The potential for white-rot fungi in the treatment of pollutants

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Lignin-degrading white-rot fungi have the unique ability to degrade/mineralize a broad spectrum of structurally diverse toxic environmental pollutants. Extracellular peroxidases are important in degrading some, but not all, xenobiotic compounds. More research is needed to realize the potential of white-rot fungi in field-scale applications. Recent progress in our knowledge of the biochemistry and molecular biology of the key enzymes involved in xenobiotic degradation should pave the way for the eventual development of rational and enhanced bioremediation strategies.

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Introduction

White-rot fungi are the most active degraders of the complex aromatic plant polymer lignin to CO_2 [1–3]. Early reports by Bumpus *et al.* [4] and Eaton [5] indicating that the white-rot fungus *Phanerochaete chrysosporium* degrades dioxins, polychlorinated biphenyls (PCBs), and other chloroorganics, propelled white-rot fungi into the forefront of bioremediation research. A large body of evidence now shows that white-rot fungi are among the most versatile of microbes in their ability to mineralize a broad range of xenobiotics. *P. chrysosporium* is the most extensively studied of the ligninolytic white-rot fungi that mineralize environmental pollutants [6–9].

The lignin-degrading enzyme systems (LDS) of *P. chrysosporium* and other white-rot fungi are relatively nonspecific, and several of the xenobic oxidations catalyzed by white-rot fungi are believed to be fortuitous side reactions of their LDS. Extracellular peroxidases — lignin peroxidases (LIPs) and manganese-dependent peroxidases (MNPs) — and laccases are key components of the LDS [1,2,10••]. The potential of the white-rot fungi for *in situ* bioremediation has been attributed to their ability to degrade a variety of xenobiotic chemicals via a free radical mechanism mediated by extracellular peroxidases. The LIPs are of particular interest from the stand-point of pollutant degradation, as these enzymes, unlike other peroxidases, have a very high oxidation–reduction potential and can potentially oxidize xenobiotics that are not attacked by other peroxidases [3,7].

White-rot fungi differ from most bacteria in their approach to the mineralization/oxidation of xenobiotics in that lignin and its degradation products, as well as the aromatic pollutants studied, are unable to serve as growth substrates. Instead, they require a primary growth substrate such as glucose or another carbon source. Furthermore, similar to ligninolysis, degradation of most xenobiotics by white-rot fungi is triggered by a limitation for nutrients and is temporally correlated to ligninolysis [1,2,3,7].

Several reasons account for the attractiveness of white-rot fungi in the decontamination of pollutant sites [9]. First, they are capable of mineralizing a wide variety of toxic xenobiotics (see below). Second, they occur ubiquitously in the natural environment. Third, they have the potential to oxidize substrates that have low solubility because the key enzymes involved in the oxidation of several pollutants are extracellular. Fourth, the constitutive nature of the key enzymes involved in lignin degradation obviates the need (in most cases) for these organisms to be adapted to the chemical being degraded. Fifth, the preferred substrates for the growth of white-rot fungi, such as corn cobs, straw, peanut shells, and sawdust, are inexpensive and easily added as nutrients to the contaminated site. Sixth, the key LDS of *P. chrysosporium* are expressed under nutrient-deficient conditions, which are prevalent in many soils. And finally, as filamentous fungi grow by hyphal extension and extend through the soil with growth, they can reach pollutants in the soil in ways that bacteria cannot.

The primary focus of this review is on xenobiotic degradation by *P. chrysosporium*, the most intensively studied of the white-rot fungi. Several recent publications have re-

Abbreviations

BPE—bleach-plant effluent; **BTEX**—benzene, toluene, ethylbenzene, and *ortho-*, *meta-*, and *para*-xylenes; **2,4-D**—2,4-dichlorophenoxyacetic acid; **LDS**—lignin-degrading enzyme system(s); **LIP**—lignin peroxidase; **MNP**—manganese-dependent peroxidase; **PAH**—polycyclic aromatic hydrocarbon; **PCB**—polychlorinated biphenyl; **PCP**—pentachlorophenol; **PCR**—polymerase chain reaction; **2,4,5-T**—2,4,5-trichlorophenoxyacetic acid; **TNT**—2,4,6-trinitrotoluene.

viewed the physiology and molecular biology of the LDS components of white-rot fungi [9,10••,11,12•,13••,14••], as well as the degradation of environmental pollutants by these organisms [5–8].

