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Decolorization of some reactive textile dyes by white rot fungi isolated in Pakistan

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Summary

Four white-rot fungi isolated in Pakistan were used for decolorization of widely used reactive textile dyestuffs. *Phanerochaete chrysosporium, Coriolus versicolor, Ganoderma lucidum* and *Pleurotus ostreatus* were grown in defined nutrient media for decolorization of Drimarene Orange K-GL, Remazol Brilliant Yellow 3GL, Procion BluePX-5R and Cibacron Blue P-3RGR for 10 days in shake flasks. Samples were removed every day, centrifuged and the absorbances of the supernatants were read to determine percentage decolorization. It was observed that *P. chrysosporium and C. versicolor* could effectively decolorize Remazol Brilliant Yellow 3GL, Procion BluePX-5R and Cibacron Blue P-3RGR. Drimarene Orange K-GL was completely decolorized (0.2 g/l after 8 days) only by *P. chrysosporium*, followed by *P. ostreatus* (0.17 g/l after 10 days). *P. ostreatus* also showed good decolorization efficiencies (0.19–0.2 g/l) on all dyes except Remazol Brilliant Yellow (0.07 g/l after 10 days). *G. lucidum* did not decolorize any of the dyestuffs to an appreciable extent except Remazol Brilliant Yellow (0.2 g/l after 8 days).

Introduction

Synthetic dyes are extensively used in the textile industry. Due to inefficiencies of the industrial dyeing process, 10–15% of the dyes are lost in the effluents of textile units, rendering them highly coloured (Vaidya & Datye 1982; Boer *et al.* 2004). It is estimated that 280,000 tons of textile dyes are discharged in such industrial effluents every year worldwide (Maas & Chaudhari 2005). Direct discharge of these effluents causes formation of toxic aromatic amines under anaerobic conditions in receiving media. In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes are toxic, mutagenic and carcinogenic (Chung & Stevens 1993). The efficient removal of dyes from textile industry effluents is still a major environmental challenge (Baldrian & Gabriel 2003).The frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies an important priority (O'Neill *et al.* 1999).

Degradation of dyes, especially azo dyes which comprise about 70% of all dyes used, is difficult, due to their complex structure and synthetic nature (Swamy & Ramsay 1999; Maas & Chaudhari 2005). Currently, various chemical, physical and biological treatment methods are used to remove colour (Pala & Toket 2002; Zhang *et al.* 2003). Because of the high cost and disposal problems, most of the chemical and physical methods for treating dye wastewater have not been widely applied in the textile industries (Robinson *et al.* 2001; Mazmanci & Ünyayar 2005). Since synthetic dyestuffs are resistant to biological degradation, colour removal by bioprocesses is also difficult (Shaul *et al.* 1991;Willmott *et al.* 1998). Decolorization generally occurs by adsorption of dyestuffs on bacteria, rather than oxidation in aerobic systems. Some bacteria can biodegrade dyestuffs by azoreductase activity. However, the effluent at the end of biotransformation of dyestuffs could be toxic (Chung & Stevens 1993; Chander *et al.* 2004). These problems limit large-scale application of bacterial decolorization.

White rot fungi, are however efficient in biodegradation of recalcitrant compounds like xenobiotics, lignin and dyestuffs by their extracellular ligninolytic enzyme system (Brodkorb & Legge 1992; Heinfling *et al.* 1997). White-rot fungi offer significant advantages over bacteria. Their extracellular enzyme systems including lignin peroxidase (LiP), Mn-dependent peroxidase (MnP), laccase and Mn-independent versatile peroxidases (VP) being non-specific can attack a wide variety of complex aromatic dyestuffs (Barclay *et al.* 1990; Nagai *et al.* 2002; Boer *et al.* 2004; Kamitsuji *et al.* 2005). Since the enzymes are extracellular, the substrate diffusion limitation into the cell, generally observed in bacteria, is not encountered. white-rot fungi do not require precondi-

tioning to particular pollutants, because enzyme secretion depends on nutrient limitation, nitrogen or carbon, rather than presence of pollutant. The extracellular enzyme system also enables white-rot fungi to tolerate high concentrations of pollutants (Knapp *et al*. 1997).

