Manganese Peroxidases of the White Rot Fungus *Phanerochaete sordida*

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The ligninolytic enzymes produced by the white rot fungus *Phanerochaete sordida* in liquid culture were studied. Only manganese peroxidase (MnP) activity could be detected in the supernatant liquid of the cultures. Lignin peroxidase (LiP) and laccase activities were not detected under a variety of different culture conditions. The highest MnP activity levels were obtained in nitrogen-limited cultures grown under an oxygen atmosphere. The enzyme was induced by Mn(II). The initial pH of the culture medium did not significantly affect the MnP production. Three MnP isozymes were identified (MnPI, MnPII, and MnPIII) and purified to homogeneity by anion-exchange chromatography followed by hydrophobic chromatography. The isozymes are glycoproteins with approximately the same molecular mass (around 45 kDa) but have different pIs. The pIs are 5.3, 4.2, and 3.3 for MnPI, MnPII, and MnPIII, respectively. The three isozymes are active in the same range of pHs (pHs 3.0 to 6.0) and have optimal pHs between 4.5 and 5.0. Their amino-terminal sequences, although highly similar, were distinct, suggesting that each is the product of a separate gene.

The lignin-degrading fungus *Phanerochaete sordida* was first described by Karsten in 1882 as *Corticium sordidum* (6) and was given its present name by Eriksson and Ryvarden (10). This fungus is found worldwide, especially in the northern hemisphere, as a saprophyte on hardwood and occasionally on softwood, on which it causes white rot decay (6). Previous work demonstrated that *P. sordida* isolate HHB-8922-sp (USDA Forest Products Laboratory designation) possesses a rapid growth rate over a wide range of temperatures and is superior in the ability to mineralize the wood preservative pentachlorophenol (PCP) in chemically defined aqueous culture compared with *P. chrysosporium* BKM-F-1767 and several other *P. sordida* isolates (30). On the basis of these results, this isolate was evaluated further for use in remediation of PCP-contaminated soils. The ability of this fungus to cause rapid and extensive decreases in PCP concentrations in both laboratory soils (30) and field soils with (9, 29) and without (28) cocontamination with creosote demonstrates its potential for use in remediation of PCP-contaminated soils.

Extracellular lignin peroxidases (LiPs), manganese peroxidases (MnPs), and laccases produced by lignin-degrading fungi have been shown to catalyze the oxidative degradation of several important pollutants (27). For example, the oxidative 4-dechlorination of PCP to chloranil (tetrachloro-*p*-benzoquinone) is catalyzed by both LiP and MnP from *P. chrysosporium* (17). These enzymes have also been shown to be involved in the initial and subsequent oxidations of 2,4-dichlorophenol and several of its transformation products that lead to complete mineralization of this compound by *P. chrysosporium* in aqueous culture (49). Fungal laccases, peroxidases, and tyrosinases have also been shown to catalyze the in vitro coupling of chlorophenols to humic materials, yielding chlorophenol-humic material hybrid polymers (44–46), a process that would eliminate the bioavailability and thus the toxicity of the chlo-

rinated compounds. Evidence that this process also occurs in soil was obtained by Qiu and McFarland (42), who reported that *P. chrysosporium* enhanced the formation of soil-bound residues of the creosote component benzo[*a*]pyrene.

Because (i) fungal extracellular lignin-degrading enzymes may play important roles in pollutant transformation in soil and (ii) there is interest in using *P. sordida* for soil remediation, the present research was undertaken to identify and characterize the extracellular lignin-degrading enzymes produced by this fungus.

MATERIALS AND METHODS

Organisms and culture conditions. *P. sordida* isolate HHB-8922-sp was obtained from the culture collection at the Center for Forest Mycology Research at the Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, Madison, Wis. The fungus was maintained in potato dextrose agar slants at 4°C. All cultures were prepared in the minimal salts medium described by Tien and Kirk (48) but with 10 mM transaconitic acid as a buffer instead of 2,2-dimethylsuccinate and without veratryl alcohol. All of the experiments described were done in shallow cultures consisting of 10 ml of medium in 125-ml Erlenmeyer flasks. They were incubated in stationary conditions at 30°C. Beginning on day 3, the flasks were flushed daily with oxygen, unless otherwise stated. All experiments were done in triplicate. Blended mycelium was used as the inoculum. For inoculum preparation, 7-day-old fungal mats were broken up in a Waring blender (five 15-s spurts at high speed with 30-s intervals to prevent warming of the mycelium). The cultures were inoculated with 0.45 mg of mycelium (dry-weight basis) ml^{-1} of medium.

