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Decolourization of Congo Red by *Hypocrea nigricans* from Forest Litter Layer

SATADRU PRAMANIK AND SUJATA CHAUDHURI*
Department of Botany, University of Kalyani, Kalyani, Nadia, W.B. Pin: 741404.

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Soil and water body contamination by high molecular weight dye effluents is a major concern worldwide. Congo Red (CR), a high molecular weight diazo dye, is extensively used in textile industries as well as in biological laboratories. In the present work fungi mediated decolourization of CR by *Hypocrea nigricans* collected from the forest litter bed of Bethuadahari Wildlife Sanctuary, Nadia, West Bengal was studied. Positive and significant decolourization (61.7%) was observed in liquid growth media supplemented with CR.

Key words: Ascomycetous Fungus, bioremediation, laccase, manganese peroxidase, textile dye,

INTRODUCTION

Dye contamination of soil and water bodies is a major concern for the environmental scientists. Dye waste water from textile and dyestuff industries is one of the most difficult industrial waste waters to treat. The waste water from these industries is characterized by high alkalinity, biological oxidation demand, chemical oxidation demand, total dissolved solids with dye concentrations generally below 1 g/dm3 (Kaushik and Malik, 2009). The synthetic origin and complex aromatic structures of dyes make them stable and difficult to be biodegraded (Fewson, 1998; Seshadri *et al.*, 1994). Dye waste water is usually treated by physical or chemical treatment processes including flocculation combined with flotation, electroflocculation, membrane filtration, electrokinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation, and katox treatment method involving the use of activated carbon and air mixtures (Banat *et al.*, 1996). But these technologies are not only ineffective in color removal but also expensive and less adaptable to a wide range of dye wastewaters (Banat *et al.*, 1996). Thus, development of efficient and environmentally friendly technologies to decrease dye content in wastewater to acceptable levels at affordable cost is of utmost importance (Couto, 2009) and the need of the hour. Biological methods are generally consid-

*Email : sujatachaudhuri@gmail.com

ered environment friendly as they can lead to complete mineralization of organic pollutants at low cost (Pandey *et al.* 2007). It is now known that several microorganisms, including fungi, bacteria, yeasts, and algae, can decolorize and even completely mineralize many azo dyes under certain environmental conditions (Pandey *et al.* 2007). The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages; the process is relatively inexpensive, the running costs are low, and the end products of complete mineralization are not toxic (Forgacs *et al.* 2004). As a best alternative, much interest is now focused on biodegradation of dyes (McMullan *et al.* 2001; An *et al.* 2002) and, thus, bioremediation may be the most effective method of treating industrial dyes wastewater (Nozaki *et al.* 2008).

Congo Red (CR) is a high molecular weight dye that is extensively used in textile dying industry. It is also used in some biological staining compositions. The molecular formula of CR is $C_{32}H_{22}N_6Na_2O_6S_2$ and the molecular weight is 696.663219. CR is carcinogenic as well as mutagenic in nature (Afkhami and Moosavi, 2010; Zvezdelina and Nedyalka 2012). Chemically CR (1-Naphthalenesulfonic acid, 3, 3'-(4, 4' biphenylene bis (azo) bis 4-amino) di sodium salt) is a benzedene based diazo dye, known to metabolize to benzedene, a known human carcinogen (Sharma and Janveja, 2008).

In the present work, the CR utilization capacity of *Hypocrea nigricans* (an ascomycetous fungus) collected from the forest soil-litter bed of Bethuadahari Wildlife Sanctuary, Nadia, West Bengal has been investigated.

MATERIALS AND METHODS

Standard methods for isolation, lignolytic enzyme assay, dye decolourization and molecular identification were followed as-

The fungus was collected from the forest litter layer and cultured in a growth medium in the laboratory. Decomposed leaf samples colonized by whitish fungal mycelium were cut into small pieces; surface sterilized with 70% alcohol, washed thoroughly and placed in Petri plates plated with potato dextrose agar (HiMedia) supplemented with 0.5% streptomycin. The plates were incubated at ±280C for 4 to 7 days. The fungus was purified and maintained in PDA slants at ± 40C.

Enzyme assay for lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase was done in HNHC medium (Katagiri *et al.* 1995) after varying periods of incubation (7, 14, 21 and 28 days). An aliqust of 10 ml of HNHC medium in culture vessel was inoculated with 1mm young growing fungal mycelial disc and incubated at ± 28^{0}C in a shaker incubator (30 rpm). The increase in growth of the fungus in HNHC medium was calculated following the measurement of the dry weight of the fungal mat at the end of the incubation period. The experiment was conducted in triplicate. Laccase activity was measured by the method of Niku-Paavola, (1990). LiP and MnP assays were done by the methods of Tien and Kirk (1988) and Glenn and Gold (1985) respectively.

