Decolorization of textile dyes by \textit{Aspergillus tamarii}, mixed fungal culture and \textit{Penicillium purpurogenum}

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This study describes degradation ability of \textit{Aspergillus tamarii}, mixed fungal culture (\textit{Trichoderma} sp., and \textit{A. flavus}) and \textit{Penicillium purpurogenum} on various synthetic dyes. \textit{A. tamarii} decolorized (> 90\%) of coomassie brilliant blue (CBB), bromophenol blue (BPB), and malachite green (MG) dyes. Mixed fungal culture decolorized: CBB, 74; BPB, 78; and MG, 45\%. \textit{P. purpurogenum} decolorized: CBB, 91; BPB, 92; and MG, 52\%. Dye decolorization was achieved by metabolism rather than by adsorption. Isolated fungal strains could effectively be used in development of alternative and eco-friendly method for removal and degradation of textile dyes.

Keywords: \textit{Aspergillus flavus}, \textit{A. tamarii}, Biodegradation, Dye decolorization, Laccase, \textit{Penicillium purpurogenum}, \textit{Trichoderma}

\section*{Introduction}

Release of toxic and recalcitrant chemicals including synthetic dyes from industries profoundly affects soil fertility and aquatic life\textsuperscript{1,2}. Use of physical and chemical methods for removal of dyes creates disposal problem of remaining dye sludge, whereas biotechnological approach provides viable, less sludge and eco-friendly method. Also, bacteria could degrade synthetic dyes at a faster rate but at the same time releases carcinogenic aromatic amines as degradation products, which severely affect animal and human health\textsuperscript{3} and water\textsuperscript{4}. White-rot fungi though effectively decolorizes dyes in simulated textile effluents but decolorization of dyes in real textile effluent is not encouraged due to less competitive ability with other fungi, grows well on wood/lignin, and also relatively low decolorization rates\textsuperscript{5}.

This study describes degradation ability of \textit{Aspergillus tamarii}, mixed fungal culture (\textit{Trichoderma} sp., and \textit{A. flavus}) and \textit{Penicillium purpurogenum} on various synthetic dyes.

\section*{Materials and Methods}

\textbf{Materials}

Coomassie brilliant blue (CBB), bromophenol blue (BPB) and malachite green (MG) were purchased from HiMedia, India. All other reagents were of analytical grade.

\section*{Isolation and Screening of Fungi}

Soil samples were collected from Botanical Garden, Ooty, Kunjappanai waterfalls (Mettupalayam) and Topslip (Pollachi, Coimbatore). Fungal isolates (65) were screened to find out ability to decolorize synthetic dyes at primary and secondary level. In preliminary screening, a clear halo on Czapek-Dox agar plates containing dyes was observed. Fungal isolates from secondary level screening, which was performed for isolates from preliminary screening, were identified by Indian Type Culture Collection (ITCC) Scientist, IARI, New Delhi. \textit{P. purpurogenum} was obtained from Department of Biotechnology, KCT, Coimbatore. Periodical sub-culturing of fungi was done on Czapek-Dox agar slants at 4°C.

\section*{Dye Decolorization in Batch Submerged Fermentation (SmF)}

About 0.2 ml (0.05\%, w/v) of sterilized dye solution was added to sterilized Czapek-Dox broth (50 ml) containing glucose (2\%, w/v) and NaNO\textsubscript{3} (0.2\%). Decolorization of CBB, BPB and MG was measured by Beckman spectrophotometer while monitoring decrease in absorbance at 553, 601 and 620 nm, respectively. Flasks inoculated with spore suspension (1 ml), were placed on an orbital shaker at 130 rpm and 30°C for 13 days. Duplicate flasks were withdrawn at every 24 h and
contents were filtered through Whatmann filter paper no. 1. Filtrate was measured for decrease in absorbance at particular wavelength for each dye and results were compared with control medium. Extent of decolorization was determined by monitoring decrease in absorbance of each dye. Dye removal (%) in test was calculated as relative to control medium for each dye.

