REVIEW ARTICLE

Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution

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One sentence summary: A number of fungal and bacterial organisms have developed both competitive and mutualistic strategies based on the enzymatic machinery and low molecular compounds, which gives these organisms not only an advantage in wood degradation processes, but potentially giving them an advantage in their ecological niches.

ABSTRACT

Extensive research efforts have been dedicated to describing degradation of wood, which is a complex process; hence, microorganisms have evolved different enzymatic and non-enzymatic strategies to utilize this plentiful plant material. This review describes a number of fungal and bacterial organisms which have developed both competitive and mutualistic strategies for the decomposition of wood and to thrive in different ecological niches. Through the analysis of the enzymatic machinery engaged in wood degradation, it was possible to elucidate different strategies of wood decomposition which often depend on ecological niches inhabited by given organism. Moreover, a detailed description of low molecular weight compounds is presented, which gives these organisms not only an advantage in wood degradation processes, but seems rather to be a new evolutionary alternative to enzymatic combustion. Through analysis of genomics and secretomic data, it was possible to underline the probable importance of certain wood-degrading enzymes produced by different fungal organisms, potentially giving them advantage in their ecological niches. The paper highlights different fungal strategies of wood degradation, which possibly correlates to the number of genes coding for secretory enzymes. Furthermore, investigation of the evolution of wood-degrading organisms has been described.

Keywords: lignin degradation; fungi; bacteria; ecology; evolution

ABBREVIATIONS

CAZy: Carbohydrate active enzymes
DyP: dye-decolorizing peroxidase
ECM: ectomycorrhizal fungi
GLOX: glyoxal oxidase
GMC: glucose-methanol-choline oxidase/dehydrogenase
HTP: heme thiolate peroxidase
LDA: lignin-degrading auxiliary enzymes
LiP: lignin peroxidase
LMCO: laccase-like multicopper oxidase
LME: lignin-modifying enzymes
LPMO: lytic polysaccharide monooxygenase
MCO: multicopper oxidase

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INTRODUCTION

Evolution created lignocellulose, a main component of woody plants’ cell wall, as a compromise between mechanical functionality of land inhabiting plants and physical protection against herbivores and saprobionts. The chemical complexity of lignin has increased during the course of evolution from ancient pteridophytes and gymnosperms to the most evolved grasses (Barceló et al. 2004). Rigid tubular structures are required in higher plants to transport water and nutrients yet, at the same time cellulose microfibrils together with lignin and hemicellulose form a natural biocomposite that allows trees to support their enormous weight. This natural biocomposite allows trees to withstand hostile weather—very strong winds and consequently live for many thousands of years. A prime example of this is the North American sequoias, which are considered the largest single living trees and one of the oldest organisms inhabiting our biosphere.

With cellulose fibrils being embedded in a lignin matrix, wooden plants cell walls have developed a very robust structural framework (Boer et al. 2005). The expansion of land plants had significant and far-reaching consequences on the current appearance of terrestrial ecosystems, and was a major evolutionary event in the history of photosynthetic organisms. Most scientists agree that the successful colonization of the terrestrial realm involved a symbiotic association with certain fungi (Taylor, Krings and Taylor 2015b).

It is believed that the lignin biosynthetic pathway is highly conserved throughout the evolution of vascular plants. Molecular-clock analyses suggest that land plants form a monophyletic group and diverged from green algae about 700 million years ago (Ma) (Qi and Palmer 1999). It is not surprising that the multifunctional basic peroxidases responsible for lignin synthesis appeared early in the evolution of land plants. It is likely that this class of enzymes conferred important adaptive traits to plants for their new life on land and the development of vascular tissues (Barceló et al. 2004). This aromatic polymer (lignin) evolved c.a. 400–450 Ma in the Late Ordovician period. The earliest unequivocal fossil evidence of lignins has been found in pteridophytes, which are widely considered to be the first vascular plants and are likely to have played a key role in the colonization of the terrestrial landmasses (Barceló et al. 2004; Weng and Chapple 2010). Phenylpropanoid pathways in plants were probably one of the most critical for the genesis of this aromatic polymer. However, monolignol biosynthesis evolved primarily for the generation of UV-protectant molecules which are more ancient than vascular plants. The ability to deposit polymers of phenylpropanoid units in the cell walls by early tracheophytes was evolutionarily very advantageous. Lignin strengthens the plant cell walls by adhesion of layers of cellulose microfibrils which allows plants to expand significantly in body size, enhance water transport, resistance to pathogens and slows the degradation of wood by microorganisms (Weng and Chapple 2010; Leisola, Pastinen and Axe 2012; Labeeuw et al. 2015).

Figure 1A and B shows a picture of the cross section of a plants’ woody cell wall, whilst Fig. 1C shows a cartoon schematic of the cell wall structural components. The lignin and hemicellulose polymers function as the recalcitrant adhesive for cellulose microfibrils. The stratified structure and the cellulose fibers orientation in the S layers are non-parallel, which gives this biocomposite additional mechanical strength. Only recently were humans able to engineer a composite with such mechanical strength using glass or carbon fibers and epoxy resins. Although macroscopically many types of wood tend to have a similar appearance, there are significant differences between the structure and chemical composition of major polymeric components of softwood and hardwood (Table 1). Softwood (coniferous) and hardwood (deciduous) fibers mainly differ in the structure and composition of hemicellulose and lignin components. For instance, glucuronoxylan comprises 15% to 35% of the secondary cell wall polysaccharides in hardwood, while galactoglucomannan is the main hemicellulose component of secondary cell walls in softwood. Moreover, softwoods contain mainly guaiacyl lignin, whereas hardwoods contain varying ratios of syringyl and guaiacyl lignins (Ek, Gellerstedt and Henriksson 2009; Suzuki et al. 2012). The recalcitrance of softwood lignocellulose to bioprocess technologies has been attributed to its higher lignin content, smaller pore size and fewer hemicellulose-derived acetyl groups in comparison with hardwood (Palonen et al. 2004; MacDonald et al. 2011).

SPECIES DECOMPOSING LIGNIN

Lignin degradation is caused by certain fungi as well as several bacterial species (Fig. 2) (Breen and Singleton 1999; Singh Arora and Kumar Sharma 2010; Bugg et al. 2011a; Huang et al. 2013). Fungi are more efficient in the breakdown of lignin than bacteria, in which delignification is slower and more limited (Table S2, Supporting Information) (Siggoillot et al. 2012).

Microbial degradation of lignin has not been intensively studied in organisms other than fungi, but there are reports of bacteria that can break down lignin (Fig. 3). These lignin-degrading bacteria represent mainly three classes: Actinomycetes, α-Proteobacteria and γ-Proteobacteria (Bugg et al. 2011a; Huang et al. 2013). This ubiquitous group of microbes is widely distributed in natural ecosystems worldwide and occurs both in terrestrial and aquatic habitats (Srinivasan, Laxman and Deshpande 1991). Their apical and branching growth, production of aerial or substrate mycelia, and morphogenetic development resemble filamentous fungi. Due to the production of secondary metabolites and the use of extracellular enzymes, these bacterial species are considered major decomposers of lignocelluloses in soils (Tiquia, Wan and Tam 2002; Bibb 2005; Sonia, Naceur and Abdennaceur 2011; Saini et al. 2015). In the prokaryotes, one of the most frequently identified and studied ligninolytic enzymes is laccase (Kuhad, Singh and Eriksson 1997; Tian et al. 2014). Phylogenetic analysis has shown that representatives of Archaea from four phyla have laccase genes. Yet the greatest numbers of strains with laccase genes were identified in Proteobacteria, Actinobacteria and Firmicutes. Within Cynobacteria and Bacteroidetes, the number of strains with identifiable laccase genes was found to be substantially lower (Tian et al. 2014). Recently, research of Rashid et al. (2015) showed that Sphingobacterium (phylum Bacteroides) produce manganese superoxide dismutase, which is able to oxidize lignin via hydroxyl radical mechanism.

Many strains from the Brucella, Ochrobactrum, Sphingobium and Sphingomonas genera, belonging to the class α-Proteobacteria, decompose lignin. Within another group of ligninolytic bacteria (γ-Proteobacteria) the best known for their degrading abilities are Pseudomonas fluorescens (producing lignin peroxidase—LiP), Ps. putida (dye-decolorizing peroxidase—DyP,
Figure 1. Scanning electron micrograph (SEM) of transverse section of xylem tissue of Pinus sylvestris. (A) Visible are both the early and the latewood tracheides, SEM – × 200; (B) SEM – ×10 000 of the indicated area of panels A and C—diagram of the woody cell walls layers (adopted from Côté 1967). ML, middle lamella; P, primary wall; S1, outer layer of the secondary wall; S2, middle layer of the secondary wall; S3, innermost layer of the secondary wall.

Table 1. Chemical composition of plant material (Adapa, Tabil and Schoenau 2009; Brosse et al. 2012; Rowell 1984; Hon and Shiraishi 2001; El-Tayeb et al. 2012; Rytiöja et al. 2014; Kumar and Shukla 2016).

| Plant material | Cellulose (%) | Lignin (%) | Hemicelluloses | Pectin, starch, ash, etc |
|----------------|---------------|------------|----------------|------------------------|
|                | Galactoglucomannan | Arabinogluconoroxylan | Glucuronoxylan | Glucomannan |
| Softwood       | 33–42         | 27–32      | 5–18           | 7–14                   | 1–3           |
| Hardwood       | 38–51         | 21–31      | 15–35          | 2–5                    | 1–4           |
| Straw           | 33.25         | 17.13      | 14.5–18.1      | 24.8–33.9              |
| Barley          | 37.60         | 12.85      |                |                        |
| Wheat           | 34.20         | 13.88      |                |                        |
| Switchgrass    | 31–32         | 20.4–25.2  | 14.5–18.1      | 24.8–33.9              |
| Miscanthus      | 43–50.3       | 9.2–12.5   |                |                        |
| Corn stalks     | 61.2          | 6.9        |                | 19.3                   |
| Cereal          | 45–55         | 16–21      |                | 26–32                  |

manganese peroxidase—MnP), Enterobacter lignolyticus (catalase/peroxidase HPI and DyP) and Escherichia coli (laccase) (Tian et al. 2014).

The representatives of Actinomycetes with the known ability to produce catabolic enzymes are Streptomyces viridosphorus, S. paucinobilis and Rhodococcus jostii (Tian et al. 2014). Several filamentous bacterial species from the genus Streptomyces have been identified as lignin degraders and less than 10 enzymes pertaining to lignin degradation have been characterized for them (Fernandes et al. 2014). Within Actinomycetes, several species producing three enzymes (laccase, LiP, MnP) which are believed to play the most important role in biodegradation of lignin are known, e.g. S. coelicolor, S. griseus and S. psammoticus (Niladevi, Sheejaidevi and Prema 2008; Le Roes-Hill, Rohland and Burton 2011). In S. cinnamomeus, laccase and LiP activity was observed (Jing and Wang 2012). The best-studied strain (S. viridosphorus) produces several extracellular peroxidases and laccase (Bugg et al. 2011a; Tian et al. 2014). Laccase activity was also observed in S. ipomoea (Niladevi, Jacob and Prema 2008; Molina-Guijarro et al. 2009; Fernandes et al. 2014). All of these findings indicate that bacterial degradation of lignin may be more important than previously thought, particularly in soil.

The number of fungal species capable of decomposing lignin is not known exactly; however, Gilbertson reported that in North America, there are ~1600 to 1700 species of wood-degrading fungi (Gilbertson 1980). Wood-degrading fungi mostly live as saprotrophs or weak parasites in natural and human-affected forest ecosystems. Lately, information about genes coding enzymes that degrade lignin has been found in Ustilago maydis, which is a biotrophic phytopathogen (Couturier et al. 2012). Also, it has been shown that coprophilic fungi (e.g. Panaeolus papilionaceus, Coprinopsis fiesii) produce ligninolytic enzymes such as laccases and/or peroxidases (Heinzkll et al. 1998). Saprotrophic fungi play important functions in the biosphere, preventing accumulation of dead plant organic matter. Generally, wood-rotting fungi are primary lignin degraders and are the only group of microorganisms capable of mineralization of this abundant plant product accounting for about 25% of removable organic matter in the biosphere (Kuhad and Singh 2007; Huang et al. 2013). Depending on the particular morphology of wood
Figure 2. Relative quantities of secondary SW (grasses) components in this representative grass are 45–60% cellulose, 20–40% hemicellulose and 5–10% lignin (Bidlack 1990). Cellulose microfibrils consist of ~40 of these bonded chains. Three or four glucan chains occur for each β1→4-D-xylopyranose chain of hemicellulose with an occasional L-arabinofuranose molecule linked α1→3 to the xylopyranose chain. The hemicellulosic xylans and arabinoxylans, represented by the actual molecules or as long, triangular rods, are hydrogen bonded to cellulose and ester or ether-bonded to non-core lignin. Ester bonds between hemicellulose and non-core lignin shown in the figure include linkage between the O-5 position of arabinose in arabinoxylan and ρ-coumaric, ferulic and diferulic acids (Scalbert et al. 1985; Mueller-Harvey and Hartley 1986), as well as a hypothetical linkage between the O-3 position of xylose and cinnamic acid. Some of these lignin monomers, such as ferulic acid (Mueller-Harvey and Hartley 1986), may be so intimately associated with the hemicellulosic fraction that they fail to cross-link to lignin. Reproduced from Bidlack J, Malone M, Benson R: Molecular structure and component integration of secondary cell walls in plants. Proc Okla Acad Sci (1992, 72:51–56) with permission granted by author and the Oklahoma Academy of Science.

decomposition, the saprophytic fungi have been divided into three main groups: (i) white rot fungi, (ii) brown rot fungi and (iii) soft rot fungi. These groups can be further broken down into (iv) litter-decomposing and (v) dung-dwelling (coprophilic) fungi that also degrade lignin (Blanchette 1995; Liers et al. 2011). All these types of fungi are able to decompose lignin, but only white rot degrade it completely to CO₂ and H₂O (Blanchette 1995).

