Folia Microbiol. **53** (6), 479–485 (2008) **http://www.biomed.cas.cz/mbu/folia/**

Implication of *Dichomitus squalens* Manganese-Dependent Peroxidase in Dye Decolorization and Cooperation of the Enzyme with Laccase

M. ŠUŠLA, Č. NOVOTNÝ, P. ERBANOVÁ, K. SVOBODOVÁ*

Laboratory of Experimental Mycology, Institute of Microbiology of the Academy of Sciences of the Czech Republic, v.v.i., 142 20 Prague, Czech Republic

fax +420 241 062 384

e-mail ksvobod@biomed.cas.cz

Received 24 January 2008
Revised version 2 June 2008

ABSTRACT. Three new chromatographic forms of *Dichomitus squalens* manganese-dependent peroxidase (MnP) were isolated from wheat-straw cultures using Mono Q and connective interaction media (CIM) fast protein liquid chromatography. Enzymes revealed identical molar mass of 50 kDa (estimated by SDS-PAGE) and p*I* values of 3.5, however, they varied in K_m values obtained for Mn^{2+} oxidation. The addition of wood and straw methanol extracts to the cultures showed that the production of MnPs in wheat-straw cultures was influenced rather by the type of cultivation than by phenolic compounds from lignocellulosic material which induced laccase production. The purified CIM1 MnP was able to decolorize selected azo and anthraquinone dyes more rapidly than laccase Lc1. *In vitro* dye decolorization showed a synergistic cooperation of MnP and laccase. In the case of CSB degradation MnP prevented from the production of a differently colored substance that could be produced after CSB degradation by laccase–HBT system.

Abbreviations

ABTS	2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
CIM	connective interaction media
CSB	Chicago Sky Blue
DMP	2,6-dimethoxyphenol
HBT	1-hydroxybenzotriazole
IEF	isoelectric focusing
LiP	lignin peroxidase
Lac	laccase
Lc1	laccase Lc1
LNMM	low nitrogen mineral medium
MnP(s)	manganese-dependent peroxidase(s)
RBBR	Remazol Brilliant Blue R
RBV5R	Remazol Brilliant Violet 5R
RO16	Reactive Orange 16

White-rot fungi have been shown to possess a remarkable potential for degradation of synthetic dyes due to the production of oxidases and peroxidases, that are highly oxidative and substrate-nonspecific (Wesenberg *et al.* 2003; Cajthaml *et al*. 2008). Studies of *in vitro* decolorization of synthetic dyes by crude or purified enzymes become important for understanding the biodegradation mechanism (Moreira *et al.* 2001; Couto *et al.* 2002; Kokol *et al.* 2007). The most important enzymes are LiP, MnP and Lac. Their ability to degrade various synthetic dyes has been reported by Torres *et al.* (2003) and Lu *et al.* (2007). Decolorization rate and dye degradation mechanism can vary among ligninolytic enzymes produced by the same fungus (Podgornik *et al.* 2001). The decolorization of Indigo carmine by *Phanerochaete chrysosporium* MnP resulted in a red product formation (λ_{max} = 550 nm) that was not observed during decolorization by LiP. However, there is only very limited information on the combined effect of both enzymes on dye decolorization (Champagne and Ramsay 2005).

White-rot fungus *Dichomitus squalens* is very effective in decolorizing a wide range of structurally diverse synthetic dyes including azo, anthraquinone, phthalocyanine, and oligocyclic aromatic dyes (Gill *et al.* 2002; Eichlerová *et al.* 2006). Although a cooperation of Lac and MnP in the decolorization was hypothesized (Eichlerová *et al.* 2005), the relative contribution of different enzymes in fungal cultures to decolorization is not yet understood.

The main objective of this work was to evaluate the contribution of ligninolytic enzymes Lac and MnP of *D. squalens* to the degradation of synthetic dyes. The purified *D. squalens* enzymes were tested for their ability to decolorize azo and anthraquinone dyes *in vitro* both individually and in combination. The inducion of ligninolytic enzyme activities on two different lignocellulosic substrates was also analyzed.

*Corresponding author.

MATERIALS AND METHODS

Microorganism. Dichomitus squalens (P. KARST.) REID CCBAS 750 was obtained from the CCBAS collection (*Institute of Microbiology*, *Academy of Sciences of the Czech Republic*, Prague, Czech Republic). The strain was maintained on MEG (in g/L: agar 20, glucose 10, malt extract 5) agar slants at 4 °C.

