J Ind Microbiol Biotechnol (2004) 31: 94–97
DOI 10.1007/s10295-004-0116-y

SHORT COMMUNICATION

M. Chander · D. S. Arora · H. K. Bath

Biodecolourisation of some industrial dyes by white-rot fungi

Received: 25 June 2003 / Accepted: 22 December 2003 / Published online: 3 February 2004

Abstract Eight white-rot fungal strains were screened for biodecolourisation of eight dyes commercially employed in various industries. Decolourisation of Poly R 478 was used as a standard to ascertain the dye-decolourisation potential of various fungi. All the fungi tested significantly decolourised Poly R 478 on solid agar medium. When tested in a nitrogen-limited broth medium, *Dichomitus squalens*, *Irpex flavus*, *Phlebia* spp. and *Polyporus sanguineus* were better industrial dye decolourisers than *Phanerochaete chrysosporium*.

Keywords Biodecolourisation · Industrial dyes · Ligninolytic enzymes · White-rot fungi · Textile effluents

Introduction

A great variety of synthetic dyes are used for textile dyeing and other industrial applications. The structural diversity of dyes is attributed to the presence of different chromophoric groups, such as azo, triphenyl methane and phthalocyanine [5, 11]. Such synthetic dyes are released into the environment from textile and other dyestuff industries. Existing wastewater treatment systems or natural microflora are unable to completely remove recalcitrant dyes from effluents [12, 16]. Anaerobic degradation of such dyes by bacteria has been reported to produce carcinogenic and/or mutagenic products [17, 20]. Further, anaerobic bacterial degradation of these dyes requires their intracellular uptake, whereas fungal systems cause dye degradation via extracellular enzymes [4, 8]. In fungi, especially white-rot organisms, their better biodegradation potential has been attributed to ligninolytic enzymes [1, 9, 14, 18], which are capable of non-specifically breaking down heterogeneous macromolecular lignin structures. This lignin biodegradation potential of fungi could prove an efficient method of biocleaning of industrial dyes and related wastes. Some work has been done in the field of fungal degradation of these dyes [2, 15, 19]. Previous studies by our team credited *Dichomitus squalens*, *Irpex flavus* and *Phlebia* spp. as better laboratory dye decolourisers than the much-studied *Phanerochaete chrysosporium* [6]. The present study aims at ascertaining the role of these white-rot fungi in decolourisation of substitutive industrial dyes, which are preferred for their economy and finishing properties. Thus, this study is an effort to widen the application of white-rot fungi in biocleaning of dye and dye-based effluents.

M. Chander · D. S. Arora (✉) · H. K. Bath
Microbial Technology Laboratory,
Department of Microbiology,
Guru Nanak Dev University,
143 005 Amritsar, Punjab, India
E-mail: daljit_02@yahoo.co.in
Tel.: +91-183-225880209
Fax: +91-183-225881920

Materials and methods

Chemicals

Poly R 478 was procured from Sigma. Industrial dyes of disperse, basic and acid complex were received from Colourtex India (Mumbai, India), Ornet Intermediates (Vatva, Ahmedabad, India) and Rathi Dye Chem (Pune, India), while all other chemicals were procured from Hi Media Chemicals (Mumbai, India).

Microorganisms

Eight white-rot fungal cultures: *Daedalea flavida* (MTCC 145), *Dichomitus squalens* (FP-105351-sp), *Irpex flavus* (MTCC 168), *Phanerochaete chrysosporium* (BKM-F 1767), *Phlebia brevispora* (HHB 7030), *Phlebia fascicularia* (FP-70880-sp), *Phlebia floridensis* (HHB 9905) and *Polyporus sanguineus* (MTCC 137) were selected for dye decolourisation. *Dichomitus squalens*, *Phanerochaete chrysosporium* and *Phlebia* spp. were a gift from T.W. Jefferies (Forest Products Laboratories, Madison, Wis.) while the remaining cultures were procured from the Microbial Type Culture Collection

(MTCC), Institute of Microbial Technology, Chandigarh, India. Cultures were maintained on yeast extract glucose agar (YGA) and stored at 4°C.