An aliquot of 10 ml of CR (1%) supplemented low nutrient PD (12 g/l, Hi Media) broth was inoculated with 1mm diameter of young growing fungal mycelium. The cultures, along with un-inoculated dye containing medium, were placed in a shaker incubator (30 rpm) at ± 28^{0}C. The cultures were incubated up to 28 days. For decolourization studies, 5 ml aliquot of the decolorized culture broth was collected and centrifuged (3000 rpm) for 5 minutes to remove any fungal mycelium. The supernatant was taken and analyzed spectrophotometrically at 497 nm. The uninoculated medium with CR dye was served as blank. The experiment sets were replicated thrice. Percentage of decolourization was measured using the following formula (Parshetti *et al.* 2007):

$$\text{Congo Red dye decolourization \%} = \frac{\text{Initial Absorbance of dye} - \text{Final Absorbance of dye} \times 100}{\text{Initial Absorbance of dye}}$$

Culture of the fungus was sent to XCeleris India for Molecular identification based on 18S rDNA region sequencing. Forward and reverse DNA sequencing reaction of PCR amplicon were carried out and a consensus sequence of 855 bp of the 18S rDNA region was generated using aligner software. Finally the consensus sequence was used to carry out BLAST with the NCBI genbank database.

RESULTS AND DISCUSSIONS

The growth rate of the fungus (Fig. 1) and the enzyme activity in HNHC media (Fig. 2) were mea-

sured for up to 28 days. The results indicated low increase in growth of the fungus (0.12 g) but a significantly high amount of laccase activity (50.08 nano katal), after 28 days. Laccase activity showed maximum rate of increase between 14th and 21st

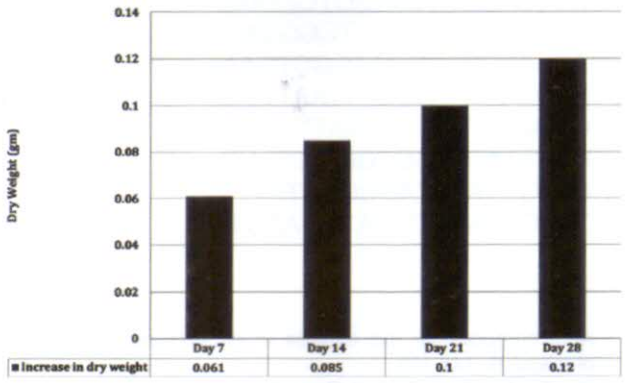

Fig. 1 : Increase in dry weight of *H. nigricans* in HNHC medium after incubation for different periods of time

days. Though the activity continued to increase after the 21st day, the comparative rate of increase was lower than during the former period. MnP activity of the fungus in the medium was found to be low (3.5 nano katal, on 28th day). No MnP activity was recorded on 7th day. This indicated that MnP production started after the 7th day. LiP activity was not recorded in culture condition.

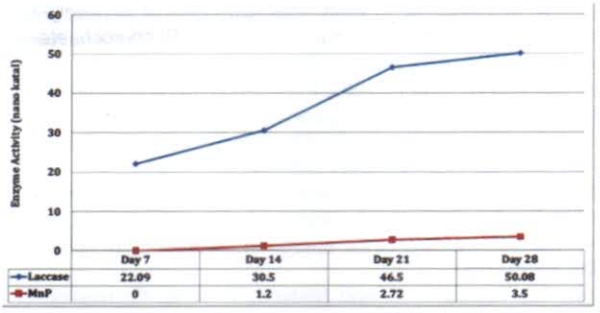

Fig. 2 : Activity of Laccase and Manganese peroxidase of *H. nigricans* in HNHC medium after incubation for different periods of time.

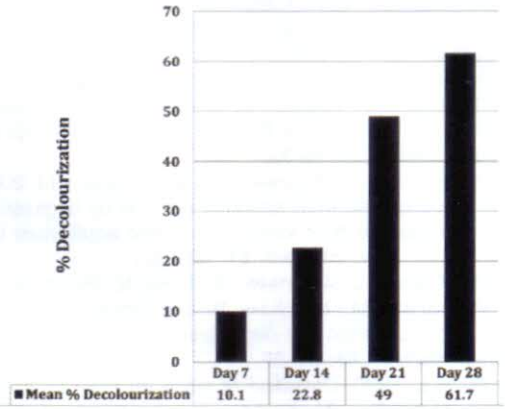

Fig. 3 : Mean decolourization percentage of the dye by *H. nigricans* at different periods of incubation in low PDB medium supplemented with 1% Congo Red.

