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Preliminary Screening for Laccase Producing Endophytic Fungi from Cupressus Torulosa D.Don

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Abstract: **Fungal laccases are unit of multicopper oxidases (MCOs) with high biotechnological potential due to their capability to oxidize a wide range of aromatic contaminants using oxygen from the air. Albeit the numerous laccase like genes described in ascomycetous fungus fungi, ascomycete laccases are less completely studied than white rot fungus laccase.0'; We have a tendency to aim here to identify the laccase type Oxidoreductases which may be concerned within the decolourization of textile dyes by using laccase producing endophytic fungi and to characterize them as potential biotechnological tools. Fungal Laccase (benzenediol: O enzyme, EC 1.10.3.2) along with ferroxidases (EC one.16.3.1) and ascorbate oxidase (EC one.10.3.3) kind the family of multicopper oxidases (MCOs), which play major role in bioremediation of these toxic dyes. In view of application of Laccase in bioremediation producing, a total of six endophytic fungi were recovered from leaves of healthy plant of Cupresustorulosa employing standard isolation methods. These endophytes were morphologically characterized by using colony appearance on PDA and Lacto phenol cotton blue staining techniques aswell as 18SrDNA sequencing methods. Endophytic fungi were screened for Laccase assay as it plays a crucial role in detoxification of toxic dye for human health. Laccase assay were performed using substrate such as Guaiacol, nephthol and ABTS, Syringeldehyd, Tannic acid. Out of six endophytic fungi only one endophytic fungus showed strong Laccase activity using Guaicol and ABTS, syringeldehyd as substrate. These results suggest that laccase producing Daldinia sp. was effective in decolorizing of synthetic dyes and promising in bioremediation of wastewater in textile, food and aquaculture industries.**

***Index Terms*—Laccase, Endophytic fungi, Daldiniasp,Guaiacol, nephthol and ABTS, Syringeldehyd, Tannic acid**

I. INTRODUCTION

In the recent years, enzymes have gained big importance in Industries; laccases are one of them that are wide found in the nature. Laccases are the oldest and most studied catalyst systems [1]. These enzymes contain 15–30% super moleculetheir molecule mass of 60–90 kDa. Laccase belongs to a small group of enzymes called the blue multicopper oxidases, having potential ability of oxidation. It belongs to enzymes, which have innate properties of reactive radical production, but its utilization in many fields has been ignored because of its unavailability in the commercial field [2]. There are diverse sources of laccase producing organisms like bacteria, fungi and plants. In fungi, laccase is present in Ascomycetes, Deuteromycetes, and Basidiomycetes and is particularly abundant in many white- rot fungi that degrade lignin. These are glycosylated polyphenol oxidases that contain 4 copper ions per molecule that carry out 1electron oxidization of phenoplast and its related compound and reduction of oxygen to water [3, 4]. Once substrate is change by a laccase, it loses one electron and frequently forms an atom which can endure more oxidation or non-enzymatic reactions as well as hydration, disproportionation, and chemical change [5]. These enzymes are chemical compound and usually contain 1 each of type 1, type 2, and type 3 copper Centre/subunit wherever the type 2 and type 3 are approximate forming a trinucleated copper cluster. Laccases are widely distributed in higher fungi, plants, bacteria, and insects. In plants, laccases are found in cabbages, turnip, potatoes, pears, apples, and alternative vegetables [6].

They have been isolated from Ascomycetes, Deuteromycteousand Basidiomycetes fungi to which more than 60 fungal strains belong [4]. The white-rot Basidiomycetes fungi with efficiency degrade the polymer as compared to Ascomycetes and class Deuteromycetes that oxidize phenolic compounds to present phenoxy radicals and quinines [7].Laccases play a crucial role in food industry, paper and pulp industry, textile industry, artificial chemistry, biodegradation, cosmetics, soilAnd bioremediation of environmental phenolic waste material and removal of endocrine disruptors [8].Laccase has potential for industrial and biotechnological applications, since laccase have ability the flexibility to degrade phenolic and nonphenolic polymer structures and also the ability to make some modification on the plant phytochemical that might produces potential pharmaceutical product. At this moment laccases have of great interest in synthetic

