SCREENING AND CHARACTERIZATION OF LACCASE FROM FUNGAL ISOLATES

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Abstract : Laccases (EC 1.10.3.2) are multi-copper oxidases that catalyze oxidation of various substituted phenolic compounds, aromatic amines and even certain inorganic compounds by using molecular oxygen as the electron acceptor. Their substrate versatility makes laccases highly interesting for various applications, including textile dye bleaching, pulp bleaching and bioremediation. This work focused on identifying and characterizing novel fungal laccases having high potential for the applications. Laccase-producing fungi were screened from various soil samples collected from Western Ghats Tamil Nadu by plate tests using guaiacol as indicator. A total of seven different samples were screened and their ability to produce laccase was studied in liquid media. One fungal strain produced significant amounts of laccase, and these enzymes were preliminarily characterized. And the optimum pH and temperature for fungal stain was found to 4.8 and 50 c. And its ability to decolorize three different dye also been studied and it's seen that it decolorize the dyes effectively.

Key Words: Laccase, oxidases, bioremediation, bleaching, guaiacol

1. INTRODUCTION

The laccase (E.C 1.10.3.2) is widely distributed throughout nature and exhibits a wide range of roles and substrate specificity. They are sometimes also referred as polyphenol oxidases (PPOs) and are part of the multi copper oxidase family. The laccase enzyme was first discovered in the tree *Rhus vernicifera* more than a hundred years ago. Both plant as well as fungal laccases is similar in that they have low substrate specificity, reacting with a host of derivative phenols with K_m of a few mill molar. This versatility due to lack of substrate specificity has allowed new roles to be found in several commercial processes including delignification, decolourization of industrial effluents, stain removal and wine clarification (Mayer and Staples , 2002). Laccase, a kind of polyphenol oxidase containing copper atoms, can oxidize an array of organic and inorganic substrates, including mono-, di-and polyphenols, aminophenols, methoxyphenols, as well as metal complexes like ferrocene, ferrocyanide or iodide, by concomitant four electron reduction of oxygen to water [6]. Laccase typically contains 15-30% carbohydrate. Laccase usually has an acidic isoelectric point and has a molecular mass of 60-90 kDa. The currently used oxidation methods in industries such as textile, food, wood processing, pharmaceutical, and chemical are not eco-friendly or economical and also produce unwanted side reactions. Hence, enzymatic oxidation is a potential alternative to chemical methods. Laccases from fungi has the capability to oxidize a wide range of industrially relevant substrates. They are being increasingly used for various industrial purposes such as paper processing, prevention of wine discoloration and detoxification of environmental pollutants [5], oxidation of dyes [9], and production of chemicals from lignin. The physical and chemical methods currently used to decolorize the textile industry effluents currently face a number of limitations, including high cost and inability to decolorize the broad range of dyes [4]. Electrophoretic separation of crude and purified laccases reveals the presence of multiple

isoforms in many fungi. Multi-copper oxidases belong to the class of enzymes containing four or more Cu centers in a protein molecule. The minimum functional unit of multicopper oxidase comprises a set of one type 1 Cu, one type 2 Cu and a pair of type 3 Cus. The type 1 cu functions as the electron mediator from substrate to the trinuclear centre composed of the type 2 Cu and type 3 Cu , where dioxygen is bound and reduced to two water molecules. All these copper sites are indispensable for the four-electron reduction of dioxygen without the release of activated oxygen species as intermediates. Attempts to develop bio-discoloration processes have largely been focused on bacterial cultures and have, in general, been successful.

2. MATERIALS AND METHODS

2.1. Screening of Fungal Producing Laccase

Soil samples were collected from Western Ghats Tamil nadu and these samples were serially diluted to 10^{-6} and they were spread on the PDA plate (Potato dextrose agar) containing 0.001% guaiacol (v\v) which is a substrate for detecting for laccase activity and plates were incubated for 3-4 days at 27°C [7].

