

Industrial Dye Decolorization by Laccases from Ligninolytic Fungi

Elizabeth Rodríguez, Michael A. Pickard, Rafael Vazquez-Duhalt

Instituto de Biotecnología UNAM, Apartado Postal 510-3, Cuernavaca, Morelos, 62250 México

Received: 26 May 1998 / Accepted: 7 August 1998

Abstract. White-rot fungi were studied for the decolorization of 23 industrial dyes. Laccase, manganese peroxidase, lignin peroxidase, and aryl alcohol oxidase activities were determined in crude extracts from solid-state cultures of 16 different fungal strains grown on whole oats. All *Pleurotus ostreatus* strains exhibited high laccase and manganese peroxidase activity, but highest laccase volumetric activity was found in *Trametes hispida*. Solid-state culture on whole oats showed higher laccase and manganese peroxidase activities compared with growth in a complex liquid medium. Only laccase activity correlated with the decolorization activity of the crude extracts. Two laccase isoenzymes from *Trametes hispida* were purified, and their decolorization activity was characterized.

Industrial dyes can be released into the environment from two major sources: as effluents from synthesis plants and from dye-using industries, such as textile factories. It is estimated that between 10 and 15% of the total dye used in the dyeing process may be found in wastewater [6]. Several of these dyes are very stable to light, temperature, and microbial attack, making them recalcitrant compounds [28]. About 50% of the industrial colorants produced in the world are azo dyes [21]. These can be transformed to carcinogenic compounds under anaerobic conditions [11].

Ligninolytic fungi have been reported to degrade xenobiotic compounds [14]. The enzymatic systems that involve the enzymes of lignin degradation are able to transform polycyclic aromatic hydrocarbons [3, 18, 24, 40], chlorinated phenols [24, 32], PCB [33, 42], dioxins [8, 38], pesticides [19, 22], explosives [17, 37], dichloroaniline [2, 25], and dyes [15, 20, 31, 34].

The white rot fungus *Phanerochaete chrysosporium* is able to decolorize several industrial dyes [20] and polymeric dyes [15]. *P. chrysosporium* cultures, extracellular fluid, and purified lignin peroxidase were able to degrade crystal violet and six other triphenylmethane dyes by sequential N-demethylations [7]. Azo dyes Orange II, Tropaeolin O, and Congo Red, and the heterocyclic dye Azure B were decolorized by cultures of *P. chrysosporium* [12]. However, crude lignin peroxidase decolorized all the dyes except Congo Red, indicating

that other enzymes must be involved in the degradation of this azo dye [12]. The role of purified lignin peroxidase in the decolorization of several azo dyes has been clearly demonstrated [30]. The different isoenzymes of lignin peroxidase produced by *P. chrysosporium* are able to decolorize several dyes with different chemical structures, including azo, triphenylmethane, heterocyclic, and polymeric dyes [27]. This decolorization was enhanced by the presence of veratryl alcohol. Manganese peroxidase from *P. chrysosporium* was also able to decolorize several azo dyes in vitro, and with both enzymes the decolorization rate was dependent on the chemical structure of the dye [29]. Dyes such as Poly R [16] and Azure B [1] have been proposed as a standard assay for determination of lignin peroxidase activity. In *P. chrysosporium* cultures, dye decolorization is not a one-step oxidation process, as several dyes are extensively mineralized [36].

Other ligninolytic fungi have shown the capacity for dye decolorization. *Pleurotus ostreatus* decolorized a polymeric dye, Poly-B411, but only when the fungus was previously cultured in lignin-containing media [31]. A 73-kDa peroxidase from *P. ostreatus* has been shown to be involved in Remazol Brilliant Blue decolorization [34]. Congo Red is readily decolorized by cultures of *Pleurotus ostreiformis* [13], and laccases from *Trametes versicolor* can use Remazol Brilliant Blue as a mediator in the oxidation of model lignin compounds [4].

The mechanism of azo dye oxidation by peroxidases

such as lignin peroxidase probably involves the oxidation of the phenolic group to produce a radical at the carbon bearing the azo linkage. Then water attacks this phenolic carbon to cleave the molecule producing phenyldiazene. The phenyldiazene can be oxidized by a one-electron reaction generating N_2 [10, 35]. For laccase oxidation of phenolic azo dyes, a similar mechanism has been proposed [9].

