

Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes

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Abstract: Paper and pulp mills, textile and dye-making industries and alcohol distilleries release highly colored effluents that are relatively difficult to decolorize by chemical and physical treatments. White-rot basidiomycetous fungi that produce lignin-degrading enzymes are reported to be the most efficient in decolorizing such effluents. We report here decolorization of all the three effluents by a marine fungal isolate, NIOCC # 2a cultured from decaying mangrove wood. The fungus also decolorized several synthetic dyes. Laccase was the most dominant lignin-degrading enzyme produced by this fungus with very low activities of manganese-dependent peroxidase and no lignin peroxidase activity. The growth and production of laccase was best in a medium prepared with sea water having salinity in the range of 25-30 ppt. The pH optimum for the laccase activity was 3.0 and 6.0 and the temperature optimum was 60°C. Laccase production was increased in the presence of phenolic and non-phenolic inducers. A several fold enhancement in laccase production was found during treatment of colored effluents from textile, paper and pulp mill and distillery waste. Industrial effluents and synthetic dyes added to the growing culture of this fungus were decolorized to a great extent. The culture supernatant without the fungal biomass was also effective in decolorization of these effluents to various degrees within 6 h of incubation. Extracellular polymeric substances (EPS) produced by this fungus were also useful in decolorization of these effluents. Thus, efficiency of this fungus in decolorization of various effluents with laccase that is active at pH 3.0 and 6.0 and 60°C in the presence of sea water has great potential in bioremediation of industrial effluents. Enhanced laccase production in the presence of industrial effluents in this fungus is an added advantage during bioremediation of effluents.

Keywords: laccase, inducers, marine fungus, mangroves, colored effluents, decolorization

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1. Introduction

Lignin peroxidase, manganese-dependent peroxidase and laccase are the three major lignin-degrading enzymes with great potential in industrial applications. Laccase (EC 1.10.3.2, benzenediol:oxygen oxidoreductase) is a multicopper blue oxidase capable of oxidizing ortho and para-diphenols and aromatic amines by removing an electron and proton from a hydroxyl group to form a free radical. These enzymes lack substrate specificity and are thus capable of degrading a wide range of xenobiotics including industrial colored wastewaters. The most efficient microorganisms to break down colored pollutants so far reported are white-rot fungi. These comprise mostly basidiomycetous fungi capable of extensive aerobic lignin degradation and mineralization. This is possible through several extracellular lignin-degrading enzymes synthesized by these fungi [1].

Paper and pulp mills, textiles and dye-making industries, alcohol distilleries and leather industries are some of the industries that discharge highly colored effluents. The paper and pulp industry release large volumes of intensely coloured black liquors containing toxic chlorinated lignin degradation products, including chlorolignins, chlorophenols, chloroguaiacols and chloroaliphatics [2]. Chlorinated organic compounds are acute or chronically toxic besides being mutagenic and carcinogenic.

Dye-making and textiles industries release industrial dyes into the environment. About 10 to 15 % of the total dye finds its way into the waste waters [3]. Several of these dyes resist microbial attack and are thus recalcitrant. These can be transformed to carcinogenic compounds under anaerobic conditions [4]. The fast coloured dyes such as azo dyes are a major source of concern to environmentalists since such pollutants, besides causing aesthetic damage to sites are also toxic and carcinogenic [5].

Distilleries producing beverage alcohol by fermentation use sugarcane molasses as the raw material. The effluents from such distilleries contain large amounts of dark brown pigments called molasses or melanoidin pigments [6]. Melanoidin pigments (MP) are the products of the "Maillard reaction" between

sugars and amino compounds produced on heating [6]. Pollution of aquatic ecosystems by molasses spent wash (MSW) is due to its intense brown colour that cuts off light, prevents photosynthesis and causes anaerobic conditions. When MSW is dispersed in soil, it acidifies the soil and thereby affects agricultural crops [7].

Besides colour, the various effluents from the above mentioned industries also contain various inorganic chemicals such as sulfides, sulfates, chlorides, carbonates, sodium hydroxide, peroxides and chlorine bleach compounds. The pH varies between a range of 7-12. These chemicals may also add taste and odors [8]. Obligate and facultative marine fungi occurring in coastal marine environments can be an important source for use in bioremediation of saline soil and waste-waters. We reported decolorization of some of these colored effluents by a lignin-degrading fungus isolated from sea grass detritus [9, 10]. Search for better and more efficient fungi from newer sources for application in bioremediation of such colored effluents, especially in the presence of high salt contents of the effluents continues.

We report here decolorization of black liquor, textile dye waste-waters and molasses spent wash by a marine fungal isolate NIOCC # 2a, producing laccase as the major lignin-degrading enzyme. Although laccase is a constitutive enzyme, its production can be stimulated by the presence of several inducing substrates, nitrogen and carbon levels [11]. We further discuss enhanced laccase production by this fungus in the presence of colored effluents and other synthetic dyes.

