Hadar, Y., and Cullen, D. (2013) Organopollutant degradation by wood decay basidiomycetes. in The Mycota, Agricultural Applications (Kempken, F. ed.), 2 Ed., Springer-Verlag, Berlin. pp 115-144; 2013

5 Organopollutant Degradation by Wood Decay Basidiomycetes

Yitzhak Hadar[1], Daniel Cullen[2]

CONTENTS

I. Introduction

Wood decay fungi are obligate aerobes, deriving nutrients from the biological 'combustion' of wood, using molecular oxygen as terminal electron acceptor (Kirk and Farrell 1987; Blanchette 1991). Non-specific extracellular enzymes are generally viewed as key components in lignin depolymerization. The major enzymes implicated in lignin degradation are lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. All three can act with low molecular weight mediators to bring about oxidation of lignin and various xenobiotics (Cullen 2002).

Saprotrophic Agaricomycotina, particularly ligninolytic 'white-rot fungi' have been extensively studied for their ability to degrade a wide range of organopollutants such as polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, pentachlorophenol (PCP), pesticides, and explosives. The unique extracellular systems of the white-rot fungi have been repeatedly invoked to explain the extraordinary oxidation potential of these microbes, but the precise mechanisms remain elusive. In addition to secreted enzyme systems, intracellular metabolic processes are responsible for further transformation, degradation, and, often, mineralization of compounds. Cytochrome P450s have been implicated in several instances, and a hallmark of most Agaricomycotina is a large number of P450 encoding genes.

The nature and extent of white-rot genetic diversity have been more fully appreciated in recent years, with the rapid increase in genome sequencing and analysis. Among the white-rot species known to degrade recalcitrant PAHs, sequences of *Phanerochete chrysosporium* (Martinez et al. 2004), *Pleurotus ostreatus* (http://genome.jgi.doe.gov/PleosPC15_2/PleosPC15_2.info.html) (Fig. 5.1), *Ceriporiopsis subvermispora* (Fernandez-Fueyo et al. 2012), *Trametes versicolor*, and *Dichomitus squalens* (Floudas et al. 2012) are now publicly available. This review provides a critical analysis of recent advances on the genetics and physiology of wood decay fungi as they relate to organopollutant degradation, and we place particular emphasis on the model experimental systems *P. chrysosporium* and *P. ostreatus*. This is not

[1]Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel; e-mail: hadar@agri.huji.ac.il
[2]USDA Forest Products Laboratory, One Gifford Pinchot Dr, Madison, WI, USA; e-mail: dcullen@wisc.edu

Agricultural Applications, 2nd Edition
The Mycota XI
F. Kempken (Ed.)

Fig. 5.1. A fruiting body of *Pleurotus ostreatus*, the oyster mushroom, a commercially important edible white-rot filamentous basidiomycete cultivated on lignocellulosic waste. This is a model fungus for the study of biochemical and molecular mechanisms involved in biodegradation of lignin and recalcitrant and toxic anthropogenic aromatic compounds. The dikaryon stage is required for the formation of the fruiting bodies

intended to be a comprehensive treatment of the voluminous physiological literature in this area. Interested readers are referred to earlier reviews (Kirk and Farrell 1987; Eriksson et al. 1990; Cullen and Kersten 2004).

II. Biochemistry of Lignin and Organopollutant Degradation

A. Lignin Peroxidase

Lignin peroxidase (LiP) will cleave Cα–Cβ bonds of lignin model compounds and partially depolymerize methylated lignin in vitro (Glenn et al. 1983; Tien and Kirk 1983, 1984; Gold et al. 1984). A variety of oxidations, all dependent on H_2O_2, have been demonstrated (Harvey et al. 1985; Kersten et al. 1985; Shoemaker et al. 1985; Hammel et al. 1986b). In short, LiP oxidizes aromatic substrates by a single electron, and the resulting aryl cation radicals undergo spontaneous degradation, yielding many different products dependent on substrate structure. The complex reactions and the role(s) of peroxidases in ligninolysis have been reviewed (Higuchi 1990; Hammel and Cullen 2008).

Given their low specificity and high oxidation potential, it is perhaps not surprising the LiPs oxidize a remarkable array of organopollutants (reviewed in Refs. Higson 1991; Hammel 1995a, b; Pointing 2001; Cullen 2002). Among these are the PAHs, pollutants from both geochemical and anthropogenic sources. PAHs consist of three or more benzene rings fused in a linear, angular, or cluster arrangement. As their molecular weight increases, water solubility and biodegradability decrease and genotoxicity increases. The biodegradation and bioremediation of these compounds have attracted much attention in recent decades. (reviewed in Refs. Peng et al. 2008; Gan et al. 2009; Haritash and Kaushik 2009; Lu et al. 2011)

***Phanerochaete chrysosporium* has been the most intensively studied white-rot fungus** for its extraordinary ability to oxidize and/or mineralize a broad range of PAHs. For example, *P. chrysosporium* degraded at least 22 PAHs, including all of the most abundant PAH components present in anthracene oil, and underwent 70–100 % disappearance during 27 days of incubation with nutrient nitrogen-limited cultures (Bumpus 1989). The mechanism(s) of degradation of PAHs is/are diverse, and can include those that are unique to ligninolytic fungi or exist in other microorganisms. Benzo (a) pyrene, anthracene, and pyrene have ionization potentials below 7.6 eV, and serve as substrates for LiP (Hammel et al. 1986a; Hammel 1995a). In

addition to PAHs, purified LiPs will transform 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 nitro-methoxybenzenes (Valli and Gold 1991; Valli et al. 1992a, b; Teunissen et al. 1998).

B. Manganese Peroxidase

Like LiPs, **manganese peroxidases (MnPs) exhibit a typical peroxidase catalytic cycle, but with Mn(II) as the substrate.** The Mn(II) is chelated by bidentate organic acid chelators such as glycolate or oxalate. Chelation is thought to stabilize Mn(III) and allow its diffusion at some distance from the enzyme (Glenn et al. 1986; Paszczynski et al. 1986). MnPs lack sufficient oxidative potential to cleave the major non-phenolic units of lignin, but can oxidize phenolic structures. The resulting phenoxy radicals undergo a variety of reactions including polymer cleavage within certain units, e.g., between Cα and aromatic rings (Wariishi et al. 1991; Tuor et al. 1992). MnPs purified from *P. chrysosporium*, *Nematoloma frowardi*, and *Phlebia radiata* have been shown to oxidize pentachlorophenol and 2,4,6-trinitrotoluene (TNT) in a Mn-dependent manner (Scheibner and Hofrichter 1998; Van Aken et al. 1999; Reddy and Gold 2000), whereas decolorization of azo dye by *Pleurotus eryngii* and *Bjerkandera adusta* MnP isozymes is Mn-independent (Heinfling et al. 1998a).

Phanerochete chrysosporium cultures will efficiently degrade the PAHs phenanthrene and fluorine, an observation difficult to explain because neither serves as a LiP or MnP substrate (George and Neufield 1989; Hammel et al. 1992; Vazquez-Duhalt et al. 1994; Bogan et al. 1996c). Alternative mechanisms must be operative. Among these, peroxidation of unsaturated lipids has been shown to generate transient lipoxyradical intermediates that oxidize non-phenolic lignin model compounds. MnP/lipid peroxidation depolymerizes phenolic- and phenol-blocked (methylated) synthetic lignins (Bao et al. 1994), as well as β-O-4 linkages of lignin model compounds (Bao et al. 1994; Kapich et al. 1999). The system has also been shown to oxidize fluorine (Bogan et al. 1996a) and phenanthrene (Moen and Hammel 1994).

Certain peroxidases 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). Designated **'versatile peroxidases'** (VPs), these enzymes typically feature Mn-binding residues as well as a conserved Trp involved in the electron transfer that enables oxidation of non-phenolic compounds. Recent work suggested a role for VP in the transformation of azo dyes (Salame et al. 2010, 2012) and carbamazepine (Golan-Rozen et al. 2011) (see below).

Less well-studied peroxidases, but potentially involved in degradation of lignin and organopollutants, are the **heme thiolate peroxidases (HTPs) and the dye decolorization peroxidases (DyPs)** (Hofrichter et al. 2010; Lundell et al. 2010). The HTPs include chloroperoxidases and peroxygenases which catalyze a wide range of reactions, including oxidations of various aliphatic and aromatic compounds (Ullrich and Hofrichter 2005; Gutierrez et al. 2011). DyPs and putative DYP-encoding genes have been identified in various fungi, and recent studies have attributed high redox potentials for the enzyme from the white-rot fungus *Auricularia auricula-judae* (Liers et al. 2010).

C. Laccase

Laccases catalyze the 1-electron oxidation of phenolics, aromatic amines, and other electron-rich substrates. Their oxidation of the phenolic units in lignin generates phenoxy radicals, which can lead to aryl-Cα cleavage (Kawai et al. 1988). Non-phenolic lignin-related substrates are oxidized in the presence of certain auxiliary substrates such as ABTS (2,2'-azinobis-3-ethylthiazoline-6-sulfonate) (Bourbonnais et al. 1997, 1998; Collins et al. 1999). For examples, in the presence of synthetic mediators, organophosphorous insecticides are degraded by *P. ostreatus* laccase (Amitai et al. 1998), and

high ionization potential PAHs are oxidized by *Coriolopsis gallica* and *T. versicolor* laccases (Johannes et al. 1996; Pickard et al. 1999). Cultures of the white-rot fungi *Pycnoporus cinnabarinus* and *Trametes versicolor* produce small molecular weight compounds thought to act as natural intermediaries for oxidation of non-phenolic lignin substructures (Eggert et al. 1996) and PAHs (Johannes and Majcherczyk 2000). Most white-rot fungi produce multiple laccase isozymes (Fukushima and Kirk 1995; Salas et al. 1995; Perie et al. 1998) but some, notably *P. chrysosporium*, produce none. Laccases and their applications have been reviewed (Giardina et al. 2010).

Both **peroxidases and laccases can degrade pentachlorophenol (PCP)**, a restricted-use wood preservative. The first step in the oxidation of such chlorophenols, the formation of para-quinones and release of a chlorine atom, can be carried out by several white-rot fungi. When *P. chrysosporium* LiP and MnP genes were expressed in *Amylomyces rouxii*, a zygomycete producing only phenoloxidases, the transformant exhibited increased activity (95 % depletion) in comparison to the wild type grown without the inducer tyrosine (45 % removal) (Montiel-Gonzalez et al. 2009).

Another mechanism for PCP detoxification could be humification of these xenobiotics via polymerization into soil organic matter. Polymerization of PCP and ferulic acid by manganese peroxidase, lignin peroxidase, and laccase converted a significant portion of the PCP into soil-bound transformation products that are not extractable with organic solvents (Ruttimann-Johnson and Lamar 1996). Using labeled PCP, highest binding to the humic substances was obtained with *P. ostreatus*, followed by *Irpex lacteus*, *T. versicolor*, and *Bjerkandera adusta*. The highest mineralization rate of 8.8 % was demonstrated for *T. versicolor*. Remediation of PCP-contaminated soils with ligninolytic fungi has been the focus of several studies, and inoculum formulation has been a central objective. (Lamar and Dietrich 1990; Lamar et al. 1990a, b, 1994; Lestan and Lamar 1996; Ford et al. 2007a, b)

In addition to PCP, **peroxidases and laccases will degrade a wide range of aromatic dyes** that can pose severe environmental problems. Their highly variable and complex chemical structures also make them difficult to remove by using conventional wastewater treatment systems. The most frequently used color-removal technologies are physical (adsorption, filtration, and flotation), chemical (coagulation, oxidation, reduction, and electrolysis), and biological (aerobic and anaerobic). Thus, color removal is one of the most difficult requirements to be faced by the textile finishing, dye manufacturing, pulp, and paper industries. These industries are major water consumers and are, therefore, a source of considerable pollution. Azo dyes, the largest class of synthetic dyes, are characterized by the presence of one or more azo bonds (-N = N-) in association with one or more aromatic systems, which may also carry sulfonic acid groups (Singh and Arora 2011).

Numerous wood-rotting fungi, including *P. chrysosporium*, *Coriolus versicolor*, *Irpex lacteus*, *P. ostreatus*, and *Ganoderma applanatum*, are able to degrade a wide range of synthetic dyes (Wesenberg et al. 2003; Kaushik and Malik 2009). Many of the decolorization studies indicate that wood degrading fungi have a potential to be developed further into industrial wastewater treatment technology. (Stolz 2001)

Dye degradation and decolorization are of particular relevance to this chapter as a result of the dual role they play in the study of ligninolytic fungi and their oxidative systems. On the one hand, dyes are targets for degradation and bioremediation as toxic pollutants, as described above. On the other hand, they have been used as model compounds to elucidate catalytic mechanisms of ligninolytic enzymes. Evidence suggests that lignin-degrading enzymes, MnP, VP, LiP, and laccases are directly involved not only in the degradation of lignin, their natural lignocellulosic substrates, but also in the degradation of dyes (Heinfling et al. 1998a, b).

Polymeric dye decolorization and ligninolytic activity of *P. chrysosporium* were correlated by comparing the effect of various physiological parameters, mutations, and inhibitors on both processes. Dye decolorization, like ligninolytic activity, appears to be a secondary metabolic process. It was repressed by nitrogen and only occurred after the nitrogen in the cultures had been consumed. Dye decolorization paralleled lignin degradation temporally. Thus it was concluded that some dyes are a good model to represent the ligninolytic system. (Glenn and Gold 1983)

Pleurotus eryngii VP-active sites were demonstrated by measuring oxidizing activity

towards high-redox-potential aromatic compounds and dyes such as Reactive Black 5 (RB5) (Camarero et al. 2005). This was later verified by site-directed mutagenesis, again using RB5 oxidation to show the importance of Trp164 in the reaction (Ruiz-Duenas et al. 2008).

Laccases are also important in dye decolorization (Giardina et al. 2010). Studies of anthraquinone and azo dye degradation by purified laccase from *Lentinus* sp., together with molecular docking of Acid Blue 80, RBBR, and Acid Red 37 onto the enzyme, confirmed the amino acid residues involved in dye oxidation (Hsu et al. 2012). The potential of *Trametes trogii* purified laccase for the decolorization of different types of recalcitrant dyes without the addition of redox mediators has been shown (Grassi et al. 2011), and random mutagenesis has been shown to improve performance of a *P. ostreatus* laccase (Miele et al. 2010).

