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Mechanisms Used by White-Rot Fungus to Degrade Lignin and Toxic Chemicals

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Abstract Wood-rotting basidiomycetous fungi are the most efficient degraders of lignin on earth. The white-rot fungus *Phanerochaete chrysosporium* has been used as a model microorganism in the study of enzymology and its application. Because of the ability of the white-rot fungus to degrade lignin, which has an irregular structure and large molecular mass, this fungus has also been studied in relation to degrading and mineralizing many environmental pollutants. The fungus includes an array of enzymes, such as lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), cellobiose:quinone oxidoreductase, and H_2O_2-producing enzymes and also produces many other components of the ligninolytic system, such as veratryl alcohol (VA) and oxalate. In addition, the fungus has mechanisms for the reduction of degradation intermediates. The ligninolytic systems have been proved to provide reductive reactions as well as oxidative reactions, both of which are essential for the degradation of lignin and organopollutants. Further study on the white-rot fungus may provide many tools to both utilize lignin, the most abundant aromatic polymer, and bioremediate many recalcitrant organopollutants.

Key words: White-rot fugus, *Phanerochaete chrysosporium*, lignin, organopollutants, ligninolytic system, peroxidases, veratryl alcohol, oxalate, manganese, oxidations, reductions

Lignin, the most abundant aromatic polymer on earth, is found in all higher plants. It gives plants physical strength and serves as a barrier against microbial attack. Lignin is also a highly irregular three-dimensional biopolymer composed of phenylpropanoid units. The heterogeneity in the linkage is a result of its synthesis by a free radical-based mechanism. The structural features of this heterogenous polymer impose unusual restrictions on its biodegradability. Although lignin is recalcitrant to most forms of microbial attack, wood-rotting basidiomycetous fungi, which cause white-rot, are the most efficient lignin degraders in nature. Due to the irregular structure and large size of the lignin molecule, the lignin degradative enzyme system of these fungi would appear to be nonspecific and extracellular. The biodegradation of lignin has been studied extensively during the last decade and the white-rot fungus *Phanerochaete chrysosporium* has been widely used as a model microorganism to investigate the physiological requirements and enzymes required for lignin degradation [82, 84, 114, 133]. This fungus also mineralizes some of the most recalcitrant environmental pollutants, such as polychlorinated biphenols (PCBs), 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT), lindane, and polycyclic aromatic hydrocarbons (PAHs) etc. as shown in Table 1. Since the cost of the treatment of hazardous environmental pollutants in the United States alone is expected to have a value of more than one trillion dollars, researchers all around the world are interested in developing white-rot fungus-based technology, both for the utilization of the lignocellulosic biomass and as a possible bioremediation technology to degrade environmental pollutants.

Lignin degradation by *P. chrysosporium* occurs during secondary metabolism triggered by nutrient limitation. The extracellular ligninolytic system of *P. chrysosporium* consists of lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), H_2O_2-producing enzymes, and the secondary metabolite veratryl alcohol (VA). The mechanism of the oxidation of the chemicals by these peroxidases is a free radical-mediated process, such that the enzyme can even catalyze the oxidation of molecules that are some distance from itself using a suitable free radical mediator. Veratryl alcohol is considered to be a physiological free radical mediator. The ability of the enzyme to produce free radicals "in solution" is considered to be related to its involvement in lignin degradation, as free radicals are generally highly reactive and nonspecific. A variety of pollutants, such as benzo(a)pyrene, 4-chloroaniline, and pentachlorophenol (PCP) are also oxidized by this enzyme. The mineralization

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Table 1. Organic compounds degraded by *Phanerochaete chrysosporium*[1].

Aromatic compounds	Chlorinated aromatic compounds
Vanilic acid	4-Chlorobenzoic acid
2,4-Dihydroxybenzoic acid	Dichlorobenzoic acid
4-Hydroxy-3-methoxybenzaldehyde	2,4,6-Trichlorophenol
Isovanillic acid	4,5-Dichloroguaiacol
Syringic acid	6-Chlorovanillin
Curcumin	5-Chlorovanillin
7-Hydroxy-4-methylcoumarin	4,5,6-Trichloroguaiacol
2,6-Dihydroxybenzoic acid	Tetrachloroguaiacol
2'-Hydroxy-3'-methoxyacetophenone	3-Chloroaniline
4'-Hydroxy-3'-methoxyacetophenone	3,4-Dichloroaniline
6,7-Dimethoxycoumarine	2,3,4,5,6-Pentachlorophenol
Gentisic acid	4-Chloroaniline
Guaiacol	
4-Hydroxy-3-methoxymandelic acid	Polycyclic chlorinated aromatic compounds
Protocatechuic acid	
3',4'-Dihydroxyacetophenone	1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane
2',3',-Dihydroxy-4'-methoxyacetophenone	2,3,7,8-Tetrachlorodibenzo-*p*-dioxine
6,7-Dihydroxy-4-methylcoumarine	3,4,3',4'-Tetrachlorobiphenyl
3,5-Dimethylcatechol	2,4,5,2',4',5'-Hexachlorobiphenyl
2',3',4'-Trihydroxyacetophenone	Arochlor 1254
Pyrogallol	
Catechol	Biopolymers
3-Methylcatechol	
3,4-Dimethylcatechol	Lignin
4-Methylcatechol	Cellulose
Benzoic acid	Kraft Lignin
Acetoguaiacone	3-Chloroaniline-lignin conjugate
Vanillin	3,4-Dichloroaniline-lignin conjugate
Veratryl alcohol	
Veratryl aldehyde	Explosives
Vanilly alcohol	
	Trinitrotoluene (TNT)
Lignin model compounds	HMX (Cyclotetramethylenetetranitramine)
	RDX (Cyclotrimethylenetrinitramine)
Veratrylglycerol-β-(*O*-methoxyphenyl)ether	
Guaiacylglycerol-β-coniferyl alcohol ether	Polycyclic aromatic compounds
Dehydrodiconiferyl alcohol	
Dehydrodivanillin	Benzo[a]pyrene
	Phenanthrene

[1]References from 7, 10, 19, 20, 27, 29, 30, 34, 46, 53, 54, 56, 101, 116, 119, 136, and 141.

of lignin and pollutants coincides with the onset of the enzyme production by the fungus [10, 82, 133]. This article summarizes current understanding of physiology, enzymology, and mechanisms of *P. chrysosporium* that are utilized to degrade lignin and various organopollutants.

BIOLOGY OF *PHANEROCHAETE CHRYSOSPORIUM*

P. chrysosporium belongs to a class of fungi referred to as basidiomycetes. Basidiomycetes include the higher fungi such as mushrooms and brackets. The life cycle of basidiomycetes begins with a microscopic vegetative state, also called the asexual, imperfect stage, or anamorph. A sexual stage (teleomorph) can also occur, during which it produces macroscopic fruit bodies (i.e., a mushroom). Reproduction occurs in the vegetative state by the asexual formation of conidiospores. In the sexual stage, the fruit body bears sexually produced basidiospores on structures termed basidia [38]. *P. chrysosporium* is subclassified as a hymenomycete. The ability to reproduce asexually allows basidiomycetes to exist and proliferate entirely in the vegetative state. Fruit bodies are generally only produced on solid substrates, and even then under a limited range of conditions [21]. Thus, the mycelial state is the most important in characterizing the physiology of *P. chrysosporium* in a submerged culture.