2. Materials and Methods

2.1. Organism and culture conditions

Decaying wood pieces from mangrove swamps from Chorao island in Goa, India (73°55' E and 15°30'N) were collected in sterile plastic bags and processed within 3 hours. They were washed free of attached soil particles and other extraneous matter using sterile seawater. The wood pieces were then incubated in sterile bags lined with moist filter paper for a fortnight. As soon as fungal

mycelia were observed to colonize the wood pieces, as little mycelia as possible were picked up using a sterile glass needle and transferred onto Boyd and Kohlmeyer (B&K) agar [12] fortified with 10% antibiotic solution to prevent bacterial growth. The stock solution of antibiotics contained 400,000 units of procaine penicillin and 1 g of streptomycin sulphate in 100 ml of sterile distilled water. B&K agar contained 10 g glucose, 2 g peptone, 1 g yeast extract and 18 g agar in 1 liter of 50% seawater. The same medium was used for maintenance of the cultures. The culture was routinely checked for purity by light microscopy.

2.2. Qualitative assay for laccase

The isolates were screened for laccase production by growing them on plates of B&K medium containing 4 mM guaiacol (S.D. Fine-Chemicals Ltd, Mumbai) or 2 mM ABTS i.e. 2,2'-azino-bis-(3-ethyl benzothiazoline-6-sulphonic acid); (Sigma chemical, USA). The production of an intense brown color under and around the fungal colony in the case of guaiacol-supplemented agar and a deep green color in the case of ABTS-supplemented plates was considered as a positive reaction for the presence of laccase activity.

2.3. Quantitative estimation of lignin-degrading enzymes

The fungal culture grown in B&K broth (20 ml in 100 ml Erlenmeyer flasks) for 7 days was blended in sterile sea water with sterilized glass beads and was used at 10% (v/v) concentration for growing stationary cultures. The culture filtrate was obtained by filtering the culture through Whatman GF/C filter paper and centrifuged at 5000 g for 10 min at 5°C. Laccase activity was assayed using ABTS substrate [13]. The enzyme units were expressed as micromole of substrate transformed per minute per liter of culture filtrate i.e. as enzyme units per liter of culture filtrate ($U L^{-1}$). In the absence of the enzyme activity, no increase in the rate of absorbance was observed.

Lignin peroxidase (LiP) activity was determined by measuring the rate of oxidation of veratryl alcohol to veratraldehyde [14]. Manganese-dependant peroxidase (MnP) activity was determined by measuring the rate of oxidation of veratryl alcohol to veratraldehyde in the presence of Mn [15].

2.4. Partial purification and optimization of laccase activity in the culture supernatant

About 500 ml of culture supernatant was concentrated to 10 ml by ultrafiltration using Amicon YM10 membrane (Millipore, USA) at 4°C. An aliquot of the concentrate was passed through an anion exchange column “Resource Q” (Amersham Biosciences, Upssala, Sweden). It was eluted using a NaCl gradient (0.1-1.0 M) at a flow rate of 0.5ml/ min. Fractions of 2 ml were collected during the elution. Fractions showing laccase activity were pooled and concentrated further using nanosep 10K Omega tubes (Paul Corporation, USA). This partially purified enzyme was used for determining the optimum temperature and pH.

The laccase activity was measured at temperatures of 5 to 90°C in an electrically controlled cell holder of a spectrophotometer (Shimadzu, UV-2450, Japan). Laccase activity was estimated at pHs 3 to 11. To obtain pH 3, glycine-HCl buffer (0.2 M), for pH 4 and 5 acetate buffer at 0.2 M concentration, for pH 5, 6 and 7 phosphate buffer (0.2 M), for pH 8 and 9 glycine-NaOH buffer (0.2 M) and carbonate-bicarbonate buffer (0.2 M) for pH 11 were used.

2.5. Effect of salinity on growth and enzyme production

The effect of salinity on growth of the marine fungal isolate was tested by preparing the B & K medium with sea water to give final concentration of 10, 15, 20, 25, 30 and 34 parts per thousand salinity. For determination of mycelial dry weight, duplicate cultures were vacuum-filtered through tarred Whatman GF/C filter papers, rinsed with 100 ml of distilled water, dried to a constant weight, and the cell mass was calculated by difference. Laccase, LIP and MNP activities were measured in the culture supernatants of the fungus grown under different salinities. The average values of duplicate cultures were plotted.

2.6. Enhancement of laccase production by inducers

In order to enhance the laccase production, various inducers like *p*-anisidine, catechol, guaiacol, ferulic acid, vanillic acid, veratryl alcohol and 2,5 dimethyl aniline were used at a final concentration of 1 mM. Cupric sulphate at a

concentration of 2 mM, indulin at 0.25 mg ml⁻¹ and a combination of 2 mM cupric sulphate and 1mM guaiacol were added to B & K medium and grown as shallow stationery cultures at room temperature (28°-30°C). Dyes like trypan blue, aniline blue and remazol brilliant blue R at a final concentration of 0.04%, methylene blue, crystal violet, brilliant green and congo red at 0.02 % and reactive orange 176 at 0.015 % final concentration were tested for their laccase induction properties. Textile effluents as well as molasses spent wash (MSW) from an alcohol distillery and black liquor from a paper mill industry were also screened for their laccase inducing properties, each at the final concentrations of 1,10 and 20 percentage. The values from triplicates were used for comparing the effect of various inducers.

