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Laccase-Mediated Detoxification of Phenolic Compounds†

J.-M. BOLLAG,* K. L. SHUTTLEWORTH, AND D. H. ANDERSON

Laboratory of Soil Biochemistry, Department of Agronomy, The Pennsylvania State University, University Park, Pennsylvania 16802

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The ability of a polyphenoloxidase, the laccase of the fungus *Rhizoctonia praticola*, to detoxify phenolic pollutants was examined. The growth of the fungus could be inhibited by phenolic compounds, and the effective concentration was dependent on the substituents of the phenol. A toxic amount of a phenolic compound was added to a fungal growth medium in the presence or absence of a naturally occurring phenol, and half of the replicates also received laccase. The medium was then inoculated with *R. praticola*, and the levels of phenols in the medium were monitored by high-performance liquid chromatography analysis. The addition of the laccase reversed the inhibitory effect of 2,6-xylenol, 4-chloro-2-methylphenol, and *p*-cresol. Other compounds, e.g., *o*-cresol and 2,4-dichlorophenol, were detoxified only when laccase was used in conjunction with a natural phenol such as syringic acid. The toxicity of *p*-chlorophenol and 2,4,5-trichlorophenol could not be overcome by any additions. The ability of the laccase to alter the toxicity of the phenols appeared to be related to the capacity of the enzyme to decrease the levels of the parent compound by transformation or cross-coupling with another phenol.

Phenolic pollutants can enter the environment from several sources, including the partial degradation of phenoxy herbicides (20), the use of wood preservatives (22), and the generation of wastes by petroleum-related industries (7). These phenolic compounds have various degrees of toxicity, and their fate in the environment is therefore important. Although many chlorophenols, cresols, and other aromatic compounds are biodegradable (6, 21), other types of biological transformations and chemical reactions can also alter the toxicity of these compounds. While mineralization is a detoxification reaction, partial degradation or transformation can either increase or decrease the toxicity of the parent compound (6).

One way to decrease the toxic effects of a compound is to bind the substance to humic acids, clays, or other materials (14). Binding can reduce the amount of material available to interact with the biota (11, 12), and as the quantity of available compound is reduced, the toxic effects also decrease. If covalent binding occurs, the compound will be less available and less likely to be released, and the change in the chemical structure may render the compound less toxic.

Several polyphenoloxidases catalyze the oxidative coupling of phenolic compounds, resulting in the formation of polymeric compounds (18). One type of polyphenoloxidase, laccase, is produced by several fungi, including *Rhizoctonia praticola* (3). Although the natural function of the laccase from this fungus is not known, it is possible that the laccase renders phenolic compounds less toxic via the polymerization reaction. Because the laccase is relatively nonspecific, it is capable of cross-coupling pollutant phenols with naturally occurring phenols (2). This phenomenon may alter the toxicity of the pollutant phenols.

The purpose of this study was to determine the ability of the laccase from *R. praticola* to decrease the toxicity of cresols and chlorophenols. Since the function of laccase is still undetermined, the results of this study may also provide insight into the biological significance of this enzyme.

* Corresponding author.

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MATERIALS AND METHODS

Organism and culture methods. Stock cultures of *R. praticola* were maintained on Czapek Dox agar slants. The medium was a modified Czapek Dox broth which consisted of the basal broth plus the following amendments (grams per liter): L-asparagine, 2.5; $CaSO_4 \cdot 5H_2O$, 0.025; $CuSO_4 \cdot 5H_2O$, 0.0015; and 1 ml of a trace element solution (19). Narrow-mouth glass reagent bottles (125 ml) containing 30 ml of medium were used in all experiments.

A uniform inoculum was obtained by growing the mycelia in tubes of nutrient broth for 2 days and then sonicating the cultures in a water bath sonicator until the mycelia were uniformly dispersed. The optical density of the suspension was measured at 380 nm, and 0.15 ml of a suspension with an absorbance of 0.1 was used as the inoculum for each bottle.