Lignin peroxidases, manganese-dependent peroxidases and laccases

LIPs, MNPs and laccases are three classes of enzyme that are important in lignin degradation by different white-rot fungi [1–3,10••,11,15]. Varying combinations of these enzymes are produced by different species of white-rot fungi. Apart from their importance in lignin biodegradation, these enzymes (especially LIPs) are the focus of intense research because of their potential applications in the detoxification of a broad range of environmental pollutants, such as PCBs and dioxins, in biopulping, and in the conversion of lignocellulosic materials to fuels and chemicals [3].

LIPs (EC 1.11.1.7) are extracellular glycosylated heme proteins that catalyze H_2O_2-dependent one-electron oxidation of a variety of lignin-related aromatic structures, resulting in the formation of aryl cation radicals which undergo various non-enzymatic reactions yielding a multiplicity of end-products [1,12•]. These enzymes are relatively non-specific, which is acceptable given the random structure of lignin. Non-specificity enables these enzymes to oxidize a variety of xenobiotic compounds that have some structural similarity to the lignin substructures. MNPs (EC 1.11.1.7) are extracellular glycosylated heme proteins that catalyze the H_2O_2-dependent oxidation of Mn(II) to Mn(III), the latter mediating the oxidation of a variety of phenolic substrates [1,14••,15]. Laccases (benzenediol/oxygen oxidoreductase [EC 1.10.3.2]) are copper-containing oxidases that utilize molecular oxygen as an oxidant and also oxidize phenolic substrates to phenoxy radicals [10••,12•,16••]. They can also oxidize non-phenolic aromatics in the presence of compounds such as 2,2′-azino-*bis*(3-ethylbenz-thiazoline-6-sulfonic acid) (reviewed in [10••]).

Other important biochemical features of LIPs, MNPs, and laccases have been reviewed recently [1,14••,15]. Most recently, the X-ray crystallographic structures of LIP and MIP from *P. chrysosporium* have been determined [17••,18,19,20••]. The overall three-dimensional structure of LIP is similar to that of cyt *c* peroxidase. Both enzymes have histidine as the proximal ligand, which accepts a proton from the peroxide, and a distal arginine that facilitates O–O bond cleavage [20••]. The structure of MNP is very similar to that of LIP, except that it has five, rather than four, disulfide bonds. Importantly, a new cation-binding site (which is the probable manganese-binding site) has been identified in the MNP crystal structure.

In the past few years, remarkable progress has been achieved in the cloning and sequencing of *lip*, *mnp*, and laccase genes from a variety of white-rot fungi (reviewed in [13••,14••,15]). Both homologous and heterologous expression of *lip* and *mnp* genes have been reported, and molecular approaches to study the regulation of expression of *lip* and *mnp* genes have been described [21•–23•].

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a large group of widespread environmental pollutants that are commonly released into air, soil, water, and marine environments, by oil drilling, coal mining and burning of fossil fuels and wood. Several of the PAHs are toxic and/or potentially carcinogenic. Sites of coke- and town-gas manufacture, where coal tar has been improperly disposed of in soil and water, are of particular concern. Also of concern are sites contaminated by coal tar distillation products, such as creosote and anthracene oil, which were traditionally used by the wood-preserving industry.

Previous studies have established that *P. chrysosporium* mineralizes a number of different PAHs [3,6,7,9]. Bumpus [24] showed that at least 22 of the PAHs, including all of the more abundant PAHs in anthracene oil, undergo 70–100% breakdown in 27 days in nitrogen-limited cultures of *P. chrysosporium*. In one week, this fungus mineralized 15% of the added ^{14}C-benzo(*a*)pyrene to CO_2 and transformed an additional 58% into water-soluble products [7]. Further studies demonstrated that some PAHs, such as benzo(*a*)pyrene, benz(*a*)anthracene, anthracene, pyrene, and perylene are directly oxidized by the LIPs of *P. chrysosporium* to quinone-type products and that the mechanism for PAH oxidation by LIP is fundamentally the same as that for lignin-substructure compounds. Veratryl alcohol, which is known to act as a mediator in ligninolytic oxidation reactions catalyzed by LIP, has also been suggested to mediate oxidation of PAHs such as benzo(*a*)pyrene. In contrast to this, other PAHs, such as benzo(*e*)pyrene, benzo(*c*)phenanthrene, phenanthrene, chrysene, and naphthalene do not serve as LIP substrates, but are mineralized by *P. chrysosporium*. Kennes and Lema [25] have reported the degradation of a mixture of naphthalene, phenanthrene, anthracene, *p*-cresol, pentachlorophenol (PCP) and phenol, which represent major components of creosotes, by *P. chrysosporium*; intermediate products, such as quinones, did not accumulate. Several investigators have shown that both ligninolytic (nutrient-limited) and non-ligninolytic (nutrient non-limited) cultures of *P. chrysosporium* mineralize phenanthrene [26–29], and the major steps in phenanthrene oxidation have been defined [26,27]. The ability of *P. chrysosporium* cultures, but not purified LIP, to oxidize phenanthrene to $^{14}CO_2$ has led to the suggestion that other enzymes, such as monooxygenases, may also