2.7. Decolorization of various effluents and dyes in the culture medium

The fungus was tested for its ability to decolorize various dyes and effluents by monitoring the change in the respective specific absorbance maxima every alternate day after the addition of the dye/ effluent over a period of 6 days. The dye was added to a six day old culture and the time of addition was considered to be the 0 day. The final concentration of the dye in the medium on day 0 was considered to be 100%. The extent of decolorization was recorded as residual color (in percentage). Mean values from triplicate cultures were used for comparing the extent of decolorization of various dyes and effluents. Trypan blue, aniline blue, remazol brilliant blue R, methylene blue, crystal violet, brilliant green, congo red and reactive orange 176 (RO 176) were monitored at their absorbance maxima at 599, 585, 597, 663, 589, 623, 486 and 499 nm respectively. Decolorization of Poly-R 478 was monitored by determining the ratio of absorbance at 513 nm versus 362 nm. (All the dyes were from Sigma Chemicals, USA; RO 176 was a gift from Dr. C. Novotny, Czech Republic). Effluents like textile effluent A, B, MSW and black liquor were also monitored at their absorbance maxima at 505, 663, 663 and 317 nm respectively. Textile effluent A (from Atul Ltd., Gujarat, India) contained mainly Azo dye- 20 and had a pH of 8.9 with 0.34% carbonate. The textile effluent B from the same source contained a mixture of dyes and had a pH of 2.5 with 21700 color units, total

solids 4.2%, Na⁺ 2440 ppm, Ca⁺ 31 ppm, SO₄ 0.6%, Cl 3.2% and PO₄ 0.5%. The dyes in this mixture were reactive blue 140 base, reactive blue 140, reactive blue 160 base, reactive blue 163, reactive red 11, reactive yellow 145, reactive green 19 and reactive blue 4. The black liquor obtained from Seshasayee Paper Mills, Tamil Nadu was from bagasse and wood chip-based newsprint manufacturing unit. As per the data provided by the paper mill, the effluent had COD of 416 mg l⁻¹ and BOD of 190 mg l⁻¹. Raw untreated molasses spent wash used in this study (obtained from Rhea Distilleries Ltd., Goa, India) was dark brown in color. According to the data provided by the unit, the raw MSW had a pH of 4.3, a BOD of 42,000 mg and COD of 80,000 mg L⁻¹.

2.8. Decolorization of effluents and dyes by using the fungus free-culture supernatant

The culture filtrate with maximum laccase activity was used as a source of enzyme to test its efficiency in decolorization of various effluents and dyes. This in brief was carried out by incubating the enzyme with effluents and dyes buffered at pH 6 for 6-12 h at 60°C in duplicates. The percentage of decolorization achieved was calculated with reference to the control samples that were not treated with the enzyme.

2.9. Decolorization of effluents and dyes by using the exopolymeric substances (EPS) produced by the fungus

The exopolymeric substance (EPS) of the fungus was prepared as follows: 1 litre of frozen culture filtrate was allowed to thaw. The EPS precipitated on thawing was removed by decantation. Methanol at a final concentration of 70% was added to the supernatant to further precipitate the remaining EPS from the culture supernatant. The precipitated EPS was collected by centrifugation and lyophilized and the dry weight determined. Carbon, nitrogen and sulfur ratio was analyzed in CNS analyzer (Model NCS 2500/S.No.980813, Thermoquest Italia, S.P.A.) using MAG-1 and Sulphan standards. Various dyes and effluents were added to 10 mg of EPS in phosphate buffer at pH 6 and incubated at 60°C in duplicates. The decolorization was monitored at 6 and 12 hours at the

absorbance maxima specific to that particular dye/ effluent. Dyes and effluents without addition of EPS were used as control.

3. Results

Out of 40 fungi isolated from decaying mangrove wood, 3 fungi showed positive reaction for laccase activity when grown in the presence of guaiacol and ABTS. One isolate designated NIOCC #2a used for the present work was deposited in the Microbial Type Culture Collection (MTCC, Chandigarh, India) under the accession number MTCC 5159 under the Budapest treaty for patent culture deposition [16]. Based on partial large subunit rRNA (D2) gene (~350 bp) alignment with GenBank database, it was shown to have 99% homology to an unidentified basidiomycete species AY187277 (MIDI LABS Inc, Newark, USA). As the fungus does not produce spores, its further identity is not possible. Using the ITS sequencing also it showed homology to an unidentified basidiomycete. The sequencing data of ITS and D2 region are deposited in the GenBank under the accession No. AY 939879 and AY 939878 respectively.

