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Isolation and Evaluation of Fungal Strains from Textile Effluent Disposal Sites for Decolorization of Various Azo Dyes

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ABSTRACT

The potential of 10 indigenous fungi isolated from soil samples of dye disposal sites was evaluated to decolorize textile azo dyes *viz*., Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B. In pure culture, it was observed that *Humicola insolens*, *H. brevis*, *Aspergillus terrus*, *A. flavus*, *A. niger* and *Rhizopus* sp. were efficient in decolorizing textile dyes. The study also depicted that *Rhizopus* sp. was highly efficient in decolorizing (81.01%) a mixture of 5 dyes used in the present investigation followed by a fungal consortium consisting of all the ten fungal strains (78.73%). Recalcitrant dye yellow M4G was also efficiently degraded by fungal consortium (55.76%) compared to pure cultures. This study reinforces the potential of indigenous adapted fungal consortia for the decolorization of textile effluents.

Keywords: decolorization, fungal consortia, indigenous fungi, recalcitrant, textile dyes

INTRODUCTION

Synthetic dyes are manufactured and consumed annually in large quantities in textile, food processing, paper and pulp, cosmetics and pharmaceutical industries. The textile industries accounts for two-third of the total dye stuff market. Dyes have been used increasingly in textile and dying industries because of their ease and cost effectiveness in synthesis, firmness and variety in colour compared to that of natural dyes. The most commonly used dyes in the textile industries are azo dyes *e.g.*, Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B.

Approximately 100,000 commercial dyes are manufactured (Zollinger 1991) and about 10,000 dyes with an annual production of over 7×10^5 metric tons are commercially available (Bajpai and Jain 2010). About 10-15% of dyes used in textile industries remains unutilized (Selvam *et al.* 2003; Wesenberg *et al*. 2003). Dyes are designed in such a way that they are resistant to light, water and oxidizing agents and, therefore, most dyes cannot be treated by conventional physical and chemical processes. Dye colours are visible in water concentration as low as 1 mg/l, whereas textile processing waste water, normally contains more than 10-200 mg/l of dye concentration (Hawkes *et al.* 1999) resulting in aesthetic problem, affecting photosynthesis in aquatic plants (McMullan *et al.* 2001) and have toxic and carcinogenic effect in mammals (Chung and Stevens 1993). Removal of dyes from the effluents or their degradation before discharge is a great environmental challenge for the industries (Baldrin and Gabriel 2003). Various physicochemical and biological processes are usually employed to remove these dyes before discharge into the environment.

Microbial communities are of primary importance in biodegradation of dye contaminated soils and water as microorganisms alter dye chemistry and mobility through reduction, accumulation, mobilization and immobilization. Among microorganisms, bacteria are most commonly used for various bioremediation processes. White rot fungi such as *Phanerochaete chrysosporium* has been used extensively for decolorization of dyes in wastewaters (Krik *et al.* 1992) and is correlated with the ability to synthesize lignin degrading exoenzymes such as lignin and manganese peroxidases (MnP) (Koichi *et al*. 2003; Sharma *et al*. 2009) or Laccases (Rodriguez *et al.* 1999; Murugesan *et al*. 2007). *Trametes versicolor* and *Bjerkandera adusta* degrade nickel based dyes, phthalocyanine, and copper containing azo-dye (Heinfling *et al*. 1997). There are several reports describing the degradation of azo dyes using monocultures (Bhatt *et al*. 2005; Kalyani *et al*. 2008; Telke *et al*. 2010; Kaushik and Malik 2011) or microbial consortia (Khehra *et al*. 2005; Moosvi *et al*. 2007).

The present investigation reports the ability of 10 fungal species for decolorization of five (Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B) frequently used textile dye besides isolation, enumeration and identification of indigenous fungal flora from dye waste disposal sites.

MATERIALS AND METHODS

Media and chemicals

The textile dyes (Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B) used for the decolorization in the present investigation was a gift from M/s Mayur Paliwal Textiles, Panipat (Haryana). All media components and chemicals used in the present study were of analytical grade and purchased from Hi-Media Laboratories (Mumbai, India).