Optimization of the culture medium for MnP production and search for LiP and laccase. In a series of experiments, different components of the culture medium were varied to find the best conditions for production of the three extracellular enzymes. Aliquots of culture supernatant fluids were taken at different times to assay for activities of MnP, LiP, and laccase.

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To study the effect of the nitrogen concentration, we prepared cultures containing 1.0 and 10.0 mM ammonium tartrate and a non-growth-limiting concentration of the carbon source (1% glucose). These cultures contained 11 μg of Mn(II) ml^{-1}. Mn(II) was always added as its sulfate form (MnSO$_4$). All of the concentrations given are for the free ion and not the salt.

The effect of oxygen was studied by flushing cultures containing 1.0 mM ammonium tartrate and 11 μg of Mn(II) ml^{-1} daily with either 100% oxygen or air, beginning on day 3 after inoculation.

The cultures used to assess the effect of Mn(II) contained 0.3, 11.0, 40.0, or 100.0 μg of Mn(II) ml^{-1} in standard medium with 1.0 mM ammonium tartrate and an oxygen atmosphere.

The effect of pH was studied by using 10 mM transaconitic acid as a buffer at pHs 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, and 6.0. The cultures contained 1.0 mM ammonium tartrate and 11 μg of Mn(II) ml^{-1}.

The effect of veratryl alcohol on LiP production was assayed by adding a 0.4 mM concentration of the alcohol to cultures that contained 1.0 mM ammonium tartrate and 11 μg of MnSO$_4$ ml^{-1}.

Cultures designed to enhance laccase production contained 10 mM MES (morpholineethanesulfonic acid) buffer (pH 6.5) in the presence of 500 μg of CuSO$_4$ per liter (15). Also, the laccase inducers p-hydroxybenzoic acid (1 mM) (15) and 2,5-xylidine (0.2 mM) (11) were added to cultures made in the standard conditions described previously.

The production of enzymes in shaking cultures was also assessed. Cultures were made in 125-ml flasks containing 30 ml of culture medium and shaken at 180 rpm. Tween 20 (0.1 or 0.5%) was also added to these cultures.

Enzyme assays. MnP activity was assayed by using vanillylacetone as the substrate, as described by Paszczynski et al. (35). Two assays for laccase activity were used. The first used 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as the substrate at pH 3.0 (3). The second used syringaldazine as the substrate at pH 6.8 (1). LiP activity was assayed as described by Tien and Kirk (47), with veratryl alcohol as the substrate.

MnP purification. Four hundred stationary 10-ml cultures of P. sordida grown in the conditions found to be best for MnP production [1 mM ammonium tartrate, 40 μg of Mn(II) ml^{-1} and an oxygen atmosphere] were harvested 6 days after inoculation. The culture supernatant solution was separated from the mycelium by filtration through cheesecloth. The fluid was frozen (-20°C) and thawed to promote precipitation of the extracellular polysaccharide, from which it was then separated and then concentrated 50-fold by ultrafiltration (10-kDa molecular-weight cutoff membrane). After dialysis against 10 mM sodium acetate (pH 6.0), the dialyzed concentrate was subjected to chromatography on an Accel (Waters, Medford, Mass.) anion-exchange column (1.0 cm^2 by 10 cm). Proteins were eluted with 0.6 M sodium acetate (pH 6.0). Fractions containing MnP activity were pooled, concentrated, and dialyzed against 10 mM sodium acetate (pH 6.0). The pooled fractions were then submitted to anion-exchange chromatography (Mono Q column; Pharmacia, Uppsala, Sweden) in a fast protein liquid chromatography system (Pharmacia). The mobile phase was 10 mM sodium acetate (pH 6.0), and the flow rate was 1.0 ml min^{-1}. Proteins were eluted with a sodium acetate gradient (0.0 to 0.45 M in 45 min, pH 6.0). A_{280} and A_{405} were continuously monitored. Three peaks containing MnP activity were obtained. Fractions corresponding to the first peak were pooled separately. Fractions corresponding to the second and third peaks were pooled together. Both pools were subjected to hydrophobic chromatography (phenyl Superose column; Pharmacia) in the fast

protein liquid chromatography system. Potassium phosphate buffer (50 mM, pH 7.0) containing 1.7 M ammonium sulfate was used as the mobile phase at a flow rate of 0.5 ml min^{-1}. The proteins were eluted with a gradient of ammonium sulfate that varied from 1.7 to 0.85 M in the first 20 min and from 0.85 to 0.0 M in the following 40 min. The MnP peaks obtained from this chromatographic step were pooled, concentrated, and dialyzed.