Laccase production and activity. Laccase was produced by induction of *R. praticola* with 1 mM *p*-anisidine as previously described (17). In brief, the fungus was grown for 9 to 11 days at 23°C, and *p*-anisidine was then added to a final concentration of 1 mM. The temperature was lowered to 15°C, and the fungus was incubated for an additional week. The extracellular laccase was harvested and partially purified by DEAE-cellulose column chromatography with 50 mM Tris (pH 7.1) as the eluting buffer (3). The enzyme was dialyzed against 10 mM sodium bicarbonate (pH 7.1) and stored at −20°C until needed. The enzyme was sterilized by passage through a 0.22-μm-pore-size cellulose triacetate membrane filter before its use.

The 2,6-dimethoxyphenol assay was used to determine enzyme activity after filtration. To obtain linear results it was necessary to dilute the enzyme. A 100-μl volume of the diluted enzyme was added to 3.4 ml of 1 mM 2,6-dimethoxyphenol in a 0.1 M sodium phosphate buffer (pH 6.9). An A_{468} change of 1 U/min is equal to 1 dimethoxyphenol (DMP) unit.

Addition of phenolic compounds and laccase. Phenolic compounds were dissolved in 95% ethanol, except for syringic acid, which was dissolved in 50% ethanol, and were sterilized by filtration through fritted glass (0.9- to 1.4-μm nominal pore size). The final concentration of ethanol in all

TABLE 1. Detoxification of methylphenols by *Rhizoctonia* laccase in the presence or absence of SYR[a]

Medium treatment	Concn (mM)[b] of remaining:		Days until:		Final biomass ± SD[c]
	Methylphenols	SYR	First growth	Confluence	
o-Cresol					
Alone	2.0		NG	NG	0
+ LAC	1.51		NG	NG	0
+ SYR	2.14	1.00	NG	NG	0
+ SYR and LAC	0.2	0.01	5–7	9–10	641 ± 8
p-Cresol					
Alone	2.0		NG	NG	0
+ LAC	0.85		5–6	11–13	633 ± 22
+ SYR	1.95	0.99	NG	NG	0
+ SYR and LAC	0.18	<0.01	7–8	12–15	644 ± 10
2,6-Xylenol					
Alone	1.0		NG	NG	0
+ LAC	<0.01		3–4	8–9	631 ± 38
+ SYR	0.88	1.01	NG	NG	0
+ SYR and LAC	<0.01	0.06	3–4	8–9	609 ± 13
SYR					
Alone		ND	2	6–7	613 ± 32
+ LAC		ND	3–4	8–9	662 ± 20
Ethanol control			2	6–7	622 ± 29
Control (no ethanol)			2	5–6	520 ± 16

[a] Abbreviations: SYR, syringic acid; LAC, laccase; ND, not determined; NG, no growth. *Rhizoctonia* laccase, 1 DMP unit per ml.
[b] Determined by HPLC analysis (1 day after addition of phenols and laccase).
[c] Dry weight in milligrams.

bottles was 1%. Preliminary experiments were completed to determine the relative toxicity of each phenol to the fungus; the lowest test concentration which completely inhibited growth of the fungus was then used for the detoxification experiments. Additions of phenolic compounds and laccase to the growth medium were made 1 day prior to fungal inoculation. After the reactants were mixed, the bottles were incubated in the dark without agitation at 23 ± 1°C to allow the enzyme reaction to proceed to completion prior to addition of the fungus. The final concentration of laccase was 0, 0.5, or 1.0 DMP units per ml of medium as noted. Naturally occurring phenolic compounds at a concentration of 0.5 or 1.0 mM were added to some bottles in an attempt to increase the level of enzymatic transformation of the selected phenol. All experiments were performed in triplicate or quadruplicate unless otherwise stated.