Faisalabad is one of the major textile cities of Pakistan. The reactive textile dyes selected in this study are extensively used in dying plants. The major objective of this study was to investigate the potential of some white-rot fungi cultures isolated in Pakistan for decolorization of the relatively less studied (Kapdan *et al*. 2000) but widely used reactive textile dyestuffs.

Materials and methods

Reactive dyestuffs

The four reactive textile dyestuff were Drimarene Orange K-GL (from Clariant), Remazol Brilliant Yellow 3GL (from DyStar), Procion Blue PX-5R (from DyStar) and Cibacron Blue P-3RGR (from Ciba), provided by the distribution offices of the respective companies at Faisalabad, Pakistan.

Fungal cultures

Pure cultures of *Phanerochaete chrysosporium, Coriolus versicolor, Ganoderma lucidum* and *Pleurotus ostreatus* were obtained from the National Fungal Culture Collection of Pakistan (NFCCP), University of Agriculture, Faisalabad, Pakistan. The cultures were grown on potato-dextrose agar (PDA) medium in petri plates for 6 days and were preserved at 4 °C. Cultures were recultivated periodically. Growth temperatures were 37 °C for *P. chrysosporium* and 30 °C for *G. lucidum*, *P. ostreatus and C. versicolor*.

Culture media

Following four nutrient media were used to select a simple and suitable medium for decolorization of the dyestuffs:

Medium I: Mainly Kirk's basal salts medium (Tien & Kirk 1988). It was composed of (g/l): ammonium tartarate, 0.22; KH_2PO_4, 0.2; $MgSO_4.7H_2O$, 0.05; $CaCl_2$, 0.01. Thiamine, 1 mg/l; 10 ml/l of 10% Tween-80 solution; 100 mM veratry1 alcohol and 10 m1/l trace elements solution was added. Trace elements solution had the following composition (g/l): 0.08 $CuSO_4$, 0.08; 0.05 H_2MoO_4, 0.05; $MnSO_4.4H_2O$, 0.07; $ZnSO_4.7H_2O$, 0.043 and $Fe_2(SO_4)_3$, 0.05.

Medium II: Same composition as Medium I excluding veratry1 alcohol and Tween-80.

Medium III: (g/l) urea 0.03; KH_2PO_4 0.2; $MgSO_4.7H_2O$ 0.1; $CaCl_2$ 0.01. No Tween-80 and veratryl alcohol were added to this medium.

Medium IV: (g/l) urea 0.04; KH_2PO_4 0.1; K_2HPO_4 0.2; $MgSO_4.7H_2O$ 0.5; $CaCl_2.2H_2O$ 0.05.

Inoculum preparation

Spore suspensions for inoculating the decolorization flasks were obtained by adding1% sterile glucose solution to fungal cultures grown on PDA slants. The slants were gently shaken to transfer spores to the liquid medium. These spore suspensions were passed through sterile glass wool columns to remove hyphal fragments. The concentrations of the eluted suspensions were determined by hemocytometer spore counting and adjusted to give 1×10^8 spores/ml^{-1} (Kay-Shoemake *et al*.1996)

Decolorization procedure

Decolorization flasks were prepared in triplicate and each contained 50 ml of the nutrient media. Glucose and dyestuff concentrations in all flasks were 5 and 0.2 g/l, respectively. The pH of all media was adjusted to 4.5 by using 2, 2-dimethylsuccinic acid. Each medium was sterilized (121 °C) in an autoclave (SANYO) for 15 min. The flasks were inoculated with 5 ml of the fungal conidial suspension and incubated for 10 days at 120 rev/min in a temperature-controlled incubator shaker (Gallenkamp). Control flasks contained only dyestuff and nutrients, but received no inoculum. Temperature was controlled at 37 °C for *P. chrysosporium and* 30 °C for *G. lucidum*, *P. ostreatus* and C. *versicolor*. The sealed flasks were oxygenated by pure oxygen for 5 min everyday. Samples were removed after every 24 h and centrifuged at 48,000× *g* for 30 min in a refrigerated centrifuge (EYLA). Supernatants were collected and analysed for residual dyestuff concentrations.

