Laccase are generally produced during the secondary metabolism of white-rot fungi growing on natural or in submerged culture [4]. Laccase catalyze the oxidation of both phenolic and non-phenolic compounds[5] and thus can mineralize a wide range of synthetic dyes. This non-specific mechanism of laccase makes it a versatile biocatalyst suitable for several applications such as industrial wastewater treatment, bioleaching and biopulping.

Laccase has been utilized in biological treatment of effluents containing a mixture of different dyes. As a result, most processes currently employed to treat dye wastewater are ineffective and uneconomical[6]

MATERIALS & METHODS

White rot fungus*, pleurotus florida* were isolated from the foothills of nilgiris and maintained on potato dextrose agar plated (PDA) at 4°C. Screening of laccase production is based on oxidation of guaiacol. White rot fungi *pleurotus florida* was inoculated into the guaiacol PDA plate and incubated at room temperature for 3days. After incubation, laccase production was observed by brown color. Growth rate and laccase activity was assayed. Pleurotus florida strain present in PDA plates were used as the inoculums. *Pleurotus florida* strain were inoculated to 100ml of medium and incubating it for ten days at 25°C. After ten days the mycelia growth in the broth was mixed well and transferred to the cultivation medium. Samples were withdrawn periodically (regularly 24hr interval) and centrifuged at 5000rpm for 5minutes. The supernatant was used as a source of enzyme. Growth rate of the fungus were measured at 520nm and the laccase activity at480nm were analyzed. The crude enzyme was characterized by analyzing its effect on pH, temperature, substrate concentration, enzyme concentration and also on organic solvents (methanol, ethanol and acetone) activator ($CuSo_4$, $MgCl_2$, $CaCl_2$, Kcl and $NaCl_2$) and inhibitor (EDTA, l-Cysteine and NaN_3) were analyzed.

(i) Determination of optimum pH

There is not much information available on the influence of pH on laccase production, but when fungi are grown in a medium where pH is optimal for growth (pH 5), the laccase will be produced in excess. The assay was performed at different pH levels pH-3.5, pH 4.5, pH 5.5, pH6.5 and pH 7.5. The

optimum pH was determined for laccase enzyme .

(ii) Determination of optimum temperature

The fungi were cultivated at temperatures between 25°C and 30°C for optimal laccase production[7]. When cultivated at temperatures higher than 30°C the activity of ligninolytic enzyme was reduced. Te optimum temperature was determined for the laccase of *pleurotus florida*. The assay was performed at different temperatures -4°C, 25°C, 37°C, 50°C and 60°C.

(iii) *Determination of substrate concentration*:

The assay was performed at different substrate concentration - 50µl, 100µl, 150µl, 200µl and 250µl.

(iv) *Determination of enzyme concentration*:

The assay was performed at different enzyme concentration -25µl, 50µl, 100µl, 125µl, and 150µl.

(v) *Effect of organic solvents on laccase activity*:

Organic solvent stability of the *pleurotus florida* was done. The crude laccase was incubated for 30minutes with organic solvent such as methanol, acetone, ethanol and chloroform at different concentration (10%, 20%, 30%, 40% and 50%). After the incubation, the laccase activity was assayed.

(vi) Effect of metal ions on laccase production:

Experiments were performed for the culture medium supplemented with 1.0mM concentration of $CuSO_4$ on second, third and fourth day of fermentation. Lack of $CuSO_4$ in the fermentation medium served as a control. For optimizing copper concentration for laccase production by this organism, the subsequent experiments were carried out at various concentrations of $CuSO_4$ (0.5-5.0 mM) on the third day of fermentation[8].

(vii) Effect of inhibitor on laccase activity

some of the inhibitor effect was studied on the *pleurotus florida* laccase. The crude laccase was incubated for 30minutes with different inhibitors such as EDTA, NaN_3 and L-Cysteine at different concentration (90.1mM, 1mM and 10mM). After incubation, the effect of inhibitor was analyzed.

(viii) Determination of laccase activity:

Assay mixture contained 3.8ml of sodium acetate buffer (10mM, pH 5.5), 150µl of guaiacol (2mM), and 0.2ml of crude enzyme. This reaction mixture was incubated at 25°c for 30minutes. The absorbance was measured at 480nm[9].

(ix) *In vitro dye decolourization using pleurotus florida*:

The decolorization effect was observed both on the commercially available synthetic dyes as well as on the textile effluent.