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chemistry as well as inexperienced chemistry within the future [9,10] These enzymes are used for pulp delignification, chemical or pesticide degradation, organic synthesis [11], waste detoxification, food technological uses, textile dye transformation and biosensor and analytical applications.[12] Recently laccases have been efficiently applied to Nano biotechnology owing to their ability to catalyze electron transfer reactions without extra compound.[13] The technique for the immobilization of biomolecule like layer-by-layer, micro patterning, and self-assembled monolayer technique is used for preserving the enzymatic activity of laccases.[14]

II. MATERIAL AND METHOD

2.1. Sample Collection and Isolation of entophytic fungi and identification

The sampling procedure was designed with the intention of isolating as many endophytic fungal species as possible from the different tissues samples. Tissues of the leaves of *C. torulosa*D. Don were cut into 5.0 mm long segments then surface sterilized [15]. Segments were surface sterilized by consecutive immersion for 1 min in 75 % Ethanol, treated for1 min in 0.1 % mercuric chloride, followed by several washing for in sterile distilled water. The time of the dilution and immersion in ethanol and Merchuric chloride varies with tissues and host (At least three washing require). Under sterile conditions, tissue segments were allowed to surface-dry before plating. Five segments were then evenly placed in each 90 mm Petri dish containing Potato dextrose agar medium. The dishes were sealed with parafilm and incubated at 27ºC ± 2˚C for 7-10 days in incubator.

2.2. Identification of Endophytic fungi

Fungal growth and sporulation was facilitated by placing the isolates onto PDA culture medium. The plates were continuously monitored for spore formation. Isolates were identified on the basis of cultural characteristics, color and morphology of fruiting bodies and spores. Fungal isolates were stained with Lacto Phenol cotton blue and examined under light microscope (Olympus, USA) [16].

2.3. Qualitative screening of endophytic fungi by laccase Assay

Laccase activity was tested by growing the endophytic fungi on GYP agar medium(peptone- 10gm/L, yeast extract-5 g/L, glucose-20 g/L, agar15g/L, (pH 7.0)amended with 1-napthol (0.005%) (pH6), ABTS (2mm) (pH 4.5), Guaicol (4mm) (pH5.4), Tannic acid (0.50µm), syringeldehyd (0.5mm) and incubated at 25±2˚C for 2 week by rapping the petri plates with black polythene bags.Have been used as a lignolytic indicator compound. The enzyme laccase production by the endophytic fungi will result in the oxidation of colorless 1-napthol to a brownish-violet, Guaicol to anintense brown, ABTS to a Ranged from bright green to dark purple, Tannic acid to a brown, syringeldehyd to a reddish brown. The fungi grown on a 1-napthhol, Guaicol, ABTS, Tannic acid free media was used as a control.

III. RESULT AND DISSCUSSION

3.1. Collection and Isolation of endophytic fungi and identification

A systematic study about the endophytic fungal biodiversity in a forest plant, *Cupressus torulosa*L. which were located in GovindBallabh Pant Engineering College Campus, Pauri Garhwal, and Uttarakhand was carried out to evaluate A total of 6 endophytic fungi were isolated from leaves of *Cupressus torulosa* L. by using different culture media.And its Morphotypic Characterization table[1]

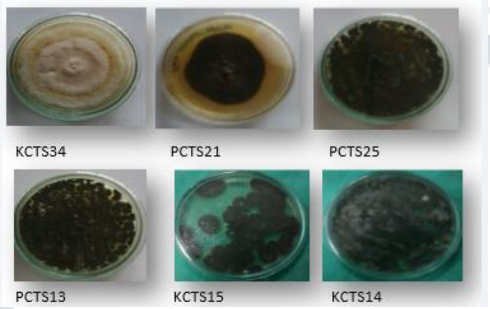

Fig. 1.Colony morphology on PDA
Morphotypic Characterization of Endophytic Fungi

Table1. 18S r DNA Sequencing of endophytic fungi isolated From Cupressus Torulosa

Sample ID	Organism name	% Identity	Accession No
PCTS13	Penicilliumoxalicum	99%	KT355727
KCTS15	Altemariaaltemata	100%	KT355727
PCTS21	Altemariaaltemata	100%	KT355727
PCTS25	Penicilliumoxalicum	99%	KT355727
KCTS34	Daldinia sp.	99%	KT355727
KCTS14	Pestalotiosisneglecta	99%	KT355727