Characterization of MnP isozymes. The molecular weights of the three isozymes were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) performed with the Phast System (Pharmacia). Gels (10 to 15%) and conditions recommended by the manufacturer were used. Staining was done with Coomassie blue. The molecular size standards used were rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), chicken egg white ovalbumin (45 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and chicken egg white lysozyme (14.4 kDa).

The isoelectric points of the proteins were determined with a Bromma 2117 Multiphor apparatus (LKB) and Servalyt Precoats polyacrylamide gels (Serva Fine Biochemicals Inc., Westbury, N.Y.) with a pH range of 3 to 6. Proteins were focused for 5 min at 400 V and then for 5 min at 800 V. The standards used were amyloglucosidase (pI 3.55), trypsin inhibitor (pI 4.55), β-lactoglobulin A (pI 5.13), and bovine carbonic anhydrase (pI 5.85). Proteins were stained with Serva Violet. MnP activity staining was done with 4-chloro-1-naphthol as the substrate. Five milligrams of the substrate was dissolved in 1.7 ml of methanol and mixed with 8.3 ml of 20 mM Tris-HCl (pH 7.5)–0.5 M NaCl. Eight milliliters of the resulting solution was mixed with 32 ml of 50 mM sodium tartrate (pH 5.0) containing 100 mM MnSO$_4$. After the gel was soaked in this solution, hydrogen peroxide was added to a final concentration of 50 μM.

N-terminal sequencing of the purified enzymes was done at the University of Wisconsin Biotechnology Center. Sequence similarity searches were performed on Swiss and PIR data bases (release 77) with DNASTAR (Madison, Wis.) software. Sequences were aligned by clustal analysis (18).

Two criteria were used to determine whether the MnP isozymes were glycosylated: affinity to concanavalin A-Sepharose and detection of sugars in glycoconjugates by an enzyme immunoassay (Glycan Detection Kit; Boehringer Mannheim). The total crude concentrate from P. sordida cultures was loaded onto a concanavalin A-Sepharose (Sigma) column equilibrated with 10 mM Na acetate (pH 6)–0.5 M NaCl–1.0 mM MnCl$_2$–1.0 mM CaCl$_2$. After loading of the protein, the column was washed with the same buffer and the fractions were checked for MnP activity. The column was then eluted with 0.5 M α-methyl-D-mannopyranoside (Sigma) in the same buffer. All of the MnP activity eluted at this step. The Glycan Detection Kit (Boehringer Mannheim catalog no. 1142 372) was used as recommended by the manufacturer. Transferrin and creatinase were used as positive and negative controls, respectively. One-microgram samples of purified MnPI, MnPII, and MnPIII, and control proteins were used.

pH-activity profiles were determined for each MnP isozyme by using 100 mM sodium tartrate buffer (final concentration in the assay medium) adjusted to pHs 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. Citrate phosphate buffer was used for pH 6.0. Assays were conducted by using the standard conditions described above.
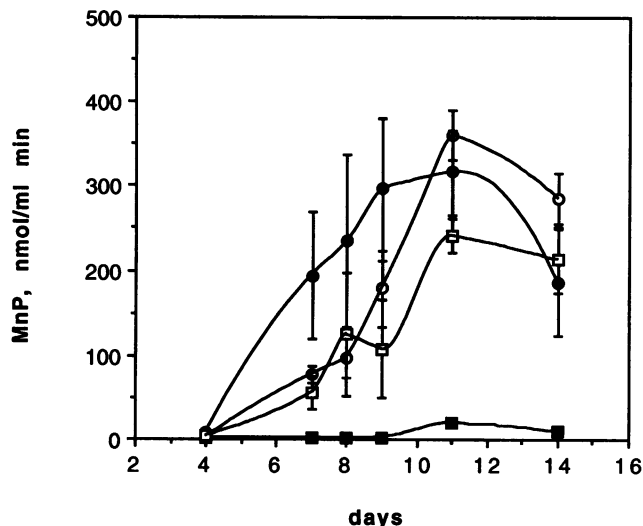
FIG. 1. Effect of Mn(II) concentration in the culture medium on MnP production by *P. sordida*. Cultures contained 0.3 (■), 11 (O), 40 (●), or 100 (□) μg of Mn(II) ml^{-1}.