Wood-decaying fungal Basidiomycota ecologically represent white and brown rot, litter-decomposing, plant-pathogenic and ectomycorrhizal (ECM) fungi, although over 90% of all known wood-rotting species belong to the white rot type. Species of Basidiomycetes occupy different ecological niches and grow on various substrates, for instance, deciduous trees, conifers, crops, forest litter, grassland soils and roots of plants (Ryttioja et al. 2014). The members of Polyporales and Agaricales, i.e. Ganoderma spp., Phlebia radiata, Lentinula edodes or Pleurotus spp., mostly represent white rot fungi. Among brown rot fungi, Gloeophyllum trabeum has been studied the most and along with Serpula lacrymans and Coniophora puteana (Boletales) plays a harmful role in relation to the wood used in construction (Blanchette 1995). In nature, white rot Basidiomycetes occur more often on hardwood of angiosperms than on softwood of gymnosperms, whereas brown rot fungi more often attack softwoods and are usually found in coniferous ecosystems (Gilbertson 1980; Hatakka 2005; Sigoillot et al. 2012).

The details of the lignin degradation mechanism have been elucidated in the white rot fungus species Phanerochaete chrysosporium (currently Phanerodontia chrysosporium)
which produces multiple isoenzymes of lignin and manganese peroxidases but does not produce laccase (Hatakka 2005; Huang et al. 2013). The fungus occurs in the later stages of wood degradation, when available nitrogen is depleted by other microorganisms (Deacon 2006).

Within litter-decomposing Basidiomycota, ligninolytic enzymes have been detected, prime examples being Agaricus bisporus producing laccase and MnP and Marasmius quercophilus producing only laccase (Hatakka 2005). ECM fungi have genes encoding enzymes that degrade lignocellulose, but their ability to decompose lignocellulose is lower than in most other wood-decaying fungi. They are able to survive in the absence of host plants by assimilating reduced carbon compounds, similarly to free-living saprotrophs. Their degradation mechanism of lignocellulose is similar that of brown rot type of decay (e.g. Paxillus), while others use a white rot type mechanism (e.g. Cortinarius) (Kohler et al. 2015; Lindahl and Tunlid 2015).

Representatives from Ascomycota mostly cause soft rot decay. The ability to degrade lignin by these fungi is limited (Cragg et al. 2015). However, members of the Xylariaceae order (from the Daldinia, Hypoxylon, Xylaria genera) currently grouped with soft rot fungi have been previously included in the white rot fungi cluster (Hatakka 2005). Anamorphic fungi, such as the commonly occurring Alternaria alternata, also cause soft rot decay (Sigoillot et al. 2012). Worrall, Anagnost and Zabel (1997) and later Pildain, Novas and Carmárán (2005) reported that a few Eutypella species produce white rot decay in the late stages of wood decay, but soft rot in the early stages. Other studies of microfungi (e.g. Penicillium chrysogenum, Fusarium solani and F. oxysporum) have shown lower degrees of lignin decomposition when compared to white rot fungi (Kirk and Farrell 1987). Production of extracellular laccases has been observed in the plant pathogenic, anamorphic fungus Botrytis cinerea, which causes soft rot like decay in many horticultural crop plants (e.g. Daucus and Cucumis) as well as the ‘noble rot’ and ‘grey rot’ of Vitis vinifera (Thurston 1994).

There is no information to be found in literature about laccase production in lower fungi (Zygomycota and Chytridiomycota) (Singh Arora and Kumar Sharma 2010), although recent research suggests that these fungi are involved in wood degradation in nature (Fukasawa, Osono and Takeda 2011). Zygomycota generally do not utilize cellulose and lignin. However, some representatives of this phylum can decompose lignin and are confined to outer layers of decaying plant tissue (Ingold 1978).

Investigation of fungal succession of woody colonization showed that Basidiomycota dominate in the earlier stages of wood decomposition, while Ascomycetes dominate in the later stages (Crawford, Carpenter and Harmon 1990; Fukasawa, Osono and Takeda 2011). Species in Xylariaceae (Ascomycota) are known as latent inhabitants of living wooden tissues and primary decomposers of partially degraded woody debris (Fukasawa, Osono and Takeda 2011). Additionally, the studies of some fungal species and their relationship with plants have shown preference to a certain type of substrate (i.e. hard-wood, softwood, sapwood, heartwood) (Hatakka 2005; Deacon 2006).

ENZYMES INVOLVED IN LIGNIN DEGRADATION

Enzymes involved in lignin degradation can generally be divided into two main groups: lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. LDA enzymes

Figure 3. Ecology (occurrence) of lignin-degrading fungi. Polyporales—Cerrena unicolor (2), Phanerochaete chrysosporium (4), Postia placenta (8). Agaricales—Armillari meleae (1), Pleurotus spp. (5), Trametes versicolor (6). Agaricus bisporus (10). Green box—soft rot fungi (SRF); orange box—litter decomposers (LD); red box—parasite.
are unable to degrade lignin on their own yet are necessary to complete the degradation process (da Silva Coelho-Moreira et al. 2013).

LME produced by different microorganisms are classified as phenol oxidase (laccases) and heme containing peroxidases (POD), namely lignin, manganese and multifunctional (versatile) peroxidase (Fig. 4).

Recently, a new group of enzymes, heme-thiolate haloperoxidases, has been suggested to have a role in lignin degradation. This class of enzymes has yet to be classified to either the LME or LDA group. These enzymes are supposed to be the most versatile biocatalysts of the hemeprotein family. Heme-thiolate peroxidases (HTPs) share catalytic properties with at least three other classes of heme-containing oxidoreductases: classic plant and fungal peroxidases, cytochrome P450 monooxygenases and catalases (Hofrichter and Ullrich 2006). These enzymes were suggested to be involved in lignin degradation in culture of Ceriporiopsis subvermispora (Fernandez-Fueyo et al. 2012b) but only were proved to oxidase aryl alcohols (Ullrich et al. 2004). Up to date, there is no experimental evidence that these enzymes alone can degrade lignin, so we concluded that they should be considered as LDA enzymes.

Auxiliary enzymes allow the lignin degradation process through the sequential action of several proteins which may include oxidative generation of H$_2$O$_2$. This group includes glyoxal oxidase (GLOX; EC 1.2.3.5), aryl alcohol oxidases (AAO; EC 1.1.3.7), pyranose 2-oxidase (FOX; EC 1.1.3.10), cellobiose dehydrogenase (CDH; EC 1.1.99.18) and glucose oxidase (EC 1.1.3.4). These enzymes are frequently found in white rot fungi secretomes (Levasseur et al. 2008).

In nature, only Basidiomycota belonging to the aerobic white rot group are able to degrade lignin completely (Dashtban et al. 2010). Anaerobic fungi lack the enzymatic machinery to catabolize/mineralize lignin. The enzymatic reaction that cleaves the aromatic ring requires oxygen or its partially reduced species (reactive oxygen species—ROS), and therefore cannot take place in an anaerobic environment (Berg and McClougherty 2014).

**MAJOR GROUPS OF LMEs**

Lignin-modifying peroxidases (LMPs: LiP, MnP and VP) also called POD (Hammel and Cullen 2008) belong to class II peroxidases within the superfamily of catalase-peroxidases (formerly called plant and fungal peroxidases) (Welinder 1992; Zamocky et al. 2014). All members of this group contain protoporphyrin IX as a prosthetic group (Pollegioni, Tonin and Rosini 2015). This superfamily can be divided into three subclasses based on their amino acid sequences similarities and catalytic properties. Class I includes catalase-peroxidases, prokaryotic and organelle-localized eukaryotic heme peroxidases. Class II

![Figure 4](https://academic.oup.com/femsre/article-abstract/41/6/941/4569254)
includes all extracellular fungal heme peroxidases and is classified as auxiliary activities (AA2) family in carbohydrate active enzyme (CAZy) (Lombard et al. 2014). Class III comprises all secreted plant heme peroxidases (Zamocky, Furtmüller, Obinger 2009; Lundell, Makela and Hilden 2010). Very recently, first in fungi then later in bacteria, the new superfamily of heme peroxidases was identified. This newly identified group of enzymes is called dye-peroxidases (DyP), originally named ‘dye-decolorizing peroxidases’ (Sugano 2009). DyP may also participate in the degradation of lignocellulose and lignin, due to their apparent LMP-like enzymatic activities. The potential for cleavage of lignin substructure linkages was recently demonstrated for a DyP enzyme from the Basidiomycete Auricularia auricula-judae (Liers et al. 2010; Lundell, Makela and Hilden 2010).

Lignin peroxidase (EC 1.11.1.14)

Lignin peroxidases were first discovered in Phanerochaete chrysosporium (Tien and Kirk 1983; Paszczynski, Huynh and Crawford 1986; Kirk and Farrell 1987) and later in Trametes versicolor (Johansson and Nyman 1993). Bjerkandera sp., and Phlebia tremellosa, which are well known white rot fungi (Aarti, Arasu and Agastian 2015). Activity of LiP has previously been detected in some bacteria, such as Acinetobacter calcoaceticus and Streptomyces viridosporus (Dashbhat et al. 2010). LiPs, capable of attacking lignin polymers, are relatively non-specific to their substrates. LiPs are known to oxidize different phenolic aromatic compounds and a variety of non-phenolic lignin model compounds along with many other organic molecules (Wong 2009). These enzymes are usually secreted by green microorganisms as a family of isozymes whose relative composition and isoelectric points (pI) vary depending on the growth medium and nutrient conditions (Santhanam et al. 2012). The globular structure of LiP isolated from P. chrysosporium is composed of eight major and eight minor α-helices with limited β-components and is organized into two domains that form an active center cavity composed of a heme-chelating single ferric ion (Choinowski et al. 1999). The LiP contains two glycosylation sites, two Ca$^{2+}$-binding sites and four disulfide bridges, all stabilizing the three-dimensional structure of this enzyme. Molecular mass of LiPs ranges from 35 to 48 kDa and it has a pI between 3.1 and 4.7. The high redox potential of LiPs (around 1.2 V at pH 3) makes these enzymes capable of oxidizing substrates that are not oxidized by other peroxidases (Siggoillot et al. 2012). The catalytic cycle of LiP resembles the catalytic mechanism common to all peroxidases. In each cycle, the native enzyme is oxidized by H$_2$O$_2$ and generates a compound I intermediate that exists as a ferric protohemin radical cation [Fe(IV) = O$^+$]. Next, the enzyme is subjected to two single-electron reduction steps by the electron donor substrate, such as veratryl alcohol (VA), leading to a transient formation of compound II [Fe(IV) = O] and a VA radical cation (VA$^+$). The compound II further oxidizes the second VA molecule, simultaneously returning to its native stage to initiate a new catalytic cycle of LiP. VA, similar to Mn(III), plays the role of being a small molecular weight redox transfer mediator between the enzyme and its polymeric substrate (Wong 2009).

Manganese-dependent peroxidase (EC 1.11.1.13)

Manganese peroxidase is another important LME secreted in multiple isoforms and was first detected in P. chrysosporium more than 30 years ago (Glenn and Gold 1985; Paszczynski, Huynh and Crawford 1986). These enzymes have also been found in other Basidiomycota species, including F. tigrinus (Lisov, Leontievsky and Golovleva 2003), Lenzites betulina (Hoshino et al. 2002), Agaricus bisporus (Lankinen et al. 2001), Bjerkandera sp. (Palma et al. 2000) and Nematomata frowardi (Hilden et al. 2008). The details about the presence of MnP in bacteria, yeast and mold are emerging in the scientific literature. The MnP activity in Bacillus pumilus, Paenibacillus sp. (de Oliveira et al. 2009) and Azospirillum brasilense (Kupriashina, Selivanov and Nikitina 2012) was studied under absence and presence of the inducers. MnP activity was also detected in the Actinobacterium S. psammaticus (Niladevi and Prema 2005). Similarly to LiP, MnP has a molecular structure composed of two α-helices domains with the heme sandwiched in between. MnP has five disulfide bridges and two Ca$^{2+}$ ions, which maintain the structure of the active enzyme (Sutherland et al. 1997). The Mn(II)-binding site consists of two glutamate and one aspartate γ-carboxylic groups and is located close to the porphyrin macrocycle (Wong 2009). The molecular mass of MnPs ranges from 38 to 62.5 kDa, which include 4%–18% glycans, and their pI ranges from 2.9 to 7.1 (Sigoillot et al. 2012). The catalytic cycles of MnPs and LiPs are very similar, but Mn-dependent peroxidases are unique in utilizing Mn(II) as their reducing substrate, generating Mn(III), which diffuses from enzymes into the lignocellulose structure. Outside of the enzymes, Mn(III) is stabilized by chelation with organic acids such as oxalate, fumarate, glyoxylate and malate (Martinez et al. 2005; Sigoillot et al. 2012) and acts as a diffusible low molecular weight redox mediator that oxidizes organic substrates (Goszczyński et al. 1994) via hydrogen or one electron abstraction. The organic acids also facilitate the release of Mn (III) from the active site of the enzyme (Niladevi 2009).

Addition of Mn(II) rapidly reduces compound I to compound II, thus forming Mn(III). A second Mn(II) is then used to reduce compound II back to the native enzyme. The conversion of compounds (I and II) can also be achieved by the addition of other electron donors, such as a variety of phenolic substances, but at a slower rate. Phenolic compounds are, however, not able to reduce efficiently MnP compound II back to the native enzyme. Mn(II) is an obligatory redox coupler for the enzyme to complete its catalytic cycle. Similar to the LiP, the excess of H$_2$O$_2$ can generate compound III which is an inactive form of the enzyme (Warishi, Akleswaran and Gold 1988; Manavalan, Manavalan and Heese 2015).

Versatile peroxidase (EC 1.11.1.16)

Versatile peroxidases (VPs) combine the molecular architecture of LiP and MnP. VP oxidizes typical LiP substrates e.g. VA, methoxybenzenes and non-phenolic model lignin compounds, as well as Mn$^{2+}$ (Hofrichter et al. 2010; Garcia-Ruiz et al. 2014). The structure of VP includes 11–12 helices, four disulfide bridges, two structural Ca$^{2+}$ sites, a heme pocket and a Mn$^{2+}$-binding site similar to that of MnP (Perez-Boada et al. 2005). The substrate specificity of VPs is similar to that of LiPs, including oxidation of high and medium redox potential compounds. Yet VP also oxidizes azo-dyes and other non-phenolic compounds with high-redox potential in the absence of mediators (Garcia-Ruiz et al. 2014). VP was detected in members of the genera Pleurotus (Pleurotus eryngii (Ruiz-Duenas et al. 1999), Pl. ostreatus (Ruiz-Duenas et al. 2001)) and Bjerkandera [Bjerkandera adusta (Heinfling et al. 1998), B. fumosa (Rodakiewicz-Nowak, Jarosz-Wilkolazka and Luterek 2006)]. VPs are glycoproteins secreted as several isoenzymes with a molecular mass ranging between 40 and 45 kDa with a pI ranging between 3.4 and 3.9 (Mester and Field 1998). The basic catalytic cycle of VPs is similar to those of other peroxidases with the two intermediary compounds I and II, but...
is more complex due to a more diversified pool of potential substrates (Sigoillot et al. 2012).