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3.2.Laccase production by fungal endophytes by using Guaiacol

Six different endophytic fungal isolates were subcultured and screened for laccase activity on GYP agar with 4mm Guaiacol. Laccase enzyme react with Guaiacol to give intense brown color product, out of the above 6 isolates 3 isolates were found to be positive. These two fungal isolates were screened by liquid assay. Fig. [2] Guaiacol substrate showed a very strong ability to facilitate the growth and the isolation of the *Centellaasiatica* with the laccase activity, [17]

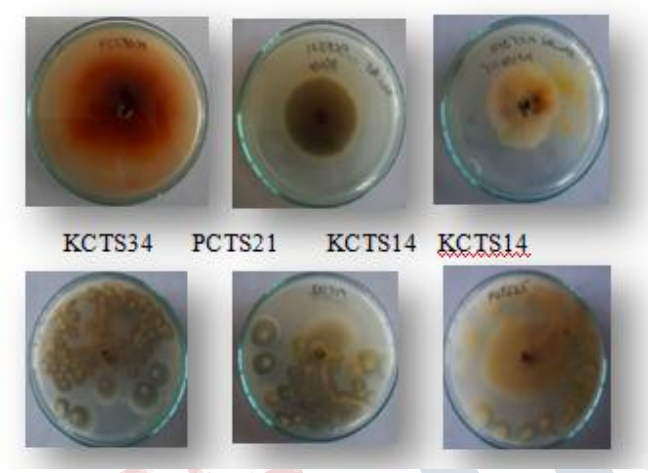

Fig.2.Laccase producing fungal endophytes using guaiacol

3.3.Laccase production by fungal endophytes by using ABTS

Six different endophytic fungal isolates were subcultured and screened for laccase activity on GYP agar with 2mm ABTS. ABTS were considered as the best substrates for laccase activity [18].Laccase enzyme react with ABTS give Purple color product, out of the above 6 isolates 3 isolates were found to be positive. These three fungal isolates were screened by liquid assay. Fig. [3]

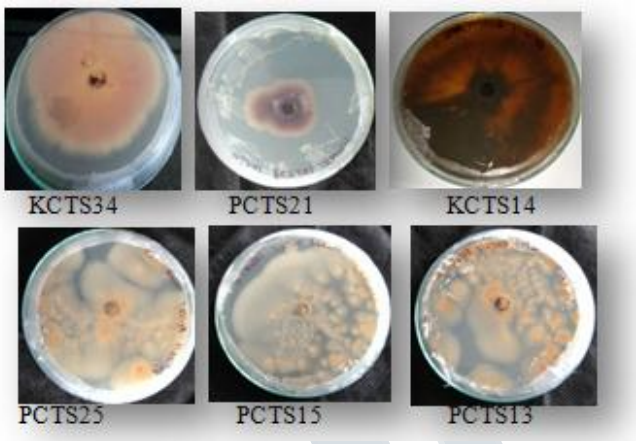

Fig.3. Laccase producing fungal endophytes using ABTS

3.4. Laccase production by fungal endophytes by using Syringeldehyd

Six different endophytic fungal isolates were subcultured and screened for laccase activity on GYP agar with 0.5mm Syringeldehyd. Syringeldehyd substrate showed a very strong ability to facilitate the growth and the isolation of the White rot fungus *P. ostreatus* was with the laccase activity [19], Laccase enzyme react with Guaiacol to give reddish brown color product, out of the above 6 isolates 3 isolates were found to be positive. These 3 to fungal isolates were screened by liquid assay. Fig. [4]

