Decolorization of textile dye by laccase from newly isolated endophytic fungus  

Sheetal Chanyal, Pavan Kumar Agrawal*  
Department of Biotechnology G.B. Pant Engineering College, Ghurdauri, Pauri, Garhwal, Uttarakhand, India  
*Corresponding author Email: p_k_agarwal@rediffmail.com  
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ABSTRACT

In view of application of laccase in bioremediation of textile dyes, a total of six endophytic fungi were isolated from needle of healthy Gymnosperm plant, *Cupressus torulosa* D. Don of Garhwal, Himalayan region. The colonization rate of these fungi on PDA was higher for various sampling sites in S1 (90) > S2(60) > S3 (40) but colonization rate of fungi in KMM was S3(73) > S1(60) > S2(40). All endophytic fungi were screened for laccase assay as it plays a crucial role in detoxification of toxic dye for human health. Out of six fungi only one endophytic fungus known as KCT34 showed significant laccase activity in solid and liquid media. Initial screening on the malt extract agar plates supplemented with 200 mg L⁻¹ of each Congo red, Rosebangal, Orange G and Rhodamine B dye showed that KCT34 was able to decolorize all of the four dyes. Quantitative analysis of the dye decolorization showed that KCT34 was able to decolorize textile dyes efficiently and dye decolorization percentage was maximum on 16th day with percentage ranging from 90.45% for Congo red, 87.91% for Rose bangal, 85.81% for Orange G and 54.39% for Rhodamine B. Molecular identification of the fungal isolate KCT34 using primer ITS1 and ITS4 showed 99% sequence similarity with *Daldinia* sp, an Ascomycetous fungus. The ability to decolorize different types of textile dyes by the isolated *Daldinia* sp. suggested a possible application of fungal endophytes in the decolorization of dyestuff effluents from textile, food and aquaculture industries.

Keywords: Decolorization, Laccase, bioremediation, textile dye, endophytic fungi, *Daldinia* sp.

INTRODUCTION

Water is basic need of life and used for number of household as well as industrial activities. Being one of the essential natural resource, it is unfortunately exploited the most. Because of its unrestricted and excessive exploitation, presently the world is facing crisis of drinking water. Increased urbanization of natural resources like, increase in textile industries, possesses a threat to the aquatic bodies as these industrial effluents with various toxic components, basically dye compounds deteriorate the quality of water and makes it harmful for further usage. Approximately 10,000 dyes are commercially available, with an annual production of over 7 x 10⁴ metric tons (Campos et al., 2001). Over 50% of these dyes are released in the industrial effluent (Zollinger, 2003). These dyes contain high amount of heavy metals and organic compounds and they contribute in textile industries water pollution as 93% of the intake water comes out as colored wastewater (Gupta et al., 2014).

Wastewater released from various textile industries are highly variable and complex mixture of many polluting substances, especially dyes, inducing color coupled with organic load which leads to disruption of the total ecological balance thus disturb the whole aquatic ecosystem (Hassani et al., 2008). The non-biodegradable nature of the dyes in the spent dye baths of textile industries constitutes a serious environmental hazard. Most of the dyes induce toxological problems in cardiovascular, dermologic, gastrointestinal, genito-urinary, hemotologic and central nervous system (Harvey and Keitt, 1983).

In general, the direct discharge of the chemicals especially dyes in the effluents from various textile industries in open environment is the principle sources of water pollution (Ramesh Babu et al., 2007). Hence, the removal of dyes and other chemical substances from industrial effluents is very important before its discharge into open water (Balaji et al., 2012).

Physical or chemical processes could not be effectively used for treatment of dyes from wastewater as those processes are not economically viable (Grag et al., 2003). Due to low biodegradability of dyes, conventional biological treatment processes are also not much successful in treating dye containing wastewater. Therefore, innovative treatment technologies such as low cost biological conversion based technologies of ecofriendly nature are needed to be investigated for adsorbing or decolorizing dyes from waste water released from textile industries. Many microorganisms including fungi and its enzymes have the ability to decolorize textile dyes (Fargacs et al., 2004). Recently, in situ degradation of dyes in wastewater using fungal metabolic activities is the subject of many research studies.

