Chapter 13
Recent Advances on the Genomics of Litter- and Soil-Inhabiting Agaricomycetes

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13.1 Introduction

Woody biomass makes up the major portion of terrestrial carbon, and forest ecosystems contain enormous reservoirs of lignocellulose belowground, in dead trees, and litter. Decomposition of this recalcitrant material and mobilization of nutrients are essential for forest health [reviewed by Boddy and Watkinson (1995)]. Although mechanisms are incompletely understood, initial decomposition of lignocellulose is efficiently carried out by certain filamentous fungi, and the genomes of representative species have been recently sequenced. This review covers these genome studies and the insight they provide regarding lignocellulose degradation. Emphasis is placed on extracellular oxidative systems which are widely thought to be involved in lignin degradation but increasingly implicated in the depolymerization of cellulose and hemicellulose. Areas of uncertainty are highlighted. Detailed descriptions of the voluminous literature are not provided. Instead, interested readers are referred to earlier reviews (Cullen and Kersten 2004; Hatakka and Hammel 2010; Kersten and Cullen 2007).

13.2 Microbiology of Woody Litter Decay

Wood cell walls represent a complex and formidable substrate. Cellulose, essentially linear chains of ß-1,4-linked cellobiose organized into microfibrils, is the major component. Where chains are tightly stacked, the polymer is crystalline and resistant to hydrolysis, Nevertheless, many microbes are capable of cellulose utilization by hydrolyzing the ß-1,4 linkages [reviewed by Baldrian and Valaskova

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(2008)]. *Hemicellulose* is also widely utilized although a more diverse set of hydrolases are needed to fully degrade the branched polymer [reviewed by van den Brink and de Vries (2011)]. In contrast to cellulose and hemicellulose, *lignin* is a complex phenylpropanoid polymer (Higuchi 1990; Ralph et al. 2004). Few microbes have the capacity to depolymerize lignin, and none have been convincingly shown to utilize native lignin as a sole carbon source (Hatakka and Hammel 2010).

Efficient wood degradation is typically attributed to Agaricomycetes, and two basic forms are recognized: white rot and brown rot. *White rot* fungi depolymerize and mineralize all cell wall components including cellulose, hemicellulose, and the more recalcitrant lignin. Decay patterns vary among fungal species and strains as well as among wood species, morphology, and composition (Blanchette 1991; Daniel 1994; Eriksson et al. 1990; Schwarze 2007). Cell wall erosion by most white rot fungi, including *"Phanerochaete chrysosporium"* involves simultaneous degradation of all three polymers, whereas *Ceriporiopsis subvermispora* selectively degrades lignin in advance of cellulose and hemicellulose (Blanchette et al. 1992, 1997; Srebotnik and Messner 1994). In this context, it should be noted that reports of lignin depolymerization should be suspect in the absence of persuasive experimental support such as cleavage of non-phenolic lignin model compounds (below) and the degradation of radiolabeled lignin or synthetic lignins. Experiments relying on commercially available "lignin" should be carefully interpreted as these preparations typically contain contaminants and sulfonated lignin of varying molecular weight.

In contrast to white rot, *brown rot* fungi modify lignin but the polymeric residue remains (Niemenmaa et al. 2007; Yelle et al. 2008, 2011). Also distinctive, brown rot fungi rapidly depolymerize cellulose (Gilbertson 1981; Kirk et al. 1991; Kleman-Leyer et al. 1992; Worrall et al. 1997) in advance of extensive weight loss, This observation, together with microscopic localization of decay and studies of cell wall porosity, strongly argue for the involvement of small molecular weight oxidants diffusing into cell walls (Blanchette et al. 1997; Cowling 1961; Flournoy et al. 1993; Srebotnik and Messner 1991; Srebotnik et al. 1988). Hydroxyl radical has been repeatedly implicated as the diffusible oxidant, and its production attributed to *Fenton* reactions ($H_2O_2 + Fe^{2+} + H^+ \longrightarrow H_2O + Fe^{3+} + \cdot OH$) (Arantes et al. 2011; Cohen et al. 2002, 2004; Xu and Goodell 2001). Typically invoked to explain brown rot, such reactive oxygen species may also be operative in white rot as recently suggested (Arantes et al. 2011; Gomez-Toribio et al. 2009). In any case, the role of hydroxyl radical in situ is unresolved, and any reasonable model must accommodate the generation of highly reactive radical at or near the substrate as well as the need for a plausible redox system [reviewed by Arantes et al. (2012) and Goodell (2003)]. The involvement of small molecular weight iron chelators (Xu and Goodell 2001), cellobiose dehydrogenase (Henriksson et al. 2000a, b), and hydroquinone redox cycling (Paszczynski et al. 1999; Suzuki et al. 2006) has been proposed.

Beyond the wood-decay Agaricomycetes, several litter-inhabiting fungi have the capacity to degrade lignin, albeit less efficiently. These include the commercial

button mushroom *Agaricus bisporus* (Durrant et al. 1991), other basidiomycetes and a few higher ascomycetes [reviewed by Eriksson et al. (1990) and Hatakka (2001)]. Certain litter-decomposing fungi likely play a crucial role in the transformation and degradation of humic substances, a major fraction of soil organic matter (Kluczek-Turpeinen et al. 2005; Snajdr et al. 2010; Steffen et al. 2002). Recently, a white rot fungus *Trametes* sp. has been shown to degrade humic substances, and a Fenton-based mechanism implicated (Grinhut et al. 2011a, b).

Unexpectedly, recent studies have also connected hydroxyl radical-based degradation of humic substances by ectomycorrhizal (ECM) fungi. Ectomycorrhiza obtain carbon from plant hosts, but under some conditions, soil organic matter may be at least partially degraded (Baldrian 2009; Cullings and Courty 2009). As described below, scant ECM genome evidence (Martin et al. 2008; Vaario et al. 2012) supports a role for facultative saprotrophy, but transcriptome analyses, together with lignin structure determinations, suggest mechanisms by which soil organic extracts could be degraded by *Paxillus involutus* (Rineau et al. 2012).

13.3 Physiology and Genetics

13.3.1 Peroxidases

Peroxidases catalyze oxidations of diverse substrates with reduction of peroxide, which groups these enzymes under EC 1.11.x [donor: hydrogen peroxide oxidoreductase] in the NC-IUBMB system of nomenclature (Fleischmann et al. 2004). Beyond this description of chemical reaction, the classification of peroxidases based on protein properties is evolving as new enzymes are discovered and structural details are delineated indicating structure–function relationships. Not only may a single peroxidase have diverse substrates, but peroxidases of distinctly different structures may catalyze the same reaction. Furthermore, peroxidases may have different modes of oxidation while catalyzing the same net overall reaction. This presents significant challenges for classification of peroxidases into groups that adequately reflects function, protein structure, and phylogenetic relatedness (Hofrichter et al. 2010).

The first opportunity to classify a selection of secreted fungal peroxidases based on their crystal structure occurred in the early 1990s; *lignin peroxidase* (LiP), *manganese peroxidase* (MnP), and *Coprinus cinereus* peroxidase (CiP) were shown to be sufficiently similar in overall 3D structure and active site to group as Class II peroxidases, distinct from Class I intracellular peroxidases, and Class III secretory plant peroxidases (Welinder 1992). Versatile peroxidase (VP), more recently discovered, shows properties of both LiP and MnP and is likewise a Class II peroxidase. However, other newly discovered heme-thiolate peroxidases (HtPs) and the dye-decolorizing peroxidases (DyPs) are clearly distinct in sequence, protein structure, and catalysis, justifying establishment of HtP-like

and DyP-like peroxidase superfamilies, separate from the Class II peroxidases, These complexities of fungal peroxidases and differences between “new” and “classic” families have been recently reviewed (Hofrichter et al. 2010). Salient properties of peroxidases as they may relate to litter and soil ecosystems are briefly summarized here.