Sampling

Soil samples and waste water samples from Mayur Paliwal Textiles (MPT), Orien Dye House (ODH) and Bharat Dying Unit (BDU), Panipat, Haryana state were collected in sterile polypropylene bags and glass bottles respectively. The soil samples were kept in a refrigerator at 4°C until the fungi were isolated. Waste water samples were immediately processed for physicochemical analysis.

Isolation and identification of dye-degrading fungi

Fungal strains native to the sampled area were isolated on Czapek

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Yeast extract Agar (CYA) by dilution plate technique (Aneja 2007). The following composition of medium was used (all in g L^{-1}) [K_2HPO_4, 1.0; $NaNO_3$, 3.0; KCL, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; Yeast extract, 5.0; Sucrose 30.0; Rose bengal, 0.03; Agar, 15.0]. Fungal strains differing in growth pattern and morphology were isolated and identified using a stereobinocular microscope (Olympus CX 41 RF, Japan) and with the help of taxonomic guides and standard procedure (Gilman 1944; Raper and Thom 1945; Barnett and Hunter 1998; Ellis 1976; Domsch *et al*. 2007). The identified fungal strains were preserved on CYA slants at 4°C in a refrigerator and served as stock cultures. Subcultures were routinely made every 30 to 60 days.

Physiochemical analysis

Temperature, pH, colour and odour of the various wastewater samples were recorded on the spot. Samples collected from the discharge sites were filtered through Whatman No. 1 filter paper and their chemical oxygen demand and biological oxygen demand was determined using standard procedures (Clesceri *et al*. 1989).

Decolorization assay

The ability of fungal strains to decolorize textile dyes was carried out in C-limited Czapek Dox broth (all in g L^{-1}) (sucrose, 5; $NaNO_3$, 2; K_2HPO_4 1; $MgSO_4 \cdot 7H_2O$, 0.5; KCL, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01) according to Yatome *et al*. (1993). Textile dyes, i.e., Reactive blue MR (λ_{max} 585 nm), Orange M2R (λ_{max} 494 nm), Yellow M4G (λ_{max} 425 nm), Black HFGR (λ_{max} 594 nm) and Red M8B (λ_{max} 545 nm) were used at 200 mg/L. Agitated cultures of fungal species were grown for 10 days in a shake incubator at 30 ± 2°C and 120 rpm. Samples were withdrawn aseptically on alternate days, centrifuged at 5000 rpm for 10 min and supernatant was scanned in a spectrophotometer at λ_{max} of the respective dye. Control flasks without dye were also maintained. Percent decolorization was calculated using the formula:

$$\text{Decolorization (\%)} = [(A_0 - A_t)/A_0] \times 100$$

where A_0 is initial absorbance of sample and A_t is the absorbance at different time intervals.

Statistical analysis

The data were analyzed as mean of triplicates ± standard deviation (SD). Duncan's multiple range test (DMRT) was employed to test the level of significance at $P < 0.05$.

RESULTS

Physicochemical characterization of effluents

The data pertaining to physicochemical characterization of effluent samples from various sites is presented in **Table 1**. Wastewater was highly colored showing high concentrations of unused dye in the effluents. The effluents were found to be highly alkaline with a pH value ranging from 8.0 to 9.5. COD values were also found to be very high (985 – 1215 mg L^{-1}) at various sites. BOD levels ranged from 365 – 480 mg L^{-1} at various sampling sites. This data is also consistent with Devi and Kaushik (2005), who also observed a high BOD (560 mg L^{-1}), COD (1418 mg L^{-1}) and pH (9.4) of effluents taken from a textile industry, SGL industry, Faridabad (Haryana). High BOD and COD levels are indicative of the fact that substances present in the effluents can be biologically degraded (Pathe *et al*. 1995). Increased pH of the effluents is due to the use of carbonate, bicarbonate, H_2O_2 and NaOH during the bleaching process in the textile industry (Wood and Kellog 1988).