FIG. 2. Effect of pH on MnP production by *P. sordida*. Cultures contained 10 mM transaconitic acid buffer at pH 4.5 (□), 5.5 (■), 6.0 (●), or 6.5 (O).

RESULTS

Extracellular enzymes secreted by *P. sordida*. Of the three enzymes for which activities were assayed, only MnP activity was detected in cultures of *P. sordida*. Lignin peroxidase and laccase activities were not detected under any of the different culture conditions evaluated. In some cases, some oxidation of the laccase substrates 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and syringaldazine was observed, but this activity disappeared after dialysis of the samples. Oxidation of these substrates was probably due to Mn(III) that was produced by oxidation of Mn(II) by MnP, both of which were present in the culture supernatant solution. Both substrates can be oxidized by Mn(III) (16, 36). Indeed, when no Mn(II) was added to the cultures, neither substrate was oxidized. Culture conditions described for laccase production by other fungi (15) were also evaluated by using MES buffer (pH 6.5) in the presence of 500 μg of CuSO$_4$ per liter with negative results. Addition of known laccase inducers including *p*-hydroxybenzoic acid (15) and 2,5-xylidine (11), to the culture medium also failed to induce laccase production by *P. sordida*. Attempts to detect LiP in the culture medium were also unsuccessful. The enzyme was not detected even under culture conditions that have been reported to enhance LiP activity in other fungi, such as addition of veratryl alcohol to the medium (25) and use of low concentrations of Mn(II) (2). No LiP activity was detected either after 20-fold concentration of the media.

Optimal conditions for MnP production by *P. sordida*. MnP was induced by Mn(II). Cultures containing 0.3 μg of Mn(II) ml^{-1} produced very low levels of MnP compared with cultures containing 11 μg of this metal ion ml^{-1} (Fig. 1). Addition of higher concentrations (40 and 100 μg ml^{-1}) of Mn(II) to the culture medium did not produce a further increase in MnP levels.

Variation of the initial pH of the culture medium between pHs 2.0 and 6.0 did not have a significant effect on MnP production (Fig. 2). The pH of the culture medium was measured at the end of the experiment (12 days). It was found that the pHs of all of the flasks were approximately the same
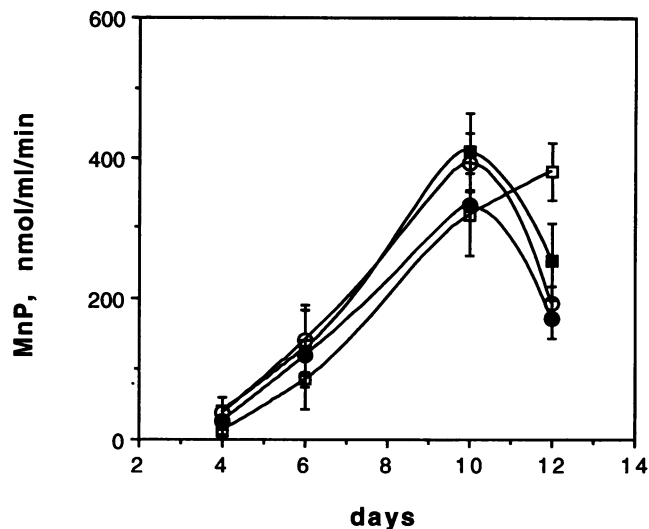
(pHs 5.2 to 5.9). This indicates that the pH of the culture medium was altered by the activity of *P. sordida*.

Production of MnP was found to be induced by nitrogen limitation. Cultures containing 1.0 mM ammonium tartrate produced, at their maxima, almost 10 times more enzyme than did cultures containing 10.0 mM ammonium tartrate (data not shown). Oxygen also enhanced the production of MnP. MnP levels in oxygen-treated cultures were around 220 nmol min^{-1} ml^{-1}, compared with 10 nmol min^{-1} ml^{-1} in air-treated cultures, on day 10 (data not shown).

