Journal of Industrial Microbiology & Biotechnology (2002) 28, 201–203
© 2002 Nature Publishing Group All rights reserved
1367-5435/02 $25.00

www.nature.com/jim

Biodecolourization of azo and triphenylmethane dyes by *Dichomitus squalens* and *Phlebia* spp.

PK Gill, DS Arora and M Chander

Microbial Technology Laboratory, Department of Microbiology, Guru Nanak Dev University, Amritsar, Punjab, 143 005 India

Nine white-rot fungal strains were screened for biodecolourization of brilliant green, cresol red, crystal violet, congo red and orange II. *Dichomitus squalens*, *Phlebia fascicularia* and *P. floridensis* decolourized all of the dyes on solid agar medium and possessed better decolourization ability than *Phanerochaete chrysosporium* when tested in nitrogen-limited broth medium.
Journal of Industrial Microbiology & Biotechnology (2002) **28,** 201–203 DOI: 10.1038/sj/jim/7000222

Keywords: dyes; biodegradation; white-rot fungi; ligninolytic enzymes

Introduction

Synthetic dyes are released into the environment from the textile and dyestuff industries [13]; azo dyes form the largest and most important group followed by triphenylmethane dyes. Some of these dyes are mutagenic and carcinogenic [3,11]. The waste water treatment systems used are unable to completely remove the recalcitrant dyes from the effluents [17,20]. Some triphenylmethane dyes have been found in the soil due to improper waste disposal [17]. Azo dyes are most resistant to microbial attack; however, ligninolytic enzymes of the white-rot fungus *Phanerochaete chrysosporium* degrade at least two azo dyes under aerobic conditions [7,14,18]. However, under anaerobic conditions, these dyes are reduced to aromatic amines that are also carcinogenic [22]. Dye decolourization by fungi has received less attention in comparison to bacteria. However, one of the major prerequisites for dye decolourization by bacteria is that the dye must first be taken up into the cells [12], whereas in fungal systems, dye decolourization is carried out entirely by extracellular enzymes [4]. Moreover, in bacterial degradation of azo dyes, biodegradation requires anaerobic conditions. Bacterial strains capable of degrading these dyes under aerobic conditions are usually specific towards a single azo dye [9,19,22]. This limits their practical applicability in the treatment of effluents emanating from dyestuff industries that contain mixtures of dyes. In recent years, attention has thus been directed towards fungal dye decolourization systems [15,16]. The present study is the first report on dye decolourization by *Dichomitus squalens* and *Phlebia* spp.

Materials and methods

Chemicals

The azo dyes, congo red and orange II (tropaeolin o), and the triphenylmethane dyes, brilliant green, cresol red and crystal violet, were selected for the present study. Cresol red and congo red were purchased from Hi Media Chemicals (Mumbai, India); crystal violet, orange II and brilliant green were purchased from Ranbaxy Laboratories (New Delhi, India), SD Fine Chemicals (Mumbai, India) and Qualigens Fine Chemicals (Mumbai, India), respectively.

Microorganisms

Nine white-rot fungal cultures, *Daedalea flavida* (MTCC 145), *D. squalens* (FP-105351-sp), *Irpex flavus* (MTCC 168), *Pha. chrysosporium* (BKM-F 1767), *Phlebia brevispora* (HHB 7030 sp), *P. fascicularia* (FP-70880 sp), *P. floridensis* (HHB-9905), *P. radiata* (MJL-1198-sp) and *Polyporus sanguineus* (MTCC 137) were selected for the present study. *D. squalens, Pha. chrysosporium* and *Phlebia* spp. were a gift from CR Bergmann (Forest Products Laboratories, Madison, WI). The remaining cultures were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (Chandigarh, India). The cultures were maintained on yeast extract glucose agar (YGA) slants and stored at 4°C.