13.3.1.1 High Oxidation Potential Class II Peroxidases (LiP, MnP, and VP)

Three Class II peroxidases, the LiPs (systematic name 1,2-bis (3,4-dimethoxyphenyl)propane-1,3-diol:hydrogen-peroxide oxidoreductase; EC 1.11.l.14), VPs (systematic name reactive-black-5:hydrogen peroxide oxidoreductase; EC 1.1 1.1.16), and MnPs (systematic name Mn(II):hydrogen peroxide oxidoreductase; EC 1.11.1.13) are able to modify lignin and other recalcitrant aromatic molecules in part due to their high oxidation capacity, in contrast to low redox potential peroxidases (e.g., CiP and NoP, also Class II peroxidases; see following section), LiP and VP are the most powerful of the peroxidases with redox potentials of approximately 1.5 V and able to oxidize non-phenolic lignin model compounds directly by one electron (Kersten et al. 1985; Kirk et al. 1986; Miki et al. 1986). Another feature of both LiP and VP is an enzyme surface tryptophan that mediates oxidation through long-range electron transfer (LRET) enabling oxidation of larger sterically hindered non-phenolic substrates (Choinowski et al. 1999; Doyle et al. 1998).

Unlike LiPs and VPs, the MnPs do not have a conserved Trp at the enzyme surface and are not able to efficiently oxidize non-phenolic aromatics directly. Instead, MnPs have a conserved solvent-exposed Mn-binding site in the vicinity of a heme propionate and therefore are able to catalyze the oxidation of Mn^{2+} to Mn^{3+} in the presence of peroxide and suitable Mn^{3+} chelators (Sundaramoorthy et al. 1994; Wariishi et al. 1992). The VPs similarly have a Mn-binding site and therefore have hybrid characteristics of both LiPs and MnPs (Ruiz-Dueñas et al. 2009). A likely, physiological Mn^{3+} chelator for the MnP- and VP-catalyzed reactions is oxalate, which is known to be secreted by these fungi (Kuan and Tien 1993). Therefore, one role of MnPs and VPs is thought to be generation of diffusible Mn^{3+} chelates, which can oxidize phenolics directly. A more complex role may also be possible via oxidation of the chelates, thus generating other oxidizing species such as superoxide and perhydroxyl radical, which initiate radical chain reactions (e.g., in the presence of lipids) to generate ligninolytic radicals (Kapich et al. 1999). Phylogenetic analyses show the rise to two groups of MnPs, the so-called long-MnPs and the short-type hybrid MnPs which are more closely related to LiPs and VPs (Lundell et al. 2010).

The oxidizing capacity of these Class II peroxidases is achieved through the classical peroxidative cycle where “resting” enzyme is oxidized with peroxide by two electrons to generate Compound I enzyme intermediate. Compound I oxidizes substrates by one electron to produce oxidized substrate and Compound II enzyme

intermediate. The Compound II also oxidizes substrate, returning the enzyme back to "resting" state. In the case of LiPs and VPs with non-phenolic aromatics, the oxidized substrate are cation radicals and, as demonstrated with various substrates, the fate of the cation radical substrate intermediates is in large part determined by the substrate structure. Importantly, these reactions result in fragmentation of lignin model compounds and lignin (Miki et al. 1986; Tien and Kirk 1983).

Genes encoding Class II peroxidases have been identified in all lignin-degrading fungi, but not in brown rot or ECM. *Trametes versicolor* and *P. chrysosporium* genomes each feature ten LiP genes, but the corresponding proteins were not identified after 5 days growth in media containing ground aspen as sole carbon source (Table 13.1). On the other hand, ground pine wood and nutrient limited defined media support high transcript levels and secretion of several *P. chrysosporium* LiP and MnP isozymes (Vanden Wymelenberg et al. 2005, 2006b, 2009, 2010, 2011). Relative to LiPs, MnP-encoding genes appear more widely distributed. Sixteen MnP genes were identified in the genome of white rot fungus *Fomitiporia mediterranea,* and the corresponding proteins of seven are secreted in aspen cultures. Two *A. bisporus* MnP genes have been identified, and significant transcript accumulation occurs for one of these in compost (Morin et al. 2012). No Class II peroxidases were detected in the *Schizophyllum commune* genome. Although often classified as a white rot fungus and thereby presumed ligninolytic, the genetic repertoire of *S. commune* is consistent with weak or nonexistent lignin degradation (Boyle et al. 1992; Schmidt and Liese 1980).

Many wood- and litter-inhabiting fungi are clearly able to transform lignin or structurally related components of humic substances, but little is known about the role, if any, of high oxidation potential peroxidases in litter and soils. ^{14}C-labeled lignin and humic acid are degraded by litter decomposers *Gymnopus erythropus* and *Hypholoma fasciculare* when cultured on sterile leaf litter. MnP activity was higher in *G. erythropus* colonized litter, but degradation was less efficient for both species on non-sterile material, presumably due to interspecific competition (Snajdr et al. 2010). Similarly, laboratory experiments demonstrated degradation of ^{14}C-labeled humic acid and production of MnP by the litter decomposer, *Collybia dryoplzila* (Steffen et al. 2002). Laboratory studies of *P.chrysosporium*-colonized wood chips and soil have quantified transcript levels of specific LiP and MnP genes (Bogan et al. 1996b; Janse et al. 1998), and MnP activity was measured in organopollutant-contaminated soil (Bogan et al. 1996a).

13.3.1.2 Low Oxidation Potential Class II Peroxidases (CiP and NoP)

"*Coprinopsis cinerea* peroxidase" or CiP (systematic name phenolic donor: hydrogen-peroxide oxidoreductase; EC 1.11.1.7) is not able to oxidize non-phenolics such as veratryl alcohol; neither does it have the manganese-binding site of VP or MnP. Rather, CiP very efficiently oxidizes phenolics as a low oxidation peroxidase. As with other Class II peroxidases, CiP is similar in structure with conserved proximal and distal histidines near the heme active site; however, when compared

Table 13.1 Agaricomycete genes potentially involved in lignocellulose degradation: mass spectrometry-identified proteins — gene number

	White rot										Brown rot						Saprotrophs		ECM
Gene	Ad	Cs	Ds	Fm	Ha	Pc	Ps	Sc	Sh	Tv	Cp	Fp	Gt	Pp	Sl	Wc	Ab	Cc	Lb
LiP	0-0	0-0	0-0	0-0	0	0-10	0-0	0	0-0	0-10	0-0	0-0	0-0	0-0	0-0	0-0	0-0	0	0-0
MnP	0-5	2-13	0-9	7-16	8	0-5	1-10	0	0-10	2-13	0-0	0-0	0-0	0-0	0-0	0-0	2	0	0-0
VP	0-0	0-2	0-3	0-0	0	0-0	0-0	0	0-0	1-2	0-0	0-0	0-0	0-0	0-0	0-0	0	0	0-0
HtP	1-16	0-9	0-4	0-4	5	0-3	0-8	3	0-10	0-3	0-2	1-4	0-6	0-5	0-3	0-5	24	8	0-5
DyP	1-11	0-0	1-1	0-3	1	0-0	1-5	0	0-2	1-2	0-0	0-0	0-0	0-2	0-0	0-0	0	4	0-2
Lac	0-7	1-7	3-11	5-10	13	0-0	2-12	2	0-15	4-7	0-6	0-5	4	0-3	1-4	1-5	12	17	0-9
GLX	0-2	0-0	0-5	0-0	0	0-1	1-3	0	0-3	1-5	0-0	0-0	0-0	0-0	0-0	0-0	3	0	0-0
Cro1	0-0	0-0	0-1	0-1	1	0-1	0-0	0	0-0	0-1	0-1	0-1	0-0	0-0	0-0	0-1	2	3	1-6
Cro2	2-3	0-2	1-1	0-1	1	0-2	1-3	1	1-3	1-1	3-4	1-1	2-1	0-1	0-1	1-1	2	2	2-2
Cro3/5	1-1	0-1	1-1	0-1	1	0-3	1-1	0	0-1	1-1	0-0	0-1	0-0	1-1	0-1	1-1	1	1	0-2
Cro6	1-2	0-1	0-1	0-1	1	0-1	0-1	1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	1	1	0-0
CDH	0-1	0-1	1-1	0-1	1	0-1	1-1	1	1-1	1-1	1-1	0-0	0-1	0-0	0-2	0-0	1	1	0-0
GH61	1-19	0-9	3-15	0-13	10	2-15	6-13	22	1-16	1-16	0-10	0-4	1-4	0-2	0-5	0-2	11	35	0-5
GH6	2-2	1-1	1-1	0-2	1	1-1	1-1	1	1-1	1-1	0-2	0-0	0-0	0-0	0-1	0-0	1	5	0-0
GH7	2-6	1-3	1-4	0-2	1	3-6	1-5	2	1-3	1-4	0-2	0-0	9-0	0-0	0-0	0-0	1	6	0-0
UP	56	98	43	20	NA	27	30	NA	36	44	49	65	53	9	NA	21	NA	NA	NA
TP	151	121	180	85	NA	90	135	NA	208	218	269	253	174	64	NA	171	NA	NA	NA