The salinity in the mangrove environment fluctuates from 5-35 parts per thousand (ppt). Therefore the effect of various salinities on growth of the fungus and production of lignin-degrading enzymes LIP, MNP and laccase were determined. The results showed that the maximum biomass of the fungus was produced at 34 ppt salinity, whereas laccase production was best at 25 ppt (Figs. 1a and 1b). The production of MNP and LIP by this fungus was negligible and this was further inhibited by sea water at all the salinities.

Among the various inducers used, CuSO_4 and guaiacol induced the maximum laccase production, individually as well as in combination (Table 1). Among the various dyes, brilliant green induced good laccase activity (Table 2). Trypan blue, methylene blue, remazol brilliant blue R, Poly R and crystal violet did not induce laccase activity as the other dyes (Table 2). Among the effluents used, textile effluent B at 1% concentration induced the highest laccase activity (Table 1), the other effluents stimulated moderate increase in laccase production. The partially purified laccase showed highest activity at pH 3 and 6 and at 60°C.

The dyes that acted as inducers of laccase production in the culture medium were in turn decolorized by the enzyme produced. Brilliant green was decolorized to the maximum by day 4 whereas RO 176 was least decolorized (Fig. 2a). Among the synthetic blue dyes, aniline blue was decolorized almost totally by day 4 (Fig. 2b). Poly R and crystal violet did not induce high laccase activity and their decolorization was also very low (Figs. 2a). On the other hand trypan blue, methylene blue and RBBR were decolorized by 60-70% but they did not induce laccase activity (Table 2). About 60 % decolorization of black liquor (used at 10 % concentration) from paper and pulp mills was achieved by day 6 (Fig. 2c). Molasses spent wash from distillery waste when used at 10 % concentration was totally decolorized by day 6 (Fig. 2c). Textile effluent B at this concentration was decolorized by 60% on day 2 and no further reduction in color was observed (Fig. 2c).

The fungus free-culture supernatant with laccase activity (18 U ml^{-1}) when incubated with various dyes at pH 6 and 60°C showed about 79% decolorization of brilliant green dye within 12 h of incubation (Table 3) and about 71 % of the color was removed from 10% solution of black liquor within 6 h (Table 3).

The fungus produced 2.3 g exopolymeric substance (EPS) L^{-1} of the culture medium. Analysis of EPS in cultures grown with 1% glucose showed CNS ratio of 4.5: 0.76: 10 respectively and therefore appeared to be a sulphated polysaccharide. We determined the efficacy of this in decolorization of various dyes and effluents. Almost total decolorization of some of the dyes and effluents was noticed within 24 h of incubation with 10 mg of EPS (Table 3).

4. Discussion

This is the first report wherein high amount of laccase (921 U L^{-1}) is reported to be produced by a marine fungus NIOCC # 2a when grown in seawater medium with peptone as a nitrogen source. On addition of 2 mM copper sulphate to this medium, ~ 100 -fold increase in laccase production (83619 U L^{-1}) was achieved. Besides, addition of aromatic compounds such as p-anisidine, catechol, guaiacol, ferulic acid, vanillic acid and veratryl alcohol also induced laccase production.

Several of the dyes tested for decolorization in fact induced laccase production. This is also the first report where textile effluent at low concentration (1%) increases laccase production by ~100-fold. Other effluents such as black liquor from paper and pulp mill and molasses spent wash from alcohol distillery also induced laccase production when used at a concentration of 1% (V/V). Using batch culture of the fungus in B & K medium with peptone and glucose as nitrogen and carbon source and 1% textile effluent we could routinely obtain laccase activity of ~80 U ml⁻¹. This is much higher than those reported in terrestrial fungi recently [17, 18, 19, 20].

The optimum pH for laccase activity reported so far in majority of the fungi is between 3-5 [11, 18, 19, 20]. Partially purified laccase of NIOCC # 2 showed biphasic pH curve having maximum activity at pH 3.0 and 6.0. The enzyme peak at pH 3.0 cannot be due to MnP activity as the enzyme assay was done without addition of Mn and H₂O₂ [18]. Optimum temperature of 60°C is reported in terrestrial fungi *Botrytis cinerea*, *Fomes fomentarius* and *Chaetomium thermophile* and a mesophilic white-rot fungus [18 and references therein]. Laccase active at high temperature may find potential application in treatment of heated industrial effluents.

Most of the dyes and effluents added to the culture medium were simultaneously decolorized and also acted as inducers. Among these, the textile effluent B containing 8 reactive dyes induced maximum laccase production. Its acidic nature (pH of 2.5) might induce more laccase activity as the laccase activity of this fungus showed peak at pH 3.0. The textile effluent A on the other hand with its alkaline pH of 8.9 induced much lower laccase activity and was decolorized to a much lesser extent (Table 1 and Fig. 2c). Similar high production of ligninolytic enzymes during treatment of paper mill effluents was reported in *Trametes versicolor* [21].