Dyestuff analysis

Absorbance measurements were done by using a UV/Visible spectrometer (Hitachi). Wavelengths resulting in maximum absorbance (λ_{max}) were used for respective dyestuff. A dilution of 1/10 was used for absorbance measurements. Drimarene Orange K-GL, Remazol Brilliant Yellow 3GL, Procion BluePX-5R and Cibacron Blue P-3RGR had λ_{max} values of 430, 420, 670 and 640 nm, respectively. The absorbance values for respective supernatants at each time period were corrected by subtracting the values for respective blanks (containing only medium but omitting the respective dye). The corrected absorbance values were used to calculate percentage decolorization.

Results and discussion

Results of the initial investigation showed that Kirk's nutrient medium was the best out of four media used for decolorization of all reactive dyes by all white-rot fungi cultures (data not shown). It was therefore, decided to use this Kirk's nutrient medium in subsequent decolorization studies.

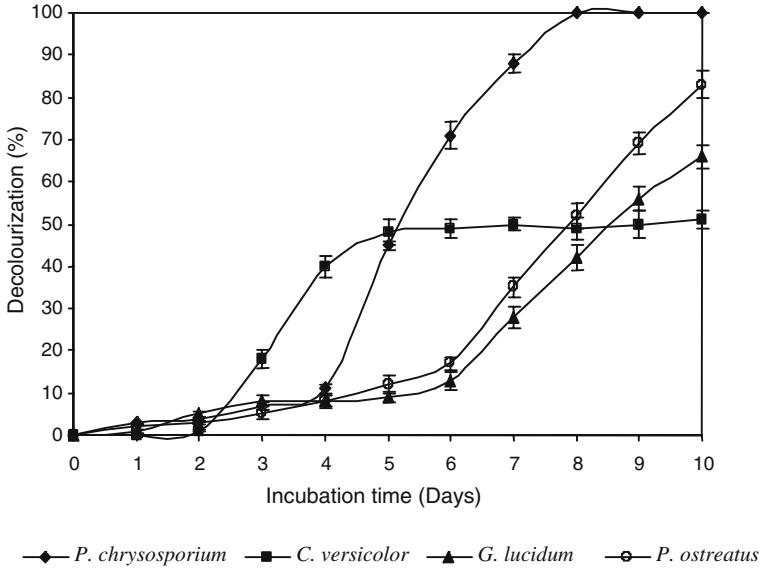

Figure 1. Decolorization of Drimarene Orange K-GL by four white rot fungi.

Results of time course studies on decolorization of Drimarene Orange K-GL are presented in Figure 1. *Phanerochaete chrysosporium* culture could bring about 100% decolorization after 8 days of incubation; although the rate of colour removal within the first 3 days was very low (5%). *P. ostreatus* caused 83 % colour removal, while *C. versicolor* was the poorest decolourizer (51%) followed by *G. lucidum* causing 66% colour loss in 10 days. Results with *P. chrysosporium* compare favourably with those of Kapdan *et al.* (2000) who found that *P. chrysosporium* MUCL could cause 100% decolorization of Drimarene Orange K-GL after 9 days of incubation.

Remazol Brilliant Yellow 3GL was effectively decolorized by most of the white-rot fungi cultures (Figure 2). Although the colour removal with *P. chrysosporium*, *C. versicolor* and *G. lucidum* cultures was not much pronounced within the first 4 days (2–41%), the decolorization proceeded rather sharply within the next three days resulting in complete decolorization in 6 days by *C. versicolor* and in 8 days by *P. chrysosporium* and *G. lucidum*. *P. ostreatus* was very poor in this case with only 52% decolorization achieved after 10 days.