be involved in the metabolism of certain PAHs by *P. chrysosporium* [26].

White-rot fungi other than *P. chrysosporium* also appear to be effective in mineralizing PAHs [26]. For example, *Trametes versicolor* and *Chrysosporium lignorum* have been reported to be as good as, or better than, *P. chrysosporium* in mineralizing phenanthrene [30]. *Pleurotus ostreatus*, *T. versicolor*, and *Coriolopsis polyzona* have been shown to oxidize anthracene to anthraquinone, and this oxidation appears to be independent of LIPs [31]. Furthermore, unlike cultures of *P. chrysosporium*, these three cultures do not accumulate anthraquinone as a product of anthracene oxidation [31]. The white-rot fungus *Bjerkandera* sp., unlike *P. chrysosporium*, has been shown to produce its ligninolytic system during primary growth under nitrogen-sufficient conditions, degrading anthracene in liquid cultures ($9\,mg\,l^{-1}\,day^{-1}$) and benzo(*a*)pyrene ($0.8\,mg\,kg^{-1}\,day^{-1}$) in a soil medium [32].

The ability of the *P. chrysosporium* to degrade PAHs has led to its use in the treatment of coal- and creosote-contaminated soils augmented with wood chips, corn cobs, or sawdust [6,33]. Substantial depletion (~10–20% of the original levels) of the PAH constituents were observed under these conditions. On the basis of these results, field-scale bioremediation was investigated using one-meter soil plots (varying in depth from 20–100 cm) in which PAH-contaminated soils were mixed with corn cobs, sawdust or bark chips and heavily inoculated (10–30% w/w) with *P. chrysosporium* grown in the laboratory on the same lignocellulosic materials. The concentration of the PAHs did not, however, change significantly in most of these experimental plots.

The above findings concur with other studies using *P. chrysosporium* where laboratory results have been encouraging, but results from full-scale trials have been variable. This arises from the difficulty of growing the organism to sufficient biomass in the soil being treated, and enabling it to compete effectively with the native soil microbial flora [33]. A recent study reports that bacteria from polluted and agricultural soils antagonize the growth of *P. chrysosporium*, but the extent of antagonism varies according to the pH, as well as the nitrogen and carbon sources used in the medium [34]. Johnston and Aust [35•] have described a polymerase chain reaction (PCR) procedure to detect and monitor the growth of *P. chrysosporium* in soils as a first step toward quantitation of survival of the organism in treated soils.

Chlorophenols

Chlorophenols are important constituents of paper-mill effluents. Previous studies (reviewed in [6,7]) have shown that *P. chrysosporium*, immobilized on rotating biological contactor disks, efficiently degrades 2,4-dichlorophenol, 2,4,6-trichlorophenol, polychlorinated guiacols, and several chlorinated vanillins. Furthermore, PCP, which is used extensively as a wood preservative and as a fungicide/herbicide, was mineralized (20–50%) in nitrogen-limited static cultures of *P. chrysosporium* [6,7]. Mineralization of both PCP and 2,4-dichlorophenol was suppressed in high-nitrogen media, suggesting the involvement of LDS in the oxidation of these pollutants. Subsequently, polychlorinated phenols have been shown to be substrates for the extracellular peroxidases (LIPs and MNPs) of *P. chrysosporium*. 2,4-Dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and PCP were all oxidized by *P. chrysosporium* to give the corresponding 1,4-benzoquinones as end products *in vitro* [7]. Studies by Valli and Gold [36] led to the conception of a multistep pathway for 2,4-dichlorophenol degradation, involving LIP and MNP, which function not just in the initial oxidation of a pollutant, but at multiple stages in the degradative pathway. The pathway for the complete degradation of 2,4,6-trichlorophenol by *P. chrysosporium* has since been elucidated [37,38]. This multistep pathway also involves cycles of peroxidase-catalyzed oxidative dechlorination reactions followed by quinone reduction reactions to yield the key intermediate 1,2,4,5-tetrahydroxybenzene, which is presumably ring-cleaved. The proposed pathway results in the removal of all three chlorine atoms before ring cleavage [37•].