Primary screening for dye decolourisation

Primary screening for dye decolourisation was carried out on nitrogen-limited mineral salt agar medium containing 10μg/ml Poly R 478. Plates were inoculated in the centre with an 8 mm disc of a fungus grown on YGA medium for 6–8 days. The relative dye decolourisation potential of each white-rot fungus was recorded on the basis of clear zones formed around the mycelial disc after incubation at its respective optimum growth temperature (37 ± 0.5°C for *Daedalea flavida, Phanerochaete chrysosporium, Polyporus sanguineus* and 25 ± 0.5°C for *Dichomitus squalens*, *I. flavus* and *Phlebia* spp.) after 8 days of incubation.

Secondary screening for dye decolourisation in nitrogen-limited broth medium

As all the fungi could decolourise most of the dyes tested in agar medium, they were grown further on mineral salts broth (MSB), containing KH_2PO_4 2 g, $MgSO_4$ 0.5 g, $CaCl_2$ 0.1 g, glucose 10 g, ammonium tartrate 0.2 g, thiamine hydrochloride 0.010 mg, 10ml trace element solution (nitrilotriacetic acid 1.5 g, $MnSO_4 \cdot H_2O$ 0.48 g, NaCl 1 g, $CoSO_4 \cdot 7H_2O$ 10 mg, $FeSO_4 \cdot 7H_2O$ 10 mg, $ZnSO_4 \cdot 7H_2O$ 10 mg, $CuSO_4 \cdot 5H_2O$ 8 mg, H_3BO_3 8 mg, $Na_2MoO_4 \cdot 2H_2O$ 8 mg, distilled H_2O 1,000 ml) in 1,000 ml distilled water and the dye under test at a concentration of 20 μg/ml. MSB (20 ml) in a 100 ml flask was autoclaved at 15 psi for 15 min and inoculated with two 8 mm fungal discs obtained from a 6- to 8-day-old culture on YGA plates and incubated at the optimum growth temperature. On the 8th day of incubation, biomass was harvested by filtering the culture through Whatman no. 1 filter paper to remove mycelium and obtain a cell-free enzyme extract (CFEE). Each dye was added separately to 20 ml CFEE in a sterile 100 ml conical flask to a final concentration of 20μg/ml. The mixture was then incubated at the optimum growth temperature and decolourisation of the dye was assayed by measuring the decrease in absorbance at the λ_{max} after a period of 0, 1, 2, 3, 5, 24 and 48 h as follows:

$$\%\ \text{decrease in absorbance} = \frac{\text{optical density at zero hour} - \text{optical density at respective hour of incubation}}{\text{optical density at zero hour}} \times 100 \quad (1)$$

Results

Primary screening of fungi for dye decolourisation on agar plates

All fungi tested decolourised Poly R 478. *Daedalea flavida* was the most efficient as it completely decolourised the dye in agar plates in 12 days, followed by *Polyporus sanguineus*, *I. flavus*, *Phlebia floridensis* and *Phanerochaete chrysosporium*, which took 15 days. *Phlebia brevispora*, *Phlebia fascicularia* and *Dichomitus squalens* decolourised the dye in 17, 18 and 19 days, respectively (Table 1). All the fungi showed luxuriant growth during first 8 days of incubation and then started dye decolourisation from the centre of the agar plates to the periphery.

Table 1 Comparative decolourisation of Poly R 478 by white-rot fungi on agar plates

Organism	Time taken for complete decolourisation (days)
Daedalea flavida	12
Dichomitus squalens	19
Irpex flavus	15
Phanerochaete chrysosporium	15
Phlebia brevispora	17
Phlebia fascicularia	18
Phlebia floridensis	15
Polyporus sanguineus	15

Secondary screening of fungi for their dye decolourisation potential in broth medium

Nitrogen-limited broth medium was used to obtain CFEE from 8-day-old cultures of each fungus. These extracts decolourised the dyes to a variable extent. Most were decolourised from 70–100% in the first 2–5 h, with a marginal increase upon prolonged incubation.