P. chrysosporium is one of an estimated 1,600 to 1,700 species of wood-rotting basidiomycetes [4]. Wood-rotting fungi are classified according to the type of decay. There

are three major types: white-, brown-, and soft-rot. White-rot is characterized by a bleaching of the wood during degradation, which is caused by the metabolism of both lignin and carbohydrates. Brown-rot occurs due to the degradation of carbohydrates while lignin is not degraded. During soft-rot degradation, the degradation of lignin is slower than that of carbohydrates. Thus, the white-rot fungi are the principal microorganisms in terrestrial carbon cycling, since they can actively metabolize lignin to CO_2 [84].

As with most other fungi, *P. chrysosporium* has similar requirements for primary growth. However, *P. chrysosporium* has some physiological aspects that are different from other white-rot fungi. The growth of *P. chrysosporium* varies little from pH 4 to pH 6 [38], yet *P. chrysosporium* has a relatively high optimal growth temperature of about 40°C [21]. Generally, secondary metabolism results from the limitation of a critical substrate such as carbon, nitrogen, or sulfur [82]. Nitrogen limitation is especially relevant to wood-rotting fungi, which appear to have adapted to the low nitrogen contents of their principal natural substrate, wood. Thus, secondary metabolism, also called idiophase, is of vital importance to white-rot fungi, including *P. chrysosporium* [38]. Lignin degradation occurs, in general, as a secondary metabolic process [84].

BIODEGRADATION OF LIGNIN BY WHITE-ROT FUNGI

The means by which lignin decomposes in the natural environment has been a topic of scientific interest for many years. Understanding how lignin is degraded is important to understanding the physiology of white-rot fungi, and is a prerequisite to elucidating the role of extracellular enzymes in the degradation process.

Lignin is found in all higher plants. The percentage of carbon present as lignin in woody tissues varies among species. Conifers (gymnosperms) have the highest content of lignin (20–35%) [84]. In the cell wall, lignin is closely associated with hemicellulose. The major component of a cell wall is cellulose (50–70%), around which the hemicellulose and lignin form a solid and protective matrix against microbial degradation [84]. Hence, researchers are very much interested in understanding the chemistry of lignin degradation for the economic use of cellulose. There are three monomeric precursors of lignin; *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, whose structures are shown in Fig. 1. Lignin synthesis involves the polymerization of these monomers by free radical mechanisms. Radical coupling can lead to different types of bonds between monomers, resulting in a complex network of the monomers as shown schematically in Fig. 2. The molecule is large in size and has an irregular structure. The predominant

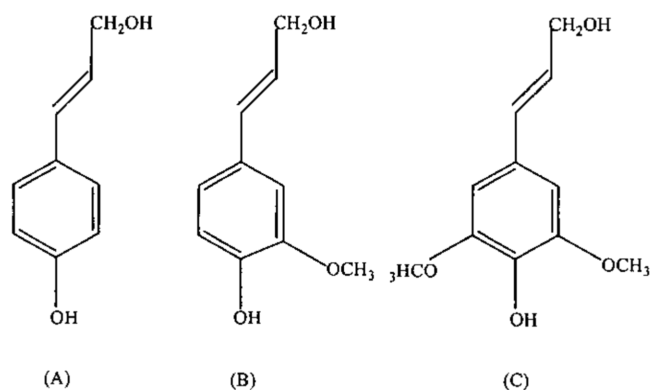


Fig 1. Some lignin precursors.
(A) *p*-coumaryl alcohol, (B) coniferyl alcohol, (C) sinapyl alcohol [82].

linkage observed is β-O-4 type, which accounts for 60% of the interunit linkage of lignin, and β-1 type [82].

Although lignin is resistant to most microbial attacks, it is degraded in nature to CO_2 primarily by white-rot fungi [82, 84]. Among the fungi previously studied, the white-rot fungus *P. chrysosporium* has beeen shown to exhibit relatively high rates of lignin degradation [139]. Although lignin is mineralized by this fungus, it cannot be used as an energy source. The fungus degrades lignin to access cellulose that serves as a carbon source. The fungus degrades lignin under ligninolytic conditions that can be induced by limiting nutrients, such as nitrogen, sulfur, or carbon [82]. Several important enzymes and secondary metabolites, such as lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), veratryl alcohol (VA), and

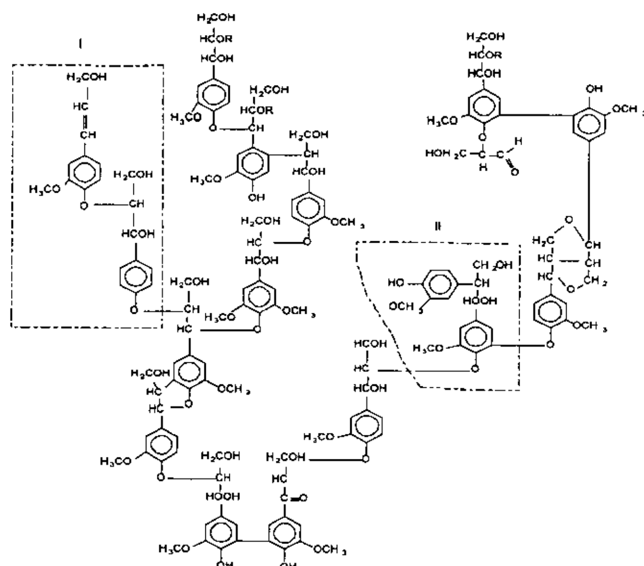


Fig. 2. Representative structure of coniferous lignin.
The unit linkages of the β-O-4 type (dashed box I) are predominant. The interunit linkages enclosed in a dashed box (II) are representative of the β-1 type linkage [82].

oxalate, are produced by *P. chrysosporium* under nutrient limiting conditions. The production of extracellular enzymes and other metabolites explains, at least in part, the ability of the fungus to degrade lignin and xenobiotics [7, 10, 133]. It would appear reasonable to expect that lignin degradation proceeds by depolymerization to smaller oligomers and eventually to monomers. Vanillic acid is a common lignin metabolite observed in cultures of white-rot fungi [84]. Whole cultures of *P. chrysosporium* [140] and LiP [83, 135] have been observed to cleave representative lignin substructure dimers. In addition to depolymerization, three of the most important steps in the white-rot decay of lignin in wood appear to be the demethoxylation of methoxy side chains, ring cleavage in the polymer, and the oxidation of aliphatic side chains. Demethoxylation is a common reaction of both white-rot and brown-rot fungi. Methanol production from demethoxylation may be important in the overall lignin decomposition process. It has been found that *P. chrysosporium* produces methanol oxidase that generates H_2O_2 upon the oxidation of methanol [84]. *P. chrysosporium* demethoxylates a number of aromatic monomers, including vanillic acid [84]. Lignin peroxidase has also been observed to demethoxylate dimethoxybenzenes [73]. Another important result of demethoxylation is that a free hydroxyl group is formed on the aromatic moiety. The introduction of such a hydroxyl group is believed to be important for subsequent ring cleavage reactions by white-rot fungi [84].

