

Screening of Laccase Producing White Rot Fungus from Decayed Wood

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ABSTRACT

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) belongs to the super family of multi-copper oxidases and is found in plants, fungi, and bacteria. Fungal laccase is reported to play roles in several processes including lignin degradation, morphogenesis, and pathogenesis. This paper describes the screening of potent extracellular- laccase producing white-rot fungus isolated from decayed wood and other soil samples. Potent fungal isolates were screened on Potato dextrose agar plate using various substrates such as 3mM guaiacol, 3m MABTS and 4mM tannic acid. In our screening, six fungal isolates from decayed wood were found to be potent laccase producer, where ABTS has proved to be well efficient substrate for laccase screening, where laccase for environmental application could be suitable for dye decolorization and removal of textile effluents.

KEYWORDS: ABTS, decayed wood, laccase, lignin degradation, white rot fungus

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1. INTRODUCTION

Laccases are copper-centred, blue-coloured polyphenolic oxidases, characterized as one of the critical enzymes or lignin degradation, bioremediation, green synthesis, sensors, food processing and paper and pulp industries. Mostly cases are a catalyst in acidic medium, but few laccases are reported to work at alkaline pH [7–9]. The laccase-catalysed redox reaction of substrates (phenolic substrates and dyes) with oxygen molecule results in the production of free radicals and water molecules. Laccases are one of the most used enzymes in most of the industries. Laccases are frequently isolated from many bacteria, fungi and plants. Laccases- producing microbes are isolated from plant tissues and animal guts. Most of the microbes produce extracellular laccases, few are intracellular, and some are on the cell wall of microbe/spore. The detailed reports on white-rot fungal laccases were abundant, but similar studies of laccase production from endophytic microbes are not frequent. Endophytic microbes first colonize and efficiently degrade host lignocellulosic waste [10]. The endophytic fungi *Monotropa*, *Phomopsis liquidambri*, *Aspergillus* sp., *Paecilomyces lilacinus* and *Lasiodiplodia theobromae* were isolated from *Cynodon dactylon*, *Bischofia polycarpam*, *Hierochloe brunonis* and *Psychotria flavid*, respectively, for the laccase production [11–14].

Laccases are remarkably non-specific regarding their reducing substrate. This range can even be widened in the presence of aromatic electron transfer or radical forming mediators (Knutson and Ragauskas, 2004; Camarero et al., 2005; Rodríguez Couto et al., 2005). Thus, in the presence of redox mediators such as 2-2'- azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) (Cantarella et al., 2003), N-hydroxybenzotriazole (HBT) (Kleen et al., 2003) and 3-hydroxyanthranilic acid (Li et al., 2001), laccases are capable to oxidise non-phenolic aromatic compounds. This makes laccases highly interesting for different industrial applications such as pulp bleaching, textile dye decolouration, wastewater treatment, bioremediation, etc. Recently, the potential applications of laccases within different industrial fields have been reviewed (Rodríguez Couto and Toca Herrera, 2006). The successful application of laccases to the above-mentioned processes requires the production of high amounts at low cost. laccase possesses broad catalytic properties; therefore it was exploited for wide biotechnological applications in various industrial areas. Laccase find application as health care field in medical diagnosis; and preparation of anticancer drug in pharmaceutical industry; synthesis of hormone derivatives, antiviral derivatives and antioxidants (Rocasalbas et al.,

2013). The most interesting study by the application of laccase is the development of newer oxygen cathode in biofuel cells, biosensors, labeling in immunoassays and organic synthesis through biocatalysis, food industry, reduction of toxicity in cosmetics and dermatological preparations.

Laccases are potential enzymes for treating various chemicals that cause environmental pollution in the processing industries. The merits of laccase suggest the researchers to produce in large scale for various applications. In our present study different subs rates are used for the screening of laccase. The fungal strain was screened for laccase production using ABTS, guaiacol and tannic acid as a substrate, which resulted positive indication for laccase production. One activity unit is defined as the amount of enzyme that oxidizes 1 mmol of ABTS per min at 25°C and the activities are expressed in U/L.