In the case of Procion BluePX-5R, *P. ostreatus* was the best with 100% colour loss observed after 6 days. *P. chrysosporium* and *C. versicolor* could reduce the colour gradually and showed complete colour removals after 7 and 8 days of incubation respectively (Figure 3). *G. lucidum* was the poorest decolorizer of Procion Blue-PX-5R; it could cause only 54% decolorization in 10 days.

Percentage colour removals versus time curves for Cibacron Blue P-3RGR are shown in Figure 4. There was a slow decolorization rate within the first 3 days for most of the white-rot fungi cultures. *P. chrysosporium* was the most effective strain causing 100% colour loss

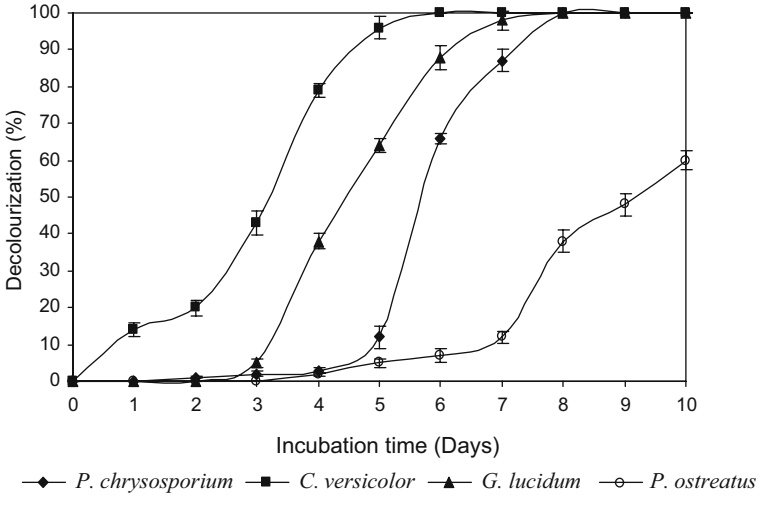

Figure 2. Decolorization of Remazol Brilliant Yellow 3GL by four white rot.

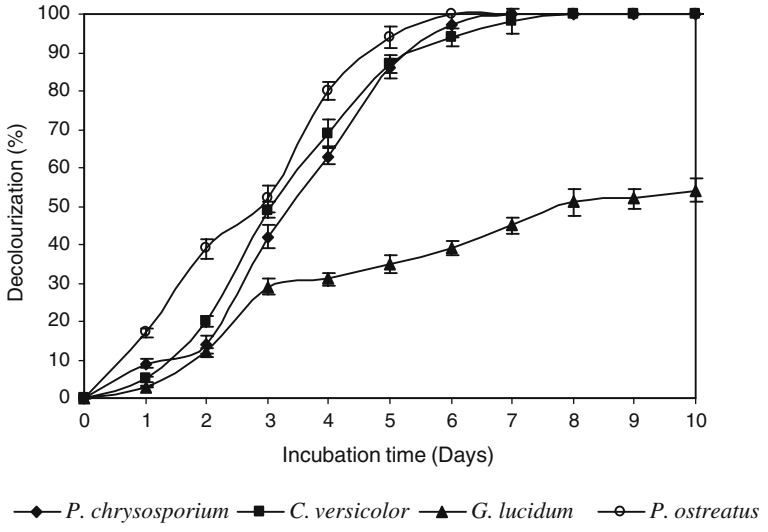

Figure 3. Decolorization of Procion Blue PX-5R by four white rot Fungi.

after 6 days of incubation. *P. ostreatus* gave a similar trend but took a longer time (8 days) for complete colour removal. The performance of *C. versicolor* (87%) was better than *G. lucidum* (44%) culture but none of them was effective enough to be of any practical application.