Approximately 10,000 different dyes and pigments are produced annually worldwide and used extensively in the dye and printing industries. It is estimated that about 10% are lost in industrial effluents [44]. Industry uses synthetic dyes with a great variety of chemical structures; thus, a biocatalyst for decolorization should be able to degrade dyes of diverse structures. In this work, 16 strains of ligninolytic fungi were examined for the decolorization of 23 industrial dyes, and an attempt was made to correlate dye decolorization with enzyme production. Two forms of laccase from *Trametes hispida*, shown to be involved in the decolorization reaction, were purified and their kinetic properties were determined.

Materials and Methods

Fungal strains. *Bjerkandera adusta* 4312, 7308, 8258; *Pleurotus ostreatus* 7964, 7972, 7980, 7988, 7989, 7992; *Phanerochaete chrysosporium* 3541, 3642; *Sporotichum pulverulentum* 4521; *Trametes hispida* 8260 and *Trametes versicolor* 8272 were obtained from University of Alberta Mold Herbarium, Edmonton, Canada. *Pleurotus ostreatus* IE8 was obtained from the Ecology Institute, Xalapa, Mexico and *Phanerochaete chrysosporium* ATCC 24725 was from the American Type Culture Collection, Rockville Pike, MD. All fungi were maintained on potato dextrose agar plates (Difco).

Chemicals. Veratryl alcohol, 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), and sodium malonate were purchased from Aldrich (Milwaukee, WI). Orisol dyes were obtained from Colorfran S.A. (Monterey, Mexico) and the other industrial dyes were obtained from BASF (Ludwigshafen, Germany). Mineral salts were obtained from J.T. Baker (Phillipsburg, NJ), and glucose, yeast extract, and malt extract were purchased from Difco Laboratories (Detroit, MI).

Enzyme production. Inocula were prepared as follows: five 5-mm disks of fungal mycelium, excised from agar plates, were inoculated in each 125-ml flask containing 50 ml of glucose-malt extract-yeast extract medium. The culture medium contained, per liter, 10 g of glucose, 2 g of yeast extract, 3.5 g of malt extract, 2 g KH_2PO_4 , 0.5 g $MnSO_4 \cdot 7H_2O$, and 1 ml of trace metals solution. The trace metals solution contained, per liter, 0.14 mg of $ZnSO_4 \cdot 7H_2O$, 0.29 mg of $CoCl_2 \cdot 6H_2O$, 0.50 mg of $FeSO_4 \cdot 7H_2O$. After 6 days of incubation in shaken flasks at 28°C, the fungal growth in liquid glucose-malt medium reached 2.02 (± 0.20) g/L of dry biomass, and 20 ml of this culture was used to inoculate 50 g of wet whole cereal grain at 30°C. After 20 days of mycelial growth in solid state fermentation, extracellular enzymes were extracted by washing three times with 100 ml of 60 mM sodium phosphate buffer, pH 6.0. The combined extracts were filtered and assayed for enzyme activities.

Enzyme assays. Laccase activity was determined spectrophotometrically as the absorbance increase at 436 nm of 0.5 mM ABTS in 100 mM

Table 1. Dye decolorization by fungal cultures on agar medium and in vitro by extracellular crude extracts from fungal cultures grown on oat grain

Dye	Metabolic decolorization		Enzymatic decolorization	
	<i>P. chrysosporium</i> (ATCC-24725)	<i>P. ostreatus</i> (IE8)	<i>P. ostreatus</i> (IE8)	<i>T. hispida</i> (8260)
BASF				
Acid black 194	+	+	+	+
Acid blue 185	-	+	+	+
Direct black 22	NA ^a	NA	-	+
Disperse blue 56	+	+	-	+
Disperse blue 79	NA	NA	-	+
Disperse orange 30	-	-	-	-
Disperse yellow 54	-	+	-	-
Disperse red 161	-	-	-	-
Reactive blue 19	-	+	-	+
Reactive blue 158	-	+	+	+
Reactive red 141	-	-	-	-
Reactive red 180	-	-	-	-
Reactive yellow 84	-	-	-	-
Sulfur black 1	+	+	-	+
Vat blue 6	+	+	-	-
Vat red 10	-	+	-	-
Vat yellow 46	-	+	-	-
COLORFAN				
Orisol black 2V	NA	NA	-	+
Orisol blue BH	+	+	+	+
Orisol orange S	-	-	-	-
Orisol scarlet 4BS	-	-	-	-
Orisol turquoise JL	-	+	+	+
Orisol yellow 4JLZ	-	-	-	-