**Decolorization of Dyes with Crude Enzyme from Solid State Fermentation (SSF)**

SSF was carried out using wheat bran as substrate for laccase production. Wheat bran (10 g) in Erlenmeyer flask was moistened with distilled water (8 ml) and sterilized for 30 min at 121°C. Sterilized flasks were inoculated separately with cultures of *A. tamarii*, mixed fungal culture (*Trichoderma* sp., and *A. flavus*) and *P. purpurogenum*. SSF was carried out for 7 days at 28°C. At the end of 7th day, 50 mM acetate buffer (20 ml, pH 5.0) was added to extract enzymes from fermented wheat bran and contents were filtered through Whatman filter paper no. 1. Filtrate was dialyzed for 12 h with intermittent change in buffer and resultant dialyzate was used as source of laccase and for decolorization studies.

CBB, BPB, and MG (3 ml each, 0.05% v/v) solutions were added to dialyzed culture filtrate (30 ml) from SSF and mixtures were incubated at 37°C for 40 h. At particular time intervals, sample (3 ml) was withdrawn and analyzed spectrophotometrically for decrease in dye level. Decrease in absorbance of CBB, BPB, and MG were measured at 553, 601 and 620 nm, respectively. For control medium, distilled water (3 ml) was added in place of enzyme.

**Direct Decolorization of CBB in SSF by *A. tamarii***

Distilled water (8 ml) was added to moisten wheat bran (10 g) in Erlenmeyer flasks and were autoclaved at 121°C for 30 min. CBB dye solution (2 ml, 0.05%) and 24 h grown *A. tamarii* mycelia (0.5 ml) were added to sterilized flasks. Flasks were stirred manually at every 12 h for 7 days to achieve uniform distribution of dye and inoculum. Flasks were kept at 30°C. After 7 days, sterile distilled water (20 ml) was added to both test and control flasks. Contents were mixed well and filtered through Whatman filter paper no. 1. Absorbance of control and tests were measured at 553 nm. For control experiments, all ingredients were added except inoculum.

**Enzyme Assay**

Laccase assay in dialyzed culture filtrate obtained from submerged fermentation (SmF) and SSF was determined using ABTS as substrate. Reaction mixture contained 50 mM ABTS (3 ml) in 50 mM sodium acetate buffer (pH 5.0) and dialyzed culture filtrate (1 ml). Changes in absorbance were recorded at 420 nm up to 3 min using UV-Visible spectrophotometer (ELICO, India). Enzyme activity was expressed in international units (IU). One unit of enzyme activity is defined as quantity of enzyme, which transforms 1 µmol min⁻¹ of ABTS.
Results and Discussion

Decolorization of Dyes in Batch Submerged Fermentation (SmF)

After 13 days, *A. tamarii* decolorized BPB (100%), CBB (93%) and MG (91%); mixed culture decolorized CBB (74%), BPB (78%), and MG (45%); and *P. purpurogenum* decolorized CBB (91%), BPB (92%) and MG (52%). *A. tamarii* and *P. purpurogenum* completely decolorized CBB after 20 days. These observations were supported by disappearance of CBB and BPB in medium as well as with mycelia of *A. tamarii* and *P. purpurogenum*. On the contrary, mixed culture failed to decolorize CBB, BPB and MG completely even after 20 days. Addition of bagasse extract to growth medium for *Pleurotus ostreatus* enhanced ligninolytic enzymes and this in turn decolorized fast green, 90%; rhodamine, 74%; and orange G, 58%. Decolorization of dyes in the medium by fungus is usually done by adsorption of dyes on fungal mycelia or biodegradation by enzymes. Results indicated that there was a decrease in a peak (\( \lambda_{max} \)) for all dyes (Fig. 1). Disappearance of dye color may be due to biodegradation of a chromophore in dye molecule.

Decolorization of Dyes Using an Enzyme Extract From SSF

After 13 days, an enzyme extract from SSF of *A. tamarii* decolorized CBB (90%), BPB (95%), and MG (94%); mixed culture decolorized CBB (77%), BPB (92%), and MG (93%); and *P. purpurogenum* decolorized CBB (81%), BPB (92%), and MG (94%). *A. tamarii* was found a better choice to mixed culture.

Direct Decolorization of CBB in SSF

Growth of fungi on solid substrate in presence of dyes also showed a reduction in the levels of dye. *A. tamarii* decolorized CBB dye to an extent of 90% after 7 days of incubation.

Conclusions

Fungi used in this study are ideal candidates for degradation of recalcitrant dyes in textile effluent. Optimization of culture conditions and gene cloning of ligninolytic enzymes may reduce long duration required by fungi for complete removal of dyes.

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References