A perusal of the data presented in Fig 3 indicated that maximum decolourization was recorded in cultures incubated for 28th day (61.7%), however, the maximum rate of decolourization was found between 14th and 21st days. This period correlated with the maximum rate of activity of laccase. Ligninolytic activity appeared to be a secondary metabolic process and it was repressed by nutrient nitrogen and occur red only after the nitrogen in the cultures had been consumed (Glenn and Gold, 1983). The present results showed that the rate of increase in laccase activity and the rate of dye utilization were high after a fortnight of incubation possibly suggesting the utilization of nitrogen source in the medium during the early period of incubation. Depletion of nitrogen in the medium might have been the cause of increased rate of enzyme activity which resulted in the decolourization of the dye. There was an appreciable reduction in the colour intensity of the dye (Fig. 4) in comparison with the untreated dye in the medium.

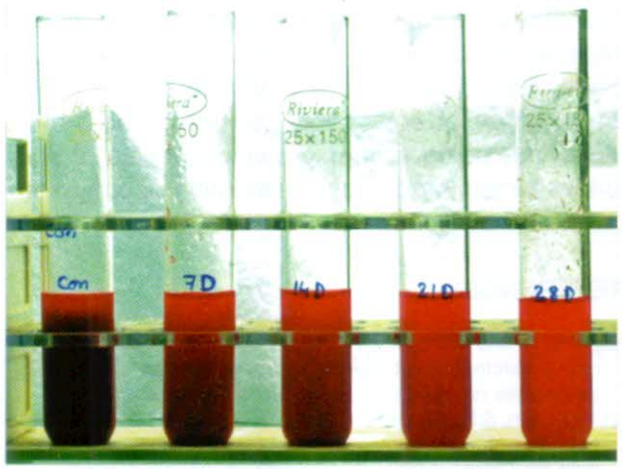

Fig. 4 : Change of colour intensity of Congo Red supplemented medium (against the control) after different time period of incubation (7 Days, 14 Days, 21 Days and 28 Days) with *H.*

The 18s rDNA sequencing data gave a consensus sequence of 855 bp as follows: The sequence analysis confirmed the fungus to be *Hypocrea nigricans* strain NBRC 31290 (GenBank Accession Number: JN941677.1) based on nucleotide homology and phylogenetic analysis.

CGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCG
TAGTTGAACCTTGGGCCTGGCTGGCCGGTCCGCCTCACCGCGTGCACTGGTCCGGCC
GGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGA
CTTTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTATGCTCGAATACATTAG
CATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTTTCTAGGACCGCCG
TAATGATTAATAGGGACAGTCGGGGGCATCAGTATTCAATTGTCAGAGGTGAAATT
CTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATT
AATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAAC
CATAAACTATGCCGACTAGGGATCGGACGATGTTACATTTTTGACGCGTTCGGCAC
CTTACGAGAAATCAAAGTGCTTGGGCTCCAGGGGGAGTATGGTCGCAAGGCTGAAA
CTTAAAGAAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGA
CTCAACACGGGGAAACTCACCAGGTCC AGACACAATGAGGATTGACAGATTGAGAG
CTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGAT
TTGTCTGCTTAATTGCGATAACGAACGAGACCTTAACCTGCTAAATAGCCCGTATT
GCTTTGGCAGTACGCCGGCTTCTTAGAGGGACTATCGGCTCAAGCCGATGGAAGTT
TGAGGCAATAACA

In the past years both physico-chemical and biological degradation studies have been carried out with CR (Chatterjee *et al* 2007; Gharbani *et al.* 2008; Lachheb *et al* 2002; Mall *et al.* 2005; Zhang *et al.* 2007; Tatarko and Bumpus 1998; Cripps *et al.* 1990; Gopinath *et al.* 2009). *Phanerochaete chrysosporium* has been reported to utilize CR extensively in agitated cultures (Tatarko and Bumpus, 1998). *Aspergillus niger* is another potent fungus that has been reported to absorb CR (Fu and Viraraghavan, 2002).

The present study indicates that *H. nigricans* is capable of utilizing congo red under cultural condition. Hence the fungus could be used for biodegradation of congo red contaminated water. Moreover, being a litter growing species, it may further be explored in bioremediation of congo red contaminated soil areas.

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