Hyphenated entries report the number of published proteins identified by mass spectrometry followed by the total number of predicted genes. Citations and abbreviations: (Floudas et al. 2012) Ad, *Auricularia delicata;* Ds, *Dichomitus squalens;* Fm, *Fomitiporia mediterranea;* Ps, *Punctularia strigosozonata;* Sh, *Stereum hirsutum;* Tv, *Trametes versicolor;* Cp, *Coniophora puteana;* Fp, *Fomitopsis pinicola;* Gt, *Gloeophyllum trabeum;* and Sc, *Wolfiporia cocos.* (Fernandez-Fueyo et al. 2012) Cs, *Ceriporiopsis subvermispora.* (Olson et al. 2012) Ha, *Heterobasidion annosum.* (Vanden Wymelenberg et al. 2011) Pc, *Phanerochaete chrysosporium.* (Ohm et al. 2010) Sc, *Schizophyllum commune.* (Martinez et al. 2009) Pp, *Postia placenta.* (Eastwood et al. 2011) Sl, *Serpula lacrymans.* (Morin et al. 2012) Ab, *Agaricus bisporus.* (Stajich et al. 2010) Cc, *Coprinopsis cinereus.* (Vincent et al. 2012) Lb, *Laccaria bicolor.* The total number of proteins identified in aspen-containing medium and those designated as uncharacterized are indicated by TP and UP, respectively. Additional abbreviations include the following: LiP, lignin peroxidase; MnP, manganese peroxidase; VP, versatile peroxidase; HTP, heme-thiol peroxidase; DyP, dye-decolorizing peroxidases; Lac, laccase; GLX, the copper radical oxidase glyoxal oxidase; CROs, copper radical oxidases most closely related to those of *P. chrysosporium* (Vanden Wymelenberg et al. 2006a); CDH, cellobiose dehydrogenase; and GH61, GH6, and GH7, members of the glycoside hydrolase families 61, 6, and 7, respectively

to LiP, the distal side substrate channel is more open to easily oxidized reducing aromatic substrates (Kunishima et al. 1994; Petersen et al. 1994). However, CiP can be engineered to have activity with veratryl alcohol, the standard high redox potential substrate for LiP, by mutations to introduce the surface Trp and negatively charged microenvironment (Smith et al. 2009). Based on sequence analysis, genes encoding such low oxidation peroxidases have also been identified in brown rot fungi *F. pinicola, P. placenta,* and *W. cocos* as well as the white rot fungi *C. subvermispora, C. strigosozonata, F. mediterranea, H. annosum, S. hirsutum* (Floudas et al. 2012), and *P. chrysosporium.* Designated NoP, the *P. chrysosporium* structure has been studied in some detail (Larrondo et al. 2005). High transcript levels and secreted proteins have not been observed, and the role of these peroxidases remains uncertain.

13.3.1.3 Heme-thiolate Peroxidases

Heme-thiolate peroxidases (HtPs) comprise a superfamily of secreted fungal peroxidases distinguished from the Class II peroxidases, not only by the proximal cysteine and distal glutamate heme ligands but also by distinctive 3D structure, protein sequence, and remarkable catalytic capacity. HtPs are classified based on dominating reactions that are consistent with *chloroperoxidase* (CPOs; systematic name chloride:hydrogen-peroxide oxidoreductase; EC 1.1 1.1.10) and aromatic peroxygenase (APOs; systematic name substrate:hydrogen peroxide oxidoreductase (RH-hydroxylating or -epoxidizing); EC 1.1 1.2.1) activities.

CPO from *Caldariomyces fumago* halogenates suitable organic substrates in the presence of peroxide and halide (Morris and Hager 1966; Shaw and Hager 1959). The primary function of CPO here is oxidization of chloride (Cl^-) to hypochlorous acid, which is a strong oxidant and able to chlorinate the organic compounds (Murali Manoj 2006). Bromide and iodide are also oxidized by CPO, but not fluoride. If halide is absent, CPO oxidizes suitably substituted phenols and anilines directly. In the case of chlorinated phenols, oxidations with CPO are much more efficient than with horseradish peroxidase, LiP or VP (Casella et al. 1994; Longoria et al. 2008; Osborne et al. 2007). Besides halogenation and phenol oxidation capability, CPO also has peroxygenase properties, resembling cytochrome P450-dependent monooxygenase (also a heme-thiolate) in catalysis and structure (Manoj and Hager 2008; Sundaramoorthy et al. 1995). CPO is able to epoxidize alkenes and hydroxylates benzylic carbons via a peroxygenase mechanism (Manoj and Hager 2008), but oxygen transfer to less-activated molecules such as alkanes or aromatic rings is not catalyzed. The proposed functional roles of the enzyme are varied, from biosynthesis of chlorinated metabolites (Morris and Hager 1966) to antimicrobial activity, due to the biocidal activity of hypochlorite (Bengtson et al. 2009). A consequence of the nonspecificity of CPOs and the reactive chemical species generated is the chlorination of lignin (Ortiz-Bermúdez et al. 2007).

In contrast to CPO, the fungal *peroxygenases* (APOs) from *Agrocybe aegerita* (AaeAPO; Ullrich et al. 2004), *Coprinellus radians* (CraAPO; Anh et al. 2007;

Aranda et al. 2009), and *Marasmius rotula* (MroAPO; Gröbe et al. 2011) are all able to catalyze oxygen transfer reactions to aromatic rings, in addition to other divers reactions. AaeAPO was first described as a haloperoxidase but with further characterization shown to be a functional hybrid with properties of both a peroxidase and a monooxygenase (Hofrichter et al. 2010). APOs have a wide variety of substrates including polyaromatics (e.g., naphthalene), recalcitrant heterocycles (e.g., pyridine), ethers (with *O*-dealkylation resulting), and alkanes (e.g., propane and hexane). The chemical transformations include hydroxylation, epoxidation, N-oxidation, sulfooxidation, bromination, and one-electron oxidations. However, the array of reactions is not shared by all APOs; the MroAPO of *M. rotula* does not have the brominating activity of AaeAPO and CraAPO (Gröbe et al. 2011).

All sequenced Agaricomycete genomes feature genes encoding HtPs, but their number and expression vary substantially. Peptides were identified in culture filtrates of *A. delicata* and *F. pinicola* (Floudas et al. 2012). LC-MS/MS could not detect HTPs in *P. chrysosporium* or *P. placenta* cultures (Vanden Wymelenberg et al. 2011), although each species exhibited significant transcript accumulation of two HtP genes in wood-containing cultures relative to glucose medium. More impressive, 24 HtP genes were predicted in the *A. bisporus* genome, and 16 of these were significantly upregulated in compost (Morin et al. 2012). This observation suggests that HtPs may play an important role in metabolism of partially decomposed litter and humic substances. A Dacrymycete classified as a brown rotter, *Dacryopinax* sp., contains six putative HtP-encoding genes, and the corresponding protein has been detected for two of these (Floudas et al. 2012).

13.3.1.4 Dye-Decolorizing Peroxidases

Prototypical DyP was isolated from the Agaricomycete *Bjerkandera adusta* [first reported as *Geotrichum candidum* Dec 1, re-identified as *Thanatephorus cucumeris* Dec 1, and then *B. adusta* (Ruiz-Dueñas et al. 2011)] because of its dye-decolorizing activities (Kim and Shoda 1999; Kim et al. 1995; Sugano 2009). Subsequent structural and sequence comparisons indicate that the peroxidase is unlike any previously characterized peroxidase (Sugano et al. 2007; Zubieta et al. 2007). DyPs (systematic name Reactive-Blue-5:hydrogen peroxide oxidoreductase; EC 1.11.1.19) have a proximal histidine, but unlike the Class II and HtP peroxidases, DyPs have a distal Asp. The model substrate, Reactive Blue 5, is converted to products by a combination of oxidation and hydrolytic steps. Other dyes, anthraquinone derivatives and typical peroxidase substrates, are also oxidized. Structural and biochemical characterization of DyP-like peroxidases from bacteria is also reported (Brown et al. 2012; Zubieta et al. 2007). In the case of *Amycolatopsis*, the DyP2 appears multifunctional, showing high peroxidase activity, manganese peroxidase activity, and also a mode of oxidase activity with 4-methoxymandelic acid (Brown et al. 2012). Liers et al. (2013) have recently compared activities of fungal DyP with heme peroxidases, and considering the

complex catalytic properties of the DyPs, the physiological roles are most likely diverse.

