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RESEARCH ARTICLE

Biodegradation of textile dyes, direct brilliant violet and direct greenish blue by *Aspergillus* spp.

D. Yamini[1], V. Sivakami[1], M. Soundarya[1] and D.J. Mukesh Kumar[2]
[1]Dept. of Biotechnology, St. Joseph's College of Engineering, Chennai
[2]Centre for Advanced Studies in Botany, Maraimalai Campus, University of Madras, Chennai, India
+91 9884553310; itsmemukesh@gmail.com

Abstract

Three dye decolorizing isolates were isolated from the textile effluent samples collected from Tiruppur, Tamil Nadu. The isolated strains were morphologically characterized as *Aspergillus* spp. and the strains were assigned as AS1, AS2 and AS3. The dye degradation ability of the isolated strains was examined against two common textile dyes, direct brilliant violet and direct greenish blue. *Aspergillus* strain AS2 showed maximum dye decolorization of brilliant violet at 78% under optimum condition. The strain AS3 was found to be more efficient in decolorization of direct greenish blue (90%). The results reported in the present investigation will help to establish the usefulness of the isolates for bioremediation and biodegradation applications such as wastewater treatment.

Keywords: *Aspergillus* spp., effluent, brilliant violet, greenish blue, decolorization, wastewater treatment.

Introduction

Textile industries are one of the dominated industries and for the wet processing of the textiles, substantial amount of water and chemicals are consumed by these industries. They are discharged into environment in the form of effluents after processing or without processing. Various contaminants are present in such effluents, such as toxic organic and inorganic compounds and colors. Among them, colors are considered as major pollutants, mainly dyes (Christian *et al.,* 2005). During the earlier days, natural dyes were used as the coloring agents but nowadays, dyes are synthetic in origin and their complex structures make them more stable and often difficult to degrade by any biological means.

In the modern era, the usage of the synthetic dyes increased rapidly for coloring materials because they are easy to prepare and give long standing appearance and quality to the materials on which they are used (Gohl and Vilensky, 1987). In India, for the annual production of approximately 30 million tonnes of textiles, 70000 tonnes of dyes are required (Sri kumaran and Dharani, 2011.). These dyes were discharged into the environment after processing causes serious environmental problems. Since the effluents of the textile industries are brightly colored, their disposal into ground water significantly affects the photosynthetic activity in aquatic life, by reducing the penetration of sunlight. Due to the presence of various aromatics compounds and heavy metals, the effluents are highly toxic to aquatic life forms, consequently to the life forms feeding on them including humans (Asamudo *et al.,* 2005).

The physico-chemical methods (Mavros *et al.,* 1994) for the removal of color from the wastewater, such as adsorption, activated carbon, reverse osmosis, ion exchange and flocculation, showed various types of difficulties such as low efficiency, high cost, limited applications and leaves out a lot of waste. As a viable alternative, biological methods for their treatment possess various advantages such as efficient degradation, environmental friendly and cost effective (Gurses, 2002). The isolation of potent organism to degrade dyes is one of the important aspects of effluent treatment. A wide variety of microorganisms capable of decolorizing various dyes including bacteria, fungi were screened. The fungi were found to be dominant among them, since they were easy to manipulate and versatile in nature (Bhole *et al.,* 2004, Amezcua-Allieri *et al.*, 2005). This study aims at investigating the potential of isolated fungal cultures for the decolonization of model textile dyes, direct brilliant violet and direct greenish blue.

Materials and methods

Textile dyes: Textile dyes such as direct brilliant violet and direct greenish blue were purchased from Sigma Alrich. The selection of dyes was based on their structural diversity and frequency of use in the local textile industries. The chemicals and medium components were of analytical grade and purchased from Hi-Media.

Effluent and soil samples: Effluent and soil samples were collected from the textile industries in Tiruppur district of Tamil Nadu, India. The samples were collected in sterile container and stored at 4°C.