2. MATERIALS AND METHODS

2.1. Chemicals

Tannic acid, Guaiacol, ABTS, Potato Dextrose Agar (PDA) medium, and agar powder. Stated all chemicals and microbial media used in this studies were procured from Sigma-Aldrich and Hi-Media, Mumbai, India and were certified reagent grade.

2.2. Sample collection

Sample collection was done from decayed wood, bark and tree from various site of Ahmedabad such as soil sample surrounding tree from Gujarat university area, decayed wood sample near Botanical garden of Gujarat Arts and Science College. The samples were collected in sterile plastic bags swapped with alcohol and were sealed and brought to the lab aseptically for further processed inside laminar air flow.



(a)



(b)

Fig - 1 : (a) and (b) sample collection from decayed wood

2.3. Isolation and Screening of fungi for laccase production

For the screening of fungal laccase, plate assay techniques was used.

2.3.1. Plate as say method

The isolated fungi were screened for laccase enzyme using ABTS (2-2'-Azino-bis-[3-ethyl benzthiazoline-6- sulfonic acid]), guaiacol, and tannic acid as a substrates. The fungus was inoculated on different PDA agar plates containing 3 mM of ABTS (Srinivasan et al., 1995; Prabu et al., 2006), 4 mM of guaiacol (Collins and Dobson, 1997) and 4 mM of tannic acid as individually and incubated at room temperature for 7 days. The culture plates were observed for color change in the media. Various substrates used for screening of *Penicillium chrysogenum* for laccase production,

Fig.2(a)&(b) front and back morphology of ABTS screening-green zone formed, Fig.2(C)&(d) front and back morphology of guaiacol screening-reddish brown oxidation zone, Fig. 2(e) & (f) front and back morphology of tannic acid screening-dark brown oxidation zone

2.3.2. Bavendamtest

For the confirmation of the laccase production Bavendem test is been used. Fungal isolates were processed for Bavendem test. Fungal isolates which were giving potential zone of oxidation during primary screening were selected from Bavendemtest.

The fungal isolates were inoculated in to test tube containing potato dextrose agar supplemented with 100 mM guaiacol. The colour change from colourless to reddish brown colour was observed in fungal culture broth for presence of laccase. (Soponsathien, 1998).

3. Laccase activity

The laccase activity was measured by spectrophotometer using guaiacol as a substrate. Crude enzyme extract from the liquid culture medium was collected under aseptic condition. The reaction mixture consists of 3 ml of 100 mM of guaiacol dissolved in 10% acetone in sodium acetate buffer and 1 ml of culture filtrate (crude laccase). The mixture was incubated at 28°C for 60 min and the absorbance was recorded at 470 nm. One unit of laccase activity was defined as the amount of enzyme catalyzing the substrate (guaiacol) for the production of 1 all of colored product per min per ml (Collins and Dobson, 1997).

Laccase activity was measured as decrease in absorbance of Guaiacol reagent (substrate) due to laccase enzyme (1ml) in 60 min (Hadri, S.H *et al.*, .1998). It was calculated by the following formula;

$$\text{U/ml} = \frac{\text{Decrease in the absorbance of guaiacol reagent}}{\text{Incubation period}} \times \text{Dilution factor}$$

4. Results

4.1. Isolation and screening of laccase producing fungi

The laccase producing fungi were isolated from various samples of decayed wood and bark of trees. It was inoculated on PDA medium supplemented with guaiacol having concentration 4mM% as a substrate. The red dish brown oxidation zone was developed around 4 fungal colonies shown in figure 4 namely SM1, SM2, SM3, SM4, as shown in figure 2.

