Production of laccase BT solid state fermentation and application in textile dye removal

Publication History
Received: 17 March 2015
Accepted: 21 April 2015
Published: 29 April 2015

Citation
PRODUCTION OF LACCASE BT SOLID STATE FERMENTATION AND APPLICATION IN TEXTILE DYE REMOVAL

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ABSTRACT

Laccase is a copper containing poly phenol oxidase that acts on a wide range of substrates. This enzyme is found in many plant species and is widely distributed in fungi including wood rot fungi, where it is often associated with the lignin peroxidase, manganese dependent peroxidase or both. Laccases have widespread applications, ranging from effluent decolouration and detoxification to pulp bleaching, removal of phenolics from wines and dye transfer blocking functions in detergents and washing powders. The biotechnological application of laccase has been expanded by the introduction of laccase mediator systems, which are able to degrade lignin in kraft pulps. Fungi were cultivated on potato dextrose agar plates containing indicator compounds namely bromophenol blue and tannic acid. It resulted in isolation of two fungal strains. Among which one of the strain was presumed to be potent depending on its growth characteristics and was used for further experiments. It was identified as belonging to Trichoderma genus. Higher level of laccase activity was observed under solid state condition production. The isolated strain can be further worked for production of laccase using banana peels and rice bran as the substrate and it could proved to be a potent enzyme for the decolorisation of Congo red.

KEYWORDS: Laccase, lignin peroxidase, manganese dependent peroxidase, tannic acid, Trichoderma, Congo red.

INTRODUCTION

Laccases (oxygen oxidoreductases, ec 1.10.3.2) are multi-copper enzymes belonging to the group of blue oxidases. They are defined as oxido reductases, which oxidizes diphenol substances. The ability of laccases to oxidize phenolic compounds as well as their ability to reduce molecular oxygen to water has led to intensive studies of these enzymes. Laccases are typically found in plants and fungi. Plant laccases participate in the radical-based mechanisms of lignin polymer formation, whereas in fungi laccases probably have more roles including morphogenesis, fungal plant–pathogen host interaction and stress defense and lignin degradation. Although there are also some reports about laccases activity in bacteria, it does not seem probable that laccases are common enzymes from certain prokaryotic groups. Most of the laccases reported thus far are of fungal origin, especially from white rot fungi.
The enzymatic catalysis by laccases in different industrial applications such as textile dye bleaching, pulp bleaching and bioremediation could serve as a more environmentally benign alternative than the currently used chemical processes. Hence, the present research work aimed at isolation of potent laccase production fungi, establishment and comparison of submerged and solid state conditions for laccase production and partial characterization of the enzyme extracted from potent strain. (Alcalde M: Laccase: biological functions, molecular structure and industrial applications)

Water used in the chemical and process industries collects various constituents which may be soluble or remain in suspension. This water is called wastewater or industrial effluent. Among these waste waters, textile industries releasing water can contain dye like congo red. These dyes when introduced into water bodies create an imbalance in mainly in aquatic life. The color and odor changes for water. These must be separated from the discharge to ensure that pollution control board’s guidelines are met. Chemical addition and reaction can often aid in the separation. As an alternative, laccases can be used to oxidize these dyes and thus decolorize the dye content.

MATERIALS AND METHOD

Isolation of laccase producing microorganisms

Banana peel from kitchen wastes and rice bran from rice mills were collected. Moisture was introduced the fungi was cultured to potato dextrose agar (PDA) media containing petriplates. Morphological and cultural examination was carried out by using fungal staining.

Confirmatory test

The laccase production fungi were screened based on the growth on potato dextrose agar (PDA) media containing specific substrate of laccase which included tannic acid (0.1, 0.2, 0.3 mg/ml) and bromo phenol blue (0.2 mg/ml). PDA plates were observed for growth development.
of brown colour precipitate in tannic acid containing plates and green hallow zones around laccase producing colonies.

**Solid state fermentation**

The solids state production was carried out using banana peel and rice bran obtained from the local market. Two conical flasks containing 50g chopped banana peel moistened with distilled water (1:1 w/v) were incubated with selected organism at 30oC in an incubator autoclaved water(10 ml) was dispensed into a PDA plates (incubated with selected organism at 30oC for 7 days) properly mixed and equal amount of water was then used for inoculating individual flasks. Crude culture filtrate was obtained by adding 50 ml of distilled water to the conical flasks and filtering through cloth. It was further centrifuged at 1000rpm for 10 min. the supernatant collected was further used as enzyme source.