Dye-decolorizing peroxidase (EC 1.11.1.19)

Dye-decolorizing peroxidases constitute a new family of heme peroxidases, which are phylogenetically unrelated to the classical LME peroxidases, including LiP, MnP and VP (Zamocky et al. 2015). The structure of DyP-type peroxidases reveals two domains that contain α-helices and anti-parallel β-sheets, and the heme cofactor is located at the cavity between the two domains (Colpa, Fraaije and van Bloois 2014). In view of the scant evolutionary relationship between DyPs and the classical LMP family, the observed similarities of the catalytic site (heme pocket) must be considered as a convergent type of evolution to provide similar reactivity of these enzymes (Linde et al. 2015). The DyP-type peroxidases were first discovered in a culture of the fungus B. adusta (previously Geotrichum candidum) and were named after their ability to decolorize a wide range of dyes (Kim and Shoda 1999; Fernandez-Fueyo et al. 2015). Ligninolytic activity of DyPs has been reported in other fungi, such as Termitomyces albuminus (Johkuma, Okhuma and Kudo 2003), Aureobasidium auricula-judae (Liers et al. 2010), Ipex lacteus (Salvachua et al. 2013) and the bacteria Rhodococcus jostii (Robert et al. 2011), Thermojigida fusca (van Bloois et al. 2010) and Pseudomonas fluorescens (Rahmanpour and Bugg 2015). Recent genome sequence analysis revealed that DyPs are prominent in bacteria, whereas only a small number are reported in fungi and higher euarkyotes. Occurrence of DyP in archaea is even more limited, suggesting that these enzymes can be regarded as the bacterial equivalent of the fungal lignin-degrading peroxidases (Colpa, Fraaije and van Bloois 2014). Based on the phylogenetic analysis of genomic sequences from fungi, bacteria and archaea, DyPs have been classified into four subfamilies: A, B, C and D. Type A include bacterial DyPs that contain a Tat-dependent signal sequence, which suggests that they function outside of the cytoplasm. The type B subfamily, all putative cytoplasmic enzymes, is also found in Ascomycota and the only DyP from a non-fungal Eukaryota (the mycetozoon Dictyostelium discoideum). Type C includes sequences from bacteria, and two sequences from B. adusta which are involved in intracellular metabolism similar to type B. Type D contains primarily fungal variants of the enzyme from Ascomycota and Basidiomycota which are linked to dye decolorization (Colpa, Fraaije and van Bloois 2014). DyPs possess low substrate specificity and oxidize all of the typical peroxidase substrates (ABTS, DMP, Congo Red, Acid Red 151, Adlerol, Mordant Black 9, Reactive Black 5, etc.). In addition to typical peroxidase activity, it has been suggested that DyP has an additional hydrolytic or oxygenase activity (Sugano et al. 2009). These enzymes are active at low pH values (pH range 3–4) and are able to degrade different dyes, particularly anthraquinone dyes (Colpa, Fraaije and van Bloois 2014). Nevertheless, the physiological role of DyPs is still unclear; although evidence is accumulating that some bacterial variants of this enzyme are involved in lignin degradation and/or as a defense mechanism against oxidative stress (Liers et al. 2013).

Laccase (Lac, benzenediol: oxygen oxidoreductases; EC 1.10.3.2)

Laccases are considered to be among the most important components of the ligninolytic complex of wood-destroying microorganisms (Morozova et al. 2007). All laccases oxidize a range of aromatic compounds, including phenolic moieties typically found in lignin, aromatic amines, benzenothiols and hydroxyindoles using molecular oxygen as an electron acceptor. Inorganic/organic metal compounds are also substrates for laccases, and it has been reported that Mn(II) is oxidized by laccase to form Mn(III) and organometallic compounds (Fe(CN)63−) to (Fe(CN)64−) (Zimmerman et al. 2008; Garcia-Ruiz et al. 2014). Laccases are widely distributed in plants, fungi (Leontievsky et al. 1997) and in some bacteria (A. lipoferum (Diamantisidis et al. 2000), B. subtilis (Martin et al. 2002), S. lavendulae (Suzuki et al. 2003) and Sinorhizobium meliloti (Pawlik et al. 2016)) and even insects (Suderman et al. 2006). The localization of laccase is associated with its different physiological functions and is dependent on the organism producing the enzyme. Laccases are extracellular, periplasmic and intracellular proteins, in contrast to the other ligninolytic enzymes mostly reported as extracellular proteins. In plants, laccases are intracellular enzymes that participate in the synthesis of lignin (Morozova et al. 2007). The majority of fungi produce both intracellular and extracellular laccases. The intracellular laccases of fungi and periplasmic bacterial laccases most likely participate in the transformation of low molecular weight phenolic compounds in the cell (Baldrian 2006). For example, the CotA protein (laccase) is involved in dark pigment (melanin) formation in B. subtilis endospores’ coat, protecting the spores against harmful UV radiation (Hullo et al. 2001).

Fungal laccases are involved in multiple processes, such as pathogenesis, detoxification, degradation of lignin (Youn, Hah and Kang 1995) and involvement in the development and morphogenesis of higher fungi (Leonowicz et al. 2001; Morozova et al. 2007). Laccases are metalloproteins belonging to the group of polyphenol oxidases containing copper atoms in the catalytic site and therefore called blue multicopper oxidases (Baldrian 2006). The active center of laccases is composed of three types of four copper atoms with differing electron paramagnetic resonance parameters (Morpurgo et al. 1980). The type 1 copper (T1Cu) is characterized by a strong absorption around 600 nm and it is responsible for the intense blue color of purified laccase. The type 2 copper (T2Cu) is colorless and exhibits only weak absorption in the visible portion of the electromagnetic spectrum and type 3 copper (T3Cu) has an absorption peak at 330 nm. T2Cu and T3Cu create a 3-nuclear copper cluster T2/T3 consisting of one T2 copper ion and two T3 copper ions (Madhavi and Lele 2009). Such an arrangement of copper atoms distributed in three domains is present in most of the bacterial and fungal laccases. However, structural research in several Actinobacteria such as S. griseus, S. cyaneus, S. coelicolor, S. ipomoea, S. sicueus and T. fusca have revealed the presence of two Cu-binding domains rather than three. The enzymes with two-domain structures have been named small laccase or small laccase-like multicopper oxidases (LMCO) (Saini et al. 2015). Laccases mainly react with free phenolic fragments of lignin due to the random polymer nature of lignin and laccases lower redox potential. However, in the presence of some low molecular mass mediators, laccase can also attack non-phenolic aromatic compounds with high redox potentials (Kunamneni et al. 2007). Substrate oxidation by laccase is a single-electron reaction generating a free radical. The initial product of the reaction is typically an unstable phenoxy radical that can be converted to quinone in a second reaction catalyzed by the enzyme (Shraddha et al. 2011; Fitigau, Peter and Boeriu 2013). Most fungi secrete several isozymes of laccases. These isozymes originate from the same or different genes encoding for the laccase enzyme. The number of isozymes found depends on species, growth conditions and the presence or lack of an inducer in the growth medium (de Souza et al. 2004; Morozova et al. 2007).
LDA ENZYMES
Glyoxal oxidase (EC 1.2.3.5)
Glyoxal oxidase generates extracellular hydrogen peroxide by the oxidation of a variety of simple dicarbonyl and hydroxycarboxyl compounds, especially glyoxal and methylglyoxal (Kersten and Cullen 1993; Yamada et al. 2014). Based on an unusual free radical-coupled copper active site, GLOX was classified in the CAzY family among other copper radical oxidases in the family AAS (Whittaker et al. 1999; Daou et al. 2016). Moreover, GLOX was suggested to regulate peroxidase activity and be activated in vitro by the presence of LiP (Kersten 1990). The mechanism of aldehyde oxidation by GLOX has not been studied in as great detail; however, it was suggested that GLOX reacts with the hydrated, gem-diol form of the substrate via an analogous mechanism to galactose oxidase (Yin et al. 2015). The best-characterized GLOX (MW = 68 kDa) is an enzyme isolated from *Phanerochaete chrysosporium*; however, the genes coding for GLOX were found in many other white rot fungi (Kersten 1990). Kersten and Cullen (2014) suggested that along with both substrates mentioned above, glycol aldehyde (derived from lignin) may be a substrate for GLOX via these reactions: glycolaldehyde → glyoxal → glyoxylic acid → oxalate. There are two other possible sources for GLOX substrates: (i) MnP-dependent lipid peroxidation of linoleic acid (Watanabe et al. 2001); (ii) abiotic oxidation of sugars and trioses in a Fenton system which produces glyoxal (Manini et al. 2006; Kersten and Cullen 2014).

Aryl alcohol oxidase (EC 1.1.3.7)
Aryl alcohol oxidase, belonging to the glucose-methanol-choline oxidase/dehydrogenase family (GMC), was originally found in many *Agaricaceae* species (Hernandez-Ortega, Ferreira and Martinez 2012) and later in *Aspergillus*, *Fusarium* and even in some bacteria (Kumar and Goswami 2006; Tamboli et al. 2011). This monomeric enzyme is composed of two domains with a non-covalently bound FAD cofactor and a molecular mass ranging from 66.7 up to 334 kDa (Guillen, Martinez and Martinez 1992; Bruckmann et al. 2002; Fernandez et al. 2009). AAO produces hydrogen peroxide by oxidative dehydrogenation of phenolic and non-phenolic aryl-alcohols, polyunsaturated (aliphatic) primary alcohols or aromatic secondary alcohols to their corresponding aldehydes (Ferreira et al. 2009). Hernandez-Ortega, Ferreira and Martinez (2012) suggested that the *H₂O₂* needed in lignolysis domains during the cycling reaction of AAO (extracellular enzyme) and aryl-alcohol dehydrogenase, which converts alcohol to aldehyde operating inside hyphae by NADP-dependent reaction. Recently described aryl-alcohol:quinone oxidoreductase from *Phanerochaete chrysosporium* was shown to have homology with AAO. The enzyme was able to reduce radical intermediates (guaiacol, sinapic acid, etc.) generated by laccase (Mathieu et al. 2016).

Heme-thiolate haloperoxidases
Two types of haloperoxidases were included in this recently discovered group of enzymes: chloroperoxidases (CPO, EC 1.11.1.10) and aromatic peroxidogenases (APO, unspecific peroxidogenases, EC 1.11.2.1). CPO was first discovered in the Ascomycete *Calderomyces fumago*. Their reaction resembles that of cytochrome P450s, consisting of epoxidation and hydroxylation of substrates such as organic sulfides, olefins and aromatic rings; however, they are not susceptible to CPO-catalyzed oxygen trans-

fer (Hofrichter and Ulrich 2006). Ortiz-Bermudez, Srebotnik and Hammel (2003) proved that CPO may not only chlorinate major structures in lignin but also cleave them.

APO has been discovered in cultures of *Agrocybe aegerita* (MW = 45 kDa) and has been shown to transfer oxygen to aromatic and aliphatic substrates similar to cytochrome P450, but instead of directly transferring molecular oxygen, these enzymes utilize hydrogen peroxide (Piontek et al. 2010). Moreover, this enzyme has a surprisingly high reactivity toward benzyl C – H and phenolic substrates compared to typical peroxidogenes or model compounds due to the basic ferryl environment of their active center (Wang et al. 2015). Piontek et al. (2013) proved that these enzymes, unlike the other classical peroxidogenes containing histidine imidazole, have a cysteine residue as the fifth ligand coordinating with the heme iron atom.

Glucose dehydrogenase (EC 1.1.99.10)
Flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase (GDH), an extracellularly secreted enzyme by fungi, is supposed to play important role in detoxification by reducing quinones produced by plants. Moreover, the enzyme was suggested to protect fungal cell from phenoxy radicals of the same origin (Sygmund et al. 2011; Piumi et al. 2014). GDH catalyzes the anemic hydroxy group of glucose while the cofactor FAD serves as the primary electron acceptor. In comparison with GOX, GDH does not use oxygen as an external electron acceptor but instead uses phenoxy radicals, quinones, redox dyes and iron complexes such as ferricyanide and ferrocenium hexafluorophosphate (Piumi et al. 2014). Although this enzyme is mostly studied in genus *Aspergillus*, recent studies showed that it may play important role in metabolism of *Py. cinnabarinus* (Piumi et al. 2014; Rola et al. 2015).

Pyranose 2-oxidase (EC 1.1.3.10)
Pyranose oxidase (pyranose: oxygen 2-oxidoreductase) catalyzes the C-2 oxidation of several aldopyranoses comitomant with the reduction of O₂ to H₂O₂ (Daniel, Volc and Kubatova 1994; de Koker et al. 2004). With a molecular weight of up to 300 kDa (homotetramer), POX is produced by a number of Basidiomycetes, especially in the order of Polyporales (Daniel, Volc and Kubatova 1994; de Koker et al. 2004). This enzyme contains three conserved domains: the FAD-binding site, prosthetic group—flavin attachment loop and the substrate-binding region. The active site loop was proved to be moving in the presence of oxygen facilitating release of sugar product (Prongjit et al. 2009; Pittsawong et al. 2010). POX is located within the periplasmic space, where it is associated with membrane-bound vesicles and other membrane structures including extracellular mucilage slime (de Koker et al. 2004).

