5 Molecular Genetics of Lignin-Degrading Fungi and Their Applications in Organopollutant Degradation

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CONTENTS

I. Introduction

This chapter provides an overview of the physiology and associated molecular genetics of wood-decaying fungi as they relate to organopollutant degradation. White-rot fungi are characterized by an ability to fragment all major structural polymers of wood including lignin. More poorly understood are the brown-rot fungi, which rapidly depolymerize cellulosic materials while only modifying lignin. Collectively, these wood- and litter-degrading fungi play a pivotal role in the carbon cycle. In fact, white-rot fungi are the only microbes known to efficiently mineralize fully lignified tissue.

The wood-rotting fungi are obligate aerobes, deriving their nourishment from the biological combustion of wood and associated materials, using molecular oxygen as a terminal electron acceptor. The focus here is on the non-specific extracellular enzyme systems of white-rot fungi. These oxidative enzymes have been shown to transform and degrade an array of organopollutants in addition to depolymerizing lignin. Intracellular metabolism of small molecular weight products is also covered, although relatively little is known of these processes. This chapter does not comprehensively review the voluminous literature on fungal degradation of lignin and related organopollutants. Recent developments are emphasized, and the reader is referred to previous review articles (Kirk and Farrell 1987; Eriksson et al. 1990; Gold and Alic 1993; Hammel 1995b; Cullen and Kersten 1996; Cullen 1997; Kirk and Cullen 1998; Cameron et al. 2000; Pointing 2001) for more detailed information. Areas of uncertainty are highlighted.

II. Physiology of Wood-Decaying Fungi

Lignin is a formidable substrate. The polymer is large and initial depolymerization must be extracellular. The structure is comprised of inter-unit carbon–carbon and ether bonds that demand oxidative rather than hydrolytic degradative mechanisms. In addition, the polymer's lack of stereoregularity requires ligninolytic agents that are less specific relative to the hydrolytic enzymes attacking cellulose. White-rot fungi have met these challenges through the evolution of extracellular peroxidases and oxidases that act non-specifically via the generation of unstable lignin free radicals, which undergo a variety of spontaneous cleavage reactions. The major enzymes acting directly or indirectly on lignin are lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Fig. 5.1). All three enzymes can act with low molecular weight mediators to

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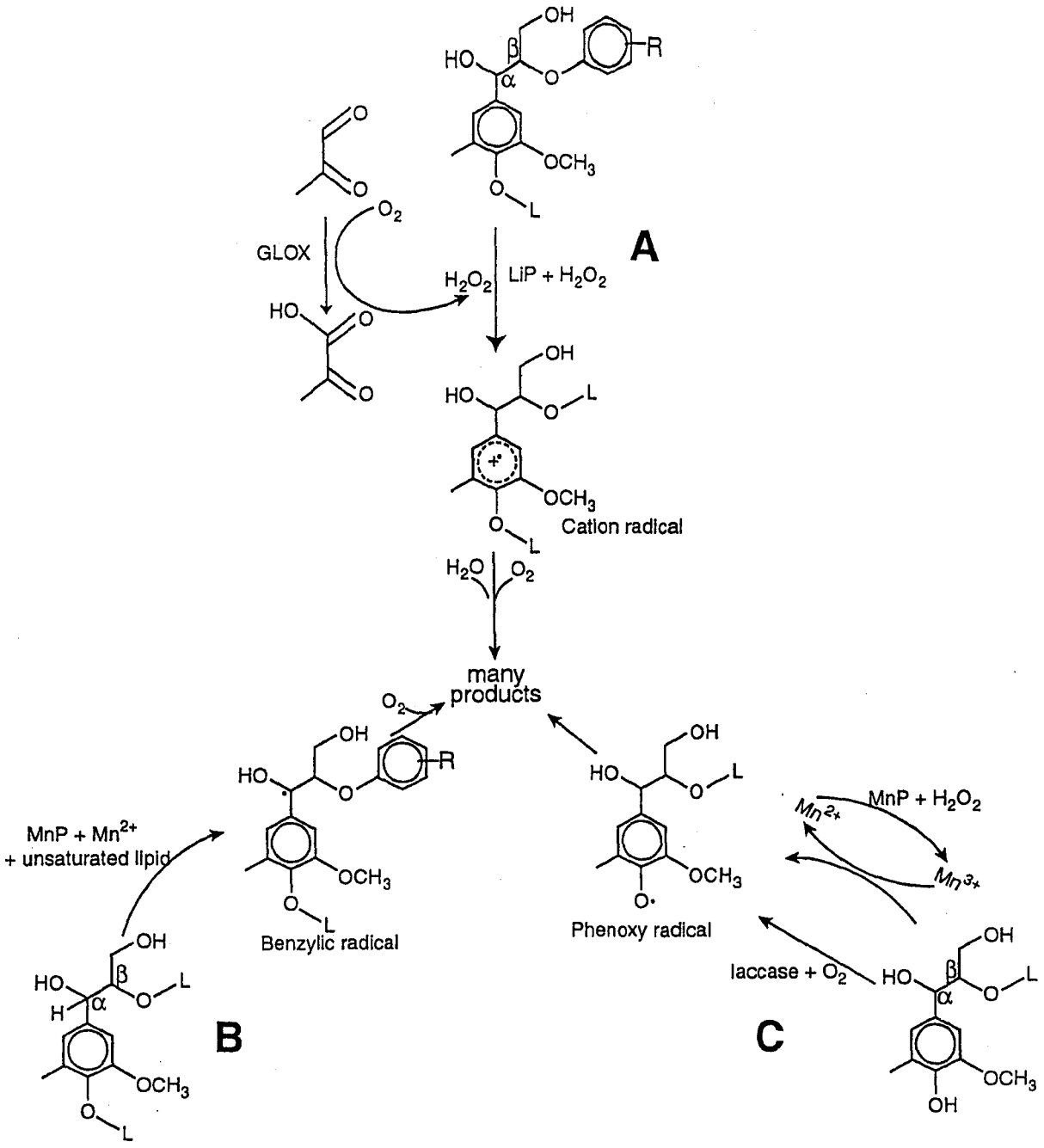

Fig. 5.1. Extracellular oxidative enzyme systems of *P. chrysosporium* and related white-rot fungi. Lignin peroxidase (LiP) and manganese peroxidase (MnP) coupled lipid peroxidation oxidize β-O-4 models as shown in reactions **A** and **B,** respectively. Laccases and Mn(III) oxidize phenols (C)

bring about oxidation of lignin as well as an impressive array of xenobiotics (Table 5.1). The microbiology and physiology of ligninolytic fungi have been reviewed (Kirk and Farrell 1987; Eriksson et al. 1990; Gold and Alic 1993; Higuchi 1993; Tuor et al. 1995; Cullen and Kersten 1996).

A. Peroxidases

1. Lignin Peroxidases

LiP was first discovered based on H_2O_2-dependent Cα–Cβ cleavage of lignin model compounds and subsequently shown to catalyze the partial

Table 5.1. Examples of organopollutants degraded by wood-decaying fungi. PCBs polychlorinated biphenyls; PAHs polycyclic aromatic hydrocarbons

Species	Organopollutant	References(s)
Bjerkandera adusta	Azo dyes	Heinfling et al. (1998)
	Phenyl urea herbicides	Khadrani et al. (1999)
	PCBs	Beaudette et al. (1998)
	PAHs	Field et al. (1992); Kotterman et al. (1998a,b)
Ceriporiopsis subvermispora	PCBs	Ferrey et al. (1994)
	Anthracene	Krivobok et al. (1998)
	Fluorene	Garon et al. (2000)
Coriolus versicolor	4-Methyldibenzothiophene	Ichinose et al. (1999)
	Textile dyes	Kapdan et al. (2000)
Corolopsis gallica	PAHs	Pickard et al. (1999)
Corolopsis polyzona	PCBs	Novotny et al. (1997)
Gloeophyllum striatum[a]	2,4,-Dichlorophenol	Schlosser et al. (2000)
	Pentachlorophenol	Fahr et al. (1999)
Gloeophyllum trabeum[a]	Pentachlorophenol	Fahr et al. (1999)
	Polyethylene glycol	Kerem et al. (1998); Kerem and Hammel (1999)
Irpex lacteus	2,4,6-Trinitrotoluene	Kim and Song (2000)
Kuehneromyces mutabilis	Phenanthrene and pyrene	Sack and Fritsche (1997); Sack et al. (1997a)
Laetiporus sulphureus[a]	Phenanthrene and pyrene	Sack and Gunther (1993); Sack et al. (1997a)
Nematoloma frowardii	PAHs	Sack and Gunther (1993); Sack et al. (1997a-c)
	2,4,6-Trinitrotoluene	Scheibner and Hofrichter (1998)
Phanerochaete chrysosporium	Alkyl halide insecticides	Kennedy et al. (1990)
	Chloroanilines	Sandermann et al. (1998)
	DDT	Bumpus and Aust (1987)
	2,4-Dichlorophenol	Valli and Gold (1991)
	Heterocyclic dyes	Cripps et al. (1990)
	Endosulfan (cyclodiene)	Kullman and Matsumura (1996)
	Various PAHs	Bumpus 1989; Hammel et al. (1991); Vazquez-Duhalt et al. (1994); Bogan et al. (1996a); May et al. (1997)
	PCBs	Bumpus et al. (1985); Eaton (1985);Thomas et al. (1992); Dietrich et al. (1995);Yadav et al. (1995); Novotny et al. (1997)
	Pentachlorophenol	Hammel and Tardone (1988); Mileski et al. (1988); Lamar et al. (1990b); Aiken and Logan (1996); Reddy and Gold (1999,2000)
	Trichloroethylene	Yadav et al. (2000)
	2,4,5-Trichlorophenol	Joshi and Gold (1993)
	2,4,6-Trichlorophenol	Armenante et al. (1994); Reddy et al. (1998)
	2,4,6-Trinitrotoluene	Fernando et al. (1990); Sublette et al. (1992); Hodgson et al. (2000)
Phanerochaete laevis	PAHs	Bogan and Lamar (1996)
Phanerochaete sordida	Creosote	Davis et al. (1993); Lamar et al. (1994)
	Pentachlorophenol	Lamar and Dietrich (1990); Lamar et al. (1990a,b,1993)
	Dioxins	Takada et al. (1996)
Phlebia radiata	2,4,6-Trinitrotoluene	Van Aken et al. (1999)
Phlebia tremellosa	PCBs	Ferrey et al. (1994)
Pleurotus eryngii	Azo dyes	Heinfling et al. (1998)
Pleurotus ostreatus	Industrial dyes	Vyas and Molitoris (1995); Rodriguez et al. (1999)
	PCBs	Beaudette et al. (1998)
	Phenanthrene	Bezalel et al. (1996)
	Phosphorothiolates	Amitai et al. (1998)
	Various PAHs	Martens and Zadrazil (1998); Novotny et al. (1999); Baldrian et al. (2000)
Pleurotus pulmonarius	Benzo[a]pyrene	Maspahy et al. (1999)
Pycnoporus cinnabarinus	Triclosan	Hundt et al. (2000)
Trametes hirsutus	Lindane	Singh and Kuhad (1999)
Trametes hispida	Industrial dyes	Rodriguez et al. (1999)
Trametes versicolor	PAHs	Majcherczyk et al. (1988); Collins et al. (1996); Johannes et al. (1996); Johannes and Majcherczyk (2000)
	PCBs	Novotny et al. (1997); Beaudette et al. (1998)
	Triclosan	Hundt et al. (2000)

[a]Brown-rot fungi. All others are classified as white-rot fungi.

depolymerization of methylated lignin in vitro (Glenn et al. 1983; Tien and Kirk 1983,1984; Gold et at. 1984; Fig. 5.1A). Lignin peroxidase catalyzes a variety of oxidations, all of which are dependent on H_2O_2. Simply stated, **LiP oxidizes** aromatic **nuclei of substrates** by one electron and the resulting aryl cation radicals degrade spontaneously via many reactions dependent on substrate structure and on the presence of reactants (Harvey et al. 1985; Kersten et al. 1985; Shoemaker et al. 1985; Hammel et al. 1986b). Reactions subsequent to cation radical formation are complex. The chemistry of LiP-catalyzed reactions has been reviewed (Higuchi 1990).

Direct involvement of LiP has been demonstrated in the degradation of a wide range of organopollutants. **Polycyclic aromatic hydrocarbons** (PAHs) with ionization potentials below approximately 7.6 eV, including benzo[a]pyrene, anthracene, and pyrene, are substrates for LiP (Hammel et al. 1986a). Similarly, free radical mechanisms involving purified LiP have been shown for chlorinated phenols (Hammel and Tardone 1988; Mileski et al. 1988; Valli and Gold 1991; Reddy and Gold 2000), tetrahydrofurans (Vazquez-Duhalt et al. 1994), dioxins (Hammel et al. 1986a; Valli et al. 1992b), methoxybenzenes (Kersten et al. 1985), and various chloro- and nitromethoxybenzenes (Valli and Gold 1991; Valli et al. 1992a,b; Teunissen et al. 1998). The biochemistry of organopollutant degradation has been reviewed (Higson 1991; Hammel 1995a,b).