Poly R 478 decolourisation

Dichomitus squalens and *Phlebia floridensis* caused decolourisation up to 98% and 89%, respectively, in 5 h, increasing to 100% by 48 h. Similarly, the extracts obtained from *Phlebia fascicularia* and *Phanerochaete chrysosporium* caused colour loss of 89% and 83%, respectively, in 2 and 5 h. *I. flavus*, *Polyporus sanguineus* and *Phlebia brevispora* caused 75–80% colour loss in 3–4 h, while *Daedalea flavida* was poorest in causing colour loss; 17.6% in 2 h with no further decolourisation upon prolonged incubation (Table 2).

Decolourisation of coracryl dyes

Coracryl green was efficiently decolourised, between 80% and 100% in the first 5 h by the majority of the fungi. *I. flavus* was the most efficient, causing 100% decolourisation in 5 h, while *Dichomitus squalens*, *Phlebia fascicularia* and *Phlebia floridensis* required 24–48 h for complete decolourisation. *Daedalea flavida* moderately decolourised coracryl green—up to 59% in 3 h with no further increase. On the other hand, *Daedalea flavida* was able to decolourise coracryl red (65% in 3 h), which was decolourised only to a limited extent (2–28%) by the remaining fungi, irrespective of the reaction time. *Phanerochaete chrysosporium* was very poor, with only 2% decolourisation after 48 h, while *Polyporus sanguineus* did not decolourise coracryl red at all. However, *Polyporus sanguineus* was the best

Table 2 Decolourisation of industrial dyes by cell-free enzyme extract (CFEE) from 8-day fungal cultures

Dye	Maximum % decolourisation (hours of incubation) by:							
	Daedalea flavida	*Dichomitus squalens*	*I. flavus*	*Phanerochaete chrysosporium*	*Phlebia brevispora*	*Phlebia fascicularia*	*Phlebia floridensis*	*Polyporus sanguineus*
Poly R 478	17.6 (2)	100 (48)	90 (3)	83 (5)	75 (5)	89 (2)	100 (48)	76 (5)
Coracryl green	59 (3)	100 (24)	100 (5)	97 (5)	96 (48)	100 (48)	100 (48)	82 (5)
Coracryl yellow	7.7 (30	26 (5)	–[a]	29 (5)	16 (5)	–	6 (3)	36 (2)
Coracryl red	65 (3)	12 (3)	8 (1)	2 (2)	5 (5)	8 (2)	28 (2)	–
Reactive red	2 (1)	94 (5)	91 (2)	91 (2)	87 (5)	95 (5)	91 (48)	70 (2)
Reactive yellow	58 (2)	68 (2)	–	62 (5)	63 (3)	–	24 (3)	48 (2)
Rathidol baurdeaux	18 (3)	92 (3)	98 (5)	91 (2)	85 (3)	77 (3)	91 (24)	77 (3)
Coloderm black	48 (2)	53 (5)	–	50 (5)	76 (48)	–	–	–
Coralene blue	43 (2)	26 (1)	–	47 (3)	76 (1)	–	–	–

[a]No decolourization in 48 h

organism for decolourisation of coracryl yellow (36% in 2 h) while *Phlebia floridensis* was the poorest, effecting only 6% decolourisation. *I. flavus* and *Phlebia fascicularia* did not decolourise coracryl yellow at all (Table 2).

Decolourisation of reactive dyes

Though most of the fungi could cause 90–95% decolourisation in up to 5 h, except *Polyporus sanguineus*, which caused a maximum of 70% decolourisation in 2 h (Table 2). *Phanerochaete chrysosporium* and *I. flavus* decolourised reactive red up to 91% in 2 h. *Daedalea flavida* was the poorest in this case, causing a negligible colour loss of only 2% even at 48 h. Reactive yellow, on the other hand, was relatively resistant and suffered decolourisation of 24–68% in 2–5 h. *Phlebia brevispora* and *Phanerochaete chrysosporium* were equally effective, causing almost equal colour loss of 63% in 3 h. *I. flavus* and *Phlebia fascicularia* did not decolourise reactive yellow (Table 2).