Dry weight determinations. A visual determination of fungal growth was made every 2 or 3 days. The day on which growth was first observed and the time necessary for the fungus to form a confluent mat on the surface of the medium were recorded. On day 21, all cultures were harvested, and dry weights were determined by filtering the cultures through a Whatman no. 4 filter in a Büchner funnel. The mycelia were washed twice with 30 ml of distilled water and dried at 90°C for 1 day. Distilled-water controls were used to correct for weight changes of the filter paper itself.

Dry weight measurements were taken at various intervals during the growth cycle and were used to compose growth curves. For this experiment, 14 replicate bottles were used for each set of conditions, and two bottles were harvested per time point.

HPLC analysis. Immediately after the fungal inoculation, 5-ml samples were removed from the replicate bottles for each set of conditions. Each sample was acidified with 50 μl of 30% trichloroacetic acid. Samples were then centrifuged, and 4 ml of the supernatant was subjected to the solid-phase extraction and high-performance liquid chromatography (HPLC) methods described previously (16). Sep-Pak C_{18} cartridges (Waters Associates, Inc., Milford, Mass.) were used for the extraction. Phenols were eluted from a 5-cm Supelcosil LC-18-DB 5-μm column (Supelco Inc., Bellefonte, Penn.) with a mobile phase composed of methanol-water-acetic acid. The proportions of methanol and water were adjusted so that the k' was always greater than 2; acetic acid in the mobile phase was always 1%. Cresols were diluted 1:2 immediately prior to the solid-phase extraction.

Preparation of synthetic humic acid. Synthetic humic acid was prepared by a modification of the method of Martin and Haider (10) with the following phenolic compounds used as substrates: 2,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid, vanillic acid, gallic acid, ferulic acid, syringic acid, caffeic acid, orcinol, phloroglucinol, resorcinol, pyrogallol, and catechol. The humic acid was prepared by dropwise addition of the phenols and the peroxide into the reaction flask containing the enzyme solution. The final preparation was dialyzed overnight against deionized water; the dialysis was completed to remove unreacted phenols and low-molecular-weight fulvic acid.

Reagents and chemicals. All chemicals were commercially available and of reagent grade, except the synthetic humic acid and the laccase. All commercial reagents were used without further purification.

RESULTS

The toxic concentration of phenol varied according to the substituent group(s) on the ring. The chlorophenols were

TABLE 2. Detoxification of chlorophenols by *Rhizoctonia* laccase in the presence or absence of SYR[a]

Medium treatment	Concn (mM)[b] of remaining:		Days until:		Final biomass ± SD[c]
	Chlorophenols	SYR	First growth	Confluence	
o-Chlorophenol					
Alone	0.87		4–6	10–13	ND
+ LAC	0.72		6–8	>16	ND
+ SYR	0.78	0.72	2–4	10–13	ND
+ SYR and LAC	0.60	0.01	4–6	10–13	ND
p-Chlorophenol					
Alone	1.01		NG	NG	0
+ LAC	0.82		NG	NG	0
+ SYR	0.95	0.75	NG	NG	0
+ SYR and LAC	0.72	<0.01	NG	NG	0
2,4-Dichlorophenol					
Alone	0.18		NG	NG	0
+ LAC	0.11		NG	NG	0
+ SYR	0.17	0.78	NG	NG	0
+ SYR and LAC	0.07	<0.01	2–4	10–13	661 ± 74
2,4,5-Trichlorophenol					
Alone	0.041		NG	NG	0
+ LAC	0.042		NG	NG	0
+ SYR	0.043	0.71	NG	NG	0
+ SYR and LAC	0.035	<0.01	NG	NG	0
4-Chloro-2-methylphenol					
Alone	0.45		NG	NG	0
+ LAC	0.01		2–4	8–10	684 ± 12
+ SYR	0.51	0.76	NG	NG	0
+ SYR and LAC	0.02	<0.01	2–4	8–10	731 ± 22
SYR					
Alone		0.74	<2	6–8	651 ± 2
+ LAC			<2	8–10	706 ± 17
Ethanol control			<2	6–8	660 ± 29