Laboratory soil studies have shown that PCP concentrations in *P. chrysosporium* and *P. sordida* decrease by 96% and 82%, respectively; pentachloroanisole is the major intermediate of PCP metabolism. Mineralization of PCP, in contrast to the high level of PCP breakdown, is relatively low (8–15%) in soil [39].

A comparison of the toxicity of PCP to several *Phanerochaete* species [39] and other selected white-rot fungi [40] showed that *T. versicolor* was the fastest-growing species that remained viable at high levels of PCP ($40\,mg\,l^{-1}$), whereas *P. chrysosporium* was moderately resistant. Alleman *et al.* [41•] have used rotating tube bioreactors to study PCP degradation, demonstrating 99% PCP removal within one day with the white-rot fungi *P. chrysosporium*, *T. versicolor*, and *Inonatus dryophilus* when grown in nitrogen-sufficient media. The highest percentage of dehalogenation of PCP is observed with *T. versicolor* (62%), followed by *P. chrysosporium* (38%) and *I. dryophilus* (21%).

In early field studies of bioremediation of PCP-contaminated soils, overall depletion of PCP by *P. chrysosporium* over 6.5 weeks was 88–91% in soil augmented with peat as a source of carbon for fungal growth [42]. Although a small percentage (8–13%) was attributed to pentachloroanisole formation, most of the PCP was converted into non-extractable soil-bound products, and only a small amount of the PCP carbon was mineralized. In further field studies, Lamar [43•] has investigated the ability of white-rot fungi to deplete PCP in two different sites contaminated with wood preservatives. Fungal inocula, consisting of wood chips heavily colonized by *P.*

chrysosporium or *P. sordida*, were added to soil at 3.3% (w/w, dry), and peat (supplemental carbon source) was added at 1.9% (w/w, dry). The results show that *P. chrysosporium* can be grown in soils contaminated with up to 100 g (g PCP)$^{-1}$ with or without creosote (4000 g [g total measured PAHs]$^{-1}$). In addition, PCP and low molecular weight PAH concentrations are highly reduced by the end of 6.5 weeks of fungal activity. *P. sordida* gave comparable results. The results of these studies, though encouraging, indicate the need for following measures: first, further improvements in producing inexpensive and effective fungal inocula; second, optimization of the parameters for better growth, survival, and activity of the fungus; third, better understanding of the fate, nature, stability, and toxicity of the pollutant in fungal-treated soils; and fourth, determination of the extent of pollutant mineralization/degradation.

Bleach plant effluents

In the production of high-grade paper, residual lignin is chemically liberated from wood pulp through the use of chlorine bleaching. As a result, the pulp and paper industry releases large volumes of intensely colored bleach plant effluents (BPEs) which contain toxic chlorinated lignin degradation products, including chlorolignins, chlorophenols, chloroguiacols and chloroaliphatics.

BPE-decolorizing and -dechlorinating activity occurs in several of the white-rot fungi (reviewed in [3]). Although the BPE-decolorizing activity of the ligninolytic white-rot fungi *P. chrysosporium* and *Trametes versicolor* has been known for some time, the enzyme systems used by these organisms to degrade BPEs have only recently been elucidated. Michel *et al.* [44] showed that MNPs play the primary role in BPE decolorization by *P. chrysosporium*, whereas LIPs have a minor role. Lackner *et al.* [45] independently confirmed the importance of MNPs in BPE decolorization and showed that oxidation of BPE was mediated by Mn(III). The decolorization activity was duplicated *in vitro* by addition of Mn(III) chelated with lactate. Purified *P. chrysosporium* MNPs also catalyzed BPE oxidation in the presence of lactate, Mn(II) and H_2O_2. These results indicate that Mn(III) chelated to lactate or other organic acids is primarily responsible for BPE decolorization *in vivo*. A report by Jaspers *et al.* [46] independently confirms the findings of Michel *et al.* [44] and Lackner *et al.* [45], showing that purified MNP of *P. chrysosporium*, but not purified LIP, is able to decolorize BPEs.