Isozymic forms of Phanerochaete chrysosporium LiPs are commonly distinguished by their pI and order of elution from a MonoQ anion-exchange column. Ten peroxidases have been separated by this method and are designated H1 through H10. Six of these, H1 (pI 4.7), H2 (pI 4.4), H6 (pI 3.7), H7 (pI 3.6), H8 (pI 3.9, and H10 (pI 3.3), have veratryl alcohol oxidation activity (Renganathan et al. 1985; Kirk et al. 1986; Leisola et al. 1987; Farrell et al. 1989). Analytical isoelectric focusing resolved 15 proteins with LiP activity (Leisola et al. 1987). The number of isozymes observed depends on the growth conditions (e.g. nitrogen- versus carbon-limited), the means of purification, and storage conditions. Proteolysis, dephosphorylation and other post-translational modifications contribute to isozyme multiplicity (Eriksson and Pettersson 1982; Kuan and Tien 1989; Dosoretz et al. 1990a,b; Datta 1992; Dass et al. 1995; Feijoo et al. 1995; Rothschild et al. 1997, 1999). Although the various Lips have similar substrate specificities towards aromatic substrates, their kinetic behavior varies more clearly when tert-butyl hydroperoxide is substituted for H_2O_2 (Farrell et al. 1989; Glumoff et al. 1990). The processing, biological roles and interactions among isozymes are poorly understood.

The precise role of LiPs in complex substrates such as wood and organopollutant-contaminated soils remains uncertain. As mentioned above, extracellular peroxidases are physically too large to enter the pores of the cell wall (reviewed by Blanchette et al. 1997). Thus, LiP has been proposed to act indirectly by oxidizing low molecular weight substrates, which in turn penetrate the wall and oxidize the polymer (reviewed by Hammel 1996). Specifically, the secondary metabolite **veratryl alcohol,** which is synthesized and secreted by *P. chrysosporium* and which is oxidized by LiP to a cation radical (Khindaria et al. 1995), was proposed to play this role (Harvey et al. 1986). However, the veratryl alcohol cation radical is too short-lived to function as a diffusible mediator (Khindaria et al. 1995). Recent studies show Lip is capable of oxidizing **ferrocytochrome c** and synthetic lignin at the protein surface by a long-range electron transfer mechanism (Wariishi et al. 1994; Johjima et al. 1999).

2. Manganese Peroxidases

Low molecular weight diffusible oxidants could be provided by **manganese peroxidases** (MnPs; Glenn et al. 1986; Paszczynski et al. 1986). Like Lips, MnPs are glycosylated and have acidic pIs and pH optima. Also like LiPs, they have a conventional peroxidase catalytic cycle, but with Mn(II) as the substrate. The Mn(II) must be chelated by bidentate organic acid chelators such as glycolate or oxalate, which stabilize the product Mn(III) and promote its release from the enzyme. The resulting Mn(III) chelates are **diffusible oxidants** that can act at some distance from the enzyme. However, they are not strong oxidants and cannot attack the non-phenolic units of lignin. Instead, they oxidize phenolic structures (Fig. 5.1C), which make up about 10% of the units in lignin. The phenoxy radicals resulting from the one-electron oxidation undergo a variety of reactions, some of which result in polymer cleavage within certain units, e.g., between the aromatic rings and Cα (Wariishi et al. 1991; Tuor et al. 1992; Fig. 5.1C). Purified MnPs from cultures of *P. chrysosporium, Nematoloma frowndi,* and

Phlebia radiata oxidize **xenobiotics** such as pentachlorophenol and 2,4,6-trinitrotoluene (TNT) in an Mn-dependent manner (Scheibner and Hofrichter 1998;Van Aken et al. 1999; Reddy and Gold 2000). In contrast, azo dye decolorization by *Pleurotus eryngii* and *Bjerkandera adusta* MnP isozymes is Mn-independent (Heinfling et al. 1998).

It seems unlikely that the MnP-catalyzed reaction should result in the extensive depolymerization of lignin or the efficient oxidation of organopollutants of relatively high ionization potential. However, certain efficient **lignin degraders,** including *Ceriporiopsis subvermispora, Phanerochaete sordida,* and *Dichomitus squalens,* lack detectable LiP but produce MnP (Perie and Gold 1991; Ruthann et al. 1992, Ruttimann-Johnson et al. 1993,1994; Hatakka 1994; Orth et al. 1994). Also, phenanthrene and fluorene are not LiP or MnP substrates and yet both are efficiently degraded by *P. chrysosporium* cultures (George and Neufield 1989; Hammel et al. 1992; Vazquez-Duhalt et al. 1994; Bogan et al. 1996a). These studies point to **alternative mechanisms,** and recent research shows that the production of diffusible oxyradicals by MnP can occur by a mechanism other than via chelates of Mn(III). In the presence of Mn(II), MnP promotes the peroxidation of unsaturated lipids. Transient lipoxyradical intermediates are generated and these have been shown to oxidize non-phenolic lignin model compounds (Fig. 5.1B). The **MnP/lipid peroxidation system** depolymerizes phenolic- and phenol-blocked (methylated) synthetic lignins (Bao et al. 1994), and oxidizes fluorene (Bogan et al. 1996a), phenanthrene (Moen and Hammel 1994), and β-O-4 linkages within lignin model compounds (Bao et al. 1994; Kapich et al. 1999). Taken together, these data suggest that lipid peroxidation may be a component of the lignin-degrading system of certain fungi. The identity of the substrate lipid(s) is under investigation (Enoki et al. 1999), but currently unknown.

MnP/lipid peroxidation models aside, LiP remains the only fungal oxidant known that can efficiently mimic, in vitro, the Cα-Cβ cleavage and extracellular cleavage of aromatic rings that is characteristic of ligninolysis by white-rot fungi. Recently identified peroxidases defy simple classification as MnP or LiP, and can oxidize Mn(II) as well as non-phenolic substrates (e.g., veratryl alcohol) in the absence of manganese (Mester and Field 1998; Camarero et al. 1999).

B. Laccases

Laccases are **blue copper** oxidases that catalyze the one-electron oxidation of phenolics, aromatic amines, and other electron-rich substrates. Like Mn(III) chelates, they oxidize the phenolic units in lignin to phenoxy radicals, which can lead to aryl–Cα cleavage (Kawai et al. 1988; Fig. 5.1C). Most white-rot fungi produce laccase but some do not, indicating that laccase is not absolutely required in lignin degradation. Isozyme multiplicity is common among basidiomycetes including *C. subvermispora* (Fukushima and Kirk 1995; Salas et al. 1995), *P. chrysosporium* (Srinivasan et al. 1995; Dittmer et al. 1997; Rodriguez et al. 1997), and *D. squalens* (Perie et al. 1998).

Laccase can oxidize non-phenolic lignin-related substrates in the presence of certain auxiliary substrates such as ABTS **(2,2'-azino-bis-3-ethylthiazoline-6-sulfonate;** Collins et al. 1996; Bourbonnais et al. 1997, 1998). For example, purified *P. ostreatus* laccase efficiently degrades organophosphorus insecticides and related nerve agents in the presence of ABTS (Amitai et al. 1998). High ionization potential PAHs are also oxidized by *C. gallica* and *T. versicolor* laccases in the presence of synthetic mediators (Johannes et al. 1996; Pickard et al. 1999). *Pycnoporus cinnabarinus* and *Trametes versicolor* cultures produce small molecular weight compounds that may act as natural intermediaries for oxidation of non-phenolic lignin substructures (Eggert et al. 1996) and PAHs (Johannes and Majcherczyk 2000). The structure and function of fungal laccases have been reviewed (Thurston 1994; Youn et al. 1995).

C. Other Enzyme Systems

Although numerous laboratory studies show extracellular peroxidases and laccases capable of oxidizing various xenobiotics, it is clear that a **multitude of additional extracellular and intracellular processes** must be involved in the transformation and/or mineralization of these compounds. For examples, O-methylation of PCP and triclosan has been observed in *P. chrysosporium* (Lamar and Dietrich 1990) and *P. cinnabarinus* (Hundt et al. 2000) cultures, respectively. Glycosyl conjugation of triclosan has also been shown in *T. versicolor* cultures (Hundt et al. 2000). Membrane-bound and intracellular enzymes have

received relatively little attention to date, but **cytochrome P-450 monooxygenases** have been implicated in bioconversions of phenanthrene by *P. ostreatus* (Bezalel et al. 1996), benzo[a]pyrene by *Pleurotus pulmunoarius* (Maspahy et al. 1999), endosulfan by *P. chrysosporium* (Kullman and Matsumura 1996), and 4-methyldibenzothiophene by Coriolus versicolor (Ichinose et al. 1999). In the case of 2,4,6-trichlorophenol (Reddy et al. 1998) and PCP (Hammel and Tardone 1988; Mileski et al. 1988; Reddy and Gold 1999,2000) degradation by *P. chrysosporium*, extracellular LiP or MnP may intitially catalyze oxidative dechlorination and the products subsequently undergo various intracellular reductive dechlorination and hydroxylation reactions.

Organopollutant degradation by **brown-rot** fungi may involve extracellular oxidative mechanisms that are **fundamentally different** from white-rot species. *Gloeophyllum* spp. and related brown-rot fungi lack detectable LiP, MnP, or laccase activities but nevertheless efficiently degrade 2,4 dichlorophenol (Fahr et al. 1999; Schlosser et al. 2000), PCP (Fahr et al. 1999), and polyethylene glycol (PEG; Kerem et al. 1998; Kerem and Hammel 1999). Hydroxyl radical, formed by a Fenton-type reaction of Fe(II) and H_2O_2, has long been suspected as the powerful oxidizing agent needed to rapidly depolymerize cellulose during brown rot (Koenigs 1974a,b). Analysis of metabolites and the identification of Fe(II) and H_2O_2 in *Gleophyllum* cultures support a Fenton-type degradation (Hyde and Wood 1997; Kerem et al. 1998; Kerem and Hammel 1999; Paszczynski et al. 1999; Schlosser et al. 2000).

Several systems have been suggested as potential sources of extracellular H_2O_2 for peroxidase activity and hydroxyl generation (Fig. 5.1A). One likely source of H_2O_2 for *P. chrysosporium* Lips is glyoxal oxidase (GLOX; Kersten and Kirk 1987). GLOX is a **radical-copper oxidase** (Whittaker et al. 1996) that utilitizes a wide variety of small aldehydes such as glyoxal and methylglyoxal (extracellular metabolites of *P. chrysosporium)* and transfers the electrons to O_2, generating H_2O_2. Another substrate, glycolaldehyde, is produced by the action of LiP on β-O-4 lignin substructures. Perhaps the most interesting property of GLOX, and of considerable physiological significance, is that in the absence of a peroxidase system, the oxidase is reversibly inactivated (Kersten 1990). The enzyme is reactivated, however, by reconstituting the complete peroxidase system, including both LiP and substrate. This suggests that the supply of H_2O_2 by GLOX is responsive to the demand of the peroxidases, thereby providing an extracellular regulatory mechanism for control of the coupled enzyme systems. GLOX is also produced by other white-rot fungi, although apparently not by all (Hatakka 1994; Orth et al. 1994).

Another likely candidate for generating H_2O_2 in some white-rot fungi, including P. chrysosporium and B. adusta, is **aryl alcohol** oxidase (AAO; Muheim et al. 1990; Asada et al. 1995b). This enzyme oxidizes benzyl alcohols to aldehydes, transferring the electrons to O_2, producing H_2O_2. Interestingly, *B. adusta* secretes chlorinated benzyl alcohols that are not substrates for LiP but are for the AAO. Because B. adusta also secretes LiP, this strategy circumvents the non-productive oxidation of the AAO substrate by LiP. *P. ostreatus* secretes a mixture of benzyl alcohols, including anisyl alcohol, and an AAO that oxidizes them (Sannia et al. 1991).

Various **sugar oxidases** have also been suggested to be producers of the required extracellular H_2O_2. Most are intracellular enzymes. Pyranose oxidase, however, which oxidizes various monosaccharides at C-2, with transfer of electrons to O_2 to produce H_2O_2, has been located in the extracellular polysaccharide matrix that coats the lumens of cells during white-rot. The enzyme has been studied in *P. chrysosporium, T. versicolor, Oudemansiella mucida,* and *Agaricus bisporus* (Daniel et al. 1994; Artolozaga et al. 1997; Volc et al. 1997). Glucose 1-oxidase activity has been reported in *P. chrysosporium* mycelium (Kelley and Reddy 1986, 1988) but appears to be less common than pyranose 2-oxidase (reviewed by Ander and Marzullo 1997).

In addition to the oxidases, extracellular H_2O_2 may also be generated by the oxidation of organic acids that are secreted by many white-rot fungi. Specifically, Mn(II)-dependent oxidation of glyoxylic and oxalic acids in *C. subvermispora* cultures generates H_2O_2 (Urzua et al. l995, 1998a,b; Aguilar et al. 1999).

Cellobiose dehydrogenase (CDH) is widely distributed among white-rot and brown-rot fungi, and may also play an important role in organopollutant degradation (Westermark and Eriksson 1974a,b, 1975; Henriksson et al. 2000a). The enzyme has two domains containing FAD or heme prosthetic groups, and these can be cleaved by *P. chrysosporium* proteases. CDH binds to cellulose

and oxidizes cellodextrins, mannodextrins, and lactose. Suitable electron acceptors include quinones, phenoxy radicals, and Fe^{3+}. (CDH can use O_2 as a poor electron acceptor, but it is still classified as a dehydrogenase, not an oxidase.)

