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RESEARCH NOTE

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Laccase Activity and Azo Dye Decolorization Potential of *Podoscypha elegans*

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ABSTRACT

Azo dyes containing effluents from different industries pose threats to the environment. Though there are physico-chemical methods to treat such effluents, bioremediation is considered to be the best eco-compatible technique. In this communication, we discuss the decolorization potentiality of five azo dyes by *Podoscypha elegans* (G. Mey.) Pat., a macro-fungus, found growing on the leaf-litter layer of Bethuadahari Wildlife Sanctuary in West Bengal, India. The fungus exhibited high laccase and very low manganese peroxidase activities under different culture conditions. Decolorization of five high-molecular weight azo dyes, viz., Orange G, Congo Red, Direct Blue 15, Rose Bengal and Direct Yellow 27 by the fungus was found to be positive in all cases. Maximum and minimum mean decolorization percentages were recorded in Rose Bengal (70.41%) and Direct Blue 15 (24.8%), respectively. This is the first record of lignolytic study and dye decolorization by *P. elegans*.

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Azo dyes represent up to 70% of dyestuffs used in textile and other industries [1]. In textile dyeing process, 280,000 tons of unbound xenobiotic dyes are discharged annually as wastewater [2]. Colored components are undesirable in water and most of the synthetic dyes being toxic, mutagenic, and carcinogenic and thus, can impart serious environmental hazard [3]. The suspended dyes in water bodies influence the aquatic ecosystem [4], pose public health risks due to bioaccumulation and cause soil contamination [5]. In addition, the aromatic amines, which are the reduced intermediates of azo dyes, are more toxic than the dyes themselves [6]. Due to cost effectiveness and eco-compatibility, bioremediation of such high-molecular weight xenobiotic dyes has come up as a promising alternative technology over the conventional physicochemical management practices [7,8].

In the last decade, some authors have argued the effectiveness of soil and water bioremediation potentialities of litter-decomposing fungi (LDF) over the widely studied white rot fungi (WRF) [9,10]. The LDFs naturally inhabit the soil-litter layers where they co-exist and compete with other microorganisms and possess similar lignolytic activity as that of the WRFs and can effectively utilize different high-molecular weight dyes from both soil and liquid conditions *in vitro* [9,11].

With this rationale, in this present study, we investigated the efficacy of *Podoscypha elegans*, a LDF, against five high-molecular weight azo dyes (Table 1) used widely in textile industries. This is the first report of the lignolytic activity and dye decolourization potentiality of the fungus.

Podoscypha elegans (G. Mey.) Pat. was collected from the litter bed of Bethuadahari Wildlife Sanctuary forest, Nadia and cultured in Potato Dextrose Agar supplemented with ampicillin 100 mgL^{-1}, nystatin 50 mgL^{-1} and incubated at 28 ± 2 °C for 5–7 days. The fungus was identified previously by the present authors [12].

Lignolytic activity was initially assayed in five growth media, viz. Kirk's medium (KM) [13], HNHC medium [14], mineral salts medium (MSB) [15], modified PDB (M-PDB) medium [16], and low-nutrient PDB (LN-PDB) medium [16]. Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase activities were measured spectrophotometrically using a spectrophotometer [17–19]. Enzyme activity was expressed in nanokatals per milliliter (nkat/ml). The fungus was grown in flasks containing 100 ml of respective growth medium for 7, 14, 21 and 28 days at 28 ± 2 °C.

Laccase activity was recorded in all the media studied while low activity of MnP was recorded only in HNHC, KM and MSB. High fungal growth was recorded in nutrient rich M-PDB and LN-PDB, low and slow growth was observed in the other three low-nutrient starvation media (Figure 1). HNHC was found to be the best inducer of laccase followed

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Table 1. Details of the different dyes.

No	Dye and their abbreviations	Molecular details	Chemical nature and use
1	Orange G (OG)	452.37 $C_{16}H_{10}N_2Na_2O_7S_2$ λmax: 482.0 nm	Azo dye used in photography, plastic, paper, textile and wood industry. It is toxic and carcinogenic.
2	Congo Red (CR)	696.7 $C_{32}H_{22}N_6Na_2O_6S_2$ λ_{max} 497 nm	Azo dyes used in fertilizer, pesticides, and optical films, highlighters, textile and is carcinogenic and cytotoxic.
3	Direct Blue 15 (DB)	992.8 $C_{34}H_{28}N_6O_{16}S_4\bullet 4Na$ λmax =610 nm	Mono azo dye and is carcinogenic.
4	Rose Bengal (RB)	1017.46 $C_{20}H_2Cl_4I_4Na_2O_5$ λmax: 545 nm	Azo dyes used in apoptosis assay, biological staining photography, recording industry, etc. The dye is genotoxic and microbial toxic.
5	Direct Yellow 27 (DY)	662.62 $C_{25}H_{20}N_4Na_2O_9S_3$ λmax: 393 nm	Azo direct dye used in textile industry.