D. Peroxide Generation

Several systems have been suggested as sources of **extracellular H_2O_2 necessary for peroxidase activity**. Considerable evidence implicates GLOX, a radical-copper oxidase (Whittaker et al. 1996) produced by *P. chrysosporium* (Kersten and Kirk 1987). GLOX utilizes a wide range of small aldehydes such as glyoxal and methylglyoxal (extracellular metabolites of *P. chrysosporium*), and transfers the electrons to O_2, generating H_2O_2. Glycolaldehyde, another substrate, is produced by the action of LiP on β-O-4 lignin substructures. This suggests a physiological connection between GLOX and LiP, and this is further supported by the reversible inactivation of GLOX in the absence of a peroxidase system (Kersten 1990). GLOX is reactivated, however, by reconstituting the complete peroxidase system, including both LiP and substrate. Thus, the supply of H_2O_2 by GLOX responds to the demand of the peroxidases, thereby providing an extracellular regulatory mechanism controlling the coupled enzyme systems. GLOX activity has been detected in several lignin-degrading fungi when grown on oak sawdust (Orth et al. 1993).

Detailed comparative studies with galactose oxidase of *Dactylium dendroides* have defined GLOX as a copper radical oxidase (Kersten and Cullen 1993; Whittaker et al. 1999; Whittaker 2002). Site-specific mutagenesis have confirmed essential residues including an internal Cys-Tyr radical forming a metalloradical complex and copper ligands Tyr135 Tyr377 and His378. (reviewed by Whittaker 2002)

Six additional copper radical oxidase genes were identified by BLAST searches of the *P. chrysosporium* genome. Residues coordinating copper and forming the radical redox site are conserved (Martinez et al. 2004; Vanden Wymelenberg et al. 2006b). Designated *cro1* through *cro6*, three of these genes (*cro3-5*) are predicted to encode repeats of an N-terminal WSC domains, which may be involved in carbohydrate binding (IPR002889; http://www.ebi.ac.uk/interpro/IEntry?ac=IPR002889). *Cro6* is most closely related to *glx* (47 % amino acid identity), but contains a ~200 amino acid N-terminal region absent from the other copper radical oxidases. The predicted *cro2* protein is only 28 % identical to GLOX but, in contrast to GLOX, the enzyme oxidized a glycolaldehyde dimer, but not methylglyoxal (Vanden Wymelenberg et al. 2006b).

Also possibly important in peroxide generation are the **glucose–methanol–choline oxidases (GMCs) which include aryl alcohol oxidase (AAO), methanol oxidase, and various sugar oxidases** (reviewed in Ref. (Hernandez-Ortega et al. 2012)). AAOs oxidize benzyl alcohols to aldehydes, transferring the electrons to O_2, producing H_2O_2 (Muheim et al. 1990; Asada et al. 1995). *P. ostreatus* secretes a mixture of benzyl alcohols that are oxidized by AAO (Sannia et al. 1991). The white-rot fungus *Bjerkandera adusta* secretes chlorinated benzyl alcohols that are substrates for AAO but not LiP. Since both LiP and AAO are produced in *B. adusta* cultures, such substrate preferences may have important physiological roles in ligninolysis.

Methanol oxidase may play an important role in generating H_2O_2 in both white-rot and brown-rot fungi. The enzyme is highly expressed and associated with hyphal cell walls in the brown-rot fungus, *Gloeophyllum trabeum* (Daniel et al. 2007). Brown-rot demethylation of lignin may provide the substrate, and the H_2O_2 produced is thought to participate in generation of highly reactive hydroxyl radical via a Fenton reaction, $H_2O_2 + Fe^{2+} + H^+ \rightarrow H_2O + Fe^{3+} + \cdot OH$. This diffusible radical will mediate the rapid depolymerization of cellulose, a central feature of brown-rot decay. High expression of methanol oxidase has also been observed in the white-rot fungus *P. chrysosporium* when grown on media containing ground wood as sole carbon source (Vanden Wymelenberg et al. 2010).

Pyranose 2-oxidase oxidizes various monosaccharides at C-2, with transfer of electrons to O_2 to produce H_2O_2. This GMC enzyme has been identified in various fungi, including *P. chrysosporium*, *T. versicolor*, *Oudemansiella mucida*, and *Agaricus bisporus* (Daniel et al. 1994; Artolozaga et al. 1997), and the catalytic mechanism has received considerable attention in recent times (Tan et al. 2010; Sucharitakul et al. 2011; Tan et al. 2011; Wongnate et al. 2011). *P. chrysosporium* mycelium also exhibits glucose 1-oxidase activity (Kelley and Reddy 1986, 1988), but this enzyme appears to be less common than pyranose 2-oxidase (reviewed by Ander and Marzullo 1997).

Cellobiose dehydrogenase (CDH) is widely distributed among fungi, but its precise role in ligninolysis and/or organopollutant degradation, if any, remains uncertain (Henriksson et al. 2000; Zamocky et al. 2006). The enzyme contains a dehydrogenase domain and a heme prosthetic group (Hallberg et al. 2000). CDH oxidizes cellodextrins, mannodextrins, and lactose, and suitable electron acceptors include quinones, phenoxyradicals, and Fe^{3+}. Interestingly, recent studies have shown that CDH will enhance cellulose depolymerization by members of the glycoside hydrolase family 61 (Harris et al. 2010; Langston et al. 2011). Previously considered to be cellulases, the latter 'hydrolases' have been recently classified as copper-dependent monooxygenases (Quinlan et al. 2011; Westereng et al. 2011).

E. Other Oxidoreductases

In addition to the extracellular peroxidases and laccases, transformation and/or complete mineralization of organopollutants involve additional extracellular and intracellular processes. Examples include glycosyl conjugation of triclosan by *T. versicolor* cultures (Hundt et al. 2000), and O-methylation of PCP and triclosan by *P. chrysosporium* (Lamar et al. 1990a) and *P. cinnabarinus* (Hundt et al. 2000) cultures, respectively.

The role of **cytochrome P450s in organopollutant degradation** remains largely unexplored, but some progress has been made. The metabolic steps in PAH degradation have occurred in both N-limited and N-sufficient culture media, and are similar to those in nonligninolytic fungi, such as *Cunninghamella elegans* (Casillas et al. 1996) and N-sufficient cultures of *P. chrysosporium*. Apparently, *P. ostreatus* acts on PAHs like nonligninolytic fungi, but is also able to mineralize PAHs (Bezalel et al. 1996a, b). Since *P. ostreatus* does not contain lignin peroxidase, and since PAH metabolism did not correlate with laccase or MnP activities, it is possible that a cytochrome P450 monooxygenase is responsible for the initial step in the attack. The ligninolytic system of *P. ostreatus* may be involved in the later steps of metabolism, such as ring cleavage, which leads to CO_2 evolution. These conclusions were based on metabolite analyses, physiological and biochemical studies (Bezalel et al. 1997).

Additional investigations suggest that *P. chrysosporium* degradation of 2,4,6-trichlorophenol (Reddy et al. 1998) and PCP (Hammel and Tardone 1988; Mileski et al. 1988; Reddy and Gold 1999, 2000) involve oxidative dechlorination by extracellular peroxidases, followed by intracellular reductive dechlorination and hydroxylation reactions. White-rot cytochrome P450s reactions also include monooxygenase bioconversions of phenanthrene by *P. ostreatus* (Syed et al. 2010), benzo(a)

pyrene by *Pleurotus pulmonarius* (Masapahy et al. 1999) and by *P. chrysosporium* (Syed et al. 2011a), 4-methyldibenzothiophene by *T. versicolor* (Ichinose et al. 1999), and endosulfan and carbamazepine (CBZ) by *P. chrysosporium*. (Kullman and Matsumura 1996; Golan-Rozen et al. 2011)

Fungi are active in **biodegradation of a wide array of pharmaceuticals and hormones**. Analyses of the degradation pathways and metabolites formation was recently reviewed (Cruz-Morato et al. 2012). As mentioned above, **cytochrome P450s and MnPs** have been implicated in the transformation and metabolism of CBZ, a drug used in large quantities worldwide for the treatment of epilepsy, and increasingly used for various psychiatric treatments (Leclercq et al. 2009). Studies in Europe and North America have shown that CBZ and CBZ metabolites are among the most frequently detected pharmaceuticals in wastewater effluents, river water, and drinking water (Heberer 2002; Miao et al. 2005; Benotti et al. 2009). CBZ is an environmentally recalcitrant compound extremely stable in water and soil (Lienert et al. 2007), mainly as a result of its remarkably high stability towards bacterial degradation. Thus, because of its very slow degradation, it has been proposed as a tracer for anthropogenic activity and contamination originated from municipal waste water (Gasser et al. 2010).

Nevertheless, CBZ modification by different white-rot fungi has been reported (Kang et al. 2008). For example, the white-rot fungi *T. versicolor* and *G. lucidum* eliminated 57 % and 46 % respectively of CBZ after 7 incubation days (Marco-Urrea et al. 2009). Similar removal efficiency of CBZ was observed by *T. versicolor* in a solid-phase bioreactor containing sewage sludge and mycelium (Rodriguez-Rodriguez et al. 2010). *P. ostreatus* strains F6, N001 (dikaryons), and PC9 (monokaryon) degraded CBZ to levels ranging from 48 % to 99 % of the initial concentration. With strain PC9, CBZ concentration was reduced from 10 mg l^{-1} to 20 μg l^{-1} within 17 days of incubation (Golan-Rozen et al. 2011). To evaluate the potential use of *P. ostreatus* to remediate contaminated water, CBZ removal was studied at its environmental relevant concentration (~1 μg l^{-1}, 4.6 nM). When optimal conditions were obtained, CBZ concentration decreased by 97.9 % to 0.093 nM (22 ppt) within 8 days. Unlike the accumulation of the metabolite 10,11EPCBZ observed at high CBZ concentration, in this experiment 10,11EPCBZ disappeared, gradually reaching a minimal concentration of 0.1 nM. These results suggested that at environmentally relevant concentrations *P. ostreatus* can not only transform CBZ to 10,11EPCBZ, but may also continue its metabolism. (Golan-Rozen et al. 2011)

Several enzymatic mechanisms have been suggested to be involved in the oxidation of CBZ. When a cytochrome P450 inhibitor was added to the growth medium, CBZ elimination by *T. versicolor* was inhibited by more than half, indicating the possible involvement of cytochrome P450 in the biodegradation process (Montiel-Gonzalez et al. 2009). When *P. ostreatus* was grown in media supporting high levels of both cytochrome P450 and manganese peroxidase (MnP), 99 % of the added CBZ was eliminated from the solution (Golan-Rozen et al. 2011). High removal of CBZ was also obtained when either MnP or CYP450 was active. When both CYP450 and MnP were inactivated, only 10–30 % of the added CBZ was removed.

In-vitro reaction between CBZ and crude lignin peroxidase produced by the fungus *P. chrysosporium* in the presence of H_2O_2 and veratryl alcohol resulted in only 5–9 % elimination. Repeated treatment with laccase from *T. versicolor* and 1-hydroxybenzotriazole (used as a redox mediator) resulted in the elimination of 20 % of the CBZ after 24 h (Hata et al. 2010). Increased removal of CBZ (about 80 %) was only observed when a lignin-derived quinone was added to the growth medium of *T. versicolor* together with ferrous oxalate to form a Fenton-like reaction (Marco-Urrea et al. 2010). This reaction facilitates the formation of hydroxyl radicals, which oxidize the CBZ molecule faster than the enzymatic reactions.

III. Comparative Genome Analysis

Knowledge of the genomes of wood decay fungi is rapidly advancing, in large part because of the support of the U.S. Department of Energy's Joint Genome Institute. An interactive MycoCosm web portal (http://genome.jgi.doe.gov/programs/fungi/index.jsf) integrates all publicly accessible fungal genomes, including those featured in this review (Grigoriev et al. 2012). Throughout the following passages, we provide protein model identification numbers that allow searches of the JGI genome portal, and thereby link to detailed protein pages, alternative models, annotation, and comparisons to other databases.

A. Gene Structure, Phylogeny, and Expression

1. Peroxidases

Based on overall sequence conservation, a **Trp active site, and the absence of Mn-binding** residues, the LiP genes identified to date are confined to lignin-degrading fungi, with ten genes present in the genomes of *P. chrysosporium* and *T. versicolor*. However, of the ten white-rot genomes analyzed and published as of July 2012, most do not have LiP genes but feature two to sixteen MnP genes. *P. chrysosporium* and *T. versicolor* contain five and thirteen MnP genes respectively (Ohm et al. 2010; Fernandez-Fueyo et al. 2012; Floudas et al. 2012; Olson et al. 2012). Genome analysis has also identified at least four, two, and three VP genes in *P. ostreatus*, *T. versicolor*, and *D. squalens* respectively. Of the eight published brown-rot genomes, none contain genes encoding LiP, MnP, or VP (Martinez et al. 2009; Eastwood et al. 2011; Floudas et al. 2012).

Early studies had also identified *P. chrysosporium* MnP genes, *mnp1*, *mnp2*, and *mnp3* (Pease et al. 1989; Pribnow et al. 1989; Orth et al. 1994; Alic et al. 1997). The draft genome revealed two new MnP genes (Martinez et al. 2004), one of which, *mnp4*, was unexpectedly localized to a region 5 kb upstream of *mnp1*. A cytochrome P450 gene lies within the *mnp4*-*mnp*1 intergenic region. The *mnp5* predicted protein corresponds to the N-terminal amino acid sequence of a MnP long ago purified from *P. chrysosporium*-colonized wood pulp (Datta et al. 1991). Most intron positions are conserved within the MnP (Larrondo et al. 2005) and LiP (Stewart and Cullen 1999) gene families.

