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ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF PROKARYOTIC LACCASE

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ABSTRACT

Laccases are multicopper oxidases enzymes which are widely distributed in fungi, plants and prokaryotes. Laccases from fungi are reported frequently and showed limited applications where harsh industrial conditions (high temperature, alkaline pH and high level of salts) are required. Prokaryotes recently emerged as the industrially suitable laccase producers as comparison to their counterpart fungi. In present study, laccase producing bacterium was isolated from the rhizosphere of paddy plant. Morphology of the organism showed this is Gram negative bacterium. It was observed newly isolated bacterium is early (12-15h) producer of intracellular laccase in cost effective liquid medium at 37C°, 7.2 pH and 150 rpm shaking conditions.

Keywords: Laccase, isolation and characterization

I. INTRODUCTION

Laccases (benzenediol: oxygen oxidoreductases; EC 1.10.3.2) exist widely in nature. Laccase was first extracted from the exudates of the Japanese lacquer tree, Rhus vernicifera by Yoshida in 1883and Bertrand in 1985 described its characteristics as a metalcontaining oxidase.

They are predominantly found in higher plants and fungi [1] .There is an increasing evidence for large distribution of laccsase and laccase like activity in bacteria but only some bacteria have been characterized. Laccase induction in bacteria is interesting and ongoing topic in microbiology because bacteria offer many advantages over fungi e.g. the faster multiplication rates resulting in early enzyme production. Fungi are usually acidophilic and psychrophilic, while bacteria can inhabit acidophilic to alkalophilic, psychrophilic to thermophilic environments making their enzymes more stable to different pH.

Among bacteria, Azospirillum lipoferum was the first bacterium in which laccase enzyme activity was found [2], followed by a report in γ-proteobacterium JB [3] and Streptomyces lavendulae [4]. Bains et al demonstrated the pH range of bacterial laccase and concluded that bacteria can grew well from pH 6 to 10 and produced laccase maximally at pH 10 .The laccases with different substrate specificities and improved stabilities such as pH and temperature is important for industrial applications. A bacterial alkalophilic laccase *γ-Proteobacterium*

was isolated,purified and characterized as they are widely used for transformation of aromatic compounds such as dyes, aromatic pollutants or in wastewater treatments [5],[6],[7]. The thermostability, pH stability and activity of laccase from bacterial origin were enhanced in the presence of Cu2O nanoparticles. The enhanced activity can be used for decolorisation of dyes [8] .A strain of Serratia marcescens having a psychotolerant nature produces laccase at very broad range of temperature i.e. 4 to 45°C and pH 3 to 14 pH [9]. In 2016 Kumar et al. [10] optimized the nutritional and cultural paramaters for laccase production.

We have isolated a bacterium producing laccase with high temperature and pH .This work reports isolation, biochemical characterization of prokaryotic laccase and its application in dye degradation like indigo carmine and malachite green.

II. MATERIALS AND METHODS

1. Medium, isolation and screening

For isolation, screening and enzyme production, M162 [11] and tryptone, yeast (TY) media were used. The soil samples were taken from paddy plant rhizosphere, garden soil and degrading wood soil region. The samples were serially diluted and spread plated on M162 agar plates containing 5 mM guaiacol as substrate. The plates were incubated at 37◦C for 48h.

2. Laccase production

Initially, M162 and TY media were used for laccase production. TY media was chosen as the best medium for enzyme production. TY (0.2% yeast extract and tryptone, pH, 7.2) media was inoculated with 1.0% of 12–14 h old inoculum of isolated bacterium grown in the same medium, and incubated at 37°C, 150 rpm for 120 h. The culture was centrifuged at 10,000 rpm at 4.0°C for 10min and crude laccase was extracted from cells after sonication (8 min, 1s and 80%).

3. Enzyme assays

For the enzyme assays, the crude intracellular cellular enzyme preparation was used. Oxidation of guaiacol by laccase results into reddish brown color is used to measure enzyme activity at 465 nm.The substrate was used at 2mM in a reaction mixture buffered with 0.1 mM phosphate buffer at pH 8.0. The reaction mixture contains 1 ml substrate (guaiacol) with 2 ml buffer and 500 microlitre intracellular enzyme.The reaction mixtures were incubated at 75°C for 20 min.A blank was also prepared that contains 1 ml buffer instead of enzyme. After the 20 min the activity of enzyme checked spectrophotometrically at 465nm. Laccase activity was determined by monitoring the oxidation of guiacol at 465 nm.