White-rot fungi can carry out ring cleavage reactions directly in the lignin polymer. *P. chrysosporium* is able to cleave catechol, and a general ring cleavage system is likely to be responsible for its ability to metabolize a wide range of phenols during secondary metabolism [84, 114]. Lignin peroxidase has been reported to catalyze several ring cleavages *in vitro*. However, the ring cleavage reactions by LiP do not appear to absolutely require the presence of phenolic hydroxyl groups in the ring. All of the cleavage substrates contain at least two ether linkages (methoxyaryl, ethoxyaryl, or aryl-aryl ether) per aromatic nucleus [114]. Ring cleavage results in the formation of aliphatic side-chains, which can undergo further oxidation and release [84].

Extracellular peroxidases do indeed play a significant role in lignin degradation by *P. chrysosporium*. Since the H_2O_2 needed for peroxidase activity is derived from oxygen, a correlation of lignin degradation to oxygen partial pressure is expected. In glucose-supplemented batch cultures, lignin mineralization by *P. chrysosporium* increased substantially with increasing oxygen partial pressures over a range from 5 to 100%. Primary growth is unaffected over the same range of oxygen concentrations [85, 112]. The morphology of the fungi is also important in lignin degradation. Earlier work with *P. chrysosporium* has shown that lignin degradation is suppressed in shaking cultures, and results in pellet formation [85]. This result indicates that pelleted morphology in liquid cultures may impose mass transfer limitations on metabolism. Subsequent studies have shown, however, that pellets of the fungi in an agitated culture are able to degrade lignin in the presence of certain detergents. The production of lignin peroxidase was also observed only in the presence of detergents [68].

ENZYMOLOGY OF LIGNIN DEGRADATION

The enzyme capable of the oxidative cleavage of C_α-C_β bond of nonphenolic aromatic lignin model compounds was first discovered by Tien and Kirk in an extracellular culture of *P. chrysosporium* [135]. They called the enzyme ligninase. Harvey *et al.* [65] first proposed that ligninase functions as a peroxidase, mediating oxidation reactions via single electron transfers. Ligninase is now referred to as lignin peroxidase (LiP). The enzymatic activity of LiP is measured by the oxidation of VA to veratryl aldehyde. Veratryl aldehyde absorbs intensively at 310 nm, whereas VA does not [135]. Lignin peroxidase is relatively nonspecific to its substrate and catalyzes the oxidation of a variety of reactions in the presence of H_2O_2 [82]. Lignin peroxidase has also been discovered from a number of other white-rot fungi [145]. While LiP is characterized by its ability to oxidize VA to veratryl aldehyde in the presence of H_2O_2, Tien and Kirk [134], and Glenn and Gold [51] reported another type of ligninolytic peroxidase from *P. chrysosporium*. This peroxidase is usually referred to as manganese-dependent peroxidase (MnP), and requires manganese for its activity. Kirk and Farrell [82] have reported that there are 10 heme peroxidases of which 6 are LiP isozymes. The other 4 isozymes have been found to be MnP. Manganese-dependent peroxidase does not oxidize VA under physiological conditions [108].

Lignin Peroxidase

LiP (EC 1.11.1.7) is a heme-containing glycoprotein that requires H_2O_2 as an oxidant [135]. LiP has been thought to oxidize nonphenolic lignin substructures by abstracting one electron and generating cation radicals [65]. The reactions of LiP using lignin model compounds and synthetic lignin have been studied. Its capability to catalyze C_α-C_β bond cleavage, ring opening, and other reactions has also been demonstrated [66, 82, 114]. Together with other enzymes, LiP is thought to constitute the major component of the lignin-degrading system of *P. chrysosporium*. However, polymerization reactions have been noticed during *in vitro* experiments and this had led to doubts about the essential role of LiP *in vivo* with LiP [53]. Meanwhile, there have been several reports that LiP is important in lignin and xenobiotics degradation by LiP-producing white-rot fungi. The enzyme can depolymerize dilute solutions of lignin *in vitro*, and can oxidize and depolymerize a variety of dimers and oligomers structurally related to lignin *in vitro*

[114]. It has also been proposed that LiP plays a more important role in the degradation of synthetic [^{14}C]-lignin to $^{14}CO_2$ than MnP [15].

Lignin peroxidase is a monomeric *N*- and probably *O*-glycosylated protein with four disulfide bonds [110]. Its pH optimum is near 2.5, which is unusually low. Lignin peroxidase contains one iron protoporphyrin IX as the prosthetic group and has the same catalytic cycle as horseradish peroxidase (HRP) as shown in Fig. 3 [147]. The reaction of the ferric enzyme with H_2O_2 yields compound I, a ferryl iron [Fe(IV)] porphyrin cation radical intermediate, which has two oxidizing equivalents above the ferric enzyme. A one-electron reduction of compound I by a reducing substrate (e.g., VA) or H_2O_2 yields the iron oxo intermediate, compound II. This intermediate still contains ferryl iron [Fe(IV)] yet no longer includes the porphyrin cation radical. Finally, a single one-electron reduction step returns the enzyme to its ferric state, completing the catalytic cycle. In the absence of a reducing substrate, compound II is further oxidized by H_2O_2 to compound III, a catalytically inactive intermediate [22]. Compound III is relatively stable in the absence of H_2O_2, yet is irreversibly inactivated in the presence of excess H_2O_2. Compound III can return to the ferric enzyme either spontaneously [22] or by oxidation with VA cation radical ($VA^{\cdot+}$) [9].

Lignin peroxidase is produced as a set of closely related isozymes with molecular weights ranging from 38 to 43 kDa, which are encoded by different genes in *P. chrysosporium*. The expression of LiP genes is regulated by an inverse function of manganese concentration [15, 98]. The manganese concentration has no influence on the amount of VA *in vivo*

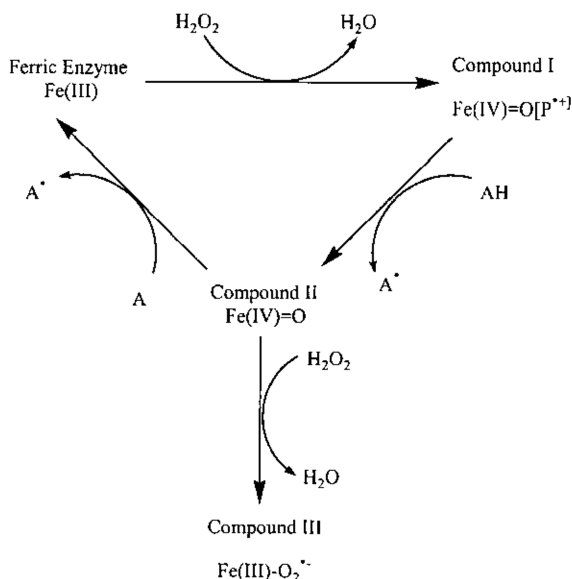


Fig. 3. Catalytic cycle of lignin peroxidase.
AH represents the enzyme substrate. the iron is in the form of protoporphyrin IX [133, 147].