Decolourisation of rathidol baurdeaux

Rathidol baurdeaux was superbly decolourised by all the fungi tested. *I. flavus* removed 98% colour in 5 h, followed by *Dichomitus squalens* and *Phanerochaete chrysosporium*, with colour loss of 92 and 91% in 3 and 2 h, respectively (Table 2). *Phlebia floridensis* gave a similar result in 24 h despite 81% colour removal in the first 5 h. *Phlebia brevispora* and *Polyporus sanguineus* showed a colour loss of 85% and 77%, respectively, in 3 h, while *Daedalea flavida* was the least effective, causing a colour loss of 18% in 3 h.

Decolourisation of coloderm black and coralene blue

Only four of the fungi tested: *Phlebia brevispora*, *Dichomitus squalens*, *Phanerochaete chrysosporium* and *Daedalea flavida*, decolourised coloderm black and coralene blue; *Phlebia brevispora* was the best for both dyes, causing a maximum colour loss of 76%. However, the former was decolourised in 48 h while coralene blue was decolourised in just 1 h (Table 2).

Discussion

In recent years, white-rot fungi have been successfully tested for dye decolourisation [2, 13] and related wastewater treatment systems, such studies are based primarily on *Phanerochaete chrysosporium* and *Trametes versicolor* [7, 11]. However, the present study shows that certain other white-rot fungi are better decolourisers than *Phanerochaete chrysosporium* for one or all of the industrial dyes tested. Moreover, in some earlier studies, dye decolourisation has been attributed to mere adsorption to mycelia [5], posing problems for quantitative decolourisation and meaningful assessment of dye removal potential. To minimise this problem, the present study used CFEE of optimally grown fungi to evaluate biocleaning potential. The present study focused on decolourisation of relatively less-studied, but widely used, industrial dyes by other white-rot fungi [2, 4].

All the fungi tested showed relatively good growth and caused dye decolourisation to variable extents on agar plates, although an earlier study had reported certain dyes to restrict fungal colony diameter [3]. When tested in MSB, Poly R 478 was efficiently decolourised by all the fungi tested, with *Dichomitus squalens* and *Phlebia floridensis* causing complete decolourisation in 48 h—20% higher than *Phanerochaete chrysosporium*. Fungi that efficiently decolourised Poly R 478 on agar plates equally efficiently decolourised industrial dyes on MSB, except *Daedalea flavida*, which was best on agar plates but lagged behind in removal of most dyes tested in broth medium, except coracryl red. The dye concentrations used in MSB were higher, which may have been more toxic for *Daedalea flavida*.

Coloderm black, a leather tanning dye and persistent colouring agent, was resistant to enzymatic attack by *I. flavus*, *Phlebia fascicularia*, *Phlebia floridensis* and *Polyporus sanguineus*, while *P. brevispora* and *Dichomitus squalens* were relatively better decolourisers.

Rathidol baurdeaux was susceptible to CFEE, while coloderm black and coralene blue were highly colourfast and resistant to decolourisation by most of the fungal extracts. In general, most colour removal was achieved in the first 5 h, with no further appreciable decolourisation upon prolonged incubation. *Dichomitus squalens* emerged as the most efficient dye decolouriser, followed by *I. flavus* and *Phlebia* spp. Furthermore, the dyes tested have diverse structures and different chromophore groups, hence no single strain could decolourise them all.

Ligninolytic white-rot fungal systems have been implicated in decolourisation of various dyes and wastewaters [6, 9, 13, 15, 19]. The present study confirms that the inherent dye decolourisation potential of less well known white-rot fungi is better than the oft-studied strains. The results using CFEE support biological decolourisation; however, some studies report non-biological colour removal by physical methods [10]. The much greater and more extensive industrial dye decolourisation potential of *Dichomitus squalens*, *I. flavus*, *Phlebia* spp. and *Polyporus sanguineus* suggests a role for these fungi in treatment and biocleaning of dye-based industrial wastewaters. Further studies are underway into treatment patterns of effluents containing mixtures of dyes, and identification of the products formed.

Acknowledgement The authors are grateful to the Council of Scientific and Industrial Research, New Delhi, India for conferring a senior research fellowship on Mr. Mukesh Chander [Grant No: 9/254 (134)/2002–EMR].