Culture conditions. For MnP isolation from wheat straw, solid state cultures, portions of 25 g air-dried wheat straw (cut to pieces 10–20 mm) were placed in 500-mL conical flasks and moistened with 85 mL deionized water. The flasks were autoclaved and inoculated with 10 discs cut from 7-d-old malt extract agar plate cultures, which were incubated at 28 °C for 14 d. The straw colonized by the fungus was cut into small pieces, soaked with 100 mL sodium acetate buffer (100 mmol/L, pH 5.0) and extracted on ice for 3 h with occasional shaking. The straw was removed by filtration and the filtrate containing the extracted enzymes was used in further purification steps. Laccase Lc1 was purified from cultures grown on pine wood cubes (1 cm^3) in LNMM (Tien and Kirk 1988); cultures were prepared according to Šušla *et al.* (2007).

For enzyme induction, the culture homogenate obtained from 7-d-old cultures grown in 20 mL of LNMM in 250-mL Erlenmeyer flasks and preinoculated from malt extract agar plates was used as inoculum. The cultures contained 100 mL of LNMM, 5 mL of inoculum, and 1.5 g of straw or pine wood chips in 500 mL Erlenmeyer flasks.

Methanol extraction of 1.5 g of straw (cut, <10 mm) or wood chips was carried out in Soxhlet apparatus for 12 h. After the extraction, methanol was evaporated, the extract was dissolved in 1 mL Me_2SO and added to the cultures. The straw or wood chips after the extraction were also used for the preparation of cultures as described *above*. The cultures were incubated at 28 °C for 25 d. All experiments were done in triplicates.

Enzyme assays. Lac activity was determined by oxidation of 5 mmol/L ABTS (Matsumura *et al.* 1986), MnP activity according to de Jong *et al.* (1994) and also by formation of Mn^{3+}–malonate complex at 270 nm (Wariishi *et al.* 1992). One unit of enzyme activity was defined as the amount of the enzyme oxidizing 1 μmol of substrate per min.

Purification of MnP and Lc1. The acetate buffer extract obtained from 14-d-old wheat-straw solid-state cultures was concentrated by ultrafiltration using 10-kDa cut-off membrane. The concentrate was filtered through a 0.2-μm cellulose nitrate filter and loaded onto a HiPREP 26/10 desalting column (*Amersham Biosciences*) equilibrated with 20 mmol/L phosphate buffer (pH 6.0). Proteins were eluted over 3 min at a flow rate of 10 mL/min. The MnP fraction was applied to a DEAE-Sepharose column (HR 10/10; *Pharmacia LKB*) equilibrated with 20 mmol/L phosphate buffer (pH 6.0). Proteins were eluted with a gradient of 0–1 mol/L NaCl over 90 min at a flow rate of 0.5 mL/min. The fractions containing MnP activity were pooled and desalted using a HiPREP 26/10 desalting column (*Amersham Biosciences*). The MnP fraction was further applied to Mono Q HR 5/5 column (*Pharmacia*) equilibrated with 20 mmol/L phosphate buffer (pH 6.0) and eluted with a linear gradient of 0–1 mol/L NaCl. Two fractions with MnP activity were obtained; they were concentrated, desalted and separately applied to a CIM QA Disk (*BIA Separation*, Slovenia) equilibrated with 20 mmol/L phosphate buffer (pH 6.0) and eluted with a linear gradient of 0–1 mol/L NaCl over 8 min at a flow rate of 4 mL/min. Purified MnP fractions were pooled and kept frozen at –18 °C.

Lc1 used for *in vitro* experiments was purified using DEAE-Sepharose column and CIM QA Disks as described by Šušla *et al.* (2007).

Physical and chemical properties of MnP. IEF was carried out with a Multiphor II electrophoresis system (*Pharmacia*). The IEF gel (7.5 %) was prepared using ampholines of p*I* 2.5–5.0 and 3.5–10.0 (*Pharmacia*). A surface electrode was used to measure the pH gradient on the gel. Gels were activity-stained with 1 mmol/L ABTS in the presence of 1 mmol/L $MnSO_4$ and 0.5 mmol/L H_2O_2. SDS-PAGE was done using 10 % polyacrylamide gels, which were stained with silver. Protein concentration was determined by the Bradford (1976) method with bovine serum albumin as standard.

MnP characterization. The effect of pH on MnP activity was examined in 50 mmol/L sodium malonate buffer (pH 2.0–6.0). The effect of chelators was monitored in 50 mmol/L acetate, lactate, malonate, and tartrate buffers (pH 4.0) in the presence of 1 mmol/L $MnSO_4$, 0.5 mmol/L H_2O_2, and 1 mmol/L DMP. The activity of MnPs towards Mn^{2+} and DMP were assayed in 50 mmol/L sodium malonate buffer (pH 4.0) at 0.04–0.15 and 0.5–2.0 mmol/L, respectively. Apparent Michaelis–Menten constants (K_m) were obtained using SoftMax Pro 4.6 software.