Isolation and identification of dye decolorizing fungi

Dye decolorizing fungi have been frequently isolated from textile effluents and soils exposed to dye wastes (Devi and Kaushik 2005; Faryal and Hameed 2005; Raju *et al*. 2007; Ponraj *et al*. 2011). In the present investigation, soil samples collected from waste disposal sites of three different textile industries were screened for the occurrence of fungi. Fungal species native to the sampling sites were isolated on CYA medium using dilution plate method. The species were identified by their morphological characteristics using a steriobinocular microscope and with the help of taxonomic keys and standard manuals. A total of 10 fungal species belonging to five genera were isolated and identified (**Table 2**). Out of these, four species *viz*., *Aspergillus niger*, *A. terrus*, *A. flavus* and *Rhizopus* sp. were ubiquitous and recovered from all the screened soil samples. The incidence of fungi in the polluted water and soil depends on the availability of nutrient, oxygen and biological, physical and chemical features of the pollutant.

Table 1 Physicochemical properties of textile waste water of different dyeing units.

Dyeing unit	BOD mg^{-L}	COD mg^{-L}	pH
Mayur Paliwal Textiles (MPT)	480 a	1215 a	9.5 a
Orien Dye House (ODH)	420 b	1017 b	9.2 a
Bharat Dying Unit (BDU)	365 c	985 b	8.0 b

Note: different letters within a column are significantly different (DMRT; $P < 0.05$); n = 5

Table 2 Fungal strains identified in different industrially polluted soil.

Organisms	MPT	ODH	BHU
Aspergillus niger	+	+	+
Aspergillus terrus	+	+	+
Aspergillus nidulans	+	-	-
Aspergillus flavus	+	+	+
Aspergillus fumigatus	+	-	+
Humicola insolens	+	+	-
Humicola brevis	-	+	+
Drechslera hawaiiensis	-	+	+
Torula herbarum	+	-	+
Rhizopus sp.	+	+	+

MPT: Mayur Paliwal Textiles, ODH: Orien Dye House, BDU: Bharat Dying Unit; + = Present, - = Absent

Decolorization of textile dyes

In the present investigation, all the ten isolated fungal strains were used to study decolorization of textile dyes *viz*., Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B at 200 mg L^{-1} in a C-limited Czapek Dox broth *in vitro*. The decolorization efficiencies were measured after 10 days of incubation under controlled conditions. The results revealed that all the isolated fungal species had variable potential to decolorize different textile dyes (**Table 3**). The most potent fungal strain in decolourizing Reactive Blue MR was *A. niger* followed by *A. terreus* > *A. fumigatus* > *A. nidulans* > *A. flavus* > *Rhizopus* sp. > *D. hawaiiensis* > *H. insolens* > *T. herbarum* > *H. brevis*. Orange M2R was most efficiently degraded by *A. flavus* followed by *A. terreus* > *A. niger* > *A. nidulans* > *A. fumigatus* > *T. herbarum* > *Rhizopus* sp. Yellow M4G was the most recalcitrant among all the dyes studied for degradation by the isolated fungi. The degradation efficiencies by different fungal strains varied from 5.14 to 39.38% (**Table 3**). From the results it was also observed that Black HFGR dye was highly degradable by all the fungal strains and the degradation efficiency varied from 28.19 to 90.26% (**Table 3**). Similarly Red M8B was degraded to 88.51% by *H. brevis*. The level of significance $P < 0.05$ using DMRT for each dye and against all test organisms is presented in **Table 3**.