MnP purification. MnP from *P. sordida* was purified from 400 10-ml shallow cultures. Efforts to produce the enzyme in submerged shaking cultures, which would have allowed the use of greater volumes of medium, were unsuccessful. The fungus grew well in shaking cultures, but it did not secrete MnP. Addition of the detergent Tween 20 to culture media has enhanced the secretion of extracellular enzymes in shaking conditions for other fungi (20). However, inclusion of Tween 20 in the *P. sordida* culture medium did not result in the appearance of MnP activity in shaking cultures.

Ion-exchange chromatography of the concentrated culture supernatant revealed the presence of three heme proteins, all of which had MnP activity (Fig. 3A). These proteins will hereafter be referred to as MnPI, MnPII, and MnPIII. MnPI was predominant over the other two. MnPII and MnPIII were present in only minor concentrations. A number of other proteins, the nature of which is unknown, were present in the anion-exchange elution profile. Fractions corresponding to MnPI were pooled separately. Those fractions corresponding to MnPII and MnPIII were pooled because their elution times were too close for efficient separation. Both pools were subjected to hydrophobic chromatography. MnPI eluted as one peak again, at 0.55 M ammonium sulfate (Fig. 3B). MnPII and MnPIII were resolved in two well-separated peaks by this type of chromatography, eluting at 0.48 and 0.27 M ammonium sulfate, respectively (Fig. 3C). Fractions corresponding to the three enzymes were then pooled separately, concentrated, and desalted. The MnPI, MnPII, and MnPIII obtained by this procedure were shown to be pure by SDS-PAGE and isoelectric focusing (see below).
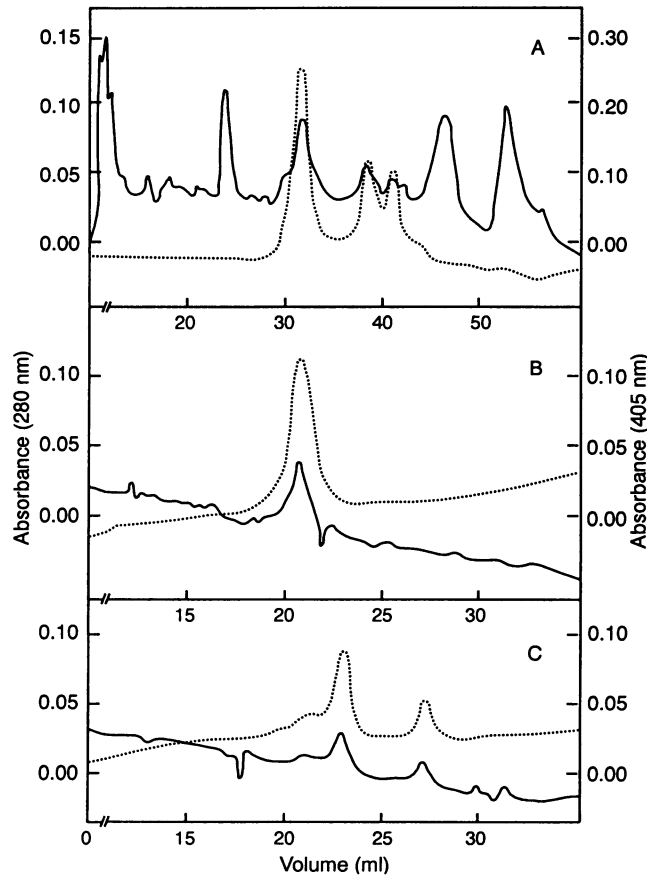
FIG. 3. FPLC profiles of *P. sordida* MnPs. (A) Ion-exchange chromatography profile of concentrated supernatant fluid from 6-day-old cultures. (B and C) Hydrophobic chromatography profiles of pools of MnPI (B) and MnPII plus MnPIII (C) obtained by anion-exchange chromatography. A_{280} (——) and A_{405} (– – –) are shown.
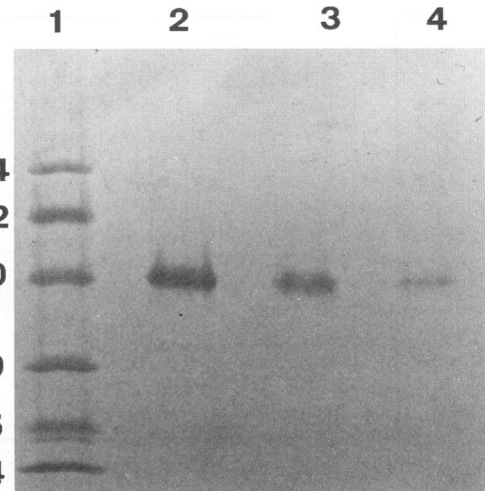