The biological function of CDH is uncertain (Eriksson et al. 1990). One model suggests that CDH generates hydroxyl radicals by Fenton-type reactions [Fe(II) and H_2O_2 reactants] as described above (Kremer and Wood 1992; Mansfield et al. 1997; Henriksson et al. 2000b). The model is consistent with many of the properties of CDH and entails the concerted efforts of additional enzymes for efficient lignin depolymerization. In addition to Fe(II), the Fenton mechanism requires a system for generating extracellular H_2O_2, and enzymes such as GLOX may be involved. Possibly, the function(s) of CDH differ among species. This issue has been recently reviewed (Henriksson et al. 2000a).

III. Molecular Genetics

The molecular genetics of lignin-degrading fungi has advanced considerably over the past 15 years. *Phanerochaete chrysosporium* has emerged as the model system, in large part due to the development of experimental procedures. Methodologies for auxotroph production, recombination analysis, rapid DNA and RNA purification, pulsed-field electrophoretic separation of chromosomes, and **genetic transformation** by auxotroph complementation and by drug resistance markers have been described (Alic et al. 1989,1990, 1991, 1993; Alic 1990; reviewed by Cullen and Kersten 1996; Cullen 1997). Another auxotroph complementation system was recently reported (Zapanta et al. 1998). No other white-rot fungi provide a range of experimental tools comparable to *P. chrysosporium*, although genetic recombination analysis (Eichlerova and Homolka 1999; Larraya et al. 19994, pulsed field **electrophoretic karyotyping** (Larraya et al. 1999b), and genetic transformation (Yanai et al. 1996; Kim et al. 1999; Honda et al. 2000) are now available for *Pleuotus ostrentus.* Most recently, transformation of Trametes versicolor has been achieved using **phleomycin resistance** as a dominant selectable marker (Bartholomew et al. 2001). Virtually nothing is known of the molecular genetics of brown-rot fungi. The molecular biology of *P. chrysosporium* has been reviewed (Alic and Gold 1991; Pease and Tien 1991; Gold and Alic 1993; Cullen and Kersten 1996; Cullen 1997; Kirk and Cullen 1998).

A. Peroxidases

1. Gene Structure

Tien and Tu (1987) were first to clone and sequence a *P. chrysosporium* cDNA encoding a **LiP.** It is now clear that *P. chrysosporium* LiPs are encoded by a **family of at least ten closely related genes** that have been designated *lipA* through *lipJ* (reviewed by Cullen and Kersten 1996). One allele, *lipI2*, is transcriptionally inactivated by an unusual class II non-autonomous transposon (Gaskell et al. 1995). Residues believed essential to peroxidase activity are conserved (Tien and Tu 1987; Schalch et al. 1989). The gene-encoding isozyme H8, *lipA*, also features a putative propeptide (Schalch et al. 1989). A similar sequence was shown in the LiP2 gene of strain OGC101, and the proenzyme was identified as an in vitro translation product (Ritch et al. 1991). The *P. chrysosporium* clones contain 8–9short introns, and the positions of six of these are highly conserved (Brown et al. 1988; Schalch et al. 1989; Ritch and Gold 1992; Gold and Alic 1993; Stewart and Cullen 1999). The **crystal structure of LIP** is similar to the overall three-dimensional structure of **cytochrome c peroxidase** (CCP), even though sequence identity is only approximately 20% (Edwards et al. 1993; Poulos et al. 1993).

Several LiP genes have been characterized from other fungal species, including Trametes versicolor genes *LPGI/LPGIV* (Jonsson and Nyman 1992,1994; Johansson and Nyman 1996), *LPGII/LPGIII* (Jonsson and Nyman 1994; Johansson and Nyman 1996), *VLG1* (Black and Reddy 1991), *LPGVI* (Johansson and Nyman 1995), *Bjerkandera adusta* clone LPO-1 (Asada et al. 1992), and Phlebia radiata *lpg3* (Saloheimo et al. 1989). Further, PCR amplification of LiP-like sequences also suggests the existence of LiP genes in *Ceriporiopsis subvermispora* and *Phanerochaete sordida* (Rajakumar et al. 1996). These results appear to contradict numerous biochemical investigations that failed to detect LiP activity in *C. subvermispora* (Ruttimann et al. 1992; Orth et al. 1993) and *P. sordida* (Ruttimann-Johnson et al. 1994) cultures.

Multiple MnP genes have been sequenced from several white-rot fungi including *P.*

chrysosporium (Pease et al. 1989; Pribnow et al. 1989; Orth et al. 1994; Alic et al. 1997), *T. versicolor* MPGI (Johansson 1994), *T. versicolor PGVII* (Johansson and Nyman 1996), *C. subvermispora* (Lobos et al. 1998; Tello et al. 2000), and *P. ostreatus* (Asada et al. 1995a). The actual number of MnP genes in these or other species remains to be established. The N-terminal amino acid sequences of *P. chrysosporium* isozymes indicate the existence of at least two more genes (Datta et al. 1991; Pease and Tien 1992).

Multiple sequence alignments reveal features that help to distinguish MnP and LiP genes (reviewed by Cullen 1997). The crystal structure of MnP shows similarities with LiP. Experimental support for Asp203 in Mn^{2+} binding has been provided by site-directed mutagenesis of the residue (Kusters-van Someren et al. 1995). Consistent with its ability to oxidize Mn^{2+} and its Mn-independent activity on aromatic substrates, the gene encoding *Pleurotus eryngii* "Mn-independent" peroxidase features putative Mn-binding ligands (Ruiz-Duenas et al. 1999b). Analogous genes have not yet been identified in *P. chrysosporium.*

2. Genomic Organization

Genetic linkage in *P. chrysosporium* has been demonstrated by restriction fragment length polymorphisms (RFLPs; Raeder et al. 1989a,b) and later refined by PCR-based segregation analysis (Gaskell et al. 1994). Recombination frequencies are based on analyses of single basidiospore cultures, which are the homokaryotic products of meiosis. **Genetic maps** are entirely consistent with physical distances established by Southern blotting of CHEF gels (Gaskell et al. 1991; Covert et al. 1992a; Stewart et al. 1992; Kersten et al. 1995) and by walking in genomic libraries (Huoponen et al. 1990; Gaskell et al. 1991).

Gene clustering and chromosome length polymorphisms are common in *P. chrysosporium.* For example, *lipA, lipB, lipC, lipE, lipG, lipH, lipI,* and *lipJ* are clustered within 3 % recombination and hybridize to a 3.5/3.7mb chromosome pair on CHEF gel Southern blots (see Stewart and Cullen 1999 and refs. cited therein). In contrast, *lipD* and *lipF* are unlinked and hybridize to 4.8/4.4mb and 1.8/2.0mb pairs, respectively (Stewart et al. 1992; D'Souza et al. 1993). The gene encoding glyoxal oxidase, *glx,* hybridizes to a 3.7/3.9 mb pair (Kersten et al. 1995). These length dimorphisms are mitotically stable in strain BKM-F-1767, but the number and sizes of bands rearrange substantially following meiosis, presumably due to recombination. Simultaneous resolution of all bands has not been achieved under a single set of electrophoretic parameters (Gaskell et al. 1991; Covert et al. 1992b; D'Souza et al. 1993). Beyond *P. chrysosporium,* little is known of the organization of peroxidase genes, although a cluster containing two LiP- and one MnP-encoding genes has been described for *T. versicolor* (Johansson and Nyman1996).

Homologous chromosomes differing in electrophoretic mobility have also been observed in numerous eukaryotes such as *Plasmodium falciparum* (Corcoran et al. 1988), dozens of fungi (reviewed in Zolan 1995), and, most recently, in the related white-rot fungus *P. ostreatus* (Larraya et al. 1999a,b). Mechanism(s) giving rise to these **chromosome length polymorphisms** (CLPs) and their possible role in genetic variability are not well understood. Intrachromosomal recombination between repetitive sequences or unequal exchange between homologues could generate shortened chromosomes (Zolan 1995; Gray 2000). The presence of repetitive elements in *P. chrysosporium* (Gaskell et al. 1995), and their apparent restriction to a single allelic homologue, has some impact on length differences. However, recent sequence analyses showed no recombination between elements (Stewart et al. 2000).

3. Regulation

LiP genes are differentially transcribed and their **expression** is dramatically **modulated by culture conditions** (Holzbaur and Tien 1988; Stewart et al. 1992; Reiser et al. 1993; Janse et al. 1998; Vallim et al. 1998; Stewart and Cullen 1999). Although the *P. chrysosporium* LiP genes are tightly clustered and structurally similar, no clear relationship between genomic organization and transcriptional regulation has been observed (Stewart et al. 1992; Stewart and Cullen 1999). A report suggesting that nutrient nitrogen limitation regulates LiP expression post-translationally by heme processing (Johnston and Aust 1994) has been directly contradicted (Li et al. 1994).

Quantitative transcript analyses by competitive RT-PCR support a role for *P. chrysosporium* peroxidases in organopollutant-contaminated soils. Consistent with an MnP-dependent lipid peroxidation mechanism, depletion of high ionization potential PAHs generally coincided with increased transcripts of *mnp1, mnp2,* and *mnp3* as well as MnP activity (Bogan et al. 1996c). In con-

trast to mnps, the transcript patterns among LiP genes varied dramatically during anthracene transformation in soils (Bogan et al. 1996b). Comparison of anthracene and PCP-contaminated soils suggests differential regulation of LiP genes in response to the particular organopollutant (Lamar et al. 1995; Bogan et al. 1996b).

Manganese peroxidase production in *P. chrrysosporium* is generally **dependent on Mn concentration** (Bonnarme and Jeffries 1990; Brown et al. 1990, 1991). Differential regulation of MnP isozymes has been observed in response to media composition for *C. subvermispora* and *P. chrysosporium* (Pease and Tien 1992; Lobos et al. 1994). Transcriptional control of MnP expression is complex. Possible transcriptional control elements, particularly metal response elements (MREs), have been identified in 5'-untranslated regions (Godfrey et al. 1990,1994; Alic and Gold 1991; Brown et al. 1993; Alic et al. 1997). Consistent with the occurrence of upstream MREs, Gettemy et al. (1998) observed upregulation of *P. chrysosporium mnp1* and *mnp2* in response to Mn^{2+} concentration in defined media, but transcript levels of *mnp3* were unaffected. In contrast, *T. versicolor mnp2* does not contain any MREs but is dramatically upregulated in response to Mn (T. Johansson and D. Cullen, unpubl.). Differential display analysis of *Coriolus versicolor* cultures exposed to PCP identified upregulated genes encoding a heat shock protein, but not MnP genes (Iimura and Tatsumi 1997).

4. Heterologous Expression

Basic biochemical investigations have been hampered by low yields and purification difficulties associated with LiP/Mnp isozymes. As a result, considerable research effort has been expended on the development of **heterologous expression systems.** Baculovirus systems have been used to produce active recombinant MnP isozyme H4 (Pease et al. 1991) and LiP isozyme H8 (Johnson and Li 1991; Johnson et al. 1992; Lin et al. 1997). Yields are relatively low, but baculovirus production may be useful for experiments requiring limited quantities of recombinant protein, e.g., site-specific mutagenesis. Techniques have also been developed for recovering active *P. chrysosporium* isozymes MnP H4 (Whitwam et al. 1995; Whitwam and Tien 1996), LiP H8 (Doyle and Smith 1996), and LiP H2 (Nie et al. 1998) from *E. coli* inclusion bodies. *P. chrysosporium* MnP and LiP can also be produced in a **"homologous expression" system** in which mnp transcriptional control is placed under the glyceraldehyde-3-phosphate dehydrogenase promoter (Mayfield et al. 1994; Sollewijn Gelpke et al. 1999). The system temporally separates the recombinant protein from other peroxidases, and it has been successfully used in site-directed mutagenesis (Kusters-van Someren et al. 1995; Kishi et al. 1997). **Efficient secretion of fully active MnP isozyme** H4 has been achieved in *A. oryzae* (Stewart et al. 1996) and *A. niger* (Conesa et al. 2000). The recombinant enzyme has served mechanistic studies (Jensen et al. 1996; Kapich et al. 1999), and *Aspergillus* expression systems have been extended to peroxidases from *C. subvermispora* [Larrondo et al. 2001, #1418], *P. eryngii* (Ruiz-Duenas et al. 1999a), and *Geotrichym candidum* (Sugano et al. 2000). Aifa et al. (1999) has recently reported low-level secretion of *P. chrysosporium* LiP by Aspergillus niger.

B. Laccases

Laccases of white-rot fungi are also encoded by complex families of structurally related genes (Kojima et al. 1990; Saloheimo et al. 1991; Coll et al. 1993; Yaver and Golightly 1996; Yaver et al. 1996; Karahanian et al. 1998; Giardina et al. 1999; Temp et al. 1999). Although laccase activity has been demonstrated in *P. chrysosporium* cultures (Srinivasan et al. 1995; Dittmer et al. 1997; Rodriguez et al. 1997), the corresponding genes have not yet been isolated (D'Souza et al. 1996). **Laccase genes are often differentially regulated** and the patterns of regulation differ substantially between species (Wahleithmer et al. 1995; Yaver and Golightly 1996; Yaver et al. 1996; Smith et al. 1998; Palmieri et al. 2000). Little information is available on the genomic organization of laccases, but *Trametes villosa* pulsed field gels suggest the possible linkage of certain laccase genes (Yaver and Golightly 1996). Basidiomycete laccases have been expressed in several systems including *A. oryzae* (Yaver et al. 1999) and *Pichia pastoris* (Otterbein et al. 2000).