III. RESULTS

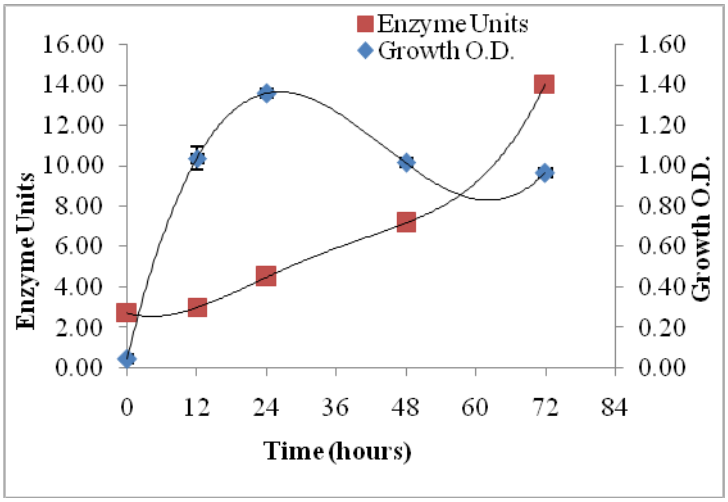

Fig. 1. Growth of bacteria and activity of laccase

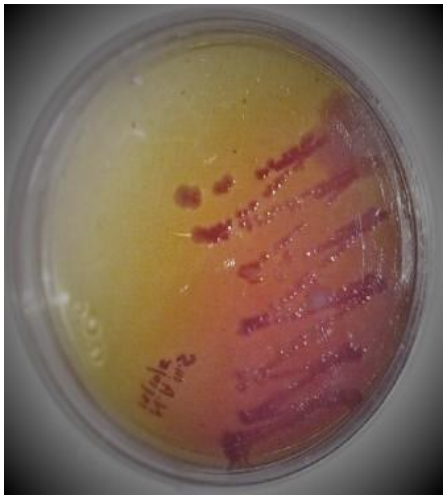

Fig.2. Brown color indicating laccase

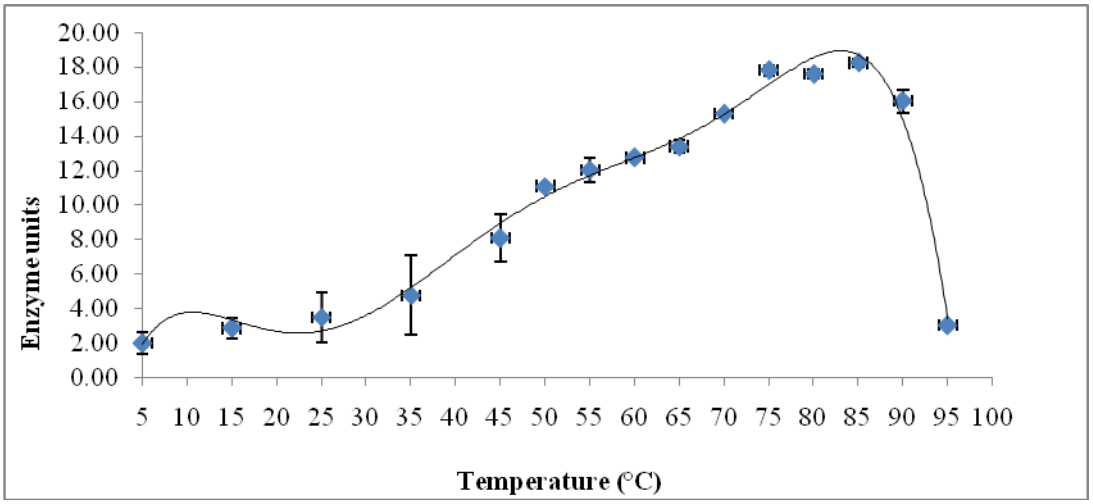

Fig.3. Effect of temperature on enzyme activity

The isolate was obtained from paddy plant rhizosphere soil sample as it gave an intense reddish-brown colony (Fig. 2) on TY agar plate and M162 agar plate containing 5.0 mM guaiacol. The growth of bacterium was observed, maximum after 24h and later growth was slightly declined after 48h in TY broth (Fig. 1) but intracellular enzyme production was highest at 72 h. When the initial pH of the medium was adjusted 7.2, the final pH was found 8.6 after 72h (data not shown). The optimum temperature range for enzyme activity is 75–85°C in a 20 min assay (Figure 3.). The enzyme shows activity in wide range of pH 3 to 9.6 (data not shown).

IV. CONCLUSIONS

A bacterial laccase has been isolated and preliminary characterized. The enzyme was found intracellular as it seems that activity is shown maximally with cells instead of extracellular. The Plate-test screening based on oxidation of guaiacol is an efficient way to discover novel laccase producers. Laccases have huge potential industrial applications including decolourization of textile dye, delignification of pulp and effluent

detoxification. It is essential to find novel, efficient enzymes to further develop these applications. The present study demonstrate that relatively simple plate test screening method can be used for discovery of novel laccases and it deserves to be extended for finding the industrial applications of this laccase in delignification of lignocellulosic biomass and pulps.

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