FIG. 4. SDS-PAGE of MnP isozymes of *P. sordida* purified by anion-exchange chromatography followed by hydrophobic chromatography. Molecular size marker proteins (lane 1), MnPI (lane 2), MnPII (lane 3), and MnPIII (lane 4) are shown. Molecular masses of marker proteins are indicated in kilodaltons on the left.

Characterization of the three MnP isozymes. MnPI, MnPII, and MnPIII were found to be glycoproteins, as shown by their affinity to concanavalin A-Sepharose and by detection of sugars in glycoconjugates by an enzyme immunoassay. They had approximately the same molecular mass, approximately 45 kDa, as determined by SDS-PAGE (Fig. 4). Their isoelectric points (pIs), however, were different. They were as follows: MnPI, pI 5.3; MnPII, pI 4.2; MnPIII, pI 3.3 (Fig. 5). Each of the enzyme preparations showed only one band when subjected to SDS-PAGE and isoelectric focusing (Fig. 4 and 5). The different pIs of the isozymes suggested that they might differ in their pH-activity profiles. Nevertheless, the optimal pHs for the three MnPs were very similar (Fig. 6). MnPI had an optimal pH of 4.5, and MnPII had an optimal pH of 4.0. MnPIII had approximately the same activity at these two pHs. The enzymes were active in a relatively narrow range of pHs (pHs 3 to 5) and were completely inactive at pHs of ≤2 and ≥6.

The N-terminal sequences of MnPI, MnPII, and MnPIII were distinct but highly similar (Fig. 7). Most mismatches involved residues with similar properties, e.g., S versus P and Q versus D. Data base searches showed no significant homology to proteins not identified as MnPs, including the LiPs. How-

ever, there was substantial homology to *P. chrysosporium* and *L. edodes* MnPs (Fig. 7).

DISCUSSION

Of the three extracellular enzymes that have been implicated in lignin degradation by white rot fungi, LiP, MnP, and laccase, only MnP activity was detected in cultures of *P. sordida* grown under a variety of different conditions. White rot fungi have been shown to produce different combinations of these three extracellular enzymes. *P. chrysosporium*, the most extensively studied white rot fungus, produces several isozymes of LiP and MnP but no laccase. Other fungi, including *Dichomitus squalens* (40), *Ceriporiopsis subvermispora* (43), *Phlebia brevispora* (43), *Rigidoporus lignosus* (15), *Panus tigrinus* (32), *Lentinus edodes* (14), and *Ganoderma colossum* (19), have been reported to possess both MnP and laccase activities but no LiP activity. In general, these two enzymes seem to be more widely
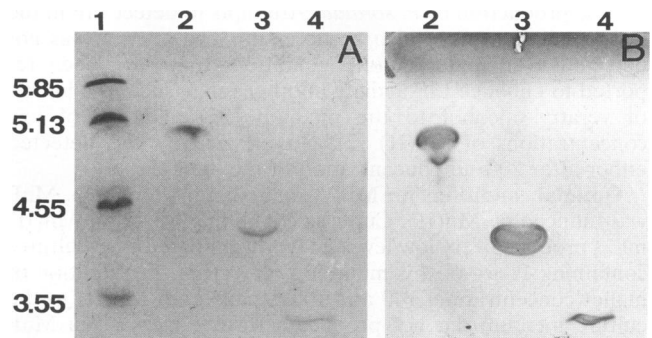