Archibald *et al.* [47] reported that laccases, and not peroxidases, play the primary role in BPE decolorization by *T. versicolor*. Archibald and Roy [48] later demonstrated that *T. versicolor* laccases, in the presence of phenolic substrates, are able to generate Mn(III) chelates similar to those produced by MNP and shown by Lackner *et al.* [45] to be responsible for the oxidation of BPEs. Thus, the potential of white-rot fungi in BPE treatment may lie not only in their ability to degrade BPEs and chlorophenols, *per se*, but also in the knowledge obtained from the study of these organisms, thereby leading to the design of effective biomimetic systems that are able to generate chelated Mn(III) for the degradation of BPEs and chlorophenolics.

Nitrotoluenes

The contamination of soil and water with nitrotoluene and other residues of explosives is a widespread problem at military facilities. Nitroaromatics are also used in the production of pesticides, pharmaceuticals and dyes, and are often found in groundwater and soils near production sites. Previous work established that *P. chrysosporium* mineralizes 2,4-dinitrotoluene, and a pathway for its degradation has been proposed [49]. Evidence was presented that *P. chrysosporium* removed both the nitro groups before ring cleavage and that both LIPs and MNPs were involved in the denitration of the pathway intermediates. *P. chrysosporium* was also shown to mineralize 30–50% of the added 2,4,6-trinitrotoduene (TNT) [50] when the TNT concentration was less than 20 mg l^{-1}. At TNT concentrations higher than 20 mg l^{-1}, however, the metabolites 2-hydroxylamino-4,6-dinitrotoluene, 4-hydroxylamino-2,6-dinitrotoluene, and 4-hydroxylamino-2,6-dinitrotoluene accumulated, inhibiting the organism [51,52].

Stahl and Aust [53] suggested that a membrane-redox system was responsible for the reduction of TNT to the corresponding amino congeners that are substrates for peroxidase-catalyzed oxidations. This membrane-associated system has since been identified as an aromatic nitroreductase that catalyzes the reduction of nitro groups in 1,3-dinitrobenzene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene, 1-chloro-2,4-dinitrobenzene, and 2,4-dichloro-1-nitrobenzene to hydroxylamines and/or amines [54•]. Rieble *et al.* [55•] have also described the purification and characterization from *P. chrysosporium* of an intracellular enzyme, 1,2,4-trihydroxybenzene-1,2-dioxygenase, that is able to oxidatively cleave the aromatic ring of trihydroxybenzene. This compound is a key intermediate during fungal degradation of a variety of aromatic pollutants, including 2,4-dinitrotoluene, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and 2,7-dichloro-dibenzo-*p*-dioxin.

Dyes

Dyes are released into the environment in effluents from textile and dyestuff industries. Azo dyes are by far the most structurally diverse and numerous of the manufactured synthetic dyes. Although azo dyes are not typically degraded by bacteria under aerobic conditions, ligni-

nolytic cultures of *P. chrysosporium* extensively degrade several of them, including orange II, azure B, tropaeolin O, Congo red, amaranth, and orange G. Non-ligninolytic cultures also degrade these dyes, albeit to a lesser extent (reviewed in [56•,57]).

The azo dyes 4-amino-1,1′-azobenzene-3,4′-disulfonic acid and sulfanilic acid, as well as their guiacol conjugates (via azo linkages), are all decolorized by ligninolytic cultures of *P. chrysosporium*, but the two guiacol-substituted dyes are decolorized more readily than the corresponding unsubstituted molecules. These results suggest that the degradability of azo dyes may be custom-enhanced by incorporating selected readily degradable substituents into a dye's structure. A variety of ^{14}C-ring-labeled azo dyes with amino, nitro, acetamido, and hydroxyl substitutions on the aromatic ring were mineralized (23–48%) by nitrogen-limited ligninolytic cultures of *P. chrysosporium* [58,59,60•]. LIPs are reported to be more important than MNPs in decolorizing these dyes, and different LIP isozymes have been shown to have varying specificities toward these dyes as substrates [61]. A new pathway for the degradation of sulfonated azo dyes has also been proposed. These data suggest that *P. chrysosporium* has the potential to clean up textile-mill effluents and bioremediate dye-contaminated soils.