Dyes and dye decolorization. Dyes (*Sigma*, USA) used:

	λ_{max}, nm		λ_{max}, nm
RO16 (azo dye)	494	CSB (azo dye)	618
RBBR (anthraquinone dye)	592	RBV5R (azo dye)	558

For *in vitro* dye decolorization by the crude culture liquid, an extract obtained from 14-d-old cultures grown on wheat straw was used. The reaction mixtures consisted of 50 mmol/L sodium malonate buffer (pH 4.5), 150 mg/L dye, 1 mmol/L $MnSO_4$, 0.5 mmol/L H_2O_2 and an aliquot of the culture extract representing 3.23 mU of MnP and 0.20 mU of Lac. Reaction mixtures with the heat-inactivated crude culture liquid served as the controls. The reaction mixtures for decolorization by purified MnP contained 50 mmol/L sodium malonate buffer (pH 4.5), 150 mg/L dye, 1 mmol/L $MnSO_4$, 0.5 mmol/L H_2O_2 and 3.23 mU of CIM1 MnP. The reaction was initiated by the addition of the enzyme and the absorbance of the reaction mixture was measured every 5 min. For the experiments with MnP and Lc1, the reaction mixtures were prepared as described *above* and contained 3.23 or 32.3 mU of Lc1. UV-VIS analysis was used to monitor the decolorization of RO16 and CSB in the presence of 2 mmol/L HBT, 34 mU Lc1 and 17 mU of CIM1 MnP. The absorbance was measured at 200–700 nm with a 2-nm step at the beginning and after 1, 6, 24, and 72 h of the reaction.

RESULTS

Effect of lignocellulosic materials on enzyme activities. Enzyme production profiles of *D. squalens* grown on wheat straw in the absence (solid-state cultures) and in the presence of liquid medium were compared. A high level of MnP (520 U/L) and a low level of Lac (17 U/L) was detected in the solid state cultures after 14 d. In contrast, a high Lac activity (up to 270 U/L after 7 d) and a low MnP activity (maximum 3 U/L after 17 d) was observed in cultures grown on wheat straw in the presence of LNMM (*data not shown*).

To determine the effect of two lignocellulosic materials (wheat straw and pine wood) on MnP and Lac production, straw and wood samples were first extracted with methanol. The obtained extracts, and the samples of wheat straw and wood before and after extraction were added to liquid cultures. Both wheat straw and wood pieces significantly induced Lac production (Fig. 1). The addition of both extracts also led to higher Lac pro duction; however, the induction effect was lower than in the case of original straw and wood sam-

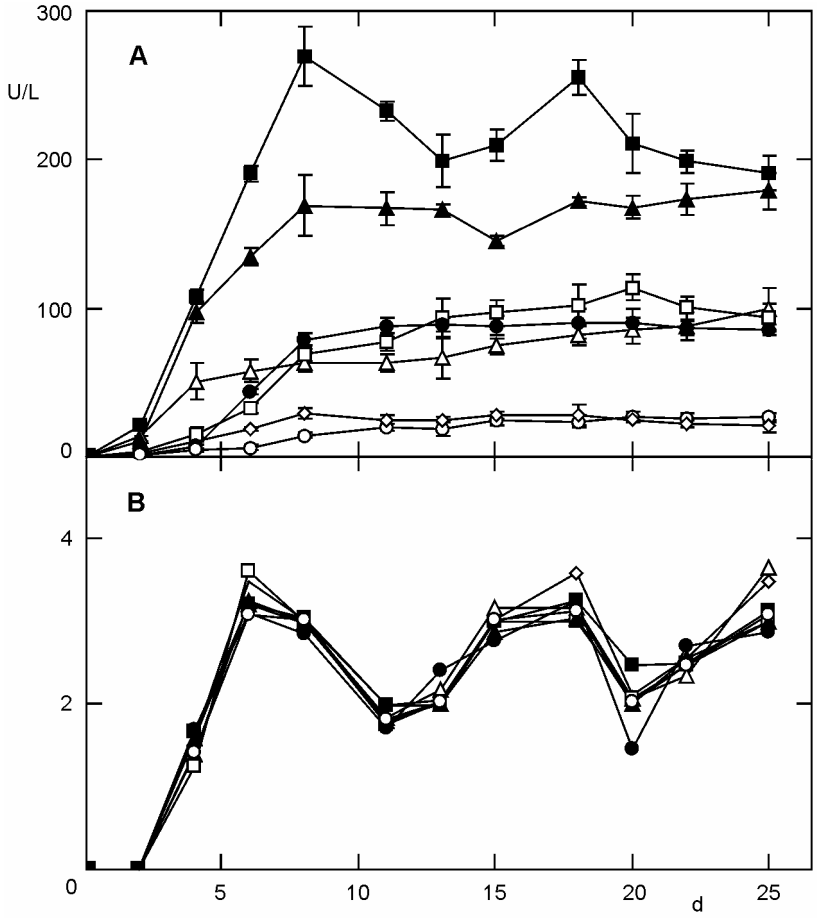

Fig. 1. Enzyme activities (U/L) produced by *D. squalens* grown in LNMM containing wheat straw (*closed squares*), pine-wood chips (*closed triangles*), wheat straw after methanol extraction (*open squares*), pine wood after methanol extraction (*open circles*) and in liquid cultures containing methanol extracts from wheat straw (*open triangles*) or pine-wood chips (*closed circles*); *diamonds* – control liquid culture; **A** – Lc1, **B** – MnP.