2.2. Sub Culturing Of Fungal Producing Laccase

The fungal isolates that shows the laccase activity were sub cultured on PDA containing guaiacol in it this was carried out for four times in order to get the pure culture and in sterilized 250-ml Erlenmeyer flask containing 10g PDB (Potato Dextrose Broth) was prepared and the pure fungal isolates were inoculated in it .Then flask were incubated at 27◦C for 6-15 days. Crude culture filter was obtained by filtering the culture in flask through cheese cloth. The filtrate was again centrifuged at 10,000 rpm for 10min.The supernatant was used as the enzyme source.

2.3. Enzyme Assay

This assay is measured based on guaiacol oxidation at 460 nm. The reactive mixture was 3 ml acetate buffer (10 mM, pH 5.0), 1 ml guaiacol (2 mM) and 1 ml culture medium [1]. The contents were mixed well and incubated at 50°C for 5 min. The brown color formed was spectrophotometrically read at 460nm using Beckman DU-530 spectrophotometer. Enzyme activity was expressed in units/ml. One unit of laccase activity is defined as the amount of enzyme required to oxidize 1µmol guaiacol per minute.

2.4. Characterization of crude enzyme

2.4.1. Effect of pH on laccase activity

The reactive mixture was 3 ml acetate buffer (10 mM) of different pH 3.6 to 5.6, 1 ml guaiacol (2 mM) and 1 ml culture medium of different age (6,10and 15 day). The contents were mixed well and incubated at 50°C for 5 min [8].The brown color formed was spectrophotometrically read at 460nm using Beckman DU-530spectrophotometer.

2.4.2. Effect of temperature on laccase activity

The reactive mixture was 3 ml acetate buffer (10 mM, pH 4.8), 1 ml guaiacol (2 mM) and 1 ml culture medium. The contents were mixed well and incubated at different temperature 30 to 90°C for 5 min [2]. The brown color formed was spectrophotometrically read at 460nm using Beckman DU-530 spectrophotometer.

2.5. Application

- **Decolourization studies**

Decolorization in solid and liquid medium was assessed by the visual disappearance of color from the plates and flask.

- **Solid plate decolorization studies**

Solid medium in petriplates were prepared in Potato dextrose broth (PDB) with 1.5% (w/v) agar, and an aliquot of an individual dye to a final concentration of 100ppm three different dyes were used (reactive red , reactive yellow, and reactive blue). Plates, each containing one of the dyes were inoculated with the culture. The culture was incubated at 27°C [3]. The plates were incubated for 10 days. Uninoculated plates served as control.

3. RESULTS AND DISCUSSION

3.1. Screening of Fungal Isolates

Intense brown colour was produced around the fungal colony in the guaiacol-containing medium which is considered as a positive reaction for the presence of laccase activity. And of seven soil sample screened four sample showed the positive result and one sample showed the effective result.

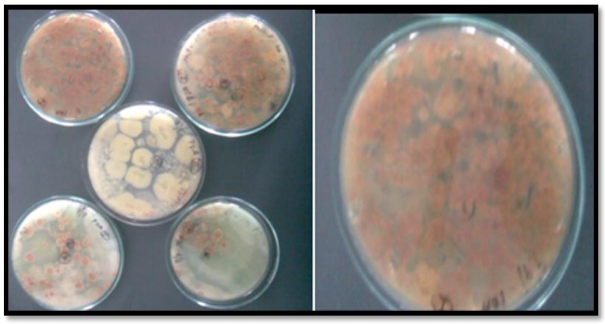


Fig 1 Screening of laccase in Potato Dextrose medium containing guaiacol

3.2. Enzyme Assay

Enzyme assay was performed at different days of inoculation (6, 10, 15 days) and crude filtrate of the sixth day showed high activity of laccase (9.33 U/ml).as shown in figure;

3.3. Characterization of Crude Enzyme

3.3.1. Effect of pH on laccase activity

The laccase enzyme activity assays was carried out at pH range from 3.6 to 5.6, using guaiacol as the substrate. The optimum pH for laccase activity was found to be at pH 4.8.