Preliminary screening for dye decolourization

Preliminary screening for decolourization of different dyes was performed on nitrogen-limited mineral salts agar medium [1] containing dyes at a final concentration of 20 μM except for cresol red, which was added at a final concentration of 40 μM. The agar plates were inoculated at the center with mycelial discs (8 mm) taken from the periphery of 6- to 7-day-old fungal cultures grown on YGA plates. The plates were analyzed for dye decolourization after 8 days of incubation at 25±1°C.

Dye decolourization studies in broth culture

On the basis of results obtained from the agar plate method, four cultures including *Pha. chrysosporium* were selected for further studies. Ten milliliters of mineral salts broth (MSB) was inoculated with two mycelial discs of the respective fungal cultures and after 6 days of growth, the dyes were added separately to each culture at

Correspondence: Dr DS Arora, Microbial Technology Laboratory, Department of Microbiology, Guru Nanak Dev University, Amritsar, Punjab 143 005, India
Received 12 July 2001; accepted 22 October 2001

Table 1 Dye decolourization by some white-rot fungi on solid media after 8 days of incubation

Organism	Dye				
	Brilliant green	Congo red	Cresol red	Crystal violet	Orange II
Da. flavida	++	+++	+	–	–
D. squalens	+++	++	+	+++	+
I. flavus	–	–	–	–	–
Pha. chrysosporium	–	++++	–	–	–
P. brevispora	+	–	+	+	–
P. fascicularia	++++	++++	++++	+++	++
P. floridensis	++++	++++	+	++++	++++
P. radiata	–	++	–	++++	+
Po. sanguineus	+	+	–	+	–

+, ++, +++, ++++ show degree of decolourization, (–) no decolourization.

the same concentration used for agar medium. The decolourization was monitored by recording the absorption spectra of each dye daily for 5 days.

Results

Dye decolourization pattern on agar plates

Different fungi showed a variable decolourization response; of the nine fungi, only *D. squalens*, *P. fascicularia* and *P. floridensis* decolourized all the dyes (Table 1). The latter two organisms were the best. While *Pha. chrysosporium*, the most studied white-rot fungus, was able to decolourize only congo red, *I. flavus* did not decolourize any of the dyes investigated.

Dye decolourization in broth cultures

In nitrogen-limited liquid cultures, extensive decolourization of all the dyes occurred. The only dye efficiently decolourized by *Pha. chrysosporium* was brilliant green; 98% of the initial colour was removed in just 2 h and complete decolourization was achieved in 48 h. *P. floridensis* and *P. fascicularia* caused 77% and 66% decolourization within the first 2 h, which reached 100% and 86%, respectively, in 48 h (Table 2). *P. floridensis*, in addition to causing a decrease in absorbance, also led to a hypochromic shift in absorbance from 626 to 416 nm. In the control, neither a shift nor a decrease in absorbance was observed. *D. squalens* was slower in its action but after 48 h, extensive decolourization set in, and by the end of 120 h, the absorbance decreased to undetectable levels.

D. squalens and *Phlebia* spp. caused extensive decolourization of crystal violet, resulting in a colour loss of more than 60% in 48 h and almost complete decolourization after 5 days. *Pha. chrysosporium*, however, did not cause more than 58% decolourization in 5 days (Table 2). *D. squalens* caused a shift in the absorbance maxima from 584 to 542 nm after 48 h of incubation.

Cresol red was most effectively decolourized by *P. fascicularia*, which caused its complete decolourization in 72 h while *D. squalens* gave a similar effect in 96 h. *P. floridensis* resulted in 81% colour removal while *Pha. chrysosporium* caused only 20% decolourization even after 5 days of incubation (Table 2).