[18]. Intracellularly, the concentration of cAMP regulates LiP gene expression at the level of transcription [16]. Both the strain and the growth conditions alter the balance of the different isozymes [81, 95, 113]. While nonphenolic compounds with high ionization potentials ($E_{1/2}$=1.06- 1.12 V *vs*. a saturated calomel electrode) are readily oxidized by either LiP, MnP, or HRP, LiP is an extraordinary peroxidase since it can oxidize nonphenolic aromatic compounds with very high ionization potentials, such as 1,2-dimethoxybenzene ($E_{1/2}$=1.5 V) and VA. Laccase, another extracellular enzyme of some white-rot fungi, can only oxidize compounds with a relatively low ionization potential, including 1,2,4,5-tetramethoxybenzene ($E_{1/2}$=0.81 V), to the corresponding cation radical [72, 108].

Manganese-Dependent Peroxidase

Manganese-dependent peroxidase (EC 1.11.1.7) was first discovered in the culture fluid of *P. chrysosporium*. MnP utilizes manganese as a redox mediator [51, 134] to oxidize a variety of phenolic compounds, polymeric dyes, lignin model compounds, and amines [51, 106]. Organic acids are good chelators for Mn(II) and Mn(III), and are required for the activity of MnP [50, 51]. Basidiomycetes are producers of oxalic acid [40], pyruvic acid [5], and malonic acid [146]. Some of the commonly used organic acids for *in vitro* experiments related to the MnP activity are tartrate, malate, and lactate [50, 51]. After oxidation by MnP, Mn(III) must form a complex with a chelator before it can oxidize substrates [146]. Furthermore, the Mn(III)-chelator complex is a freely diffusible oxidant (mediator), which has been proposed to oxidize lignin within the wood matrix [106]. The purified enzyme can catalyze the depolymerization of synthetic lignin [148] and also degrade high molecular mass chlorolignins [91]. MnP is the predominant enzyme involved in kraft pulp bleaching [148] and the decolorization of bleach plant effluents [98].

MnP is a glycoprotein (M.W. 46,000) and contains one iron protoporphyrin IX prosthetic group [51, 106]. The enzyme exists as several closely related isozymes and is encoded by several different genes in *P. chrysosporium* [95, 107]. The expression of MnP genes is regulated by a direct function of the Mn(II) concentration [15, 18, 98]. Intracellularly, the concentration of cAMP regulates MnP gene expression at the level of transcription [16]. Both the spectral and the catalytic characteristics of MnP are very similar to LiP and HRP. However, chelated Mn(II) is required for reducing compound II back to the ferric enzyme in order to complete the catalytic cycle. In the absence of a reducing substrate MnP can also react with H_2O_2 like LiP [146]. Unexpectedly, Sutherland *et al.* [129] have shown that LiP compound I can also oxidize Mn(II), whereas LiP compound II cannot. Thus, the compound II is formed in the presence of excess H_2O_2. This suggests that MnP is specialized in the oxidation of Mn(II).

Cellobiose:Quinone Oxidoreductase

Before LiP and MnP were discovered, it was known that *P. chrysosporium* also synthesizes and produces several other extracellular enzymes, including cellulolytic enzymes [43], proteases [42], and cellobiose:quinone oxidoreductase (CBQase) [43, 99]. Furthermore, the fungus produces exoglucanases and endoglucanases [43]. These cellulolytic enzymes work synergistically in cellulose degradation to primarily form cellobiose. The β-glucosidase, another cellulolytic enzyme, catalyzes the hydrolysis of cellobiose into glucose [120]. CBQase catalyzes the oxidation of cellobiose to reduce quinones to hydroquinones. This enzyme has been found to be produced by many white-rot fungi [43, 99]. Quinones are products of lignin degradation and fairly reactive, as such they can polymerize with other aromatics [67]. CBQase is believed to be involved in the prevention of the repolymerization of lignin degradation products. The reduction of quinones to phenolic compounds by CBQase has also been proposed to be a prerequisite to ring cleavage by other enzymes [43]. Morpeth and Jones [99] discovered that CBQase can catalyze the oxidation of cellobiose to reduce oxygen to H_2O_2.

VARIOUS OTHER COMPONENTS OF THE LIGNIN-DEGRADING SYSTEM

Veratryl Alcohol

Veratryl alcohol (VA) appears to be the preferred natural substrate of LiP [115]. Consequently, the LiP-catalyzed oxidation of VA has been studied extensively. The reaction of LiP ferric enzyme with H_2O_2 yields compound I. The rate of the formation of compound I is pH-independent with a second-order rate constant of $0.5-2\times10^6\ M^{-1}s^{-1}$. In contrast, the reductions of compound I to compound II and compound II to the ferric enzyme are dependent on pH [63]. Koduri and Tien [86] published the rate constants for both the conversion of compound I to compound II ($1.5\times 10^5\ M^{-1}s^{-1}$) and the conversion of compound II to the ferric enzyme ($2.3\times10^3\ M^{-1}s^{-1}$) by VA at pH 3.5. The latter rate was extracted from a curve where the rate showed a concentration dependence on VA. Initially, it was thought that VA is stoichiometrically converted to veratryl aldehyde. However, detailed studies on the product formation in the LiP-catalyzed oxidation of VA indicate that veratryl aldehyde (70–90%) is not the sole product of the reaction. Quinones (10%) and ring opened products (up to 20% yield) have also been isolated and identified [53, 138]. The mechanism of the formation of these products is readily rationalized via the intermediacy of $VA^{\cdot+}$ [114]. The $VA^{\cdot+}$ is a highly acidic species that easily loses a proton [52]. Evidence of the formation of a neutral VA radical, formed after a proton loss from the initially formed $VA^{\cdot+}$, was obtained in oxygen consumption studies. Oxygen from air reacts rapidly with the hydroxy-substituted benzyl radical to form aldehyde and $O_2^{\cdot-}$ [14].

The production of VA by *P. chrysosporium* and the onset of ligninolysis are closely linked. Both appear at the beginning of idiophase. Moreover, culture conditions that stimulate lignin degradation, such as an elevated oxygen tension, enhance the production of VA. *P. chrysosporium* biosynthesizes this compound via the *L*-phenylalanine-cinnamate pathway, which includes *L*-phenylalanine, 3,4-dimethoxycinnamyl alcohol, and veratryl glycerol [82]. The enzyme phenylalanine ammonia-lyase is common among basidiomycetes fungi [143]. Its action results in the recycling of nitrogen by removing ammonia from phenylalanine [82].

The effect of VA on the induction of the ligninolytic system has been studied by adding VA to growing cultures. Cultures of *P. chrysosporium* supplemented with exogenous VA demonstrated an earlier appearance of lignin degradation and a higher lignin degradation rate [94]. The addition of VA would seem to induce LiP production in *P. chrysosporium* [44]. However, recent research has shown that there is no relationship between VA addition and the increase of LiP mRNA. This seems to exclude an inductive role of VA in LiP synthesis [23, 133].

