SSCREENING OF FUNGAL ISOLATES PRODUCING LACCASES FROM SOIL SOURCES AND TREE BARKINGS

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Abstract:

The enzyme laccase (p-diphenol: oxygenoxidoreductase; EC 1.10.3.2) is known to degrade many phenolic aromatic compounds. This enzyme is found in many plant species and is widely distributed in fungi including wood-rotting fungi where it is often associated with lignin peroxidase or manganese dependent peroxidase, or both. Fungi can exploit marginal living conditions in large part because they produce unusual enzymes capable of performing chemically difficult reactions In present study, 13fungal strains were isolated on PDA plate containing the 0.02% guaiacol. Out of these 13 isolates, only 10 fungal isolates showed reddish brown zones on the medium. The most potent fungal strain for laccase production was screened on 0.06% guaiacol containing PDA medium.

Keywords:

Laccases, Fungal strains, Guaiacol, Potato Dextrose Agar.

1. Introduction

Laccases belongs to multinuclear copper-containing oxidase and can act on a variety of aromatic and non-aromatic compounds. Due to their broad substrate specificity, they are considered as a promising candidate in various industrial and biotechnological sectors. Laccases are *p*-diphenol:dioxygen oxidoreductase belonging to the family of multi-copper proteins. Laccases have the ability to oxidize a wide range of aromatic and non-aromatic compounds which includes substituted phenols, some inorganic ions, and variety of non-phenolic compounds. Laccases have high catalytic efficiency and are used for technical applications in various industrial and biotechnological domains [1],which includes improving properties of fibers, bio-synthesis, energy exploitation, environmental protection, bio-detection, degradation of synthetic dyes, printing and dyeing industry, bio-pulping in paper industry, conversion of aromatic compounds , and removal of phenols which causes cancer and teratogenicity when present in waste water [2].

Laccase is most widely distributed in a wide range of higher plants, fungi and bacteria[3]. Fungi can exploit marginal living conditions in large part because they produce unusual enzymes capable of performing chemically difficult reactions . Laccases are secreted out in the medium extracellulary by several fungi during the secondary metabolism but not all fungal species produce laccase such as Zygomycetes and Chytridiomycetes . Fungi belong to Deuteromycetes, Ascomycetes as well as Basidiomycetes are known producers of laccase[4].

The enzyme required for the industrial application should have the ability to tolerate wide range of pH and temperature and it needs to be operational under industrial conditions. Thus, alkaline-tolerant and thermo-stable laccase are desired by the industries, but due to limited information, its application has not been feasible at a commercial scale. However, these limitations can be overcome by the use of various immobilization techniques which can help to enhance the stability of laccase, thus enabling its use at industrial scale[5]. The current work was designed to isolate and identify the potential fungus and characterize the organism with reverence to laccase production.

2. Materials and Methods

2.1 Isolation of laccase producing fungi from the soil sample

Soil samples and tree bark scrapings were collected from Nagercoil area, Kanyakumari Dstrict . About 500 gm of soil with bark scrapings was collected with a sterile trowel from a depth of about 5 – 10 cm at each site. The samples were placed in polythene bags, aseptically air dried and stored at 4°C till further processing [6].

2.2 Enumeration of fungi [7]

The enumeration of fungi was carried out by Pour plating method. Samples were spread on Potato dextraose agar medium that were supplemented with 0.02% guaiacol) and 0.01% (w/v) chloramphenicol (to avoid bacterial growth), adjusted at pH 5.5. The plates were incubated aerobically at 25°C - 27°C for 5 days for fungal growth. The observed colonies were counted and expressed as colony forming units per gram ($cfug^{-1}$). Fungal cultures were purified by point inoculation / streaking and disc transfer method. Pure cultures were maintained on PDA slants at 27ºC for further studies [8].

2.3 Characterization and Identification

Direct Plating methods on Potato Dextrose Agar were used for isolation of microorganisms (Masilamani Selvam, 2010). A total of fifteen isolates were grown on Potato Dextrose Agar plates. The fungal isolates were identified morphologically, culturally and microscopically according to Cheesbrough (2005) and Raper and Thom (1949). Identification of fungal isolates was accomplished depending on colonial characters of the pure culture, microscopic characters and dimensions of informative characters of each fungal isolate. Identification of fungi was done by mounting process using Lactophenol Cotton Blue stain.

2.4 Selection of potent fungal strain and identification

The most potent laccase producing fungal strain was screened according to its capability of strain to grow on PDA medium supplemented with guaicaol. Guaiacol degrading fungal strain showed considerable growth and reddish brown zone on medium supplemented with different concentrations of guaiacol with 0.02%, 0.04% and 0.06%. Potent fungus was identified on the basis of its morphological characteristics i.e. hyphae, sporulating structure, sporocarp, arrangement of conidia and cultural characteristics i.e. colony colour, front and bottom view and texture.

3. Results and Discussion

In the present investigation, the fungal isolates of three sites were enumerated and ten different morphological features of isolates were determined. All the ten isolates exhibit good growth characters and morphological features. The endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic associations with higher life forms and may produce beneficial substances for host[9]. Fungal isolates have received considerable attention after they were found to protect their host against many organisms. Maximum number of fungi was isolated from soil and *Aspergillus sps* were found to be dominant among the various fungal species isolated [13, 14.].

It was found that, *Aspergillus* was the only genus represented in the soil sample of all twenty four localities but their counts and frequency were not similar from place to place being collected[7]. In the present investigation, the enumeration of the isolates in different sites were expressed in cfu/gm and tabulated in Table 1.