Deviations from these simple classifications have been noted. Certain MnPs vary in length and have been classified accordingly (Ruiz-Duenas et al. 2011; Fernandez-Fueyo et al. 2012). On the basis of homology modeling and the conservation of specific residues essential for catalysis, *C. subvermispora* protein models #118677 and #99382 were initially classified as LiP and VP genes respectively. Consistent with these designations, the corresponding proteins were capable of oxidizing nonphenolic model compounds and synthetic lignin. However, the putative VP was unable to oxidize Mn as predicted. Moreover, both enzymes exhibited catalytic properties intermediate between conventional LiPs and MnP (Fernandez-Fueyo et al. 2012).

Clustering of *P. chrysosporium* genes, especially those encoding LiPs and MnPs, is a well-known phenomenon. Prior to genome sequencing, the ten *P. chrysosporium* LiP genes had been designated *lipA* through *lipJ* (Gaskell et al. 1994), and eight of these LiP genes were mapped within 3 % recombination (Gaskell et al. 1994; Stewart and Cullen 1999). The genome sequence confirmed the genetic multiplicity and verified the overall organization, with the eight LiP genes located within 100 Kb on scaffold 19. Genes designated *lipD* and *lipF* were localized to scaffolds 11 and 9 respectively.

Beyond *P. chrysosporium*, genome sequence analysis has revealed clustering of LiP and MnP genes in most white-rot fungi. Typically, this involves simple tandem arrangements and occasionally a third or fourth gene more distantly positioned. For examples, *T. versicolor* LiP genes encoding protein models #133326, #134250, and #52333 are located on scaffold 12, and models #43576, #43578, #114944, and #112835 lie on scaffold 2. In both instances, the genes are located within a ~15 kb region. *T. versicolor* MnP genes encoding proteins #51455, #74179, and #51457 are tightly clustered within 7 Kb on scaffold 10. The VP-encoding genes of *T. versicolor* are located on scaffold 2, but very distant from LiP genes. No remarkable linkage is observed among the nine, thirteen and five MnP genes of *D. squalens*, *C. subvermispora*, and *P. ostreatus* respectively. As in the case of *T. versiolor*, the *D. squalens* and *P. ostreatus* VPs show no significant linkage to each other or to the MnP genes. In contrast, the abovementioned intermediary LiP–MnP genes of *C. subvermispora* lie within 9 Kb on scaffold 20.

The **regulation of LiP gene expression**, particularly in *P. chrysosporium*, has received considerable attention. Culture conditions substantially influence *lip* transcript levels (Holzbaur and Tien 1988; Stewart et al. 1992; Reiser et al. 1993; Janse et al. 1998; Vallim et al. 1998; Stewart and Cullen 1999; Belinky et al. 2003; Vanden Wymelenberg et al. 2009; Hiscox et al. 2010; Sakamoto et al. 2010). LiP genes within clusters may be differentially regulated but, to date, no clear relationship between organization and regulation has been reported (Stewart et al. 1992; Stewart and Cullen 1999; Macdonald

et al. 2011). In *P. chrysosporium* soil cultures, LiP transcript patterns shift depending upon the pollutant, e.g., anthracene versus PCP (Lamar et al. 1995; Bogan et al. 1996b).

In recent years, **LC-MS/MS and high throughput transcript analyses** have provided insight into the expression of specific LiP genes. Extracellular proteins corresponding to *lipC* were identified only in nitrogen-limited medium, whereas the *lipD* and *lipE* products were more abundant in carbon-limited cultures (Vanden Wymelenberg et al. 2006a, 2009). These results were consistent with transcript levels measured by Northern blots (Holzbaur and Tien 1988), by quantitative RT-PCR (qRT-PCR) (Stewart and Cullen 1999) and, more recently, by whole-genome expression microarrays (Vanden Wymelenberg et al. 2009). None of the *P. chrysosporium* LiP genes exhibited elevated transcript levels in medium containing microcrystalline cellulose (Avicel, Fluka Chemical) relative to glucose-grown cultures, but peptides corresponding to *lipD* were detected in the cellulose medium (Vanden Wymelenberg et al. 2005). In submerged medium containing ball-milled aspen (BMA) as sole carbon source, transcripts of *lipA* and *lipH* accumulated relative to glucose medium, but no extracellular peroxidase was detected by LC-MS/MS (Vanden Wymelenberg et al. 2010). On the other hand, significant transcript levels of *lipD, lipE* and *lipB* were measured in similar experiments using red oak (Sato et al. 2009). More perplexing, *lipD* and *lipE* transcript levels were lowest among all LiP genes in colonized aspen wood chips (Janse et al. 1998). Transcriptome studies of *Phanerochaete carnosa* suggest that wood species substantially influence LiP transcript levels (Macdonald et al. 2011; Macdonald and Master 2012; MacDonald et al. 2012).

As in the case of LiP-encoding genes, **media composition, especially manganese concentration, has a dramatic effect on MnP regulation** (Bonnarme and Jeffries 1990; Brown et al. 1990, 1991; Pease and Tien 1992; Lobos et al. 1994). Most studies have focused on transcriptional control, but recent results using *C. subvermispora* suggest that Mn concentration may also influence MnP secretion (Mancilla et al. 2010). Mechanisms of transcriptional regulation remain uncertain, but much attention has focused on promoters and the putative role of metal response elements (MREs) (Godfrey et al. 1990, 1994; Alic and Gold 1991; Brown et al. 1993; Alic et al. 1997). Gettemy et al. (1998) reported that *P. chrysosporium mnp1* and *mnp2* were substantially upregulated in response to Mn^{2+} concentration, and later deletion analysis identified an upstream Mn-responsive element (Ma et al. 2004). In contrast, dramatic upregulation of *T. versicolor mnp2* by Mn appears not to involve any MREs (Johansson et al. 2002). Transcripts of *P. chrysosporium mnp1* accumulate in carbon- or nitrogen-starved defined medium relative to replete medium, and the corresponding protein has been identified by LC-MS/MS under such nutrient limitation (Ravalason et al. 2008; Vanden Wymelenberg et al. 2009). Upregulation of *mnp2* transcripts has been observed in cultures that were nitrogen-starved but not carbon-starved (Vanden Wymelenberg et al. 2009).

Complex patterns of *P. chrysosporium* MnP gene expression have been observed in colonized wood and soil. *mnp4* is actively transcribed when *P. chrysosporium* is grown on wood-containing soil samples (Stuardo et al. 2004), and *mnp1*, *mnp2*, and *mnp3* transcripts are easily detected in colonized aspen wood chips (Janse et al. 1998). Transcripts corresponding to *mnp1* were detected in red oak medium (Sato et al. 2009). In *P. chrysosporium* soil cultures, the depletion of fluorine roughly correlates with transcript levels of *mnp1*, *mnp2* and *mnp3* (Bogan et al. 1996c). Degradation of this high oxidation potential PAH is consistent with a mechanism involving lipid peroxidation.

Simultaneous accumulation of transcripts corresponding to *C. subvermispora* MnPs and genes putatively involved in lipid biosynthesis (Watanabe et al. 2010) also support a **role for lipid peroxidation** (Fernandez-Fueyo et al. 2012). In line with this view, recent microarray and mass spectrometry data (Fernandez-Fueyo et al. 2012) revealed simultaneous upregulation of genes encoding MnP genes as well as those involved in lipid biosynthesis after 5 days growth in medium containing BMA. Interestingly, the *C. subvermispora* MnP genes exhibiting significant accumulation of transcripts in BMA relative to glucose (models #117436 and #49863) or secreting detectable protein (models #157986, #116608, #50297) were all classified as

Table 5.1. Number of genes encoding oxidoreductases implicated in ligninolysis and degradation of various organopollutants

	White-rot fungi					
	Phach	Pleos	Cersu	Trave	Dicsq	Brown-rot[a]
LiP	10	0	0	10	0	0
MnP	5	5[b]	13[c]	13	9	0
VP	0	4[b]	2	2[c]	3	0
HTP	3	3[b]	9	3	4	5.2 (2–6)
DYP	0	4[b]	0	2[c]	1[c]	0.3 (0–2)
Laccase	0	12[d]	7[c]	7[c]	11[c]	4.5 (3–6)[c]
GLOX	1	4	0	5[c]	5[c]	0
CRO1	1	1	0	1	1	0.8 (0–2)
CRO2	1	2	1	1[c]	1[c]	1.5 (0–4)[c]
CRO3-5	3	3	1	1[c]	1[c]	0.7 (0–1)[c]
CRO6	1	6	1	1	1	1
CDH	1	1	1	1[c]	1[c]	0.7 (0–2)[c]
GH61[e]	15[c]	29	9	18[c]	15[c]	4.5 (2–10)[c]
P450s	149	141[f]	222[c]	190	187	196

[a]The average number and range of genes in six phylogenetically related brown-rot fungi included as point of comparison. Brown-rot genomes analyzed were: *Serpula lacrymans* (Eastwood et al. 2011), *Postia placenta* (Martinez et al. 2009), *Coniophora puteana, Wolfiporia cocos, Gloeophyllum rabeum*, and *F. pinicola* (Fernandez-Fueyo et al. 2012). White-rot abbreviations: Phach, *Phanerochaete chrysosporium* (Martinez et al. 2004); Pleos, *Pleurotus ostreatus* (http://genome.jgi.doe.gov/PleosPC15_2/PleosPC15_2.home.html); Cersu, *Ceriporiopsis subvermispora* (Fernandez-Fueyo et al. 2012); Trave, *Trametes versicolor* and Dicsq, *Dichomitus squalens* (Floudas et al. 2012)

[b]See reference (Ruiz-Duenas et al. 2011)

[c]NanoLC-MS/MS unambiguously identified at least one protein in media containing ground aspen as sole carbon source. See Supplemental files in published accounts for *C. subvermispora and P. chrysosporium* (Fernandez-Fueyo et al. 2012), and for *T. versicolor*, *D. squalens*, and the six brown-rot fungi mentioned above (Floudas et al. 2012)

[d]See reference (Castanera et al. 2012)

[e]Lytic polysaccharide monooxygenase

[f]Enumerated using Cytochrome P450 Database http://genome.jgi.doe.gov/PleosPC15_2/PleosPC15_2.home.html. All others derived from published accounts

'extra long' MnPs (Fernandez-Fueyo et al. 2012). Two 'short' MnP proteins were detected by LC-MS/MS in the extracellular filtrates of *T. versicolor* (Floudas et al. 2012).

VP-encoding genes have not been identified in the genomes of any brown-rot fungi, but 2–4 genes are present in *P. ostreatus* (Ruiz-Duenas et al. 2011), *C. subvermispora* (Fernandez-Fueyo et al. 2012), *T. versicolor* and *D. squalens* (Floudas et al. 2012) (Table 5.1). Global transcriptome studies have not yet been reported for these fungi, although it is clear that *P. ostreatus* VP transcription is modulated by Mn (Cohen et al. 2001, 2002a, b), and recent genetic studies persuasively show its importance in dye decolorization (below). The four *P. ostreatus* genes are not tightly linked. The abovementioned 'transitional' or 'intermediary' LiP-MnP genes of *C. subvermispora* are closely linked on scaffold 20, but regulated expression and protein secretion have not been observed in a shake flask containing BMA suspensions (Fernandez-Fueyo et al. 2012). The VP genes of *T. versicolor* are unlinked, and peptides corresponding to protein model #26239 have been detected in BMA cultures. The three *D. squalen* VP genes are unlinked and LC-MS/MS failed to detect the proteins in the extracellular filtrates of the same medium (Floudas et al. 2012).

Genes encoding **putative heme-thiolate peroxidases (HTPs) are widespread** within the genomes of white-rot and brown-rot fungi (Table 5.1) although experimental affirmation of their expression is limited. *C. subvermispora, D. squalens*, and *T. versicolor* genomes feature nine, four, and three HTP genes respectively, but systematic studies of transcriptional regulation have not been reported, and LC-MS/MS has not detected any of the corresponding peptides in media containing BMA (Fernandez-Fueyo

et al. 2012; Floudas et al. 2012). Similarly, no extracellular protein has been observed for *P. chrysosporium* models #1710, #3274, and #34295. Transcripts corresponding to #34295 are upregulated in Avicel medium relative to glucose (Vanden Wymelenberg et al. 2009). Interestingly, relative to glucose medium, transcripts encoding #34295 accumulate in BMA medium but not in ball-milled pine medium. Possibly reflecting substrate-based differential regulation, transcripts from #3274 show the opposite pattern, i.e., upregulated in pine but not aspen (Vanden Wymelenberg et al. 2011). With the exception of tandemly arranged *T. versicolor* genes encoding protein models #154915 and #23785, none of the above mentioned HTP genes exhibit close linkage.

The **dye-decolorizing peroxidases (DyPs) are sporadically distributed among genomes of wood decay fungi**. With the exception of *Gloeophyllum trabeum* no DyP genes have been detected in brown-rot genomes. Among white-rot fungi, HTP genes are absent from *P. chrysosporium* and *C. subvermispora* whereas *P. ostreatus*, *T. versicolor*, and *D. squalens* contain four, two and one gene respectively. The *T. versicolor* gene encoding protein #48874 and #48870 lie within a ~15 kb region of scaffold 7. LC-MS/MS analysis of filtrates from BMA medium indicates that *D. squalens* protein #150405 and *T. versicolor* #48870 are particularly abundant, making up 1.3 % and 2.2 % of the total spectra (Fernandez-Fueyo et al. 2012).

Development of efficient systems for production of **recombinant peroxidases has been a key factor** in furthering biochemical investigations improving enzyme properties and assessing applications related to organopollutant degradation. Until relatively recently, production of active LiP in foreign hosts has been challenging. Yields were low in Baculovirus (Johnson and Li 1991; Johnson et al. 1992). Successful expression has also been reported using *Pichia* (Wang et al. 2004; Wang and Wen 2009) and *S. cerevisiae* (Ryu et al. 2008a, b) and, in the former reports, the catalytic ability using 2,4-dichlorophenol (DCP) has been examined. Although complicated by inclusion bodies, techniques for recovering fully active enzyme from *E. coli* have been developed (Doyle and Smith 1996; Nie et al. 1998), and the approach is now well-established for LiP, VP, and MnP (Miki et al. 2009; Ruiz-Duenas et al. 2009).