The azo dye, orange II, was decolourized most efficiently by *P. floridensis* with 72% colour removal after 24 h and complete decolourization after 72 h. *D. squalens* and *P. fascicularia* were comparatively slower in bringing about complete colour loss, which was achieved after 5 days. *Pha. chrysosporium*, however, achieved only 53% colour removal. Complete decolourization of congo red was achieved only with *P. fascicularia*, while other organisms decolourized the dye at a slower rate, resulting in 84–98% removal in 5 days (Table 2). Congo red was also decolourized

Table 2 Dye decolourization by some white-rot fungi in liquid medium

Dye	Incubation period (h)	% Dye decolourization by			
		Pha. chrysosporium	*D. squalens*	*P. fascicularia*	*P. floridensis*
Brilliant green	2	93.5	0	75.5	77
	48	100	72	86.3	97
	120	–	100	98	100
Crystal violet	2	28.3	20.7	26.6	0
	48	56.6	60.2	65	75.5
	120	58.3	100	100	95.2
Cresol red	2	0	0	50.5	15.6
	48	10	45.6	96.8	50
	120	20	100	100	81.4
Congo red	2	0	0	0	0
	48	45	65	29.7	67.5
	120	84	96	100	98
Orange II	2	0	0	0	0
	48	44.4	32.4	19	96
	120	52.9	100	100	100

to a limited extent even in the control set. Marked changes in the absorbance maxima of the dye were observed with *P. floridensis*. Moreover, a considerable amount of congo red remaining, which adsorbed to the fungal mycelium, was clearly visible. This disappeared in some cultures on continued incubation beyond 5 days, whereas in others, it still remained even after 10 days. A very slight degree of dye adsorption was noted for cresol red and crystal violet, which was also reversed after 5 days of incubation. No adsorption was observed with orange II and brilliant green.

Discussion

In recent years, dye decolourization studies have centered around *Pha. chrysosporium* [5,6,18,21]. However, in the present study, *Pha. chrysosporium* did not decolourize any dye, except congo red, when added to solid agar medium. There are only a few reports on dye decolourization by *Pha. chrysosporium* on agar plates [8]. Most of the dyes restrict fungal growth. In the agar plate method, the dye has to be added before the initiation of growth and this could be one of the factors which restricts the dye decolourization potential of *Pha. chrysosporium*. In the present report, dye decolourization in broth cultures clearly revealed *D. squalens* and *Phlebia* spp. to be much more efficient than *Pha. chrysosporium* as these caused total or near total decolourization of all the dyes in 120 h. Although congo red was decolourized to 84% by *Pha. chrysosporium*, a part of it remained adsorbed to the mycelium, which is in consonance with earlier studies [7]. Such adsorption was also observed with other fungi but was reversed by the 5th day. Though congo red has been reported to be the most resistant azo dye [7], in the present study it was almost completely decolourized by *Phlebia* spp.

Pha. chrysosporium brought about complete and efficient decolourization of brilliant green only after 48 h and at a higher concentration than reported by previous workers [5]. Cresol red and crystal violet are completely decolourized by *Pha. chrysosporium* in 24 h [5] but the higher concentration used in the present study substantially reduced the rate of decolourization. In contrast, *D. squalens* and *Phlebia* spp. were able to carry out the decolourization at a higher concentration of crystal violet and cresol red (20 and 40 μM, respectively). A hypochromic shift in the absorbance maxima of brilliant green, crystal violet and congo red is in line with earlier studies on crystal violet [5,10]. Nonbiological decolourization, as evident from control studies (except for congo red), and pH change were not observed during the entire incubation period.

Ligninolytic enzymes have been implicated in the decolourization of dyes by white-rot fungi, and the fungal cultures used in the present study have been confirmed to have a better ligninolytic enzyme production potential as compared to *Pha. chrysosporium* [1,2]. The extensive dye-decolourizing abilities of *D. squalens* and *Phlebia* spp. suggest that they are promising fungal strains for the treatment of textile industry waste waters. Further work is needed to optimize the production of ligninolytic enzymes and dye-decolourizing ability of these fungal strains.