Fungal laccases are known to be effective to degrade azo dyes (Blanquez et al., 2004). Laccases are used in commercial textile applications to improve the whiteness in conventional bleaching of cotton process. Laccases of fungi are of particular interest with respect to potential industrial applications as a result of their capability to oxidize a good range of industrially relevant substrates. Oxidization reactions are comprehensively utilized in industrial processes, for instance, within the textile, food, wood process and pharmaceutical and chemical industries (Onuki et al., 2000). Laccases from fungi provide many advantages and thus are of great interest in biotechnological applications of industrial effluent treatment. Laccases exhibit broad substrate specificity. They can bleach Kraft paper pulp or detoxify agricultural byproducts as well as olive mill wastes or even coffee pulp (Watanabe et al., 2007).

In this context, the present study is aimed to isolate the laccase producing endophytic fungi from leaves of *Cupressus torulosa* D. Don from different sites of Pauri, Garhwal region, Uttarakhand, India and to check its decolorizing ability for different dyes used in textile industries.

MATERIAL AND METHODS

Isolation and sterilization of endophytic fungi: Mature leaves and twigs of *C. torulosa* tree were collected from
different sites of GBPEC, College campus, Ghurdauri, Pauri, located at latitude 30°18'35"N and longitude 78°69'30"E. The plant was taxonomically identified and authenticated by Botanical survey of India, Dehradun. The voucher specimen was deposited there with accession number 115744. The plant materials were transported to the lab in sterile polythene bags and stored at 4°C until processed.

Isolation of endophytic fungi were carried out using the protocol given by Atalla et al. (2010) with slight modifications as described by Sharma et al. (2016). In short, the healthy C. torulosa plant tissues were washed under running tap water and samples (leaves and twigs) were cut into size of an inch. Samples were then surface sterilized sequentially with 70% alcohol for 3 min, 0.5% sodium hypochlorite for 1 min, rinsed twice with sterile water followed by drying on sterile filter paper (Arnold et al., 2000) and excess water was drained from the surface of the sample by touching to the sides of the beaker before placing them on the Potato dextrose Agar (PDA) media supplemented with antibiotic streptomycin (200 mg/L).

The petri dishes were sealed with parafilm and incubated at 28°C ± 2°C for 2-4 weeks in incubator. Fungal growth was observed from the tissue segments inoculated on the water media plates as colored cottony outgrowth. Hyphal tips, from germinating fungi, were isolated, sub cultured onto PDA and brought into pure culture by incubating at 28°C for 5-7 days. The purified fungal isolates were maintained on Potato dextrose agar slant at 4°C in refrigerator for further studies.

Morphotypic identification of endophytic fungi: Fungal growth and sporulation was facilitated by placing the isolates onto PDA growth medium. The plates were continuously visually monitored for spore formation. Fungal 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 for morphology of fruiting bodies and spores. Fungal isolates were brought into pure culture by incubating at 28°C for 5-7 days. The purified fungal isolates were maintained on Potato dextrose agar slant at 4°C in refrigerator for further studies.

Molecular Identification of the endophytic fungi: The isolated endophytic fungus was initially identified through 18S rRNA sequence. For molecular identification, total genomic DNA of the endophytic fungus was isolated directly from actively growing mycelium growing in potato dextrose broth (PDB), using DNA extraction kit, Genei. Fungal DNA was extracted in the laboratory using the protocol of Kariyawasam et al. (2012). The extracted DNA was subjected to the polymerase chain reaction (PCR) using primers ITS1: T C C G T A T G G A C C T T C G and ITS4: TCCTCCGCTTGATATGC (White et al., 1990; Sharma et al., 2016). Amplified DNA was subjected to DNA sequencing and this DNA sequence was compared with already existing DNA sequences in NCBI GenBank database (http://www.ncbi.nlm.nih.gov/blast) to identify the fungi. PCR and DNA sequencing was done by the Gujarat State Biotechnology Mission, India. The obtained sequence was submitted in National Centre for Biotechnology Information with accession number KT355731.