The mechanism of laccase-catalysed dye decolorization can differ depending upon dye structure. The white-rot fungus *Trametes versicolor* was shown to use anthraquinone dyes as enzyme substrates that were oxidized by its

laccase whereas decolorization of azo and indigo dyes depended upon metabolites having small molecular weights secreted in the culture medium [22]. These small molecular weight metabolites mediated the interactions between the dyes and the enzyme. Thus, the decolorization rate of the non-substrate dyes was limited by the concentrations of mediating compounds than laccase activity in the culture filtrate [22]. In our studies we found that the synthetic dyes, brilliant green and aniline blue induced the highest laccase production and were also decolorized much more than other dyes (see Table 2; Figs. 2a and 2b). Poly R and crystal violet induced lowest laccase activity and their decolorization was also lower than all the other dyes (see Table 2 and Fig. 2a). On the other hand, reactive orange (RO 176), an azo dye induced laccase activity moderately but its decolorization was much lower than the other dyes (compare Table 2 and Fig. 2a).

Presence of LiP and MnP, the other lignin-degrading enzymes play an important role in decolorization of dyes. In NIOCC # 2a, laccase was the major lignin-degrading enzyme as is reported in *Pycnoporus cinnabarinus* [23] *Phlebia tremellosa* [24] and *Pleurotus sojarcaju* [25]. Poly R and crystal violet are generally decolorized by white-rot fungi producing MnP and LiP [19]. Decolorization of textile dye industrial effluents by white-rot fungus producing laccase as the major lignin-degrading enzyme as seen in our culture was reported in the white-rot fungus *Clitocybula duseinii* [26].

Decolorization of several dyes and effluents was achieved within 6-12 h by incubating the culture supernatant (without the fungal biomass) containing laccase enzyme (Table 3). However, on incubation of the culture supernatant with textile effluent A and B, reduction in color was seen in the initial 2 h after which there was no further reduction in color. Addition of redox mediators may improve this situation as was reported in the case of pure laccase from a commercial formulation [27]. The exopolymeric substance produced by the fungus was more efficient in decolorization of most of the dyes and the effluents than the culture supernatant (Table 3). Even RO 176, Poly R 478 that were not decolorized when added to the culture medium were totally decolorized by the

EPS. This might be due to the combined effect of adsorption and enzymatic decolorization. The EPS produced by white-rot fungi is reported to play an active role in lignin degradation [28].

In conclusion, the unidentified marine basidiomycetous fungus NIOCC # 2a decolorized several synthetic dyes when added to the growing fungal culture medium or cell-free culture supernatant containing laccase or EPS precipitated from the culture medium. It decolorized textile effluents, black liquor from paper and pulp mill and molasses spent wash from alcohol distillery in the presence of sea salts. The fungus grew and produced laccase in sea water medium of 25 parts per thousand containing 1% textile effluent B. The synthetic dyes and textile effluent acted as laccase inducers when added to the growing culture of the fungus. The fungus with biphasic pH optima and temperature optimum of 60°C thus appears to be a good candidate organism for industrial application in bioremediation of colored waste waters in the presence of chlorides and sulphates.

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TABLE 1. Enhancement of laccase production by various inducers and effluents.

LACCASE ACTIVITY (UL ⁻¹)							
INDUCERS	DAYS						
	3	6	9	12	15	18	21
B & K medium (control)	0	0	404 ± 95.5	166 ± 21.7	158 ± 58.6	921 ±166.7	838 ±286
<i>p</i> -Anisidine (1mM)	73 ±8.3	92 ±60	32 ±13.3	35 ±23.6	24 ±10.9	1200 ±250.8	1351 ±323.2
Catechol (1mM)	21 ±6.4	36 ±2	53 ±27.3	27 ±25.3	27 ±1.1	1246 ±109	1675 ±78.5
Copper sulphate (2mM)	16 ±2.5	197 ±67.2	185 ±104.8	119 ±60.2	36571 ±30.7	42933 ±76.8	83619 ±134
Guaiacol (1mM)	0	85 ±41.3	979 ±132	384 ±51.6	344 ±196.3	1258 ±904.7	21112 ±961.8
Copper sulphate (2mM) & Guaiacol (1mM)	36 ±6.5	485 ±88.3	14078 ±970.9	21347 ±447.8	44457 ±273	45257 ±30.3	46781 ±64.9
Ferulic Acid (1mM)	9 ±4.9	70 ±48.5	7502 ±255.8	1407 ±1280.1	1755 ±721	873 ±377	780 ±142
Indulin (0.25%)	0	63 ±29.9	79 ±9.6	66 ±32	62 ±29	894 ±50.6	1174 ±10.6
Vanillic acid (1mM)	75 ±8.6	265 ±47	68 ±30.5	48 ±18.8	32 ±14.9	1599 ±493.4	1359 ±172.8
Veratryl alcohol (1mM)	14 ±5.1	112 ±14.9	768 ±273.6	395 ±33.2	332 ±61.4	1889 ±420.6	1931 ±636.3
2,5 dimethyl aniline (1mM)	0	232 ±30.4	133 ±95.3	177 ±11.8	128 ±43.6	548 ±143.2	633 ±153.2
Textile effluent A (1 %)	25 ±2.2	113 ±59.7	937 ±323.9	440 ±230	404 ±70.9	2036 ±97.8	1886 ±171.5
Textile effluent B (1 %)	7 ±2.6	358 ±66.3	4545 ±503.2	85829 ±71.5	19086 ±255.7	42248 ±116.1	266 ±59.1
Molasses spent wash (1 %)	0	159 ±38	1566 ±83.8	563 ±87.9	505 ±97.2	1756 ±298.1	2075 ±167.3
Black liquor (1 %)	2 ±3.6	139 ±27.1	738 ±123.1	296 ±38.9	268 ±134.1	1299 ±242.1	1156 ±79.8