References

1 Arora DS and PK Gill. 2000. Laccase production by some white-rot fungi under different nutritional conditions. *Biores Technol* 73: 283–285.
2 Arora DS and PK Gill. 2001. Comparison of two assay procedures for lignin peroxidase. *Enzyme Microb Technol* 28: 602–605.
3 Au W, MA Butler, SE Bloom and TS Matmey. 1979. Further study of the genetic toxicology of gentian violet. *Mutat Res* 66: 103–112.
4 Buckley KF and DW Dobson. 1998. Extracellular ligninolytic enzyme production and polymeric dye decolourization in immobilized cultures of *Chrysosporium lignorum* CL1. *Biotechnol Lett* 20: 301–306.
5 Bumpus JA and BJ Brock. 1988. Biodegradation of crystal violet by the white-rot fungus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 54: 1143–1150.
6 Capalash N and P Sharma. 1992. Biodegradation of textile azo dyes by *Phanerochaete chrysosporium*. *World J Microbiol Biotechnol* 8: 309–312.
7 Cripps C, JA Bumpus and SD Aust. 1990. Biodegradation of azo dyes by *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 56: 1114–1118.
8 Heinfling A, M Bergbauer and U Szewzyk. 1997. Biodegradation of azo and phthalocyanine dyes by *Trametes versicolor* and *Bjerkandera adjusta*. *Appl Microbiol Biotechnol* 48: 261–266.
9 Kulla HG. 1981. Aerobic bacterial degradation of azo dyes. *FEMS Symp* 12: 371–385.
10 Kwasniewska K. 1985. Biodegradation of crystal violet (hexamethyl-*p*-rosaniline chloride) by oxidative red yeasts. *Bull Environ Contam Toxicol* 34: 323–330.
11 McCann J and BN Ames. 1975. Detection of carcinogens as mutagens in the *Salmonella* microsome test. Assay of 300 chemicals: discussion. *Proc Natl Acad Sci USA* 73: 950–954.
12 Mechsner K and K Whurmann. 1982. Cell permeability as a rate limiting factor in the microbial reduction of sulphonated azo dyes. *Eur J Appl Microbiol Biotechnol* 15: 123–126.
13 Meyer U. 1981. Biodegradation of synthetic organic colorants. *FEMS Symp* 12: 371–385.
14 Michaels GB and DL Lewis. 1985. Sorption and toxicity of azo and triphenylmethane dyes to aquatic microbial populations. *Environ Toxicol Chem* 4: 45–50.
15 Miyata N, K Iwahori and M Fujita. 1998. Manganese independent and dependent decolourization of melanoidin by extracellular hydrogen peroxide and peroxidases from *Coriolus hirsutus* pellets. *J Ferment Bioeng* 85: 550–553.
16 Moreira MT, I Mielgo, G Feijoo and JM Lema. 2000. Evaluation of fungal strains in the decolourization of synthetic dyes. *Biotechnol Lett* 22: 1499–1503.
17 Nelson CR and RA Hites. 1980. Aromatic amines in and near the Buffalo river. *Environ Sci Technol* 14: 1147–1149.
18 Ollikka P, K Alhonmaki, VM Leppanen, T Glumoff, T Raijola and I Suominen. 1993. Decolourization of azo, triphenylmethane, heterocyclic and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 59: 4010–4016.
19 Pagga U and D Brown. 1986. The degradation of dyestuffs: II. Behaviour of dyestuffs in aerobic biodegradation tests. *Chemosphere* 15: 479–491.
20 Shaul GM, TJ Holdsworth, CR Dempsey and KA Dostal. 1991. Fate of water soluble azo dyes in the activated sludge process. *Chemosphere* 22: 107–119.
21 Spadro JT, MH Gold and V Renghanathan. 1992. Degradation of azo dyes by lignin-degrading fungus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 58: 2397–2401.
22 Zimmerman T, HC Kulla and T Leisinger. 1982. Properties of purified orange II azoreductase, the enzyme initiating azo dye degradation by *Pseudomonas KF 46*. *Eur J Biochem* 129: 197–203.