ples.Wheat straw after extraction showed a similar effect to its methanol extract. Wood chips after extraction had no effect on Lac production. No effect on MnP production was observed using either straw or pine wood, or their methanol extracts.

MnP isolation and characterization (Table I). Purification of the enzyme on a Mono Q column resolved two MnP activity peaks (Mono Q1 and Mono Q2) which were further purified using a CIM monolithic column. Three different chromatographic MnP forms (CIM1, CIM2, CIM3) were obtained in the last step of purification. Each chromatographic form exhibited a single band when subjected to SDS-PAGE. The molar mass of MnP forms was 50 kDa under denaturing conditions, the isoelectric points were estimated as 3.5. All chromatographic forms revealed the same pH optimum of 4.0 for Mn^{2+} oxidation (Table II). In the presence of various chelators, the highest MnP activity was obtained with lactate, very low activity was exhibited by acetate, which is not an effective chelator of Mn^{2+} (*data not shown*). For apparent K_m values for Mn^{2+} and 2,6-DMP *see* Table II.

Table I. Purification of MnP

Purification step	Total volume mL	MnP activity[a] U/mL	Protein µg/mL	Specific activity U/µg	Yield %
Crude medium	1200	0.520	2.81	0.185	100
DEAE Sepharose	11	9.17	12.5	0.733	16
Mono Q1	5	6.42	7.38	0.870	5
Mono Q2	2	2.60	1.16	2.25	1
CIM QA disk 1	5	1.97	0.79	2.48	2
CIM QA disk 2	8	1.86	0.54	3.43	2
CIM QA disk 3	3	1.24	1.01	1.23	1

[a]DMP assay.

Table II. Molar mass, p*I*, apparent K_m (Mn^{2+}, 2,6-dimethoxyphenol), and pH optimum of isolated chromatographic forms of *D. squalens* MnP (CIM1, CIM2, CIM3)

Quantity	Unit	CIM1	CIM2	CIM3
M	kDa	50	50	50
p*I*	pH	3.5	3.5	–
K_m[a]	µmol/L	24	44	47
K_m[b]	µmol/L	417	216	516
pH_{opt}	pH	4.0	4.0	4.0

[a]Mn^{2+}. [b]DMP.

Dye decolorization. Treatment of CSB and RBBR with the crude extract from wheat-straw cultures containing the same total amount of MnP resulted in the same decolorization as treatment using purified MnP (Fig. 2), decolorization by the crude extracts being dependent on the presence of Mn^{2+} and H_2O_2 in the reaction mixture. In the case of RBV5R, a higher decolorization rate was recorded using the crude extract; no RO16 decolorization was observed (Fig. 2).

MnP decolorized CSB (azo dye), and RBBR (anthraquinone dye) more rapidly and to a greater extent than Lc1 (Fig. 3). In contrast to MnP, no decolorization by Lc1 was achieved for RBV5R (azo dye); neither MnP alone nor Lc1 alone was able to decolorize RO16 (azo dye). However, 8 % of RO16 was decolorized using a combination of the enzymes (Fig. 3). The decolorization rates for all tested dyes were higher when both enzymes were present. However, the sum of the rates of each enzyme alone did not correspond to the decolorization rate in the presence of both enzymes. Thus the combined effect of the two enzymes is synergistic rather than additive.

Decolorization of CSB was monitored by UV-VIS analysis over 3 d (Fig. 4). The Lc1–HBT system decolorized the dye completely within 1 h. However, a regressive increase of absorbance of the reaction mixture in the visible range (370–500 nm) was observed when incubated over longer periods. No differently colored products yielded from CSB degradation by the Lc1–HBT system were detected in the presence of MnP.