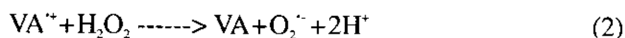
Purified LiP is irreversibly inactivated in the presence of excess H_2O_2 [28, 137, 149, 150]. Therefore, it is suspected that this enzyme may be inactivated by H_2O_2 under physiological conditions. Haemmerli *et al.* [54] were the first to propose that VA has a stabilizing effect on LiP. VA can conserve LiP activity by maintaining the catalytic cycle within the ferric enzyme, compound I, and compound II, thereby avoiding compound III formation [22]. Compound III of the peroxidases is formed by the complexation of $O_2^{\cdot-}$ with the ferric peroxidase or the reaction of compound II with H_2O_2 [22]. The second-order rate constant for the conversion of compound II to compound III has been estimated as $150\ M^{-1}\ s^{-1}$ at pH 3.5. This suggests that even during LiP catalysis in the presence of VA, a relatively significant portion of the enzyme is converted to compound III. The conversion rate of compound II to compound III increases with increasing concentrations of H_2O_2 [22]. Thus, it is conceivable that the fungus may have mechanisms to revert compound III to the ferric enzyme. It has also been proposed that VA itself may revert compound III to the ferric enzyme [149, 150]. However, Barr *et al.* [9] have shown that the cation radical of VA and methoxybenzenes, such as 1,2,4,5-tetramethoxybenzene, are the species involved in the conversion of compound III to the ferric enzyme.

Until the early 1990s, the general conception of the field was that LiP oxidizes nonphenolic compounds and MnP oxidizes phenolic compounds. This view may have originated not only because MnP cannot oxidize VA under physiological conditions but because LiP is easily inactivated during the oxidation of phenolic compounds. Harvey and Palmer [62] have shown that LiP is inactivated during the oxidation of

phenolic compounds. They suggested that this inactivation is associated with accumulation of compound III. It was also thought that $VA^{\cdot+}$ enhances the reduction of compound II to ferric enzyme over the alternative reaction with H_2O_2, which prevents the formation of compound III. In contrast, phenoxyl radicals or phenols are unable to promote the reduction of compound II over its reaction with H_2O_2, thereby resulting in the facile formation of compound III, an inactive form of LiP. However, Chung and Aust [28] showed that phenolic compounds are equally as good reducing substrates for compound II as VA, however phenols or phenoxyl radicals can not revert compound III to the ferric enzyme unlike $VA^{\cdot+}$. Thus, compound III accumulates during the catalysis due to the reaction of compound II with H_2O_2. This result indicates that VA has a dual role: prevention of the inactivation and the mediation of the oxidation of other compounds, as described in the next section.

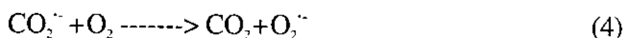
VA has a relatively high redox potential of about 1.45 V *vs.* a normal hydrogen electrode at pH3 in an aqueous buffer [45]. Because of its high redox potential, VA can participate in a variety of free radical reactions with the form of $VA^{\cdot+}$. Recent research has shown that $O_2^{\cdot-}$ and $HO^{\cdot}$ species can be formed *in vitro*. During the oxidation of VA by LiP, the formation of $O_2^{\cdot-}$ has been suggested as mentioned before (reaction (1)) [14]. The dismutation of $O_2^{\cdot-}$ results in the generation of H_2O_2. It has been found that Mn(II) strongly stimulates the reduction of $O_2^{\cdot-}$ to H_2O_2, increasing the veratryl aldehyde/H_2O_2 ratio significantly above 1. In the presence of excess H_2O_2, LiP catalyzes the one-electron oxidation of H_2O_2 to produce $O_2^{\cdot-}$ and VA via $VA^{\cdot+}$ (reaction (2)) [11]. Upon dismutation, a net production of O_2 and simultaneous decrease of H_2O_2 is the result. In reactions [3] and [4], $O_2^{\cdot-}$ is formed [109, 117, 118, 121].

$$VA^{\cdot}+O_2 \text{ -----> Veratryl aldehyde} + O_2^{\cdot-} + H^+ \quad (1)$$



$$VA^{\cdot+}+H_2O_2 \text{ ------> } VA+O_2^{\cdot-}+2H^+ \quad (2)$$

$$VA^{\cdot+}+\text{oxalate ------> } VA+CO_2^{\cdot-}+CO_2 \quad (3)$$



$$CO_2^{\cdot-}+O_2 \text{ -------> } CO_2+O_2^{\cdot-} \quad (4)$$

Barr *et al.* [12] recently showed the generation of $HO^{\cdot}$ *in vitro* under physiological pH 5 (Fig. 4). $HO^{\cdot}$ reacts with almost all biological molecules [55]. This radical may be involved in lignin degradation since Gierer *et al.* [48] showed that $HO^{\cdot}$ can react with both phenolic and nonphenolic compounds. The most important reactions include oxidative coupling, demethoxylation, and the hydroxylation of phenolic and/or nonphenolic compounds. The Fenton reaction can generate $HO^{\cdot}$, although there is controversy over whether or not this reaction occurs under physiological conditions.

The above results suggest that VA has an important role in the degradation of lignin and organopollutants by *P. chrysosporium*. Thus, the oxidized veratryl aldehyde should

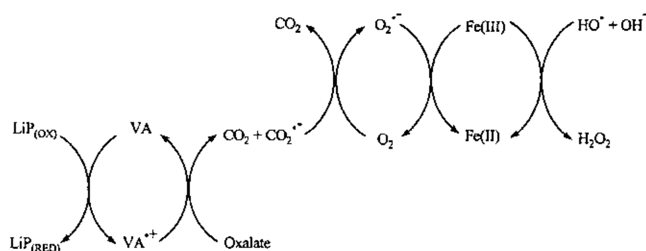


Fig. 4. Scheme to explain the production of hydroxyl radical ($HO^{\cdot}$) by lignin peroxidase [12].

be recycled. A culture of *P. chrysosporium* reduces both nonphenolic (veratric acid and veratryl aldehyde) and phenolic (vanillate and vanillin) compounds to their corresponding aldehydes and alcohols: an intracellular aryl alcohol dehydrogenase (EC 1.1.1.91) from *P. chrysosporium* has been characterized [100]. The enzyme reduces veratryl aldehyde to VA using NADPH as a cofactor. However, it is uncertain whether this dehydrogenase is responsible for the reduction of veratryl aldehyde to VA *in vivo*.

Oxalate

Oxalate is an ubiquitous compound produced as a secondary metabolite of wood-rotting fungi [5, 12, 40, 121]. Barr *et al.* [12] reported that oxalate is mineralized in a low nitrogen culture, and that the concentrations of oxalate in the cultures decreases during the period when maximum LiP activity was detected. Glyoxylate oxidase [1] and oxaloacetase [2] are responsible for the production of oxalate from glyoxylate and oxaloacetate, respectively. Recently, the biochemical role of oxalate has received attention in relation to the degradation of lignin and organopollutants by white-rot fungi. Oxalate is easily oxidized in the presence of metals and can act as an electron donor [12].