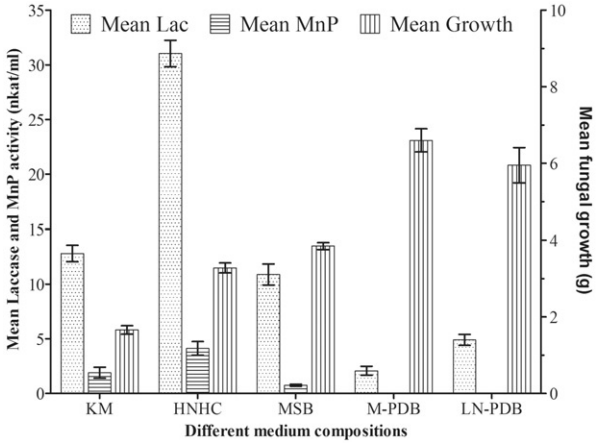

Figure 1. Enzyme activity and fungal growth of *P. elegans* in different media compositions.

by KM and MSB. M-PDB and LN-PDB were found to be poor inducer of laccase activity. No LiP activity was recorded in any of the growth medium studied. Most lignin degrading LDFs produce two main extracellular ligninolytic enzymes, MnP, and laccase [9] and LiP is not common in LDFs.

Podoscypha elegans showed significant laccase activity (31.04 nkat/ml), but a low MnP activity (4.13 nkat/ml) activity in HNHC (Figure 2). Maximum laccase activity in HNHC medium was recorded at 28 days. However, it was observed that though there was a steep increase in the rate of laccase production up to 21 days, the rate of increase decreased after 21 days, possibly because of accumulation of other fungal metabolites in the medium.

Since HNHC medium showed the best result of laccase induction, the different parameters (pH, temperature, rotation speed) and effect of different concentrations of veratryl alcohol (VA) were standardized using this growth medium. A perusal

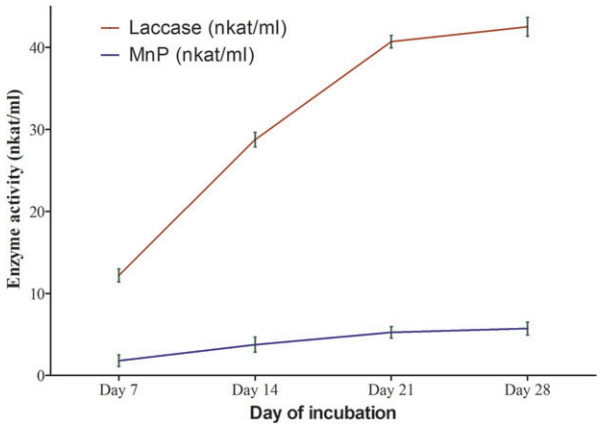

Figure 2. Laccase and MnP activities in HNHC medium at different days of incubation.

of the results indicated that all the four cultural parameters had significant effect on the production of laccase (Figure 3(A–D)). *Podoscypha elegans* produced maximum laccase at a slightly acidic pH 6.5, though significant activity was recorded at a pH range of 5.5–7.5 (Figure 3(A)). Fungal laccases typically exhibit pH optima in the acidic pH range [20]. Not only the rate of oxidation but also the reaction products can differ according to pH as pH may affect abiotic follow-up reactions of primary radicals formed by laccase. The stability of fungal laccases is generally higher at acidic pH [21], although exceptions exist [22]. Laccase stable at elevated pH (8–8.5) is of special importance in bleaching of pulp [23]. In view of this, the laccase activity of *P. elegans* should further be examined in alkaline pH range.

Laccase activity was observed in the 25–35 °C temperature range, the most effective being around 30 °C (Figure 3(B)).Temperature profiles of laccase activity are not different from other extracellular ligninolytic enzymes with optima between 50° and 70 °C [20]. Few enzymes with optima below 35 °C have been described, e.g., the laccase from *Ganoderma lucidum* has its highest activity at 25 °C [24]. Rotation speed was another factor that determined the production of laccase under cultural conditions. Aeration and its circulation (Figure 3(C)) played an important role in boosting laccase activity in comparison to the activity in still cultures. The optimum enzyme activity was noted at rotation speed range of 50–75 rpm.