C. Peroxide-Generating Enzymes

Glyoxal oxidase (GLOX) of *P. chrysosporium* is encoded by a single gene with two alleles (Kersten and Cullen 1993; Kersten et al. 1995). The deduced amino acid sequence lacks clear homology with

any other known proteins (Kersten and Cullen 1993), although Bork and Doolittle (1994) have identified a 50-residue 'kelch' motif in a variety of database sequences including glyoxal oxidase and galactose oxidase. On the basis of catalytic similarities with *Dactylium dendroides* galactose oxidase, potential copper ligands were tentatively identified at Tyr135, Tyr377, His378, and His 471(Whittaker et al. 1996). The deduced amino acid sequences of allelic variants differ by a single residue (Lys308<<Thr308), which may explain the two isozyme forms observed on isoelectric focusing gels (Kersten and Kirk 1987; Kersten 1990). Southern blot analysis of CHEF gels and segregation analysis show that *glx* is unlinked to MnP or LiP genes (Gaskell et al. 1994; Orth et al. 1994; Kersten et al. 1995). The GLOX gene *(glx)* is transcriptionally regulated in *P. chrysosporium* (Kersten and Cullen 1993). Consistent with a close physiological relationship between GLOX and LiP, *glx* transcript appearance in defined media (Stewart et al. 1992; Kersten and Cullen 1993), soil (Bogan et al. 1996b) and in wood chips (Janse et al. 1998) is coincident with *lip* and *mnp.* Production of mutant GLOX in *Aspergillus nidulans* and *Pichia pastoris* has confirmed the identity of catalytic residues (Kersten et al. 1995; Whittaker et al. 1999).

Beyond GLOX, relatively little is known of the molecular genetics of white-rot oxidases. Although numerous studies implicate pyranose 2-oxidase in lignin degradation, the corresponding gene has only been isolated from *Coriolus versicolor* (Nishimura et al. 1996). The sequence shows weak similarity to a short region within the FAD domain of several cellobiose dehydrogenase genes. Glucose 1-oxidase genes have not been isolated from any basidiomycete. Martinez and coworkers have cloned and sequenced aryl **alcohol oxidase** (AAO) encoding genes from *Pleurotus pulmunarius* (Varela et al. 2000) and *Pleurotus eryngii* (GenBank accession No. AF064069). The predicted proteins are over 90% identical, and show substantial sequence similarity to *A. niger* glucose 1-oxidase (35% identity). Blast searches clearly show that the *P. pulmunarius* sequence is related to an array of fungal "oxidases'' including *P. pastoris* alcohol oxidase (24%), and the FAD domain of cellobiose dehydrogenases from *P. chrysosporium* (25%), *Trametes versicolor* (25%), and *Pyconporus cinnabarinus* (24%). We have recently cloned and partially sequenced cDNAs from *P. chrysosporium* colonized wood that exhibit striking similarity to these FAD oxidases and to GLOX (T. Johansson and D. Cullen, unpubl.). Taken together, these results suggest involvement of a wide range of sugar oxidoreductases, aryl alcohol oxidases, and glyoxal oxidase.

D. Cellobiose Dehydrogenase

Genes encoding CDH have been cloned from several fungi including the white-rot fungi *P. chrysosporium* (Rakes et al. 1995; Li et al. 1996), *T. versicolor* (Dumonceaux et al. 1998), and *P. cinnabarinus* (Moukha et al. 1999). Sequences are highly conserved. All share a common architecture with separate FAD, heme, and cellulose-binding domains (CBD), although the latter domain has no obvious structural similarity to functionally similar bacterial or fungal CBDs. Li et al. (1997) characterized both allelic variants of *P. chrysosporium cdh.* Northern blots show upregulation of *cdh* in cellulose-containing media (Li et al. 1996; Moukha et al. 1999), and competitive RT-PCR revealed transcripts in *P. chrysosporium* colonized wood (Vallim et al. 1998). Renganathan and coworkers (1985) temporally altered expression of *P. chrysosporium* CDH by fusing cdh with the promoter of the highly expressed glyceraldehyde-3-phosphate dehydrogenase gene (Li et al. 2000). No evidence for CDH gene multiplicity has been reported.

IV. Conclusions and Future Prospects

Experiments with purified enzymes, analysis of metabolites in whole cultures, and field trials demonstrate efficient degradation of a wide range of organopollutants by wood-decaying fungi. Oxidative mechanisms involving extracellular peroxidases and laccases have been repeatedly shown, although their relative importance in complex substrates such as organopollutant-contaminated soils remains uncertain. Fenton-type reactions and an array of membrane-bound and intracellular enzyme systems probably augment or supersede peroxidases and laccases in certain circumstances.

Establishing the role of specific genes and enzymes in degradation pathways requires ex-

perimental tools that have only recently become available. Biochemical analysis in cell-free systems is greatly facilitated by the increasing availability of pure recombinant enzymes. Quantitative analysis of transcripts and organopollutant transformations in complex substrates provides further evidence for the involvement of specific genes. A more direct experimental approach might be disruption or replacement of individual genes, and transformation systems are now available for *Phanerochaete chrysosporium, Pleurotus ostreatus,* and *Trametes versicolor.* Gene replacement through homologous recombination occurs at low frequency in *P. chrysosporium* (Alic et al. 1993), but the technique has not yet been brought to bear on questions of organopollutant degradation. *Agrobacterium*-mediated gene transfer systems may facilitate gene disruption experiments in related wood-decaying fungi (Covert et al. 2001 #1538; Chen et al. 2000 #531; de Groot et al. 1998 #1537).

Current genomics research offers significant opportunities for elucidating the roles and interactions of known genes and for identifying new and novel genes involved in degrading lignin and organopollutants. The US Department of Energy's Joint Genome Institute (JGI) has completed whole genome shotgun sequencing of *P. chrysosporium.* A draft assemb1y is current1y available for Blast searching at <url>http://www.jgi.doe.gov/programs/whiterot/whiterot_mainpage.html</url>. Our laboratory and the JGI are collaborating on sequencing ESTs from *P. chrysosporium* colonized wood, and all sequences should be publicly available by 2002. Clearly, these efforts will reveal an enormous number of genes of potential relevance to organopollutant degradation. When coupled to transcript profiling, heterologous expression, and gene disruptions, these investigations will elucidate major degradation pathways, and answer fundamental questions concerning lower eukaryotic genome organization.

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References

Aguilar C. Urzua U, Koenig C, Vicuna R (1999) Oxalate oxidase from *Ceriporiopsis subvermispora:* biochemical and cytochemical studies. Arch Biochem Biophys 366:275-232

Aifa SM, Sayadi S, Gargouri A (1999) Heterologous expression of lignin peroxidase of *Phanerochaete chrysosporium* in *Aspergillus* niger. Biotechnol Lett 21:849-853

Aiken BS, Logan BE (1996) Degradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium* grown in ammonium lignosulphonate media. Biodegradation 7:175-182

Alic MM (1990) Mating system and DNA transformation of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Diss Abstr Int B 51:3681

Alic M, Gold M (1991) Genetics and molecular biology of the lignin-degrading Basidiomycete *Phanerochaete chrysosporium.* In: Bennett J, Lasure L (eds) More gene manipulations in fungi. Academic Press, New York, pp 319-341

Alic M, Akileswaran L, Gold M (1993) Gene replacement in the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Gene 136:307-311

Alic M, Akileswaran L, Gold M (1997) Characterization of a gene encoding manganese peroxidase isozyme 3 from *Phanerochaete chrysosporium.* Biochim Biophys Acta 1338:l-7

Alic M, Clark EK, Kornegay JR, Gold MH (1990) Transformation of *Phanerochaete chrysosporium* and *Neurospora crassa* with adenine biosynthetic genes from *Schizophyllum commune.* Curr Genet 17:305-311

Alic M, Kornegay JR, Pribnow D, Gold MH (1989) Transformation by complementation of an adenine auxotroph of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Appl Environ Microbiol 55:406-411

Alic M, Mayfield MB, Akileswaran L, Gold M (1991) Homologous transformation of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Curr Genet 19:491-494

Amitai G, Adani R, Sod-Moriah G, Rabinovitz I, Vincze A, Leader H, Chefetz B, Leibovitz-Persky L, Friesem D, Hadar Y (1998) Oxidative biodegradation of phosphorothiolates by fungal laccase. FEBS Lett 438: 195-200

Ander P, Marzullo L (1997) Sugar oxidoreductases and veratryl alcohol oxidase as related to lignin degradation. J Biotechnol 53:115-131

Armenante PM, Pal N, Lewandowski G (1994) Role of mycelium and extracellular protein in the biodegradation of 2,4,6-trichlorophenol by *Phanerochaete chrysosporium.* Appl Environ Microbiol 60:1711-1718

Artolozaga MJ, Kubatova E, Volc J, Kalisz HM (1997) Pyranose 2-oxidase from *Phanerochaete chrysosporium* - further biochemical characterisation. Appl Microbiol Biotechnol 47:508-514

Asada Y, Kimura Y, Oka T, Kuwahara M (1992) Characterization of a cDNA and gene encoding a lignin peroxidase from the lignin-degrading Basidiomycete *Bjerkandera adusta.* In: Kuwahara M, Shimada M (eds) Biotechnology in the pulp and paper industry. University Publishers, Tokyo, pp 421-426

Asada Y, Watanabe A, Irie T, Nakayama T, Kuwahara M (1995a) Structures of genomic and complementary DNAs coding for *Pleurotus ostreatus* manganese (II) peroxidase. Biochim Biophys Acta 1251:205-209

Asada Y, Watanabe A, Ohtsu Y, Kuwahara M (19951b) Purification and characterization of an aryl-alcohol oxidase from the lignin-degrading basidiomycete Phanerochaete chrysosporium. Biosci Biotech Biochem 59:1339-1341

Baldrian P, in Der Wiesche C, Gabriel J, Nerud F, Zadrazil F (2000) Influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil. Appl Environ Microbiol 66:2471-2478

Bao W, Fukushima Y, Jensen KA, Moen MA, Hammel KE (1994) Oxidative degradation of non-phenolic lignin during lipid peroxidation by fungal manganese peroxidase. FEBS Lett 354:297-300

Bartholomew K, Dos Santos G, Dumonceaux T, Charles T, Archibald F (2001) Genetic transformation of *Trametes versicoior* to phleomycin resistance with the dominant selectable marker shble. Appl Microbiol Biotechnol 56:201-204

Beaudette LA, Davies S, Fedorak PM, Ward OP, Pickard MA (1998) Comparison of gas chromatography and mineralization experiments for measuring loss of selected polychlorinated biphenyl congeners in cultures of white-rot fungi. Appl Environ Microbiol 64:2020-2025

Bezalel L, Hadar Y, Fu PP, Freeman JP, Cerniglia CE (1996) Metabolism of phenanthrene by the white rot fungus *Pleurotus ostreatus.* Appl Environ Microbiol 62: 2547-2553

Black AK, Reddy CA (1991) Cloning and characterization of a lignin peroxidase gene from the white-rot fungus Trametes versicolor. Biochem Biophys Res Comm 179:428-435

Blanchette R, Krueger E, Haight J, Akhtar M, Akin D (1997) Cell wall alterations in loblolly pine wood decayed by the white-rot fungus, *Ceriporiopsis subvermispora.* J Biotechnol 53:203-213

Bogan BW, Lamar RT (1996) Polycyclic aromatic hydrocarbon-degrading capabilities of *Phanerochaete laevis* HHB-1625 and its extracellular ligninolytic enzymes Appl Environ Microbiol 62:1597-1603

Bogan B, Lamar R, Hammel K (1996a) Fluorene oxidation in vivo by Phanerochaete chrysosporium and in vitro during manganese peroxidase-dependent lipid peroxidation. Appl Environ Microbiol 621788-1792

Bogan B, Schoenike B, Lamar R, Cullen D (1996b) Expression of lip genes during growth in soil and oxidation of anthracene by Phanerochaete chrysosporium. Appl Environ Microbiol 62:3697-3703

Bogan B, Schoenike B, Lamar R, Cullen D (1996c) Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with Phanerochaete chrysosporium. Appl Environ Microbiol 622381-2386

Bonnarme P, Jeffries T (1990) Mn(II) regulation of lignin peroxidases and manganese-dependent peroxidase from lignin-degrading white-rot fungi. Appl Environ Microbiol 56:210-217

Bork P, Doolittle RS (1994) *Drosophila kelch* motif is derived from a common enzyme fold. J Mol Biol 236:1277-1282

Bourbonnais R, Paice MG, Freiermuth B, Bodie E, Borneman S (1997) Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. Appl Environ Microbiol 63:4627-4632

Bourbonnais R, Leech D, Paice MG (1998) Electrochemical analysis of the interactions of laccase mediators with lignin model compounds. Biochim Biophys Acta 1379:381-390

Brown A, Sims PFG, Raeder U, Broda P (1988) Multiple ligninase-related genes from *Phanerochaete chrysosporium.* Gene 73:57-85