Cellbiose dehydrogenase (EC 1.1.99.18)
Cellbiose dehydrogenase (cellbiose: [acceptor] 1-oxidoreductase) is secreted mainly by white rot fungi such as *P. chrysosporium*, *Trametes versicolor*, *Py. sanguineus* and *I. lacteus*. The brown rot fungi do not produce CDH with the only known exception being *Coniophora puteana* (Schmidhalter and Caneva 1993; Kajsa et al. 2004). CDH was also isolated from the soil plant pathogen *Sclerotium rolfsii* and detected in the ECM fungi *Pisolithus tinctorius*, *Suillus variegatus* and *Cortinarius* sp. (Balasri and Valaskova 2008). The presence of CDH in bacteria has not been confirmed yet. Recent research shows...
that the biological function of CDHs is associated with the cellulose degradation system (Beeson et al. 2015). However, CDHs can also reduce $\text{Fe}^{3+}$ to $\text{Fe}^{2+}$ and $\text{O}_2$ to $\text{H}_2\text{O}_2$. Though, ferrous ion and hydrogen peroxide are Fenton reagents found to be major players in lignocellulose degradation by brown rot fungi (Henriksson, Johansson and Pettersson 2000).

CDH is an extracellular glycoprotein detected in the secreto- mases of white rot and some brown rot pathogenic and saprotrophic fungi from the dicaryotic phyla of Basidiomycota and Ascomycota (Zamocky et al. 2006). CDH has a bipartite domain organization containing a C-terminal flavodehydrogenase domain with non-covalently bound FAD connected via a flexible linker region to an electron mediating, heme b (proto- porphyrin IX) containing N-terminal cytochrome like domain (Hallberg et al. 2002). As a flavocytochrome, CDH exhibits a unique architecture for extracellularly secreted enzymes (Kracher et al. 2015). CDH was identified as the first non-hydrolytic enzyme involved in the breakdown of cellulose. It effi- ciently oxidizes cellulose and other $\beta$-1,4-linked disaccharides and oligosaccharides at the C-1 position to their corresponding lactones using a wide spectrum of electron acceptors including quinones, phenoxyradicals, $\text{Fe}^{3+}$, $\text{Cu}^{2+}$, cytochrome c or trioxide ion (Henriksson, Johansson and Pettersson 2000). Lactones created in reactions catalyzed by CDH are converted into aldonic acids by spontaneous or enzymatic hydrolysis with lactonase (Beeson et al. 2011). The oxidation of the substrate by CDH leads to the reduction of FAD to FADH$_2$ (Zamocky et al. 2006). CDH be- longs to the AA3 enzymes group in the CAZy database and be- longs to the GMC oxidoreductases family. Based on the available sequences CDHs were divided into three classes. Class I repres- ents CDHs secreted by Basidiomycota with shorter amino acid sequences and the optimum acidic pH. Besides the catalytic do- main, the enzyme contains conserved cellulose binding module (CBM) characteristic to cellulolytic enzymes. Enzymes belong- ing to class I show a strong preference for cellulose and cello- oligosaccharides substrates, while glucose and other monosaccharides are very weak substrates for class I CDH (Zamocky et al. 2004). Class II that has the ability to accept glucose as a substrate and exhibit neutral pH optima is encompassing the larger and more complex Ascomycota CDHs. Class II enzymes are subdi- vided into class IIA enzymes containing a type 1 CBM and class IIB enzymes that lack such a domain (Zamocky et al. 2006). The secretion of class III CDHs has not yet been confirmed (Harrei- ther et al. 2011).

LOW MOLECULAR WEIGHT SYSTEMS

Low molecular weight compounds play important roles in all stages of wood decay, acting as diffusible oxidative agents and electron shuttles for enzymatic systems. These low molecular weight fungal metabolites are important during the early stages of decay because fungal enzymes are too large to penetrate the intact wood cell wall. Therefore, the initial stages of decay in- volve direct and indirect non-enzymatic oxidative and reductive reactions of ROS, the phenoxyl radicals, organic acids and vari- ous transition metals coordination complexes as well as species produced in secondary radical cascades (Cowling 1961; Goodell et al. 1997; Arantes and Milagres 2009). These diffusible media- tors, rather than the enzymes themselves, are thought to react directly with lignin to generate radical sites within the polymer and initiate a cascade of bond scissions, ultimately leading to the depolymerization of lignin.

The main groups of these low molecular weight oxidants are extracellular aromatic compounds, which are the key electron carriers between enzymes and substrates. These extracellular aromatic compounds are present in plants such as flavonoids, tannins and lignans (Gross 2008). They can also originate from lignin degradation products such as benzoic acids (Chen, Chang and Kirk 1982, 1983), veratryl (3,4-dimethoxybenzyl) alcohol (Harvey, Schoemaker and Palmer 1986) and are found in humus (Kuiters and Dannemann 1987; Suominen, Kitunen and Smolander 2003). Very recently, long range electron transfer mecha- nism was proved to play a crucial role in reactions catalyzed by LiP and VP while degrading lignin polymer. A buried trypto- phan (Trp244), a neighbor phenylalanine (Phe198) and surpahse tryptophan appeared to be the most important in this process (Acebes et al. 2017). The same mechanism was already described for lytic polysaccharide monoxygenase (LPMO) in degradation of cellulose while insoluble high molecular weight lignin served as an electron reservoir. The electrons can be transferred by soluble low molecular weight compounds present in plant cell walls, or secreted by hyphae, for example, 3-hydroxyanthranilic acid produced by Pycnoporus cinnabarinus (Westergen et al. 2015). Preferential oxidation of phenolic lignin units and side-chain oxidation by white rot fungi leads to the release of pheno- lic residues with oxidized side chains (Canas and Camarero 2010). In addition to the phenolic hydroxyl group(s), these compounds may contain additional functional groups, such as methoxyls, amines, ketones, aldehydes and carboxylic acids. Phenolic substances are oxidized by fungal oxidoreductases (peroxidases, dehydrogenases, laccase) to phenoxyl radicals, which could oxidize non-phenolic residues of lignin through the H-abstraction mechanism (d’Acunzo et al. 2006). Studies of spruce wood lignin breakdown by Phanerochaete chrysosporium have identified low molecular weight products such as aromatic carboxylic acids and derivatives of benzoic acid, indic- ating that they had arisen from $\text{Cu}^{2+}$ oxidative cleavage of lignin components and the compounds included a biphenyl di- carboxylic acid, and a diphenyl ether dicarboxylic acid, derived from the biphenyl and diphenyl ether components of lignin, re- spectively (Chen, Chang and Kirk 1982, 1983). Similar phenolic products have been observed after treatments of lignocellulose by bacterial lignin degraders. Prime examples include benzoic acids with hydroxyl(s), methoxyl(s), carboxyl(s) substitutions (Aneurinibacillus aneurinilyticus, Pseudomonas putid; benzalde- hyde with hydroxyl, methoxy, trimethoxy substitutions (Bacillus sp., Streptomyces paucimobilis); and cinnamic acid with hydroxy, methoxy substitutions (Bacillus sp., Ps. putid). (Masai, Katayama and Fukuda 2007; Ahmad et al. 2010; Bugg et al. 2011a).

The second group of low molecular weight oxidants includes ROS such as hydroxyl radical ($\text{OH}^\cdot$), superoxide anion radia- n (O$_2$$^\cdot$) and hydrogen peroxide (H$_2$O$_2$). The main source of these ROS are enzymatic reactions (e.g. glucose oxidase, CDH, laccase, catalase and quinone reductases) and Fenton reac- tions detected mainly in brown rot fungi (Guileen et al. 2000; Martinez et al. 2009). The hydroxyl radical is the most power- ful non-specific oxidant present in biological systems, with a very short half-life, playing very important roles in the initial non-enzymatic attack of the wood cell wall in brown rot fungi (Halliwell and Gutteridge 1999; Goodell 2003). These hydro- xyl radicals are powerful oxidants that can both de- polymerize polysaccharides by hydrogen abstraction and at- tack lignin by demethylation/demethoxylation (Niemenmaa, Uusi-Rauva and Hatakka 2008; Arantes and Milagres 2009). For the Fenton reaction, Fe(II) ions and hydrogen perox- ide are needed. The main source of Fe(II) ions is insol- uble Fe(III) oxyhydroxide complexes, which must be solubi- lized and reduced to ferrous ion. The reductive solubiliza- tion is most likely facilitated by oxalic acid, accumulated ex- tracellularly by both brown and white rot fungi, leading to
a lowering of the pH in close proximity to hyphae in the lumens of decaying wood cells (Goodell 2003). It is believed that the main source of hydrogen peroxide in brown rot fungi is enzymatic oxidation of methanol (by alcohol oxidases). Methanol is generated through demethylation of the lignin polymer (Postia placenta, Gloeophyllum trabeum) (Daniel et al. 2007). In white rot fungi, 

\[ \text{H}_2\text{O}_2 \] 

is generated by different oxidases, such as GLOX or AAO (Guillen et al. 2000). During this stage, specific mechanisms of ferric iron chelation and reduction to its ferrous state are required and most likely involve extracellular low molecular weight phenolic compounds. The first group is made up of plant-derived lignin degradation products such as syringyl-type phenolic compounds (Eggert et al. 1996; Nousiainen et al. 2009). The next group are phenolic compounds produced by fungi, such as 2,5-dimethoxy-1,4-benzoquinone and 4,5-dimethoxy-1,2-benzoquinone (Po. placenta, G. trabeum, Serpula lacrymans) (Enoki, Itakura and Tanaka 1997; Paszczynski et al. 1999; Newcombe et al. 2002; Shimokawa et al. 2004; Suzuki et al. 2006). This phenolate and hydroquinone aromatic compounds substituted with electron-donating functional groups, such as –OCH₃ oxidize rapidly and in the presence of ferric ions generate ferrous ions (Suzuki et al. 2006). A redox cycling process has been proposed in G. trabeum, using two quinones that are produced extracellularly, 2,5-dimethoxy-1,4-benzoquinone and 4,5-dimethoxy-1,2-benzoquinone (Jensen et al. 2001). Each hydroquinone is able to reduce chelated Fe(III) to Fe(II). The ferrous iron-oxalate complex then reacts with hydrogen peroxide to generate hydroxyl radicals, which attack lignin. The oxidized quinone is then recycled by reduction of O₂ or Fe⁴⁺ to the hydroquinone. Besides the role as Fe (II) chelator, oxalic acid has other roles both in cellulose and lignin degradation, e.g. chelation and stabilization of Mn(II/III), acting as phenolic oxidants and as a buffer in the natural soil environment (Perez and Jeffries 1993; Hyde and Wood 1997; Shimada et al. 1997). Oxalic acid was detected in cultures of brown and white rot fungi but the main producers of this simple dicarboxylic acid are brown rot fungi (Shimada et al. 1997; Graz and Jarosz-Wilkolazka 2011). The low molecular Fe(II) reductants can diffuse through the woods cell wall and mediate the Fenton reaction (Goodell et al. 1997; Varela and Tien 2003; Wang and Gao 2003). There is also an alternative mechanism for ferric iron reduction via the activity of cellulose dehydrogenase but this enzyme has not been found in brown rot fungi (Mason, Nicholls and Wilson 2003). The other postulated mechanism of Fe(III) reduction involves extracellular low molecular weight NADH-dependent glycoalkylates identified in cultures of Po. placenta (brown rot) (Hammel et al. 2002; Goodell 2003). During ligninolysis, other substrate-derived oxidants are produced such as alkoxyl (• OR) and peroxyl (• OOR) radicals but they are less reactive than Fenton reagents (Hammel et al. 2002). Superoxide anion radicals produced by white rot fungi can be converted to 

\[ \text{H}_2\text{O}_2 \] 

via both the dismutation and the oxidation of Mn(II) to Mn(III) and can also be involved in the Haber-Weiss reaction (Barr et al. 1992). Furthermore, by reacting with phenoxyl radicals formed during lignin degradation, superoxide ions contribute to oxidative depolymerization of lignin and its degrada-

porting Information (Riley et al. 2014). Moreover, the genes GH74 xyloglucanase, GH10 endo-xylanase, GH79 ß-glucuronidase, CE1 acetyl xylan esterase and CE15 glucurononyl methyltransferase tend to appear more frequently in white rot fungi compared to brown rot Basidiomycetes. A key component of redox cycles supporting Fenton chemistry, quinone reductase activity, was found only in brown rot fungi (Alfaro et al. 2014) even if its gene homologs were detected in the white rot fungi (Floudas et al. 2012). These findings may indicate that hydrolytic and oxidative reactions of cellulose degradation are characteristic only of white rot fungi (Hori et al. 2013; Alfaro et al. 2014). However, there are exceptions, Fis-

trilina hepatica (brown rot) still possesses genes related to cellulose degradation described as characteristically plesiomorphic and are shared with its white rot ancestors.

Some species of Coniophora and Serpula are able to degrade cellulose similarly to white rot species (Nilsson 1974; Nilsson and Ginn 1979; Redhead and Ginn 1985; Floudas et al. 2015). The genes encoding DyP and HTP are less numerous as compared to white rot fungi, but generic peroxidase genes are numerous in brown rot genomes, which are characterized by low redox potential and are incapable of degrading lignin (Ruiz-Duenas et al. 2013). Certain fungi, for example, Schizophyllum commune, lack genes encoding POD and DyP and have only two laccase genes, which is more similar to brown rot fungi than white rot fungi (Ohm et al. 2010; Floudas et al. 2015). The historical line between white rot and brown rot is blurred now as some researchers suggested that Sc. commune is an evolutionary intermediate between white and brown rot species (Riley et al. 2014). A number of so-called litter decomposers (LDs) are capable of degrading lignocellulose. One of the LD fungi, Agaricus bisporus, has only two MnPs, which are both upregulated when grown on straw medium. It is possible that lignin degradation by Ag. bisporus MnPs is supported by 24 HTP genes, which is probably the highest number described to date (Morin et al. 2012). Kohler et al. (2015) showed that a higher number of POD or LPMO genes are present within white rot fungi, which evolved the ability to degrade wood first during the evolution of life on Earth. The taxonomic group Boletales have lower numbers of these genes and consequently their mode of wood degradation became more like that of brown rot. It seems that we have the possibility to observe the real time evolution of wood-decaying mechanisms in the genomes of these transitory species.