Polychlorinated biphenyls

PCBs are a family of compounds with a wide range of industrial applications, primarily as heat transfer, dielectric and hydraulic fluids. PCBs are manufactured as mixtures under the trade names Aroclor, Clophen and Delor. Commercial PCBs consist of a mixture of congeners which differ in the number and position of chlorines on the biphenyl nucleus. Arochlors 1242, 1254 and 1260 contain 42%, 54% and 60% chlorine by weight and an average of three, five and six chlorines per biphenyl molecule, respectively. Some PCB congeners have been shown to be transformed by aerobic bacteria; however, higher chlorinated congeners are generally not degraded [62]. Also, PCBs with *ortho* substitution are generally recalcitrant to anaerobic dechlorination. Unlike bacteria, ligninolytic cultures of *P. chrysosporium* can mineralize low levels (<10%) of individual tetrachloro- and hexachloro-substituted PCB congeners as well as Aroclor 1254, albeit at low initial concentrations of 0.04–1.6 ppm [63]. Recent studies show that *P. chrysosporium* degrades higher levels (10 ppm) of Aroclor 1242, 1254 and 1260 by 82%, 31% and 18%, respectively [64•]. Congener analysis shows that this organism exhibits no congener specificity among *ortho, meta* and *para* chlorine substitutions in the Arochlors. Furthermore, degradation does not require biphenyl induction and occurs in high nitrogen or malt-extract media in which LIPs and MNPs are not known to be produced [64•].

Other environmental pollutants

In addition to the major classes of xenobiotic described above, *P. chrysosporium* and other white-rot fungi metabolize a variety of other significant environmental pollutants. Of these, the metabolism of tetrachlorodibenzo-*p*-dioxin, and 1,1-*bis*(4-chlorophenyl)-2,2,2-trichloroethane (or DDT) has been reviewed previously [3,4,9]. More recently, Valli *et al.* [65] have proposed a detailed pathway for the degradation of 2,7-dichlorodibenzo-*p*-dioxin in which LIPs, MNPs, and intracellular enzymes are involved. As in the case of 2,4-dichlorophenol metabolism described above, both aromatic chlorines are removed before ring cleavage in this unique pathway.

In the past, the alkyl halides aldrin, dieldrin, heptachlor, chlordane, lindane and mirex were used extensively as insecticides, but their application is now discontinued or minimal owing to environmental persistence [66]. Of the above compounds, only ^{14}C-lindane and ^{14}C-chlordane are substantially mineralized (9–23%) to $^{14}CO_2$ by *P. chrysosporium*. The other four compounds are poorly mineralized, but do undergo substantial bioconversion, as indicated by substrate disappearance and metabolite formation [66].

Atrazine (2-chloro-4-ethylamine-6-isopropylamino-1,3,4-triazine), a chlorinated triazine, is one of the most widely used herbicides worldwide. This compound is only slowly metabolized in the soil and is considered recalcitrant. *P. chrysosporium* has been shown to effect a 48% decrease in atrazine concentration after four days incubation in a nitrogen-limited medium [67•]. In this study, ^{14}C-labeled in the ethyl position, was mineralized to $^{14}CO_2$, whereas ^{14}C-ring-labeled atrazine was not significantly mineralized. Analysis of the spent growth medium showed the presence of hydroxylated and/or *N*-dealkylated metabolites of atrazine. Similar results have been obtained with another white-rot fungus, *Pleurotus pulmonarius*, except that a novel metabolite, 2-chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine was also shown to be produced [68].

A recent paper by Ferrey *et al.* [69] has shown that the white-rot fungi *Ceriporiopsis subvermispora*, *Phlebia tremellosa* and *P. chrysosporium* mineralize ring-^{14}C-labeled alachlor (2-chloro-*N*-[2,6-diethylphenyl]-*N*-[methoxymethyl]-acetimide), a widely used herbicide, by 14%, 12% and 6.3%, respectively. The brown-rot fungus *Fomitopsis pinicola* did not mineralize alachlor under these conditions.

Organophosphorus compounds are extensively used in agriculture as insecticides. As a group, organophosphorus insecticides are quickly degraded in the environment, but some are moderately persistent. Chlorpyrifos, fonofos, and terbufos, which are widely used, are mineralized (27.5%, 12.2%, and 16.6%, respectively) by *P. chrysosporium* when incubated for 18 days in nitrogen-limited cultures [70•].