(x) Dye decolorization on agar plate:

Decolorization in solid medium was assayed by visual disappearance of color from the plate. The *pleurotus florida* was inoculated into the PDA plate. 300mg of malachite green, orange G and crystal violet were added to their respective plate. Percentage of decolorization was calculated by using this formula.

$$\text{Decolorization of dye} = \frac{\text{OD of test}}{\text{OD of control}} \text{x} 100$$

(xi) Synthetic dye decolorization in liquid culture:

The *pleorotus florida* was inoculated into the laccase production medium containing 270mg of Orange G, malachite green and crystal violet in separate conical

flask. Periodically samples were collected and dye decolorization in liquid culture was measured colorimetrically at the maximum visible wavelength of absorbance (480nm for orange G, 620nm for malachite green and crystal violet).

(xii) Synthetic dye decolorization of textile effluent:

Textile effluent was collected in and around tirupur district. The wastewater is not changed with respect to temperature, light. It was found to be slightly alkaline and characterized by reddish color. The crude filtrate of *pleurotus florida* was inoculated into the textile effluent and its diluted variants (1:10). The percentage of decolorization was measured[10].

RESULTS

1. Laccase production and optimization:

Figure 1 shows the screening of laccase enzyme and figure 2 illustrates the laccase production. The maximum level of laccase activity was produced by white rot fungus, *pleurotus florida* on 26th day culture. Highest level of growth was observed on 24th day

.Screening and production of laccase enzyme

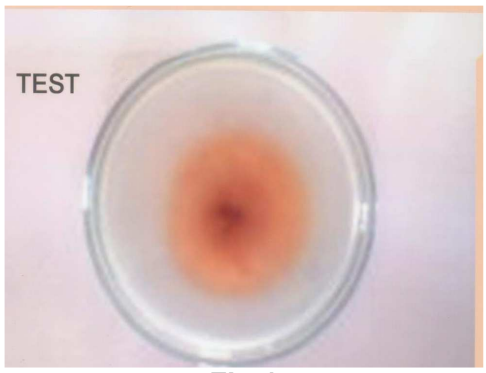

Fig 1
Laccase screening

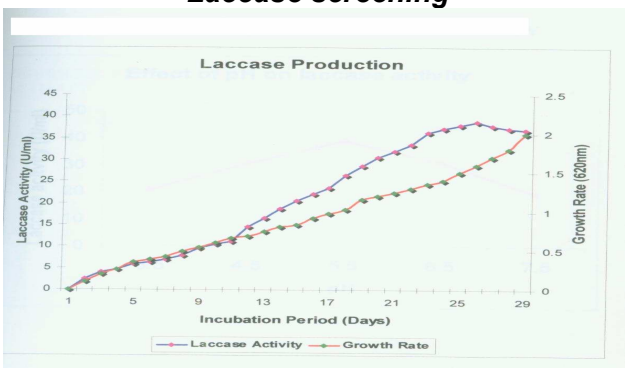

Fig 2
Growth rate and activity of laccase enzyme

The optimum pH(5.5), temperature(37°c), substrate concentration(1.0ml),enzyme concentration(0.2ml) and effect of organic solvents showed that 10% methanol has the maximum activity (32.5U/ml) compared to ethanol and acetone. Effect of metal ion and effect of inhibitors results that laccase was completely inhibited by 0.1mM sodium azide compared to 1mM L-cysteine, and EDTA.

2. ***Decolorization of synthetic dye using PDA media***:

Figure 3, 4, 5 shows the decolorization activity increases in the following order crystal violet, orange G, malachite green

. **Results of decolorization test for crystal violet, Orange G, malachite green**