Isolation and identification of fungi: One gram of the soil sample was suspended in 100 mL of 0.85% saline solution and kept in shaker (125 rpm) for 30 min. Ten mL of the above suspension was mixed with 50 mL of textile effluent and 40 mL of Czapex-dox broth in a 250 mL conical flask. The mixture was incubated in rotary shaker at 120 rpm for 4 d. About 0.2 mL of the enriched medium was spreaded on Czapex-dox agar plates incorporated with textile effluent and incubated for 4 d (Xiao *et al.,* 2006). The fungal colonies showing maximum zone of clearances were selected and maintained as pure culture in Potato dextrose Agar (PDA). The morphological identification of the isolated fungal strains was determined using Lacto-phenol cotton blue staining. The identification was based on the spore and hyphae morphology of the isolated fungal strains with respect to the standards (Thomas and Kuriakose, 1990).

Decolorization studies: In order to perform the discolorations studies, a synthetic waste water medium (Sri kumaran and Dharani, 2011) was prepared with following composition (g/l): Glucose-5, KH_2PO_4-0.099, $MgSO_4$ -0.001, trace element solution-1. Trace element solution (g/l): $CuSO_4.7H_2O$-0.08, $MnSO_4.7H_2O$-0.04, $ZnSO_4.7H_2O$-0.04. The pH of the synthetic wastewater medium was adjusted to 7 and autoclaved. Decolorization studies were carried out in separate synthetic wastewater medium amended with 1% inoculum and respective dyes (0.0025% of direct brilliant violet and 0.0003% of direct greenish blue dye) under orbital shaking condition (150 rpm/min) at room temperature. At the intervals of every 48 h, 5 mL of the samples were centrifuged and the clear supernatant was subjected for its absorbance measurements at 420 nm for direct brilliant violet and 540 nm for direct greenish blue using spectrophotometer. Medium without dye and inoculum was used as blank and medium with dye but without inoculum was used as control. The efficiency of the decolorization was expressed as decolorization percentage.

$$\text{Decolorization}\,\% = \left(\frac{\text{Initial Absorbance - Final Absorbance}}{\text{Initial Absorbance}}\right) \times 100$$

Result and discussion

The present study dealt with screening of the microbes resulted in isolation of fungi, capable of decolorizing the textile dye. Textile dye effluent and soil samples were collected from the disposal site of effluent for the isolation of dye degrading fungi. The sample collection indicates the natural adaptation of these microorganisms to survive in the presence of toxic dyes (Khadijah *et al.,* 2009). It has been estimated that about 700 dyeing and bleaching units located in and around areas of Tiruppur district. The removal of the polluting dyes from those industries is the major problem faced by the small scale textile industries. Their economic status does not allow them to treat their wastewater properly before disposal and they have no choice other than dumping the effluent into water resources (Christian *et al.,* 2005).

Microbial decolorization/degradation of dyes is an eco-friendly and cost-effective method, alternative to other chemical decomposition processes (Verma and Madamwar, 2003). The enrichment medium was designated to improve the decolorizing ability of the fungal strains. The dye decolorizing ability of the fungal strains were enriched by cultivating them in the minimal medium with textile effluent as substrate and glucose as carbon source. Upon plating, the formation of clear zones around the colony indicates that the isolate is able to utilize the dye for its metabolism. The clear zones around the fungal colony might be due to the production of extracellular enzymes by the fungus, for the up taking of tested dyes (data not shown). Of the colonies developed, 3 fungal colonies were found to possess clear zones were purified and maintained in PDA slants. The fungal strains were grown as white mold covered by dark spores. The strains were confirmed as *Aspergillus niger* by its septated hyphae with conidiophores and designated as strains AS1, AS2 and AS3.

Fig. 1. Decolorization of brilliant violet dye by *Aspergillus* spp.