VA has been reported as a laccase inducer in ascomycete *Botryosphaeria* sp. [25] and in *Trametes versicolor* [26], while it was found to have no effect on laccase production in *Penicillium chrysogenum*, [27]. VA was a positive inducer of laccase production by *P. elegans* (Figure 3(D)). All concentrations, from 0.2 to 0.8 mM, showed enhancement of laccase activity. However, 0.6 and 0.8 mM VA showed the best results.

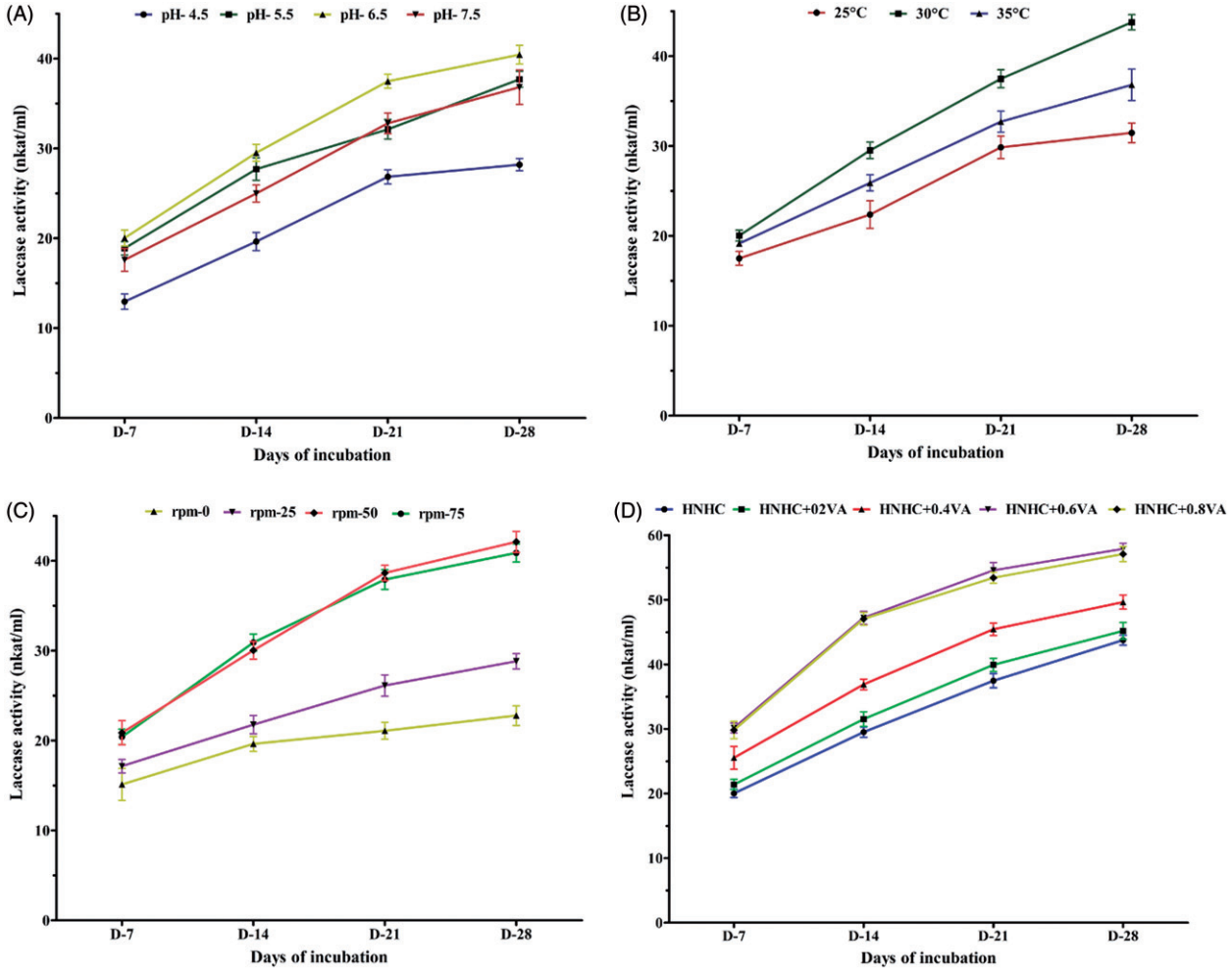

Figure 3. Effect of different parameters on laccase activity in HNHC medium. (A) pH. (B) Temperature. (C) Rotation speed. (D) VA concentrations.