Laccase activity was measured in the culture supernatant using ABTS substrate at pH 3.0.

TABLE 2. Enhanced laccase production by various dyes.

LACCASE ACTIVITY (UL ⁻¹)								
INDUCER	DAYS							
	3	6	8	10	12	14	16	18
Trypan Blue (0.04%)	21 ±4.5	1 ±2.5	149 ±15.2	747 ±103.7	844 ±155.8	897 ±189.5	812 ±125.8	505 ±156.9
Aniline Blue (0.04%)	12 ±4.5	67 ±13.5	21 ±2.1	825 ±67.9	1051 ±118.5	1166 ±178.2	1211 ±101	1048 ±97.8
Methylene Blue (0.02%)	0	51 ±15.9	93 ±16.7	634 ±205.7	783 ±200.3	651 ±189.5	656 ±109.4	666 ±98.9
RBBR (0.04%)	3 ±2.7	43 ±7.9	0	0	0	0	0	0
Crystal Violet (0.02%)	4 ±2.5	104 ±19.9	0	581 ±106.8	825 ±189.5	819 ±111.5	671 ±105.8	745 ±98.6
Brilliant Green (0.02%)	13 ± 8.8	53 ±11.8	0	1852 ±79.6	1821 ±157.9	2271 ±259.6	2191 ±205.2	2156 ±189.3
Poly-R 478 (0.02%)	17 ±2.5	36 ±7.9	0	187 ±15.9	248 ±78.5	241 ±67.9	266 ±56.9	325 ±47.6
Congo Red (0.02%)	5 ±3.3	5 ±2.2	115 ±12.9	955 ±12.9	966 ±89.9	1363 ±126.9	1389 ±187.6	1241 ±198.4
RO 176 (0.015%)	21 ±10.2	8 ±3.3	335 ±57.9	949 ±112.3	904 ±89.9	1158 ±89.9	1108 ±125.9	1180 ±118.6

Laccase activity was measured in the culture supernatant using ABTS substrate at pH 3.0.

TABLE 3. Decolorization of dyes and effluents using fungus free-culture supernatant and EPS produced by the fungus.

DYE	% decolorization by the culture supernatant		% decolorization by the EPS of the fungus	
	Hours		Hours	
	6	12	12	24
Trypan Blue (0.04%)	22	25	20	79
Aniline Blue (0.04%)	55	40	46	75
Methylene Blue (0.02%)	3	5	4	6
RBBR (0.04%)	67	46	19	100
Crystal Violet (0.02%)	44	54	45	80
Brilliant Green (0.02%)	72	79	2	90
Poly-R 478 (0.02%)	21	43	33	90
Congo Red (0.02%)	54	47	18	29
RO 176 (0.015%)	ND	ND	35	100
Effluents				
Textile effluent A (10 %)	9	11	11	100
Textile effluent B (10 %)	14	22	35	100
Molasses spent wash (10 %)	34	33	12	100
Black liquor (10 %)	71	59	41	100

500 μ l of culture supernatant having 18 U ml⁻¹ laccase activity was incubated with 500 μ l of dye solution at pH 6.0 and 60°C. The absorbance was measured at appropriate wavelengths to calculate the % of decolorization after 6 and 12 hours. 10 mg of freeze-dried EPS of the fungus was incubated with dye solutions prepared in phosphate buffer pH 6.0 at 60°C. Decolorization was measured at 6 and 12 h at the absorbance maxima specific to the dye and effluents. The % decolorization was calculated based on the initial readings. All the values are mean of 2 replicates.

Legends to the figures

Fig. 1a. Fungal biomass (dry weight in mg) in 20 ml of B & K medium at various salinities. The values are mean of 2 replicates.

Fig. 1b. Laccase activity in the culture supernatant of the fungus grown in B&K medium of different salinities. The values are mean of 2 replicates.

