Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 3 (3): 204-208, 2008
ISSN 1816-4935
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Dye Degrading Mycoflora from Industrial Effluents

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Abstract: The present study deals with the distribution of fungal species in Cuddalore dye industrial waste (Lat 11°42'N; Long 79°46'E) and their dye degrading activity. Totally 13 species under 17 genera were isolated and screened for their decolorization activity against methylene blue, gentian violet, crystal violet, cotton blue, Sudan black, malachite green, methyl red and corbol fushion in mineral salt medium and Czepex-Dox broth. In agar medium, decolorization began with the formation of zone of clearance around the colonies. *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* decolorized maximum number of dyes to a great extent. *Mucor racemosus*, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* did not decolorize any of the dyes tested. In liquid medium, decolorizing activity was measured spectrophotometrically. *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* registered maximum color reduction, where as *Mucor racemosus*, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* expressed very low amount of color reduction. Biomass and the extent of dye removal are directly propositional. Among the 13 species of fungal isolates, *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Fusarium moniliforme* and *Penicillium citrinum* seems to be potential candidates for dye degradation. These strains can be used for the bioremediation of environs polluted with dye effluents.

Key words: Hyphomycetes, biodegradation, decolorization, bioremediation

INTRODUCTION

Explosion of population coupled with industrial revolution results in pollution of water, air and soil. The discharge of pollutants from various industries poses threat to the biodiversity of the earth. Among various types of environmental pollution, water pollution is of major concern in which, dye based industries are first and foremost. Dyes are released into the environment through industrial effluents from three major sources such as textile, dyestuff manufacturing and paper industries (Cripps *et al*., 1990). One of the most pressing environmental problems related to dye effluents is the improper disposal of waste water from dyeing industry. With regard to their color removal by conventional treatment methods lead to severe water pollution, thus developing cost effective clean-up operations (Willmott *et al*., 1998). Globally, it is estimated that, 10% of the total dyestuffs used (Maguire, 1992), or about 7×10^5 tones per annum are released into the environment. Not only the color is aesthetically unacceptable, it also affect aquatic ecosystem by decreasing the light penetration and solubility of gases (Saranaik and Kanekar, 1995). Further, some synthetic dyes, such as azo dyes are carcinogenic or mutagenic (Spadaro *et al*., 1992). Microbial degradation seems to be promising compared to other organisms and the method of application are simpler compared to other available methods. Several combined anaerobic and aerobic microbial treatments had been suggested to enhance the degradation of textile dyes (Haug *et al*., 1991). A great number of white rot fungi had been reported to produce various lignin degrading enzymes like laccase, lignin peroxidase and manganese peroxidase

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or at least any one of these enzymes (Dey *et al*., 1994). To degrade the complex lignin molecule, white rot fungi produce various extra cellular enzymes with low specificity and strong oxidative activity. Numbers of laboratories are investigating the ability of the white rot fungus like *Phanerochaete chrososporium* to degrade xenobiotic compounds. The present work is an earnest attempt to assess the ability of various fungi in degradation of commonly used dyes and develop potential biobleaching agents from superior catabolic strains.

MATERIALS AND METHODS

The fungal strains were isolated from industrial effluent water and sediment samples collected from SIPCOT (Small Industries Promotion Corporation of Tamil Nadu, India) effluent discharge points (Lat 11°42'N; Long 79°46'E) located in Uppanar estuary at December 2005 work was carried out in CAS in Marine Biology. Diluted sample was taken from 10^{-3} and 10^{-4} dilutions and plated on potato dextrose agar (g L^{-1} potato infusion 200, dextrose 20, agar 15, Distilled water 1000 mL) and incubated at 28°C for 3 to 8 days. To eliminate the bacterial contamination 8 mL of 1% streptomycin was added to the medium. Isolated fungal strains were tested for decolorization activity against commercially important dyes such as methylene blue, gentian violet, crystal violet, cotton blue, Sudan black, malachite green, methyl red and corbol fuchsion in broth cultures as well as in agar medium. Dye degradation in broth culture was done by following the method described by Jothimani and Prabakaran (2003). The fungal cultures were inoculated into the potato dextrose broth and incubated at 27°C for 3 days. After 3 days, 1 mL of the culture suspension was transferred to 50 mL of Czepex-Dox broth (g L^{-1} Sucrose 30, Sodium nitrate 2, K_2HPO_4 1 g, $MgSO_4$ 0.5, KCl 0.5, $FeSO_4$ 0.01 and Distilled water 1000 mL) in 250 mL Erlenmeyer flasks. These flasks were incubated in a shaker at 200 rpm at room temperature for 4-8 days. On the fourth day, an aliquot of one of the aforementioned dye (20 mg) was added in to broth culture and incubated for 5 days. Uninoculated flasks served as controls to assess the abiotic decolorization. OD values were measured spectrophotometrically at 594 nm to estimate the decolorization process. The rate of decolorization was calculated using the following formula as described by Sani and Banerjee (1999).