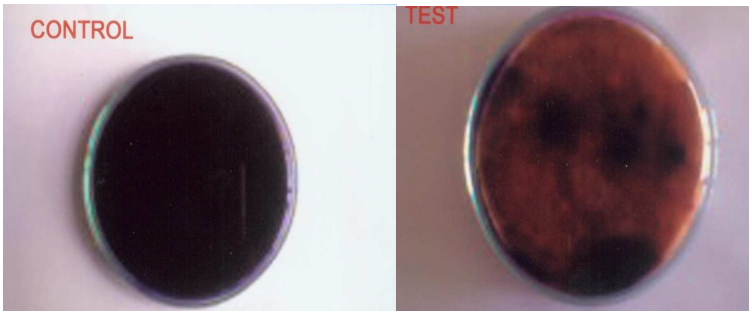

Fig 3
Decolorization of crystal violet

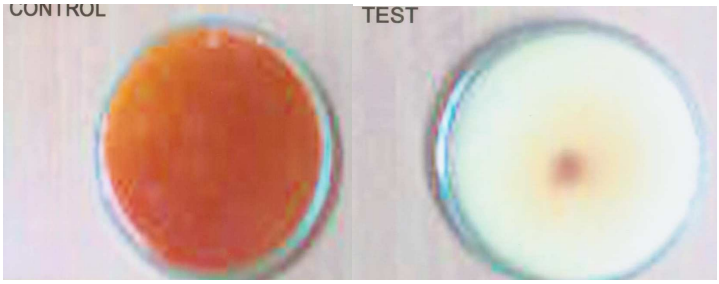

Fig 4
Decolorization of orange G

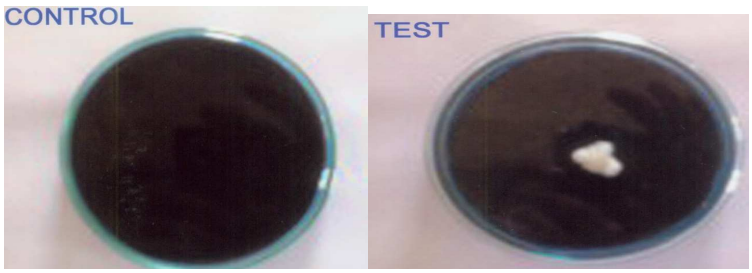

Fig 5
Decolorization of malachite green

3. ***Liquid broth synthetic dye decolorization***:
   Fig 6 shows the decolorization of synthetic dyes in liquid broth.

Synthetic dye decolorization in liquid broth

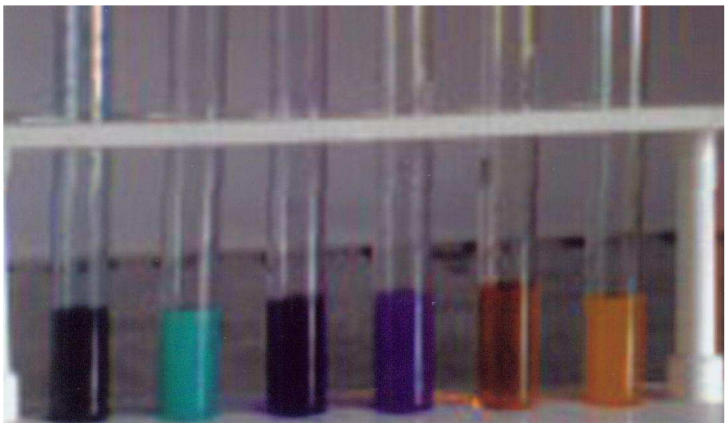

Fig 6
Decolorization of synthetic dye after 6hours incubation

4. ***Decolorization of textile effluent by tube method:***
   The organism decolorizes both the crude textile effluent and diluted (1:10) within 24 hours the results are shown in figure 7.

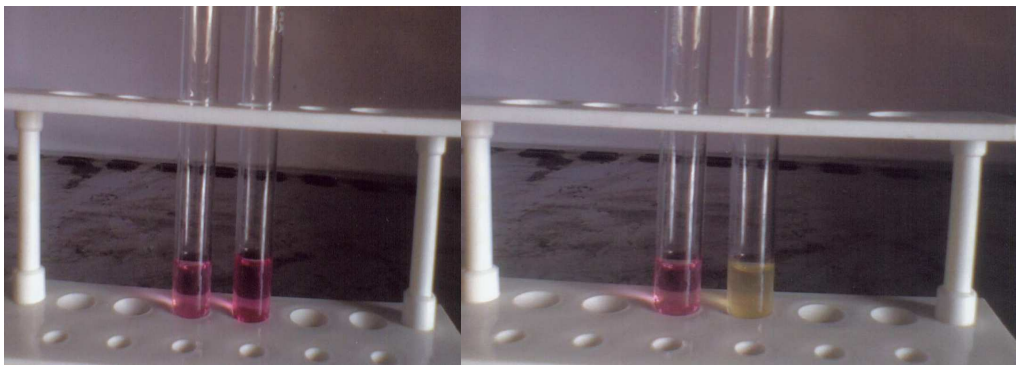

Fig 7
Control tubes and decolorizatiom of effluent after 16 hours incubation with laccase enzyme

Above results proved that laccase has better effect to decolorize the textile effluents.

DISCUSSIONS

Results showed that *pleurotus florida* is a white rot fungi, which produces laccase and the maximum level of laccase production was viewed on 22nd day of incubation. Laccase

production is optimum at pH 5.5 and 37°C.the tested synthetic dyes crystal violet and orange G showed 100% and malachite green 90% of decolorization on the PDA media. The orange G decolorize very slowly in the tube method when compared to malachite green and crystal violet. But the textile effluents are very effectively decolorized by laccase within 24hours. When compared with synthetic dye.

CONCLUSION

The orange G decolorize very slowly in the tube method when compared to malachite green and crystal violet. But the textile effluents are very effectively decolorized by laccase within 24hours when compared with synthetic dye.From the above results we can conclude the laccase can be used as decolorizing agent in the treatment of textile effluent.

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