In addition to *E. coli*, *P. chrysosporium* MnP has been successfully produced by *A. oryzae* (Stewart et al. 1996) and *A. niger* (Conesa et al. 2000) transformants. Peroxidases from *C. subvermispora* (Larrondo et al. 2001), *P. eryngii* (Ruiz-Duenas et al. 1999), *P. eryngii* (Eibes et al. 2009) and from *Geotrichym candidum* (Sugano et al. 2000) have also been expressed in *Aspergillus*. In one case (Cortes-Espinosa et al. 2011), a MnP-expressing *A. niger* transformant exhibited enhanced phenanthrene degradation in soil relative to the parent strain.

Expression involving **native promoters has proven useful for production of peroxidases** for several white-rot fungi. For examples, significant increases in VP expression were achieved in *P. ostreatus* transformants relative to the parental strain (Tsukihara et al. 2006, 2008). A similar strategy of homologous expression was previously reported for producing *P. chrysosporium* MnP (Ma et al. 2003) and LiP (Sollewijn Gelpke et al. 1999, 2002).

2. Laccases

Among the multicopper oxidases, **multiple genes encoding laccase *sensu stricto*** (Hoegger et al. 2006) are, with the exception of *P. chrysosporium*, a common feature of white-rot genomes (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). Laccase multiplicity is somewhat reduced in brown-rot fungi, and none have been detected in *Dacryopinax* sp. (Floudas et al. 2012). Prior to genome sequence, relatively little information was available on the organization of laccases, but *Trametes villosa* pulsed field gels suggested the possible linkage of certain laccase genes (Yaver and Golightly 1996). Seven laccase genes were identified in the *T. versicolor* genome, and close linkage was observed for those encoding proteins #47314 and #37188. Based on the percentage of total mass spectra (2.8 %), the latter protein is the most abundant

of 218 proteins identified in the extracellular filtrate of BMA medium. *D. squalens* laccases #67925, #169869 and #176907 were detected at more modest levels in the same medium, and no close linkage was observed among the 11 genes (Table 5.1). The seven genes of *C. subvermispora* are distantly linked, and transcripts corresponding to protein model #118801 were significantly upregulated in BMA medium relative to glucose. The protein was detected by LC-MS/MS analysis of BMA medium filtrates. The *P. ostreatus* strain PC15 genome contains 12 laccase genes (Castanera et al. 2012) and, with the exception of the adjacent models #1077328 and #1119530, all are distantly linked.

Laccase genes are often differentially regulated in response to culture conditions, and the patterns of regulation differ substantially between fungal species (Wahleithmer et al. 1995; Yaver and Golightly 1996; Yanai et al. 1996; Smith et al. 1998; Palmieri et al. 2000). Their regulation has been recently reviewed (Piscitelli et al. 2011b). Potential ACE response elements have been identified in *C. subvermispora* and may be responsible, in part, for copper induction of genes encoding laccases and MnP. (Alvarez et al. 2009)

Several systems have proven useful for production of recombinant laccases. For example, *P. ostreatus* laccase is produced in *Kluyveromyces lactis* and *S. cerevisiae* (Piscitelli et al. 2011b), and the latter system led to improved temperature and pH stability via mutagenesis (Piscitelli et al. 2011a). *Aspergillus* systems have been used to produce laccases from *C. subvermispora* (Larrondo et al. 2003), *T. villosa* (Yaver et al. 1996), and *Coprinus cinereus* (Yaver et al. 1999). More recently, *Pichia* spp. expression has been used to investigate the potential of various white-rot laccases for dye decolorization and PAH degradation (Guo et al. 2008; Lu et al. 2009; Wong et al. 2012).

3. Peroxide-Generating Copper Radical Oxidases

Discovered in *P. chrysosporium* (Kersten and Kirk 1987; Kersten 1990), **glyoxal oxidase (GLX) is encoded by a single gene** (Kersten and Cullen 1993; Kersten et al. 1995). Supporting an important role in ligninolysis, GLX homologs have been identified in the genomes of most white-rot fungi, but not in the brown-rotters (Fernandez-Fueyo et al. 2012; Floudas et al. 2012) (Table 5.1). In line with a physiological connection between peroxidases and GLX, coordinate increases in their transcript levels and/or protein secretion are observed under nutrient starvation (Stewart et al. 1992; Vanden Wymelenberg et al. 2006a, 2009), in colonized wood (Janse et al. 1998; Sato et al. 2009), and in soil (Bogan et al. 1996b).

Little is known concerning the expression of GLX genes from other white-rot fungi. Three of the five *T. versicolor* genes are distantly linked on scaffold #3, of which peptides corresponding to GLX protein model #118266 have been identified by LC-MS/MS in BMA-containing medium. In *D. squalens*, genes encoding GLX proteins #104366 and #126455 are tandemly arranged on scaffold 10, but none of the five GLX proteins have been detected in BMA medium. No linkage has been observed among the five *P. ostreatus* GLX genes and, to date, nothing has been published regarding their expression.

Beyond GLX, a total of six CRO genes have been identified in the *P. chrysosporium* genome. Interestingly, the WSC-containing genes *cro3*, *cro4*, and *cro5* lie within the LiP gene cluster on scaffold 19 (Cullen and Kersten 2004). The clustering of *lip* and *cro* genes seems consistent with a physiological connection between peroxidases and peroxide-generating oxidases. Relatively little data is available on the expression of *P. chrysosporium* GLX genes, although transcripts of all CRO genes have been measured over time in colonized wood wafers (Vanden Wymelenberg et al. 2006b), and the CRO2 protein has been shown in medium containing ball-milled pine (Vanden Wymelenberg et al. 2011).

Systems for heterologous expression of CROs include *Aspergillus nidulans* and *Pichia pastoris*. These have been used to confirm catalytic residues (Kersten et al. 1995; Whittaker et al. 1999) of GLX, and *A. nidulans* production of *P. chrysosporium cro2* revealed differences in the substrate preferences of GLX and CRO2 (Vanden Wymelenberg et al. 2006b).

This observation may explain how the highly efficient and **selective lignin degrader, *C. subvermispora*, lacks a clear GLX homolog** (Fernandez-Fueyo et al. 2012) (Table 5.1). Possibly, functionally related CROs fulfill the same role and/or are better suited for a spectrum of small molecular weight substrates unique to the ligninolytic system of *C. subvermispora*. Experimental support for this hypothesis is limited, but genome analysis has identified CROs in various fungi (Table 5.1), including at least three in the *C. subvermispora* genome. Further, transcriptome analysis showed upregulation of a *cro2*-like gene as well as several MnP genes in *C. subvermispora* cultures containing BMA as sole carbon source (Fernandez-Fueyo et al. 2012). Separate LC-MS/MS studies have identified *cro2* and WSC-containing CRO genes (*cro4*, *cro5*) in BMA culture filtrates of *T. versicolor* and *D. squalens* (Floudas et al. 2012).

4. Peroxide-Generating GMC Oxidoreductases

Genome analysis has greatly expanded knowledge of the distribution and diversity of GMC oxidases, particularly AAO-encoding genes. Based largely on recently published genomes (Fernandez-Fueyo et al. 2012; Floudas et al. 2012), Hernandez-Ortega et al. (2012) and coworkers describe relationships among 40 genes from various wood decay fungi. The **AAO genes were widely distributed among white-rot and brown-rot taxa**, although at least one white-rot fungus, *Auricularia delicata*, and three brown-rot fungi, *Coniophora puteana*, *Wolfiporia coccos*, and *Dacryopinax* sp. have no detectable AAO gene (Floudas et al. 2012). Transcript levels in nutrient-starved medium, Avicel medium, and BMA medium were modest for *C. subvermispora* and *P. chrysosporium* (Vanden Wymelenberg et al. 2009). One of the three *P. chrysosporium* putative AAO proteins (#135972) and two of the five *C. subvermispora* proteins (#117387, #84544) were detected by LC-MS/MS in glucose-replete media, but not in BMA. On the other hand, AAO-derived peptides were unambiguously identified in BMA cultures of *T. versicolor* (#133945 and #176148) and *D. squalens* (#160546 and #171752) (Floudas et al. 2012). Little is known regarding the expression of the *P. ostreatus* AAO genes, but sequence comparisons with other wood-decay fungi have been reported (Hernandez-Ortega et al. 2012). With the exception of the tandemly arranged *D. squalens* genes encoding proteins #102587 and #153908, close linkage has not been observed within the AAO gene families.

Recent genome analysis of **methanol oxidase (MOX), another potentially important GMC oxidase, shows a wide distribution among white and brown-rot fungi**. Among the white-rot fungi considered here (Table 5.1), *D. squalens*, *T. versicolor*, and *P. ostreatus* genomes each contained at least four unlinked genes. The *P. ostreatus* secretome has not yet been reported, and none of the *D. squalens* and *T. versicolor* MOX proteins were detected in by LC-MS/MS in BMA medium (Floudas et al. 2012). In contrast, *P. chrysosporium* MOX protein #126879 was identified in BMA culture filtrates, and the corresponding transcripts were significantly upregulated relative to glucose medium (Vanden Wymelenberg et al. 2010). Surprisingly, the same medium showed decreased transcript levels of *C. subvermispora* MOX #80773, and no LC-MS/MS evidence for MOX in BMA medium (Fernandez-Fueyo et al. 2012). The apparent absence of soluble MOX protein in filtrates should be carefully interpreted, as cell-wall associations seem likely (Daniel et al. 2007).

Several studies implicate pyranose 2-oxidase in lignin degradation. The corresponding gene has been isolated from *T. versicolor* (Nishimura et al. 1996), *P. chrysosporium* (de Koker et al. 2004), and *G. trabeum* (Dietrich and Crooks 2009), and obvious homologs are absent from most of the recently sequenced genomes. In *P. chrysosporium*, transcripts are upregulated under ligninolytic conditions (de Koker et al. 2004; Vanden Wymelenberg et al. 2009, 2010), and the extracellular protein has been identified in culture filtrates carbon-starved cultures (Vanden Wymelenberg et al. 2010) and in BMA medium (Vanden Wymelenberg et al. 2011). The *P. chrysosporium* and *G. trabeum*

pyranose 2-oxidases have been successfully expressed in *E. coli*. (Dietrich and Crooks 2009; Pisanelli et al. 2009).

Prior to the increase in genome data, genes encoding CDH were cloned from several fungi, including the white-rot fungi *P. chrysosporium* (Raices et al. 1995; Li et al. 1996), *T. versicolor* (Dumonceaux et al. 1998), and *P. cinnabarinus* (Moukha et al. 1999) All **white-rot genomes have a single CDH gene**. On the other hand, brown-rot fungal genomes have none (*P. placenta*, *F. pinicola*, *W. cocos*), one (*Coniophora puteana*, *G. trabeum*), or two (*S. lacrymans*) copies of the CDH gene. Sequences are highly conserved, and share a common architecture with separate FAD, heme, and cellulose-binding domains (CBD).

Northern blots had shown 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). Later microarray and LC-MS/MS investigations have shown that *P. chrysosporium* CDH transcripts accumulated in media containing Avicel relative to glucose as the sole carbon source (Vanden Wymelenberg et al. 2009). Transcripts have also been detected in red oak medium (Sato et al. 2009), and upregulation observed in BMA medium relative to glucose medium (Vanden Wymelenberg et al. 2010). The CDH protein has been identified in various culture filtrates, including those that are nutrient-starved (Vanden Wymelenberg et al. 2009), contain microcrystalline cellulose, or contain complex lignocellulose (Sato et al. 2009; Vanden Wymelenberg et al. 2011). The wood species used as substrate alters expression, with higher transcript and protein levels in mediums containing ball-milled pine relative to ball-milled aspen. (Vanden Wymelenberg et al. 2011)

Irrespective of the *P. chrysosporium* culture conditions, CDH transcripts and secretion are typically mirrored by expression of aldose 1-epimerase (#138479) (Vanden Wymelenberg et al. 2005; Sato et al. 2009; Vanden Wymelenberg et al. 2011). This coordinate expression may indicate a physiological coupling via generation of the cellobiose b-anomer, the preferred CDH substrate (Higham et al. 1994). Co-expression of CDH and genes encoding members of the CAZy glycoside 'hydrolase' family GH61 has also been observed. Now classified as copper-dependent monooxygenases (Quinlan et al. 2011; Westereng et al. 2011), GH61s will boost cellulose depolymerization by CDH (Harris et al. 2010; Langston et al. 2011). In addition to cellulose, xylan has been shown to increase secretion of CDH and GH61 (Hori et al. 2011). Further supporting these associations, of five recently sequenced wood decay fungi (*T. versicolor*, *D. squalens*, *Punctularia strigoso-zonata*, *Stereum hirsutum*, *Coniophora puteana*), all but *P. strigoso-zonata* simultaneously secreted ALE and CDH in BMA medium. Excluding the brown-rotter *C. puteana*, at least one GH61 monooxygenase was secreted by each of these same fungi (Floudas et al. 2012). The roles(s) and interaction(s) between these genes, if any, remain unclear.

Several systems are available for heterologous expression. Homologous expression of *P. chrysosporium* CDH was achieved by fusing *cdh* with the promoter of the highly expressed glyceraldehyde-3-phosphate dehydrogenase gene (Li et al. 2000). Expression in *Pichia* spp. has also been reported (Yoshida et al. 2001; Zamocky et al. 2008; Bey et al. 2011), and *E. coli* was used to isolate the flavin domain.

5. Cytochrome P450s

Prior to 2004, the involvement of cytochrome 450s in xenobiotic degradation by *P. chrysosporium* was well-established, but the extent of genetic diversity was not fully appreciated until the genome became available (reviewed by (Syed and Yadav 2012)). Approximately **150 *P. chrysosporium* P450 genes were identified**, and close linkage and tandem arrangements were observed (Martinez et al. 2004). Such organizational tendencies were subsequently shown among many of the 222 and 254 P450 genes of *C. subvermispora* (Fernandez-Fueyo et al. 2012) and *P. placenta* (Martinez et al. 2009) respectively. Most recent analyses of Agaricomycotina genomes reiterate the impressive genetic diversity and complex organization (Floudas et al. 2012). The distribution into families and clans has shown no clear trends related to phylogeny or to ecological role, i.e., brown-rot versus white-rot.