FIG. 5. Isoelectric focusing of the purified isozymes MnPI (lane 2), MnPII (lane 3), and MnPIII (lane 4). pI standards were run in lane 1. Gel A was stained for protein with Serva Violet, and gel B was stained for MnP activity by using 4-chloro-1-naphthol as the substrate. pIs of standard proteins are indicated on the left.
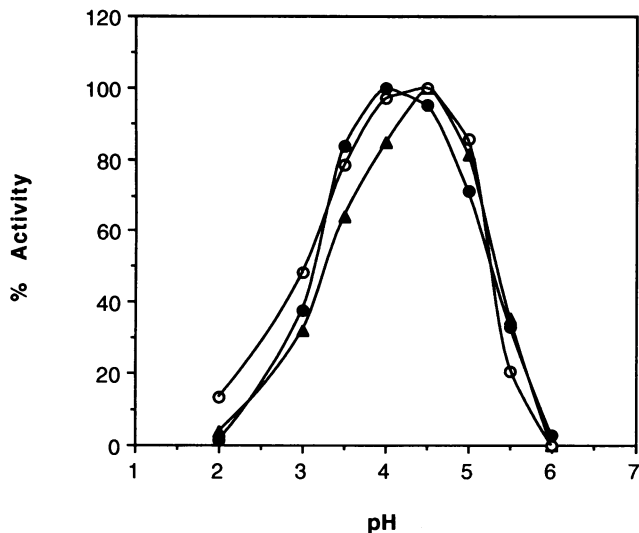
FIG. 6. pH-activity profiles of MnPI (▲), MnPII (●), and MnPIII (○). Assays were done in standard conditions with sodium tartrate buffer for pHs 2.0 to 5.5 and citrate phosphate buffer for pH 6.0.

distributed among white rot fungi than is LiP. *Phlebia radiata* (23, 33) and *Coriolus versicolor* (22) are unique in that they produce LiP in addition to MnP and laccase. Recently, two white rot fungi, *Phlebia ochraceofulva* and *Junghunia separabilima*, that produce LiP and laccase but not MnP have been described (50). *J. separabilima* also produces a novel enzyme that apparently possesses both oxidase and peroxidase activities (50). In *P. sordida* cultures, no LiP or laccase could be detected, even under conditions in which secretion of these enzymes by other fungi is optimized. However, it is not possible to rule out the production of LiP. Genes like those that encode LiP have been found in this organism by using Southern blot techniques (7), and therefore it is possible that the enzyme can be induced in other culture conditions, for example, when growing on wood or in soil.

The culture conditions for optimum production of MnP by *P. sordida* are the same as those that have been described for optimum production of this enzyme by *P. chrysosporium* (12, 25). When grown in the optimal culture conditions, the levels of MnP produced by *P. sordida* are in the same range as those

described for other fungi that secrete this enzyme. The highest MnP levels are produced in nitrogen-limiting conditions, that is, during secondary metabolism. This is true for most of the white rot fungi that have been studied, including *P. chrysosporium* (21, 24), *P. radiata* (33), *P. brevispora* (43), and *C. versicolor* (22). However, some exceptions have been described. For example, *C. subvermispora* produces higher levels of MnP and laccase when grown under nitrogen- and carbon-sufficient conditions (43), and *D. squalens* degrades lignin and therefore expresses its lignin-degrading enzymes under nitrogen-sufficient as well as nitrogen-limiting conditions (40).

Oxygen and Mn(II) also enhanced the production of MnP by *P. sordida*. It has been reported that for *P. chrysosporium* lignin mineralization, as well as LiP and MnP production, is enhanced by an oxygen atmosphere (12). Oxygen had a very pronounced inducing effect on the MnP of *P. sordida*. Nevertheless, this does not seem to be a universal feature for all white rot fungi, since it did not improve the production of ligninolytic enzymes by *C. subvermispora* (43). It is known that Mn(II) induces the production of MnP in other white rot fungi (2, 5, 39, 40, 43). For *P. chrysosporium* it has been demonstrated that the inducing effect of the Mn(II) cation is directly on the transcription of the MnP gene (4). MnP production by *P. sordida* was induced by 11 μg of Mn(II) ml^{-1}. This is comparable to the concentration (10 μg ml^{-1}) found to give maximum production of MnP by *P. chrysosporium* (4). Also, as with *P. chrysosporium* (4), higher concentrations of Mn(II) did not enhance MnP levels further.