References

1. Arora DS, Chander M, Gill PK (2002) Involvement of lignin peroxidase, manganese peroxidase and laccase in degradation and selective ligninolysis of wheat straw. Int Biodeterior Biodegradr 50:115–120
2. Bakshi RK, Gupta KG, Sharma P (1999) Enhanced biodecolourisation of synthetic textile dye effluent by *Phanerochaete chrysosporium* under improved culture conditions. World J Microbiol Biotechnol 15:507–509
3. Bragulat MR, Abarca ML, Bruguera MT, Cabanes FJ (1991) Dyes as fungal inhibitors: effect on colony diameter. Appl Environ Microbiol 57:2777–2780
4. Buckley KF, Dobson DW (1998) Extracellular ligninolytic enzyme production and polymeric dye decolourisation in immobilized cultures of *Chrysosporium lignorum* CL 1. Biotechnol Lett 20:301–306
5. Cripps C, Bumpus JA, Aust SD (1990) Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. Appl Environ Microbiol 56:1114–1118
6. Gill PK, Arora DS, Chander M (2002) Biodecolourisation of azo and triphenylmethane dyes by *Dichomitus squalens* and *Phlebia* spp. J Ind Microbiol Biotechnol 28:201–203
7. Heinflig A, Bergbauer M, Szewzyk U (1997) Biodegradation of azo and phthalocyanine dyes by *Trametes versicolor* and *Bjerkandera adusta*. Appl Microbiol Biotechnol 48:261–266
8. Heinflig A, Martinez MJ, Martinez AT, Bergbauer M, Szewzyk U (1998) Transformation of industrial dyes by manganese peroxidases from *Bjerkandera adusta* and *Pleurotus eryngii* in a manganese independent reaction. Appl Environ Microbiol 64:2788–2793
9. Moreira MT, Mieglo I, Feijoo G, Lema JM (2000) Evaluation of fungal strains in the decolourisation of synthetic dyes. Biotechnol Lett 22:1499–1503
10. Namasivayam C, Kavitha D (2002) Removal of congo red from water by adsorption onto activated carbon prepared from coir pith, an agricultural solid waste. Dyes Pigm 54:47–58
11. Ollika P, Alhonmaki K, Leppanen VM, Glumoff T, Raijola T, Suominen I (1993) Decolourisation of azo, triphenylmethane, heterocyclic and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. Appl Environ Microbiol 59:4010–4016
12. Pagga U, Brown D (1986) The degradation of dyestuffs: II. Behaviour of dyestuffs in aerobic biodegradation tests. Chemosphere 22:479–491
13. Reddy CA (1995) The potential of white-rot fungi in the treatment of pollutants. Curr Opin Biotechnol 6:320–328
14. Rivela I, Couto SR, Sanroman A (2000) Extracellular ligninolytic enzyme production by *Phanerochaete chrysosporium* in a new solid state bioreactor. Biotechnol Lett 22:1443–1447
15. Robinson T, Chandran B, Nigam P (2001) Studies on the decolourisation of an artificial textile effluent by white-rot fungi in N-rich and N-limited media. Appl Microbiol Biotechnol 57:810–813
16. Shaul GM, Holdsworth TJ, Dempsey CR, Dostal KA (1991) Fate of water soluble azo dyes in activated sludge process. Chemosphere 22:107–119
17. Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56:69–80
18. Swamy J, Ramsay JA (1999) Effects of Mn^{2+} and NH_4^+ concentrations on laccase and manganese peroxidase production and amaranth decolourisation by *Trametes versicolor*. Appl Microbiol Biotechnol 51:391–396
19. Wesenberg D, Buchon F, Agathos SN (2002) Degradation of dye containing textile effluent by agaric white-rot fungi *Clitocybula dusenii*. Biotechnol Lett 24:989–993
20. Zimmerman T, Kulla HC, Leisinger T (1982) Properties of purified orange II azoreductase, the enzyme initiating azo dye degradation by *Pseudomonas* KF 46. Eur J Biochem 129:197–203