Functional analyses of P450s, especially those derived from *P. chrysosporium*, have advanced significantly in recent years. Syed and coworkers reported the identification and functional characterization of **P450 monooxygenases capable of oxidizing different ring-size PAHs using a genome-to-function strategy** (Syed et al. 2010). A P450 microarray screen (Doddapaneni and Yadav 2005), first identified six P450 genes (Pc-pah1–Pc-pah6) induced by PAHs of varying ring size. The cDNAs of the six P450 monooxygenases were cloned and co-expressed in *Pichia pastoris* along with a P450 reductase partner. Each of the six recombinant P450 monooxygenases showed PAH-oxidizing activity (Syed et al. 2010). In separate studies, the P450 monooxygenase CYP5136A3 showed common responsiveness and catalytic versatility towards endocrine-disrupting alkylphenols and PAHs. The recombinant CYP5136A3 possessed oxidation activity towards alklyphenols with varying alkyl side-chain length (C3–C9), in addition to PAHs (3–4 ring size) (Syed et al. 2011b).

A P450 monooxygenase involved in anthracene metabolism by *P. chrysosporium* was identified by a combination of functional screening and a microarray system (Chigu et al. 2010). A wide variety of compounds were screened, and resulted in characterization of novel cytochrome P450 functions and discovery of a versatile cytochrome P450 that exhibit broad substrate profiles. The authors (Hirosue et al. 2011) suggested that multifunctional properties of the versatile cytochrome P450s would play crucial roles in diversification of fungal metabolic systems involved in xenobiotic degradation.

B. Experimental Systems

Comparative analysis of the genomes of wood decay fungi has provided considerable insight into oxidative systems. Interpretations are relatively clear in some cases, such as the importance of class II ligninolytic peroxidases (LiP, MnP, VP) in white-rot, but not brown-rot, decay. Likewise, the diminished repertoire of cellulases in brown-rot relative to white-rot genomes is consistent with a mechanism of cellulose depolymerization involving hydroxyl radicals. Nevertheless, the roles and interactions of thousands of genes remain uncertain.

High throughput transcriptome and secretome approaches rarely provide complete functional understanding. Instead, the methods allow the number of gene models to be filtered to a more manageable subset that is worthy of more detailed investigations. Whole genome microarrays and RNAseq have been used extensively to assess transcript levels and regulation, principally under conditions favoring lignocellulose degradation. Microarrays representing *P. chrysosporium* P450 genes have identified those induced by various organopollutants (Doddapaneni and Yadav 2005). Proteome analysis has involved mass spectrometry-based identification of 2DE-separated proteins (Abbas et al. 2004; Shimizu et al. 2005; Ravalason et al. 2008; Hori et al. 2011), of peptides tagged for iTRAQ quantitation (Manavalan et al. 2011), and of concentrated total extracellular proteins (Vanden Wymelenberg et al. 2005, 2006a, 2009, 2010, 2011).

A disconcerting aspect of these studies has been the imposing numbers of highly expressed and/or regulated genes encoding proteins of unknown function. Considering *P. chrysosporium* grown under nutrient starvation or in Avicel medium, 193 upregulated genes are predicted to encode 'hypothetical proteins'. Of these, 54 were unambiguously detected in extracellular filtrates by nanoLC-MS/MS (Vanden Wymelenberg et al. 2009). A total of 55, 32, and 14 'unknown proteins' were also identified in cultures of *P. chrysosporium, P. placenta*, and *C. subvermispora* respectively, containing complex lignocellulose substrates. Functional analysis of these hypothetical proteins represents a daunting challenge.

1. Genetic Tools

A major obstacle to research has been the lack of refined genetic tools for functional analysis and, potentially, for strain improvement. In the absence of monokaryons, genome assembly and annotation can be substantially

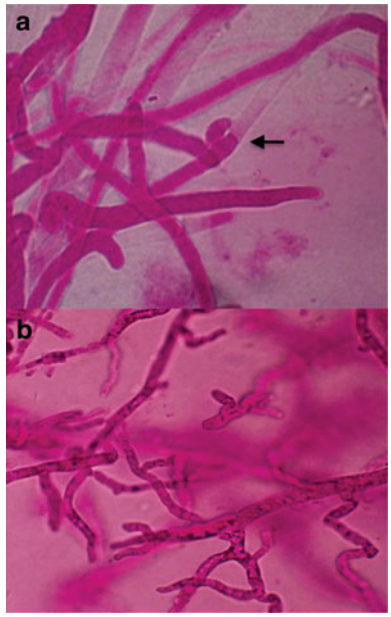

Fig. 5.2. Light microscopy of phloxine-stained *Postia placenta* hyphae. *Panel A*: sequenced parental dikaryon showing a clamp connection (*arrow*) that are commonly, but not always, observed in dikaryons. *Panel B*: typical of monokaryotic derivatives, no clamp connections are observed in the single basidiospore progeny

complicated by dikaryosis (Martinez et al. 2009), the typical nuclear conditions of agaricomycetes (Fig. 5.2). Genetic transformation for *P. chrysosporium* includes auxotroph complementation (Alic et al. 1989, 1990, 1991; Alic 1990; Randall et al. 1991; Akileswaran et al. 1993; Zapanta et al. 1998) and by drug resistance markers (Randall et al. 1989, 1991; Randall and Reddy 1992; Gessner and Raeder 1994). Transformation efficiencies are low, and gene targeting difficult (Alic et al. 1993). Still, reporters for studying gene expression have been described (Gettemy et al. 1997; Birch et al. 1998; Ma et al. 2001), homologous gene expression has proven useful, and RNA interference has been used to suppress Mn-dependent superoxide dismutase gene expression (Matityahu et al. 2008).

Beyond *P. chrysosporium*, ***P. ostreatus* offers transformation protocols** (Yanai et al. 1996; Honda et al. 2000; Irie et al. 2001; Sunagawa and Magae 2002) as well as methodology for physical (Larraya et al. 1999) and genetic mapping (Eichlerova-Volakova and Homolka 1997; Eichlerova and Homolka 1999; Larraya et al. 2000, 2002). *Trametes versicolor* has also been transformed with drug resistance vectors (Bartholomew et al. 2001; Kim et al. 2002), and gene disruptions have been reported (Dumonceaux et al. 2001). Recently, RNAi targeting of *P. ostreatus mnp3* was shown to suppress azo dye decolorization (Salame et al. 2010, 2011). RNAi provides a powerful tool, but suppression is often incomplete and the results further confounded by ectopic integration events.

In contrast to yeasts and many ascomycetes, filamentous basidiomycetes generally give low frequencies of homologous recombination. Recent advances have been made with *C. cinereus* (Nakazawa et al. 2011) and *Schizophyllum commune* (de Jong et al. 2010) strains in which nonhomologous end joining has been impaired by Ku knockouts. This approach substantially enhances the efficiency of gene targeting, but the species are inefficient lignin degraders.

Addressing this issue, **a Δ*ku80* strain has been constructed in *P. ostreatus*** (Salame et al. 2012). The recipient strain is similar to the parent with respect to growth, ligninolytic potential, and mating ability. Gene replacement showed 100 % homologous recombination, and the transformants remained stable in the absence of drug selection (Salame et al. 2012). By inactivation of a VP (*mnp4*), the enzyme was proven to be a major component of the ligninolytic system under Mn limitation. Thus, the system facilitates the efficient gene replacement in *P. ostreatus* and complements RNAi approaches.

2. Biochemical Tools

Efficient heterologous expression systems have been key to advancing our understanding of gene function, especially those encoding low levels of closely related proteins in the native systems. In particular, *E. coli* production and activation of peroxidases have been critical for evaluating catalytic properties (Doyle and Smith 1996; Nie et al. 1998). Similarly, co-expression of membrane-bound P450 monooxygenases with a reductase partner in *Pichia* has been essential for identifying P450s with activity against PAHs and

other compounds (Syed et al. 2010). Garcia-Ruiz and co-workers demonstrated the usefulness of *S. cerevisiae* for directed evolution of *P. eryngii* VP, and achieved improvements in secretion and activity. Additional rounds of evolution have enhanced VP stability in terms of temperature-, peroxide- and alkaline pH-tolerance (Garcia-Ruiz et al. 2012). Also using *S. cerevisiae* as host, directed evolution has improved a basidiomycete laccase. Enzyme stability related to temperature, pH, and organic solvents has been enhanced through a strategy that combines directed evolution with rational approaches (Mate et al. 2010).

IV. Current Research and Future Prospects

The advances made in recent years in biochemistry, genomics, and genome function studies of white-rot fungi provide a large amount of information on the mechanisms of degradation of a wide range of natural and xenobiotic aromatic hazardous compounds.

However, the conversion of this vast theoretical knowledge into practical biotechnology is limited. It is thus challenging to bridge this gap by identifying and resolving bottlenecks. The availability of increasing numbers of fungal genomes is an important step forward, but at the same time presents new challenges related to gene modeling, annotation, and meaningful phylogenetic comparisons. **Functional analyses of the hypothetical proteins remain a particularly daunting task.** Genetic approaches and possibly biochemical analysis of purified protein might be helpful, but the latter approach generally assumes that assays are available.

In addition to new discoveries related to the pathways of xenobiotic degradation, it could be important to identify new genes with traits that can support growth and activity of the fungi under environmental stress, and provide the ability to compete with bacteria. This may help moving from controlled sterile conditions to natural environments. In this connection, metatranscriptomics offer exciting new opportunities for identifying microbes and genes in organopollutant-contaminated soils (Damon et al. 2012; de Menezes et al. 2012).

Such investigations could lead to new strategies for improving the fitness of bioremediation strains via specialized inoculum preparation and/or genetic alterations. The latter might be augmented by altering expression of genes directly involved in xenobiotic degradation, such as specific peroxidases and CyP genes. Further strain improvements might focus on the expression of genes indirectly influencing oxidative enzyme systems. Examples include H_2O_2-generating enzymes that could enhance peroxidase catalysis or supply reactants for Fenton chemistry.

Genome analysis of wood-decay fungi contributes to our fundamental understanding of lignin degradation, a pivotal but incompletely understood, element of the carbon cycle. Ultimately, increasing genome resources will elucidate mechanisms of ligninolysis, and simultaneously serve as a framework for development of effective bioremediation and related bioprocesses.

References

Abbas A, Koc H et al (2004) Fungal degradation of wood: initial proteomic analysis of extracellular proteins of *Phanerochaete chrysosporium* grown on oak substrate. Curr Genet 47:49–56

Akileswaran L, Alic M et al (1993) Isolation and transformation of uracil auxotrophs of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Curr Genet 23:351–356

Alic MM (1990) Mating system and DNA transformation of the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Diss Abstr Int B 51(8):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, New York, pp 319–341

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

Alic M, Clark EK et al (1990) Transformation of *Phanerochaete chrysosporium* and *Neurospora crassa* with adenine biosynthetic genes from *Schizophyllum commune*. Curr Genet 17:305–311

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

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

Alic M, Akileswaran L et al (1997) Characterization of the gene encoding manganese peroxidase isozyme 3 from *Phanerochaete chrysosporium*. Biochim Biophys Acta 1338(1):1–7

Alvarez JM, Canessa P et al (2009) Expression of genes encoding laccase and manganese-dependent peroxidase in the fungus *Ceriporiopsis subvermispora* is mediated by an ACE1-like copper-fist transcription factor. Fungal Genet Biol 46(1):104–111

Amitai G, Adani R et al (1998) Oxidative biodegradation of phosphorothiolates by fungal laccase. FEBS Lett 438(3):195–200

Ander P, Marzullo L (1997) Sugar oxidoreductases and veratryl alcohol oxidase as related to lignin degradation. J Biotechnol 53(2–3):115–131

Artolozaga MJ, Kubatova E et al (1997) Pyranose 2-oxidase from *Phanerochaete chrysosporium*–further biochemical characterisation. Appl Microbiol Biotechnol 47(5):508–514

Asada Y, Watanabe A et al (1995) Purification and characterization of an aryl-alcohol oxidase from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Biosci Biotech Biochem 59:1339–1341

Bao W, Fukushima Y et al (1994) Oxidative degradation of non-phenolic lignin during lipid peroxidation by fungal manganese peroxidase. FEBS Lett 354:297–300

Bartholomew K, Dos Santos G et al (2001) Genetic transformation of *Trametes versicolo*r to phleomycin resistance with the dominant selectable marker shble. Appl Microbiol Biotechnol 56(1–2):201–204

Belinky PA, Flikshtein N et al (2003) Reactive oxygen species and induction of lignin peroxidase in *Phanerochaete chrysosporium*. Appl Environ Microbiol 69(11):6500–6506

Benotti MJ, Trenholm RA et al (2009) Pharmaceuticals and endocrine disrupting compounds in U. S. drinking water. Environ Sci Technol 43 (3):597–603

Bey M, Berrin JG et al (2011) Heterologous expression of *Pycnoporus cinnabarinus* cellobiose dehydrogenase in *Pichia pastoris* and involvement in saccharification processes. Microb Cell Fact 10:113

Bezalel L, Hadar Y et al (1996a) Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62(1):292–295

Bezalel L, Hadar Y et al (1996b) Metabolism of phenanthrene by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62(7):2547–2553

Bezalel L, Hadar Y et al (1997) Enzymatic mechanisms involved in phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 63(7):2495–2501

Birch PR, Sims PF et al (1998) A reporter system for analysis of regulatable promoter functions in the basidiomycete fungus *Phanerochaete chrysosporium*. J Appl Microbiol 85(3):417–424

Blanchette R (1991) Delignification by wood-decay fungi. Ann Rev Phytopath 29:381–398

Bogan B, Lamar R et al (1996a) Fluorene oxidation in vivo by *Phanerochaete chrysosporium* and in vitro during manganese peroxidase-dependent lipid peroxidation. Appl Environ Microbiol 62:1788–1792

Bogan BW, Schoenike B et al (1996b) Expression of *lip* genes during growth in soil and oxidation of anthracene by *Phanerochaete chrysosporium*. Appl Environ Microbiol 62(10):3697–3703

Bogan B, Schoenike B et al (1996c) Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with *Phanerochaete chrysosporium*. Appl Environ Microbiol 62:2381–2386

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

Bourbonnais R, Paice MG et al (1997) Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. Appl Environ Microbiol 63:4627–4632

Bourbonnais R, Leech D et al (1998) Electrochemical analysis of the interactions of laccase mediators with lignin model compounds. Biochim Biophys Acta 1379(3):381–390