Akamatsu *et al.* [3] first reported that oxalate noncompetitively inhibites VA oxidase activity of LiP, while the acid is simultaneously decomposed to CO_2 by $VA^{\cdot+}$. Recently, the formate radical ($CO_2^{\cdot-}$, carboxylate anion radical), an intermediate produced during the oxidation of oxalate to CO_2, has also received much attention concerning the reduction of dioxygen to form $O_2^{\cdot-}$, as shown in the previous section [77, 79, 109, 117, 121]. $CO_2^{\cdot-}$ has a reduction potential of −1.9 V and can easily reduce oxygen and a number of chemicals. $CO_2^{\cdot-}$ reduces dioxygen to form $O_2^{\cdot-}$ at a near-diffusion controlled rate (2.4×10^9 M^{-1} s^{-1}) [77, 116]. Shah *et al.* [117] reported that the VA oxidase activity of LiP is also inhibited by ethylenedinitrilotetraacetic acid (EDTA) (an organic acid). The resulting $CO_2^{\cdot-}$ is involved in the reductive reactions. Shimada *et al.* [121] demonstrated that the CO_2 production from oxalate by the LiP/VA/H_2O_2 system is potentially inhibited by phenolic compounds, including vanillic acid and guaiacol. It is also speculated that $VA^{\cdot+}$ does not oxidize oxalate in the presence of phenolic

compounds. This suggests that $VA^{\cdot+}$ oxidizes phenolic compounds in preference to oxalate and that the phenoxyl radical formed cannot oxidize oxalate. As will be discussed later, oxalate-dependent reductions are important in the degradation of lignin and organopollutants. Therefore, it is believed that the fungi include a mechanism to control the concentration of phenolic compounds during the degradation (i.e., methylation of phenolic compounds).

The chemical oxidation of oxalate to CO_2 by Mn(III) has been reported by Launer [93]. The plant peroxidase system is also known to decompose oxalate in the presence of manganese ions with dioxygen being concomitantly consumed [74]. Wariishi *et al.* [146] and Kuan and Tien [90] reported that oxalate, serving as a manganese chelator at a concentration of less than 0.5 mM, stimulates the MnP activity. Kuan and collegues [89, 90] also reported that Mn(II), which is chelated by oxalate in a ratio of 1:1, is oxidized by the MnP compounds I and II. The resulting Mn(III)-oxalate complexes with another oxalate ligand under physiological concentrations of oxalate (2.5 mM). Khindaria *et al.* [79] also showed that one of the oxalate ligands reduces Mn(III) to Mn(II) while being oxidized to the free radical of the organic acid. The free radical decomposes and results in the formation of $CO_2^{\cdot-}$ and CO_2. This $CO_2^{\cdot-}$ can reduce O_2 to $O_2^{\cdot-}$, which subsequently reduces Fe(III) to Fe(II) under aerobic conditions. They also observed that only catalytic amounts of H_2O_2 are needed to initiate the reactions for Fe(III) reduction, after which no exogenous H_2O_2 is required. This result suggests that the dismutation of $O_2^{\cdot-}$ results in the production of H_2O_2 that can further participate in MnP-catalyzed reactions.

Manganese

The previous section discussed the importance of manganese in lignin degradation by white-rot fungi. Many of these fungi are known to leave black spots in decayed wood, which have been found to be associated with the selective delignification (removal of lignin in preference to cellulose). Blanchette *et al.* [13] identified these spots as deposits of manganese oxides. Changes in the distribution of manganese have been observed in various areas of decayed wood. Mn(III) in an uncomplexed form is unstable in an aqueous solution. It has a high redox potential of 1.5 V. However, under normal physiological conditions the redox potential of chelated Mn(III) is 0.9~1.2 V [108].

H_2O_2-Producing Enzymes

Prior to the discovery that extracellular peroxidases are produced by *P. chrysosporium*, it was shown that H_2O_2 generation correlates with ligninolytic activity [47]. In fact, the association of H_2O_2 with lignin degradation led to the discovery of LiP [82, 135]. It has also been reported that lignin degradation is inhibited upon the addition of catalase [97]. In more recent research, H_2O_2 has been shown to be produced by many other extracellular and intracellular oxidases. Extracellular glyoxal oxidase is thought to be very important for H_2O_2 generation [72]. MnP can generate H_2O_2 by catalyzing the oxidation of NAD(P)H [6]. Several intracellular enzymes producing H_2O_2 have been reported from white-rot fungi, including glucose-1-oxidase [70], fatty acyl-CoA oxidase [52], methanol oxidase [41], and pyranose-2-oxidase [36].

MECHANISMS OF LIGNIN AND XENOBIOTICS DEGRADATION USED BY WHITE-ROT FUNGI

Veratryl Alcohol-Mediated Oxidation

Lignin has an irregular structure such that it is resistant to microbial attack [82]. Thus, the degradation of lignin requires a nonspecific degradation process. Many studies have shown that the lignin-degrading system of white-rot fungi is nonspecific such that the fungi can also degrade and mineralize a host of structurally diverse organopollutants (Table 1) [7, 10]. As discussed earlier, white-rot fungi produce several components such as LiP, VA, oxalate, and H_2O_2-producing enzymes [70, 133]. LiP is a major component of the lignin-degrading system. Because LiP has a relatively higher redox potential than other peroxidases, such as HRP [71], purified LiP itself can oxidize chemicals that are not oxidized by other peroxidases. Hammel and Tardone [56] showed that 2,4,6-trichlorophenol is oxidized to 2,6-dichlorobenzoquinone by LiP. They proposed that the chlorophenol is oxidized to its cation radical and subject to the nucleophilic attack of water molecules. Therefore, the chemical is dechlorinated. Chemicals that can be directly oxidized include PCBs [27, 56, 141], PAHs [19], cyanide [119], benzo(a)pyrene [54], dibenzodioxin [57], and various dyes [34]. These compounds are also mineralized by whole fungal cultures (Table 1) [7, 10]. Even though these chemicals can be oxidized directly by LiP, their oxidation is significantly increased by the addition of VA [10].

The oxidation of 1,2,4-aminotriazole [136] and anisyl alcohol [64, 65, 86, 142] increases when VA is added to the reaction mixture. Similar phenomena have been found with several other organopollutants, such as phenol [29], PCP [27], heterocyclic and polymeric dyes [10, 101], and aromatic sulfides [8]. Several hypotheses have been proposed to explain the phenomena. Recent data from studies on the crystal structure of LiP have indicated that the direct interaction of polymeric lignin with the heme in the active site is very unlikely [110]. In addition, polymeric lignin is only degraded in the presence of VA [56]. Thus, it has been proposed that VA, from *P. chrysosporium* and many other white-rot fungi, functions as a mediator between the enzyme and lignin [64, 65]. Intensive studies on the oxidation of anisyl alcohol has been conducted to show the involvement

of VA as a mediator. However, it has been demonstrated that anisyl alcohol is not a substrate of compound II and that it is not oxidized via mediation. As mentioned earlier, LiP compound II reacts with H_2O_2 to form compound III, a catalytically inactive form. The formation of compound III is dependent upon the H_2O_2 concentration [22]. Koduri and Tien [86] showed that anisyl alcohol reacts with compound I but not with compound II. This may be because anisyl alcohol has a higher redox potential than VA. They also showed that when VA is added to the reaction mixture containing LiP, anisyl alcohol, and H_2O_2, the oxidation of anisyl alcohol increases because VA, as a substrate, rescues the enzyme by converting compound II to active ferric enzyme.