The number of DyP-encoding genes varies significantly among Agaricomycetes. Certain efficient lignin degraders such as *P. chrysosporium* and *C. subvermispora* have none, while 1 I genes are predicted in the *A. delicata* genome (Table 13.1). Of eight white rot cultures subjected to LC-MS/MS analysis, four contained the corresponding peptides. Five brown rot fungi have no DyP genes. Only one brown rot fungus, *P. placenta,* is predicted to have DyP genes, but the corresponding proteins were not detected in aspen-containing medium (Vanden Wymelenberg et al. 2011). Significant protein levels occur in ground wood culture filtrates of *T. versicolor, D. squalens, A. delicata,* and *P. strigozonata* where a single DyP constituted 2.2 %, 1.3 %, 0.1 %, and 0.08 %, respectively, of the total spectra (Floudas et al. 2012).

13.3.2 Extracellular Peroxide Generation

It is readily apparent that extracellular peroxide is a key player in lignocellulose biotransformation in view of the importance of peroxide-dependent peroxidases secreted by fungi (previous section). Even prior to the discovery of these peroxidases, a correlation was observed between peroxide production and the physiology of ligninolysis by *P. chrysosporium* (Faison and Kirk 1983; Forney et al. 1982; Keyser et al. 1978). There appears to be several possible mechanisms for peroxide production, including by MnP with fungal metabolites oxalate and glyoxylate; peroxide is evidently generated as a consequence of reactions of oxygen with carbon-centered radical substrate intermediates (Kuan and Tien 1993; Urzúa et al. 1998). Oxidases that catalyze reduction of oxygen to peroxide, and where evidence indicates an extracellular role in supporting peroxidase activities, are briefly summarized here.

13.3.2.1 Copper Radical Oxidases

Glyoxal oxidase (GLX) was first reported in cultures of *P. chrysosporium* where its activity was correlated with substrates glyoxal and methylglyoxal in culture, and with LiP activity (Kersten and Kirk 1987). Importantly, the activity of GLX was activated by interaction with LiP (Kersten 1990). Sequence comparisons and spectroscopic comparisons indicate that GLX has a similar active site to that of the copper radical oxidase (CRO) galactose oxidase (Kersten and Cullen 1993; Whittaker et al. 1996, 1999). Copper radical oxidases have two one-electron acceptors: a copper (II) metal at the center of the active site and a Cys-Tyr radical forming a metallo-radical complex (Whittaker 2005). Alignment of galactose oxidase with GLX indicates that the catalytic domain of GLX supplies copper ligands Tyr135 Tyr377 and His378 while Cys70 is cross-linked with Tyr135 to

form an internal radical cofactor of the sevenfold ß-barrel domain (also described as a super-barrel or ß-flower). The C-terminal domain supplies His471 copper ligand on a loop through the center of the catalytic barrel. Analysis of the *P. chrysosporium* genome indicates multiple CROs where predicted mature protein sequences diverge substantially from one another, but the residues coordinating copper and constituting the radical redox site are conserved (Vanden Wymelenberg et al. 2006a).

More recent analysis of Agaricomycete genomes has revealed widespread distribution of GLX and related *copper radical oxidases* (Table 13.1). GLX homologs are lacking from brown rot fungi, *C. cinereus* and *L. bicolor,* but present in most lignin-degrading fungi, Notable exceptions include *C. subvermispora,* a selective and highly efficient lignin degrader. Possibly, the related copper radical oxidases *cro2, cro3,* and *cro6* compensate by oxidizing an array of metabolites unique to *C. subvermispora* decay. Along these lines, elevated transcript levels of the *C. subvermispora cro2* gene were observed in wood-containing medium, and peptides corresponding to CRO5 were detected in medium using microcrystalline cellulose as sole carbon source (Fernandez-Fueyo et al. 20 12). Earlier reports showed that the substrate preference of *P. chrysosporium* CRO2 differed sharply from GLX (Vanden Wymelenberg et al. 2006a).

13.3.2.2 GMC Oxidoreductases

Another oxidase produced by *P. chrysosporium* and a select number of other white and brown rot fungi is *pyranose 2-oxidase* (P2O), which oxidizes glucose at C-2 to produce D-*arabino*-hexos-2-ulose (glucosone) with reduction of oxygen to peroxide (Baute and Baute 1984; de Koker et al. 2004; Dietrich and Crooks 2009; Giffhorn 2000). The protein is a large homotetrameric flavoprotein with a subunit size of' about 70·kDa (Hallberg et al. 2004). It is a member of the glucose-methanol-choline oxidoreductase family (Albrecht and Lengauer 2003), a superfamily of proteins including *Drosophila melanogaster* glucose dehydrogenase, *Aspergillus niger* glucose oxidase, *Hansenula polymorpha* methanol oxidase, and *Escherichia coli* choline dehydrogenase (Cavener 1992). The periplasinic and extracellular distribution of pyranose 2-oxidase in wood decayed by *P. chrysosporium* is consistent with that of MnP, suggesting a role in extracellular peroxide generation (Daniel et al. 1994). An alternative role for pyranose 2-oxidase is the synthesis of the antibiotic cortalcerone (Koths el al. 1992), but many fungi that have pyranose 2-oxidase do not have aldo-2-ulose dehydratase required for cortalcerone synthesis (Baute and Baute 1984). Quinones are alternate electron acceptors in place of oxygen, and therefore, the oxidase may have a role in redox cycling during lignocellulose degradation (Pisanelli et al. 2009). P2O-encoding genes are predicted in the genomes of brown rot fungi *G. trabeum* and *S. lacrymans* as well

as the white rot fungi *A. delicata*, *P. strigosozonata*, *T. versicolor,* and *P. chrysosporium.*

Another GMC oxidoreductase involved in peroxide generation is the extracellular monomeric *aryl-alcohol oxidase* (AAO) [recently reviewed by Hernández-Ortega et al. (2012)]. AAO substrates may be both lignin-derived metabolites, as well as aromatic fungal metabolites synthesized de novo (de Jong et al. 1994; Gutiérrez et al. 1994). The aromatic alcohol substrates are oxidized to the corresponding aldehydes by AAOs, and these aldehydes reduced back to alcohols by intracellular aryl-alcohol dehydrogenases, thus establishing a redox cycle for the generation of extracellular peroxide using reducing equivalents derived from intracellular metabolism. A possible role for AAO in preventing polymerization of lignin fragments by reduction of quinone and phenoxyradicals is also described (Marzullo et al. 1995). The AAO from *P. eryngii* has been heterologously expressed in *E. coli* (Ruiz-Dueñas et al. 2006) and crystal structure determined (Fernandez et al. 2009). The GMC oxidoreductase *methanol oxidase* is also proposed to have a role in peroxide production using methanol released from lignin methoxyls (Nishida and Eriksson 1987). Although a signal peptide is not evident from gene structure, the oxidase appears to have an extracellular role in wood decay with *G. trabeum* (Daniel et al. 2007). Putative methanol oxidase- and AAO-encoding genes have been identified in a wide range of white rot and brown rot fungi (Hernández-Ortega et al. 2012). In *C. subvermispora* and *P. chrysosporium,* the AAO genes show no transcript accumulation in medium containing ground wood relative to glucose-containing media (Fernandez-Fueyo et al. 2012; Vanden Wymelenberg et al. 2011).