Brown JA, Glenn JK et al (1990) Manganese regulates expression of manganese peroxidase by *Phanerochaete chrysosporium*. J Bacteriol 172(6):3125–3130

Brown J, Alic M et al (1991) Manganese peroxidase gene transcription in *Phanerochaete chrysosporium*: activation by manganese. J Bacteriol 173:4101–4106

Brown J, Li D et al (1993) Heat shock induction of manganese peroxidase gene transcription in *Phanerochaete chrysosporium*. Appl Environ Microbiol 59:4295–4299

Bumpus JA (1989) Biodegradation of polycyclic hydrocarbons by *Phanerochaete chrysosporium*. Appl Environ Microbiol 55(1):154–158

Camarero S, Sarkar S et al (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(15):10324–10330

Camarero S, Ibarra D et al (2005) Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. Appl Environ Microbiol 71(4):1775–1784

Casillas RP, Crow SA Jr et al (1996) Initial oxidative and subsequent conjugative metabolites produced during the metabolism of phenanthrene by fungi. J Ind Microbiol 16(4):205–215

Castanera R, Perez G et al (2012) Transcriptional and enzymatic profiling of *Pleurotus ostreatus* laccase genes in submerged and solid-state fermentation cultures. Appl Environ Microbiol 78(11):4037–4045

Chigu NL, Hirosue S et al (2010) Cytochrome P450 monooxygenases involved in anthracene metabolism by

the white-rot basidiomycete Phanerochaete chrysosporium. Appl Microbiol Biotechnol 87(5): 1907–1916

Cohen R, Hadar Y et al (2001) Transcript and activity levels of different *Pleurotus ostreatus* peroxidases are differentially affected by Mn^{2+}. Environ Microbiol 3(5):312–322

Cohen R, Persky L et al (2002a) Mn^{2+} alters peroxidase profiles and lignin degradation by the white-rot fungus Pleurotus ostreatus under different nutritional and growth conditions. Appl Biochem Biotechnol 102–103(1–6):415–429

Cohen R, Yarden O et al (2002b) Lignocellulose affects Mn^{2+} regulation of peroxidase transcript levels in solid-state cultures of *Pleurotus ostreatus*. Appl Environ Microbiol 68(6):3156–3158

Coll P, Tabernero C et al (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 PJ, O'Brien MM et al (1999) Cloning and characterization of a cDNA encoding a novel extracellular peroxidase from *Trametes versicolor*. Appl Environ Microbiol 65(3):1343–1347

Conesa A, van den Hondel CA et al (2000) Studies on the production of fungal peroxidases in *Aspergillus niger*. Appl Environ Microbiol 66(7):3016–3023

Cortes-Espinosa DV, Absalon AE et al (2011) Heterologous expression of manganese peroxidase in *Aspergillus niger* and its effect on phenanthrene removal from soil. J Mol Microbiol Biotechnol 21(3–4):120–129

Cruz-Morato C, Rodriguez-Rodriguez CE et al (2012) Biodegradation of pharmaceuticals by fungi and metabolites identification. In: Vincent T (ed) Emerging organic contaminants in sludges: analysis, fate and biological treatment. Springer, Berlin, pp 1–49

Cullen D (2002) Molecular genetics of lignin-degrading fungi and their application in organopollutant degradation. In: Kempken F (ed) The Mycota, vol XI. Springer, Berlin, pp 71–90

Cullen D, Kersten PJ (2004) Enzymology and molecular biology of lignin degradation. In: Brambl R, Marzulf GA (eds) The Mycota III biochemistry and molecular biology. Springer, Berlin, pp 249–273

Damon C, Lehembre F et al (2012) Metatranscriptomics reveals the diversity of genes expressed by eukaryotes in forest soils. PLoS One 7(1):e28967

Daniel G, Volc J et al (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

Daniel G, Volc J et al (2007) Characteristics of *Gloeophyllum trabeum* alcohol oxidase, an extracellular source of H_2O_2 in brown rot decay of wood. Appl Environ Microbiol 73(19):6241–6253

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

de Jong JF, Ohm RA et al (2010) Inactivation of ku80 in the mushroom-forming fungus *Schizophyllum commune* increases the relative incidence of homologous recombination. FEMS Microbiol Lett 310(1):91–95

de Koker TH, Mozuch MD et al (2004) Pyranose 2-oxidase from *Phanerochaete chrysosporium*: isolation from solid substrate, protein purification, and characterization of gene structure and regulation. Appl Environ Microbiol 70:5794–5800

de Menezes A, Clipson N et al (2012) Comparative metatranscriptomics reveals widespread community responses during phenanthrene degradation in soil. Environ Microbiol 14(9):2577–2588

Dietrich D, Crooks C (2009) Gene cloning and heterologous expression of pyranose 2-oxidase from the brown-rot fungus, *Gloeophyllum trabeum*. Biotechnol Lett 31(8):1223–1228

Doddapaneni H, Yadav JS (2005) Microarray-based global differential expression profiling of P450 monooxygenases and regulatory proteins for signal transduction pathways in the white rot fungus *Phanerochaete chrysosporium*. Mol Genet Genomics 274:454–466

Doyle WA, Smith AT (1996) Expression of lignin peroxidase H8 in *Escherichia coli*: folding and activation of the recombinant enzyme with Ca^{2+} and haem. Biochem J 315(Pt 1):15–19

Dumonceaux TJ, Bartholomew KA et al (1998) Cloning and sequencing of a gene encoding cellobiose dehydrogenase from *Trametes versicolor*. Gene 210:211–219

Dumonceaux T, Bartholomew K et al (2001) Cellobiose dehydrogenase is essential for wood invasion and nonessential for kraft pulp delignification by *Trametes versicolor*. Enzyme Microb Technol 29:478–489

Eastwood DC, Floudas D et al (2011) The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science 333(6043):762–765

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

Eibes GM, Lu-Chau TA et al (2009) Effect of culture temperature on the heterologous expression of *Pleurotus eryngii* versatile peroxidase in *Aspergillus* hosts. Bioprocess Biosyst Eng 32(1):129–134

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(4):321–327

Eichlerova-Volakova I, Homolka L (1997) Variability of ligninolytic enzyme activities in basidiospore isolates of the fungus *Pleurotus ostreatus* in comparison with that of protoplast-derived isolates. Folia Microbiol 42(6):583–588

Eriksson K-EL, Blanchette RA et al (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin

Fernandez-Fueyo E, Ruiz-Duenas FJ et al (2012) Comparative genomics of *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium* provide insight into selective ligninolysis. Proc Natl Acad Sci USA 109(14):5458–5463

Floudas D, Binder M et al (2012) The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336(6089):1715–1719

Ford CI, Walter M et al (2007a) Fungal inoculum properties: extracellular enzyme expression and pentachlorophenol removal by New Zealand *trametes* species in contaminated field soils. J Environ Qual 36(6):1749–1759

Ford CI, Walter M et al (2007b) Fungal inoculum properties: extracellular enzyme expression and pentachlorophenol removal in highly contaminated field soils. J Environ Qual 36(6):1599–1608

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

Gan S, Lau EV et al (2009) Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). J Hazard Mater 172(2–3):532–549

Garcia-Ruiz E, Gonzalez-Perez D et al (2012) Directed evolution of a temperature-, peroxide- and alkaline pH-tolerant versatile peroxidase. Biochem J 441(1):487–498

Gaskell J, Stewart P et al (1994) Establishment of genetic linkage by allele-specific polymerase chain reaction: application to the lignin peroxidase gene family of *Phanerochaete chrysosporium*. Biotechnology 12:1372–1375

Gasser G, Rona M et al (2010) Quantitative evaluation of tracers for quantification of wastewater contamination of potable water sources. Environ Sci Technol 44(10):3919–3925

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

Gessner M, Raeder U (1994) A histone promoter for expression of a phleomycin-resistance gene in *Phanerochaete chrysosporium*. Gene 142:237–241

Gettemy JM, Li D et al (1997) Truncated-gene reporter system for studying the regulation of manganese peroxidase expression. Curr Genet 31(6):519–524

Gettemy JM, Ma B et al (1998) Reverse transcription-PCR analysis of the regulation of the manganese peroxidase gene family. Appl Environ Microbiol 64(2):569–574

Giardina P, Palmieri G et al (1999) Protein and gene structure of a blue laccase from *Pleurotus ostreatus1*. Biochem J 341(Pt 3):655–663

Giardina P, Faraco V et al (2010) Laccases: a never-ending story. Cell Mol Life Sci 67(3):369–385

Glenn JK, Gold MH (1983) Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 45(6):1741–1747

Glenn JK, Morgan MA et al (1983) An extracellular H_20_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 et al (1986) Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. Arch Biochem Biophys 251:688–696

Godfrey BJ, Mayfield MB et al (1990) Characterization of a gene encoding a manganese peroxidase from *Phanerochaete chrysosporium*. Gene 93(1):119–124

Godfrey B, Akileswaran L et al (1994) A reporter gene construct for studying the regulation of manganese peroxidase gene expression. Appl Environ Microbiol 60:1353–2358

Golan-Rozen N, Chefetz B et al (2011) Transformation of the recalcitrant pharmaceutical compound carbamazepine by *Pleurotus ostreatus*: role of cytochrome P450 monooxygenase and manganese peroxidase. Environ Sci Technol 45(16):6800–6805

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

Grassi E, Scodeller P et al (2011) Potential of *Trametes trogii* culture fluids and its purified laccase for the decolorization of different types of recalcitrant dyes without the addition of redox mediators. Biodeterior Biodegradation 65:635–643

Grigoriev IV, Nordberg H et al (2012) The genome portal of the Department of Energy Joint Genome Institute. Nucleic Acids Res 40(Database issue): D26–D32

Guo M, Lu F et al (2008) Purification of recombinant laccase from *Trametes versicolor* in *Pichia methanolica* and its use for the decolorization of anthraquinone dye. Biotechnol Lett 30(12):2091–2096

Gutierrez A, Babot ED et al (2011) Regioselective oxygenation of fatty acids, fatty alcohols and other aliphatic compounds by a basidiomycete heme-thiolate peroxidase. Arch Biochem Biophys 514(1–2):33–43

Hallberg BM, Bergfors T et al (2000) A new scaffold for binding haem in the cytochrome domain of the extracellular flavocytochrome cellobiose dehydrogenase. Structure Fold Des 8(1):79–88

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: Young LY, Cerniglia CE (eds) Microbial transformation and degradation of toxic organic chemical. Wiley-Liss, New York, pp 331–346

Hammel KE, Cullen D (2008) Role of fungal peroxidases in biological ligninolysis. Curr Opin Plant Biol 11(3):349–355

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

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

Hammel KE, Gai WZ et al (1992) Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 58(6):1832–1838

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169(1–3):1–15

Harris PV, Welner D et al (2010) Stimulation of lignocellulosic biomass hydrolysis by proteins of glycoside hydrolase family 61: structure and function of a large, enigmatic family. Biochemistry 49(15):3305–3316

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

Hata T, Shintate H et al (2010) Elimination of carbamazepine by repeated treatment with laccase in the presence of 1-hydroxybenzotriazole. J Hazard Mater 181(1–3):1175–1178

Heberer T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 131(1–2):5–17

Heinfling A, Martinez MJ et al (1998a) Purification and characterization of peroxidases from the dye-decolorizing fungus *Bjerkandera adusta*. FEMS Microbiol Lett 165(1):43–50

Heinfling A, Martinez MJ et al (1998b) Transformation of industrial dyes by manganese peroxidases from *Bjerkandera adusta* and *Pleurotus eryngii* in a manganese-independent reaction. Appl Environ Microbiol 64(8):2788–2793

Henriksson G, Johansson G et al (2000) A critical review of cellobiose dehydrogenases. J Biotechnol 78(2):93–113

Hernandez-Ortega A, Ferreira P et al (2012) Fungal aryl-alcohol oxidase: a peroxide-producing flavoenzyme involved in lignin degradation. Appl Microbiol Biotechnol 93(4):1395–1410

Higham CW, Gordon-Smith D et al (1994) Direct 1H NMR evidence for conversion of beta-D-cellobiose to cellobionolactone by cellobiose dehydrogenase from *Phanerochaete chrysosporium*. FEBS Lett 351 (1):128–132

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 24(1):23–63

Hirosue S, Tazaki M et al (2011) Insight into functional diversity of cytochrome P450 in the white-rot basidiomycete *Phanerochaete chrysosporium*: involvement of versatile monooxygenase. Biochem Biophys Res Commun 407(1):118–123

Hiscox J, Baldrian P et al (2010) Changes in oxidative enzyme activity during interspecific mycelial interactions involving the white-rot fungus *Trametes versicolor*. Fungal Genet Biol 47(6):562–571

Hoegger PJ, Kilaru S et al (2006) Phylogenetic comparison and classification of laccase and related multicopper oxidase protein sequences. FEBS J 273 (10):2308–2326

Hofrichter M, Ullrich R et al (2010) New and classic families of secreted fungal heme peroxidases. Appl Microbiol Biotechnol 87(3):871–897

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

Honda Y, Matsuyama T et al (2000) Carboxin resistance transformation of the homobasidiomycete fungus *Pleurotus ostreatus*. Curr Genet 37(3):209–212

Hori C, Igarashi K et al (2011) Effects of xylan and starch on secretome of the basidiomycete *Phanerochaete chrysosporium* grown on cellulose. FEMS Microbiol Lett 321(1):14–23

Hsu CA, Wen TN et al (2012) Biological degradation of anthroquinone and azo dyes by a novel laccase from *Lentinus* sp. Environ Sci Technol 46 (9):5109–5117

Hundt K, Martin D et al (2000) Transformation of triclosan by *Trametes versicolor* and *Pycnoporus cinnabarinus*. Appl Environ Microbiol 66(9):4157–4160

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

Irie T, Honda Y et al (2001) Efficient transformation of filamentous fungus *Pleurotus ostreatus* using single-strand carrier DNA. Appl Microbiol Biotechnol 55(5):563–565

Janse BJH, Gaskell J et al (1998) Expression of *Phanerochaete chrysosporium* genes encoding lignin peroxidases, manganese peroxidases, and glyoxal oxidase in wood. Appl Environ Microbiol 64 (9):3536–3538