Endophytic fungal diversity analysis: The Colonization rates of fungi were calculated as the percentage of segments colonized by one or more isolate(s) from the total number of segments of each tissue plated following the method of Petriini and Fisher (1988). Samples were incubated and growth was analyzed regularly for 6 weeks and colonizing rate was calculated.

\[
\text{Colonization Rate (%)} = \frac{\text{Total no. of segments infected by fungi}}{\text{Total no. of segments analyzed}} \times 100
\]

Evaluation of laccase activity in solid media: The fungal isolates were screened for laccase production by growing them on plates of GYP medium containing 4mM guaiacol, 2mM ABTS, 0.5mM syringaldehyde, 0.5µM Tannic acid (Kumar et al., 2011). The petri dishes were inoculated with the isolated fungus and were incubated at 28°C in dark for 2 weeks. The production of intense brown color, reddish brown colour, purples colour under and around the fungal colony was considered as a laccase positive reaction resulting from respectively guaiacol and tannic acid, syringaldehyde and ABTS oxidation.

Evaluation of laccase activity in liquid media: Laccase activity was assayed at 28°C using 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)1 or guaiacol or syringaldehyde as substrates as follows. (a) The assay mixture contained 2 mM ABTS and 0.1 M sodium citrate buffer, pH 3.0. Oxidation of ABTS was followed by absorbance increase at 420 nm (ε=36,000 M⁻¹ cm⁻¹). Enzyme activity was expressed in international units (IU). (b) The assay mixture contained 4mM guaiacol, and the McIlvaine’s citrate-phosphate buffer adjusted to pH 6.0. Oxidation was followed by the absorbance increase at 460 nm. (ε=6740 M⁻¹ cm⁻¹) One unit of activity is the amount of enzyme producing a 1.0 A increase/min. (c) The assay mixture contained 0.5 mM syringaldehyde (dissolved in ethanol) and 50 mM phosphate buffer, pH 6. Oxidation of syringaldehyde was followed by an absorbance increase at 525 nm (ε=65,000 M⁻¹ cm⁻¹). Enzyme activity was expressed in IU.

Enzyme Assay: For this purpose following relation was employed

\[
\text{Enzyme activity (U/ml)} = \Delta A_{470} \text{ nm/min} \times 4 \times Vt \times \text{dilution factor/} \epsilon \times Vs
\]

Where, Vt=final volume of reaction mixture (ml)=5
Vs=sample volume (ml)=1
\epsilon=extinction coefficient of guaiacol=6,740/M/cm
4=derived from unit definition and principle

Evaluation of dye decolorization activity in solid media: Fungal endophytes were grown onto Glucose Minimal (GM) agar plates and initially screened for their ability to decolorize azo dyes eg. Orange G, Congo red, and rhodamine and rose bangal (Ngien et al., 2013). The GM agar media contained (g/L): K2HPO4, 1; ZnSO4.7H2O, 0.01; CuSO4.5H2O, 0.05; MgSO4.7H2O, 0.5; FeSO4.7H2O, 0.01; KCl, 0.5; glucose, 10; NaNO3, 3; and agar, 20. The pH of the agar medium was adjusted to 5.5 before being autoclaved at 121°C for 15 min. Dyes were added into the agar media from a sterile stock solution to a final concentration of 200 mg L⁻¹. The fungi were
cultivated during 20 days at 28°C in the dark. Uninoculated plates with the respective dyes were used as negative control. Observation for the rate of decolorization was made twice every week (Narkhede et al., 2013). The decolorizing activity was described as positive when the solid media exhibited partial or total loss of color imparted by the respective dyes. The fungal strains with decolorizing activity were further selected for the liquid media evaluation.

Evaluation of dye decolorizing activity in liquid media:
Dyes were added to the 50 mL GM liquid medium in 250 mL Erlenmeyer flask to a final concentration of 50 mg L⁻¹. Each flask was inoculated with 2 pieces of 5mm agar plugs from a 7 day old fungal culture and incubated in the dark at room temperature under static condition. Flask with the respective dye and no fungal inoculum was used as control. Each culture condition was prepared in triplicate, incubated for a period of 16 days, and sampled at 4-day interval. During the sampling, each culture was harvested and centrifuged at 6000 rpm for 10 minutes to separate the fungal mycelium from the culture medium. Fungal biomass was determined by drying the fungal mycelium to a constant weight at 70°C. Dye decolorization by the isolated fungus was measured by monitoring the absorbance of each dye in the culture medium at its respective maximum absorption wavelength (475 nm for Orange G, 497 nm for Congo red, 590 nm Rhodamine, and 549 nm for rose bengal) using a UV-Vis spectrophotometer (Lab India). Percentage of decolorization was calculated according to the following formula:

\[
\% \text{Dye Decolorization} = \frac{A_i - A_s}{A_i} \times 100
\]

where \( A_i \) is the absorbance at the maximum absorption wavelength of dye in the control flask at time, \( t \) and \( A_s \) is the absorbance at the maximum absorption wavelength of dye in the sample flask at time, \( t \) (Ozsoy et al., 2005).