A potentially important oxidoreductase, *cellobiose dehydrogenase* (CDH), oxidizes cellodextrins, mannodextrins, and lactose. In addition to the dehydrogenase, the mature protein contains a heme prosthetic group and a cellulose-binding module (Hallberg et al. 2000). Electron acceptors include quinones, phenoxyradicals, and Fe^{3+}, and involvement in hydroxyl radical generation has been proposed. CDH genes are widely distributed (Table 13.1) as are “glycoside hydrolase” family 61 (GH61) genes. Recently shown to act as *copper-dependent monooxygenases* (Quinlan et al. 2011; Westereng et al. 2011), the GH61s can act together with CDHs to boost cellulose depolymerization (Harris et al. 2010; Langston et al. 2011). The precise roles(s) and interaction(s) between these genes remain to be clarified.

While the abovementioned oxidoreductases can be functionally categorized on the basis of sequence conservation, many variants defy simple classification. For example, peptides corresponding to *S. hirsutum* protein model #118344 (http://genome.jgi.doe.gov/Stehil/Stehil.home.html) constitute 5.3 % of the total spectra observed in wood-containing medium. The protein features a secretion signal and InterPro domains that point toward a GMC oxidoreductase, but little additional information allows a firm definition.

13.3.3 Laccases

Laccases are diverse in origin (plants, fungi, and bacteria) and properties [see review (Thurston 1994)]. Brief description of fungal laccase is presented here to highlight the essential properties distinguishing it from the enzymes in the foregoing sections. Laccase (systematic name benzenediol:oxygen oxidoreductase; EC 1.10.3.2) catalyzes the four-electron reduction of oxygen to water with the electrons derived by four one-electron oxidations of substrate (typically phenols or aryl amines). The four-electron reduction is achieved with four copper ions at three enzyme sites: the T1 site contains a type I copper in tight coordination with cysteine which gives laccase its blue color, the T2 site has a type 2 copper with characteristic EPR signal, and the T3 site has a pair of strongly coupled EPR-silent type 3 coppers (Bertrand et al. 2002). The T1 site mediates oxidations with transfer of electrons to the T2/T3 trinuclear center where electrons are transferred to oxygen. The capacity for single-electron oxidations by laccase from *T. versicolor*, in comparison with LiP and Class III HRP, was demonstrated with 1,2,4,5-tetramethoxybenzene producing the corresponding cation radical as immediate product (Kersten et al. 1990). Although the laccase oxidized this methoxybenzene congener, it did not have the same capacity as the peroxidases to oxidize methoxybenzenes of higher potential. Laccase oxidation of phenols generates intermediates which may undergo further enzyme-catalyzed oxidation (e.g., generating quinones) or the unstable intermediates may undergo nonenzymatic reactions such as polymerizations (Thurston 1994). Laccase genes are widely distributed among fungi (Table 13.1) but not essential for ligninolysis as demonstrated by the lack of the enzyme in *P. chrysosporium* (Martinez et al. 2004). Differential expression among paralogs is commonly observed (see, e.g., Castanera et al. 2012; Floudas et al. 2012), although the role of genetic multiplicity is poorly understood, Excluding *P. chrysosporium*, considerable evidence suggests that laccase may have a role in lignin modification or plant litter decay (Bourbonnais et al. 1997; Kellner et al. 2007).

13.3.4 Hypothetical and Uncharacterized Proteins

A persistent concern has been the incomplete understanding of predicted proteins lacking significant homology to those of known function (herein referred to as “hypothetical”), some of which are translated and secreted (herein referred to as “uncharacterized”). The dimensions of this issue are staggering. For example, 21 % of the 13,761 *C. puteana* protein models showed no significant similarity to NCBI NR database entries (Floudas et al. 2012). Mass spectrometry analysis of filtrates from aspen-containing media identified 269 separate proteins, and 49 of these were designated “uncharacterized” (Table 13.1). High expression levels, conserved domains, and/or structural features are sometimes observed for these

uncharacterized proteins. Thus, C. puteana protein #125481 (http://genome.jgi.doe.gov/Conpul/Conpul.home.html) constitutes 1.9 % of total spectra and has a predicted secretion signal and an InterPro conserved domain of unknown function (DUF 1793).

13.4 Challenges and Future Prospects

The daunting number of hypothetical proteins and the lack of appropriate experimental tools present significant obstacles to progress in this area. Transcript and *secretome* profiles provide clues, but detailed functional analysis requires biochemical characterization of heterologously expressed proteins and/or targeted gene replacement/suppression. The latter goal has been particularly difficult to achieve because genetic transformation of filamentous basidiomycetes has typically involved low rates of homologous recombination. Recently, this obstacle has been overcome by isolating Ku knockouts that impair nonhomologous end joining in *S. commune* (de Jong et al. 2010), *C. cinereus* (Nakazawa et al. 2011), and *P. ostreatus* (Salame et al. 2012). Demonstrating the power of the approach, Salame et al. demonstrated the importance of *P. pleurotus* MnP4 by successfully inactivating the VP gene. Likely, this experimental approach will be applied to additional Agaricomycetes in the future.

Availability of increasing numbers of genome sequences facilitates high-throughput approaches for elucidating community structure and physiological processes in soils. In addition to the widely used ribosomal DNA and/or the internal transcribed spacer region (ITS) (Buee et al. 2009), the distribution of highly conserved genes such as those encoding laccases and cellobiohydrolases gauge fungal diversity in different soils and soil horizons (Baldrian et al. 2012; Luis et al. 2005). When combined with rRNA sequence, a more complete picture of microbial populations and active metabolism emerges. Degenerate primers amplified cDNAs corresponding to basidiomycete laccases, MnPs, and HtPs (Kellner et al. 2010), and more recent “metatranscriptomic” investigations have provided a more global view of transcript levels (Simon and Daniel 2011). Examples include the assessment of soil gene expression in response to phenanthrene contamination (de Menezes et al. 2012) and the measurements of transcript and populations in various forest soils (Damon et al. 2012). Both investigations identified transcripts corresponding to Agaricomycete degradative enzymes, and the latter study also employed 18S rRNA sequencing to assign broad taxonomic affiliations (Damon et al. 2012). *Metatranscriptome* approaches can be enhanced or extended to functional analysis by expression of full-length genes in *Saccharomyces cerevisiae* (Bailly et al. 2007; Damon et al. 2011; Kellner et al. 2011).

Also promising are the prospects for direct detection of fungal proteins and enzyme activities in natural substrates and/or field soils. Immobilization of fluorescent substrates allows visualization of hydrolytic enzymes on decaying litter, leaves, and wood (Baldrian and Vetrovsky 2012). In addition to such localization,

metabolite identification could play a key role in defining the processes involved in decomposition. Thus, NMR and GC/MS of field-collected decayed wood provided evidence for Fenton-based brown rot (Martinez et al. 2011). Fenton chemistry was also implicated in laboratory studies of *P. involutus* cultured on soil extracts using FTIR and GC/MS coupled to enzyme activities and to mRNAseq (Rineau et al. 2012). *Metaproteomics* [reviewed by Hettich et al. (2012)] offer unparalleled opportunities for understanding microbial processes as demonstrated by recent mass spectrometry of soil (Keiblinger et al. 2012) and leaf litter samples (Schneider et al. 2012). Although challenging technical issues remain, these experimental approaches are beginning to shed light on the roles and interactions of fungi in forest soils.

