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Assessment Of Laccase Activity In Wood Inhabiting Aphyllorphorales.

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Abstract:

This study aimed to find out appropriate laccase producing fungi. In this study wood inhabiting Aphyllorphorales fungal samples collected from different areas of Chhatrapati Sambhajnagar and isolated on malt extract agar plate. Isolated sample Screened for laccase activity using plate assay technique. Enzyme substrate oxidation study of forty two fungal isolates were carried out by using different substrate like Tannic acid, Guaiacol, α Naphthol, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) for laccase production out of which forty samples show substrate oxidation zone. The difference in the pattern of substrate oxidation by laccase from different species of fungi is paid attention in the study. The potent enzyme producing fungal isolates were identified by the screening strategies and the potent isolates were taken for enzyme production studies.

Keywords: Aphyllorphorales, Laccase, Wood rotting fungi, Enzyme Screening.

Introduction:

Laccases catalyze a single-electron oxidation and four electron reduction of molecular oxygen to water, found in various organisms including plants and many microorganisms where they play diverse physiological functions (Mot & Silaghi-Dumitrescu, 2012). These microbial enzymes are resistant to extreme conditions like high pH and extreme temperature (Shubha and Srinivas 2017). Fungal Laccases have higher redox potential, broad substrate specificity, stability than bacterial Laccases and are easy to handle and grow at room temperature due to this more preferred for biotechnological applications like bioremediation and dye biotransformation, so fungal laccases have attracted great interest (Mate & Alcalde, 2017). The high demand for laccase has intensified the research for laccase homologs with unique properties since Laccases from different species have different properties and diverse applications. Basidiomycetes a group of fungi, produce a wide variety of hydrolytic extracellular enzymes including efficient laccases and other ligninolytic enzymes that are used in many industrial processes. However, reports indicated that members of white-rot Basidiomycetes are the most efficient ligninolytic enzymes producers in response to lignin degradation (Baldrian, 2006). The diversity of fungi offers both an opportunity and a challenge for discovering potential laccase producing strains. Laccases from different fungal species have differences in catalytic activity, substrate preference, response to activators and inhibitors, and stability, which reflects variation in protein structures, Therefore search for fungal strains producing laccase continue to be important, to add to the understanding of structure function relationship of the enzyme, and inventory of fungal laccases to choose from for a certain application (Agrawal et al., 2018; Baldrian, 2006). Screening of microorganisms with significant lignin degradation ability permits uncover the opportunity to discover and characterize novel enzymes that potentially allow low-cost biological processes.

Method:

Isolation of White Rot Fungi (WRF)

2% Malt extract agar (MEA) were used to isolate fungi. Streptomycin and Benomyl, a benzimidazole fungicide were added to the media in order to select for wood decay fungi. Benomyl was autoclaved together with the media, whereas Streptomycin added to the media after autoclaving. Collected fungal samples cultured on MEA plate by tissue culture technique.

Qualitative assay for laccase production

Qualitative assay for laccase production was done by plate test assay method described by Daasi et al. with slight modification. For the assay Fungal isolates cultivated on Malt extract agar media containing Different indicator compounds in order to detect microbes that produced ligninolytic enzymes. ABTS, Guaiacol, α – Naphthol and tannic acid. Plug of fungal culture lawns on MEA plates were transferred aseptically to the center of plates containing the laccase test substrates. The plates were incubated for 3 to 6 days at room temperature. The appearance surrounding the fungal colony of a brownish red halo for Guaiacol or blue green halo for ABTS, dark brown colored for tannic acid signaled a positive test for laccase production (Vu et al., 2020).

Result and Discussion:

Qualitative determination of laccase

White rot species of Basidiomycetes were qualitative screened for their ability to produce laccase using effective plate test assay method. Based on the hypothesis that wood rotting fungi may be laccase producers, the study evaluated the capability of the selected strains to produce hydrolyzing enzymes, showing promising results for reducing enzyme costs in lignocellulosic bio refinery processes and discovering new enzymes with novel activities for the valorization of not only woody biomass but also biological waste (Vu et al., 2020). However discovering lignin-degrading microorganisms with a high performance largely depends on the screening strategy. The best laccase activity was determined with fungal growth and oxidation scale (Yang et al., 2014, Daâssi et al., 2016). This study showed that relatively simple plate test screening method can be used for discovery of novel laccases.

In this study, total 42 white rot fungi potential laccase producer collected and isolated from various samples of decayed wood and bark of trees. Culture obtained by tissue culture technique on malt extract agar media then screened for their extracellular laccase enzyme production in order to trace out the potentiality of isolates. Fungal samples were cultivated on Malt extract agar supplemented with substrate indicator compounds resembling lignin fragments such as Guaiacol, ABTS, Tannic acid, 1Naphthol that are structural analogues of lignin a laccase enzyme substrate that enabled the detection of laccases (Sivakumar et al., 2010). Laccase have capability of oxidation of indicator compounds and from colored zones in the vicinity of fungal colony on the plates as specific color reactions leads to formation of reddish brown/ green / brown /blue color respectively (fig 1) The TABLE 1 gives the results on the qualitative assay, results revealed that in total 42 fungal strains were analyzed for the production of laccase and 39 fungal isolates showed maximum zone of oxidation clearly indicates that their higher potential extracellular laccase activity. The fungus S1AP03, S1AP05, S2AP21, S11AP71, S8AP79, S8AP89, S8AP92 AND S8AP146 showing potential oxidation zone in all four substrates suggesting higher laccase activity and broad substrate specificity. Sample S1AP06, S2AP17 and S13AP114 exhibit minor activity. The results of screening with indicators were also compared in order to note down the different laccase producing fungal strains react differentially on indicator compound, some shows strong activity on a substrate and other gave relatively weak activity, Notably S5AP80 was positive only on ABTS and showed maximum zone of oxidation demonstrated strong laccase activity on ABTS and no detectable activity with other three substrate. Guaiacol was identified as the most sensitive substrates for the enzyme, showing relatively high catalytic efficiency (Baldrian, 2006). The results showed that among the 42 cultures 38 shows Guaiacol oxidation shown in table 1. α Naphthol gave relatively weak positive reactions with many samples seemed to be less specific. However, the ability of most positive microbes to form color weakened during sub culturing, some strains showed similar substrate specificity for the four investigated substrates, which were in the order of Guaiacol > tannic acid > ABTS > α Naphthol. These results corroborated (Wang et al., 2010). Based on assay these fungal isolates were selected for the further study.