The initial pH of the culture medium did not have a major effect on the MnP levels produced by *P. sordida*. This may be related to the ability of the fungus to alter the pH of the culture medium. The pHs of all of the cultures 12 days after inoculation of the fungus were around 5.5, regardless of the initial pH. It has been shown that *P. chrysosporium* also changes the pH of the medium, bringing it to around 5.0 after 9 days of growth (for cultures with initial pHs of 4.5 to 6.0) (26). The same investigators showed that the pH of the medium is critical for lignin degradation, with an optimum at pH 4.5. This fact may be related to the optimal pH for activity of the ligninolytic enzymes rather than to the amount of enzymes produced at different pHs. The MnPs of *P. sordida* have optimal pHs between 4.0 and 4.5.

The three MnP isozymes isolated from *P. sordida* are glycosylated heme proteins with molecular masses of around
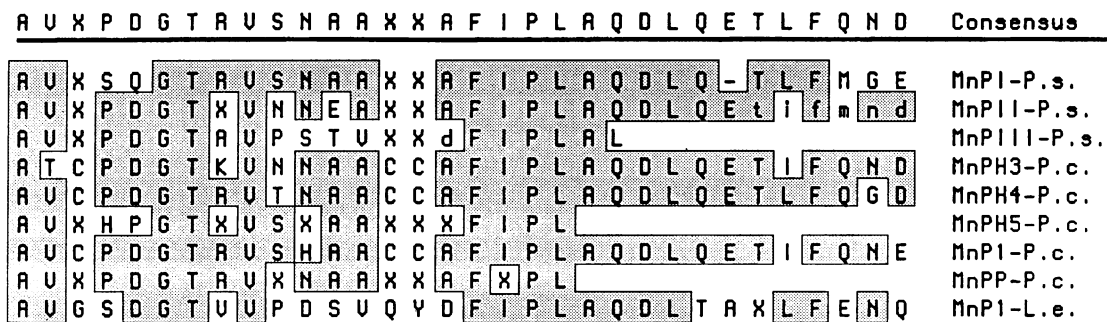

FIG. 7. N-terminal sequence alignments of MnP isozymes from *P. sordida*, *P. chrysosporium*, and *L. edodes*. The following three sequences were deduced from *P. chrysosporium* cDNA sequences: MnPH3-P.c. (34), MnPH4-P.c. (37), and MnP1-P.c. (41). Experimentally determined sequences were those of *P. chrysoporium* isozyme H5 (MnPH5-P.c.) (38), a *P. chrysosporium* isozyme purified from colonized pulp (MnPP-P.c.) (8), and the *L. edodes* MnP (MnP1-L.e.) (14). Lowercase letters indicate less confident assignments. Unknown residues are represented by the letter X. A gap was introduced in MnPI-P.s. to optimize the alignment. Residues matching the consensus are shaded.

45 kDa. They were separated on the basis of their different pIs (by anion-exchange chromatography) and further purified on the basis of their different hydrophobicities. The differences in the pIs (3.3, 4.2, and 5.3 for MnPI, MnPII, and MnPIII, respectively) did not reflect differences in their pH-activity profiles. The existence of a number of different MnP, as well as LiP, isozymes has been described for other white rot fungi (13, 22, 33, 34). The functional significance for the presence of these multiple isozymes is not clear. It has been shown that for *P. chrysosporium* the pattern of excreted LiP (31) and MnP (38) isozymes is influenced by the nutrient status of the culture. Isozyme profiles of *P. chrysosporium* were also shown to be influenced by culture age (13). Similar regulations of isozyme production may also exist for the MnPs of *P. sordida*. The ability to manipulate the production of LiP and MnP isozymes with greater oxidation potentials through nutritional or other regulatory mechanisms could provide a method for enhancing the pollutant-degrading abilities of *P. sordida* and other white rot fungi. Therefore, mechanisms that regulate isozyme patterns deserve further study.

The N-terminal sequences of MnPI, MnPII, and MnPIII, although distinct, are highly similar. The isozymes probably represent separate gene products, but allelic variants cannot be excluded in a basidiomycete such as *P. sordida*. A multiplicity of MnP-encoding genes has been demonstrated in *P. chrysosporium* (34, 38). Alignments of *P. sordida*, *P. chrysosporium*, and *L. edodes* MnP N-terminal sequences (Fig. 7) show highly conserved regions. For example, the sequence FIPLAQ is present in all of the isozymes, including those of another white rot fungus, *C. subvermispora* (51). These consensus sequences may facilitate cloning.

The extent to which MnPI, MnPII, and MnPIII are involved in xenobiotic transformation by *P. sordida* remains to be established.

ACKNOWLEDGMENTS

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