DISCUSSION

D. squalens is known to secrete Lac and MnP in shaken and stationary liquid cultures (Périé and Gold 1991). Both enzymes have been isolated from liquid cultures and characterized (Périé *et al.* 1996, 1998). Cultivation of the fungus on pine wood cubes in LNMM led to a significantly higher production of Lac in comparison with liquid cultures (Šušla *et al.* 2007). In contrast, *D. squalens* immobilized on the wheat straw sub-

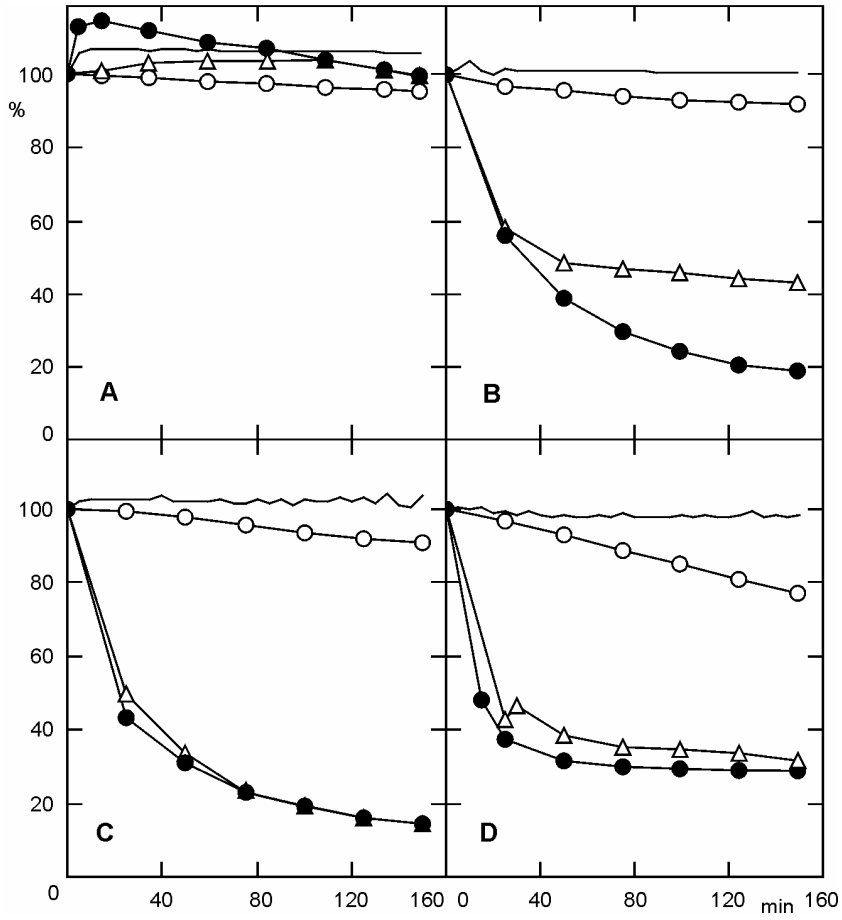

Fig. 2. Decolorization of dyes (%) by an extract from wheat straw cultures and purified MnP isolated from *D. squalens* wheat-straw cultures; *open circles* – straw extract, *closed circles* – straw extract in the presence of H_2O_2 and Mn^{2+}, *triangles* – purified MnP in the presence of H_2O_2 and Mn^{2+}, *no symbols* – heat-inactivated straw extract; error bars do not exceed the dimensions of the symbols; **A** – RO16, **B** – RBV5R, **C** – RBBR, **D** – CSB.