Conclusion:

This work state that the qualitative plate test assay can be effective for predicting extracellular laccase producing strains. Fungal isolate was able to oxidize phenolic Chromogenic indicator substrates and from color that are more easily detectable and these compounds can thus reliably be used for laccase activity screening. Our results suggest, different laccase producing fungal strains react differentially on indicator compound. The isolates S1AP03, S1AP05, S2AP21, S11AP71, S8AP79, S8AP89, S8AP92 AND S8AP146 was particularly observed for forming colored zone around colony on media supplemented with different substrate however, that In view of the results obtained, it can be concluded that the This study also indicate that new novel laccase producers could be discovered from environmental samples by very simple plate-test screening method. In future we are interested to scale up the production of laccase by using various agro residues in solid state fermentation, these strain seems to be a prospective organism for further biotechnological exploitation.

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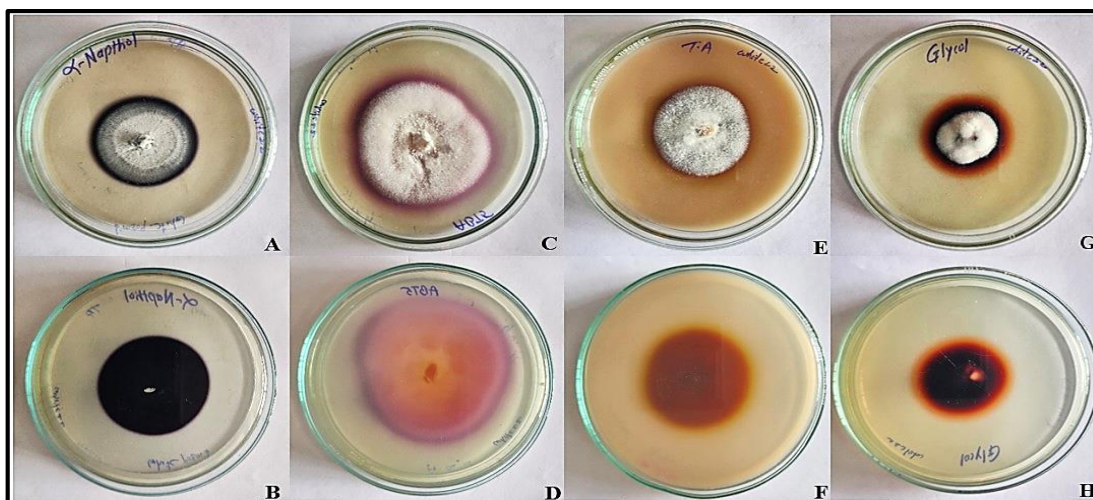


Figure 1. Screening for laccase. Malt extract agar medium with different substrate. Front and back view of α -Naphthol (A B), ABTS (C D), Tannic acid (E F), Guaiacol (G H) plate.

Table 1: Qualitative screening for laccase using different substrates.

Sr. No	Sample	ABTS	α Naphthol	Guaiacol	Tannic acid
1	S1AP01	+++	+++	+++	+++
2	S1AP03	++++	++++	++++	++++
3	S1AP05	++++	++++	++++	++++
4	S1AP06	-	-	-	-
5	S1AP09	++	+	++	+
6	S1AP15	+	+	+	-
7	S14AP15	+++	+++	++++	+++
8	S2AP17	-	-	-	-
9	S2AP21	++++	+++	++++	+++
10	S2AP22	+++	+++	+++	+++
11	S2AP37	+	++	+	-
12	S2AP49	+	+	++	+
13	S5AP69	+	++	+++	+++
14	S11AP71	++++	+++	++++	++++
15	S5AP77	+++	++	+++	++
16	S5AP79	++++	+++	++++	++++
17	S5AP80	++++	-	-	-
18	S7AP82	++	++	+++	++
19	S5AP83	+++	++	+++	+++
20	S8AP86	++	++	++	+++
21	S8AP87	+++	+++	+++	+++
22	S8AP89	++++	+++	++++	+++
23	S8AP90		++	++	+++
24	S8AP92	++++	++++	++++	++++
25	S9AP92	+++	+++	+++	+++
26	S12AP100	+	+	+++	+
27	S12AP102	+++	++	+++	++
28	S5AP109	+++	++	+++	+++
29	S13AP114	-	-	-	-
30	S13AP116	++	+	++	++

31	S13AP117	++	+	++	++
32	S14AP123	++	++	+++	++
33	S14AP124	++	+++	+++	+++
34	S13AP126	+++	+++	+++	+++
35	S14AP127	+++	+++	++++	+++
36	S5AP128	++	+	++	++
37	S5AP130	+++	+++	+++	++++
38	S12AP132	-	-	+	+
39	S5AP133	+++	++	+++	++
40	S8AP135	+++	+++	++++	+++
41	S8AP138	++	++	++	++
42	S14AP146	++++	+++	++++	+++

Colour zone +++++Excellent ++++very good, +++ good, ++small, + very small