It has been reported that veratryl aldehyde formation is suppressed during the oxidation of phenolic compounds [60, 62]. A similar inhibition has also been observed during the oxidation of several dyes [105, 132] and 1,2,4-aminotriazole [136]. Several scientists have proposed that the inhibition of veratryl aldehyde formation is due to the high affinity of phenolic compounds to LiP, and thus phenolic compounds are preferentially oxidized in the presence of VA, even though the concentration of VA is higher [62]. However, Chung and Aust [28] showed that phenolic compounds and VA have a similar affinity toward the LiP intermediate. They found that the addition of VA increases the extent of phenol oxidation, and that phenol is first oxidized and veratryl aldehyde formation is inhibited during this phenol oxidation (Fig. 5). While LiP is rapidly inactivated to compound III during the oxidation of phenol in the presence of VA, compound II is observed as soon as VA oxidation begins. The kinetics of phenol oxidation in the presence of VA are similar to those of VA oxidation. In addition, they observed that compound III does revert to the ferric enzyme in the presence of VA upon the addition of phenol. As such, $VA^{\cdot+}$ is necessary to revert compound III to the ferric enzyme [9]. These results suggest that VA is first oxidized by LiP to $VA^{\cdot+}$, thereafter phenol is oxidized to phenoxyl radical while $VA^{\cdot+}$ is reduced back

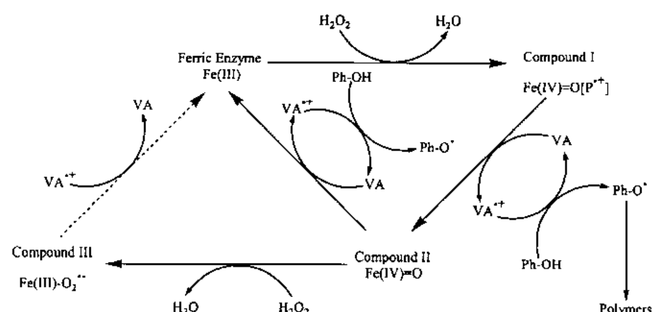


Fig. 5. Mechanism to explain the VA-mediated indirect oxidation of phenol by LiP.
Ph-OH is phenol, Ph-O$^{\cdot}$ a phenoxyl radical, and $VA^{\cdot+}$ a veratryl alcohol cation radical [27].

to VA. Thus, veratryl aldehyde formation is inhibited. LiP activity is lost as compound III is accumulated, since $VA^{\cdot+}$ is consumed by the oxidation of phenol and phenol or the phenoxyl radical was unable to revert compound III to the ferric enzyme. After all the phenol is oxidized, $VA^{\cdot+}$ becomes available to convert compound III back to the ferric enzyme. Subsequently, many researchers have shown that the oxidation of chemicals by mediation is common with LiP [27, 29, 130, 131].

While MnP can not oxidize PCP, PCP is oxidized to 2,3,5,6-tetrachloro-*p*-benzoquinone (TCBQ) by LiP in the absence of VA [10]. This suggests that LiP may have a significant role in the degradation of PCP by *P. chrysosporium in vivo*. However, the enzyme is inactivated quickly during the oxidation of PCP [29]. It is thus speculated that the fungi may include a mechanism to reduce this inactivation *in vivo*. Chung and Aust [29] reported on the VA-mediated indirect oxidation of PCP by LiP and the resulting enhancement of PCP oxidation by addition of VA. It has also been found that PCP is oxidized indirectly via $VA^{\cdot+}$. TCBQ is the major product during PCP oxidation in the presence of VA, thereby suggesting that whether PCP is oxidized directly by LiP or via mediation, the same product, TCBQ, is formed by 4-dechlorination. PCP has been found to be as good a substrate of LiP as VA yet it can not revert compound III to the ferric enzyme, like phenol. However, the reversion of compound III to the ferric enzyme in the presence of VA is not prevented by the addition of PCP, and compound II has been observed during the oxidation of PCP in the presence of VA. This may be because $VA^{\cdot+}$ oxidizes PCP sufficiently slowly such that some $VA^{\cdot+}$ becomes available to revert compound III to the ferric enzyme. Another example of this mediation phenomenon can be found in the LiP-catalyzed reduction of chemicals, which has been shown by Shah *et al.* [117]. They demonstrated that an electron donor such as EDTA is oxidized via $VA^{\cdot+}$ by LiP.

Harvey *et al.* [61] reported that VA can mediate oxidations during the LiP-catalyzed oxidation of 4-methoxymandelic acid. They observed that, although VA is the preferred substrate, the oxidation of VA to veratryl aldehyde is completely inhibited by 4-methoxymandelic acid. They speculated that even though 4-methoxymandelic acid has a higher redox potential than VA itself, the irreversible decarboxylation of 4-methoxymandelic acid may provide the thermodynamic driving force for the oxidation by $VA^{\cdot+}$.

All the proposed mechanisms above indicate that the reactivity of both the substrate with LiP compound II and the corresponding substrate radical with compound III are important to determine the kinetics of LiP-catalyzed oxidation. Evidently, further detailed kinetic studies are needed to distinguish between the postulated mechanisms. There are several indications that $VA^{\cdot+}$ is formed in the LiP-catalyzed oxidation of VA. The production of several quinones and

lactones in VA oxidation can only be explained when VA^{+} is produced [111, 122]. Chung and Aust [31] proposed that quinones or hydroquinones may be involved in the reductive reactions in the presence of MnP. It has been shown that radical intermediates are produced during the LiP-catalyzed oxidation of VA with nuclear magnetic resonance spectroscopy [49]. Recently, Khindaria *et al.* [75, 76, 78] demonstrated the formation of VA^{+} during LiP turnover using electron spin resonance spectroscopy. In addition, Candeias and Harvey [24] have shown that VA^{+} has a lifetime of 59 ms and can diffuse up to 7 μm in an aqueous environment, using pulse radiolysis. They also observed the oxidation of a lignin model compound (Poly-R478) by VA^{+} using the same technique.