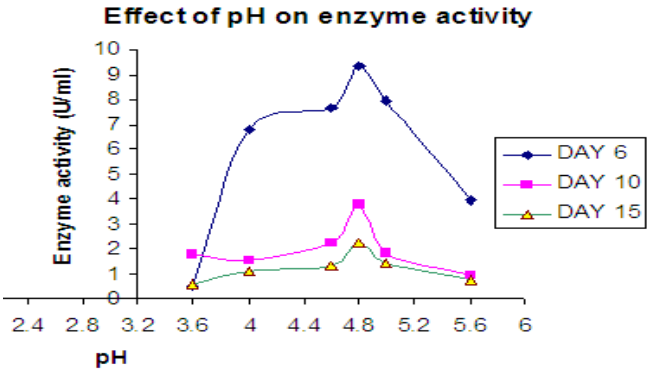


Fig 2 Effect of pH on laccase activity

3.3.2. Effect of temperature on laccase activity

The laccase enzyme activity assays was carried out at a temperature range from 30 – 90°C, using guaiacol as the substrate. The optimum temperature for laccase activity was found to be at 50°C.

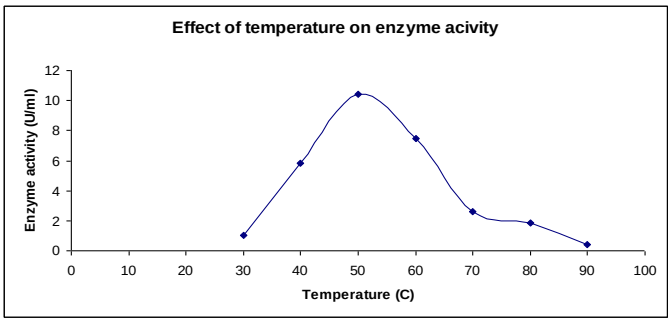


Fig 3 Effect of temperature on laccase activity

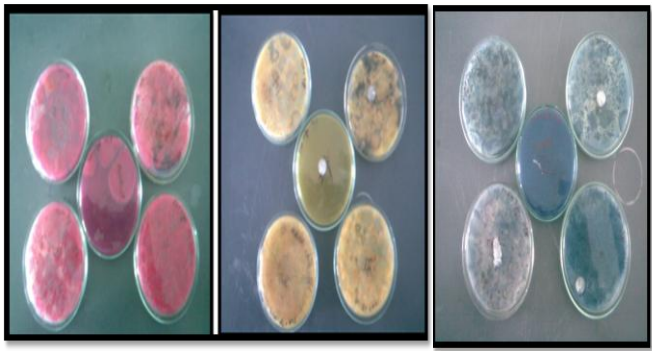
3.4. Applications

3.4.1 Solid plate decolorization studies

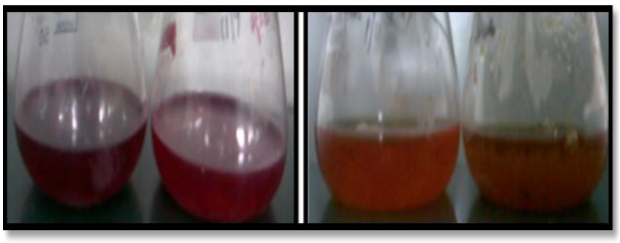
Dye decolorization studies were carried out with three different dyes and the results were seen after ten days and it was seen effective dye decolourization in the plates with the help of the control.

3.4.2 Liquid dye decolourization

Liquid dye decolourization was monitored with two different dyes reactive red and reactive yellow and decolourization was seen after ten days with the help of control and effective decolourization was seen in both the dye.



Reactive red Reactive yellow Reactive blue



Reactive red Reactive yellow

Fig 4 Decolorization Studies

4. CONCLUSIONS

The effective screening of the fungal isolates producing laccase was done effectively and the pure culture was obtained. And It was cultured and the characterization was carried out and the laccase shows maximum enzyme activity an sixth day, pH-4.8 and Temperature of 50˚C and cross checking was done for its application in dye decolourization it shown relatively good result and thus these results are for crude enzyme and thus we purify it means it may be an effective enzyme for dye decolourization.

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