$$\text{Decolurisation (\%)} = \frac{\text{Intial absorbance-after decolorization absorbance}}{\text{Initial absorbance}} \times 100$$

Dye degradation in solid medium was done by adopting the method of Swamy and Ramsay (1999). Solid mineral salt media (g L^{-1} sucrose 30, sodium nitrate 2, KH_2PO_4 1, $MgSO_4$ 0.5, KCl 0.5, $FeSO_4$ 0.01, Thiamine hydrochloride 0.01, distilled water 1000 mL and micronutrient solution 1.0 mL (mg L^{-1} $B_4O_7Na_2$ 100, $MnSO_4$ 10, $(NH_4)_6Mo_7O_{24}$ 10, Na_2SO_4 10, $CuSO_4$ 50 and distilled water) was prepared and 20 mg of filter sterilized dye was added after sterilization of the medium. Fungal strains were inoculated in to the plates and incubated at 28°C for 8 days along with control plates. The extent of clear zone formation around the colonies was observed and recorded. For biomass estimation mycelial mat recovered from the Whatman No.1 filter paper was washed with distilled water and dried at 70°C for 48 h and weighed (Yesilada and Ozcan, 1998). Biomass was expressed as dry weight.

RESULTS AND DISCUSSION

Total number of fungal species isolated included 2 members of Zygomycotina represented by *Mucor racemosus* and *Rhizopus stolonifer* and 11 species from 5 genera of Deuteromycotina represented by Hyphomycetes such as, *Aspergillus niger*, *A. fumigatus*, *A. japanicus*, *A. ochraceus*

Table 1: Decolorization of dyes

Organisms	MB	GV	CV	CB	SB	MG	MR	CF
Rhizopus stolonifier	22.4	24.6	23.7	19.9	22.3	21.7	18.2	17.2
Mucor racemosus	8.9	7.2	6.6	7.3	6.1	4.5	6.6	7.4
Aspergillus flavus	42.3	44.1	45.8	26.5	22.3	32.2	28.5	42.7
A. fumigatus	36.1	38.6	38.3	36.1	32.7	28.8	26.1	22.1
A. japonicus	42.2	62.8	44.6	32.9	34.8	22.0	63.2	53.9
A. niger	63.8	61.6	59.6	58.3	57.1	58.1	57.0	43.5
A. ochraceus	82.3	80.2	65.6	78.6	81.8	84.2	74.4	65.9
A. terreus	85.5	86.3	78.6	85.8	80.6	81.9	75.7	79.7
Cladosporium cladosporiodes	6.5	4.1	8.4	5.8	6.3	7.7	6.6	5.1
Fusarium moniliforme	52.1	71.7	63.3	73.6	74.3	78.4	34.2	36.6
Penicillium oxalicum	12.2	8.4	8.9	6.7	4.2	7.9	8.8	11.3
P. citrinum	72.6	74.1	63.8	73.8	79.6	81.9	32.2	36.7
Trichoderma viride	11.3	9.6	8.9	6.5	7.7	6.2	7.7	5.1

Table 2: Growth of fungal strains in different dyes

Organisms	MB	GV	CV	LCB	SB	MG	MR	CF
Rhizopus stolonifier	0.38	0.36	0.28	0.36	0.32	0.31	0.26	0.25
Mucor racemosus	0.28	0.24	0.22	0.20	0.19	0.21	0.22	0.23
Aspergillus flavus	0.44	0.38	0.46	0.24	0.33	0.33	0.25	0.35
A. fumigatus	0.40	0.42	0.47	0.33	0.32	0.31	0.33	0.26
A. japonicus	0.46	0.45	0.42	0.31	0.33	0.24	0.47	0.38
A. niger	0.50	0.49	0.48	0.46	0.47	0.48	0.45	0.39
A. ochraceus	0.55	0.54	0.51	0.50	0.50	0.54	0.49	0.50
A. terreus	0.53	0.55	0.52	0.53	0.49	0.51	0.50	0.51
Cladosporium cladosporiodes	0.18	0.12	0.14	0.16	0.17	0.19	0.15	0.18
Fusarium moniliforme	0.50	0.49	0.47	0.48	0.48	0.49	0.49	0.48
Penicillium oxalicum	0.16	0.19	0.15	0.16	0.18	0.17	0.18	0.18
P. citrinum	0.52	0.50	0.49	0.48	0.51	0.46	0.47	0.47
Trichoderma viride	0.19	0.13	0.22	0.20	0.19	0.21	0.21	0.24