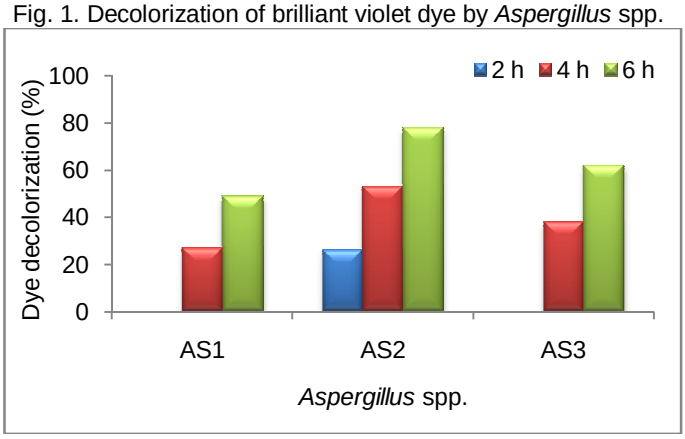
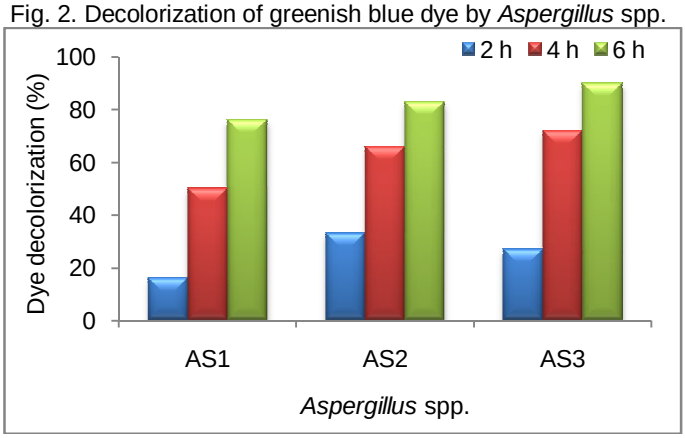


Fig. 2. Decolorization of greenish blue dye by *Aspergillus* spp.



Bioremediation of commercially available textile dyes namely direct brilliant violet and direct greenish blue was studied in batch culture using different fungal isolates. In order to test the activity of the selected *Aspergillus* spp. on different dyes, the experiments were carried out on flasks. The strain AS2 was found to be the most effective decolorizer of direct brilliant violet (Fig. 1).

The range of activity on decolorization of direct brilliant violet was 49%, 78% and 62% with AS1, AS2 and AS3 respectively. Also strain AS3 was found to be the most effective decolorizer of direct greenish blue (Fig. 2). The range of activity on decolorization of direct greenish blue was 76%, 83% and 90% with AS1, AS2 and AS3 respectively. Adsorption of dyes to the microbial cell surface is the primary mechanism of decolorization (Knapp *et al*., 1995).

The dye degradation/decolorization of this study might be due to the biosorption of the fungal hyphae as previously reported by various researchers (Sumathi and Manju, 2000; Ali *et al.*, 2008). Decolorization of various dyes is related to the various processes of extracellular oxidases, such as manganese peroxideases (Gold *et al.*, 1988). Liginin peroxidise (Lip), manganese dependant peroxidase (MnP) and laccase, all of them were involved in lignin degradation (Vyas and Molitores, 1995). The fungi *Aspergillus niger* have been previously reported to be having decolourizing activity over other textile dyes including Anthroquinone (Andleeb *et al.,* 2010), Azo dyes (Manikandan *et al.,* 2012), Reactive violet 2RL (Agnes Mariya Dorthy *et al.,* 2012), Reactive red HE7B, Yellow FN2R (Balaji *et al.,* 2012), etc. From these evidences and also our results clearly elucidates the dye degrading ability of *A. niger*. The dyedecolorizing/degrading activity of the fungi can be further analyzed deeper in order to develop an eco-friendly remediation technique for the remediation of textile dyes.

Conclusion

The study clearly indicates the role of selected fungal isolates of *Aspergillus* spp. for achieving enhanced decolorization/degradation of direct brilliant violet and direct greenish blue stains. This study suggests that the isolated fungal strains belong to *Aspergillus* spp. and possess a significant dye degradation capacity and can be applied in bioremediation of toxic industrial dyes in near future.

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