Until recently, it was assumed that efficient lignin degraders have different combinations of PODs (Floudas et al. 2012; Fernandez-Fuego et al. 2012a; Hori et al. 2014). While POD genes were found in brown rot fungi, GLOX genes were not (Floudas et al. 2012), implying that this enzyme might have an important role in lignin degradation, similar to POD or DyP. Hernandez-Ortega, Ferreira and Martinez (2012) suggested that AAO may be the main enzyme generating hydrogen peroxide in white rot fungi. Floudas et al. (2012) proved the existence of AAO genes in 24 analyzed white rot fungal genomes (except Auricularia delcata), whereas other H₂O₂-producing GMC are less abundant. However, it seems that gene annotation may be misleading in some cases. Mathieu et al. (2016) described the gene in Pycnoporus cinnabarinus with sequence homology to fungal AAO which was later expressed heterologously showing biochemical properties of aryl alcohol quinone oxidoreductase. The number of laccase genes is fewer in genomes of brown rot fungi. However, even among white rot fungi the number of laccase genes differs significantly among species, e.g Phanerochaete chrysosporium (0), Phlebia radiata (2), Cerrena sp. (9) and Pleurotus sp. (12) (Janusz et al. 2013; Kuuskeri et al. 2016; Yang et al. 2016) suggesting that laccase role in lignin degradation is not clear. It is well known that

**GENOMICS ANALYSIS**

Genomic analysis suggests that brown rot evolved from white rot fungi. Over time, brown rot lost genes coding for ligninolytic class II peroxidases and the cellobiohydrolase-encoding genes are generally absent or lacking a CBM1 domain (Table S3, Sup-
P. chrysosporium has 15 POD genes, composed only of MnP and LiP. On the other hand, VFs are common in the Pleurotus genus (Ruiz-Duenas et al. 2001; Gao et al. 2016) suggesting that different fungi use different strategies for wood degradation. However, the species mentioned above are ecologically different. Interestingly, being a saprotroph, P. chrysosporium only degrades dead wood, whereas Pleurotus sp. is rather parasitic compared to other saprotrophic species. Another conifer degrading fungus, Heterobasidion annosum, has no genes coding for LiP, VF or GLOX; therefore, implying its ability to degrade lignin is based on eight MnPs and 13 laccase genes (Olson et al. 2012). Fernandez-Fueyo et al. (2014) proved that the ability of Pleurotus ostreatus to degrade lignocellulose at different pHs and temperatures may rely on a number of MnP and VF isoenzymes.

The GLOX enzyme seems to be highly important in LDs as it is upregulated in the fungus Ag. bisporus when grown in compost medium. Other potential H$_2$O$_2$-generating extracellular enzymes include various glucose-methanolcholine oxidoeductases and likely include an AAO and a highly expressed methanol oxidase (Morin et al. 2012). Comparing genomes of P. chrysosporium and P. carnosa CAZymes belong to families GH5, GH79, CE16, PL14 and genes encoding MnP and LiP enzymes identified as responsible for efficient softwood degradation by P. chrysosporium by Suzuki et al. (2012). Phlebia radiata employs oxidoeductases (lignin attacking and auxiliary) during the first weeks of lignin degradation, followed by the production of cellulose and hemicellulose-degrading enzymes (Kuuskeri et al. 2016). It is possible that fungi are capable of applying flexible biochemical mechanisms to degrade different kinds of wood. Liers et al. (2011) showed that 7/11 fungal species produced mainly MnP and VP, 8/11 produced laccase and only 3/11 synthesized AAO when growing on Fagus sylvatica wood. Only one fungus produced DyP and LiP was not detected in any (Liers et al. 2011). The surprisingly efficient lignin-degrading fungus, P. chrysosporium, has no DyP genes in its genome, whereas several DyP genes are highly expressed in Trametes versicolor and Phlebiopsis gigantea (Floudas et al. 2012; Hori et al. 2014).

In the fungal kingdom, plant pathogens were determined to have the largest secretomes, animal pathogens the smallest and saprotrophs have intermediate secretomes (Krüger et al. 2014). Possessing a great number of genes coding for wood-degrading enzymes, pathogenic and saprotrophic fungi are able to easily adapt to attack vast numbers of different tree species (https://nt.ars-grin.gov/fungalDATABASES/). Raffaello et al. (2014) proved that a number of all uniquely upregulated genes in H. annosum are greater than a number of genes coding for wood-degrading enzymes. Moreover, the number of upregulated genes during sapwood degradation is almost three times higher than when H. annosum was cultivated on hardwood (Raffaello et al. 2014) which suggest that we have long way to go to fully understand this process. Besides the wood-degrading enzymes found in Ophiostoma piceae, during growth on natural substrates, there is upregulated expression of genes coding for the following transporters, allantois, urea, hexose, iron and sugars in addition to major facilitator superfamily transporters (Haridas et al. 2013). These findings suggest that fungal metabolism should be analyzed more globally when grown on natural substrates.

Based on genomic research, bacterial strategies to degrade lignin seem to be equally sophisticated as those of fungi. Klebsiella sp. strain BRL6–2 was proved to have genes coding for glutathione peroxidases, DyP-type peroxidases and catalases/peroxidases, catalase genes and no laccase genes. In contrast, microbiome analysis showed that laccase seems to be responsible for lignin degradation by Pseudomonas putida in guts of giant panda (Fang et al. 2012). Moreover, multiple cytochrome oxidase genes were found in its genome as was previously observed for a related isolate Enterobacter lignolyticus SCF1 (Deangelis et al. 2013; Woo et al. 2014). In the Rhodococcus jostii RHA1 genome sequence, 26 peripheral pathways and 8 central pathways are involved in the catabolism of aromatic compounds including modifications by monoxygenases and dioxygenases (McLeod et al. 2006; Martinkova et al. 2009). The genome of Amycolatopsis sp. 75iv2 (formerly Streptomycetes setonii and S. griseus 75iv2) was proved to comprise genes coding for heme peroxidases, laccases and cytochrome P450 (Brown et al. 2011). Besides a suite of oxidative enzymes that could modify lignin for breakdown by hydroxylation or demethylation, one interesting example, a gene encoding a non-heme iron dioxygenase fused to a carbohydrate-binding domain was found in Streptomyces sp. SirexAA-E (Bianchetti et al. 2013). Rhodococcus jostii RHA1, known producer of DyP, was proved to be able to degrade lignin without hydrogen peroxide via the β-ketoadipate pathway (Ahmad et al. 2010).

It seems like bacteria may employ similar sets of enzymes to cope with so complex polymer as lignin is. Moreover, it is possible that bacteria may evolve other lignolytic enzymes beyond the canonical enzymes used by fungi, and therefore their strategies to decompose lignin may differ significantly comparing to fungi.

**EVOLUTION**

It is believed that the increase in fungal presence at particular biostratigraphic levels coincides with periods of rapid fungal evolution and diversity (Taylor, Krings and Taylor 2015a). The application of evolutionary genomics together with evolutionary and ecological genetics and the evaluation of fossils have allowed scientists to determine the oldest fungal ancestor—chrytid-like form. Molecular clock data obtained using fossilized fungi suggest that terrestrial fungi diverged from Chytridiomycota ~550 Ma (Taylor, Krings and Taylor 2015b). Employing an approach that did not utilize fossils, the fungi appear to be much older, with the origin of the soil-born Glomeromycota appearing to have arisen between 1400 and 1200 Ma and the separation of Ascomycota and Basidiomycota around 1200 Ma (Heckman et al. 2001; Taylor, Krings and Taylor 2015b) (Fig. 5). According to Lücking et al. (2009), the saprophytic Ascomycota and Basidiomycota originated much later at around 500 Ma, more or less parallel to the first appearance of primitive land plants. The diversification of the higher Ascomycetes, the Pezizomycotina, is estimated to be around 320 Ma, which correlates with the diversification of vascular plants as documented by the fossil record (Lücking et al. 2009). Further molecular clock analyses revealed that the Pezizomycotina and Agaricomycetes are ~400 and 300 million years old, respectively (Kohler et al. 2015). To place the origin of lignin degradation in the context of geologic time, Floudas et al. (2012) exploited Bayesian relaxed molecular clock analyses with fossil-based calibrations, which revealed the mean age of the Agaricomycetes at c.a. 290 Ma. Using this approach, the mean age of Agaricomycotina was determined at c.a. 430 to 470 Ma, which coincide with earlier findings (Floudas et al. 2012).

It should be noted, however, that the accumulation of the vast coal deposits around the globe formed during the Carboniferous–Permian age (359–252 Ma) has historically been approached, the mean age of Agaricomycotina was determined at c.a. 290 Ma. Using this approach, the mean age of Agaricomycotina was determined at c.a. 430 to 470 Ma, which coincide with earlier findings (Floudas et al. 2012).
Figure 5. Timetree of plants and fungi combined and simplified from various sources (Heckman et al. 2001; Hullo et al. 2001; Lücking et al. 2009; Moore, Robinson and Trinci 2011; Floudas et al. 2012; Schirrmeister et al. 2013; Taylor, Krings and Taylor 2015). In addition, there are extensive coal deposits that formed during the Permain (298–252 Ma) and Cretaceous (145–66 Ma), as well as in other time periods, and their presence seems to refute the hypothesis that Carboniferous coals formed due to a lack of wood-decaying organisms at that time (Taylor, Krings and Taylor 2015). Researchers concluded that lignin degradation evolved only once and within the Agaricomycotina some members lost the ability altogether and evolved other methods to degrade plant organic matter, e.g. brown rot and ECM (Floudas et al. 2012). It has been suggested that diversification in fungal nutritional preferences occurred alongside diversification of angiosperms and gymnosperms. Additionally, the chronology of diversifications in the fungal nutritional lifestyle is compatible with the predicted major diversification in conifers, suggesting that the boreal forest (taiga) biome may have its origin in parallel genetic coevolution of plants and fungi (Eastwood et al. 2011). Genome information indicates that brown rot fungi evolved several times from ancestral white rot species, which makes this group rather genetically heterogeneous, and to present some of them resemble white rot fungi (Floudas et al. 2012). Thus, the brown rot classifier denotes the properties of decay in plant tissues rather than phylogeny (Kaffengerber and Schilling 2015).

Recently, it has been suggested that Agaricomycetes capable of lignin degradation may have been present before the Carboniferous and lignin degradation was likely never restricted to this class of fungi (Kohler et al. 2015; Nelsen et al. 2016). The Carboniferous–Permian peak of the coal accumulation was probably the result of a unique combination of tropical conditions and tectonics during the assembly of Pangea and fungal community composition bared no direct relevance. This hypothesis is based on the fact that lignin modification is known to occur via other enzymatic mechanisms in other fungal lineages and these alternate mechanisms may have evolved deeper in the phylogeny (Nelsen et al. 2016). Evolutionary and functional analysis of mycorrhizal Basidiomycota and Ascomycota, using comparative analysis of 18 newly sequenced genomes, revealed that mycorrhizal symbioses arose repeatedly during fungal evolution from functionally diverse saprotrophic ancestors. During the process, distinct suites of plant cell-wall-degrading enzymes were retained thus suggesting that ECM fungi possess diverse abilities to decompose lignocellulose (Kohler et al. 2015). The hypothesis concerning multiple ECM origins has been questioned by Wolfe, Tulloss and Pringle (2012), who demonstrated a single origin of ECM mutualism in Amanita. However, Wolfe et al.’s theory is supported by the loss of various crucial cellulase genes, which suggests that Amanita can no longer decompose complex organic matter and thus must rely on carbon sources from the host plant via ECM symbiosis (Wolfe, Tulloss and Pringle 2012). It was demonstrated that the loss of aggressive ligninolysis in Boletales might have permitted the brown rot species transition to biotrophic ECM symbiont, which is promoted in soils impoverished of nitrogen by brown rot enrichment and by the nutritional advantage conferred by the connection to a mycorrhizal network (Eastwood et al. 2011). Comparative analysis of Fistulina hepatica and Cylindrobasidium torrendii proved that brown rot fungi evolved from white rot as a result of multiple genes losses (Floudas et al. 2015). According to Martinez et al. (2009),...
brown rot fungi diverged from white rot fungi by losing the capacity to degrade lignin, particularly by losing extracellular peroxidases. The transition from white rot toward brown rot could have taken place multiple times across the Agaricomycotina lineage (Floudas et al. 2012). Transitions back to a white rot lifestyle, from either a brown rot or a mycorrhizal fungal lifestyle, have been observed, which imply that fungi with non-ligninolytic properties would have retained genes for lignin-degrading enzymes (Morgenstern, Klopman and Hibbett 2008). Analysis of the functional diversity of forest fungi revealed that brown rot fungi have the fewest oxidoreductases, not because of gene losses but because of gene duplications in white rot species, and alternative mechanisms evolved (Eastwood et al. 2011). Some fungal wood degraders act in an unclassified and diverse way (dual life style between white rot and brown rot), which indicate that wood decay mechanisms as well as fungal classification based on decay strategy are not that reliable (Floudas et al. 2015).