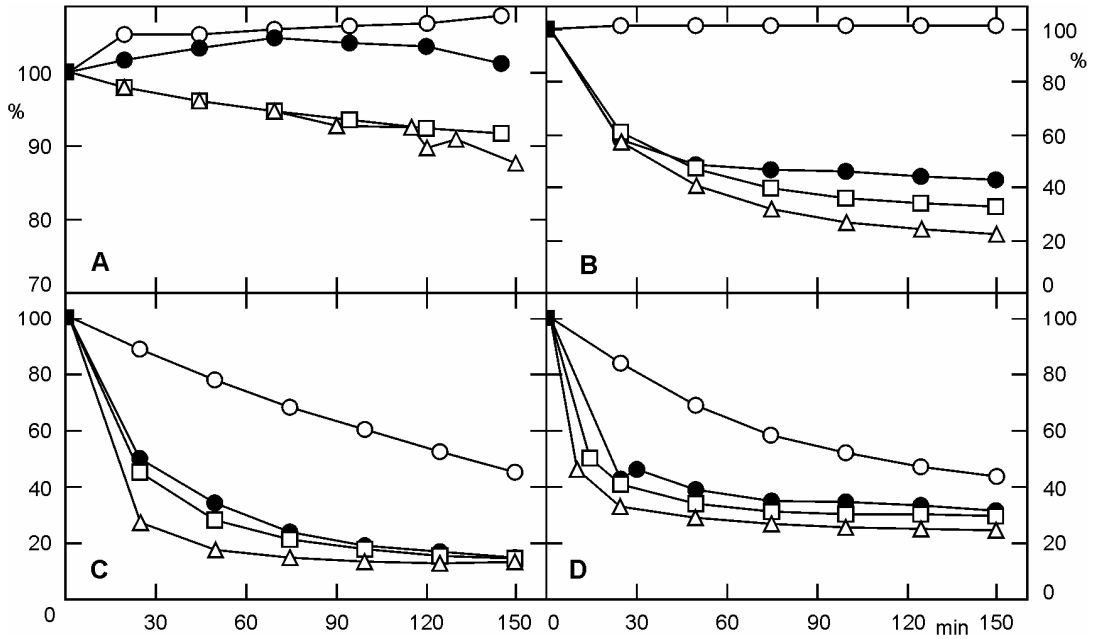

Fig. 3. Decolorization of dyes (%) by *D. squalens* Lc1 and MnP; *open circles* – Lc1, *closed circles* – MnP, *squares* – Lc1 and MnP (1 : 1), *triangles* – Lc1 and MnP (10 : 1); error bars do not exceed the dimensions of the symbols; **A** – RO16, **B** – RBV5R, **C** – RBBR, **D** – CSB.

strate produced a high level of MnP (Lang *et al.* 1998). Our results indicate that wheat straw significantly induced MnP production in the absence of free liquid which is in accordance with Lang *et al.* (1998). However, no MnP induction by wheat straw or wheat-straw extract was observed in cultures with LNMM (Fig. 1) which means that the method of cultivation is a factor determining the expression of the enzyme rather than phenolic compounds extractable from the growth material with methanol. Similarly, the activities of *P. chrysosporium* and *Lentinus edodes* MnPs in solid state fermentation were far higher than those in submerged fermentation of plant raw material (Fujian *et al.* 2001; Elisashvili *et al.* 2008).

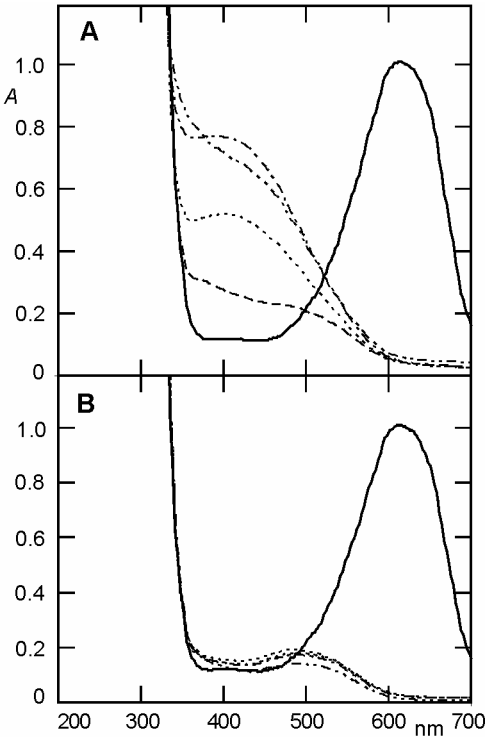

Fig. 4. UV-VIS spectra (absorbance *A*; wavelength in nm) of azo dye CSB during decolorization with Lc1 + HBT (**A**) or Lc1 + MnP + HBT (**B**); *full lines* – 0 h, *dashed lines* – 1 h, *dotted lines* – 6 h, *dash-and-dot lines* – 1 d, *dash-and-double-dot lines* – 3 d.

Our experiments revealed that pine wood as well as wheat straw methanol extracts contained Lac inducers but no MnP ones. We used methanol extraction as this solvent has been shown to extract phenolic compounds from wood (Martínez *et al*. 2005) which could act as enzyme inducers in the fungal cultures. Production of Lac in *D. squalens* cultures was significantly induced by pine wood chips and wood methanol extract. As the effect of pine wood chips on Lac production was removed by methanol extraction, the induction of enzyme activity can be attributed to phenolic compounds extracted from wood. In the case of wheat straw, straw samples after extraction partially kept their induction effect on Lac production. It shows that the induction of Lac by wheat straw could result from the effect of phenolic compounds extracted from straw combined with the effect of culture conditions (*e.g.*, the presence of liquid medium in cultures, and size of straw pieces).

Three chromatographic forms of *D. squalens* MnP were isolated and characterized from solid-state wheat-straw cultures. The enzymes differed from the two MnP isoezymes isolated from liquid cultures by Périé *et al.* (1996) in isoelectric points, pH optimum, and apparent K_m values suggesting an isolation of new forms of enzymes. *D. squalens* Lc1 was obtained from pine wood cultures with high Lac activity, its isolation being consistent with the results of Šušla *et al.* (2007).

The fact that CSB and RBBR degradation by culture extract was Mn-dependent indicates the involvement of MnP in the decolorization process. A synergistic action of the enzymes present in the crude extract could be possibly responsible for the higher decolorization rate of the extract observed in the case of RBV5R. Similar findings have been reported by Podgornik *et al.* (2001) working with Indigo carmine dye.

