Indian J.Sci.Res. 13 (2): 188-191, 2017 ISSN: 0976-2876 (Print)
ISSN: 2250-0138 (Online)

LIGNOCELLULOLYTIC FUNGAL ISOLATION AND SCREENING FOR THEIR LACCASE PRODUCING ABILITY

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ABSTRACT

The current study includes isolation and screening of laccase producing lignocellulolyticfungi from banana waste soil. In this study twenty three fungal isolates were isolated by serial dilution technique. They were cultivated on Potato Dextrose Agar (PDA) plates. For their laccase producing ability isolates were qualitatively screened by guaiacol plate assay on Potato Dextrose Agar medium plates which contains 0.02% Guaiacol in the medium. Guaiacol is a substrate for laccase producing fungi. After 5 days incubation eightfungal isolates were shows laccaseproducing ability. Fungi oxidize Guaiacoland produce reddish brown color halo on the plates. Five fungal isolate (BWC, BWJ, BWM, BWW and BWY) had shown maximum quantitative laccase production in Potato Dextrose Broth after 15 days of incubation at room temperature.

KEYWORDS: Laccase, Guaiacol, Indicator, Qualitatively, Quantitative

Laccase (p-diphenol: Oxygenoxireductase EC 1.10.3.2) enzyme degrade many phenolic compounds(Hoegger*et al*., 2006; Alcalde, 2007). It also known as multi-copper blue oxidizes, it belongs to oxidoreductase group of enzyme. 50 kDa and 130 kDa are their biochemically glycoprotein carrying molecular mass (Morozova*et al*., 2007).A fungus produces more than one isoform of laccase (Hoshida*et al*., 2001). Fungal laccase are multinuclear, extracellular, mostly inducible, monomeric glycoproteins with 10-20% of carbohydrate contents. It makes laccase to high stable (Mayer and Staples, 2002). Fungal laccases have play more important roles in fungal plant pathogen/host interaction, stress defense, morphogenesis and lignin degradation (Thurston, 1994). Fungi belonging to deuteromycetes, basidiomycetes and ascomycetes are known to laccase producers of biotechnological important and as well as ecological important such as bioremediation and biodegradation (Mayer and Staples, 2002; Morozova*et al*., 2007; Desai and Nityanand, 2011; Shraddha*et al.,* 2011). Laccase are used as new biocatalysts for organic synthesis (Milstein *et al*., 1989; Mayer and Staples, 2002). They are applied for the modification and appearance of beverages or foods. It eliminates the undesirable phenolic compounds, which responsible for the haze formation, turbidity and browning in clear beer, wine and fruit juice (Rodriguez and Toca, 2006). It is capable to depolymerize and de-lignify wood pulp fiber and use in chlorine-free in bio-palpation process (Camarero*et al*., 2004; Rodriguez and Toca, 2006; Vikineswary*etal*., 2006). There potential application in textile and dye industries for the enzymatic modification of bleaching dye (Abadulla*et al*., 2000; Kunamneni*et al*., 2008). Laccase degrade recalcitrant and xenobiotic compounds, it is a major contamination source in soil (Rodriguez and Toca, 2006). Laccasealso degrade PHAs, which shows mutagenic, carcinogenic and cytotoxic properties that responsible for risk to human health (Bamforth and Singleton, 2006).

MATERIALS AND METHODS

Materials

The reagent grade chemicals Potato Dextrose Agar, Potato Dextrose Broth, Guaiacol, Sodium Acetate Buffer and Streptomycin were procured from Hi-Media, Mumbai (India).

Collection of Soil Sample and Isolation of Fungi

Sample of banana waste soil was collected in sterile plastic bag from Purai Road Utai, DistrictDurg (C.G.) and subject to isolation of fungi by serial dilution method. 1 gram of soil sample was added into 10ml sterile water and mixed. The suspension was serially diluted 10^{-1} to 10^{-5} dilution. After dilution 1ml of each dilution was spread on sterile plate and adds Potato Dextrose Agar medium which containing 0.01% Streptomycin poured in plates and plates rotate clock-wise and anti-clock wise. Streptomycinwas used for inhibition of bacterial contaminants. After solidification of medium plates were incubated at 28^0C for 7 days (Waskman, 1922).

Qualitative Screening

The fungal strains were inoculated in 0.02% Guaiacol containing Potato Dextrose Agar plates. The plates were incubated at 30^0C for 5 days. After 5 days of incubation the laccase producing fungal strains showed

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reddish brown color halo in plates containing Guaiacol supplement indicate a positive laccase secretion. Positive fungal culture was taken for the quantitative estimation (Adiveppa*et al*., 2015).

Quantitative Screening

For quantitative screening positive fungal cultures were carried out in Erlenmeyer flask (250ml) which containing 100ml Potato Dextrose Broth. Flasks were incubated at room temperature for 15 days.