Reconstruction of the evolution of degradation capabilities was proposed based on phylogenetic analyses applied for different genes encoding for plant cell-wall-degrading enzymes (Morgenstern, Klopman and Hibbett 2008; Castilho et al. 2009; Floudas et al. 2012; Cázares-García, Vázquez-Garcidueñas and Vázquez-Marrufo 2013; Ruiz-Duenas et al. 2013; Kohler et al. 2015). Establishing a topological hierarchy regarding the genetic and evolutionary relationships of laccase genes based on their amino acid sequences suggests that fungal laccases of the Ascomycetes and Basidiomycetes clearly belong to two distinct clusters (Castilho et al. 2009). Contrary to other findings, lignin is the major precursor of coal so the finding that the origin of AA2 genes roughly coincided with the end of Carboniferous supported the view that the origin of white rot fungi impacted the global carbon cycle (Nagy et al. 2015). Furthermore, the reconstruction of the evolution of 18 gene families showed an increase in the copy number and the diversification of genes responsible for lignocellulose decomposition after the divergence of Auricularia, which refines previous hypotheses on the origin of white rot (Floudas et al. 2012). The authors speculate that the diversity of oxidative enzymes present in white rot fungi emerged after the origins of the first white rot species (Nagy et al. 2015). A detailed analysis using amino acid sequences data generated from 11 species of wood-decaying Agaricomycetes revealed a detailed picture of the fungal peroxidases phylogeny (Morgenstern, Klopman and Hibbett 2008). This distinctive and ecologically important class of proteins has diversified extensively especially in Basidiomycetes, after the split of Basidiomycetous and Ascomycetous lineages of fungi. The sequences of extracellular fungal peroxidases including MnPs, LiPs and VPs constitute a monophyletic gene family within the superfamily of plant and microbial peroxidases. LiPs evidently occur and diversified only in members of the Polyporales, which harbors many white rot taxa. According to Morgenstern, Klopman and Hibbett (2008), MnPs and VPs are more widespread and may have multiple origins. MnPs, LiPs and VPs were identified in only three orders, although their degenerate forms were found in different Pezizomycota (Passardi et al. 2007). The study provided by Zhou, Wei and Dai (2014) gave essential evidence for the explanation of the evolutionary dynamics of peroxidases and thus gaining insights into the cause for the alternation of white rot and brown rot fungi in their common lineage; these enzymes originated many times at the level of orders independently, and thus they did not exist in all taxonomic groups of Basidiomycetes. According to the study, the occurrence of enzymes is limited to Rus-sulales, Agaricales and Polyporales. Also, the evolutionary order of peroxidase appearance was proposed; peroxidases without any obvious lignin-degrading activity and MnPs were the primary groups of enzymes to evolve, following VPs and LiPs, which secured complete lignin degradation. This hypothesis also indicates that the gene duplication events probably occurred before the divergence of Ascomycetes and Basidiomycetes and the duplicated enzymes were passed down to newborn species or genera (Zhou, Wei and Dai 2014). It has been proven that MnPs are phylogenetically older than LiPs and thus duplicated and diversified before the split of major lineages of Agaricomycetes. However, the VPs of Agaricales and Polyporales do not exhibit a close relationship but seem to have evolved independently in different clades, which suggests that VPs may have arisen relatively early in the evolutionary history of fungal peroxidases. According to the organismal phylogeny calibrated with the split between Ascomycota and Basidiomycota, the first ligninolytic MnP arose at about 295 Ma, which is slightly earlier than (and therefore consistent with) the oldest definitive white rot fossils from the Permian (c.a. 260 Ma) and Triassic (c.a. 230 Ma) (Floudas et al. 2012). It has been confirmed that a common ancestor of Agaricales possesses a sophisticated enzymatic system for utilization of crystalline cellulose and cellubiose, and all oxidative gene families, including POD and DyPs, similar to contemporary white rot species of Agaricales (Floudas et al. 2015).

Over the course of the evolutionary process, laccases have maintained a high degree of similarity in terms of amino acid sequence and three-dimensional structure. In order to track the evolutionary history of fungal laccases, the reconstruction of the gene loci evolution inferred from the comparative analysis of 48 different sequences was applied. The phylogenetic relations indicated that most laccases from the Ascomycota and the Basidiomycota divisions formed independent clades, consistent with taxonomy. It has been shown that a single monophyletic branch exists for fungal laccases and that laccase isozyme genes may have evolved independently, possibly through duplication–divergence events. Bearing in mind that the enzyme activity is linked to three different copper sites, it must have been a very early biological event. Therefore, laccases are probably ancient enzymes from an evolutionary point of view (Valderrama et al. 2003). Also, the reconstruction of MLCo phylogeny in Trichoderma reesei, a model organism of cellulose and hemicellulose degradation, has been undertaken by Levasseur et al. (2010). MLCo and classical laccases were clearly divided into two distinct groups containing the laccases of Basidiomycota and Ascomycota, having two different evolutionary histories leading to high-sequence divergence between homologs. A comparative in silico analysis of the identified genes of laccase in Trichoderma performed elsewhere (Cázares-García, Vázquez-Garcidueñas and Vázquez-Marrufo 2013) allows for clear differentiation between extracellular and intracellular enzymes. Additionally, phylogenetic analysis supports the hypothesis of two laccase genes in the common Trichoderma ancestor with later events of reten-

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elsewhere (Castilho et al. 2009). The LMCO classification proposed by Hoegger et al. (2006) lead to the creation of a database for a systematic sequence-based classification and analysis of the multicopper oxidase protein family—LccED (Laccase and Multicopper Oxidase Engineering Database). In the beginning, the classification was based on 10 protein superfamilies (named from A to J) and one more superfamily (K), established for two-domain bacterial LMCO (Fernandes et al. 2014). Presently, LccED lists the number of these superfamilies growth to have reached 16 (https://lcced.biocatnet.de/). This classification seems to be an important step toward understanding the evolutionary relations between this large group of fungal enzymes. Phylogenetic distribution of lignin-degrading prokaryotes, widely discussed by Tian et al. (2014), demonstrated clear clustering of the laccase-encoding species.

Until recently, the enzymes responsible for degradation of lignin in prokaryotes were poorly understood (Bugg et al. 2011b; Tian et al. 2014), but peroxidases from the Dyp family have been shown to be active in lignin degradation. Very few MnPs were described for bacteria, whereas most of the identified LiP sequences are putative. Bacterial catabolic pathways are still poorly understood, and our current knowledge is not sufficiently advanced enough to establish a phylogenetic overview of all ligninolytic prokaryotic species (Tian et al. 2014).

Phylogenetic studies proved to be an important tool for the characterization of proteins responsible for wood degradation and it became the basis for its division into families and subfamilies (Hoegger et al. 2006; Passardi et al. 2007; Morgenstern, Klopmann and Hibbett 2008; Lundell, Makela and Hilden 2010; Zhou, Wei and Dai 2014; Zamocky et al. 2015). However, there is a huge knowledge gap concerning lignin degradation. The role of laccases in decomposition of lignin is still questionable, since there are non-lignin-degrading fungi which may contain several laccase encoding genes (Hatakka and Hammel 2011). Recently, several novel clades with putative MnP and LiP members were described in various Ascomycetes unable to decay wood (Zamocky et al. 2015). Also, lignin is degraded after nutrient depletion and lignin degradation is too slow to serve as a source of metabolic energy (Leisola, Pastinen and Axe 2012). Genome studies of wood-inhabiting Basidiomycetes show that there is a need for a more detailed classification of the rot types, since some fungi do not fulfill the traditional criteria for dichotomous grouping (Riley et al. 2014; Rytioja et al. 2014; Floudas et al. 2015). Additionally, the presence of extensive coal deposits formed during the Permian and Cretaceous periods seems to refute the hypothesis that Carboniferous coals formed due to a lack of wood-decaying organisms at that time (Taylor, Kring and Taylor 2015a).

CONCLUDING REMARKS

Even if fungal organisms are considered to be one of the first eukaryotic ones, their evolution could not be perceived as finished. They are still evolving new sophisticated metabolic solutions to grow faster, cover more areas, spend less energy and adopt novel ecological niches. Many of them easily adapted to live in an environment near humans, which may be harsh conditions for other species. For many years, white rot fungi were suggested to be the most efficient wood degraders. However, recent data suggest that Nature may have an alternative solution—brown rot fungi, which are capable of depolymerizing holocellulose and extensively modifying lignin. They are thought to have evolved from ancestral forms of saprotrrophic white rot ancestors, possessing the whole lignocellulose-degrading system, both enzymatic and non-enzymatic (Martinez et al. 2009). The evolution of brown rot saprotrophy was accompanied by reductions and losses in key enzymes implicated in biomass breakdown in white rot, especially cellulases and LMEs. Low molecular weight compounds most likely originated from microbial metabolism or from plant biomass and act as diffusible oxidizing agents and electron shuttles. Therefore, non-enzymatic degradation processes are expected to be considerably less energetically expensive than biosynthesis and secretion of complex enzymatic proteins.

Lignin degradation is a complex process and multidimensional scientific survey which must be undertaken to understand its mechanisms and evolution. This must include the vast distribution among phylogenetic lineages and wood-decaying mechanisms. It seems that the diversity in the fungal kingdom allowed these microorganisms to be successful in inhabitation of different ecological niches from rotting plant material in water, to forests and even to houses made of wood material. It is hard to perceive how the evolution of wood roting fungi will look in the future. We believe that fungal organisms are well adapted and prepared for changes in the environment. However, rapidly changing climate may have had the greatest impact on the evolution of fungi.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSRE online.

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Conflict of interest. None declared.

REFERENCES

Aarti C, Arasu MV, Agastian P. Lignin degradation: A microbial approach. Indian J Biol Sci 2015;1:119–27.

Acebes S, Ruiz-Duenas FJ, Toubes M et al. Mapping the long-range electron transfer route in ligninolytic peroxidases. J Phys Chem B 2017;121:3946–54.

Adapa P, Tabil L, Schoenau G. Compaction characteristics of barley, canola, oat and wheat straw. Biosyst Eng 2009;104:335–44.

Ahmad M, Taylor CR, Pink D et al. Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Mol BioSyst 2010;6:815–21.

Alfaro M, Oguiza JA, Ramirez I et al. Comparative analysis of secretomes in basidiomycete fungi. J Proteomics 2014;102:28–43.

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REFERENCES

Aarti C, Arasu MV, Agastian P. Lignin degradation: A microbial approach. Indian J Biol Sci 2015;1:119–27.

Acebes S, Ruiz-Duenas FJ, Toubes M et al. Mapping the long-range electron transfer route in ligninolytic peroxidases. J Phys Chem B 2017;121:3946–54.

Adapa P, Tabil L, Schoenau G. Compaction characteristics of barley, canola, oat and wheat straw. Biosyst Eng 2009;104:335–44.

Ahmad M, Taylor CR, Pink D et al. Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Mol BioSyst 2010;6:815–21.

Alfaro M, Oguiza JA, Ramirez I et al. Comparative analysis of secretomes in basidiomycete fungi. J Proteomics 2014;102:28–43.
Arantes V, Milagres AME. Relevance of low molecular weight compounds produced by fungi and involved in wood biodegradation. *Quimica Nova* 2009;32:1586–95.

Arnold CA, On Callixylon Newberryi (Dawson) Elkins Et Wieland. Detroit, USA: University of Michigan Press, 1931.

Baldrian P. Fungal laccases - occurrence and properties. *FEMS Microbiol Rev* 2006;30:215–42.

Baldrian P, Valaskova V. Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol Rev* 2008;32:501–21.

Barceló AR, Gómez Ros LV, Gabaldón C et al. Basic peroxidases: The gateway for lignin evolution? *Phytochem Rev* 2004;3:61–78.

Barr DP, Shah MM, Grover TA et al. Production of hydroxyl radical by lignin peroxidase from *Phanerochaete chrysosporium*. Arch Biochem Biophys 1992;298:480–5.

Beeson WT, Iavarone AT, Hausmann CD et al. Extracellular aldonolactonase from *Myeliophthora thermophila*. *Appl Environ Microb* 2011;77:650–6.

Beeson WT, Vu VV, Span EA et al. Cellulose degradation by polysaccharide monooxygenases. *Annu Rev Biochem* 2015;84:923–46.

Berg B, McClaugherty C. Decomposer organisms. In: Plant Litter: Decomposition, *Humus Formation, Carbon Sequestration*. Berlin, Heidelberg: Springer Berlin Heidelberg, 2013;35–52.

Bianchetti CM, Harmann CH, Takasuka TE et al. Fusion of dioxygenase and lignin-binding domains in a novel secreted enzyme from cellulolytic Streptomyces sp. *SirexAA-E*. *J Biol Chem* 2013;288:18574–87.

Bijb MJ. Regulation of secondary metabolism in streptomycetes. *Curr Opin Microbiol* 2005;8:208–15.

Bidlack JE. Cell-wall components and lignin biosynthesis in forages volume Ph.D. Dissertation. Ames, IA: Iowa State University, 1990.

Blanchette RA. Degradation of the lignocellulose complex in wood. Can J Bot 1995;73:999–1010.

Boer WD, Folman LB, Summerbell RC et al. Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiol Rev* 2005;29:795–811.

Breen A, Singleton FL. Fungi in lignocellulose breakdown and biopulping. *Curr Opin Biotechnol* 1999;10:252–8.

Brosse N, Dufour A, Meng X et al. Breen A. *Comparative Biochemistry of the Decay of Sweetgum Sapwood by White-Rot and Brown-Rot Fungi*. Washington: U. S. Department of Agriculture; 1961.

Bragg SM, Beckham GT, Bruce NC et al. Lignocellulose degradation mechanisms across the Tree of Life. *Curr Opin Chem Biol* 2015;29:108–19.

Crawford RH, Carpenter SE, Harmon ME. Communities of filamentous fungi and yeast in decomposing logs of *Pseudotsuga menziesii*. *Int J Biochem Mol Biol* 2013;47:1–11.

Cragg SM, Beckham GT, Bruce NC et al. Lignocellulose degradation mechanisms across the Tree of Life. *Curr Opin Chem Biol* 2015;29:108–19.

Couturier M, Navarro D, Olive C et al. Post-genomic analyses of fungal lignocellulosic biomass degradation reveal the unexpected potential of the plant pathogen *Ustilago maydis*. *BMC Genomics* 2012;13:57.

Cowling EB. *Comparative Biochemistry of the Decay of Sweetgum Sapwood by White-Rot and Brown-Rot Fungi*. Washington: U. S. Department of Agriculture; 1961.

Daniel G, Volc J, Filonova L et al. Mechanistic and steric issues in the oxidation of phenolic and non-phenolic compounds by laccase or laccase-mediated systems. The case of bifunctional substrates. *New J Chem* 2006;30:583–91.

Daou M, Piumi F, Cullen D et al. Characteristic of two glyoxal oxidases from *Phanerochaete chrysosporium*. *Holzforschung* 2009;63:486–95.

Daou M, Piumi F, Cullen D et al. Characteristic of two glyoxal oxidases from *Phanerochaete chrysosporium*. *Holzforschung* 2009;63:486–95.

Dashtban M, Schraft H, Syed TA et al. Fungal biomass degradation and enzymatic modification of lignin. *Int J Biochem Mol Biol* 2010;1:36–50.

de Koker TH, Mozuch MD, Cullen D et al. Isolation and purification of pyranose 2-oxidase from *Phanerochaete chrysosporium* and characterization of gene structure and regulation. *Appl Environ Microb* 2004;70:5794–800.
de Oliveira PL, Duarte MC, Ponezi AN et al. Purification and partial characterization of manganese peroxidase from Bacillus pumilus and Paenibacillus sp. Braz J Microbiol 2009;40:818–26.

de Souza CG, Tychanowicz GK, de Souza DF et al. Production of laccase isoforms by Pleurotus pulmonarius in response to presence of phenolic and aromatic compounds. J Basic Microbiol 2004;44:129–36.