Brown J, Alic M, Gold M (1991) Manganese peroxidase gene transcription in *Phanerochaete chrysosporium:* activation by manganese. J Bacterial 173:4101-4106

Brown J, Li D, Alic M, Gold M (1993) Heat shock induction of manganese peroxidase gene transcription in Phanerochaete chrysosporium. Appl Environ Microbiol 59:4295-4299

Brown JA, Glenn JK, Gold MH (1990) Manganese regulates expression of manganese peroxidase by *Phanerochaete chrysosporium.* J Bacteriol 172:3125-3130

Bumpus JA (1989) Biodegradation of polycyclic hydrocarbons by Phanerochaete chrysosporium. Appl Environ Microbiol 55:154-158

Bumpus JA, Aust SA (1987) Biodegradation of DDT by the white rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 53:2001-2007

Bumpus JA, Tien M, Wright D, Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434-1436

Camarero S, Sarkar S, Ruiz-Duenas FJ, Martinez MJ, Martinez AT (1999) Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. J Biol Chem 274:10324-10330

Cameron MD, Timofeevski S, Aust SD (2000) Enzymology of *Phanerochaete Chrysosporium* with respect to the degradation Of recalcitrant compound and xenobiotics. Appl Microbiol Biotechnol 54:751-758

Chen X, Stone M, Schlagnhaufer C, Romaine CF (2000) A fruiting body tissue method for efficient agrobacterium-mediated transformation of *Agaricus bisporus.* Appl Environ Microbiol 66:4510-4513

Coll P, Tabernero C, Santamaria R, Perez P (1993) Characterization and structural analysis of the laccase I gene from the newly isolated ligninolytic basidiomycete PM1 (CECT 2971). Appl Environ Microbiol 59: 4129-4135

Collins P, Kotterman M, Field J, Dobson A (1996) Oxidation of anthracene and benzo[a]pyrene by laccases from *Trametes versicolor.* Appl Environ Microbial 62: 4563-4567

Conesa A, van den Hondel CA, Punt PJ (2000) Studies on the production of fungal peroxidases in Aspergillus niger. Appl Environ Microbiol 66:3016-3023

Corcorall LM, Thompson JK, Walliker D, Kemp DJ (1988) Homologous recombination within subtelomeric repeat sequences generates chromosome size polymorphisms in *P. falciparum.* Cell 53:807-813

Covert S, Bolduc J, Cullen D (1992a) Genomic organization of a cellulase gene family in *Phanerochaete chrysosporium.* Curr Genet 22:407-413

Covert S, Vanden Wymelenberg A, Cullen D (1992b) Structure, organization and transcription of a cellobiohydrolase gene cluster from *Phanerochaete chrysosporium.* Appl Environ Microbiol 55:2168-2175

Covert SF, Kapoor P, Lee M-H, Briley A, Nairn CJ (2001) *Agrobacterium tumefaciens*-mediated transformation of *Fusarium circinatum.* Mycol Res 105:259-264

Cripps C, Bumpus JA, Aust SD (1990) Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium.* Appl Environ Microbiol 56:1114-1118

Cullen D (1997) Recent advances on the molecular genetics of ligninolytic fungi. J Biotechnol 53:273–289

Cullen D, Kersten PJ (1996) Enzymology and molecular biology of lignin degradation. In: Bramble R, Marzluf G (eds) The Mycota, vol III. Springer, Berlin Heidelberg New York, pp 297–314

Daniel G, Volc J, Kubatova E (1994) Pyranose oxidase, a major source of H_2O_2 during wood degradation by Phanerochaete chrysosporium, Trametes versicolor, and *Oudemansiella mucida.* Appl Environ Microbiol 60:2524–2532

Dass SB, Dosoretz C, Reddy CA, Grethlein H (1995) Extracellular proteases produced by the wood-degrading fungus *Phanerochaete chrysosporium* under ligninolytic and non-ligninolytic conditions. Arch Microbiol 163:254–258

Datta A (1992) Purification and characterization of a novel protease from solid substrate cultures of *Phanerochaete chrysosporium.* J Biol Chem 267:728–732

Datta A, Bettermann A, Kirk TK (1991) Identification of a specific manganese peroxidase among ligninolytic enzymes secreted by *Phanerochaete Chrysosporium* during wood decay. Appl Environ Microbiol 57:1453–1460

Davis MW, Glaser J, Evans J, Lamar RT (1993) Field evaluation of the lignin-degrading fungus *Phanerochaete sordida* to treat creosote-contaminated soil. Environ Sci Technol 27:2572–2576

de Groot MJ, Bundock P, Hooykaas PJ, Beijersbergen AG (1998) *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi [published erratum appears in Nat Biotechnol 1998 16:1074]. Nat Biotechnol 16:839-42

Dietrich D, Hickey WJ, Lamar R (1995) Degradation of 4,4'-dichlorobipheny-I,3,3',4,4'-tetrachlorobiphenyl, and 2,2',4,4',5,5'-hexachlorobiphenyl by the white rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 61:3904–3909

Dittmer J, Patel N, Dhawale S, Dhawale S (1997) Production of multiple laccase forms by *Phanerochaete chrysosporium* grown under nutrient sufficiency. FEMS Microbiol Lett 149:65–70

Dosoretz C, Dass B, Reddy CA, Grethlein H (1990a) Protease-mediated degradation of lignin peroxidase in liquid cultures of *Phanerochaete chrysosporium.* Appl Environ Microbiol 56:3429–3434

Dosoretz CD, Chen H-C, Grethlein HE (1990b) Effect of environmental conditions on extracellular protease activity in lignolytic cultures of *Phanerochaete chrysosporium.* Appl Environ Microbiol 56:395-400

Doyle WA, Smith AT (1996) Expression of lignin peroxidase H8 in *Escherichia coli:* folding and activation of the recombinant enzyme with Ca and haem. Biochem J 315:15–19

D'Souza TM, Dass SB, Rasooly A, Reddy CA (1993) Electrophoretic karyotyping of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Mol Microbiol 8:803–807

D'Souza TM, Boominathan K, Reddy CA (1996) Isolation of laccase gene-specific sequences from white-rot and brown-rot fungi by PCR. Appl Environ Microbiol 62: 3139–3744

Dumonceaux TJ, Bartholomew KA, Charles TC, Moukha SM, Archibald FS (1998) Cloning and sequencing of a gene encoding cellobiose dehydrogenase from *Trametes versicolor.* Gene 210:211–219

Eaton DC (1985) Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium.* Enzyme Microb Technol 7:194–196

Edwards S, Raag R, Wariishi H, Gold M, Poulos T (1993) Crystal structure of lignin peroxidase. Proc Natl Acad Sci USA 90:750–754

Eggert C, Temp U, Dean J, Eriksson K-E (1996) A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase. FEBS Lett 391:144–148

Eichlerova I, Homolka L (1999) Preparation and crossing of basidiospore-derived monokaryons - a useful tool for obtaining laccase and other ligninolytic enzyme higher-producing dikaryotic strains of *Pleurotus ostreatus.* Antonie Van Leeuwenhoek 75:321–327

Enoki M, Watanabe T, Nakagame S, Koller K, Messner K, Honda Y, Kuwahara M (1999) Extracellular lipid peroxidation of selective white-rot fungus, *Ceriporiopsis subvermispora.* FEMS Microbiol Lett 180:205–211

Eriksson K-E, Pettersson B (1982) Purification and partial characterization of two acidic proteases from the white rot fungus *Sporotrichium pulverulentum.* Eur J Biochem 124:635–642

Eriksson K-EL, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin Heidelberg New York

Fahr K, Wetzstein HG, Grey R, Schlosser D (1999) Degradation of 2,4-dichlorophenol and pentachlorophenol by two brown rot fungi. FEMS Microbiol Lett 175: 127–132

Farrell RL, Murtagh KE, Tien M, Mozuch MD, Kirk TK (1989) Physical and enzymatic properties of lignin peroxidase isozymes from *Phanerochaete chrysosporium.* Enzyme Microb Technol 11:322–328

Feijoo G, Rothschild N, Dosoretz C, Lema J (1995) Effects of addition of extracellular culture fluid on ligninolytic enzyme formation by *Phanerochaete chrysosporium.* J Biotechnol 40:21–29

Fernando T, Bumpus JA, Aust SD (1990) Biodegradation of TNT (2,4,6-trinitrotoluene) by *Phanerochaete chrysosporium.* Appl Environ Microbiol 56:1666–1671

Ferrey ML, Koskinen WC, Blanchette RA, Burnes TA (1994) Mineralization of alachlor by lignin-degrading fungi. Can J Microbiol 40:795–798

Field JA, de Jong E, Feijoo Costa G, de Bont JA (1992) Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. Appl Environ Microbiol 58:2219–2226

Fukushima Y, Kirk TK (1995) Laccase component of the *Ceriporiopsis subvermispora* lignin-degrading system. Appl Environ Microbiol 61:872-876

Garon D, Krivobok S, Seigle-Murandi F (2000) Fungal degradation of fluorene. Chemosphere 40:91–97

Gaskell J, Dieperink E, Cullen D (1991) Genomic organization of lignin peroxidase genes of *Phanerochaete chrysosporium.* Nucleic Acids Res 19:599-503

Gaskell J, Stewart P, Kersten P, Covert S, Reiser J, Cullen D (1994) Establishment of genetic linkage by allele-specific polymerase chain reaction: application to the lignin peroxidase gene family of *Phanerochaete chrysosporium.* Bio/Technology 12:1372–1375

Gaskell J, Vanden Wymelenberg A, Cullen D (1995) Structure, inheritance, and transcriptional effects of Pce1, an insertional element within *Phanerochaete chrysosporium* lignin peroxidase gene *lipI.* Proc Natl Acad Sci USA 92:1465–7469

George EJ, Neufield RD (1989) Degradation of fluorene in soil by fungus *Phanerochaete chrysosporium.* Biotechnol Bioengin 33:1306–1310

Gettemy JM, Ma B. Alic M, Gold MH (1998) Reverse transcription-PCR analysis of the regulation of the manganese peroxidase gene family. Appl Environ Microbiol 64:569–574

Giardina P, Palmieri G, Scaloni A, Fontanella B, Faraco V, Cennamo G, Sannia G (1999) Protein and gene structure of a blue laccase from *Pleurotus ostreatus.* Biochem J 341:655-663

Glenn JK, Morgan MA, Mayfield MB, Kuwahara M, Gold MH (1983) An extracellular H_2O_2-requiring enzyme preparation involved in lignin biodegradation by the white-rot basidiomycete *Phanerochaete chrysosporium.* Biochem Biophys Res Comm 114:1077–1083

Glenn JK, Akileswaran L, Gold MH (1986) Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete Chrysosporium.* Arch Biochem Biophys 251:688-696

Glumoff T, Harvey PJ, Molinari S, Goble M, Frank G, Palmer JM, Smit JDG, Leisola MSA (1990) Lignin peroxidase from *Phanerochaete chrysosporium:* molecular and kinetic characterization of isozymes. Eur J Biochem 187:515–520

Godfrey BJ, Mayfield MB, Brown JA, Gold M (1990) Characterization of a gene encoding a manganese peroxidase from *Phanerochaete chrysosporium.* Gene 93:119-124

Godfrey B, Akileswaran L, Gold M (1994) A reporter gene construct for studying the regulation of manganese peroxidase gene expression. Appl Environ Microbiol 60:1353–1358.

Gold M, Alic M (1993) Molecular biology of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Microbiol Rev 57:605-622

Gold MH, Kuwahara M, Chiu AA, Glenn JK (1984) Purification and characterization of an extracellular H_2O_2-requiring diarylpropane oxygenase from the white rot basidiomycete, *Phanerochaete chrysosporium.* Arch Biochem Biophys 234:353–362

Gray YH (2000) It takes two transposons to tango: transposable-element-mediated chromosomal rearrangements. Trends Genet 16:461-468

Hammel KE (1995a) Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. Environ Health Perspect 103:Suppl 5:41-43

Hammel KE (1995b) Organopollutant degradation by fungi. In: Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss, New York, pp 331–346

Hammel KE (1996) Fungal degradation of lignin. In: Gadisch G, Giller G (eds) Driven by nature: plant litter quality and decomposition, CAB International, Wallingford, Oxon, pp 33-45

Hammel KE, Tardone PJ (1988) The oxidative 4-dechlorination of polychlorinated phenols is catalyzed by extracellular fungal lignin peroxidases. Biochemistry 27:6563-6568

Hammel KE, Green B, Gai W (1991) Ring fission of anthracene by a eukaryote. Proc Natl Acad Sci USA 88:10605-10508

Hammel KE, Gai WZ, Green B, Moen MA (1992) Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 58:1832–1838

Hammel KE, Kalyanaraman B, Kirk TK (1986a) Oxidation of polycyclic hydrocarbons and dibenzo[p]dioxins by *Phanerochaete chrysosporium* ligninase. J Biol Chem 261:16948-16952

Hammel KE, Kalyanaraman B, Kirk TK (1986b) Substrate free radicals are intermediates in ligninase catalysis. Proc Natl Acad Sci USA 83:3808–3812

Harvey PJ, Schoemaker HE, Bowen RM, Palmer JM (1985) Single-electron transfer processes and the reaction mechanism of enzymic degradation of lignin. FEBS Lett 183:13–16

Harvey PJ, Schoemaker HE, Palmer JM (1986) Veratryl alcohol as a mediator and the role of radical cations in lignin biodegradation by *Phanerochaete chrysosporium.* FEBS Lett 195:242–246

Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. FEMS Microbiol Rev 13:125–135

Heinfling A, Martinez MJ, Martinez AT, Bergbauer M, Szewzyk U (1998) Transformation of industrial dyes by manganese peroxidases from *Bjerkandera adusta* and *Pleurotus eryngii* in a manganese-independent reaction. Appl Environ Microbiol 64: 2788–2793

Henriksson G, Johansson G, Pettersson G (2000a) A critical review of cellobiose dehydrogenases J Biotechnol 7893–113

Henriksson G, Zhang L, Li J, Ljungquist P, Reitberger T, Pettersson G, Johansson G (2000b) Is cellobiose dehydrogenase from *Phanerochaete chrysosporium* a lignin degrading enzyme? Biochim Biophys Acta 1480:83–91

Higson FK (1991) Degradation of xenobiotics by white rot fungi. Rev Environ Contam Toxicol 122:111–152

Higuchi T (1990) Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 2423–63

Higuchi T (1993) Biodegradation mechanism of lignin by white-rot basidiomycetes. J Biotechnol 30:1-8

Hodgson J, Rho D, Guiot SR, Ampleman G, Thiboutot S, Hawari J (2000) Tween 80 enhanced TNT mineralization by *Phanerochaete chrysosporium.* Can J Microbiol 46:110–118

Holzbaur E, Tien M (1988) Structure and regulation of a lignin peroxidase gene from *Phanerochaete chrysosporim.* Biochem Biophys Res Commun 155:626-633

Honda Y, Matsuyama T, Irie T, Watanabe T, Kuwahara M (2000) Carboxin resistance transformation of the homobasidiomycete fungus *Pleurotus ostreatus.* Curr Genet 37:209–212

Hundt K, Martin D, Hammer E, Jonas U, Kindermann MK, Schauer F (2000) Transformation of triclosan by *Trametes versicolor* and *Pycnoporus cinnabarinus.* Appl Environ Microbiol. 66:4157-4160

Huoponen K, Ollikka P, Kalin M, Walther I, Mantsala P, Reiser J (1990) Characterisation of lignin peroxidase-encoding genes from lignin-degrading basidiomycetes. Gene 89:145–150

Hyde SM, Wood PM (1997) A mechanism for production of hydroxyl radicals by the brown rot fungus *Coniophora putenna:* Fe(III) reduction by cellobiose dehydrogenase and Fe(II) oxidation at a distance from the hyphae. Microbiology 143:259–266

Ichinose H, Wariishi H, Tanaka H (1999) Bioconversion of recalcitrant 4-methyldibenzothiophene to water-extractable products using lignin-degrading basidiomycete *Coriolus versicolor.* Biotechnol Prog 15:706–714

Iimura Y, Tatsuini K (1997) Isolation of mRNAs induced by a hazardous chemical in white-rot fungus, *Coriolus versicolor,* by differential display. FEBS Lett 412:370–374

Janse B, Gaskell J, Ahktar M, Cullen D (1998) *Phanerochaete chrysosporium* genes encoding lignin peroxidases, manganese peroxidases and glyoxal oxidase in wood. Appl Environ Microbiol 64:3536–3538

Jensen K, Bao W, Kawai S, Srebotnik E, Hammel K (1996) Manganese-dependent cleavage of nonphenolic lignin structures by *Ceriporiopsis subvermispora* in the absence of lignin peroxidase. Appl Environ Microbiol 62:3679–3686

Johannes C, Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl Environ Microbiol 66:524–528

Johannes C, Majcherczyk A, Huttermann A (1996) Degradation of anthracene by laccase of Trametes versicolor in the presence of different mediator compounds. Appl Microbiol Biotechnol 46:313–317

Johansson T (1994) Manganese(II) peroxidase and lignin peroxidase from the white-rot fungus Trametes versicolor. PhD Thesis, University of Lund, Lund

Johansson T, Nyman PO (1995) The gene from the white-rot fungus Trametes versicolor encoding the lignin peroxidase isozyme LP7. Biochim Biophys Acta 1263: 71–74

Johansson T, Nyman P (1996) A cluster of genes encoding major isozymes of lignin peroxidase and manganese peroxidase from the white-rot fungus *Trametes versicolor.* Gene 17031–38

Johjima T, Itoh N, Kabuto M, Tokimura F, Nakagawa T, Wariishi H, Tanaka H (1999) Direct interaction of lignin and lignin peroxidase from *Phanerochaete chrysosporium.* Proc Natl Acad Sci USA 96:1989–1994

Johnson T, Pease E, Li J, Ten M (1992) Production and characterization of recombinant lignin peroxidase isozyme H2 from *Phanerochaete chrysosporium* using recombinant baculovirus. Arch Biochem Biophys 296:660-666

Johnson TM, Li JK (1991) Heterologous expression and characterization of an active lignin peroxidase from *Phanerochaete chrysosporium* using recombinant baculovirus. Arch Biochem Biophys 291:371–378

Johnston CG, Aust SD (1994) Transcription of ligninase H8 by *Phanerochaete chrysosporium* under nutrient nitrogen sufficient conditions. Biochem Biophys Res Commun 200:108–112

Jonsson L, Nyman P (1992) Characterization of a lignin peroxidase gene from the white-rot fungus *Trametes versicolor*. Biochimie 74:177–182

Jonsson L, Nyman P (1994) Tandem lignin peroxidase genes from the fungus *Trametes versicolor*. Biochim Biophys Acta 218:408-412

Joshi D, Gold M (1993) Degradation of 2,4,5-trichlorophenol by the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Appl Environ Microbiol 59:1779–1785

Kapdan IK, Kargia F, McMullan G, Marchant R (2000) Effect of environmental conditions on biological decolorization of textile dyestuff by *C. versicolor.* Enzyme Microb Technol 26:3381–387

Kapich AN, Jensen KA, Hammel KE (1999) Peroxyl radicals are potential agents of lignin biodegradation. FEBS Lett 461:115–119

Karahanian E, Corsini G, Lobos S, Vicuna R (1998) Structure and expression of a laccase gene from the lignin-olytic basidiomycete *Ceriporiopsis subvermispora.* Biochim Biophys Acta 1443:65–74

Kawai S, Umezawa T, Higuchi T (1988) Degradation mechanisms of phenolic β-1 lignin substructure and model compounds by laccase of *Coriolus versicolor.* Arch Biochem Biophys 262:99–110

Kelley RL, Reddy CA (1986) Purification and characterization of glucose oxidase from ligninolytic cultures of *Phanerochaete chrysosporium.* J Bacteriol 166:269–274

Kelley RL, Reddy CA (1988) Glucose oxidase of *Phanerochaete chrysosporium.* Methods Enzymol 161:307–316

Kennedy DW, Aust SD, Bumpus JA (1990) Comparative biodegradation of alkyl halide insecticides by the white rot fungus, *Phanerochaete chrysosporium* (BKM-F-1767). Appl Environ Microbiol 56:2347–2353

Kerem Z, Hammel KE (1999) Biodegradative mechanism of the brown rot basidiomycete *Gloeophyllum trabeum:* evidence for an extracellular hydroquinone-driven fenton reaction. FEBS Lett 446:49–54

Kerem Z, Bao W, Hammel KE (1998) Rapid polyether cleavage via extracellular one-electron oxidation by a brown-rot basidiomycete. Proc Natl Acad Sci USA 95:10373–10377

Kersten P, Cullen D (1993) Cloning and characterization of a cDNA encoding glyoxal oxidase, a peroxide-producing enzyme from the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Proc Natl Acad Sci USA 90:7411–7413

Kersten PJ (1990) Glyoxal oxidase of *Phanerochaete chrysosporium;* its characterization and activation by lignin peroxidase. Proc Natl Acad Sci USA 87: 2936–2940

Kersten PJ, Kirk TK (1987) Involvement of a new enzyme, glyoxal oxidase, in extracellular H_2O_2 production by *Phanerochaete chrysosporium.* J Bacteriol 169:2195–2201

Kersten PJ, Tien M, Kalyanaraman B, Kirk TK (1985) The ligninase of *Phanerochaete chrysosporium* generates cation radicals from methoxybenzenes. J Biol Chem 260:2609–2612

Kersten PJ, Witek C, Vanden Wymelenberg A, Cullen D (1995) *Phanerochaete chrysosporium* glyoxal oxidase is encoded by two allelic variants: structure, genomic organization and heterologous expression of *glx1* and *glx2.* J Bacteriol 177:6106-6110

KhadraniA,Seigle-MurandiF,Steiman R, Vroumsia T (1999) Degradation of three phenylurea herbicides (chlortoluron, isoproturon and diuron) by micromycetes isolated from soil. Chemosphere 38:3041–3050

Khindaria A, Grover TA, Aust S (1995) Evidence for formation of the veratryl alcohol cation radical by lignin peroxidase. Biochemistry 34:6020-6025

Kim HY, Song HG (2000) Comparison of 2,4,6-trinitrotoluene degradation by seven strains of white rot fungi. Curr Microbiol 41:317–320

Kim BG, Magae Y, Yoo YB, Kwon ST (1999) Isolation and transformation of uracil auxotrophs of the edible basidiomycete *Pleurotus ostreatus.* FEMS Microbiol Lett 181:225–228.

Kirk TK, Cullen D (1998) Enzymology and molecular genetics of wood degradation by white-rot fungi. In: Young RA, Akhtar M (eds) Environmentally friendly technologies for the pulp and paper industry. Wiley, New York, pp 273–308

Kirk TK, Farrell RL (1987) Enzymatic "combustion": the microbial degradation of lignin. Ann Rev Microbiol 41:465–505

Kirk TK, Croan S, Tien M, Murtagh KE, Farrell RL (1986) Production of multiple ligninases by *Phanerochaete chrysosporium:* effect of selected growth conditions and use of a mutant strain. Enzyme Microb Technol 8:27–32

Kishi K, Hildebrand D, Kuster-van Someren M, Gettemy J, Mauk A, Gold M (1997) Site-directed mutations at phenylalanine-190 of manganese peroxidase: effects on stability, function, and coordination. Biochemistry 36:4268-4277

Koenigs JW (1974a) Hydrogen peroxide and iron: a proposed system for decomposition of wood by brown rot basidiomycetes. Wood Fiber 6:66–79

Koenigs JW (1974b) Production of hydrogen peroxide by wood rotting fungi in wood and its correlation with weight loss, depolymerization and pH changes. Arch Microbiol 99:129–145

Kojima Y, Tsukuda Y, Kawai Y, Tsukamoto A, Sugiura J, Sakaino M, Kita Y (1990) Cloning, sequence analysis, and expression of ligninolytic phenoloxidase genes of the white-rot basidiomycete *Coriolus hirsutus.* J Biol Chem256:15224–15230

Kotterman MJ, Rietberg HJ, Hage A, Field JA (1998a) Polycyclic aromatic hydrocarbon oxidation by the white-rot fungus *Bjerkandera* sp. strain BOS55 in the presence of nonionic surfactants. Biotechnol Bioeng 57:220–227

Kotterman MJ, Vis EH, Held JA (1998b) Successive mineralization and detoxification of benzo[a]pyrene by the white rot fungus *Bjerkandera* sp. strain BOS55 and indigenous microflora. Appl Environ Microbiol 64: 2853–2858

Kremer SM, Wood PM (1992) Production of Fenton's reagent by cellobiose oxidase from cellulolytic cultures of *Phanerochaete chrysosporium.* Eur J Biochem 208:807–814

Krivobok s, Miriouchkine E, Seigle-Murandi F, Benoit-Guyod JL (1998) Biodegradation of anthracene by soil fungi. Chemosphere 37:523–530

Kuan IC, Tien M (1989) Phosphorylation of lignin peroxidase from *Phanerochaete chrysosporium.* J Biol Chem 264:20350-20355

Kullman SW, Matsumura F (1996) Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of the cyclodiene pesticide endosulfan. Appl Environ Microbiol 62:593-600

Kusters-van Someren M, Kishi K, Ludell T, Gold M (1995) The manganese binding site of manganese peroxidase: characterization of an Aspl79Asn site-directed mutant protein. Biochemistry 34:10620–10627

Lamar R, Dietrich D (1990) In situ depletion of pentachlorophenol from contaminated soil by *Phanerochaete* spp. Appl Environ Microbiol 56:3093–3100

Lamar RT, Glaser JA, Kirk TK (1990a) Fate of pentachlorophenol (PCP) in sterile soils inoculated with the white-rot basidiomycete *Phanerochaete chrysosporium;* mineralization, volatilization and depletion of PCP. Soil Biol Biochem 22:433-440

Lamar RT, Larsen MJ, Kirk TK (1990b) Sensitivity to and degradation of pentachlorophenol by *Phanerochaete* spp. Appl Environ Microbiol 56:3519–3526

Lamar RT, Evans JE, Glaser JA (1993) Solid-phase treatment of a pentachlorophenol-contaminated soil using lignin-degrading fungi, Environ Sci Technol 27:2566–2571

Lamar RT, Davis MW, Dietrich D, Glaser JA (1994) Treatment of a pentachlorophenol- and creosote-contaminated soil using the lignin-degrading fungus *Phanerochaete chrysosporium:* a field demonstration. Soil Biol Biochem 26:1603–1611