Fig. 2a. Decolorization of synthetic dyes measured as percentage of residual color with reference to untreated dyes. The results are shown as percent residual color with reference to the color measured immediately after addition of the dyes to the fungal culture (day zero).

Fig. 2b. Decolorization of some more synthetic dyes by the fungus measured as percentage of residual color with reference to untreated dyes. The values represent mean of 3 replicates. The SD values were below 5% in all the cases.

Fig. 2c. Decolorization of paper mill black liquor, molasses spent wash from alcohol distillery and textile mill effluent A and B used at 10 % concentration. The values represent mean of 3 replicates. The SD values were below 5% in all the cases.

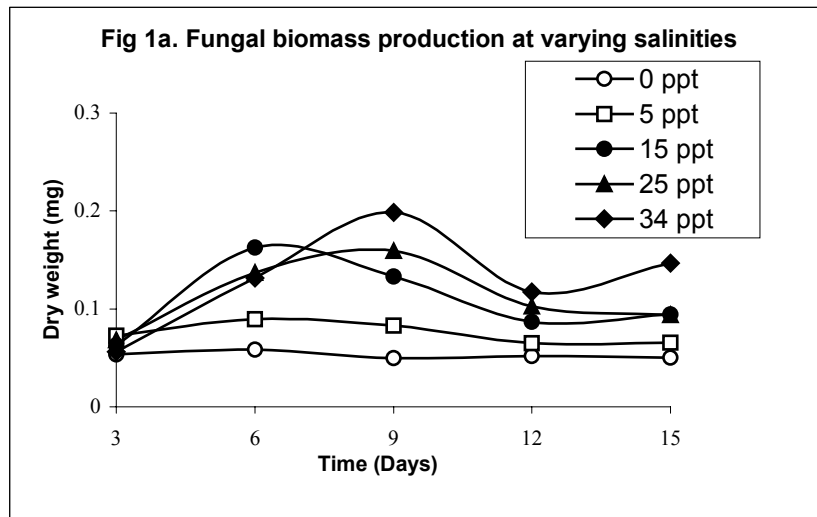


Fig. 1a. Fungal biomass (dry weight in mg) in 20 ml of B & K medium at various salinities. The values are mean of 2 replicates.

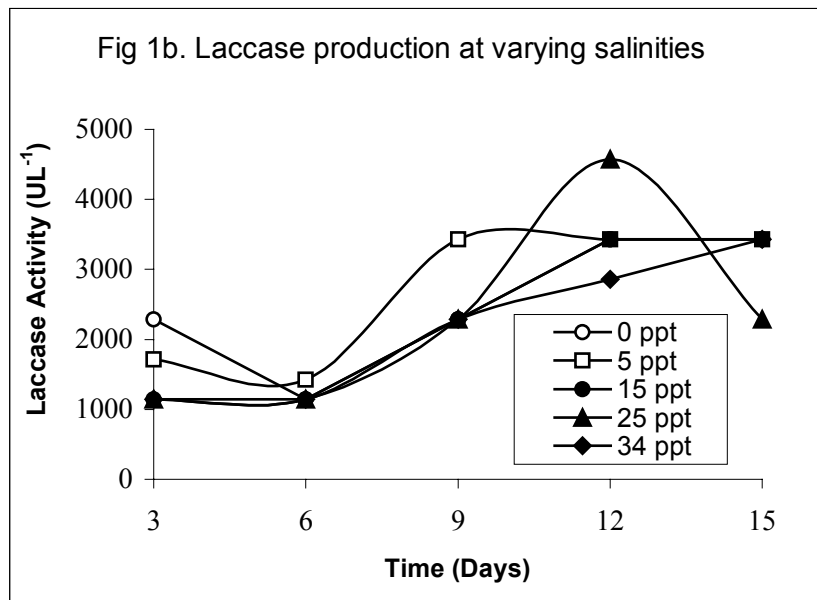


Fig. 1b. Laccase activity in the culture supernatant of the fungus grown in B&K medium of different salinities. The values are mean of 2 replicates.