Deacon JW. Fungal Biology: 4th edn. Malden, MA: Blackwell Publisher, 2006.

Deangelis KM, Sharma D, Varney R et al. Evidence supporting dissimilatory and assimilatory lignin degradation in Enterobacter lignolyticus SCF1. Front Microbiol 2013;4:280.

Desai SS, Nityanand C. Microbial laccases and their applications: a review. Asian J Biotechnol 2011;3:98–124.

Diamantidis G, Effosse A, Potier P et al. Purification and characterization of the first bacterial laccase in the rhizospheric bacterium Azospirillum lipoferum. Soil Biol Biochem 2000;32:919–27.

Eastwood DC, Floudas D, Binder M et al. The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. Science 2011;333:762–5.

Eggert C, Temp U, Dean JF et al. A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase. FEBS Lett 1996;391:144–8.

Ek M, Gellerstedt G, Henriksson G. Wood Chemistry and Wood Biotechnology. Berlin: Walter De Gruyter Inc, 2009.

El-Tayeb TS, Abdelhafez AA, Ali SH et al. Effect of acid hydrolysis and fungal biotreatment on agro-industrial wastes for obtaining of free sugars for bioethanol production. Braz J Microbiol 2012;43:1523–35.

Enoki A, Itakura S, Tanaka H. The involvement of extracellular enzymes of forest fungi. J Biotechnol 1991;5:365–72.

Fang W, Fang Z, Zhou P et al. Evidence for lignin oxidation by the giant panda fecal microbiome. PLoS One 2012;7:e50312.

Fernandes TAR, da Silveira WB, Passos FML et al. Laccases from Actinobacteria. What we have and what to expect. Adv Microbiol 2014;4:285–96.

Fernandez IS, Ruiz-Duenas FJ, Santillana E et al. Novel structural features in the GMC family of oxidoreductases revealed by the crystal structure of fungal aryl-alcohol oxidase. Acta Crystallogr D 2009;65:1196–205.

Fernandez-Fueyo E, Castanera R, Ruiz-Duenas FJ et al. Ligninolytic peroxidase gene expression by Pleurotus ostreatus: differential regulation in lignocellulose medium and effect of temperature and pH. Fungal Genet Biol 2014;72:150–61.

Fernandez-Fueyo E, Linde D, Almendral D et al. Description of the first fungal dye-decolorizing peroxidase oxidizing manganese(II). Appl Microbiol Biot 2015;99:8927–42.

Fernandez-Fueyo E, Ruiz-Duenas FJ, Ferreira P et al. Comparative genomics of Ceriporiopsis subvermispora and Phanerochaete chrysosporium provide insight into selective ligninolysis. P Natl Acad Sci USA 2012a;109:5458–63.

Fernandez-Fueyo E, Ruiz-Duenas FJ, Miki Y et al. Lignin-degrading peroxidases from genome of selective ligninolytic fungus Ceriporiopsis subvermispora. J Biol Chem 2012b;287:16503–16.

Ferreira P, Hernandez-Ortega A, Herguedas B et al. Kinetic and chemical characterization of aldehyde oxidation by fungal aryl-alcohol oxidase. Biochem J 2010;425:585–93.

Fitigau IF, Peter F, Boeriu CG. Oxidative polymerization of lignins by laccase in water-acetone mixture. Acta Biochim Pol 2013;60:817–22.

Floudas D, Binder M, Riley R et al. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 2012;336:1715–9.

Floudas D, Held BW, Riley R et al. Evolution of novel wood decay mechanisms in Agaricales revealed by the genome sequences of Fistulina hepatica and Cylindrobasidium torrendii. Fungal Genet Biol 2015;76:78–92.

Fukasawa Y, Osono T, Takeda H. Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood. Mycologia 2011;103:474–82.

Gao Y, Zheng L, Li J et al. Insight into the impact of two structural calcium ions on the properties of Pleurotus eryngii versatile ligninolytic peroxidase. Arch Biochem Biophys 2016;612:9–16.

Garcia-Ruiz E, Mate DM, Gonzalez-Perez D et al. Directed evolution of ligninolytic oxidoreductases: from functional expression to stabilization and beyond. In: Fessner W (ed.) Cascade Biocatalysis. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2014, 1–22.

Gilbertson RL. Wood-rotting fungi of North-America. Mycologia 1980;72:1–49.

Glenn JK, Gold MH. Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, Phanerochaete chrysosporium. Arch Biochem Biophys 1985;242:329–41.

Goodell B. Brown-rot fungal degradation of wood: Our evolving view. In Goodell Barry, Darrel D. Nicholas, Tor P. Schultz, (eds). Wood Deterioration and Preservation. Washington, DC: American Chemical Society 2003;845:97–118.

Goodell B, Jellison L, Liu J et al. Low molecular weight chelators and phenolic compounds isolated from wood decay fungi and their role in the fungal biodegradation of wood. J Biotechnol 1997;53:133–62.

Goszczyński S, Paszczynski A, Pasti-Grigsby MB et al. New pathway for degradation of sulfonated azo dyes by microbial peroxidases of Phanerochaete chrysosporium and Streptomyces chromofuscus. J Bacteriol 1994;176:1339–47.

Graz M, Jarosz-Wilkolazka A. Oxalic acid, versatile peroxidase secretion and chelating ability of Bjerkandera fumosa in rich and limited culture conditions. World J Microbiol Biot 2011;27:1885–91.

Gross GG. From lignins to tannins: forty years of enzyme studies on the biosynthesis of phenolic compounds. Phytochemistry 2008;69:3018–31.

Guillén F, Gomez-Toribio V, Martinez MJ et al. Production of hydroxyl radical by the synergistic action of fungal laccase and aryl alcohol oxidase. Arch Biochem Biophs 2000;383:142–7.

Guillén F, Martinez AT, Martinez MJ. Substrate specificity and properties of the aryl-alcohol oxidase from the ligninolytic fungus Pleurotus eryngii. Eur J Biochem 1992;209:603–11.

Hallberg BM, Henriksson G, Petersson G et al. Crystal structure of the flavoprotein domain of the extracellular flavocytochrome cellobioside dehydrogenase. J Mol Biol 2002;315:421–34.

Hallingberg BM, Hertting T, Petersson G et al. The genome and transcriptome of the pine saprophyte Ophiostoma piceae, and a comparison with the bark beetle-associated pine pathogen Grosmannia clavigera. BMC Genomics 2013;14:373.
Harreither W, Sygmond C, Augustin M et al. Catalytic properties and classification of cellobiose dehydrogenases from ascomycetes. Appl Environ Microb 2011;77:1804–15.

Harvey PJ, Schoemaker HE, Palmer JM. Veratryl alcohol as a mediator and the role of radical cations in lignin biodegradation by Phanerochaete chrysosporium. FEBS Lett 1986;195:242–6.

Hatakka A. Biodegradation of lignin. In: Alexander S, (ed.) Biopolymers Online, Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2005.

Hatakka A, Hammel KE. Fungal biodegradation of lignocelluloses. In: Hofrichter M (ed.) Industrial Applications. Berlin, Heidelberg: Springer Berlin Heidelberg, 2011, 319–40.

Heckman DS, Geiser DM, Eddel BR et al. Molecular evidence for the early colonization of land by fungi and plants. Science 2001;293:1129–33.

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

Heinrichs J, Geipel I, Halkier T et al. Genomewide analysis of protein sequences. FEBS J 2008;273:2308–26.

Hofrichter M. Review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb Technol 2002;30:354–66.

Hofrichter M, Ullrich R. Heme-thiolate haloperoxidases: versatility and classification of cellobiose dehydrogenases from wood-rotting fungi (family Corinaceae). Appl Environ Microb 1998;64:1601–6.

Henrikkson G, Johansson G, Pettersson G. A critical review of cellobiose dehydrogenases. J Biotechnol 2000;78:93–113.

Hernandez-Ortega A, Ferreira P, Martinez AT. Fungal aryl-alcohol oxidase: a peroxide-producing flavoenzyme involved in lignin degradation. Appl Microbiol Biot 2012;93:1395–410.

Hilden KS, Bortfeldt R, Hofrichter M et al. Molecular characterization of the basidiomycete isolate Nematoloma frowardii b19 and its manganese peroxidase places the fungus in the corticoid genus Phlebia. Microbiology 2008;154:2371–9.

Heoegger PJ, Kilaru S, James TY et al. Phylogenetic comparison and classification of laccase and related multicopper oxidase protein sequences. FEBS J 2006;273:2308–26.

Kaffenberger JT, Schilling JS. Comparing lignocellulose physiochemistry after decomposition by brown rot fungi with distinct evolutionary origins. Environ Microbiol 2015;17:4885–97.

Kajisa T, Yoshida M, Igarashi K et al. Characterization and molecular cloning of cellobiose dehydrogenase from the brown-rot fungus Coniophora puteana. J Biosci Bioeng 2004;98:57–63.

Kersten P, Cullen D. Copper radical oxidases and related extra-cellulose oxidoreductases of wood-decay Agaricomycetes. Funct Genet Biol 2014;72:124–30.

Kersten P. Glyoxal oxidase of Phanerochaete chrysosporium: its characterization and activation by lignin peroxidase. P Natl Acad Sci USA 1990;87:2936–40.

Kersten PJ, Cullen D. Cloning and characterization of cDNA encoding glyoxal oxidase, a H2O2-producing enzyme from the lignin-degrading basidiomycete Phanerochaete chrysosporium. P Natl Acad Sci USA 1993;90:7411–3.

Kim SJ, Shoda M. Purification and characterization of a novel peroxidase from Geotrichum candidum dec 1 involved in decolorization of dyes. Appl Environ Microb 1999;65:1029–35.

Kirk TK, Farrell RL. Enzymatic “combustion”: the microbial degradation of lignin. Annu Rev Microbiol 1987;41:465–505.

Kohler A, Kuo A, Nagy LG et al. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat Genet 2015;47:410–5.

Kračer D, Zahma K, Schulz C et al. Inter-domain electron transfer in cellobiose dehydrogenase: modulation by pH and divalent cations. FEBS J 2015;282:3136–48.

Krieger J-J, Thon MR, Deising HB et al. Compositions of fungal secretomes indicate a greater impact of phylogenetic history than lifestyle adaptation. BMC Genomics 2014;15:1–19.

Kuhad RC, Singh A. Lignocellulose Biotechnology: Future Prospects. New Delhi: I.K.International (P) Ltd., 2007.

Kuhad RC, Singh A, Eriksson KE. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biot 1997;57:45–125.

Kuipers AT, Denneman CAJ. Water-soluble phenolic substances in soils under several coniferous and deciduous tree species. Soil Biol Biochem 1987;19:765–9.

Kumar AK, Goswami P. Functional characterization of alcohol oxidases from Aspergillus terreus MTCC 6324. Appl Microbiol Biot 2006;72:906–11.

Kumar S, Shukla SK. A review on recent gasification methods for biomethane gas production. Int J Energy Eng 2016;6:32–43.
Kunamneni A, Ballesteros A, Plou FJ et al. Fungal laccase—a versatile enzyme for biotechnological applications, in Méndez-Vilas A, (ed.) Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Badajoz, Spain: Formatex Research Center. 2007, 233–45.

Kupriashina MA, Selivanov N, Nikitina VE. Isolation and purification of Mn-peroxidase from Azospirillum brasilense Sp245. Prikl Biokhim Mikrobiol 2012;48:23–6.

Kuskeri J, Hakkinen M, Laine P et al. Time-scale dynamics of proteome and transcriptome of the white-rot fungus Phlebia radiata: growth on spruce wood and decay effect on lignocellulose. Biotechnol Biofuels 2016;9:192.

Labelew U, Martone PT, Boucher Y et al. Ancient origin of the biosynthesis of lignin precursors. Biol Direct 2015;10:23.

Lankinen VP, Bonnen AM, Anton LH et al. Characteristics and N-terminal amino acid sequence of manganese peroxidase from solid substrate cultures of Agaricus bisporus. Appl Microbiol Biot 2001;55:170–6.

Le Roes-Hill M, Rohland J, Burton S. Actinobacteria isolated from the ligninolytic fungus choderma reesei. Appl Environ Microb 2011;77:3211–8.

McLeod MP, Warren RL, Hsiao WW et al. The complete genome of Rhodococcus sp. RHA1 provides insights into a catalobic powerhouse. P Natl Acad Sci USA 2006;103:15582–7.

Madhavi V, Lele SS. Laccase: properties and applications. BioResources 2009;4:1694–17.

Manavalan T, Manavalan A, Heese K. Characterization of lignocellulolytic enzymes from white-rot fungi. Curr Microbiol 2015;70:485–98.

Manini P, La Pietra P, Panzella L et al. Glyoxal formation by Fenton-induced degradation of carbohydrates and related compounds. Carbohydr Res 2006;341:1828–33.

Martinez AT, Speranza M, Ruiz-Duenas FJ et al. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int Microbiol 2005;8:195–204.

Martinez D, Challacombe J, Morgenstern I et al. Genome, transcriptome, and secretome analysis of wood decay fungus Postia placenta supports unique mechanisms of lignocellulose conversion. P Natl Acad Sci USA 2009;106:1954–9.

Martinkova L, Uhnakova B, Patek M et al. Biodegradation potential of the genus Rhodococcus. Environ Int 2009;35:162–77.

Martinso LO, Soares CM, Pereira MM et al. Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the Bacillus subtilis endospore coat. J Biol Chem 2002;277:18849–59.

Masai E, Katayama Y, Fukuda M. Genetic and biochemical investigations on bacterial catalobic pathways for lignin-derived aromatic compounds. Biosci Biotech Bioch 2007;71:1–15.

Mason MG, Nicholls P, Wilson MT. Rotting by radicals—the role of cellulose oxidoreductase? Biochem Soc Trans 2003;31:1335–6.

Mathieu Y, Piumi F, Valli R et al. Activities of secreted aryl alcohol oxidase oxidoreductases from Pycnoporus cinnabarinus provide insights into fungal degradation of plant biomass. Appl Environ Microb 2016;82:2411–23.