[a] Abbreviations: SYR, syringic acid; LAC, laccase; NG, no growth; ND, not determined. *Rhizoctonia* laccase, 1 DMP unit per ml.
[b] Determined by HPLC analysis (1 day after addition of phenols and laccase).
[c] Dry weight in milligrams.

more toxic than the cresols. No toxicity was observed for *m*-cresol at concentrations of up to 2 mM, although both *o*- and *p*-cresol inhibited fungal growth at this concentration. The most toxic compound tested in this study was 2,4,5-trichlorophenol, which completely inhibited growth at 0.05 mM. Synthetic humic acid at a concentration of 0.5 mg/ml also inhibited the growth of *R. praticola*. Growth was first visible in control cultures on day 2, and a confluent mat was formed on day 7; however, when 0.5 mg of synthetic humic acid per ml was present, there was a 7- to 9-day lag until first visible growth and the mycelia were not confluent until day 14.

The toxicity of the methylphenols was decreased when laccase was added to the medium (Table 1); however, the presence of polymerized products or the residual unreacted cresols apparently caused a slight inhibition of fungal growth as indicated by an increased lag time. Both *p*-cresol and 2,6-xylenol (2,6-dimethylphenol) were made less toxic by the addition of laccase alone and by the addition of laccase and syringic acid. On the other hand, *o*-cresol was detoxified only when both syringic acid and laccase were added (Table 1). Detoxification as measured by the removal of growth inhibition of the fungus corresponded to the relative ability of the laccase to transform the phenolic substrate. The concentration of free *o*-cresol was reduced by approximately 25% when this compound was incubated with laccase alone. However, a 90% reduction in *o*-cresol occurred when 1 mM syringic acid was added in addition to the laccase (Table 1).

The ethanol which was used to dissolve the phenolic compounds may have been slightly inhibitory (Table 1). However, more noteworthy was the increase in the dry weight of the ethanol controls relative to the controls without ethanol (Table 1), indicating that the ethanol was used as a carbon source by the fungus.

The effects of laccase and laccase plus syringic acid on the toxicity of chlorophenols are presented in Table 2. Insufficient *p*-chlorophenol and 2,4,5-trichlorophenol were transformed by the laccase to allow growth of the fungus in either the presence or absence of syringic acid. In contrast, laccase was able to polymerize sufficient 4-chloro-2-methylphenol to remove growth inhibition either with or without syringic acid present (Table 2). The toxicity of 2,4-dichlorophenol was reduced when cross-coupled with syringic acid in the presence of laccase, although 2,4-dichlorophenol continued to inhibit fungal growth after exposure to laccase alone. Little if any difference was observed between the various treatments with *o*-chlorophenol, which by itself caused only a 2-to 4-day increase in lag time at the concentrations tested.

R. praticola was able to grow in the presence of 2,4-dichlorophenol, which was cross-coupled to any of the

TABLE 3. Detoxification of DCP by *Rhizoctonia* laccase in the presence of naturally occurring phenols[a]

Medium treatment	Concn (mM)[b] of remaining:		Days until:		Final biomass ± SD[c]
	DCP	Natural phenol	First growth	Confluence	
SYR					
Alone		0.47	<3	7	622 ± 14
+ LAC		0.01	2–6	7–10	668 ± 16
+ DCP	0.19	0.51	NG	NG	0
+ DCP and LAC	0.09	<0.01	3–6	12–14	631 ± 72
Ferulic acid					
Alone		0.56	<3	7	628 ± 29
+ LAC		<0.01	<3	7–10	631 ± 32
+ DCP	0.18	0.51	NG	NG	0
+ DCP and LAC	0.1	<0.01	7–10	14–17	627 ± 31
Vanillic acid					
Alone		0.72	<3	7	620 ± 24
+ LAC		<0.01	<3	7–10	611 ± 10
+ DCP	0.18	0.76	NG	NG	0
+ DCP and LAC	0.1	<0.01	8–12	14–17	557 ± 16
P-Coumaric acid					
Alone		0.52	<3	7	613 ± 10
+ LAC		<0.01	<3	7–10	625 ± 22
+ DCP	0.17	0.48	NG	NG	0
+ DCP and LAC	0.12	0.02	13–17	>19	40 ± 41
Guaiacol					
Alone		0.46	<3	7–10	579 ± 33
+ LAC		0.01	<3	7–10	614 ± 19
+ DCP	0.17	0.47	NG	NG	0
+ DCP and LAC	0.13	<0.01	10–12	17–19	509 ± 111
DCP control	0.15		NG	NG	0
DCP + LAC	0.19		NG	NG	0
Ethanol control			<3	7	637 ± 22