Some papers on azo and anthraquinone dye decolorization by purified fungal MnPs have been published (Svobodová *et al.* 2006; Xiaobin *et al.* 2007; Kariminiaae-Hamedaani *et al.* 2007). *D. squalens* MnP decolorized azo dyes CSB and RBV5R, and anthraquinone dye RBBR more rapidly than *D. squalens* Lc1 per unit of enzyme activity (Fig. 3).

RO16 could be decolorized neither by *D. squalens* MnP nor by Lc1 (Fig. 3). However, Šušla *et al.* (2007) showed that RO16 was efficiently decolorized by whole cultures under conditions of MnP and Lac production. That could be connected with lipid peroxidation or other redox mediator mechanisms enhancing the oxidation potential of MnPs and Lac under *in vivo* conditions (Harazono *et al.* 2003). In the presence of both *D. squalens* enzymes (MnP and Lc1) without any redox mediator, 8 % RO16 decolorization was observed under *in vitro* conditions indicating a synergistic action of the enzymes. Synergistic contribution of both enzymes was proved also for other dyes (Fig. 3). In contrast, the rate of amaranth decolorization by *Trametes versicolor* MnP and Lac was additive (Champagne and Ramsay 2005). Our results also demonstrated that the presence of MnP prevented the coupling of reaction products resulting from Lc1 degradation of RO16 (*data not shown*) and it decolorized differently colored products produced by the Lc1–HBT system during CSB decolorization (Fig. 4).

Eichlerová *et al.* (2005, 2006) stated that *D. squalens* efficiently decolorized various synthetic dyes but only Lc1 has been reported to decolorize RBBR and RO16 so far (Šušla *et al.* 2007). We describe three new different forms of *D. squalens* MnP and their possible synergistic cooperation with Lc1 in dye decolori-

zation. In contrast to Lc1, production of MnP in wheat-straw cultures of *D. squalens* was influenced rather by the type of cultivation than by the phenolic compounds from straw material.

The work was supported by projects *GAČR* 526/06/P102, *IGA AV* no. IAA 602 0411, FRVS G4 359/2007 and by the *Institutional Research Concept* no. AV0Z 5020 0510.

REFERENCES

BRADFORD M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analyt.Biochem.* **72**, 248–254 (1976).

CAJTHAML T., ERBANOVÁ P., KOLLMANN A., NOVOTNÝ Č., ŠAŠEK V., MOUGIN C.: Degradation of PAHs by ligninolytic enzymes of *Irpex lacteus*. *Folia Microbiol.* **53**, 289–294 (2008).

CHAMPAGNE P.P., RAMSAY J.A.: Contribution of manganese peroxidase and laccase to dye decolorization by *Trametes versicolor*. *Appl.Microbiol.Biotechnol.* **69**, 276–285 (2005).

COUTO S.R., DOMÍNGUEZ A., SANROMÁN M.A.: Production of manganese-dependent peroxidase in a new solid-state bioreactor by *Phanerochaete chrysosporium* grown on wood shavings. Application to the decolorization of synthetic dyes. *Folia Microbiol.* **47**, 417–421 (2002).

EICHLEROVÁ I., HOMOLKA L., LISÁ L., NERUD F.: Orange G and Remazol Brilliant Blue R decolorization by white rot fungi *Dichomitus squalens*, *Ischnoderma resinosum* and *Pleurotus calyptratus*. *Chemosphere* **60**, 398–404 (2005).

EICHLEROVÁ I., HOMOLKA L., NERUD F.: Synthetic dye decolorization capacity of white rot fungus *Dichomitus squalens*. *Biores.Technol.* **97**, 2153–2159 (2006).

ELISASHVILI V., PENNINCKX M., KACHLISHVILI E., TSIKLAURI N., METREVELI E., KHARZIANI T., KVESITADZE G.: *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. *Biores.Technol.* **99**, 457–462 (2008).

FUJIAN X., HONGZHANG C., ZUOHU L.: Solid-state production of lignin peroxidase (LiP) and manganese peroxidase (MnP) by *Phanerochaete chrysosporium* using steam-exploded straw as substrate. *Biores.Technol.* **80**, 149–151 (2001).

GILL P.K., ARORA D.S., CHANDER M.: Biodecolourization of azo- and triphenylmethane dyes by *Dichomitus squalens* and *Phlebia* spp. *J.Ind.Microbiol.Biotechnol.* **28**, 201–203 (2002).

HARAZONO K., WATANABE Y., NAKAMURA K.: Decolorization of azo dye by the white-rot basidiomycete *Phanerochaete sordida* and by its manganese peroxidase. *J.Biosci.Bioeng.* **95**, 455–459 (2003).

DE JONG E., CAZEMIER A.E., FIELD J.A., DE BONT J.A.M.: Physiological role of chlorinated aryl alcohols biosynthesis *de novo* by the white rot fungus *Bjerkandera* sp. strain BOS55. *Appl.Environ.Microbiol.* **60**, 271–277 (1994).