Common herbicides, such as acylanilides and phenylurea, are derivatives of chloroanilines, and microbial metabolism of these compounds often leads to the formation of toxic aniline derivatives. *P. chrysosporium* has been shown to degrade 4-chloroaniline to CO_2; however, toxic oligomers of 4-chloroaniline are formed when the compound is incubated with LIP both *in vivo* and *in vitro*. Chang and Bumpus [71] reported that the toxic oligomers formed as intermediates are subsequently degraded by the fungus. LIP from *P. chrysosporium* has also been shown to transform 3,4-dichloroaniline to the dimerization product 3,4,3′,4′-tetrachloroazobenzene, a substance comparable to 2,3,7,8-tetrachloro-*p*-dibenzodioxin in structure and toxicity [72]. Simultaneous occurrence of competing toxifying and detoxifying reactions in relation to chloroaniline metabolism by *P. chrysosporium* requires further scrutiny from the stand-point of biotechnological applications.

Benzene, toluene, ethylbenzene, and *ortho*-, *meta*-, and *para*-xylenes (BTEX) compounds are an important family of toxic organopollutants. They are components of gasoline and aviation fuels and frequently enter soil, sediments, and groundwater because of leakage from underground storage tanks and pipelines, accidental spills, and improper waste-disposal practices. *P. chrysosporium* efficiently degrades BTEX components individually or in mixtures, both in ligninolytic and in non-ligninolytic cultures (i.e. not producing LIPs or MNPs). The fungus carries out substantial mineralization of ^{14}C-ring-labeled benzene and toluene to $^{14}CO_2$ [73•]. It is also able to degrade, individually or in combination, relatively high concentrations of *p*-cresol (150 mg l^{-1}) and phenol (50 mg l^{-1}), which are also often encountered in wastewaters originating from petroleum-related industries [74].

Two of the most extensively used phenoxyalkanoic herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are mineralized by *P. chrysosporium* under nitrogen-limited as well as nitrogen-rich conditions, indicating that LIPs and MNPs are not important in the mineralization of these compounds [75•,76]. A higher rate of mineralization of 2,4-D and 2,4,5-T is observed when these compounds are added as a mixture to cultures of *P. chrysosporium* than when they are added singly.

Intensive use of chlorobenzenes as solvents, degreasers, odorizers, and as intermediates in the synthesis of various pesticides and dyes has resulted in their widespread release into the environment. *P. chrysosporium* has been shown to extensively mineralize both chlorobenzenes and *ortho*-, *meta*- and *para*-dichlorobenzenes [77•]. Maximal degradation and mineralization of chlorobenzene is observed in malt-extract cultures in which LIP and MNP production is not seen. These cultures are able to simultaneously degrade chloro- and methyl-substituted benzenes.

In addition to the above compounds, recent studies show that *P. chrysosporium* can carry out extensive degradation of trichloroethene and the widely used anionic surfactant, linear alkylbenzene sulfonate; however, mineralization of the latter is negligible (JS Yadav, CA Reddy, C Bethea, unpublished data).

Conclusions and future directions

White-rot fungi are unique among eukaryotic or prokaryotic microbes in possessing powerful oxidative enzyme systems (as exemplified by LIPs) which have a broad substrate specificity and are able to oxidize several environmental pollutants. The vast range of toxic environmental pollutants that are mineralized/degraded by white-rot fungi also makes these organisms unique and attractive for the bioremediation of polluted sites. The degree of success achieved in the laboratory in the mineralization by *P. chrysosporium*, of major pollutants, such as chlorophenols, PAHs, and PCBs, has not, however, been realized in field-scale studies. Much research is needed to study the factors involved in limiting the effectiveness of the fungus in field-scale applications. Also, further research is needed to identify the white-rot fungi that are dominant in agricultural and polluted soils and to determine if some of these organisms are better suited for bioremediation applications.

Progress is being made on the comparative biology of the LDS produced by various white-rot fungi to identify fungal strains that may be superior to the widely studied fungus *P. chrysosporium* for bioremediation purposes. Knowledge is accumulating in several aspects of the enzymology and molecular biology of the LDS: the major genes encoding LIPs, MNPs, and laccases have now been cloned and sequenced; regulation of expression of the key ligninolytic genes has been studied; and X-ray crystallographic structures of the LIP and MNP isozymes are now available. Continuing progress in these areas should lead to the successful genetic engineering of white-rot fungi and their enzyme systems to enable the design and application of optimal bioremediation strategies for treating contaminated sites.

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