International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 4; October 2017; Page No. 83-84

Isolation and screening of *Sclerotium rolfsii* for laccase production

VR Mhaske, MS Wadikar
Department of Botany, Vinayakrao Patil College, Vaijapur, Aurangabad, Maharashtra, India

Abstract
In the present investigation an attempt has been made for the isolation of potent laccase producing fungi *i.e*. *Sclerotium rolfsii*. About twenty five fungi were isolated from different soil samples and screen for laccase production on potato dextrose agar media amended with different laccase substrates like Guaiacol, ABTS and Tannic acid. *Sclerotium rolfsii* shows oxidation of all substrates.

Keywords: laccase, guaiacol, ABTS, tannic acid and *sclerotium rolfsii*

Introduction
Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) is a multi-copper-bearing lignolytic enzyme, which catalyzes the one-electron oxidation of many phenolics compounds with concomitant reduction of oxygen to water. It is widely distributed in the higher plants, some insects, a few bacteria, and fungi. Most of the known laccases are of fungal origin, in particular from the white rot fungi Saito *et al*., (2003) [7]. Laccase has a number of environmental or biotechnological applications that include waste water treatment, biopulping, biobleaching, and also soil bioremediation. Considerable concern recently has been expressed over the biodegradation of such man-made estrogenic chemicals as bisphenol A (2, 2-bis (4-hydroxyphenyl) propane; BPA) and nonylphenol (NP) by oxidative enzymes from lignin-degrading fungi Tsutsumi (2001) [8].

Materials and Methods: Collection of soil samples
Soil samples were collected from different localities *Viz*. Badnapur, Karmad, Shendra MIDC and Aurangabad. The soil samples were collected by digging 10 to 15 cm depth using sterile spatulas and five soil samples were randomly collected approximately 100gm from each sampling site in pre sterilized zip-lock bags.

Isolation of fungi
Isolation of fungi were done by dilution plate method (Waksman, 1916), one gram of each soil sample were taken and added in test tube containing 10ml of sterile distilled water. The suspension was serially diluted five times 10-1 to 10-5 in sterile distilled water. The Suspension obtained after vigorous mixing of the soil samples were allow to stand for 20 min. 0.5ml of each aqueous dilutions of soil suspensions were inoculated on Petri plates amended with Potato dextrose agar. Trace amount of Streptomycin was added in PDA media to avoid bacterial contamination. Spread plate and Pour plate techniques were used. The inoculated plates were incubated at 30°C for 5 days. Different fungal colonies were observed and sub cultured to obtain pure cultures.

Identification of fungi
Identification of fungi were done on the basis of morphological and microscopic observations like colony colour, margin of the colony, growth pattern, sporulation and pigment production. The identification and further confirmation fungi was made by preparing slides of the fungal growth and observing them under compound microscope and slide culture technique. The identification was made with the help of manuals, The Illustration of fungi by Mukadam *et al*., (2005) [5]. A manual of soil fungi by Gilman (2001) [3], A hand book of soil fungi by Nagmani and Kunwar (2005) [6]. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.

Screening of laccase producing fungi
The screening of laccase producing fungi was done by using plate assay method. For screening of laccase three different substrates were used like, Guaiacol, Tannic Acid and ABTS (2, 2'-azino–bis (3-ethylbenzothiazoline)-6-sulphonic acid). The ability of the fungal strains to secrete extracellular laccase was visualized by oxidation of substrate used in media. 0.02% Guaiacol added in potato dextrose agar media according to Buddolla *et al.*, (2008) [1], 0.02% ABTS added in potato dextrose agar media according to Kalyani *et al.*, (2012) [2] and sterilized in autoclave at 1200c 15 lb pressure for 20 minutes. 0.5% Tannic acid was separately autoclaved and added in autoclaved potato dextrose agar media according to Kiiskinen *et al.*, (2004) [4]. Media were poured in sterilized Petri plates and seven day old pure cultures of fungi were inoculated on plates for laccase screening. The plates were incubated at 300 C for 1–7 days. The presence of brick red colour, dark brown and green colour aroundthe mycelium was considered as Guaiacol, Tannic acid and ABTS oxidizing laccase secreting organism.