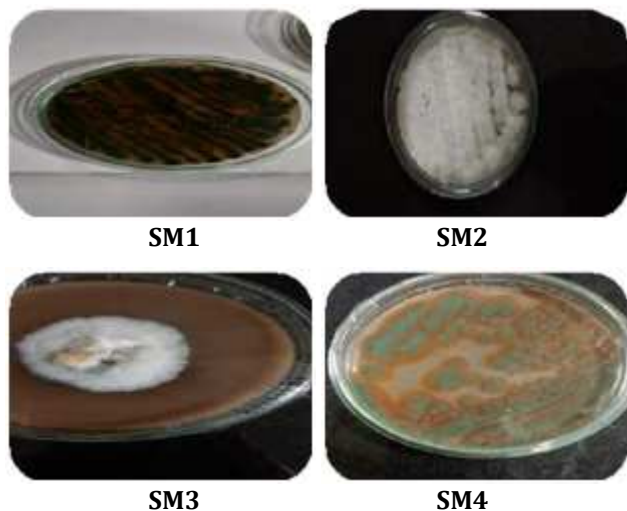


Fig 2- Isolation of fungal laccases from decayed wood

4.2. Screening of laccase using different substrates

As shown in figure 1, isolation was carried out using plate assay techniques on Potato Dextrose agar medium. For the screening of fungal laccases substrates such as 3 mM % ABTS (2-2'-Azino-bis-(3-ethyl benzthiazoline- 6-sulfonic acid)) and 4mM % tannic acid were used. The fungus SM3 showing potential oxidation zone was further screened for laccase production using as above substrates. In plate assay method the dark green colour was developed by all the three isolates in medium containing ABTS as a substrate and dark brown colour was developed for plate containing tannic acid as a substrate as shown in fig. The screening results confirm that isolated fungal strains are able to produce laccase enzyme.

Whereas it was also obtained that screening result using ABTS substrate provides efficient oxidation zone with lower time of incubation compared to guaiacol and tannic acid proving ABTS as potent substrate for laccase screening.

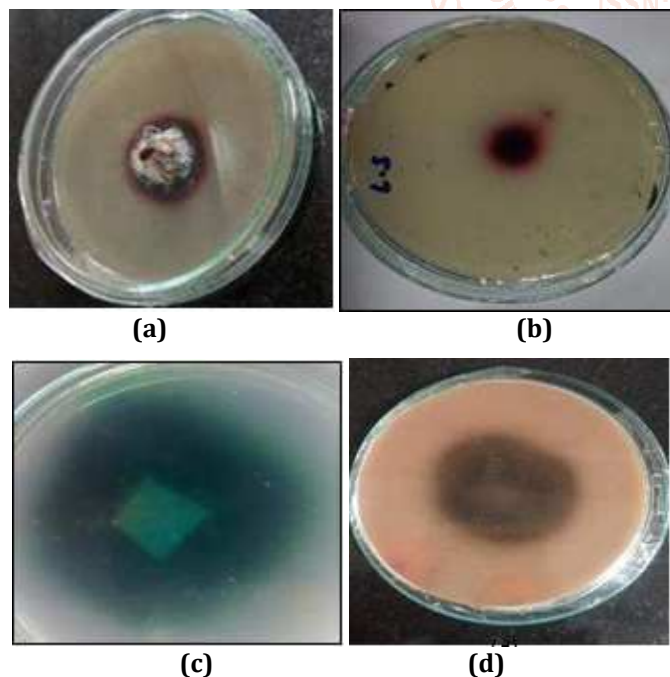


Fig-3 : Various substrates used for screening of laccases, a, b) front and back morphology of guaiacol screening- reddish brown oxidation zone c) back morphology of ABTS screening dark green zone d) back morphology of tannic acid screening-dark brown oxidation zone

5. Conclusion

Based on the screening results using various substrates of ABTS, guaiacol and tannic acid, laccase positive test were confirmed. While potent oxidation zone were obtained using various substrates which states that laccase has broader specificity towards different substrate such as ABTS, tannic acid and guaiacol. It can also be concluded that laccase enzyme show broader specificity towards wide range of substrate. This property makes laccase efficient candidate for the degradation of various phenolic and non-phenolic substrate.

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