[a] Abbreviations: DCP, 2,4-dichlorophenol; LAC, laccase; NG, no growth; SYR, syringic acid. *Rhizoctonia* laccase, 0.5 DMP unit per ml.
[b] Determined by HPLC analysis (1 day after addition of phenols and laccase).
[c] Dry weight in milligrams.

following naturally occurring phenolic compounds: syringic acid, ferulic acid, vanillic acid, or guaiacol (Table 3). Again, the lower toxicity appeared to correlate with the decrease in free 2,4-dichlorophenol remaining in the medium. None of the naturally occurring phenols was toxic at the concentrations tested, either in the free form or after laccase polymerization (Table 3).

The only observed toxic effect was an increase in lag time, and there did not appear to be any large differences in growth rates as determined by the number of days between first growth and confluency (Tables 1–3). The increase in lag time was clearly demonstrated by the time course experiment done with 2,4-dichlorophenol (Fig. 1). Low dry weight values such as those for 2,4-dichlorophenol incubated with guaiacol and laccase apparently reflected the increased lag time, i.e., the fungus had not reached the stationary phase at the time of sampling (day 21).

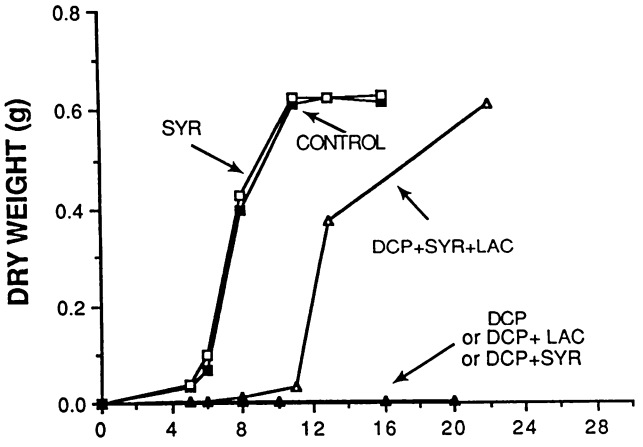

FIG. 1. Growth of *R. praticola* in the presence of 2,4-dichlorophenol and 2,4-dichlorophenol cross-coupled with syringic acid. The reagents used were 2,4-dichlorophenol (DCP), 0.2 mM; syringic acid (SYR), 0.5 mM; and laccase (LAC), 0.5 DMP units per ml. Points are averages of duplicate samples.

DISCUSSION

The concentrations of phenolic compounds found to be toxic to the fungus in this study were 10 to 1,000 times higher than the concentrations of phenols reported at such sites as a sawmill (22) and an oil sands extraction wastewater pond (9) but below those reported for shale oil and solvent-refined

coal liquid (7), some petroleum industry discharges (1), and coal conversion wastewater effluents (5). Thus, a chemical spill of certain wastes may indeed inhibit the growth of fungi. However, detoxification reactions may prevent an accumulation of phenolics, thus allowing continued fungal growth.

Many other fungi are also inhibited by similar concentrations of *p*-chlorophenol (1 to 2 mM), whereas a wide range of MICs has been reported for 2,4-dichlorophenol (0.06 mM for *Saccharomyces cerevisiae* and 1 mM for *Candida krusei* [15]). It is generally accepted that toxicity increases as the degree of chlorination increases (21), and the results of this study confirm this hypothesis, e.g., a lower concentration of 2,4,5-trichlorophenol than mono-substituted chlorophenols was needed to inhibit *R. praticola*.