^a Not assayed.

sodium acetate buffer, pH 4.5 ($\epsilon_{436} = 29.30 \text{ mm}^{-1} \text{ cm}^{-1}$) [26]. Aryl alcohol oxidase (AAO) activity was estimated by the oxidation of 5 mM veratryl alcohol (3,4-dimethoxybenzyl alcohol) to veratraldehyde in 100 mM sodium phosphate buffer, pH 6.0 ($\epsilon_{310} = 9.30 \text{ mm}^{-1} \text{ cm}^{-1}$) [26]. Manganese peroxidase (MnP) activity was assayed by the oxidation of 1 mM $MnSO_4$ in 50 mM sodium malonate, pH 4.5, in the presence of 0.1 mM H_2O_2 . Manganic ions, Mn^{+3} , form a complex with malonate, which absorbs at 270 nm ($\epsilon_{270} = 11.59 \text{ mm}^{-1} \text{ cm}^{-1}$) [41]. Lignin peroxidase activity was determined as the oxidation of 4 mM veratryl alcohol to veratraldehyde in 20 mM succinate buffer pH 4.0, in the presence of 0.4 mM of H_2O_2 [39]. One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 μmol of substrate min^{-1} . Decolorization activity was determined by measuring the decrease of the dye absorbance at their maximum visible absorbance wavelength. Dye concentration in the reaction mixture was adjusted to 1.0 absorbance unit at the maximum wavelength in the visible spectrum.

Laccase purification. Twenty-day-old cultures of solid-state fermentation of *Trametes hispida* 8260 on oats were each extracted three times with 100 mM phosphate buffer (pH 6.0), and the extracellular liquid was filtered and concentrated by ultrafiltration (Amicon PM10). The concentrated extract was applied to an anion exchange column (diethylaminoethyl cellulose DE52, Whatman). The column was equilibrated with 10 mM sodium phosphate buffer (pH 6.0) and eluted with a 0–0.6 M NaCl gradient in the same buffer. This column served to remove the majority

Table 2. Enzymatic activities and dye decolorization^a of crude extracts from *Pleurotus ostreatus* IE8 grown in different media

Enzymatic activity	Liquid		Wheat		Oat	
	U/flask	U/mg protein	U/flask	U/mg protein	U/flask	U/mg protein
Laccase	410	15	2462	106	3052	181
Manganese peroxidase	ND ^b	ND	19	1	17	2
Aryl alcohol oxidase	ND	ND	ND	ND	ND	ND
Lignin peroxidase	ND	ND	ND	ND	ND	ND
Reactive blue 158	102	11	2244	96	2418	149
Acid blue 185	32	4	654	28	684	42
Acid black 194	34	4	78	3	126	7

^a Units for dye decolorization activity were estimated as the $\Delta A/\text{min}$ at the maximum visible absorbance (see Materials and Methods).

^b ND, not detected.

of the pigment. Fractions containing laccase activity were pooled, concentrated, and dialyzed by ultrafiltration. The enzyme was then applied to a Sephadex-G100 column (Sigma) and eluted with 60 mM sodium phosphate buffer pH 6.0. Fractions containing laccase activity were pooled and concentrated. The enzyme was applied a second time on a DE52 column under the same conditions to remove any residual pigment. Fractions with laccase activity were concentrated and applied to an Econo-Pac Q, strong anion exchanger (Bio-Rad), eluted with a gradient from 0 to 100% of 1 M NaCl in a 10-mM sodium phosphate buffer pH 6.0. Two peaks containing laccase activity were detected and called laccase I and II according to elution time. Laccase I and II showed specific activities of 168 U/mg and 170 U/mg, respectively.