In waste water effluents, we seldom get a single dye rather a mixture of dyes is present. Similarly pure cultures of microorganisms are never present in natural ecosystems. Thus, in the present investigation a fungal consortium consisting of all the ten fungal strains and a mixture of all the five dyes was also studied to evaluate the degradation efficiencies of isolated strains *in vitro*. It was noted that the

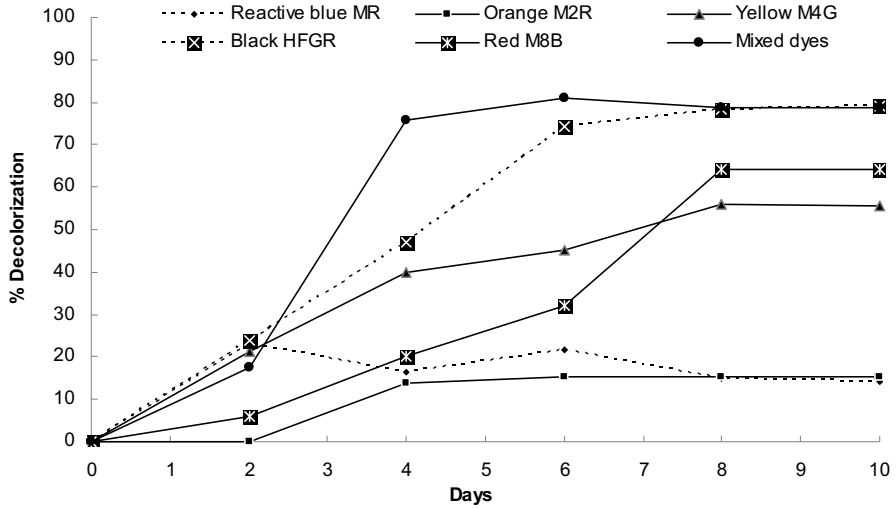

Fig. 1 Decolorization kinetic of various dyes by mixed microbial consortia.

Table 3 Per cent decolorization of textile dyes by isolated fungal strains after 10 days of incubation.

Organism	Decolorization (%)					
	Reactive Blue MR	**Orange M2R**	**Yellow M4G**	**Black HFGR**	**Red M8B**	**Mixture of all dyes**
Aspergillus niger	88.36 ± 1.34 a	51.31 ± 0.66 b	39.38 ± 0.33 c	78.79 ± 1.49 a	22.27 ± 0.33 d	30.71 ± 0.38 d
Aspergillus terrus	81.53 ± 1.55 a	55.68 ± 0.71 b	-	90.26 ± 1.72 a	83.76 ± 1.55 a	34.34 ± 0.38 c
Aspergillus nidulans	70.98 ± 1.20 a	43.92 ± 0.43 b	8.69 ± 0.19 c	78.15 ± 1.49 a	23.51 ± 0.25 d	35.18 ± 0.35 b
Aspergillus flavus	57.67 ± 0.75 a	82.63 ± 0.75 b	13.54 ± 0.24 c	88.56 ± 1.74 b	58.23 ± 0.70 a	21.94 ± 0.29 c
Aspergillus fumigatus	72.30 ± 1.30 a	36.13 ± 0.36 b	22.10 ± 0.35 c	65.27 ± 0.85 a	13.72 ± 0.19 d	26.16 ± 0.35 c
Humicola insolens	26.37 ± 0.37 a	-	12.77 ± 0.26 b	90.35 ± 1.70 c	9.33 ± 0.13 b	14.09 ± 0.21 b
Humicola brevis	22.18 ± 0.34 a	-	13.36 ± 0.30 a	82.19 ± 1.52 b	88.51 ± 1.68 b	18.73 ± 0.31 a
Drechslera hawaiiensis	42.08 ± 0.43 a	-	13.71 ± 0.21 b	28.94 ± 0.38 c	38.56 ± 0.35 a	28.01 ± 0.38 c
Torula herbarum	24.70 ± 0.34 a	33.89 ± 0.39 b	5.14 ± 0.10 c	57.88 ± 0.80 d	10.84 ± 0.20 c	16.28 ± 0.27 a
Rhizopus sp.	53.59 ± 0.68 a	20.89 ± 0.34 b	25.96 ± 0.35 b	41.98 ± 0.43 c	2.79 ± 0.07 d	81.01 ± 1.52 e
Fungal consortium*	14.26 ± 0.23 a	15.29 ± 0.24 a	55.76 ± 0.72 b	79.09 ± 1.53 c	64.27 ± 0.86 d	78.73 ± 1.52 c