References

Albrecht M, Lengauer T (2003) Pyranose oxidase identified as a member of the GMC oxidoreductase family, Bioinformatics 19:1216–1220

Anh DH, Ullrich R, Benndorf D et al (2007) The coprophilous mushroom *Coprinus radians* secretes a haloperoxidase that catalyzes aromatic peroxygenation. Appl Environ Microbiol 73:5477-5485

Aranda E, Kinne M, Kluge M et al (2009) Conversion of dibenzothiophene by the mushrooms *Agrocybe aegerita* and *Coprinellus radians* and their extracellular peroxygenases. Appl Microbiol Biotechnol 82: 1057–1066

Arantes V, Milagres AM, Filley TR, Goodell B (2011) Lignocellulosic polysaccharides and lignin degradation by wood decay fungi: the relevance of nonenzymatic Fenton-based reactions. J Ind Microbiol Biotechnol 38:541-555

Arantes V, Jellison J, Goodell B (2012) Peculiarities of brown-rot fungi and biochemical Fenton reaction with regard to their potential as a model for bioprocessing biomass. Appl Microbiol Biotechnol 94:323–338

Bailly J, Fraissinet-Tachet L, Verner MC et a1 (2007) Soil eukaryotic functional diversity, a metatranscriptomic approach. ISME J 1:632-642

Baldrian P (2009) Ectomycorrhizal fungi and their enzymes in soils: is there enough evidence for their role as facultative soil saprotrophs? Oecologia 161:657-660

Baldrian P, Valaskova V (2008) Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32:501–521

Baldrian P, Vetrovsky T (2012) Scaling down the analysis of environmental processes: monitoring enzyme activity in natural substrates on a millimeter resolution scale. Appl Environ Microbiol 78:3473–3475

Baldrian P, Kolarik M, Stursova M et al (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J 6:248-258

Baute M-A, Baute R (1984) Occurrence among macrofungi of the bioconversion of glucosone to cortalcerone. Phytochemistry 23:271–274

Bengtson P, Bastviken D, De Boer W, Öberg G (2009) Possible role of reactive chlorine in microbial antagonism and organic matter chlorination in terrestrial environments. Environ Microbiol 11:1330-1339

Bertrand T, Jolivalt C, Briozzo P et al (2002) Crystal structure of a four-copper laccase complexed with an arylamine: insights into substrate recognition and correlation with kinetics. Biochemistry 41:7325-7333

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

Blanchette RA, Burnes TA, Eerdmans MM, Akhtar M (1992) Evaluating isolates of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* for use in biological pulping processes. Holzforschung 46:109-115

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

Boddy L, Watkinson SC (1995) Wood decomposition, higher fungi, and their role in nutrient redistribution. Can J Bot 73:S1377-S1883

Bogan B, Schoenike B, Lamar R, Cullen D (1996a) Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with *Phanerochaete chrysosporium.* Appl Environ Microbiol 62:2381-2386

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

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

Boyle CD, Kropp BR, Reid ID (1992) Solubilization and mineralization of lignin by white rot fungi. Appl Environ Microbiol 58:3217-3224

Brown ME, Barros T, Chang MCY (2012) Identification and characterization of a multifunctional dye peroxidase from a lignin-reactive bacterium. ACS Chem Biol 7:2074–2081

Buee M, Reich M, Murat C et al (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184:449-456

Casella L, Gullotti M, Selvaggini C et al (1994) The chloroperoxidase-catalyzed oxidation of phenols. Mechanism, selectivity, and characterization of enzyme-substrate complexes. Biochemistry 33:6377-6386

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

Cavener DR (1992) GMC oxidoreductases: a newly defined family of homologous proteins with diverse catalytic activities. J Mol Biol 223:811-814

Choinowski T, Blodig W, Winterhalter KH, Piontek K (1999) The crystal structure of lignin peroxidase at 1.70 A resolution reveals a hydroxy group on the cbeta of tryptophan 171: a novel radical site formed during the redox cycle. J Mol Biol 286:809–827

Cohen R, Jensen KA, Houtman CJ, Hammel KE (2002) Significant levels of extracellular reactive oxygen species produced by brown rot basidiomycetes on cellulose. FEBS Lett 531:483-488

Cohen R, Suzuki MR, Hammel KE (2004) Differential stress-induced regulation of two quinone reductases in the brown rot basidiomycete *Gloeophyllum trabeum.* Appl Environ Microbiol 70:324–331

Cowling EB (1961) Comparative biochemistry of the decay of sweetgum sapwood by white-rot and brown-rot fungi. Technical Bulletin No. 1258, U.S. Department of Agriculture, Washington, DC

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

Cullings K, Courty PE (2009) Saprotrophic capabilities as functional traits to study functional diversity and resilience of ectomycorrhizal community. Oecologia 161:661-664

Damon C, Vallon L, Zimmermann S et al (2011) A novel fungal family of oligopeptide transporters identified by functional metatranscriptomics of soil eukaryotes. ISME J 5:1871-1880

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

Daniel G (1994) Use of electron microscopy for aiding our understanding of wood biodegradation. FEMS Microbiol Rev 13:199-233

Daniel G, Volc J, Kubatova E (1994) Pyranose oxidase, a major source of H2O2 during wood degradation by *Phanerochaete chrysosporium, Trametes versicolor,* and *Oudemansiella mucida.* Appl Environ Microbiol 60:2524–2532

Daniel G, Volc J, Filonova L et al (2007) Characteristics of *Cloeophyllum trabeum* alcohol oxidase, an extracellular source of H2O2 in brown rot decay of wood. Appl Environ Microbiol 73:6241-6253

de Jong E, Cazemier AE, Field JA, de Bont JAM (1994) Physiological role of chlorinated aryl alcohols biosynthesized de novo by the white rot fungus *Bjerkandera* sp. Strain BOS55. Appl Environ Microbiol 60:271-277

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

de Koker TH, Mozuch MD, Cullen D et al (2004) Isolation and purification of pyranose 2-oxidase from *Phanerochaete chrysosporium* and characterization of gene structure and regulation. Appl Environ Microbiol 70:5794–5800

de Menezes A, Clipson N, Doyle E (201 2) Comparative metatranscriptomics reveals widespread community responses during phenanthrene degradation in soil. Environ Microbiol 14:2577–2588

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

Doyle WA, Blodig W, Veitch NC et al (1998) Two substrate interaction sites in lignin peroxidase revealed by site-directed mutagenesis. Biochemistry 37:15097-15105

Durrant AJ, Wood DA, Cain RB (1991) Lignocellulose biodegradation by *Agaricus bisporus* during solid substrate fermentation. J Gen Microbiol 137:751-755

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

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

Faison BD, Kirk TK (1983) Relationship between lignin degradation and production of reduced oxygen species by *Phanerochaete chrysosporium.* Appl Environ Microbiol 46:1140-1145

Fernandez IS, Ruiz-Duenas FJ, Santillana E et al (2009) Novel structural features in the GMC family of oxidoreductases revealed by the crystal structure of fungal aryl-alcohol oxidase. Acta Crystallogr D Biol Crystallogr 65:1196-1205

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

Fleischmann A, Darsow M, Degtyarenko K et al (2004) IntEnz, the integrated relational enzyme database. Nucleic Acids Res 32:D434–D437

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

Flournoy D, Paul J, Kirk TK, Highley T (1993) Changes in the size and volume of pore in sweet gum wood during simultaneous rot by *Phanerochaete chrysosporium.* Holzforschung 47:297-301

Forney LJ, Reddy CA, Pankratz HS (1982) U1trastructural localization of hydrogen peroxide production in ligninolytic *Phanerochaete chrysosporium* cells. Appl Environ Microbiol 44:732-736

Giffhorn F (2000) Fungal pyranose oxidases: occurrence, properties and biotechnical applications in carbohydrate chemistry. Appl Microbiol Biotechnol 54:727–740

Gilbertson RL (198 1) North American wood-rotting fungi that cause brown rots. Mycotaxon 12:372-416

Gomez-Toribio V, Garcia-Martin AB, Martinez MJ et al (2009) Induction of extracellular hydroxyl radical production by white-rot fungi through quinone redox cycling. Appl Environ Microbiol 75:3944–3953

Goodell B (2003) Brown rot fungal degradation of wood: our evolving view. In: Goodell B et al (eds) Wood deterioration and preservation. American Chemical Society, Washington, DC, pp 97–118

Grinhut T, Salame TM, Chen Y, Hadar Y (2011a) Involvement of ligninolytic enzymes and Fenton-like reaction in humic acid degradation by *Trametes* sp. Appl Microbiol Biotechnol 91:1131–1140

Grinhut T, Hertkorn N, Schmitt-Kopplin P et al (2011b) Mechanisms of humic acids degradation by white rot fungi explored using 1H NMR spectroscopy and FTICR mass spectrometry. Environ Sci Technol 45:2748–2754

Gröbe G, Ullrich R, Pecyna M et al (2011) High-yield production of aromatic peroxygenase by the agaric fungus *Marasmius rotula.* AMB Express 1:1-11

Gutiérrez A, Caramelo L, Prieto A et al (1994) Anisaldehyde production and aryl-alcohol oxidase and dehydrogenase activities in ligninolytic fungi of the genus *Pleurotus.* Appl Environ Microbiol 60:1783-1788