**Ammonium sulphate precipitation**

Clarify the enzyme solution by centrifugation. Transfer the supernatant into an ice cold beaker. Keep the beaker chilled by placing it in an ice tray. Weigh the amount of ammonium sulphate to be added 28.35g of salt per of the crude enzyme containing 15% saturation of ammonium sulphate to 20%. The amount depends on the volume of the solution and the percentage saturation of the salt needed. Slowly add the ammonium sulphate with stirring. One needs to be careful as the addition of salt should be very slow. Add a small amount at a time and then allow it to dissolve before further addition. Keep on stirring for 20 minutes precipitation to occur in ice. Centrifuge at 10,000 rpm for 10 minute. The pellet contains the precipitated protein which could be dissolved in a phosphate buffer for further analysis and purification. For a second round of precipitation of a different protein, the supernatant is again used and the above same steps are followed. Gram percentage salt needed in litre of crude enzyme to bring the solution containing s1% saturation of (NH4)2SO4 S2% saturation is given by equation,

- S2% saturation = 533(s2-s1)/(100-35)
- S2=20, s1=15
Preparation of dialysis tubing

Boil the tubing in a 4l volume of 2%(w/v) sodium bicarbonate and 1Mm EDTA Ph 8.0. Rise the tubing in distilled water thoroughly. Boil for 10 minutes in 1Mm edta (PH 8.0). Allow tubing to cool then store it in ethanol at 4oC with the tubing submerged. Before use wash out tubing with distilled water. The enzyme solution is placed in dialysis bag. The dialysis bag is placed in a large volume of buffer and stirred for many hours (16 to 24 hours) on a magnetic stirrer and changed several times over a 24 hour period. Allow the solution inside the bag to equilibrate with the solution outside the bag with respect to ion concentration.

Immobilization of enzyme

Dissolve 30g of sodium alginate in 1l to make a 3% solution. Mix approximately 1 ml of enzyme with 10ml of 3%(wt) sodium alginate solution. The concentration of sodium alginate can be varied between 6-12% depending on the desired hardness. The beads are formed by dripping the polymer solution from a height of approx. 20 cm into an excess (100ml) of stirred 0.2 M CaCl2 solution with a syringe and a needle at room temperature. Leave the beads in CaCl2 solution to cure 0.5-3 hrs.

Preparation of standard congo red solution

Stock solution of congo red (0.01mg/ml) was prepared and using spectrophotometer the absorbance at 498nm was found. Add about 10 to 15 enzyme immobilized beads to a fresh congo red solution. Solution was then kept for about ten days for decolourisation of the dye to make place.

RESULTS AND DISCUSSION

Screening of sample for laccase producing fungi

The organisms that were isolated from organisms from samples as Laccase producers, as all them oxidized tannic acid present in the screening medium. It was also identified by development of dark brown precipitate below the colonies. For both the strains obtained from banana and rice bran bromo phenol blue was also positive. Trichoderma species was found to be bright green and white conidial filaments and were repetitively branched in appearance under fungal staining.
Production of laccase under solid state condition

It has been reported that fungi grow at faster rate and produces good biomass on solid substrates. It was cultivated on two different natural substrates, banana peel and rice bran. The enzyme activity of organism grown on rice bran was higher (1507U/ml) than on banana peel (11.59U/ml) which was found using Lowry’s assay. It was observed that fungal laccase activities in solid media were higher when compared with those extracts of liquid media. This result may be related to i) lower deactivation of enzymes after adsorption or immobilization on agricultural residues in solid media, and/or ii) more stimulation and production of enzymes in solid media.

Concentration of congo red versus absorbance at 498nm

<table>
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<th>Sample Number</th>
<th>Absorbance at 498nm</th>
</tr>
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<tbody>
<tr>
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<tr>
<td>S1</td>
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<tr>
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<td>S5</td>
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Absorbance of treated congo red using enzyme

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana peel</td>
<td>1.25</td>
</tr>
<tr>
<td>Rice bran</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Comparison of enzyme treated dye with blank

REFERANCE