Johannes C, Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl Environ Microbiol 66(2):524–528

Johannes C, Majcherczyk A et al (1996) Degradation of anthracene by laccase of *Trametes versicolor* in the presence of different mediator compounds. Appl Microbiol Biotechnol 46(3):313–317

Johansson T, Nyman PO et al (2002) Differential regulation of mnp2, a new manganese peroxidase-encoding gene from the ligninolytic fungus

Trametes versicolor PRL 572. Appl Environ Microbiol 68(4):2077–2080

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

Johnson T, Pease E et al (1992) Production and characterization of recombinant lignin peroxidase isozyme H2 from *Phanerochaete chrysosporium* using recombinant baculovirus. Arch Biochem Biophys 296:660–666

Kang SI, Kang SY et al (2008) Identification of fungal metabolites of anticonvulsant drug carbamazepine. Appl Microbiol Biotechnol 79(4):663–669

Kapich AN, Jensen KA et al (1999) Peroxyl radicals are potential agents of lignin biodegradation. FEBS Lett 461(1–2):115–119

Karahanian E, Corsini G et al (1998) Structure and expression of a laccase gene from the ligninolytic basidiomycete *Ceriporiopsis subvermispora*. Biochim Biophys Acta 1443(1–2):65–74

Kaushik P, Malik A (2009) Fungal dye decolourization: recent advances and future potential. Environ Int 35(1):127–141

Kawai S, Umezawa T et al (1988) Degradation mechanisms of phenolic b-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(1):269–274

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

Kersten PJ (1990) Glyoxal oxidase of *Phanerochaete chrysosporium*: its characterization and activation by lignin peroxidase. Proc Natl Acad Sci USA 87 (8):2936–2940

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, 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 et al (1985) The ligninase of *Phanerochaete chrysosporium* generates cation radicals from methoxybenzenes. J Biol Chem 260:2609–2612

Kersten PJ, Witek C et al (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

Kim K, Leem Y et al (2002) Transformation of the medicinal basidiomycete *Trametes versicolor* to hygromycin B resistance by restriction enzyme mediated integration. FEMS Microbiol Lett 209 (2):273–276

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

Kojima Y, Tsukuda Y et al (1990) Cloning, sequence analysis, and expression of ligninolytic phenoloxidase genes of the white-rot basidiomycete *Coriolus hirsutus*. J Biol Chem 256:15224–15230

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

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 et al (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(4):433–440

Lamar RT, Larsen MJ et al (1990b) Sensitivity to and degradation of pentachlorophenol by *Phanerochaete* spp. Appl Environ Microbiol 56(11):3519–3526

Lamar RT, Davis MW et al (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 et al (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

Langston JA, Shaghasi T et al (2011) Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61. Appl Environ Microbiol 77(19):7007–7015

Larraya LM, Perez G et al (1999) Molecular karyotype of the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 65(8):3413–3417

Larraya LM, Perez G et al (2000) Genetic linkage map of the edible basidiomycete *Pleurotus ostreatus*. Appl Environ Microbiol 66(12):5290–5300

Larraya LM, Idareta E et al (2002) Quantitative trait loci controlling vegetative growth rate in the edible basidiomycete *Pleurotus ostreatus*. Appl Environ Microbiol 68(3):1109–1114

Larrondo LF, Lobos S et al (2001) Isoenzyme multiplicity and characterization of recombinant manganese peroxidases from *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium*. Appl Environ Microbiol 67(5):2070–2075

Larrondo LF, Avila M et al (2003) Heterologous expression of laccase cDNA from *Ceriporiopsis*

subvermispora yields copper-activated apoprotein and complex isoform patterns. Microbiology 149 (Pt 5):1177–1182

Larrondo L, Vicuna R et al (2005) *Phanerochaete chrysosporium* genomics. In: Arora DK, Berka R (eds) Applied mycology and biotechnology, vol 5. Elsevier, Amsterdam, pp 315–352

Leclercq M, Mathieu O et al (2009) Presence and fate of carbamazepine, oxcarbazepine, and seven of their metabolites at wastewater treatment plants. Arch Environ Contam Toxicol 56(3):408–415

Lestan D, Lamar RT (1996) Development of fungal inocula for bioaugmentation of contaminated soils. Appl Environ Microbiol 62(6):2045–2052

Li B, Nagalla SR et al (1996) Cloning of a cDNA encoding cellobiose dehydrogenase, a hemoflavoenzyme from *Phanerochaete chrysosporium*. Appl Environ Microbiol 62(4):1329–1335

Li B, Rotsaert FA et al (2000) Homologous expression of recombinant cellobiose dehydrogenase in *Phanerochaete chrysosporium*. Biochem Biophys Res Commun 270(1):141–146

Lienert J, Gudel K et al (2007) Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. Environ Sci Technol 41 (12):4471–4478

Liers C, Bobeth C et al (2010) DyP-like peroxidases of the jelly fungus *Auricularia auricula-judae* oxidize nonphenolic lignin model compounds and high-redox potential dyes. Appl Microbiol Biotechnol 85(6):1869–1879

Lobos S, Larrain J et al (1994) Isozymes of manganese-dependent peroxidase and laccase produced by the lignin-degrading basidiomycete *Ceriporiopsis subvermispora*. Microbiology 140:2691–2698

Lu L, Zhao M et al (2009) Production and synthetic dyes decolourization capacity of a recombinant laccase from *Pichia pastoris*. J Appl Microbiol 107 (4):1149–1156

Lu XY, Zhang T et al (2011) Bacteria-mediated PAH degradation in soil and sediment. Appl Microbiol Biotechnol 89(5):1357–1371

Lundell TK, Makela MR et al (2010) Lignin-modifying enzymes in filamentous basdiomycetes-ecological, functional and phylogenetic review. J Basic Microbiol 50:4–20

Ma B, Mayfield MB et al (2001) The green fluorescent protein gene functions as a reporter of gene expression in *Phanerochaete chrysosporium*. Appl Environ Microbiol 67(2):948–955

Ma B, Mayfield MB et al (2003) Homologous expression of *Phanerochaete chrysosporium* manganese peroxidase, using bialaphos resistance as a dominant selectable marker. Curr Genet 43(6):407–414

Ma B, Mayfield MB et al (2004) Novel promoter sequence required for manganese regulation of manganese peroxidase isozyme 1 gene expression in *Phanerochaete chrysosporium*. Eukaryot Cell 3(3):579–588

Macdonald J, Master ER (2012) Time-dependent profiles of transcripts encoding lignocellulose-modifying enzymes of the white rot fungus *Phanerochaete carnosa* grown on multiple wood substrates. Appl Environ Microbiol 78(5):1596–1600

Macdonald J, Doering M et al (2011) Transcriptomic responses of the softwood-degrading white-rot fungus *Phanerochaete carnosa* during growth on coniferous and deciduous wood. Appl Environ Microbiol 77:3211–3218

MacDonald J, Suzuki H et al (2012) Expression and regulation of genes encoding lignocellulose-degrading activity in the genus *Phanerochaete*. Appl Microbiol Biotechnol 94(2):339–351

Manavalan A, Adav SS et al (2011) iTRAQ-based quantitative secretome analysis of *Phanerochaete chrysosporium*. J Proteomics 75(2):642–654

Mancilla RA, Canessa P et al (2010) Effect of manganese on the secretion of manganese-peroxidase by the basidiomycete *Ceriporiopsis subvermispora*. Fungal Genet Biol 47(7):656–661

Marco-Urrea E, Perez-Trujillo M et al (2009) Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by *Trametes versicolor*. Chemosphere 74(6):765–772

Marco-Urrea E, Radjenovic J et al (2010) Oxidation of atenolol, propranolol, carbamazepine and clofibric acid by a biological Fenton-like system mediated by the white-rot fungus *Trametes versicolor*. Water Res 44(2):521–532

Martinez D, Larrondo LF et al (2004) Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. Nat Biotechnol 22:695–700

Martinez D, Challacombe J et al (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. Proc Natl Acad Sci USA 106(6):1954–1959

Masapahy S, Lamb DC et al (1999) Purification and characterization of a benzo[a]pyrene hydroxylase from *Pleurotus pulmonarius*. Biochem Biophys Res Commun 266(2):326–329

Mate D, Garcia-Burgos C et al (2010) Laboratory evolution of high-redox potential laccases. Chem Biol 17(9):1030–1041

Matityahu A, Hadar Y et al (2008) Gene silencing by RNA Interference in the white rot fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 74 (17):5359–5365

Mester 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

Miao XS, Yang JJ et al (2005) Carbamazepine and its metabolites in wastewater and in biosolids in a

municipal wastewater treatment plant. Environ Sci Technol 39(19):7469–7475
Miele A, Giardina P et al (2010) Random mutants of a *Pleurotus ostreatus* laccase as new biocatalysts for industrial effluents bioremediation. J Appl Microbiol 108(3):998–1006
Miki Y, Morales M et al (2009) *Escherichia coli* expression and in vitro activation of a unique ligninolytic peroxidase that has a catalytic tyrosine residue. Protein Expr Purif 68(2):208–214
Mileski GJ, Bumpus JA et al (1988) Biodegradation of pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 54(12):2885–2889
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
Montiel-Gonzalez AM, Fernandez FJ et al (2009) Increased PCP removal by *Amylomyces rouxii* transformants with heterologous *Phanerochaete chrysosporium* peroxidases supplementing their natural degradative pathway. Appl Microbiol Biotechnol 84(2):335–340
Moukha SM, Dumonceaux TJ et al (1999) Cloning and analysis of *Pycnoporus cinnabarinus* cellobiose dehydrogenase. Gene 234(1):23–33
Muheim A, Leisola MSA et al (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(2–3):159–167
Nakazawa T, Ando Y et al (2011) Efficient gene targeting in DeltaCc.ku70 or DeltaCc.lig4 mutants of the agaricomycete *Coprinopsis cinerea*. Fungal Genet Biol 48(10):939–946
Nie G, Reading NS et al (1998) Expression of the lignin peroxidase H2 gene from *Phanerochaete chrysosporium* in *Escherichia coli*. Biochem Biophys Res Commun 249(1):146–150
Nishimura I, Okada K et al (1996) Cloning and expression of pyranose oxidase cDNA from *Coriolus versicolor* in *E. coli*. J Biotechnol 52:11–20
Ohm RA, de Jong JF et al (2010) Genome sequence of the model mushroom *Schizophyllum commune*. Nat Biotechnol 28(9):957–963
Olson A, Aerts A et al (2012) Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytol 194(4):1001–1013
Orth A, Royse D et al (1993) Ubiquity of lignin-degrading peroxidases among various wood-degrading fungi. Appl Environ Microbiol 59:4017–4023
Orth A, Rzhetskaya M et al (1994) Characterization of a cDNA encoding a manganese peroxidase from *Phanerochaete chrysosporium*: genomic organization of lignin and manganese peroxidase genes. Gene 148:161–165
Palmieri G, Giardina P et al (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Appl Environ Microbiol 66 (3):920–924
Paszczynski A, Huynh V-B et al (1986) Comparison of ligninase-I and peroxidase-M2 from the white-rot fungus *Phanerochaete chrysosporium*. Arch Biochem Biophys 244:750–765
Pease E, Tien M (1992) Heterogeneity and regulation of manganese peroxidases from *Phanerochaete chrysosporium*. J Bacteriol 174:3532–3540
Pease EA, Andrawis A et al (1989) Manganese-dependent peroxidase from *Phanerochaete chrysosporium*. Primary structure deduced from complementary DNA sequence. J Biol Chem 264 (23):13531–13535
Peng RH, Xiong AS et al (2008) Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol Rev 32(6):927–955
Perie FH, Reddy GV et al (1998) Purification and characterization of laccases from the white-rot basidiomycete *Dichomitus squalens*. Arch Biochem Biophys 353(2):349–355
Pickard MA, Roman R et al (1999) Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by *Coriolopsis gallica* UAMH 8260 laccase. Appl Environ Microbiol 65(9):3805–3809
Pisanelli I, Kujawa M et al (2009) Pyranose 2-oxidase from *Phanerochaete chrysosporium*–expression in *E. coli* and biochemical characterization. J Biotechnol 142(2):97–106
Piscitelli A, Del Vecchio C et al (2011a) Fungal laccases: versatile tools for lignocellulose transformation. C R Biol 334(11):789–794
Piscitelli A, Giardina P et al (2011b) Induction and transcriptional regulation of laccases in fungi. Curr Genomics 12(2):104–112
Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57(1–2):20–33
Pribnow D, Mayfield MB et al (1989) Characterization of a cDNA encoding a manganese peroxidase, from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J Biol Chem 264(9):5036–5040
Quinlan RJ, Sweeney MD et al (2011) Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. Proc Natl Acad Sci USA 108(37):15079–15084
Raices M, Paifer E et al (1995) Cloning and characterization of a cDNA encoding a cellobiose dehydrogenase from the white rot fungus *Phanerochaete chrysosporium*. FEBS Lett 369(2–3):233–238
Randall TA, Reddy CA (1992) The nature of extrachromosomal maintenance of transforming plasmids in the filamentous basidiomycete *Phanerochaete chrysosporium*. Curr Genet 21:255–260
Randall T, Rao TR et al (1989) Use of a shuttle vector for the transformation of the white-rot basidiomycete, *Phanerochaete chrysosporium*. Biochem Biophys Res Commun 161:720–725

Randall T, Reddy CA et al (1991) A novel extrachromosomally maintained transformation vector for the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. J Bacteriol 173(2):776–782

Ravalason H, Jan G et al (2008) Secretome analysis of *Phanerochaete chrysosporium* strain CIRM-BRFM41 grown on softwood. Appl Microbiol Biotechnol 80(4):719–733

Reddy GV, Gold MH (1999) A two-component tetrachlorohydroquinone reductive dehalogenase system from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Biochem Biophys Res Commun 257(3):901–905

Reddy GV, Gold MH (2000) Degradation of pentachlorophenol by *Phanerochaete chrysosporium*: intermediates and reactions involved. Microbiology 146(Pt 2):405–413

Reddy GV, Gelpke MD et al (1998) Degradation of 2,4,6-trichlorophenol by *Phanerochaete chrysosporium*: involvement of reductive dechlorination. J Bacteriol 180(19):5159–5164