In summary, the *P. chrysosporium* culture was the most effective on the all dyestuffs tested followed by *C. versicolor* and *P. ostreatus*. Complete colour removals were observed with *P. chrysosporium* cultures for all dyestuffs within 6–8 days of incubation. *C. versicolor* culture showed better efficiency than the *P. ostreatus* on Remazol Brilliant yellow and Procion Blue dyestuffs. On the other hand *P. ostreatus* performed better on Procion BluePX-5R and Cibacron Blue P-3RGR. Decolorization of some dyes took longer for some cultures as compared to others, and in some cases it was incomplete even at the end of the 10-day study period. However, the time taken by these local isolates to achieve 100% decolorization compares favourably with reports on other white-rot fungi which required 7–20 days for 90% decolorization of a diverse range of synthetic dyes (Kargi *et al*., 2000; Kirby *et al*. 2000; Boer *et al*. 2004). *Ganoderma lucidum* culture was the least versatile of all the white-rot fungal cultures. It could completely decolorize only Remazol Brilliant yellow 3GL to an appreciable extent. In this regard it compares favourably with some other *G. Lucidum* strains that required 9–15 days to achieve substantial dye decolorization (Fu & Viraraghavan 2001). Physiological differences among the four white-rot fungal cultures may account for differences in their decolorization abilities (Reddy 1995; Chander *et al.* 2004). The complex enzymatic system responsible for dye degradation and pattern of its expression may also vary among the white-rot fungi (Nagai *et al*. 2002; Boer *et al*. 2004; Mazmanci & Ünyayar 2005); however, the relative rates of decolorization for the four reactive dyes cannot be easily

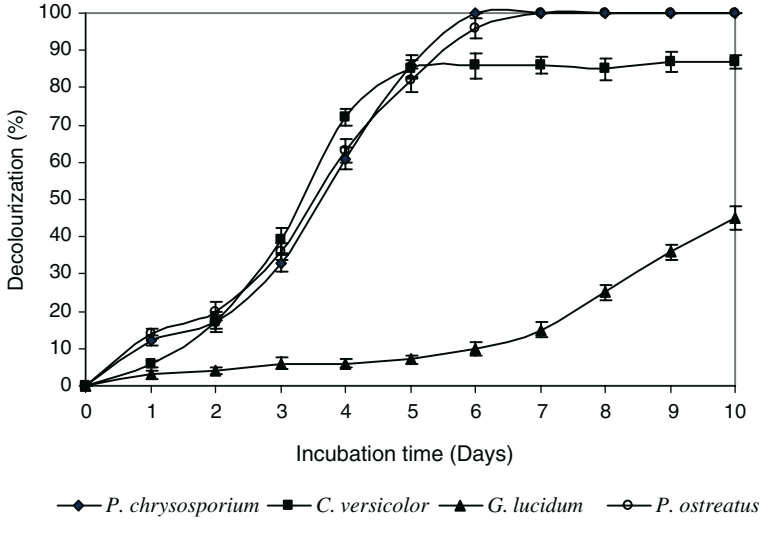

Figure 4. Decolorization of Cibacron Blue P-3RGR by four white rot fungi.

explained. Degradation of a dye involves aromatic ring cleavage, which is dependent on the identity of the ring substituents with the presence of phenolic, amino, acetamido, 2-methoxyphenol, or other easily biodegradable functional groups resulting in a greater extent of degradation (Spadaro *et al.* 1992; Chander *et al.* 2004). In the present study, different dyes were found to be decolorized to different extents by the individual fungal cultures. Also, different decolorization patterns for individual dyes were observed for the four white-rot fungi cultures. Dyes that are structurally similar may still be differently biodegraded by the white-rot fungi (Knapp *et al.* 1997). However, overall complexity of structure alone is not an indicator of the difficulty of decolorization of a particular dye (Toh *et al.* 2003; Maas & Chaudhari 2005).

Conclusions

White rot fungal cultures isolated in Pakistan showed reasonably good potential for decolorization of relatively less studied reactive textile dyestuffs. The results of the study will form the basis for development of cost-effective and robust indigenous technology for bioremediation of these reactive dyes and dye-based effluents.

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