KARIMINIAAE-HAMEDAANI H.R., SAKURAI A., SAKAKIBARA M.: Decolorization of synthetic dyes by a new manganese peroxidase-producing white rot fungus. *Dyes Pigments* **72**, 157–162 (2007).

KOKOL V., DOLIŠKA A., EICHLEROVÁ I., BALDRIAN P., NERUD F.: Decolorization of textile dyes by whole cultures of *Ischnoderma resinosum* and by purified laccase and Mn-peroxidase. *Enzyme Microb.Technol.* **40**, 1673–1677 (2007).

LANG E., NERUD F., ZADRAŽIL F.: Production of ligninolytic enzymes by *Pleurotus* sp. and *Dichomitus squalens* in soil and lignocellulose substrate as influenced by soil microorganisms. *FEMS Microbiol.Lett.* **167**, 239–244 (1998).

LU L., ZHAO M., ZHANG B.B., YU S.Y., BIAN X.J., WANG W., WANG Y.: Purification and characterization of laccase from *Pycnoporus sanguineus* and decolorization of an anthraquinone dye by the enzyme. *Appl.Microbiol.Biotechnol.* **74**, 1232–1239 (2007).

MARTÍNEZ A.T., SPERANZA M., RUIZ-DUENAS F.J., FERREIRA P., CAMARERO S., GUILLÉN F., MARTÍNEZ M.J., GUTIERREZ A., DEL RIO J.C.: Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *Internat.Microbiol.* **8**, 195–204 (2005).

MATSUMURA E., YAMAMOTO E., NUMATA A., KAWANO T., SHIN T., MURAO S.: Structure of the laccase-catalyzed oxidation products of hydroxy-benzoic acids in the presence of ABTS (2,2′-azino-di-(3-ethylbenzothiazoline-6-sulfonic acid)). *Agr.Biol.Chem.* **50**, 1355–1357 (1986).

MOREIRA M.T., PALMA C., MIELGO I., FEIJOO G., LEMA M.J.: *In vitro* degradation of a polymeric dye (Poly R-478) by manganese peroxidase. *Biotechnol.Bioeng.* **75**, 362–368 (2001).

PÉRIÉ F.H., GOLD M.H.: Manganese regulation of manganese peroxidase expression and lignin degradation by the white rot fungus *Dichomitus squalens*. *Appl.Environ.Microbiol.* **57**, 2240–2245 (1991).

PÉRIÉ F.H., SHENG D., GOLD M.H.: Purification and characterization of two manganese peroxidase isoenzymes from the white-rot basidiomycete *Dichomitus squalens*. *Biochim.Biophys.Acta* **1297**, 139–148 (1996).

PÉRIÉ F.H., REDDY G.V.B., BLACKBURN N.J., GOLD M.H.: Purification and characterization of laccases from the white-rot basidiomycete *Dichomitus squalens*. *Arch.Biochem.Biophys.* **353**, 349–355 (1998).

PODGORNIK H., POLJANŠEK I., PERDIH A.: Transformation of Indigo carmine by *Phanerochaete chrysosporium* ligninolytic enzymes. *Enzyme Microb.Technol.* **29**, 166–172 (2001).

ŠUŠLA M., NOVOTNÝ Č., SVOBODOVÁ K.: The implication of *Dichomitus squalens* laccase isoenzymes in dye decolorization by immobilized fungal cultures. *Biores.Technol.* **98**, 2109–2115 (2007).

SVOBODOVÁ K., ERBANOVÁ P., SKLENÁŘ J., NOVOTNÝ Č.: The role of Mn-dependent peroxidase in dye decolorization by static and agitated cultures of *Irpex lacteus*. *Folia Microbiol.* **51**, 573–578 (2006).

TIEN M., KIRK T.K.: Lignin peroxidase from *Phanerochaete chrysosporium*. *Methods Enzymol.* **161**, 238–248 (1988).

TORRES E., BUSTOS-JAIMES I., LE BORGNE S.: Potential use of oxidative enzymes for the detoxification of organic pollutants. *Appl. Catal B* **46**, 1–15 (2003).

WARIISHI H., VALLI K., GOLD M.H.: Manganese(II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. *J.Biol.Chem.* **267**, 23688–23695 (1992).

WESENBERG D., KYRIAKIDES I., AGASTHOS S.N.: White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol.Adv.* **22**, 161–187 (2003).

XIAOBIN C., RONG J., PINGSHENG L., SHIQIAN T., QIN Z., WENZHONG T., XUDONG L.: Purification of a new manganese peroxidase of the white-rot fungus *Schizophyllum* sp. F17, and decolorization of azo dyes by the enzyme. *Enzyme Microb.Technol.* **41**, 258–264 (2007).