Statistical analysis: All experiments were carried out in triplicate and the results obtained were statistically analysed.

RESULT AND DISCUSSION

Collection and isolation of endophytic fungi and identification: A systematic study about the endophytic fungal biodiversity in a forest plant, C. torulosa which were growing in Govind Ballabh Pant Engineering College Campus, Pauri, Garhwal, and Uttarakhand was carried out to evaluate its bioremediation potential for removal of various textile dyes. A total of six endophytic fungi were isolated from the leaves of C. torulosa by using different culture media and were morphotypically characterized (Fig. 1 and Table 1) (Williamson, 1994).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Code of isolate</th>
<th>Colony characteristics on PDA media</th>
<th>Probable endophytic fungus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PCTS13</td>
<td>Appears olivaceous brown in colour</td>
<td>Penicillium sp</td>
</tr>
<tr>
<td>2.</td>
<td>PCTS21</td>
<td>Appears greyish green in colour</td>
<td>Alternaria alternata</td>
</tr>
<tr>
<td>3.</td>
<td>PCTS25</td>
<td>Appears olive green in colour initially but colony becomes white after 10 days</td>
<td>Penicillium sp</td>
</tr>
<tr>
<td>4.</td>
<td>KCTS14</td>
<td>Appears whitish gray in colour</td>
<td>Pestalotiopsis sp</td>
</tr>
<tr>
<td>5.</td>
<td>KCTS15</td>
<td>Appears grey in colour</td>
<td>Alternaria alternata</td>
</tr>
<tr>
<td>6.</td>
<td>KCTS34</td>
<td>Appears cottony white in colour</td>
<td>Unidentified</td>
</tr>
</tbody>
</table>

In Korea, total 59 isolates and 19 species of endophytic fungi were isolated from the leaves of Juniperus rigida, Larix kaempferi and Pinus densiflora and identified using morphological and molecular characteristics (Kim et al.).

Table 2: Colonization rate of endophytic fungi on different culture media

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Collection of leaves from various sites</th>
<th>No of fungi colonized on segment in KMM</th>
<th>Colonization Rate on KMM</th>
<th>No of fungi colonized on segment in PDA</th>
<th>Colonization Rate on PDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S1</td>
<td>6</td>
<td>40±0.4</td>
<td>4</td>
<td>40±0.4</td>
</tr>
<tr>
<td>2.</td>
<td>S2</td>
<td>4</td>
<td>73.3±1.15</td>
<td>4</td>
<td>40±0.1</td>
</tr>
</tbody>
</table>

The Majority of the recovered endophytes belong to the Ascomycota. Fungal endophytes are especially common among the Ascomycota, representing at least five classes, dozens of families, and large numbers of previously unknown species (Gehlot et al., 2008). Only one species from the collected isolates in this study belong to the Dothideomycetes (Petrini, 1986).
Endophytic fungi isolated from healthy plant parts such as stem, root, rhizome of seven plant species and one thousand five hundred and twenty-nine fungal isolates were obtained from 5200 fragments. Stem fragments harbored more endophytes (80.37%) than roots (19.22%). 31 fungal taxa comprised of coelomycetes (65%), hyphomycetes (32%), and ascomycetes (3%). Fungal isolates such as *Fusarium*, *Acremonium*, *Colletotrichum*, *Chaetomium*, *Myrothecium*, *Phomopsis*, and *Pestalotiopsis* were commonly isolated (Nalini et al., 2014).

**Laccase production by fungal endophytes by solid state fermentation:** Six different endophytic fungal isolates were sub-cultured and screened for laccase activity on Glucose Yeast Extract Peptone Agar with Guaiacol, ABTS, Tannic acid and Syringaldehyde. Laccase enzyme react with Guaiacol to give intense brown color product (Fig. 3), ABTS give Purple color product (Fig. 4), Tannic acid give dark brown product (Fig 5), Syringaldehyde to give intense brown color product (Fig. 6). Out of the above six fungal isolates, only three such as KCTS34, KCTS14 and PCTS21 exhibited significant laccase activity (Table 3). The obtained results are in conformity with the report of Kiiskinen et al. (2004) for the isolation of laccase producing fungus. Muthezhilan et al. (2014) reported various morphotypes of endophytic fungal strains from different segments (leaf, stem, and root) of sand dune plants and were qualitatively screened for laccase production by agar well diffusion method. Among that, four strains namely, AEF17, AEF19, AEF22 and AEF25 have showed maximum laccase production, and they were cultured for laccase production in solid state and submerged fermentation. Laccase activity was shown by *Pleurotus* sp.