Laccase was unequivocably capable of detoxifying some of the phenolic compounds tested. The ability of the enzyme to detoxify the particular phenol appeared to be directly related to its ability to transform the compound, as demonstrated by disappearance of the parent phenol. Detoxification of *o*-cresol and 2,4-dichlorophenol required cross-coupling with another phenol. This phenomenon is apparently related to the amount of phenol which could be transformed by the laccase, since previous studies have shown that the amount of 2,4-dichlorophenol transformed by *R. praticola* laccase is a function of the concentration of syringic acid (16).

Other groups have also attempted to detoxify aromatic compounds with phenoloxidases. Popoff et al. (13) stated that liovil, a phenolic compound, was detoxified by the laccase of the fungus *Fomes annosus* (*Heterobasidion annosum*), but the evidence to support this claim was only circumstantial. Similarly, Bordeleau and Bartha (4) maintained that formation of an aniline dimer (3,3′,4,4′-tetrachloroazobenzene) from 3,4-dichloroaniline was a detoxification mechanism. However, the dimer was still highly toxic to the fungi used in the study, and the results were further complicated by the fact that the fungi were grown in cocultures. Thus, it is not clear that the dimer was actually less toxic than the parent compound (propanil) or the primary degradation product (3,4-dichloroaniline), or both.

In contrast to authors claiming that phenoloxidases have a detoxifying effect, Haars and Hüttermann (8) reported that laccase activity increased the toxicity of phenols. In their study, catechol and guaiacol strongly inhibited the growth of the fungus *F. annosus* (*H. annosum*) when the fungus produced extracellular laccase. However, addition of a copper-chelating agent such as thioglycolate, which inhibited laccase activity, reduced the toxicity of catechol and guaiacol. They concluded that the toxic effect of the phenols was related to their oxidation by laccase. Thus, the ability of laccase to detoxify a given phenol may be dependent on the phenolic compound tested, the test organism, other environmental parameters, or a combination of these factors.

Interestingly, *R. praticola* was inhibited by synthetic humic acid polymerized from various phenolic compounds with peroxidase, but the polymerized products of such compounds as ferulic acid and *p*-coumaric acid were apparently not inhibitory. There are several possible explanations for this result. First, the concentrations of polymerized products are most likely not the same as the concentration of the synthetic humic acid. Second, the synthetic humic acid was made from a mixture of 13 phenolic compounds, whereas the products in the detoxification tests were made from only 1 or 2 phenolic compounds. Thus, their chemical structures most likely are substantially different from that of the synthetic humic acid. Third, and most important, the synthetic humic acid was soluble, whereas a large amount of precipitate was generated when laccase was added to bottles containing *p*-cresol, 2,6-xylenol, 4-chloro-2-methylphenol, and each of the phenolic acids. Thus, the detoxification phenomenon may be a result not only of an alteration in the chemical form of the pollutant phenol but also of the physical removal by precipitation of the complexed products.