Analytical procedures. Protein concentration was determined by the Bio-Rad protein assay. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels was performed by the method of Laemmli [23] with lysozyme (14.3 kDa), β -lactalbumin (18.4 kDa), carbonic anhydrase (29 kDa), ovalbumin (43 kDa), bovine serum albumin (68 kDa), phosphorylase B (97.4 kDa), myosin (H-chain; 200 kDa) as molecular mass standards (Gibco BRL).

Results

Cultures of the white-rot fungi *Phanerochaete chrysosporium* (ATCC 24725) and *Pleurotus ostreatus* (IE8), growing on agar complex medium containing one of 23 industrial dyes tested, showed that *P. ostreatus* is able to decolorize 12 of 23 industrial dyes in vivo, while *P. chrysosporium* decolorized only 5 dyes (Table 1). These industrial dyes were selected on the basis of their stability to a range of pH (pH 3–11), thermostability, and stability under culture conditions in noninoculated flasks. This decolorization capacity was associated with extracellular enzymes; nevertheless, the crude extracellular extracts from *P. ostreatus* were able to decolorize only five dyes (Table 1), showing that other enzymatic mechanisms could be involved in the dye decolorization in vivo experiments. Extracellular crude extracts from *Trametes hispidia* grown on oat grain were able to decolorize in vitro 11 of 23 industrial dyes (Table 1).

With the aim of finding higher decolorization activity, and because extracellular ligninolytic enzymes have

Table 3. Enzymatic activities of crude extracts from solid cultures of different fungi grown on oat grains

Strains	Volumetric activity (U/L) ^a		
	Laccase	Mn-peroxidase	Veratryl alcohol oxidase
<i>B. adusta</i> (4312) ^b	ND ^c	ND	54–160
<i>B. adusta</i> (7308)	5–7	66–70	ND
<i>B. adusta</i> (8258)	6–18	126–226	ND
<i>P. ostreatus</i> (7964)	83–181	59–75	ND
<i>P. ostreatus</i> (7972)	151–223	21–81	ND
<i>P. ostreatus</i> (7980)	109–421	47–61	24–97
<i>P. ostreatus</i> (7988)	235–588	77–157	ND
<i>P. ostreatus</i> (7989)	287–427	97–108	41–42
<i>P. ostreatus</i> (7992)	134–215	78–253	ND
<i>P. ostreatus</i> (IE8)	403–1272	49–67	ND
<i>P. chrysosporium</i> (3541)	ND	ND	ND
<i>P. chrysosporium</i> (3642)	ND	ND	ND
<i>P. chrysosporium</i> (ATCC-24725)	ND	ND	ND
<i>S. pulverulentum</i> (4521)	ND	ND	ND
<i>T. hispidia</i> (8260)	1184–1766	78–99	ND
<i>T. versicolor</i> (8272)	86–1042	39–96	ND

^a Activity range from three independent replicates. Volumetric activity found in 300 ml extract from solid-state fermentation after 20 days' growth on 50 g of oat grains.

^b Strain number from the University of Alberta Mold Herbarium.

^c ND, not detected.

been shown to be induced by growth on natural lignin substrates, *Pleurotus ostreatus* IE8, used as an indicator strain from preliminary experiments, was grown in media containing different ligninocellulosic substrates. Solid-state cultures with whole wheat and oats were compared with submerged culture in the complex medium. These natural substrates induced the production of the ligninolytic enzymes laccase and manganese peroxidase, and the crude extracellular extract showed higher dye decolorization capacity than that obtained from the complex medium (Table 2). *P. ostreatus* IE8, grown on oats,

Table 4. Dye decolorization by crude extracts from solid cultures of different fungi grown on oat grains