**Table 3:** Laccase enzyme activity of fungal isolates from *Cupressus torulosa*

<table>
<thead>
<tr>
<th>Sample</th>
<th>guaiacol</th>
<th>ABTS</th>
<th>Tannic acid</th>
<th>Syringaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCTS34</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PCTS21</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PCTS25</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PCTS13</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>KCTS14</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>KCTS15</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

![Fig. 2: Colonization rate of endophytic fungi](image)

![Fig. 3: Laccase producing fungal endophytes A: PCTS13, B: PCTS21, C: PCTS25, D: KCTS14, E: KCTS15, F: KCTS34 on using Guacol containing 0.02% guaiacol since laccase catalyzes the decolorization of textile dye by laccase from newly isolated endophytic fungus *Daldinia* sp.](image)

![Fig. 4: Laccase producing fungal endophytes A: PCTS13, B: PCTS21, C: PCTS25, D: KCTS14, E: KCTS15, F: KCTS34 using ABTS](image)
oxidative polymerization of guaiacol to form reddish brown zones in the medium (Kumar, 2012). Syringaldehyde substrate showed a very strong ability to facilitate the growth and the isolation of the White rot fungus P. ostreatus with the laccase activity (Palmieri et al., 1997).

**Quantitative estimation of laccase production:** Fungal endophytes that showed positive reaction in the plate test for the secretion of laccase, were further analyzed in liquid culture for the quantitative estimation. Laccase activity was calculated with respect to the incubation time. Maximum secretion of laccase was seen in 10th day of the culture age. Among them fungal isolates, KCTS34 produced the maximum laccase activity (57 U/mL) by using Guaicol as substrate, 47 U/mL by using ABTS and least 32.12 U/mL by syringaldehyde (Fig. 7). Initial screening of crude enzyme showed complete oxidation of ABTS and Guaiacol after 7 days of incubation. *Alternaria arborescens* showed maximum production of laccase enzyme (800U/L) at 30°C in 4.5 pH followed by *Fusarium oxysporium* (JQ 950134) with 600 U/L at 45°C in 5 pH after 15 days of incubation (Christie and Shanmugam, 2012).

Desai et al., (2011) reported that when *Tricoderma genus* was cultivated on potato dextrose agar (PDA) plates containing guaiacol and tannic acid as indicator compounds, higher level of laccase activity was observed under solid state condition production (15.7 U/mL) in comparison to submerged condition (10.68 U/mL). It was also found out that the laccase was active over broad range of temperature and pH, with optimum temperature of 45°C and pH 5. The isolated strain can be further evaluated for other characteristics and it could prove to be a potent source of enzyme for lignin degradation.

**Dye decolourization studies by endophytic fungal isolate:** Textile dyes Congo red, Rose bangal, Orange G and Rhodamine B were used to study the ability of laccase activity.

![Fig 5: Laccase producing fungal endophytes A: PCTS13, B: PCTS21, C: PCTS25, D. KCTS14, E: KCTS15, F: KCTS34 using Tannic acid](image)

![Fig 6: Laccase producing fungal endophytes using A: PCTS13, B: PCTS21, C: PCTS25, D: KCTS14, E: KCTS15, F: KCTS34 using syringaldehyde](image)

![Fig 7: Quantitative estimation of laccase production](image)
producing endophytic fungi KCT 34 to decolorize textile dye. As per the result of decolorization obtained in the malt extract media supplemented with textile dye, KCT 34 was able to decolorize all the four different dyes tested (Fig. 8). Further, KCT34 was used for detailed study using spectrophotometry method. Poojary et al., (2012) conducted a similar study and isolated two fungal strains capable of dye decolorization. Another study by Levin et al., (2004) also showed the dye decolorizing ability of fungi isolated in Argentina. Decolorization of textile dyes by a Clitocybula dusenii, white-rot fungus producing laccase was reported by Wesenberg et al., (2002). Liu et al., (2004) also reported that laccase is solely responsible for the decolorization and degradation of dyes.