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LITERATURE CITED

1. **Baird, R. B., L. G. Carmona, and R. L. Jenkins.** 1977. The direct-injection GLC analysis of xylenols in industrial wastewaters. Bull. Environ. Contam. Toxicol. **17**:764–767.
2. **Bollag, J.-M., and S.-Y. Liu.** 1985. Copolymerization of halogenated phenols and syringic acid. Pestic. Biochem. Physiol. **23**: 261–272.
3. **Bollag, J.-M., R. D. Sjoblad, and S.-Y. Liu.** 1979. Characterization of an enzyme from *Rhizoctonia praticola* which polymerizes phenolic compounds. Can. J. Microbiol. **25**:229–233.
4. **Bordeleau, L. M., and R. Bartha.** 1971. Ecology of a herbicide transformation: synergism of two soil fungi. Soil Biol. Biochem. **3**:281–284.
5. **Fedorak, P. M., and S. E. Hrudrey.** 1985. Batch anaerobic methanogenesis of phenolic coal conversion wastewater. Water Sci. Technol. **17**:143–154.
6. **Fewson, C. A.** 1981. Biodegradation of aromatics with industrial significance, p. 141–179. *In* T. Leisinger, R. Hütter, A. M. Cook, and J. Nuesch (ed.), Microbial degradation of xenobiotics and recalcitrant compounds. Academic Press, Inc., New York.
7. **Guenther, F. R., R. M. Parris, S. N. Chesler, and L. R. Hilpert.** 1981. Determination of phenolic compounds in alternate fuel matrices. J. Chromatogr. **207**:256–261.
8. **Haars, A., and A. Hüttermann.** 1980. Function of laccase in the white-rot fungus *Fomes annosus*. Arch. Microbiol. **125**:233–237.
9. **Hargesheimer, E. E., R. T. Coutts, and M. D. MacKinnon.** 1984. Characterization of simple phenols in oil sands extraction-process water. Environ. Technol. Lett. **5**:433–440.
10. **Martin, J. P., and K. Haider.** 1980. A comparison of the use of phenolase and peroxidase for the synthesis of model humic acid-type polymers. Soil Sci. Soc. Am. J. **44**:983–988.
11. **McCarthy, J. F., and B. D. Jimenez.** 1985. Reduction in bioavailability to bluegills of polycyclic aromatic hydrocarbons bound to dissolved humic material. Environ. Toxicol. Chem. **4**: 511–521.
12. **Ogram, A. V., R. E. Jessup, L. T. Ou, and P. S. C. Rao.** 1985. Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy) acetic acid in soils. Appl. Environ. Microbiol. **49**:582–587.
13. **Popoff, T., O. Theander, and M. Johansson.** 1975. Changes in sapwood of roots of Norway spruce attacked by *Fomes annosus*. II. Organic chemical constituents and their biological effects. Physiol. Plant. **34**:347–365.
14. **Richards, D. J., and W. K. Shieh.** 1986. Biological fate of organic priority pollutants in the aquatic environment. Water Res. **20**:1077–1090.
15. **Ruckdeschel, G., and G. Renner.** 1986. Effects of pentachlorophenol and some of its known and possible metabolites on fungi. Appl. Environ. Microbiol. **51**:1370–1372.
16. **Shuttleworth, K. L., and J.-M. Bollag.** 1986. Soluble and immobilized laccase as catalysts for the transformation of substituted phenols. Enzyme Microb. Technol. **8**:171–177.
17. **Shuttleworth, K. L., L. Postie, and J.-M. Bollag.** 1986. Production of induced laccase by the fungus *Rhizoctonia praticola*. Can. J. Microbiol. **32**:867–870.
18. **Sjoblad, R. D., and J.-M. Bollag.** 1981. Oxidative coupling of

aromatic compounds by enzymes from soil microorganisms, p. 113–152. *In* E. A. Paul and J. N. Ladd (ed.), Soil biochemistry, vol. 5. Marcel Dekker, Inc., New York.

19. **Sjoblad, R. D., R. D. Minard, and J.-M. Bollag.** 1976. Polymerization of 1-naphthol and related phenolic compounds by an extracellular fungal enzyme. Pestic. Biochem. Physiol. **6**:457–463.
20. **Smith, A. E.** 1985. Identification of 4-chloro-2-methylphenol as a soil degradation product of ring-labelled [^{14}C]Mecoprop. Bull. Environ. Contam. Toxicol. **34**:646–660.
21. **Steiert, J. G., and R. L. Crawford.** 1985. Microbial degradation of chlorinated phenols. Trends Biotechnol. **3**:300–305.
22. **Valo, R., V. Kitunen, M. Salkinoja-Salonen, and S. Räisänen.** 1984. Chlorinated phenols as contaminants of soil and water in the vicinity of two Finnish sawmills. Chemosphere **13**:835–844.