Strains	Decolorization activity ^a ($\Delta A \text{ min}^{-1} \text{ l}^{-1}$)				
	Reactive blue 158	Acid blue 185	Acid black 194	Orisol blue	Orisol turquoise
<i>B. adusta</i> (4312) ^b	ND ^c	ND	ND	ND	ND
<i>B. adusta</i> (7308)	940–1800	520–667	ND	ND	133–180
<i>B. adusta</i> (8258)	ND	ND	ND	ND	ND
<i>P. ostreatus</i> (7964)	1300–2220	440–580	ND	ND	560–1040
<i>P. ostreatus</i> (7972)	1040–3320	140–460	0–160	0–180	400–480
<i>P. ostreatus</i> (7980)	8080–8420	1440	0–100	0–400	2040–3000
<i>P. ostreatus</i> (7988)	4180–10160	1580–3500	0–360	180–600	1800–6060
<i>P. ostreatus</i> (7989)	3860–6660	880–1960	0–460	160–320	1060–2580
<i>P. ostreatus</i> (7992)	1580–1820	60–600	0–40	0–260	0–780
<i>P. ostreatus</i> (IE8)	1060–9500	1160–2500	130–500	650–900	1200–2300
<i>P. chrysosporium</i> (3541)	ND	ND	ND	ND	ND
<i>P. chrysosporium</i> (3642)	ND	ND	ND	ND	ND
<i>P. chrysosporium</i> (ATCC-24725)	ND	ND	ND	ND	ND
<i>S. pulverulentem</i> (4521)	ND	ND	ND	ND	ND
<i>T. hispida</i> (8260)	14020–21700	2640–12840	420–740	240–780	2840–3720
<i>T. versicolor</i> (8272)	3480–14540	400–1220	0–840	360–840	480–900

^a Decolorization activity is estimated as the decrease in absorbance at the maximum visible wavelength for each dye.

^b Strain number from the University of Alberta Mold Herbarium.

^c ND, not detected.

showed the highest volumetric and specific enzyme production and dye decolorization activities. Laccase activity production was 7.5 times higher in oat cultures than in liquid cultures, and the decolorization activity against Reactive blue 158 from solid phase oat extracts was 23 times higher than that from complex medium culture supernatant. No lignin peroxidase or aryl alcohol oxidase activity was detected in any of the culture supernatants of *P. ostreatus* IE-8.

After this, all fungi were grown on oats in solid-state fermentation. Extracellular extracts were tested for enzyme production (Table 3) and dye decoloration (Table 4). Lignin peroxidase, manganese peroxidase, laccase, and veratryl alcohol oxidase activities were determined in the crude extracts from all 16 fungal strains. All strains of *P. ostreatus* were active to various levels in decolorizing the five dyes tested, but *T. hispida* showed the highest volumetric activity. *Bjerkandera adusta* strains showed high manganese peroxidase but low laccase and decolorizing activity, and *Phanerochaete chrysosporium*, well known as a producer of ligninolytic enzymes under low-nitrogen growth, produced none of the enzymes nor decolorized the dyes under these growth conditions. Of the four enzyme activities assayed in these extracts, only laccase seems to be correlated with the dye decolorization (Fig. 1), and *Trametes hispida* showed the highest laccase activity production, which is consistent with the highest dye decolorization activity. No lignin peroxidase activity could be detected in any of the fungi strains cultured under our growth conditions.

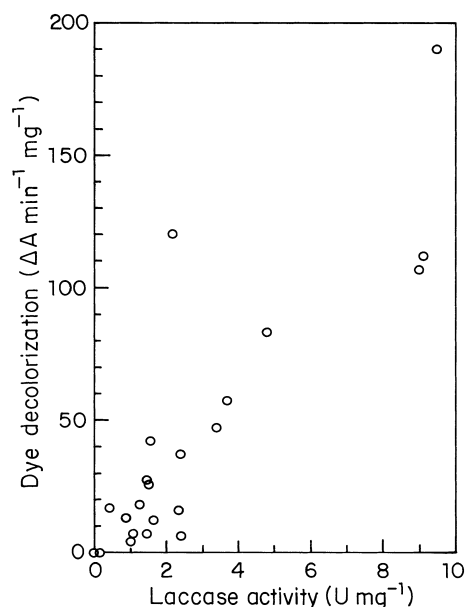


Fig. 1. Correlation between laccase activity and decolorization capacity of Reactive blue 158 dye by crude extracellular extract from 16 different fungal strains grown on oat grains.