Lamar RT, Schoenike B, Vanden Wymelenberg A, Stewart P, Dietrich DM, Cullen D (1995) Quantitation of fungal mRNAs in complex substrates by reverse transcription PCR and its application to *Phanerochaete chrysosporium*-colonized soil. Appl Environ Microbiol 61:2122–2126

Larraya L, Penas MM, Perez G, Santos C, Ritter E, Pisabarro AG, Ramirez L (1999a) Identification of incompatibility alleles and characterisation of molecular markers genetically linked to the A incompatibility locus in the white rot fungus *Pleurotus ostreatus.* Curr Genet 34:486-493

Larraya LM, Perez G, Penas MM, Baars JJ, Mikosch TS, Pisabarro AG, Ramirez L (1999b) Molecular karyotype of the white rot fungus *Pleurotus ostreatus.* Appl Environ Microbiol 65:3413–3417

Larrondo LF, Lobos S, Stewart P, Cullen D, Vicuna R (2001) Isozyme multiplicity and characterization of recombinant manganese peroxidases (rMnPs) from *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium.* Appl Environ Microbiol Appl Environ Microbiol 67:2070-2075

Leisola MSA, Kozulic B, Meusdoerffer F, Fiechter A (1987) Homology among multiple extracellular peroxidases from *Phanerochaete chrysosporium.* J Biol Chem 262:419424

Li B, Nagalla S, Renganathan V (1996) Cloning of a cDNA encoding cellobiose dehygrogenase, a hemoflavoenzyme from *Phanerochaete chrysosporium.* Appl Environ Microbiol 62:1329–1335

Li B, Nagalla S, Renganathan V (1997) Cellobiose dehydrogenase from *Phanerochaete chrysosporium* is encoded by two allelic variants. Appl Environ Microbiol 63:796–799

Li B, Rotsaert FA, Gold MH, Renganathan V (2000) Homologous expression of recombinant cellobiose dehydrogenase in *Phanerochaete chrysosporium.* Biochem Biophys Res Commun 270:141–146

Li D, Alic M, Gold M (1994) Nitrogen regulation of lignin peroxidase gene transcription. Appl Environ Microbiol 60:3447–3449

Lin Q, Li JK, Lam HY (1997) Improved heterologous expression of the white-rot fungal ligninase H8 by crossover linker mutagenesis. Appl Biochem Biotechnol 66:269–279

Lobos S, Larrain J, Salas L, Cullen D, Vicuna R (1994) Isozymes of manganese-dependent peroxidase and laccase produced by the lignin-degrading basidiomycete *Ceriporiopsis subvermispora.* Microbiology 140:12691–2698

Lobos S, Larrondo L, Salas L, Karahanian E, Vicuna R (1998) Cloning and molecular analysis of a cDNA and the Cs-mnp1 gene encoding a manganese peroxidase isoenzyme from the lignin-degrading basidiomycete *Ceriporiopsis subvermispora.* Gene 206:185–193

Majcherczyk A, Johannes C, Huttermann A (1988) Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of *Trametes versicolor.* Enzyme Microb Technol 22:335–341

Mansfield SD, de Jong E, Saddler JN (1997) Cellobiose dehydrogenase, an active agent in cellulose depolymerization. Appl Environ Microbiol 63:3804–3809

Martens R, Zadrazil F (1998) Screening of white-rot fungi for their ability to mineralize polycyclic aromatic hydrocarbons in soil. Folia Microbiol 43:97–103

Maspahy S, Lamb DC, Kelly SL (1999) Purification and characterization of a benzo[a]pyrene hydroxylase from *Pleurotus pulmonarius.* Biochem Biophys Res Commun 266:326–329

May RG, Schroder I, Hogue E, Sandermann H (1997) An ex-situ process for treating PAH-contaminated soil with *Phanerchaete chrysosporium.* Environ Sci Technol 31:2626–2633

Mayfield MB, Katsuyuki K, Alic M, Gold MH (1994) Homologous expression of recombinant manganese peroxidase in *Phanerochaete chrysosporium.* Appl Environ Microbiol 60:4303-4309

Mater T, Field JA (1998) Characterization of a novel manganese peroxidase-lignin peroxidase hybrid isozyme produced by *Bjerkandera* species strain BOS55 in the absence of manganese. J Biol Chem 273:15412–15417

Mileski GJ, Bumpus JA, Jurek MA, Aust SD (1988) Biodegradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 542885–2889

Moen M, Hammel K (1994) Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl Environ Microbiol 60:1956–1961

Moukha SM, Dumonceaux TJ, Record E, Archibald FS (1999) Cloning and analysis of *Pycnoporus cinnabarinus* cellobiose dehydrogenase. Gene 234:23–33

Muheim A, Leisola MSA, Schoemaker HE (1990) Aryl-alcohol-oxidase and lignin-peroxidase from the white-rot fungus *Bjerkandera adusta:* comparison with Phanerochaete chrysosporium lignin-peroxidase for reactivity with veratryl alcohol, homoveratric acid and alpha-benzyl veratryl alcohol. J Biotechnol 13:159–167

Nie G, Reading NS, Aust SD (1998) Expression of the lignin peroxidase H2 gene from *Phanerochaete chrysosporium* in *Escherichia coli.* Biochem Biophys Res Commun 249:146–150

Nishimura I, Okada K, Koyama Y (1996) Cloning and expression of pyranose oxidase cDNA from *Coriolus versicolor* in *E. coli.* J Biotechnol 52:11–20

Novotny C, Vyas BR, Erbanova P, Kubatova A, Sasek V (1997) Removal of PCBs by various white rot fungi in liquid cultures. Folia Microbiol 42:136–140

Novotny C, Erbanova P, Sasek V, Kubatova A, Cajthaml T, Lang E, Krahl J, Zadrazil F (1999) Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi. Biodegradation 10:159–168

Orth A, Royse D, Tien M (1993) Ubiquity of lignin-degrading peroxidases among various wood-degrading fungi. Appl Environ Microbiol 59:4017-4023

Orth A, Rzhetskaya M, Cullen D, Tien M (1994) Characterization of a cDNA encoding a manganese peroxidase from *Phanerochaete chrysosporium:* genomic organization of lignin and manganese peroxidase genes. Gene 148:161–165

Otterbein L, Record E, Longhi S, Asther M, Moukha S (2000) Molecular cloning of the cDNA encoding laccase from *Pycnoporus cinnabarinus* 1-937 and expression in *Pichia pastoris.* Eur J Biochem 267: 1619–1625

Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus.* Appl Environ Microbiol 66:920–924

Paszczynski A, Huynh V-B, Crawford RL (1986) Comparison of ligninase-I and peroxidase-M2 from the white-rot fungus *Phanerochaete chrysosporium.* Arch Biochem Biophys 244:750–765

Paszczynski A, Crawford R, Funk D, Goodell B (1999) De novo synthesis of 4,5-dimethoxycatechol and 2,5-dimethoxyhydroquinone by the brown rot fungus *gloeophyllum trabeum.* Appl Environ Microbiol 65:674–679

Pease EA, Tien M (1991) Lignin-degrading enzymes from the filamentous fungus *Phanerochaete chrysosporium.* In: Dordick JS (ed) Biocatalysts for industry. Plenum Press, New York, pp 115–135

Pease EA, Tien M (1992) Heterogeneity and regulation of manganese peroxidases from Phanerochaete chrysosporium. J Bacteriol 174:3532–3540

Pease EA, Andrawis A, Tien M (1989) Manganese-dependent peroxidase from *Phanerochaete chrysosporium.* Primary structure deduced from complementary DNA sequence. J Biol Chem 264:13531–13535

Pease EA, Aust SD, Tien M (1991) Heterologous expression of active manganese peroxidase from *Phanerochaete chrysosporium* using the baculovirus expression system. Biochem Biophys Res Commun 179:897–903

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

Perk FH, Reddy GV, Blackburn NJ, Gold MH (1998) Purification and characterization of laccases from the white-rot basidiomycete *Dichomitus squalens.* Arch Biochem Biophys 353:349–355

Pickard MA, Roman R, Tinoco R, Vazquez-Duhalt R (1999) Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by *Coriolopsis gallica* UAMH 8260 laccase. Appl Environ Microbiol 65:3805–3809

Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–33

Poulos T, Edward S, Wariishi H, Gold M (1993) Crystallographic refinement of lignin peroxidase at 2A. J Biol Chem 268:4429-4440

Pribnow D, Mayfield MB, Nipper VJ, Brown JA, Gold MH (1989) Characterization of a cDNA encoding a manganese peroxidase, from the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* J Biol Chem 264:5036–5040

Raeder U, Thompson W, Broda P (1989a) Genetic factors influencing lignin peroxidase activity in *Phanerochaete chrysosporium* ME446. Mol Microbiol 3:919–924

Raeder U, Thompson W, Broda P (1989b) RFLP-based genetic map of *Phanerochaete chrysosporium* ME446: lignin peroxidase genes occur in clusters. Mol Microbiol 3:911–918

Raices M, Paifer E, Cremata J, Montesino R, Stahlberg J, Divne C, Szabo I, Henriksson G, Johansson G, Pettersson G (1995) Cloning and characterization of a cDNA encoding a cellobiose dehydrogenase from the white rot fungus *Phanerochaete chrysosporium.* FEBS Lett 369:233–238

Rajakumar S, Gaskell J, Cullen D, Lobos S, Karahanian E, Vicuna R (1996) *lip*-like genes in *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora*, white-rot fungi from which lignin peroxidase has not been detected. Appl Environ Microbiol 62:2660–2663

Reddy GV, Gold MH (1999) A two-component tetrachlorohydroquinone reductive dehalogenase system from the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Biochem Biophys Res Commun 257:901–905

Reddy GVB, Gold MH (2000) Degradation of pentachlorophenol by *Phanerochaete chrysosporium:* intermediates and reactions involved. Microbiology 146:405-413

Reddy GV, Gelpke MD, Gold MH (1998) Degradation of 2,4,6-trichlorophenoI by *Phanerochaete chrysosporium:* involvement of reductive dechlorination. J Bacteriol 180:5159–5164

Reiser J, Walther I, Fraefel C, Fiechter A (1993) Methods to investigate the expression of lignin peroxidase genes by the white-rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 592897–2903

Renganathan V, Miki K, Gold MH (1985) Multiple molecular form of diarylpropane oxygenase, an H_2O_2-requiring, lignin-degrading enzyme from *Phanerochaete chrysosporium.* Arch Biochem Biophys 241:304–314

Ritch TG, Gold MH (1992) Characterization of a highly expressed lignin peroxidase-encoding gene from the basidiomycete *Phanerochaete chrysosporium.* Gene 118:73-80

Ritch TG, Nipper NV, Akileswaran L, Smith AJ, Pribnow DG, Gold MH (1991) Lignin peroxidase from the basidiomycete *Phanerochaete chrysosporium* is synthesized as a preproenzyme. Gene 107:119–126

Rodriguez CS, Santro R, Cameselle C, Sanroman A (1997) Laccase production in semi-solid cultures of *Phanerochaete chrysosporium.* Biotechnol Lett 19:995–998

Rodriguez E, Pickard MA, Vazquez-Duhalt R (1999) Industrial dye decolorization by laccases from ligninolytic fungi. Curr Microbiol 38:27–32

Rothschild N, Hadar Y, Dosoretz C (1997) Lignin peroxidase isozymes from *Phanerochaete chrysosporium* can be enzymatically dephosphorylated. Appl Environ Microbiol 63:857–861

Rothschild N, Levkowitz A, Hadar Y, Dosoretz C (1999) Extracellular mannose-6-phosphatase of *Phanerochaete chrysosporium:* a lignin peroxidase-modifying enzyme. Arch Biochem Biophys 372107–111

Ruiz-Duenas FJ, Martinez MJ, Martinez AT (1999a) Heterologous expression of *Pleurotus eryngii* peroxidase confirms its ability to oxidize Mn(2+) and different aromatic substrates. Appl Environ Microbiol 65:4705–4707

Ruiz-Duenas FJ, Martinez MJ, Martinez AT (1999b) Molecular characterization of a novel peroxidase isolated from the ligninolytic fungus *Pleurotus eryngii.* Mol Microbiol 31:223–235

Ruttimann C, Schwember E, Salas L, Cullen D, Vicuna R (1992) Ligninolytic enzymes of the white rot basidiomycetes *Phlebia brevispora* and *Ceriporiopsis subvermispora.* Biotechnol Appl Biochem 16:64–76

Ruttimann-Johnson C, Salas L, Vicuna R, Kirk K (1993) Extraoeilular enzyme production and synthetic lignin mineralization by *Ceriporiopsis subvermispora.* Appl Environ Microbiol 59:1792–1797

Ruttimann-Johnson C, Cullen D, Lamar R (1994) Manganese peroxidases of the white-rot fungus *Phanerochaete sordida.* Appl Environ Microbiol 60: 599–605

Sack U, Fritsche W (1997) Enhancement of pyrene mineralization in soil by wood decaying fungi. FEMS Microbiol Ecol 22:77–83

Sack U, GuntherT (1993) Metabolism of PAH by fungi and correlation with extracellular enzymatic activities. J Basic Microbiol 33:269–277

Sack U, Heinze TM, Deck J, Cerniglia CE, Martens R, Zadrazil F, Fritsche W (1997a) Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. Appl Environ Microbiol 63:3919–3925