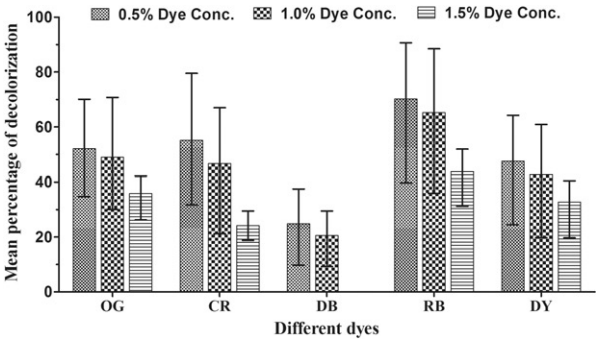

Figure 4. Decolorization percentage of different dyes over 28 days.

Based on these results, the best condition for laccase production was found to be HNHC supplemented with 0.6 mM VA at pH 6.5 and incubation at 30 ± 2 °C at 50 rpm. This condition was thus selected for dye decolorization studies.

To test the decolorization potential, HNHC was selected and the different factors, viz, pH, temperature, rotation speed, and different concentrations of VA, a laccase inducer, were optimized. Decolorization test of five high-molecular weight dyes (Table 1) was carried out in 100 ml of HNHC supplemented with 0.6 mM VA (pH 6.5) and the dyes. Three concentrations (0.5%, 1.0%, and 1.5%) of each dye were tested. Each treatment was inoculated with the fungus and incubated in a shaker incubator (50 rpm) for 28 days at 28 ± 2 °C keeping three replications and uninoculated control sets. Absorbance was measured in spectrophotometer at 7 days interval and the decolorization percentage was determined using the formula:

$$\text{Decolorization\%} = 100 \times \frac{\text{A(ini)} - \text{A(fin)}}{\text{A(ini)}}$$

[Where A (ini) = Absorbance of un-inoculated dye; A (fin) = Final absorbance of dye after inoculation and incubation.]

Two-way ANOVA was performed to test the significance of combination effect of days of incubation and dye concentrations on decolorization, using IBM SPSS Statistics 23. Significance was determined at 5% level.

The results indicated that the different dyes were utilized by *P. elegans* at different rates (Figure 4). The concentration of dye in the medium was crucial for decolourization by the fungus. Thus, the maximum decolorization was observed in the 0.5% concentration treatment for all the dyes (Figure 4).

Increase in concentration affected the process of dye utilization by the fungus. The rate of decolourization was low at the concentration of 1.5% in all dyes and no decolourization was observed where there was poor fungal growth as in Direct Blue 15 (DB).

Rose Bengal (RB) showed the maximum decolorization and the mean decolourization percentages were 70.41, 65.21, and 43.78 at 0.5, 1.0, and 1.5% dye concentrations, respectively. The lowest decolourization (24.8 and 20.52% at 0.5 and 1.0%, respectively) was recorded in DB. No decolorization was recorded in 1.5% experimental sets supplemented with DB indicating that the dye concentration was too high.

The maximum utilization of all the dyes were found between 0 to 21 days. At 0.5% concentration of Congo Red (CR) and Orange G (OG), maximum utilization by the fungus occurred within 14 to 21 days of incubation whereas, while Direct Yellow (DY) utilization was maximum between 7 to 14 days at concentration of 1.5%. These results indicate the fungus utilized the different dyes at different rates depending on their concentration in the culture medium. Both the independent effects of incubation period and initial dye concentration on decolorization were statistically significant ($p < .05$). The interaction of incubation period and initial dye concentration on decolorization was also significant ($p = .001$).

Thus, the present study establishes that LDFs with their lignolytic enzyme resource are capable of replacing their wood rotting kin in soil remediation programs. This is the first report of *P. elegans,* a litter decomposing macro fungus, exhibiting significant laccase activity and efficiently decolorizing high-molecular weight azo dyes under cultural conditions. Moreover, the laccase showed significant activity at higher pH. Thus, soil litter being a natural habitat, *P. elegans* can be used in studies of dye contaminated soil bioremediation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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