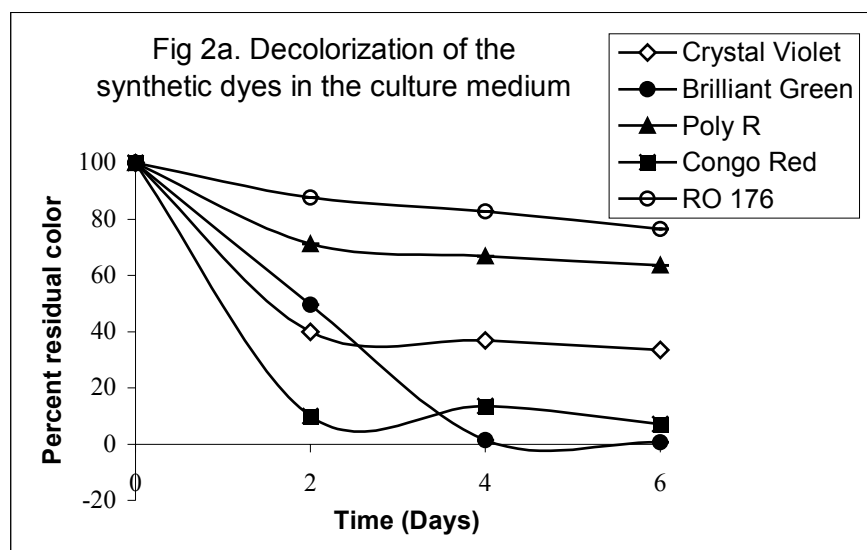


Fig. 2a. Decolorization of synthetic dyes measured as percentage of residual color with reference to untreated dyes. The results are shown as percent residual color with reference to the color measured immediately after addition of the dyes to the fungal culture (day zero).

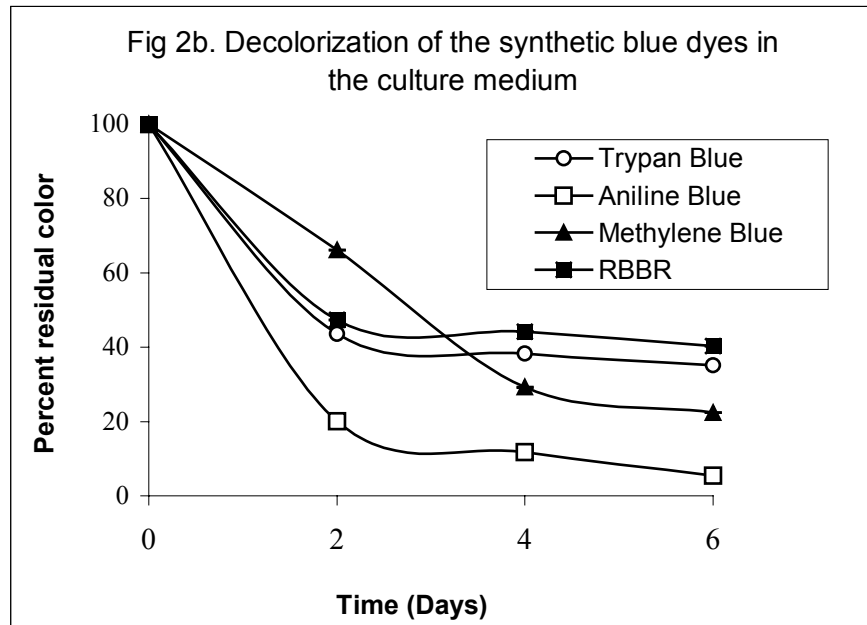


Fig. 2b. Decolorization of some more synthetic dyes by the fungus measured as percentage of residual color with reference to untreated dyes. The values represent mean of 3 replicates. The SD values were below 5% in all the cases.

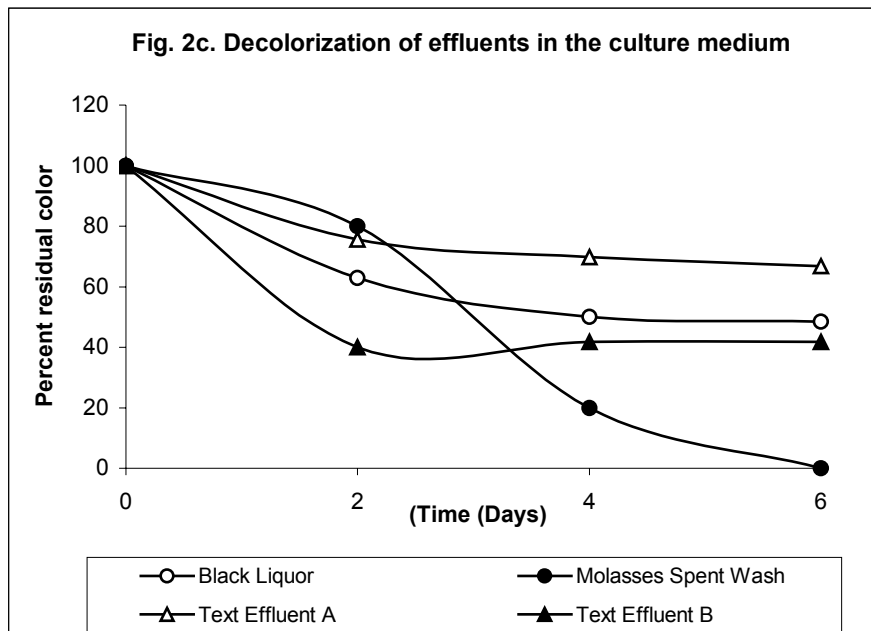


Fig. 2c. Decolorization of paper mill black liquor, molasses spent wash from alcohol distillery and textile mill effluent A and B used at 10 % concentration. The values represent mean of 3 replicates. The SD values were below 5% in all the cases.