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

Hallberg BM, Leitner C, Haltrich D, Divne C (2004) Crystal structure of the 270 kDa homotetrameric lignin-degrading enzyme pyranose 2-oxidase. J Mol Biol 341:781-796

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

Hatakka A (2001) Biodegradation of lignin. In: Hofrichter M, Steinbuchel A (eds) Lignin, humic substances and coal. Wiley-VCH, Weingeim, pp 129-180

Hatakka A, Hammel KE (2010) Fungal biodegradation of lignocelluloses. In: Hofrichter M (ed) Industrial, applications. Springer, Berlin

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

Henriksson G, Zhang L, Li J et al (2000b) Is cellobiose dehydrogenase from *Phanerochaete chrysosporium* a lignin degrading enzyme? Biochim Biophys Acta 1480:83–91

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

Hettich RL, Sharma R, Chourey K, Giannone RJ (2012) Microbial metaproteomics: identifying the repertoire of proteins that microorganisms use to compete and cooperate in complex environmental communities. Curr Opin Microbiol 15:373–380

Higuchi T (1990) Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 24:23–63

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

Janse BJH, Gaskell J, Akhtar M, Cullen D (1998) Expression of *Phanerochaete chrysosporium* genes encoding lignin peroxidases, manganese peroxidases, and glyoxal oxidase in wood. Appl Environ Microbiol 64:3536-3538

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

Keiblinger KM, Wilhartitz IC, Schneider T et al (2012) Soil metaproteomics - comparative evaluation of protein extraction protocols. Soil Biol Biochem 54:14-24

Kellner H, Luis P, Buscot F (2007) Diversity of laccase-like multicopper oxidase genes in Morchellaceae: identification of genes potentially involved in extracellular activities related to plant litter decay. FEMS Microbiol Ecol 61:153–163

Kellner H, Zak DR, Vandenbol M (2010) Fungi unearthed: transcripts encoding lignocellulolytic and chitinolytic enzymes in forest soil. PLoS One 5:e10971

Kellner H, Luis P, Portetelle D, Vandenbol M (2011) Screening of a soil metatranscriptomic library by functional complementation of *Saccharomyces cerevisiae* mutants. Microbiol Res 166:360–368

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

Kersten PJ, Cullen D (1993) Cloning and characterization of a cDNA encoding glyoxal oxidase, a H_2O_2-producing enzyme from the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Proc Natl Acad Sci U S A 90:7411–7413

Kersten P, Cullen D (2007) Extracellular oxidative systems of the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Fungal Genet Biol 44:77-87

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

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

Kersten PJ, Kalyanaraman B, Hammel KE et al (1990) Comparison of lignin peroxidase, horse-radish peroxidase, and laccase in the oxidation of methoxybenzenes. Biochem J 268:475–480

Keyser P, Kirk TK, Zeikus JG (1978) Ligninolytic enzyme system of *Phanerochaete chrysosporium:* synthesized in the absence of lignin in response to nitrogen starvation. J Bacteriol 135:790–797

Kim SJ, Shoda M (1999) Purification and characterization of a novel peroxidase from *Geotrichum candidum* Dec 1 involved in decolorization of dyes. Appl Environ Microbiol 65:1029-1035

Kim SJ, Ishikawa K, Hirai M, Shoda M (1995) Characteristics of a newly isolated fungus, *Geotrichum candidum* Dec 1, which decolorizes various dyes. J Ferment Bioeng 79:601–607

Kirk TK, Tien M, Kersten PJ et al (1986) Ligninase of *Phanerochaete chrysosporium.* Mechanism of its degradation of the non-phenolic arylglycerol beta-aryl ether substructure of lignin. Biochem J 236:279–287

Kirk TK, Ibach R, Mozuch MD et al (1991) Characteristics of cotton cellulose depolymerized by a brown-rot fungus, by acid, or by chemical oxidants. Holzforschung 45:239–244

Kleman-Leyer K, Agosin E, Conner AH, Kirk TK (1992) Changes in molecular size distribution of cellulose during attack by white rot and brown rot fungi. Appl Environ Microbiol 58:1266-1270

Kluczek-Turpeinen B, Steffen KT, Tuomela M et al (2005) Modification of humic acids by the compost-dwelling deuteromycete *Paecilomyces inflatus.* Appl Microbiol Biotechnol 66:443-449

Koths K, Halenbeck R, Moreland M (1992) Synthesis of the antibiotic cortalcerone from -glucose using pyranose 2-oxidase and a novel fungal enzyme, aldos-2-ulose dehydratase. Carbohydr Res 232:59–75

Kuan IC, Tien M (1993) Stimulation of Mn peroxidase activity: a possible role for oxalate in lignin biodegradation. Proc Natl Acad Sci U S A 90: 1242–1246

Kunishima N, Fukuyama K, Matsubara H et al (1994) Crystal structure of the fungal peroxidase from Arthromyces ramosus at 1.9 Å resolution: structural comparisons with the lignin and cytochrome c peroxidases. J Mol Biol 235:331-344

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

Larrondo L, Gonzalez A, Perez_Acle T et al (2005) The nop gene from *Phanerochaete chrysosporium* encodes a peroxidase with novel structural features. Biophys Chem 116:167–173

Liers C, Pecyna MJ, Kellner H et al (2013) Substrate oxidation by dye-decolorizing peroxidases (DyPs) from wood- and litter-degrading agaricomycetes compared to other fungal and plant heme-peroxidases. Appl Microbiol Bioteclinol 97(13):5839-5849

Longoria A, Tinoco R, Vázquez-Duhalt R (2008) Chloroperoxidase-mediated transformation of highly halogenated monoaromatic compounds. Chemosphere 72:485-490

Luis P, Kellner H, Zimdars B et al (2005) Patchiness and spatial distribution of laccase genes of ectomycorrhizal, saprotrophic, and unknown basidiomycetes in the upper horizons of a mixed forest cambisol. Microb Ecol 50:570–579

Lundell TK, Mäkelä MR, Hildén K (2010) Lignin-modifying enzymes in filamentous basidiomycetes - ecological, functional and phylogenetic review. J Basic Microbiol 50:5–20

Manoj KM, Hager LP (2008) Chloroperoxidase, a janus enzyme. Biochemistry 47:2997–3003

Martin F, Aerts A, Ahren D et al (2008) The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452:88–92

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

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

Martinez AT, Rencoret J, Nieto L et al (2011) Selective lignin and polysaccharide removal in natural fungal decay of wood as evidenced by in situ structural analyses. Environ Microbiol 13:96-107

Marzullo L, Cannio R, Giardina P et al (1995) Veratryl alcohol oxidase from *Pleurotus ostreatus* participates in lignin biodegradation and prevents polymerization of laccase-oxidized substrates. J Biol Chem 270:3823–3827

Miki K, Renganathan V, Gold MH (1986) Mechanism of beta-aryl ether dimeric lignin model compound oxidation by lignin peroxidase by *Phanerochaete chrysosporium.* Biochemistry 25:4790-4796

Morin E, Kohler A, Baker AR et al (2012) Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc Natl Acad Sci U S A 109:17501–17506

Morris DR, Hager LP (1966) Chloroperoxidase: I. Isolation and properties of the crystalline glycoprotein. J Biol Chem 241:1763-1768

Murali Manoj K (2006) Chlorinations catalyzed by chloroperoxidase occur via diffusible intermediate(s) and the reaction components play multiple roles in the overall process. Biochim Biophys Acta 1764:1325-1339

Nakazawa T, Ando Y, Kitaaki K et al (2011) Efficient gene targeting in DeltaCc.ku70 or DeltaCc.lig4 mutants of the agaricomycete *Coprinopsis cinerea.* Fungal Genet Biol 48:939–946

Niemenmaa O, Uusi-Rauva A, Hatakka A (2007) Demethoxylation of [O(14)CH (3)]-labelled lignin model compounds by the brown-rot fungi *Gloeophyllum trabeum* and *Poria (Postia) placenta.* Biodegradation 19:555–565

Nishida A, Eriksson K-E (1987) Formation, purification, and partial characterization of methanol oxidase, a H_2O_2-producing enzyme in *Phanerochaete chrysosporium.* Biotechnol Appl Biochem 9:325–338

Ohm RA, de Jong JF, Lugones LG et al (2010) Genome sequence of the model mushroom *Schizophyllum commune.* Nat Biotechnol 28:957–963