Sack U, Hofrichter M, Fritsche W (1997b) Degradation of phenanthrene and pyrene by Nematolorna frowardii. J Basic Microbiol 37:287–293

Sack U, Hofrichter M, Fritsche W (1997c) Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of Nematoloma frowardii. EMS Microbiol Lett 152:227–234

Salas C, Lobos S, Larrain J, Salas L, Cullen D, Vicuna R (1995) Properties of laccase isoenzymes produced by the basidiomycete *Ceriporiopsis subvermispora.* Biotechnol Appl Biochem 21:323—333

Saloheimo M, Barajas V, Nikupaavola ML, Knowles JKC (1989) A lignin peroxidase-encoding cDNA from the white-rot fungus *Phlebia radiata* - characterization and expression in *Trichoderma reesei.* Gene 85:343–351

Saloheimo M, Niku-Paavola M, Knowles JKC (1991) Isolation and structural analysis of the laccase gene from the lignin-degrading fungus *Phlebia radiata.* J Gen Microbiol 137:537–1544

Sandermann H Jr, Heller W, Hertkorn N, Hoque E, Pieper D, Winkler R (1998) A new intermediate in the mineralization of 3,4-dichloroaniline by the white rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 64:3305–3312

Sannia G, Limongi P, Cocca E, Buonocore F, Nitti S, Giardina P (1991) Purification and characterization of a veratryl alcohol oxidase enzyme from the lignin degrading basidiomycete *Pleurotus ostreatus.* Biochim Biophys Acta 1073:114—119

Schalch H, Gaskell J, Smith TL, Cullen D (1989) Molecular cloning and sequences of lignin peroxidase genes of *Phanerochaete chrysosporium.* Mol Cell Biol 9:2743–2747

Scheibner K, Hofrichter M (1998) Conversion of aminonitrotoluenes by fungal manganese peroxidase. J Basic Microbiol 3851–59

Schlosser D, Fahr K, Karl W, Wetzstein HG (2000) Hydroxylated metabolites of 2,4-dichlorophenol imply a Fenton-type reaction in *Gloeophyllum striatum.* Appl Environ Microbiol 66:2479–2483

Shoemaker HE, Harvey PJ, Bowen RM, Palmer JM (1985) On the mechanism of enzymatic lignin breakdown. FEBS Lett 183:7–12

Singh BK, Kuhad RC (1999) Biodegradation of lindane (gamma-hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus.* Lett Appl Microbiol 28:238–241

Smith M, Shnyreva A, Wood DA, Thurston CF (1998) Tandem organization and highly disparate expression of the two laccase genes *lcc1* and *lcc2* in the cultivated mushroom *Agaricus bisporus.* Microbiology 144: 1063–1069

Sollewijn Gelpke MD, Mayfield-Gambill M, Lin Cereghino GP, Gold MH (1999) Homologous expression of recombinant lignin peroxidase in *Phanerochaete* chrysosporium. Appl Environ Microbiol 65:1670–1674

Srinivasan C, D'Souza T, Boominathan K, Reddy C (1995) Demonstration of laccase in the white-rot basidiomycete *Phanerochaete chrysosporium* BKM-F-1767. Appl Environ Microbiol 61:4274-4277

Stewart P, Cullen D (1999) Organization and differential regulation of a cluster of lignin peroxidase genes of *Phanerochaete chrysosporium.* J Bacteriol 181:3427–3432

Stewart P, Gaskell J, Cullen D (2000) A homokaryotic derivative of a *Phanerochaete chrysosporium* strain and its use in genomic analysis of repetitive elements. Appl Environ Microbiol 66:1629–1633

Stewart P, Kersten P, Vanden Wymelenberg A, Gaskell J, Cullen D (1992) The lignin peroxidase gene family of *Phanerochaete chrysosporium:* complex regulation by carbon and nitrogen limitation, and the identification of a second dimorphic chromosome. J Bacteriol 1745036–5042

Stewart P, Whitwam R, Kersten P, Cullen D, Tien M (1996) Efficient expression of a Phanerochaete chrysosporium manganese peroxidase gene in Aspergillus oryzae. Appl Environ Microbiol 62:860–864

Sublette KL, Ganapathy EV, Schwartz S (1992) Degradation of munitions wastes by *Phanerochaete chrysosporium.* Appl Biochem Biotechnol 34:709–723

Sugano Y, Nakano R, Sasaki K, Shoda M (2000) Efficient heterologous expression in *Aspergillus oryzae* of a unique dye-decolorizing peroxidase, DyP, of *Geotrichum candidum.* Appl Environ Microbiol 66:1754–1758

Takada S, Nakamura M, Matsueda T, Kondo R, Sakai K (1996) Degradation of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by the white rot fungus *Phanerochaete sordida* YK-624. Appl Environ Microbiol 62:4323-4328

Tello M, Corsini G, Larrondo LF, Salas L, Lobos S, Vicuna R (2000) Characterization of three new manganese peroxidase genes from the ligninolytic basidiomycete *Ceriporiopsis subvermispora.* Biochim Biophys Acta 1490:137–144

Temp U, Zierold U, Eggert C (1999) Cloning and characterization of a second laccase gene from the lignin-degrading basidiomycete *Pycnoporus cinnabarinus.* Gene 236:169–177

Teunissen PJ, Sheng D, Reddy GV, Moenne-Loccoz P, Field JA, Gold MH (1998) 2-Chloro-l,4-dimethoxybenzene cation radical: formation and role in the lignin peroxidase oxidation of anisyl alcohol. Arch Biochem Biophys 360:233–238

Thomas DR, Carswell KS, Georgiou G (1992) Mineralization of biphenyl and PCBs by the white rot fungus *Phanerochaete chrysosporium.* Biotechnol Bioeng 40:1395–1402

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140:19–26

Tien M, Kirk TK (1983) Lignin-degrading enzyme from the Hymenomycete *Phanerochaete chrysosporium* Burds. Science 221:661–663

Tien M, Kirk TK (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium:* purification, characterization, and catalytic properties of a unique H_20_2-requiring oxygenase. Proc Natl Acad Sci USA 81:2280-2284

Tien M, Tu C-PD (1987) Cloning and sequencing of a cDNA for a ligninase from *Phanerochaete chrysosporium.* Nature 326:520–523

Tuor U, Wariishii H, Schoemaker H, Gold M (1992) Oxidation of phenolic β-aryl ether lignin model compounds by mangansese peroxidase from *Phanerochaete chrysosporium:* oxidative cleavage of an α-carbonyl model compound. Biochemistry 31:4986-4995

Tuor U, Winterhalter K, Fiechter A (1995) Enzymes of white-rot fungi involved in lignin degradation and ecological determinants for wood decay. J Biotechnol 41:1–17

Urzua U, Fernando Larrondo L, Lobos S, Larrain J, Vicuna R (1995) Oxidation reactions catalyzed by manganese peroxidase isoenzymes from *Ceriporiopsis subvermispora.* FEBS Lett 371:132–136

Urzua U, Kersten PJ, Vicuna R (1998a) Kinetics of Mn3+-oxalate formation and decay in reactions catalyzed by manganese peroxidase of *Ceriporiopsis subvermispora.* Arch Biochem Biophys 360:215–222

Urzua U, Kersten PJ, Vicuna R (1998b) Manganese peroxidase-dependent oxidation of glyoxlic and oxalic acids synthesized by *Ceriporiopsis subvermispora* produces extracellular hydrogen peroxide. Appl Environ Microbiol 6468–73

Valli K, Gold MH (1991) Degradation of 2,4-dichlorophenol by the lignin-degrading fungus *Phanerochaete chrysosporium.* J Bacteriol 173:345–352

Valli K, Brock J, Joshi D, Gold M (1992a) Degradation of 2,4-dinitrotoluene by the lignin-degrading fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 58:221–228

Valli K, Wariichi H, Gold M (1992b) Degradation of 2,7-dichlorodibenzo-p-dioxin by the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* J Bacteriol 174:2131–2137

Vallim M, Janse B, Gaskell J, Pizzirani-Kleiner A, Cullen D (1998) *Phanerochaete chrysosporium* cellobiohydrolase and cellobiose dehydrogenase transcripts in wood. Appl Environ Microbiol 64:1924–1928

Van Aken B, Hofnchter M, Scheibner K, Hatakka AI, Naveau H, Agathos SN (1999) Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete *Phlebia radiata.* Biodegradation 1083–91

Varela E, Bockle B, Romero A, Martinez AT, Martinez MJ (2000) Biochemical characterization, cDNA cloning and protein crystallization of aryl-alcohol oxidase from *Pleurotus pulmonarius.* Biochim Biophys Acta 1476:129–138

Vazquez-Duhalt R, Westlake DWS, Fedorak PM (1994) Lignin peroxidase oxidation of aromatic compounds in systems containing organic solvents. Appl Environ Microbiol 60:459-466

Volc J, Kubatova E, Wood DA, Daniel G (1997) Pyranose 2-dehydrogenase, a novel sugar oxidoreductase from the basidiomycete fungus *Agaricus bisporus.* Arch Microbiol 167:119–125

Vyas BR, Molitoris HP (1995) Involvement of an extracellular H_2O_2-dependent ligninolytic activity of the white rot fungus *Pleurotus ostreatus* in the decolorization of Remazol brilliant blue R. Appl Environ Microbiol 61:3919-3927

Wahleithmer JA, Xu F, Brown K, Brown S, Golightly E, Halkier T, Kauppinen S, Pederson A, Schneider P

(1995) The identification and characterization of four laccase genes from the plant pathogenic fungus *Rhizoctonia solani.* Curr Genet 29:395403

Wariishi H, Valli K, Gold MH (1991) *In vitro* depolymerization of lignin by manganese peroxidase of *Phanerochaete chrysosporium.* Biochem Biophys Res Commun176:269–275

Wariishi H, Sheng D, Gold MH (1994) Oxidation of ferrocytochrome c by lignin peroxidase. Biochemistry 33: 5545–5552

Westermark U, Eriksson K-E (1974a) Carbohydrate-dependent enzymatic quinone reduction during lignin degradation. Acta Chem Scand 28:204–208

Westermark U, Eriksson K-E (1974b) Cellobiose:quinone oxidoreductase, a new wood-degrading enzyme from white-rot fungi. Acta Chem Scand 28:209–214

Westermark U, Eriksson K-E (1975) Purification and properties of cellobiose:quinone oxidoreductase from *Sporotrichum pulverulentum.* Acta Chem Scand, B 29:419-424

Whittaker M, Kersten P, Nakamura N, Sanders-Loehr J, Schweizer E, Whittaker J (1996) Glyoxal oxidase from *Phanerochaete chrysosporium* is a new radical-copper oxidase. J Biol Chem 271:681–687

Whittaker MM, Kersten PJ, Cullen D, Whittaker JW (1999) Identification of catalytic residues in glyoxal oxidase by targeted mutagenesis. J Biol Chem 274:36226–36232

Whitwam R, Tien M (1996) Heterologous expression and reconstitution of fungal Mn peroxidase. Arch Biochem Biophys 333:439-446

Whitwam R, Gazarian I, Tien M (1995) Expression of fungal Mn peroxidase in E. coli and refolding to yield active enzyme. Biochem Biophys Res Commun 216 1013–1017

Yadav JS, Quensen JF III, Tiedje JM, Reddy CA (1995) Degradation of polychlorinated biphenyl mixtures (Aroclors 1242, 1254, and 1260) by the white rot fungus *Phanerochaete chrysosporium* as evidenced by congener-specific analysis. Appl Environ Microbiol 61:2560–2565

Yadav JS, Bethea C, Reddy CA (2000) Mineralization of trichloroethylene (TCE) by the white rot fungus *Phanerochaete chrysosporium.* Bull Environ Contam Toxicol 67:28–34

Yanai K, Yonekura K, Usami H, Hirayama M, Kajiwara S, Yamazaki T, Shishido K, Adachi T (1996) The integrative transformation of *Pleurotus ostreatus* using bialaphos resistance as a dominant selectable marker. Biosci Biotechnol Biochem 60:472-475

Yaver D, Golightly E (1996) Cloning and characterization of three laccase genes from the white-rot basidiomycete *Trametes villosa:* genomic organization of the laccase gene family. Gene 181:95–102

Yaver D, Xu F, Golightly E, Brown K, Brown S, Rey M, Schneider P, Halkier T, Mondorf K, Dalbage H (1996) The purification, characterization, molecular cloning and expression of two laccase genes from the white-rot basidiomycete *Trametes villosa.* Appl Environ Microbiol 62:834–841

Yaver DS, Ovejero MD, Xu F, Nelson BA, Brown KM, Halkier T, Bernauer S, Brown SH, Kauppinen S (1999) Molecular characterization of laccase genes from the basidiomycete *Coprinus cinereus* and heterologous expression of the laccase *lcc1*. Appl Environ Microbiol 65:4943-4948

Youn H, Hah Y, Kang S (1995) Role of laccase in lignin degradation by white-rot fungi. FEMS Microbiol Lett 132:183–188

Zapanta LS, Hattori T, Rzetskaya M, Tien M (1998) Cloning of *Phanerochaete chrysosporium leu2* by complementation of bacterial auxotrophs and transformation of fungal auxotrophs. Appl Environ Microbiol 64:2624–2629

Zolan M (1995) Chromosome-length polymorphisms in fungi. Microbiol Rev 59:686-698