Because the crude extract from *T. hispida* cultures showed the highest laccase and decolorization activities, laccase isoenzymes from *T. hispida* were purified and their kinetics constants determined with ABTS and Reactive blue 158 (Remazol brilliant blue, CI 61200) as substrates. Specific activities of the two purified enzymes were 168 U mg^{-1} for laccase I and 170 U mg^{-1} for

Table 5. Kinetic constants of laccase isoenzymes for ABTS and Reactive blue 158 oxidations

Enzyme	ABTS			Reactive blue		
	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($s^{-1} mM^{-1}$)	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($s^{-1} mM^{-1}$)
Laccase I	16	16	986	46	3500	13
Laccase II	178	235	759	25	3900	7

laccase II. The kinetic constants, determined by double reciprocal plots (Table 5), showed that the k_{cat} for ABTS oxidation is 10 times higher in laccase II than those found with laccase I. However, the K_M value of laccase II is also higher than that of laccase I, making no significant difference in the catalytic efficiency values (k_{cat}/K_M) for ABTS. On the other hand, kinetic constants for the Reactive blue 158 as substrate were similar for both laccases. The molecular weights determined by SDS-PAGE electrophoresis showed that both laccase I and laccase II are 68-kDa proteins, and both enzymes were stable, showing no decrease in activity during 30 days at room temperature under aseptic conditions. Although data are presented for only five dyes, *T. hispidia* laccase was able to decolorize in vitro 11 of the 23 industrial, while *P. ostreatus* laccase was able to oxidize in vitro only five of the same dyes. These results also show that there are other enzymatic systems involved in dye decolorization in in vivo cultures.

Discussion

Ligninocellulosic materials were able to induce ligninolytic enzyme production in many fungi. The increase of enzyme activities is correlated with the decolorization capacity of extracellular medium (Table 2). Lignin peroxidase has been shown to be involved in dye decolorization, mainly in *P. chrysosporium* cultures [7, 12, 27, 30, 44]. However, none of the strains tested under our conditions produced detectable amounts of lignin peroxidase (Table 3), while most of the extracellular extracts from these cultures were able to decolorize several dyes (Table 4). Manganese peroxidase [1, 16, 29] and laccase also have been reported to decolorize some synthetic dyes. From the strains we tested, it seems that laccase is the main enzyme involved in dye decolorization: this activity is clearly correlated with the decolorization capacity (Fig. 1), and the purified preparations are able to perform this decolorization reaction in vitro.

While laccases from different sources have many similar properties, there are also catalytic differences. We found that the molecular weight of both laccases from *T. hispidia* was 68 kDa, in the same range reported for

laccases I and II found in *Pleurotus ostreatus*, 64 kDa [43]; *Trametes versicolor*, 67 kDa [5]; and *Pleurotus eryngii*, 65 and 61 kDa respectively [26]. However, the laccases from *T. hispidia* and *P. ostreatus* (IE-8) exhibited different substrate specificities. *T. hispidia* laccase was able to decolorize in vitro 11 of the 23 industrial dyes. On the other hand, *P. ostreatus* laccase was able to oxidize only five dyes in vitro, even if it was capable of oxidizing 11 industrial dyes in in vivo experiments. The differences of substrate specificity between purified laccases from both microorganisms could be explained by the differences of amino acid sequences [26] and seems not to be related to the culture conditions. These results also show that there are other enzymatic systems in *P. ostreatus* involved in dye decolorization in in vivo cultures, such as cytochromes P450 or peroxidases [34].

In conclusion, several industrial dyes were decolorized biocatalytically by extracellular enzymes from different strains of white-rot fungi grown on oats in solid-state fermentation. This decolorization capacity was correlated with the laccase activity levels. *Trametes hispidia* showed the highest volumetric decolorization activity, and purified laccases from *T. hispidia* were able to decolorize several synthetic dyes in vitro. This enzymatic system appears to be a good candidate for immobilization and use as a bioreactor for effluent treatment from the dye and printing industries.

ACKNOWLEDGMENTS

This work was funded by a DGAPA-UNAM Grant IN 220597 and by the National Council for Science and Technology of Mexico (Grant 25376-A).