Table 1: Enumeration of colony forming units (cfu) in different Sites

Isolates	Site 1	Site 2	Site 3
	Colony forming units (cfu/gm)	Colony forming units (cfu/gm)	Colony forming units (cfu/gm)
1.	$50x10^3$	$35x10^3$	$30x10^3$
2.	$10x10^3$	$7x10^3$	$7x10^3$
3.	$32x10^3$	$42x10^3$	$29x10^3$
4.	$13x10^3$	$21x10^3$	$19x10^3$
5.	$24x10^3$	-	$20x10^3$
6.	$14x10^3$	$27x10^3$	-
7.	-	$39x10^3$	-
8.	$39x10^3$	-	$4x10^3$
9.	$10x10^3$	-	$31x10^3$
10.	-	$20x10^3$	-

Table 2: Presence of fungal isolates in different sites

Isolates	Microorganisms	Site 1	Site 2	Site 3
1.	*Aspergllus niger*	P	P	P
2.	*Mucor sps*	P	P	A
3.	*Aspergillus flavus*	P	P	P
4.	*Penicillium sps*	A	P	P
5.	*Aspergillus fumigatus*	P	P	P
6.	*Fusarium* sps	P	A	P
7.	*Rhizopus* sps	P	A	A
8.	*Melanospora sps*	P	A	A
9.	*Aspergillus sulphurous*	P	P	P
10.	*Cladosporium sps*	P	P	A
11.	*Alternaria sps*	P	A	A
12.	*Aspergillus terrus*	A	P	P
13.	*Penicillium regulosm*	P	A	A

In the present work, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Aspergillus sulphurous* were present most frequently in the plant ecosystem while other organisms like *Penicillium sps, Cladosporium sps, Mucor sps, Fusarium sps* were less dominant. *Rhizopus sps* and *Cauvularia sps* occurred less frequently in the soil areas. This results were depicited in Table 2. Totally twenty two fungal species were isolated from the leaf surface mycoflora of green, senescent and brown leaves of *Avicinnea marina* by plating of leaf surface washings and washed leaf bits [15]. A study of endophyte biodiversity of the two dry and moisture of mangrove forest in Ramanathapuram District, Karnataka was conducted [14]. They have reported diversity of fungal species ranging from 10 to 26 in the host.

Table 3: Identification of fungal isolates

Isolates	Macroscopic Observation	Microscopic Observation	Identified Organism
1.	Black and powdery	Non septate smooth walled conidiophores	*Aspergllus niger*
2.	White colonies with the centre black or dark grey cotton like colonies	Non septate, Round conidia; No rhizoids	*Mucor sps*
3.	Light green and powdery	Long, erect, septate conidiophore	*Aspergillus flavus*
4.	Brown and cotton like	Long erect conidiophores with round conidia	*Penicillium sps*
5.	Grey green fluggy colonies	Long erect non septate conidiophores	*Aspergillus fumigatus*
6.	Yellowish pink creamy colonies	Cylindrical to ovoid conidia with septate conidiophores	*Fusarium* sps
7.	Yellow brown cotton like colonies	Rhizoids present, Non septate hyphae	*Rhizopus* sps
8.	Dark olive grey colonies	Branched conidiophores	*Curvularia sps*
9.	Yellow colonies	Smooth conidiophores with thick walled globose conidia	*Aspergillus sulphureus*
10.	Greenish colonies	Erect conidiophores with olive coloured conidia	*Cladosporium sps*

According to the microscopical and macroscopical observations, the fungal strains were identified as *Aspergllus niger, Mucor sps, Aspergillus flavus, Penicillium sps, Aspergillus fumigatus, Fusarium* sps, *Rhizopus* sps, *Cauvularia sps, Aspergillus sulphurous, Cladosporium sps.*and tabulated in Table 3. Twenty two species of fungi were isolated in course of four samplings [15]. They include *Absidia glauca, Acrphialophora fusipora, Alternaria alternate, Aspergillus candidus, A. flavus, A.luchueusis, A.niger, A.sydowi, A.fumigatus, A.sulphureus, Cladosporium sps, Cunninghamella sps, Curvularia sps, Gliocladium sps, Fusarium sps, Melanospora sps, Myrothecium sps, Nigrospora sphaerica, Pestalotia, Penicillium sps, Rhizopus sps, Trichoderma viridae.*

All the fungal colonies were screened for laccase production and better results were obtained. The white rot fungus is the most common laccase producers among all fungi [16, 14] .All the fungal isolates were screened for laccase production on PDA medium supplemented with 0.02% guaiacol as substrate. Almost all fungal isolates developed reddish brown zones in the medium due to oxidative polymerization of guaiacol, which marked the presence of laccase. Guaiacol concentration was increased from 0.02% to 0.04% and 0.06% on different PDA plates and developed reddish brown zones in the medium containing 0.04% guaiacol. These results are showed that many of *Trichoderma* sp [17], extensively studied as sources of cellulases also have been reported as sources of laccases. Moreover, *T. harzianum* and *Trichoderma longibrachiatum* are the sources of laccases production respectively [18, 19, 20].

4. Conclusion

The enzyme laccases have been widely studied for various applications, like functionalization of lignocellulosic materials, wood fiber modification, remediation of soil and contaminated effluents as well as their use in biosensors. Thus, it has a great potential application in environment protection. So, this enzyme must be produced in large quantities with less expensive. Hence, the present study clearly shows the diverse presence of fungal isolates in the soil and tree barkings with the potential of laccase enzyme production. Further research should be carried out to make use of laccase in industrial purposes.

5. References

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