Oxalate-Dependent Reduction Mechanism

Most organopollutants, such as DDT, Lindane, and PCBs, are highly chlorinated and thus very electron-deficient [118]. Even though LiP and MnP cannot oxidize these chemicals, they are degraded to CO_2 by ligninolytic cultures of *P. chrysosporium* [10]. Therefore, it is believed that these organopollutants should first be reduced prior to further oxidation by peroxidases. In the previous section, it was shown that VA^{+} can initiate free radical reactions that lead to the reduction of other chemicals. Shah *et al.* [117, 118] showed that in the presence of LiP, VA, and H_2O_2, the resulting VA^{+} reacts with EDTA to form an EDTA radical, which results in the production of $CO_2^{\cdot-}$. This anion radical has been shown to reduce cytochrome c, nitroblue tetrazolium, dioxygen, and carbon tetrachloride. Khindaria *et al.* [77] reported that in the presence of LiP, VA, and H_2O_2, oxalate can act as an electron donor and mediate the reductive dehalogenation of other haloorganics, including trichloroethylene (TCE) and chloroform. They also showed that CCl_4 and TCE are mineralized under ligninolytic cultures of *P. chrysosporium*. Previously, these halocarbons have been reported to be degraded only under anaerobic conditions, and incomplete reductive dehalogenation results in the accumulation of dichloroethane, $CHCl_3$, and CH_2Cl_2 [144]. Thus, the reductive dehalogenation by this fungus under aerobic conditions is striking. MnP has also been shown to have a reductive capability in the presence of oxalate to reduce dioxygen and Fe(III) to $O_2^{\cdot-}$ and Fe(II), respectively [79]. It has also been shown that $CO_2^{\cdot-}$ is involved in this process. However, the reductive dehalogenation of halocarbons and the generation of $OH^{\cdot}$ in the $MnP/H_2O_2/$oxalate system has yet to be investigated.

Plasma-Membrane-Dependent Reductions Mechanism

Kohler *et al.* [88] found that DDT disappears yet is not mineralized under nonligninolytic cultures of *P. chrysosporium*. Subsequently, it has been shown that some highly oxidized organopollutants, including 2,4,6-trinitrotoluene (TNT) [46] and DDT [20], disappear before the production of LiP. Thus, it is conceivable that this fungus may have a mechanism that does not include LiP or MnP so as to metabolize those organopollutants. Sollod *et al.* [126] demonstrated that several redox dyes are efficiently reduced by a plasma-membrane redox system by several fungi. The fungi generate a proton gradient across the plasma-membrane such that the electromotive potential can reduce chemicals. Stahl and Aust [127, 128] reported that TNT is reduced to 2- and 4-aminodinitrotoluene under nutrient-sufficient and nutrient-deficient cultures. The amino congeners of TNT are less toxic to the fungus such that further degradation of a high concentration of TNT is achieved by the fungus.

Methylation of Phenolic Compounds

Many secondary metabolites from white-rot fungi contain methoxy groups, indicating the presence of a methylating system in white-rot fungi [39]. Methylation is also a common reaction in the catabolism of aromatic compounds by white-rot fungi [86, 87]. The demethoxylation and ether cleavage of lignin and lignin model compounds catalyzed by LiP generate phenolic compounds [4, 84], whose further oxidation to a phenoxyl radical can result in the formation of polymerized products [53]. Phenolic compounds are not ideal substrates for LiP. Thus, LiP is easily inactivated during the oxidation of phenolic compounds [62]. The fungi can reduce the concentration of phenolic compounds by methylation such that better lignin degradation can be achieved. In addition, polychlorinated phenols such as PCP have been shown to be methylated to pentachloroansiole (PCA) by *P. chrysosporium* [30, 92]. The methylation of PCP to PCA may be a detoxification mechanism since PCA is not an inhibitor of oxidative phosphorylation [35] and is less toxic to wood-rotting fungi, other microbes, and fish [36].

Harper *et al.* [59] reported that white-rot fungi can methylate a host of phenolic compounds. A methyl donor used by some basidiomycetes in the biosynthesis of methoxy groups and esters is chloromethane (CH_3Cl), which has a primary role in the methylation of aromatic compounds such as acids or phenolic compounds. Although CH_3Cl may serve as a methyl donor in the biosynthesis of VA, *P. chrysosporium* does not emit any detectable amount of CH_3Cl [58]. In *P. chrysosporium* two distinct systems have been reported to be simultaneously involved in the methylation system, one using *S*-adenosyl methionine and the other using CH_3Cl as a methyl donor [32]. A *S*-adenosyl methionine-dependent *O*-methyltransferase has been purified from cell extracts of *P. chrysosporium* [33]. This enzyme catalyzes the para-specific methylation of vanillate and syringate. It can also methylate 2,4-dichlorophenol. Accordingly, it has been suggested that CH_3Cl-dependent *O*-methyltransferase is membrane bound and/or part of a

multienzyme complex. A CH_3Cl-dependent *O*-methyltransferase has not yet been isolated and characterized [32]. It is still uncertain whether these enzymes are responsible for methylation of PCP to PCA.

Conclusions

Many bacteria have also an ability to degrade various organopollutants [25, 69, 102, 103, 104, 125, 151]. However, the scope of degradation by each bacterium is often limited to one or a few kind of pollutants. Considering the complex nature of organopollutants in the contaminated field, *P. chrysosporium*, a white-rot fungus, may have an advantage over bacteria since this fungus can degrade a host of pollutants as shown in Table 1. Some fungi can utilize cellulosic materials for an energy source yet cannot degrade lignin [96, 124, 152, 153, 154]. In contrast, *P. chrysosporium* have the ability to degrade lignin as well as to utilize cellulosic materials [17, 43, 99, 120]. Thus, a cheap materials, such as corncob, straw, or wood chip, can be employed to support the growth of this fungus. In addition, a number of the other white-rot fungi has also shown to have an ability to degrade organopollutants as well as lignin, although their physiology, enzymolgoy, and mechanisms are often somewhat different from those of *P. chrysosporium* [66, 67, 80, 82, 92, 97, 132].

Although the ligninolytic system of *P. chrysosporium* has initially been studied to elucidate the degradation mechanism of lignin, more attention has recently been given to the enzymolgy and biochemical mechanisms for the degradation of organopollutants. It is believed that the elucidated mechanisms for the degradation of organopollutants be also applicable to those for the degradation of lignin. Even though much knowledge on the physiology, biochemistry, and molecular biology of *P. chrysosporium* has been accumulated until now [7, 10, 17, 64, 82, 84, 133], more studies should be done to efficiently utilize the capacity of various white-rot fungi, since each strains may have its own strong points. More investment on the biochemistry and molecular biology of white-rot fungi may provide the ground for both the economical use of lignocellulosic materials and the bioremediation of recalcitrant organopollutants.

Abbreviations: cAMP, cyclic-3', 5'-adenosine monophosphate; CBQase, cellobiose:quinone oxidoreductase; DDT, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane; EDTA, ethylenedinitrilotetraacetic acid; HRP, horseradish peroxidase; LiP, lignin peroxidase; MnP, manganese-dependent peroxidase; PAHs, polycyclic aromatic hydrocarbons; PCA, pentachloroansiole; PCBs, polychlorinated biphenols; PCP, pentachlorophenol; TCBQ, 2,3,5,6-tetrachloro-*p*-benzoquinone; TNT, 2,4,6-trinitrotoluene; VA, veratryl alcohol.

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