A. flavus, A. terreus, *Penicillium oxalicum*, *P. citrinum*, *Fusarium moniliforme*, *Cladosporium cladosporioides* and *Trichoderma viride*. Total number of fungi isolated in the sediment sample was relatively higher than water sample indicating that, the sediments act as reservoir of nutrients for fungal growth. Complete decolorization was represented by the total disappearance of color without any visible sorption into the biomass (Table 1). *Mucor racemosus*, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* did not decolorize any of the dyes tested. During 8 days of incubation period, it was found that, *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* decolorized maximum number of dyes to a greater extent and the percentage of color removal increased with incubation period. It was similar to the earlier findings of Jothimani and Prabakaram (2003) and Ryu and Weon (1992). From the results, it was noted that, at least 48 h of contact time is required for the initial acclimatization of the fungal species to establish in the stimulated environment with dyes. Growth study revealed that, biomass and dye removal are directly proportional, which may be attributed to the fact that, the increase of biomass gave more surface area for sorption of the dye molecules available (Table 2). *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *A japonicus*, *A. flavus*, *A. fumigatus*, *Rhizopus stolonifer*, *Penicillium citrinum*, *P. oxalicum*, *Fusarium moniliforme*, *Mucor racemosus*, *Cladosporium cladosporioides* and *Trichoderma viride* shows good decolorization activity against all the dyes tested (i.e.) methylene blue, gentian violet, crystal violet, cotton blue, Sudan black, malachite green, methyl red and corbol fuchsion. Results obtained in this study are testimony to the potential biodegrading capability of these strains. Decolorization of azo, anthroquinone, heterocyclic, triphenylmethane and polymeric dyes with this fungus had been reported (Paszczynski and Crawford, 1992), among the fungal strains tested,

Table 3: Decolorization of synthetic dyes by Deuteromycotina and Zygomycotina in agar medium

Organisms	MB	GV	CV	CB	SB	MG	MR	CF
Zygomycotina								
Rhizopus stolonifer	+	+	-	+	+	+	-	-
Mucor racemosus	-	-	-	-	-	-	-	-
Deuteromycotina								
Aspergillus flavus	+	+	+	-	+	+	-	-
A. fumigatus	+	+	+	+	+	+	+	-
A. japonicus	+	++	+	+	+	-	++	+
A. niger	++	++	+	+	+	+	+	+
A. ochraceus	+++	+++	++	++	+++	+++	++	++
A. terreus	+++	+++	+	+++	+++	+++	++	++
Cladosporium cladosporioides	-	-	-	-	-	-	-	-
Fusarium moniliforme		++	++	+	++	++	+++	+
Penicillium oxalicum	-	-	-	-	-	-	-	-
P. citrinum	++	++	++	++	++	++	+	+
Trichoderma viride	-	-	-	-	-	-	-	-

+: - Zone of clearance 0.2 to 0.5 mm, ++: - Zone of clearance 0.6 to 0.8 mm and +++, - Zone of clearance 0.9 to 1.2 mm

Aspergillus ochraceus and *A. terreus* were potential against the dyes, which are confirmed by the larger zone of clearance in the dye containing medium. *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *F. moniliforme* and *P. citrinum* were able to decolorize methylene blue, sudan black, cotton blue, malachite green and gentian violet (Table 3). However, with synthetic dyes like methyl red, corbol fuchsion and crystal violet a complete decolorization could not be achieved. This may be due to the chemical functionalities of dyes and a clear relationship between dye structures and fungal dye biodegradability had not been established so far (Fu and Viraraghavan, 2001). Among the fungal strains *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* showed maximum decolorization (80%), where as *Mucor racemosus*, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* showed very low decolorization (25%). Among the dyes used, cotton blue, methylene blue, gentian violet, sudan black and malachite green were completely decolorized, where as 40-60% decolorization was observed with crystal violet, methyl red and corbol fuchsion. Based on the mycelial weight, *Aspergillus ochraceus*, *A. terreus*, *A. niger*, *Penicillium citrinum* and *Fusarium moniliforme* were well grown in dye containing liquid medium, where as *Rhizopus stolonifer*, *Mucor racemosus*, *A. flavus*, *A. fumigatus* and *A. japonicus* showed limited growth. Finally, *Cladosporium cladosporioides*, *Penicillium oxalicum* and *Trichoderma viride* were found with very low or negligible growth in dye containing liquid medium. In liquid culture, rapid dye decolorization by the fungal strain was observed within 24 h. It was mainly due to the high adsorption of the dye in the mycelium. In subsequent days, dye decolorization may be due to production of extra cellular enzyme (Haglund, 1999). However further works on their enzyme systems are needed to understand the decolorizing mechanism of there fungal isolates. The study concluded that, these fungal consortia on their own can offer a cost effective, easily applicable and an environmentally sound solution to dye effluents.

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