Mester T, Field JA. Characterization of a novel manganese peroxidase-lignin peroxidase hybrid isozyme produced by Bjerkandera species strain BOS55 in the absence of manganese. J Biol Chem 1998;273:15412–7.

Molina-Guijarro JM, Perez J, Munoz-Dorado J et al. Detoxification of azo dyes by a novel pH-versatile, salt-resistant laccase from Streptomyces ipomoea. Int Microbiol 2009;12:13–21.

Moore D, Robson GD, Trinci APJ. 21st Century Guidebook to Fungi. Cambridge. Cambridge University Press, 2011.

Morgenstern I, Klopman S, Hibbett DS. Molecular evolution and diversity of lignin degrading heme peroxidases in the Agaricomycetes. J Mol Evol 2008;66:243–57.

Morin E, Kohler A, Baker AR et al. Genome sequence of the button mushroom Agarius bisporus reveals mechanisms governing adaptation to a humic-rich ecological niche. P Natl Acad Sci USA 2012;109:17501–6.

Morozova OV, Shumakovich GP, Gorbacheva MA et al. “Blue” laccases. Biochemistry (Mosc) 2007;72:1136–50.

Morphuro L, Graziani MT, Finazzi-Agro A et al. Optical properties of Japanese-lacquer-tree (Rhus vernicifera) laccase depleted...
of type 2 copper(II). Involvement of type-2 copper(II) in the 330nm chromophore. Biochem J 1980;187:361–6.

Mueller-Harvey J, Hartley RD. Linkage of \( \rho \)-coumaroyl and feruloyl groups to cell-wall polysaccharides of barley straw. Carbohydr Res 1986;148:71–85.

Nagy LG, Riley R, Tritt A et al. Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities. Mol Biol Evol 2015;33:959–70.

Nelsen MP, DiMichele WA, Peters SE et al. Delayed fungal evolution did not cause the Paleozoic peak in coal production. P Natl Acad Sci USA 2016;113:2442–7.

Newcombe D, Paszczynski A, Gajewska W et al. Production of small molecular weight catalysts and the mechanism of trinitrotoluene degradation by several Gloeophyllum species. Enzyme Microb Tech 2002;30:506–17.

Niemenmoo O, Uusi-Rauva A, Hatakka A. Demethylation of [O14CH3]-labelled lignin model compounds by the brown-rot fungi Gloeophyllum trabeum and Poria (Postia) placenta. Biodegradation 2008;19:555–65.

Niladevi KN. Ligninolytic enzymes. In: Singh-Nee Nigam P, Nagy LG, Riley R, Tritt A et al. Linkage of manganese-oxidizing peroxidases: a comparison between the model mushroom Bjerkandera fumosa and other diatrypaceous fungi from Argentina. J Agric Technol 2005;1:81–96.

Piontek K, Strittmatter E, Ulrich R et al. Structural basis of substrate conversion in a new aromatic peroxygenase: cytochrome P450 functionality with benefits. J Biol Chem 2013;288:34767–76.

Piontek K, Ulrich R, Liers C et al. Crystallization of a 45 kDa peroxygenase/peroxidase from the mushroom Agrocybe aergerita and structure determination by SAD utilizing only the haem iron. Acta Crystallogr F 2010;66:693–8.

Pitsawong W, Sucharitakul J, Prongjit M et al. A conserved active-site threonine is important for both sugar and flavin oxidations of pyranose 2-oxidase. J Biol Chem 2010;285:9697–705.

Piumi F, Levasseur A, Navarro D et al. A novel glucose dehydrogenase from the white-rot fungus Pseudonocorpa cinnabarinus: production in Aspergillus niger and physicochemical characterization of the recombinant enzyme. Appl Microbiol Biot 2014;98:10105–18.

Pollegioni L, Tonin F, Rosini E. Lignin-degrading enzymes. FEBS J 2015;282:1190–213.

Prongjit M, Sucharitakul J, Wongmate T et al. Kinetic mechanism of pyranose 2-oxidase from Trametes multicolor. Biochemistry 2009;48:4170–80.

Qiu Y-L, Palmer JD. Phylogeny of early land plants: insights from genes and genomes. Trends Plant Sci 1999;4:26–30.

Raffaello T, Chen H, Kohler A et al. Transcriptomic profiles of Heterobasidion annosum under abiotic stresses and during saprotrophic growth in bark, sapwood and heartwood. Environ Microb 2014;16:1654–67.

Rahmanpour R, Bugg TD. Characterisation of Dyp-type peroxidases from Pseudomonas fluorescens Pf-5: Oxidation of Mn(ll) and polymeric lignin by Dyp1B. Arch Biochem Biophys 2015;574:93–8.

Rashid GM, Taylor CR, Liu Y et al. Identification of manganese superoxide dismutase from Sphingobacterium sp. T2 as a novel bacterial enzyme for lignin oxidation. ACS Chem Biol 2015;10:2286–94.

Redhead SA, Ginns JH. A reappraisal of Agaric genera associated with brown rots of wood. J Mycol Soc Jpn 1985;26:349–81.

Riley R, Salamov AA, Brown DW et al. Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. P Natl Acad Sci USA 2014;111:9923–8.

Roberts JN, Singh R, Grigg JC et al. Characterization of dye-decolorizing peroxidases from Rhodococcus jostii RHA1. Biochemistry 2011;50:5108–19.

Rodakiewicz-Nowak J, Jarosz-Wilkolazka A, Laterek J. Catalytic activity of versatile peroxidase from Bjerkandera fumosa in...
aqueous solutions of water-miscible organic solvents. Appl Catal A-Gen 2006;308:56–61.

Rola B, Pawlik A, Frac M et al. The phenotypic and genomic diversity of Aspergillus strains producing glucose dehydrogenase. Acta Biochim Pol 2015;62:747–55.

Rowell RM. The Chemistry of Solid Wood. Advances in Chemistry Series. Washington, D.C.: American Chemical Society, 1984.

Ruiz-Duenas FJ, Camarero S, Perez-Boada M et al. A new versatile peroxidase from Pleurotus. Biochem Soc Trans 2001;29:116–22.

Ruiz-Duenas FJ, Guillen F, Camarero S et al. Regulation of peroxidase transcript levels in liquid cultures of the ligninolytic fungus Pleurotus eryngii. Appl Environ Microb 1999;65:4458–63.

Ruiz-Duenas FJ, Lundell T, Floudas D et al. Lignin-degrading peroxidases in Polyporales: an evolutionary survey based on 10 sequenced genomes. Mycologia 2013;105:1428–44.

Rytioja J, Hilden K, Yuzon J et al. Plant-poly saccharide-degrading enzymes from Basidiomycetes. Microb Mol Biol Rev 2014;78:614–49.

Saini A, Aggarwal NK, Sharma A et al. Actinomycetes: a source of lignocellulolytic enzymes. Enzyme Res 2015;2015:279381.

Salvachua D, Prieto A, Martinez AT et al. Characterization of a novel dye-colorizing peroxidase (DyP)-type enzyme from Irpex lacteus and its application in enzymatic hydrolysis of wheat straw. Appl Environ Microb 2013;79:4316–24.

Sánchez O, Rocío Sierra R, Alméciga-Díaz CJ. Delignification process of agro-industrial wastes an alternative to obtain fermentable carbohydrates for producing fuel. In: Manzanera M (ed.) Alternative Fuel. London, UK: InTech, 2011.

Santhanam N, Badri DV, Decker SR et al. Lignocellulose decomposition by microbial secretions. In: Vivanco MJ, Baluška F (eds.) Secretions and Exudates in Biological Systems. Berlin, Heidelberg: Springer Berlin Heidelberg, 2012, 125–53.

Scalbert A, Monties B, Lallemand JY et al. Plant-polysaccharide-degrading enzymes from Basidiomycetes. Fungal hydro-quinones contribute to brown rot of wood. Environ Microbiol 2006;8:2214–23.

Suzuki T, Endo K, Ito M et al. A thermostable laccase from Streptomyces lavendulae REN-7: purification, characterization, nucleotide sequence, and expression. Biosci Biotech Bioch 2003;67:2167–75.

Sygmund C, Staudigl P, Klausberger M et al. Comparative genomics of the white-rot fungi, Phanerochaete carnosa and P. chrysosporium, to elucidate the genetic basis of the distinct wood types they colonize. BMC Genomics 2012: 13:444.

Thurston CF. The structure and function of fungal laccases. Appl Microbiol Biotechnol 2006;79:353–65.

Taylor TN, Krings M, Taylor EL. 2 - How fungal fossils are formed and studied. In: Taylor T, Krings M, Taylor E (eds.) Fossil Fungi. San Diego: Academic Press, 2015a, 13–26.

Taylor TN, Krings M, Taylor EL. 3 - How old are the fungi? In: Taylor T, Krings M, Taylor E (eds.) Fossil Fungi. San Diego: Academic Press, 2015b, 27–39.

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

Tian JH, Pournier AM, Bouchez T et al. Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. Appl Microbiol Biotechnol 2014;98:9527–44.

Tien M, Kirck TK. Lignin degrading enzyme from the Hypomyces Phanerochaete chrysosporium Burds. Science 1983;221:661–3.

Tiquia SM, Wan JHC, Tam NFY. Microbial population dynamics and enzyme activities during composting. Compost Sci Util 2002;10:150–61.

Ulrich R, Nuske J, Scheiner K et al. Novel haloperoxidase from the agaric basidiomycete Agrocybe aegerita oxidizes aryl alcohols and aldehydes. Appl Environ Microb 2004;70:4575–81.
Valderrama B, Oliver P, Medrano-Soto A et al. Evolutionary and structural diversity of fungal laccases. Anton Leeuw 2003;84:289–99.

van Bloois E, Torres Pazmino DE, Winter RT et al. A robust and extracellular heme-containing peroxidase from Thermobifida fusca as prototype of a bacterial peroxidase superfamily. Appl Microbiol Biot 2010;86:1419–30.

Varela E, Tien M. Effect of pH and oxalate on hydroquinone-derived hydroxyl radical formation during brown rot wood degradation. Appl Environ Microb 2003;69:6025–31.

Wang W, Gao PJ. Function and mechanism of a low-molecular-weight peptide produced by Gloeophyllum trabeum in biodegradation of cellulose. J Biotechnol 2003;101:119–30.

Wang X, Ullrich R, Hofrichter M et al. Heme-thiolate ferryl of aromatic peroxxygenase is basic and reactive. P Natl Acad Sci USA 2015;112:3686–91.

Wariishi H, Akleswaran L, Gold MH. Manganese peroxidase from the basidiomycete Phanerochaete chrysosporium: spectral characterization of the oxidized states and the catalytic cycle. Biochemistry 1988;27:5365–70.

Watanabe T, Shirai N, Okada H et al. Characterization of the oxidized states and the catalytic cycle of fungal peroxidase-catalase superfamily. J Biol Chem 1995;270:18561.

Welinder K. Plant peroxidases: structure-function relationships. In: Penel C, Gaspar T, Greppin H (eds.) Plant Peroxidases, Topics and Detailed Literature on Molecular, Biochemical and Physiological Aspects. Geneve: Universit´e de Gen¨eve, 1992.

Weng JK, Chapple C. The origin and evolution of lignin biosynthesis. New Phytol 2010;187:273–85.

Westereng B, Cannella D, Wittrup Agger J et al. Enzymatic cellulose oxidation is linked to lignin by long-range electron transfer. Sci Rep 2015;5:18561.

Whittaker MM, Kersten PJ, Cullen D et al. Identification of catalytic residues in glyoxal oxidase by targeted mutagenesis. J Biol Chem 1999;274:36226–32.

Wolfe BE, Tulloss RE, Pringle A. The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis. PLoS One 2012;7:e39597.

Wong DW. Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotech 2009;157:174–209.

Woo HL, Ballor NR, Hazen TC et al. Complete genome sequence of the lignin-degrading bacterium Klebsiella sp. strain BRL6-2. Stand Genomic Sci 2014;9:19.

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

Yamada Y, Wang J, Kawagishi H et al. Improvement of ligninolytic properties by recombinant expression of glyoxal oxidase gene in hyper lignin-degrading fungus Phanerochaete sordida YK-624. Biosci Biotech Bioch 2014;78:2128–33.

Yang J, Xu X, Ng TB et al. Laccase gene family in Cerrena sp. HYB07: sequences, heterologous expression and transcriptional analysis. Molecules 2016;21:1–16.

Yin DT, Uresti S, Lafond M et al. Structure-function characterization reveals new catalytic diversity in the galactose oxidase and glyoxal oxidase family. Nat Commun 2015;6:10197.

Youn HD, Hah YC, Kang SO. Role of laccase in lignin degradation by white-rot fungi. FEMS Microbiol Lett 1995;132:183–8.

Zamocky M, Furtmüller PG, Obinger C. Two distinct groups of fungal catalase/peroxidases. Biochem Soc Trans 2009;37:772–7.

Zamocky M, Gasselhuber B, Furtmüller PG et al. Turning points in the evolution of peroxidase-catalase superfamily: molecular phylogeny of hybrid heme peroxidases. Cell Mol Life Sci 2014;71:4681–96.

Zamocky M, Hallberg M, Ludwig R et al. Ancestral gene fusion in cellulobiose dehydrogenases reflects a specific evolution of GMC oxidoreductases in fungi. Gene 2004;338:1–14.

Zamocky M, Hofbauer S, Schaffner I et al. Independent evolution of four heme peroxidase superfamilies. Arch Biochem Biophys 2015;574:108–19.

Zamocky M, Ludwig R, Peterbauer C et al. Cellulobiose dehydrogenase–a flavocytochrome from wood-degrading, phytopathogenic and saprotrophic fungi. Curr Protein Pept Sci 2006;7:255–80.

Zhou L-W, Wei Y-L, Dai Y-C. Phylogenetic analysis of ligninolytic peroxidases: preliminary insights into the alternation of white-rot and brown-rot fungi in their lineage. Mycology 2014;5:29–42.

Zimmerman AR, Kang DH, Ahn MY et al. Influence of a soil enzyme on iron-cyanide complex speciation and mineral adsorption. Chemosphere 2008;70:1044–51.