Reiser J, Walther I et al (1993) Methods to investigate the expression of lignin peroxidase genes by the white-rot fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 59:2897–2903

Rodriguez-Rodriguez CE, Marco-Urrea E et al (2010) Degradation of naproxen and carbamazepine in spiked sludge by slurry and solid-phase *Trametes versicolor* systems. Bioresour Technol 101(7):2259–2266

Ruiz-Duenas FJ, Martinez MJ et al (1999) Heterologous expression of *Pleurotus eryngii* peroxidase confirms its ability to oxidize Mn(2+) and different aromatic substrates. Appl Environ Microbiol 65 (10):4705–4707

Ruiz-Duenas FJ, Morales M et al (2008) Site-directed mutagenesis of the catalytic tryptophan environment in *Pleurotus eryngii* versatile peroxidase. Biochemistry 47(6):1685–1695

Ruiz-Duenas FJ, Morales M et al (2009) Substrate oxidation sites in versatile peroxidase and other basidiomycete peroxidases. J Exp Bot 60(2):441–452

Ruiz-Duenas FJ, Fernandez E et al (2011) *Pleurotus ostreatus* heme peroxidases: an in silico analysis from the genome sequence to the enzyme molecular structure. C R Biol 334(11):795–805

Ruttimann-Johnson C, Lamar RT (1996) Polymerization of pentachlorophenol and ferulic acid by fungal extracellular lignin-degrading enzymes. Appl Environ Microbiol 62(10):3890–3893

Ryu K, Hwang SY et al (2008a) Functionality improvement of fungal lignin peroxidase by DNA shuffling for 2,4-dichlorophenol degradability and H2O2 stability. J Biotechnol 133(1):110–115

Ryu K, Kang JH et al (2008b) Expression in yeast of secreted lignin peroxidase with improved 2,4-dichlorophenol degradability by DNA shuffling. J Biotechnol 135(3):241–246

Sakamoto T, Kitaura H et al (2010) Transcriptional effect of a calmodulin inhibitor, W-7, on the ligninolytic enzyme genes in *Phanerochaete chrysosporium*. Curr Genet 56(5):401–410

Salame TM, Yarden O et al (2010) *Pleurotus ostreatus* manganese-dependent peroxidase silencing impairs decolourization of Orange II. Microb Biotechnol 3(1):93–106

Salame TM, Ziv C et al (2011) RNAi as a potential tool for biotechnological applications in fungi. Appl Microbiol Biotechnol 89(3):501–512

Salame TM, Knop D et al (2012) Predominance of a Versatile-Peroxidase-Encoding Gene, *mnp4*, as Demonstrated by Gene Replacement via a Gene Targeting System for *Pleurotus ostreatus*. Appl Environ Microbiol 78(15):5341–5352

Salas C, Lobos S et al (1995) Properties of laccase isoenzymes produced by the basidiomycete *Ceriporiopsis subvermispora*. Biotechnol Appl Biochem 21:323–333

Saloheimo M, Niku-Paavola M et al (1991) Isolation and structural analysis of the laccase gene from the lignin-degrading fungus *Phlebia radiata*. J Gen Microbiol 137:1537–1544

Sannia G, Limongi P et al (1991) Purification and characterization of a veratryl alcohol oxidase enzyme from the lignin degrading basidiomycete *Pleurotus ostreatus*. Biochim Biophys Acta 1073:114–119

Sato S, Feltus FA et al (2009) The first genome-level transcriptome of the wood-degrading fungus *Phanerochaete chrysosporium* grown on red oak. Curr Genet 55(3):273–286

Scheibner K, Hofrichter M (1998) Conversion of aminonitrotoluenes by fungal manganese peroxidase. J Basic Microbiol 38(1):51–59

Shimizu M, Yuda N et al (2005) Metabolic regulation at the tricarboxylic acid and glyoxylate cycles of the lignin-degrading basidiomycete *Phanerochaete chrysosporium* against exogenous addition of vanillin. Proteomics 5(15):3919–3931

Shoemaker HE, Harvey PJ et al (1985) On the mechanism of enzymatic lignin breakdown. FEBS Lett 183:7–12

Singh K, Arora S (2011) Removal of synthetic textile dyes from wastewaters: a critical review on present treatment technologies. Crit Rev Environ Sci Technol 41:807–878

Smith M, Shnyreva A et al (1998) Tandem organization and highly disparate expression of the two laccase genes *lcc1* and *lcc2* in the cultivated mushroom *Agaricus bisporus*. Microbiology 144(Pt 4):1063–1069

Sollewijn Gelpke MD, Mayfield-Gambill M et al (1999) Homologous expression of recombinant lignin peroxidase in *Phanerochaete chrysosporium*. Appl Environ Microbiol 65(4):1670–1674

Sollewijn Gelpke MD, Lee J et al (2002) Lignin peroxidase oxidation of veratryl alcohol: effects of the mutants H82A, Q222A, W171A, and F267L. Biochemistry 41(10):3498–3506

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, Kersten P et al (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 174:5036–5042

Stewart P, Whitwam RE et al (1996) Efficient expression of a *Phanerochaete chrysosporium* manganese peroxidase gene in *Aspergillus oryzae*. Appl Environ Microbiol 62(3):860–864

Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56(1–2):69–80

Stuardo M, Vasquez M et al (2004) Molecular approach for analysis of model fungal genes encoding ligninolytic peroxidases in wood-decaying soil systems. Lett Appl Microbiol 38(1):43–49

Sucharitakul J, Wongnate T et al (2011) Hydrogen peroxide elimination from C4a-hydroperoxyflavin in a flavoprotein oxidase occurs through a single proton transfer from flavin N5 to a peroxide leaving group. J Biol Chem 286(19):16900–16909

Sugano Y, Nakano R et al (2000) Efficient heterologous expression in *Aspergillus oryzae* of a unique dye-decolorizing peroxidase, DyP, of *Geotrichum candidum*. Appl Environ Microbiol 66(4):1754–1758

Sunagawa M, Magae Y (2002) Transformation of the edible mushroom *Pleurotus ostreatus* by particle bombardment. FEMS Microbiol Lett 211 (2):143–146

Syed K, Yadav JS (2012) P450 monooxygenases (P450ome) of the model white rot fungus *Phanerochaete chrysosporium*. Crit Rev Microbiol 38 (4):339–363

Syed K, Doddapaneni H et al (2010) Genome-to-function characterization of novel fungal P450 monooxygenases oxidizing polycyclic aromatic hydrocarbons (PAHs). Biochem Biophys Res Commun 399(4):492–497

Syed K, Kattamuri C et al (2011a) Cytochrome b(5) reductase-cytochrome b(5) as an active P450 redox enzyme system in *Phanerochaete chrysosporium*: atypical properties and in vivo evidence of electron transfer capability to CYP63A2. Arch Biochem Biophys 509(1):26–32

Syed K, Porollo A et al (2011b) A fungal P450 (CYP5136A3) capable of oxidizing polycyclic aromatic hydrocarbons and endocrine disrupting alkylphenols: role of Trp(129) and Leu(324). PLoS One 6(12):e28286

Tan TC, Pitsawong W et al (2010) H-bonding and positive charge at the N5/O4 locus are critical for covalent flavin attachment in *Trametes* pyranose 2-oxidase. J Mol Biol 402(3):578–594

Tan TC, Haltrich D et al (2011) Regioselective control of beta-d-glucose oxidation by pyranose 2-oxidase is intimately coupled to conformational degeneracy. J Mol Biol 409(4):588–600

Temp U, Zierold U et al (1999) Cloning and characterization of a second laccase gene from the lignin-degrading basidiomycete *Pycnoporus cinnabarinus*. Gene 236(1):169–177

Teunissen PJ, Sheng D et al (1998) 2-Chloro-1,4-dimethoxybenzene cation radical: formation and role in the lignin peroxidase oxidation of anisyl alcohol. Arch Biochem Biophys 360(2):233–238

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

Tien M, Kirk TK (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proc Natl Acad Sci USA 81:2280–2284

Tsukihara T, Honda Y et al (2006) Exclusive overproduction of recombinant versatile peroxidase MnP2 by genetically modified white rot fungus, *Pleurotus ostreatus*. J Biotechnol 126(4):431–439

Tsukihara T, Honda Y et al (2008) Mechanism for oxidation of high-molecular-weight substrates by a fungal versatile peroxidase, MnP2. Appl Environ Microbiol 74(9):2873–2881

Tuor U, Wariishii H et al (1992) Oxidation of phenolic b-aryl ether lignin model compounds by manganese peroxidase from *Phanerochaete chrysosporium:* oxidative cleavage of an a-carbonyl model compound. Biochemistry 31:4986–4995

Ullrich R, Hofrichter M (2005) The haloperoxidase of the agaric fungus *Agrocybe aegerita* hydroxylates toluene and naphthalene. FEBS Lett 579(27):6247–6250

Valli K, Gold MH (1991) Degradation of 2,4-dichlorophenol by the lignin-degrading fungus *Phanerochaete chrysosporium*. J Bacteriol 173(1):345–352

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

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

Vallim MA, Janse BJ et al (1998) *Phanerochaete chrysosporium* cellobiohydrolase and cellobiose dehydrogenase transcripts in wood. Appl Environ Microbiol 64(5):1924–1928

Van Aken B, Hofrichter M et al (1999) Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete *Phlebia radiata*. Biodegradation 10(2):83–91

Vanden Wymelenberg A, Sabat G et al (2005) The *Phanerochaete chrysosporium* secretome: database predictions and initial mass spectrometry peptide identifications in cellulose-grown medium. J Biotechnol 118(1):17–34

Vanden Wymelenberg A, Minges P et al (2006a) Computational analysis of the *Phanerochaete chrysosporium* v2.0 genome database and mass spectrometry identification of peptides in ligninolytic cultures reveals complex mixtures of secreted proteins. Fungal Genet Biol 43:343–356

Vanden Wymelenberg A, Sabat G et al (2006b) Structure, organization, and transcriptional regulation of a family of copper radical oxidase genes in the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 72:4871–4877

Vanden Wymelenberg A, Gaskell J et al (2009) Transcriptome and secretome analysis of *Phanerochaete chrysosporium* reveal complex patterns of gene expression. Appl Environ Microbiol 75:4058–4068

Vanden Wymelenberg A, Gaskell J et al (2010) Comparative transcriptome and secretome analysis of wood decay fungi *Postia placenta* and *Phanerochaete chrysosporium*. Appl Environ Microbiol 76:3599–3610

Vanden Wymelenberg A, Gaskell J et al (2011) Significant alteration of gene expression in wood decay fungi *Postia placenta* and *Phanerochaete chrysosporium* by plant species. Appl Environ Microbiol 77(13):4499–4507

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

Wahleithmer JA, Xu F et al (1995) The identification and characterization of four laccase genes from the plant pathogenic fungus *Rhizoctonia solani*. Curr Genet 29:395–403

Wang W, Wen X (2009) Expression of lignin peroxidase H2 from *Phanerochaete chrysosporium* by multicopy recombinant *Pichia* strain. J Environ Sci (China) 21(2):218–222

Wang H, Lu F et al (2004) Heterologous expression of lignin peroxidase of *Phanerochaete chrysosporium* in *Pichia methanolica*. Biotechnol Lett 26(20):1569–1573

Wariishi H, Valli K et al (1991) In vitro depolymerization of lignin by manganese peroxidase of *Phanerochaete chrysosporium*. Biochem Biophys Res Comm 176:269–275

Watanabe T, Tsuda S et al (2010) Characterization of a Delta12-fatty acid desaturase gene from *Ceriporiopsis subvermispora*, a selective lignin-degrading fungus. Appl Microbiol Biotechnol 87(1):215–224

Wesenberg D, Kyriakides I et al (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 22(1–2):161–187

Westereng B, Ishida T et al (2011) The putative endoglucanase PcGH61D from *Phanerochaete chrysosporium* is a metal-dependent oxidative enzyme that cleaves cellulose. PLoS One 6(11): e27807

Whittaker J (2002) Galactose oxidase. Adv Protein Chem 60:1–49

Whittaker MM, Kersten PJ et al (1996) Glyoxal oxidase from *Phanerochaete chrysosporium* is a new radical-copper oxidase. J Biol Chem 271(2):681–687

Whittaker MM, Kersten PJ et al (1999) Identification of catalytic residues in glyoxal oxidase by targeted mutagenesis. J Biol Chem 274(51):36226–36232

Wong KS, Huang Q et al (2012) Biodegradation of dyes and polyaromatic hydrocarbons by two allelic forms of *Lentinula edodes* laccase expressed from *Pichia pastoris*. Bioresour Technol 104:157–164

Wongnate T, Sucharitakul J et al (2011) Identification of a catalytic base for sugar oxidation in the pyranose 2-oxidase reaction. Chembiochem 12 (17):2577–2586

Yanai K, Yonekura K et al (1996) The integrative transformation of *Pleurotus ostreatus* using bialaphos resistance as a dominant selectable marker. Biosci Biotechnol Biochem 60(3):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 et al (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, Overjero MD et al (1999) Molecular characterization of laccase genes from the basidiomycete *Coprinus cinereus* and heterologous expression of the laccase *lcc1*. Appl Environ Microbiol 65 (11):4943–4948

Yoshida M, Ohira T et al (2001) Production and characterization of recombinant *Phanerochaete chrysosporium* cellobiose dehydrogenase in the methylotrophic yeast *Pichia pastoris*. Biosci Biotechnol Biochem 65(9):2050–2057

Zamocky M, Ludwig R et al (2006) Cellobiose dehydrogenase–a flavocytochrome from wood-degrading, phytopathogenic and saprotropic fungi. Curr Protein Pept Sci 7(3):255–280

Zamocky M, Schumann C et al (2008) Cloning, sequence analysis and heterologous expression in *Pichia pastoris* of a gene encoding a thermostable cellobiose dehydrogenase from *Myriococcum thermophilum*. Protein Expr Purif 59(2):258–265

Zapanta LS, Hattori T et al (1998) Cloning of *Phanerochaete chrysosporium leu2* by complementation of bacterial auxotrophs and transformation of fungal auxotrophs. Appl Environ Microbiol 64 (7):2624–2629