Note: ± = standard deviation, - = no decolorization, *Fungal consortium consisted of all 10 fungal strains; different letters within a row are significantly different (DMRT; $P < 0.05$)

fungal consortium was able to degrade Black HFGR (79.09%) and Red M8B (64.27%) most efficiently. Degradation kinetics of fungal consortium revealed that maximum degradation was achieved after 8 days of incubation and further incubation had no effect on the degradation of dyes (**Fig. 1**). Similarly with regard to pure cultures degrading mixture of dyes, it was observed that *Rhizopus* sp. was able decolorize the mixture of dyes more efficiently (81.01%) as compared to individual dye (**Table 3**). Furthermore, the recalcitrant Yellow M4G was also more efficiently degraded by fungal consortium (55.76%) as compared to pure cultures (**Table 3**).

DISCUSSION

Decolorization of textile dye effluents is a serious environmental problem. While physical and chemical methods of dye removal are expensive and result in high sludge problem, biological methods are environmentally safe and convert organic compounds completely into water and carbon dioxide, are cost effective and easy to use (Ponraj *et al*. 2011). In the present investigation, textile effluents from Mayur Paliwal Textiles was highly coloured and had BOD, COD and pH of 480 mg^{-L}, 1215 mg^{-L} and 9.5, respectively. These results are in agreement with earlier reports from textile effluents (Devi and Kaushik 2005). High pH is mainly due to the use of carbonate, bicarbonate, H_2O_2 and NaOH during bleeching of the textile (Wood and Kellog 1988). *Aspergillus* spp. are well adapted to textile waste water and are frequently isolated from effluents and dye contaminated soils (Devi and Kaushik 2005; Faryal and Hameed 2005; Raju *et al*. 2007; Ponraj *et al*. 2011). However, as far as *Humicola insolens*, *H. brevis* and *Rhizopus* species are concerned there is hardly any literature available about their use for decolorization of dyes. Ten fungal species were successfully identified using taxonomic guides and standard procedures. *Aspergillus niger*, *A. terrus*, *A. flavus* and *Rhizopus* sp. were the most important species and recovered from all the sites studied (**Table 2**). From the results of the present study, it is also clear that these species were also found to be efficient degrader of textile dyes. Most of the isolated fungal species were able to decolorize textile dyes (200 mg^{-L}) within 6-10 days under static culture condition. In our study, it was observed that fungal consortium consisting of all the ten fungal isolates was able efficiently degrade a mixture of dyes as compared to individual dyes (**Table 3**). Earlier such attempts were made to decolorize textile dyes using bacterial consortia (Khehra *et al*. 2005). Moosvi *et al*. (2005) used a microbial consortium JW-2 consisting of *Paenibacillus polymyxa*, *Micrococcus luteus* and *Micrococcus* sp. The concerted metabolic activity of these isolates led to complete decolorization of Reactive Violet 5R (100 ppm) within 36 h whereas individual isolates could not show decolorization even on extended incubation. The consortium had the ability to decolorize nine different dyes tested. During investigation, it was also observed that yellow M4G and Orange M2R were recalcitrant to decolorization. Orange M2R has a complex structure having three aromatic rings and one sulphonic group and it is difficult to degrade such dyes at higher concentrations. The possible mechanism of decolorization may be biosorption by fungal biomass or oxidative degradation/reduction of dye (Fu and Viraraghvan 2002). Ronkarappa *et al*. (2006) used *A. niger* and *A. nidulans* for biosorption of Congo Red. They observed that dead biomass of *A. niger* is most efficient in biosorption compared to the living biomass. Furthermore, process optimization may result in enhanced dye removal (Kaushik and Malik 2011).

Based on these findings, it can be suggested that azo dye contaminated sites can potentially be reclaimed by low cost bioremediation process using a consortia of native fungal species isolated from the dye disposal sites. These fungal strains may also have a potential for use in bioreactors for industrial discharge treatment, through biotechnological approaches to colour removal.

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