A decolorization test using Basic fuchsia, Congo red, Crystal violet, Malachite green and Orange G was carried out using partially purified immobilized laccase enzyme of Trametes hirsuta, in which maximum decolorization was observed in Malachite green and Crystal violet in 15 days (Rasheeda et al., 2014). Congo red showed 86% decolorization, followed by Orange G (71%) and Rhodamine B (52%). Another decolorization test using the same dyes along with fast green was carried out which showed decolorization rate of 93% with Congo red, followed by 87% with fast green, 81% with Orange G and 71 % with Rhodamine B (Kumar, 2012). This study results were supported by Eggert et al., (1996), who showed the decolorization ability of laccase in Phlebia tremmellosa (Robinson et al., 2001) and Pleurotus sajor-caju (Chagas et al., 2001).

Quantitative assay for synthetic textile dye decolorization: All of the 4 textile dyes were tested for decolorization by KCT34 in Malt extract broth. Fungal isolate showed variable potential to decolorize Congo red, Rosebangal, Orange G and Rhodamine B in liquid medium. All the selected dyes were degraded by Daldinia sp. fungal strains but per cent decolorization was different for all dyes. Per cent of dye decolorization by KCT34, showed minimum dye decolorization percentage on 4th day 56.20% at 497 nm, 7.38% at 475 nm, 4.42% nm at 590, 4.11% at 549 nm and maximum on 16th day 90.45%, 85.81%, 54.39%, 87.91%. Congo red, Orange G, Rhodamine B, Rose bangal, respectively (Fig. 9).

Dye decolorization activity of endophytic fungi were screened using an agar plate based and liquid culture based method. Maximum decolorization exhibited by the liquid method in comparison of agar media method. Only one endophyte, KCT34 was able to decolorize all the four different dyes tested (Congo red, Rosebangal, Rhodamine and Orange G), after 16 days of fungal incubation. Alternaria alternata CMERI F6 decolorized 99.99% of 600 mg/L Congo red within 48 h in yeast extract-glucose medium at 25°C, pH 5 and 150 rpm (Chakraborty et al., 2013). Decolorization of textile dye industrial effluents by white-rot fungus producing laccase as the major lignin-degrading enzyme as seen in this study was reported in white-rot fungus, Clitocybula dusenii by Wesenberg et al., (2002). The results of this study lead to a conclusion that endophytic fungi having potential ligninolytic enzymes can be used in industrial effluent treatments.

Identification of endophytic fungi: On PDA medium, the isolated fungal culture KCT34 appeared cottony white in colour (Fig. 10). The slide cultures prepared from this fungus showed the presence of stolons and pigmented rhizoids, multi-spores, generally globose sporangia. In addition to the morphological characterization, molecular methods were carried out to confirm the identification of most promising endophytic fungal strain KCTS34 isolated from the C. torulosa D. Don.
Molecular identification techniques were used to determine the identity of the fungus to generic level. On the basis of its 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence and 28S ribosomal RNA gene, partial sequence, it can be concluded that the fungus KCTS34 belongs to the genus Daldinia sp. The obtained sequence was submitted in National Centre for Biotechnology Information with accession number KT355731. The percentage of similarity between the fungus and database suggests it to be a novel strain of species of genus Daldinia, an Ascomycetous fungus.

**CONCLUSION**

The direct discharge of the chemicals, basically dye containing industrial effluents in open environment is the principle source of water pollution. So, the removal of dyes and other chemical substances from industrial effluents is a major concern before its discharge into open water bodies. There are different chemio-physical and biological methods used for the treatment of dye. Dye decolorization with microorganisms is cost effective and environment friendly method for controlling pollution generated by textile industries. In this work, six strains of endophytic fungi from C. torulosa were recovered and screened out for laccase activity. Out of them, 3 fungal strains, namely Alternaria alternata, Pestalotiotis neglecta and Daldinia sp. exhibited their ability to secrete laccase comparable to other lignin-degrading enzymes by 10th day of incubation. Out of these Daldinia was selected for textile dye decolorization test because of better showing in comparison in this regard. This is the first preliminary report on endophytic Daldinia sp. from Gymnospermous tree C. torulosa growing in Garhwal region.

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