Olson A, Aerts A, Asiegbu F et al (2012) Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytol 194: 1001-1013

Ortiz-Bemúdez P, Hirth KC, Srebotnik E, Hammel KE (2007) Chlorination of lignin by ubiquitous fungi has a likely role in global organochlorine production. Proc Natl Acad Sci U S A 104:3895–3900

Osborne RL, Coggins MK, Terner J, Dawson JH (2007) *Caldariomyces fumago* chloroperoxidase catalyzes the oxidative dehalogenation of chlorophenols by a mechanism involving two one-electron steps. J Am Chem Soc 129:14838-14839

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

Petersen JFW, Kadziola A, Larsen S (1994) Three-dimensional structure of a recombinant peroxidase from Coprinus cinereus at 2.6 Å resolution. FEBS Lett 339:291–296

Pisanelli I, Kujawa M, Spadiut O et al (2009) Pyranose oxidase from *Phanerochaete chrysosporium* - expression in *E. coli* and biochemical characterisation. J Biotechnol 142:197–106

Quinlan RJ, Sweeney MD, Lo Leggio L et al (2011) Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. Proc Natl Acad Sci U S A 108:15079-15084

Ralph J, Lundquist K, Brunow G et al (2004) Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids. Phytochem Rev 3:29–60

Rineau F, Roth D, Shah F et al (2012) The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. Environ Microbiol 14:1477-1487

Ruiz-Dueñas FJ, Ferreira P, Martínez MJ, Martínez AT (2006) In vitro activation, purification, and characterization of *Escherichia coli* expressed aryl-alcohol oxidase, a unique H2O2-producing enzyme. Protein Expr Purif 45:191-199

Ruiz-Dueñas FJ, Pogni R, Morales M et al (2009) Protein radicals in fungal versatile peroxidase: catalytic tryptophan radical in both compound I and compound II and studies on W164Y, W164H and W164S variants. J Biol Chem 284:7986–7994

Ruiz-Dueñas FJ, Fernández E, Martínez MJ, Martínez AT (2011) Pleurotus ostreatus heme peroxidases: an in silico analysis from the genome sequence to the enzyme molecular structure. C R Biol 334:795–805

Salame TM, Knop D, Tal D et al (2012) A gene-targeting system for *Pleurotus ostreatus:* demonstrating the predominance of versatile-peroxidase (mnp4) by gene replacement. Appl Environ Microbiol 78:5341-5352

Schmidt O, Liese W (1 980) Variability of wood degrading enzymes of *Schizophyllum commune*. Holzforschung 34:67–72

Schneider T, Keiblinger KM, Schmid E et al (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. ISME J 6:1749-1762

Schwarze FW (2007) Wood decay under the microscope. Fungal Biol Rev 21:133-170

Shaw PD, Hager LP (1959) Biological chlorination: III. ß-Ketoadipate chlorinase: a soluble enzyme system. J Biol Chem 234:2565–2569

Simon C, Daniel R (2011) Metagenomic analyses: past and future trends. Appl Environ Microbiol 77:1153-1161

Smith AT, Doyle WA, Dorlet P, Ivancich A (2009) Spectroscopic evidence for an engineered, catalytically active Trp radical that creates the unique reactivity of lignin peroxidase. Proc Natl Acad Sci U S A 106:16084-16089

Snajdr J, Steffen KT, Hofrichter M, Baldrian P (2010) Transformation of 14C-lableled lignin and humic substances in forest soil by the saprobic basidiomycetes *Gymnopus erythropus* and *Hypholoma fasciculare.* Soil Biol Biochem 42:1541-1548

Srebotnik E, Messner KE (1991) Immunoelectron microscopical study of the porosity of brown rot wood. Holzforschung 45:95–101

Srebotnik E, Messner K (1994) A simple method that uses differential staining and light microscopy to assess the selectivity of wood delignification by white rot fungi. Appl Environ Microbiol 60: 1383–1386

Srebotnik E, Messner K, Foisner R, Petterson B (1988) Ultrastructural localization of ligninase of *Phanerochaete chrysosporium* by immunogold labeling. Curr Microbiol 16:221-227

Stajich JE, Wilke SK, Ahren D et al (2010) Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea (Coprinus cinereus).* Proc Natl Acad Sci U S A 107:11889-11894

Steffen KT, Hatakka A, Hofrichter M (2002) Degradation of humic acids by the litter-decomposing basidiomycete *Collybia dryophila.* Appl Environ Microbiol 68:3442–3448

Sugano Y (2009) DyP-type peroxidases comprise a novel heme peroxidase family. Cell Mol Life Sci 66:1387-1403

Sugano Y, Muramatsu R, Ichiyanagi A et al (2007) DyP, a Unique Dye-decolorizing peroxidase, represents a novel heme peroxidase family: ASP I7 I replaces the distal histidine of classical peroxidases. J Biol Chem 282:36652−36658

Sundaramoorthy M, Kishi K, Gold MH, Poulos TL (1994) The crystal structure of manganese peroxidase from *Phanerochaete chrysosporium* at 2.06-A resolution. J Biol Chem 269:32759-32767

Sundaramoorthy M, Terner J, Poulos TL (I 995) The crystal structure of chloroperoxidase: a heme peroxidase−cytochrome P450 functional hybrid. Structure 3: 1367−1378

Suzuki MR, Hunt CG, Houtman CJ et al (2006) Fungal hydroquinones contribute to brown rot of wood. Environ Microbiol 8:2214−2223

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

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

Ullrich R, Nüske J, Scheibner K et al (2004) Novel haloperoxidase from the agaric basidiomycete *Agrocybe aegerita* oxidizes aryl alcohols and aldehydes. Appl Environ Microbiol 70:4575-4581

Urzúa U, Kersten PJ, Vicuña R (1998) Kinetics of Mn^{3+}-oxalate formation and decay in reactions catalyzed by manganese peroxidase of *Ceriporiopsis subvermispora.* Arch Biochem Biophys 360:215-222

Vaario LM, Heinonsalo J, Spetz P et al (2012) The ectomycorrhizal fungus *Tricholoma matsutake* is a facultative saprotroph in vitro. Mycorrhiza 22:409-418

van den Brink J, de Vries RP (2011) Fungal enzyme sets for plant polysaccharide degradation. Appl Microbiol Biotechnol 91:1477-1492

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

Vanden Wymelenberg A, Sabat G, Mozuch M et al (2006a) 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, Minges P, Sabat G et al (2006b) 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, Gaskell J, Mozuch MD 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, Mozuch MD 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, Mozuch M et al (2011) Significant alteration of gene expression in wood decay fungi *Postia placenta* and *Phanerochaete chrysosporium* by plant species. Appl Environ Microbiol 77:4499-4507

Vincent D, Kohler A, Claverol S et al (2012) Secretome of the free-living mycelium from the ectomycorrhizal basidiomycete *Laccaria bicolor.* J Proteome Res 11:157-171

Wariishi H, Valli K, Gold MH (1992) Manganese(II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium.* Kinetic mechanism and role of chelators. J Biol Chem 267:23688−23695

Welinder KG (1992) Superfamily of plant, fungal and bacterial peroxidases. Curr Opin Struct Biol 2:388−393

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

Whittaker JW (2005) The radical chemistry of galactose oxidase. Arch Biochem Biophys 433:227-239

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

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

Worrall JJ, Anagnost SE, Zabel RA (1997) Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199-219

Xu G, Goodell B (2001) Mechanisms of wood degradation by brown-rot fungi: chelator-mediated cellulose degradation and binding of iron by cellulose. J Biotechnol 87:43–57

Yelle DJ, Ralph J, Lu F, Hammel KE (2008) Evidence for cleavage of lignin by a brown rot basidiomycete. Environ Microbiol 10: 1844–1849

Yelle DJ, Wei D, Ralph J, Hammel KE (201 I) Multidimensional NMR analysis reveals truncated lignin structures in wood decayed by the brown rot basidiomycete *Postia placenta.* Environ Microbiol 13:1091–1100

Zubieta C, Joseph R, Sri Krishna S et al (2007) Identification and structural characterization of heme binding in a novel dye-decolorizing peroxidase, TyrA. Protein Struct Func Bioinform 69:234–243