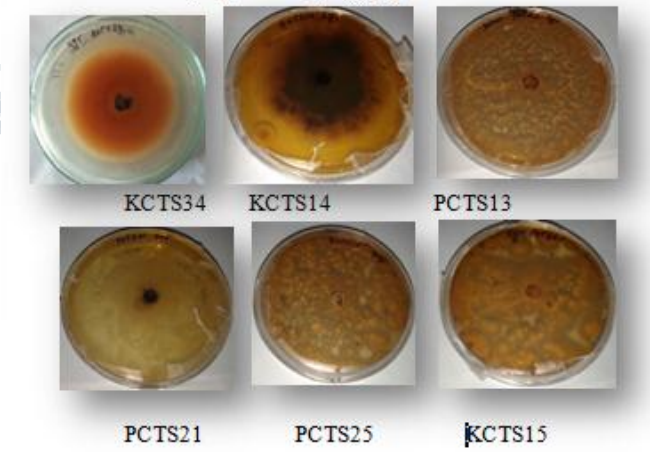

Fig.4.Laccaseproducing fungal endophytesusing syringeldehyd

3.5. Laccaseproduction by fungal endophytes by using Tannic acid

Six different endophytic fungal isolates were subcultured and screened for laccase activity on GYP agar with 0.5µM Tannic acid. However, tannic acid cannot be used alone to confirm that the produced enzyme is laccase,

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and should be used with some doubt since it is not specific to anyone of the laccase, besides, the production of a brown oxidation zone might be similar to many naturally produced fungal pigments. [17] Laccase enzyme react With Tannic acid give dark brown product, out of the above 6 isolates 3 isolates were found to be positive. These three fungal isolates were screened by liquid assay. Fig. [5]

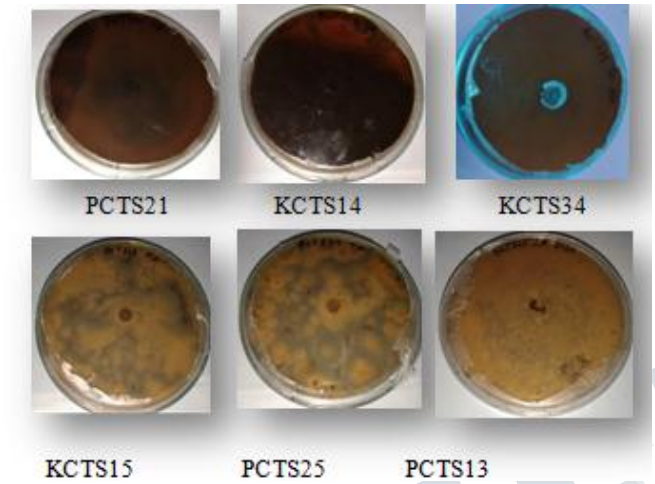

Fig.5.Laccase producing fungal endophytes using Tannic acid

Laccase enzyme activity of fungal isolates from *Cupressus torulosa.*

Table2. Laccase activity of fungal isolates from Cupressus torulosa

Sample	guaiacol	ABTS	Tannic acid	syringeldehyd
KCTS34	++++	++++	++++	++++
PCTS21	++	++	++	++
PCTS25	_	_	+	+
PCTS13	_	_	+	++
KCTS14	++ +	+++	+++	+++
KCTS15	_	_	+	+

(++++)-High activity, (+++)-Good activity, (++)-Medium activity, (+) - Low activity, (-) – No activity

IV. CONCLUSION

Laccases are receiving much attention from researchers around the globe because of their specific nature. They have many industrial applications because of its innate ability of oxidation of a broad range of phenolic and non-phenolic compounds. The biotechnological significance of laccase enzymes has led to a drastic increase in the demand for these enzymes in the recent time. Laccase are promising to replace the conventional chemical processes of several industries. The introduction of the laccase-mediator system provides a biological alternative to traditional chlorine bleaching processes. The present research work aimed at isolation of laccase producing fungi to be used in the Textile dye Decolorization isolated especially from *Cupressus torulosa*. The study investigated the possibility to isolate a white rot fungi from natural sources too. During this study, different cultivation techniques have been also done in order to facilitate efficient screening. Guaiacol, ABTS, Syringeldehyd, Tannic acid substrate also showed strong result of laccase activity, and the microscopic identification also show the characteristics of the white rot fungi. The isolated fungi characterized as Daldinia sp by 18 S rDNA sequencing which showed promised activity to be used for the main purpose of bioremediation of textile dye. Laccase producing fungi have many biotechnological applications and the isolated fungi might be used for further research projects for decolorization of various textile dye.

V. ACKNOLEDEGMENT

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