Results and Discussion
In the present study about twenty five different fungi were isolated and identified on the basis of morphological and colony characters and by slide culture technique. Out of that

three fungi shows laccase activity only on tannic acid and two fungi shows positive activity on Guaiacol and one fungi shows laccase activity on ABTS. *Sclerotium rolfsii* shows laccase activity on all tested substrates. Vantamuri and Kaliwal (2015) [11] screen different wood decaying fungi, soil and mushrooms on different substrates like ABTS, Guaiacol, Syringadalzine and Tannic acid and found more or less similar results. Pundir *et al.*, (2016) [8] studied Apophysomyces sp. novel laccase producing fungi on PDA media amended with Guaiacol and shows positive activity.

Table 1: Screening of fungi on different laccase substrates

S. No	Name of Fungi	ABTS	Guaiacol	Tannic acid
1	*Aspergillus fumigatus*	Negative	Negative	Positive
2	*Sclerotium rolfsii*	Positive	Positive	Positive
3	*Fusarium oxysporium*	Negative	Positive	Positive

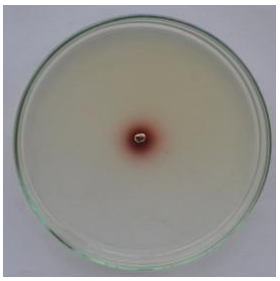

Fig 1: Screening on Guaiacol

Fig 2: Screening on ABTS

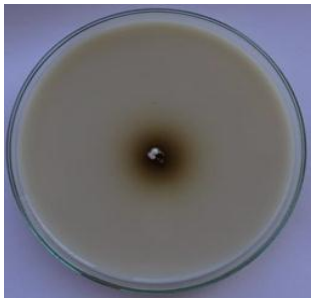

Fig 3: Screening on Tannic acid

Fig 4: Pure culture of *Sclerotium rolfsii*

Conclusion

In the present investigation it is recorded that *Sclerotium rolfsii* is highly potent fungi for laccase production which shows rapid oxidation of all substrates and can be used for large scale laccase production which is having wide industrial applications.

References

1. Buddolla Viswanath M, Subhosh Chandra H, Pallavi, Rajasekhar Reddy B. Screening and assessment of laccase producing fungi isolated from different environmental samples. African Journal of Biotechnology. 2008; 7(8):1129-1133.
2. Dayanand Kalyani, Saurabh Sudha Dhiman, Hoon Kimc, Marimuthu Jeya, In-Won Kima, Jung-Kul Leea. Characterization of a novel laccase from the isolated Coltricia perennis and its application to detoxification of biomass Process Biochemistry. 2012; 47(2012):671-678
3. Gilman JC. A Manual of soil fungi second Indian edition, Biotech books Delhi, 2001.
4. Kiiskinen LL, Ra¨tto M. Kruus K. Screening for novel laccase-producing microbes. Journal of Applied Microbiology. 2004; 97:640-646.
5. Mukadam DS, Chavan AM, Patil MS, Patil A. Illustration of fungi Kailas publication, Aurangabad, 2005.
6. Nagmani A, Kunwar IK, Manoh arachary C. Hand book of soil fungi. Published by K. International Pvt. Ltd New Delhi, 2005.
7. Pundir RK, Mishra VK, Rana S, Lakhani M. Screening of Laccase producing fungi from soil samples – An In Vitro Study. Electronic Journal of Biology. 2016; 12(3):254-257.
8. Saito T, Hong P, Kato K, Okazaki M, Inagaki H, Maeda S, Yokogawa Y. Purification and characterization of an extracellular laccase of a fungus (family Chaetomiaceae) isolated from soil. Enzyme and Microbial Technology. 2003; 33:520-526.
9. Tsutsumi Y, Haneda T, Nishida T. Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading basidiomycetes. Chemosphere. 2001; 42:271-6.
10. Vantamuri AB, Kaliwal BB. Isolation, screening and identification of Laccase producing fungi. International Journal of Pharma and Bio Sciences. 2015; 6(3):(B)242-250.