Literature Cited

1. Archibald FS (1992) A new assay for lignin-type peroxidase employing the dye Azure B. *Appl Environ Microbiol* 58:3110–3116
2. Arjmand M, Sandermann H (1985) Mineralization of chloraniline/lignin conjugates and of free chloranilines by white rot fungus *Phanerochaete chrysosporium*. *J Agric Food Chem* 33:1055–1060
3. Bogan BW, Lamar RT (1996) Polycyclic aromatic hydrocarbon-degrading capabilities of *Phanerochaete leavis* HHB-1625 and its extracellular ligninolytic enzymes. *Appl Environ Microbiol* 62:1597–1603
4. Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates. *FEBS Lett* 267:99–102
5. Bourbonnais R, Paice MG, Reid ID, Lanthier P, Yaguchi M (1995) Lignin oxidation by laccase isoenzymes from *Trametes versicolor* and role of the mediator 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) in Kraft lignin depolymerization. *Appl Environ Microbiol* 61:1876–1880
6. Brown DH, Hitz HR, Schafer L (1981) The assessment of the possible inhibitory effect of dyestuffs on aerobic wastewater bacteria. Experience with a screening test. *Chemosphere* 10:245–261
7. Bumpus JA, Brock BJ (1988) Biodegradation of crystal violet by white rot fungus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 54:1143–1150