Extracellular Enzyme Activity

After incubation crude extract were filtered in Whatman no 1 filter paper. Laccase activities were assayed by using 10mM Guaiacol and 100mM sodium acetate buffer (pH 5.0) at room temperature. The reaction mixture contains 1ml Guaiacol, 3ml sodium acetate buffer and 1ml enzyme source. Reaction mixtures were incubated at 30^0C for 10 minutes. Laccase activity is measured U/ml that define the amount of enzyme production one micromole colored product in per minute per ml. The absorbance of reaction mixture was monitored at 470 nm by using UV Spectrophotometer (Adiveppaand Basappa, 2015).

$$\text{Volume activity (U/ml)} = \frac{\Delta A470nm/min \times 4 \times Vt \times \text{dilution factor}}{€ \times Vs}$$

Calculation

Where,

Vt = final volume of reaction mixture (ml) = 5.0

Vs = sample volume (ml) = 1

€ = extinction co-efficient of Guaiacol = 6,740/M/cm

4 = derived from unit definition & principle

Table 1:- Primary screening (Qualitative screening) shown by the isolated fungal strains by Guaiacol plate assay

Isolates	Guaiacol indicator oxidation
BWA	Negative
BWB	**Positive**
BWC	**Positive**
BWD	**Positive**
BWE	Negative
BWF	Negative
BWG	Negative
BWH	Negative
BWJ	**Positive**
BWK	Negative
BWM	**Positive**
BWN	Negative
BWO	Negative
BWP	Negative
BWR	Negative
BWS	Negative
BWT	Negative
BWU	Negative
BWV	Negative
BWW	**Positive**
BWY	**Positive**
BWBB	Negative
BWEE	**Positive**

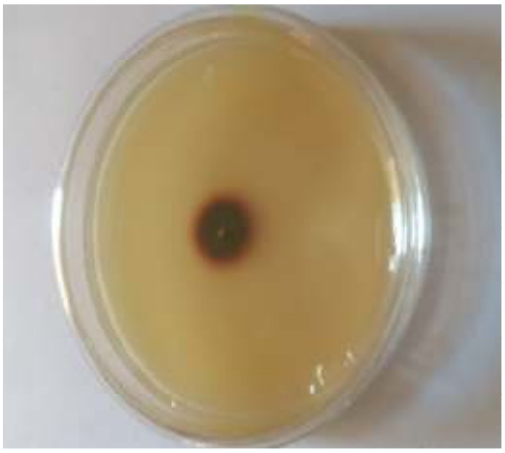
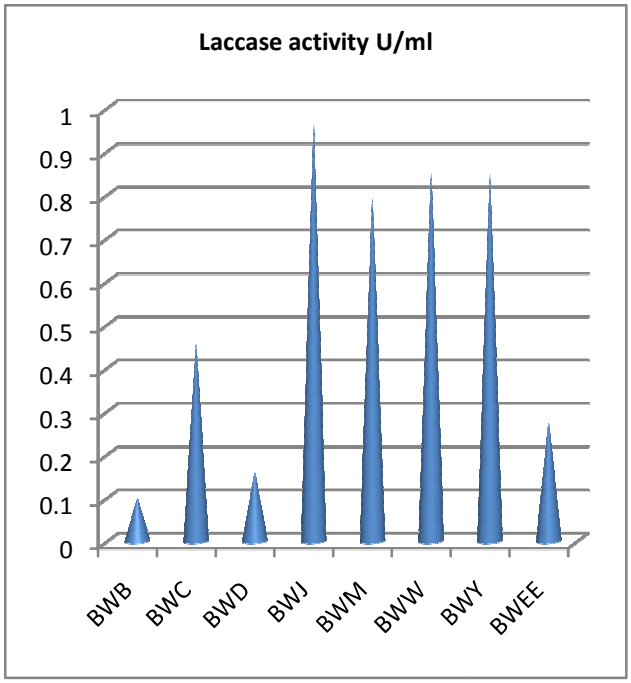


Figure 1:- Shows reddish brown color halo around the plates which contain 0.02% Guaiacol in Potato Dextrose Agar plate



Graph 1:-Quantitative screening for laccase production by the different fungal isolates

RESULTS

The current study mainly based on isolation and screening oflaccase producing lignocellulolyticfungi from banana waste soil from Purai Road, Utai (Chhattisgarh). Twenty three fungal colonies were isolated from this soil sample. Potato Dextrose Agar medium were used for maintain of fungal isolates. They were screened for their potential laccase producing ability using Guaiacol as a substrate. All fungal isolates were inoculated in Potato Dextrose Agar plates which contain 0.02% Guaiacol and plates were incubated at 30^0C for 5 days. The results of screening tests are given in Table,1. After 5days of incubation fungal colony showed reddish brown colored halo formation around the plates. It shows positive reaction for production of laccase enzyme and absence of color means negative reaction for laccase producing ability by the isolates. Eight fungal isolates shows positive reaction with Guaiacol plate assay and Five (BWC, BWJ, BWM, BWW and BWY) had shown maximum quantitative laccase production in Potato dextrose broth after 15 days of incubation at room temperature.(Graph 1& Figure 1)

CONCLUSION

In this study twenty three lignocellulolytic fungal strains were isolated from banana waste soil sample. After screening of all isolates only eight isolated has ability to produced laccase enzyme qualitatively in Guaiacol plate assay and five had ability the maximum laccase production quantitatively in Potato Dextrose Broth by spectrophotometrically after 15 days of incubation. The result clearly showed that five fungal isolates have potential for the large production of laccase enzyme.

ACKNOWLEDGMENT

The authors are thankful to Department of Microbiology and Biotechnology, St Thomas College, Bhilai and Department of Microbiology, Shri Shankaracharya Mahavidyalay, Bhilai for providing the necessary research facilities and also thankful to my lab mates.

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