8. Bumpus JA, Tien M, Wright D, Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. *Science* 228:1434–1435
9. Chivukula M, Renganathan V (1995) Phenolic azo dye oxidation by laccase from *Pyricularia oryzae*. *Appl Environ Microbiol* 61:4374–4377
10. Chivukula M, Spadaro JT, Renganathan V (1995) Lignin peroxidase-catalyzed oxidation of sulfonated azo dyes generates novel sulfophenyl hydroperoxide. *Biochemistry* 34:7765
11. Chung K-T, Stevens E (1992) The reduction of azo dyes by the intestinal microflora. *Crit Rev Microbiol* 18:175–190
12. Cripps C, Bumpus JA, Aust SD (1990) Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 56:1114–1118
13. Dey S, Maiti TK, Bhattacharyya BC (1994) Production of some extracellular enzymes by a lignin peroxidase-producing brown rot fungus, *Pleurotus ostreiformis*, and its comparative abilities for lignin degradation and dye decolorization. *Appl Environ Microbiol* 60:4216–4218
14. Field JA, de Jong E, Feijoo-Costa G, de Bont JAM (1993) Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. *Trends Biotechnol* 11:44–49
15. Glenn JK, Gold MH (1983) Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 45:1741–1747
16. Gold MH, Glenn JK, Alic M (1988) Use of polymeric dyes in lignin biodegradation assays. *Methods Enzymol* 161:74–78
17. Gorontzy T, Drzyga O, Kahl MW, Bruns-Nagel D, Breitung J, von Loew E, Blotevogel KH (1994) Microbial degradation of explosives and related compounds. *Crit Rev Microbiol* 20:265–284
18. Hammel KE, Kalyanaraman B, Kirk TK (1986) Oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]dioxins by *Phanerochaete chrysosporium* ligninase. *J Biol Chem* 261:16948–16952
19. Kennedy DW, Aust SD, Bumpus JA (1990) Comparative biodegradation of alkyl halide insecticides by the white rot fungus *Phanerochaete chrysosporium* (BKM-F-1767). *Appl Environ Microbiol* 56:2347–2353
20. Kirby N, McMullan G, Marchant R (1995) Decolourisation of an artificial textile effluent by *Phanerochaete chrysosporium*. *Biotechnol Lett* 17:761–764
21. Kulkarni SV, Blackwell CD, Blackard AL, Stackhose CW, Alexander MW (1985) Textile dyes and dyeing equipment, classification, properties and environmental aspects. U.S. Environmental Protection Agency, Research Triangle Park, NC. EPA-600/2-85/010
22. Kullman SW, Matsumura F (1996) Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of cyclodiene pesticide Endosulfan. *Appl Environ Microbiol* 62:593–600
23. Laemmli UK (1970) Change of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
24. Lewandowski GA, Armenante PM, Pak D (1990) Reactor design for hazardous waste treatment using a white rot fungus. *Water Res* 24:75–82
25. Morgan P, Lewis ST, Watkinson RJ (1991) Comparison of abilities of white-rot fungi to mineralize selected xenobiotics compounds. *Appl Microbiol Biotechnol* 34:693–696
26. Muñoz G, Gillén F, Martínez AT, Marínez MJ (1997) Laccase isoenzymes of *Pleurotus eryngii*: characterization, catalytic properties, and participation in activation of molecular oxygen and Mn²⁺ oxidation. *Appl Environ Microbiol* 63:2166–2174
27. Ollikka P, Alhonenmaki K, Leppanen V-M, Glumoff T, Raijola T, Suominen Y (1993) Decolorization of azo, triphenyl methane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 59:4010–4016
28. Pagga U, Brown DH (1986) The degradation of dyestuffs, part II. Behavior of the dyestuffs in aerobic biodegradation test. *Chemosphere* 15:479–491
29. Pasti-Grigsby MB, Paszczynski A, Goszcynski S, Crawford DL, Crawford RL (1992) Influence of aromatic substitution patterns on azo dye degradability by *Streptomyces spp.* and *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 58:3605–3613
30. Paszczynski A, Crawford RL (1991) Degradation of azo compounds by ligninase from *Phanerochaete chrysosporium*: involvement of veratryl alcohol. *Biochem Biophys Res Commun* 178:1056–1063
31. Platt MW, Hadar Y, Chet H (1985) The decolorization of the polymeric dye Poly-blue (polyvinylamine sulfonate-anthraquinone) by lignin degrading fungi. *Appl Microbiol Biotechnol* 21:394–396
32. Ruckenstein E, Wang X-B (1994) Production of lignin peroxidase by *Phanerochaete chrysosporium* immobilized on porous poly(styrene-divinylbenzene) carrier and its application to the degrading of 2-chlorophenol. *Biotechnol Bioeng* 44:79–86
33. Sasek V, Volfova O, Erbanova P, Vyas BRM, Matucha M (1993) Degradation of PCBs by white rot fungi, methylotrophic and hydrocarbon utilizing yeasts and bacteria. *Biotechnol Lett* 15:521–526
34. Shin KS, Oh IK, Kim CJ (1997) Production and purification of Remazol Brilliant Blue R decolorization peroxidase from the culture filtrate of *Pleurotus ostreatus*. *Appl Environ Microbiol* 63:1744–1748
35. Spadaro JT, Renganathan V (1994) Peroxidase-catalyzed oxidation of azo dyes: mechanism of Disperse Yellow 3 degradation. *Arch Biochem Biophys* 312:301–307
36. Spadaro JT, Gold MH, Renganathan V (1992) Degradation of azo dyes by lignin-degrading fungus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 58:2397–2401
37. Spiker JK, Crawford DL, Crawford RL (1992) Influence of 2,4,6-trinitrotoluene (TNT) concentration on the degradation of TNT in explosive contaminated soil by white rot fungus. *Appl Environ Microbiol* 58:3199–3202
38. Takada S, Nakamura M, Matsueda T, Kondo R, Sakai K (1996) Degradation of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans by the white rot fungus *Phanerochaete sordida* YK-624. *Appl Environ Microbiol* 62:4323–4328
39. Tien M, Kirk TK (1983) Lignin degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of unique H₂O₂-requiring oxygenase. *Proc Natl Acad Sci USA* 81:2280–2284
40. Vazquez-Duhalt R, Westlake DWS, Fedorak PM (1994) Lignin peroxidase oxidation of aromatic compounds in systems containing organic solvents. *Appl Environ Microbiol* 60:459–466
41. Wariishi H, Valli K, Gold MH (1992) Manganese (II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. *J Biol Chem* 267:23688–23695
42. Yadav JS, Quensen JF, Tiedje JM, Reddy CA (1995) Degradation of polychlorinated biphenyl mixtures (Aroclors 1242, 1254, and 1260) by the white rot fungus *Phanerochaete chrysosporium* as evidenced by congener-specific analysis. *Appl Environ Microbiol* 61:2560–2565
43. Youn HD, Kim KJ, Han YH, Jeong IB, Jeong G, Kang SO, Hah YC (1995) Single electron transfer by an extracellular laccase from the white-rot fungus *Pleurotus ostreatus*. *Microbiology* 141:393–398
44. Young L, Yu J (1997) Ligninase-catalyzed decolorization of synthetic dyes. *Water Res* 31:1187–1193