

5 Biodegradation of Lignin

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AAO	aryl alcohol oxidase
ABTS	2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate)
CDH	cellobiose dehydrogenase
CBQ	cellobiose quinone oxidoreductase
CcP	cytochrome <i>c</i> peroxidase
CP-MAS	cross polarization/magic angle spinning
3-D	three dimensional
DHP	dehydrogenation polymerizate, synthetic lignin
EDTA	ethylenediaminetetraacetic acid
EPR	electron paramagnetic resonance
FAD	flavin adenine dinucleotide
G	guaiacyl
GLOX	glyoxal oxidase
GPC	gel permeation chromatography
GS	guaiacyl-syringyl
GSH	glutathione
3-HAA	3-hydroxyanthranilate
HBT	1-hydroxybenzotriazole
HP-SEC	high performance size exclusion chromatography
LiP	lignin peroxidase
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
MHQ	methoxyhydroquinone
MnP	manganese peroxidase
PAHs	polycyclic aromatic hydrocarbons
QR	quinone reductase
S	syringyl
THB	1,2,4-trihydroxybenzene
VA	veratryl alcohol
VH	vanillate hydroxylase

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Introduction

Lignin is an amorphous three-dimensional substance the molecular weight of which is difficult to determine because lignins are highly polydisperse materials (Argyropoulos and Menachem, 1997). The chemical structure of lignin has also been difficult to determine, and even very recently, new bonding patterns have been described in softwood lignin, e.g., dibenzodioxocin struc-

tures. Also, the isolation of native lignin is complicated if possible at all (Buswell and Odier, 1987). Therefore, lignin model compounds, e.g., dimeric β -O-4 model compounds and synthetic lignin (dehydrogenation polymerizate, DHP), are commonly used in microbiological studies. The results obtained using these substrates are not easy to extrapolate to natural conditions, and thus little is known what happens when microorganisms, such as white-rot fungi, degrade lignin in wood.

Wood-rotting basidiomycetous fungi that cause white rot in wood are the most efficient lignin degraders in nature (Kirk and Farrell, 1987; Eriksson et al., 1990), and they are perhaps nature's major agents for recycling the carbon of lignified tissues. No other microorganisms as pure culture have been described to mineralize lignified tissues as efficiently (Kirk and Cullen, 1998). They are a group of taxonomically heterogeneous higher fungi, characterized by their unique ability to depolymerize and mineralize lignin using a set of extracellular ligninolytic enzymes. Lignin degradation by white-rot fungi has been intensively studied during the last thirty years in relation to biotechnical applications such as biopulping, biobleaching, treating of pulp mill effluents, and soil bioremediation (Akhtar et al., 1992, 1998; Lamar et al., 1992; Messner and Srebotnik, 1994). The enzymology and molecular biology of lignin degradation has been mainly studied in *Phanerochaete chrysosporium* (Gold and Alic, 1993; Cullen, 1997; Kirk and Cullen, 1998). However, many other species of white-rot fungi degrade lignin as efficiently as *P. chrysosporium* (Hatakka, 1994). Moreover, several fungi show better selectivity for lignin removal (Eriksson et al., 1990; Blanchette et al., 1992; Messner and Srebotnik, 1994).

Physiological conditions for lignin degradation, as well as secretion patterns of the ligninolytic enzymes, vary between different fungal species (Hatakka, 1994). Various authors have tried to establish correlations between ligninolytic enzymes and lignin degradation (Käärik, 1965; Ander and Eriksson, 1976, 1977; Eriksson et al., 1990; Hatakka, 1994). However, many of the enzymes necessary for lignin degradation were not characterized before the beginning of the 1980s when virtually only laccase had been known. Since the discovery of two important peroxidases in the beginning of the 1980s, namely lignin peroxidases (LiPs) in 1983 and

manganese peroxidases (MnPs) in 1984 (Kirk and Farrell, 1987), an array of enzymes have been isolated from fungi and characterized in detail. Although basidiomycetous white-rot fungi and related litter-decomposing fungi are the most efficient degraders of lignin, mixed cultures of fungi, actinomycetes, and bacteria in soil and compost can also mineralize lignin (Tuomela et al., 2000). These soil-inhabiting microorganisms may have applications in bioremediation, and their enzymes may have interesting properties, e.g., high thermostability and nearly neutral pH optimum, not usually found in the enzymes of wood-inhabiting fungi.

In this review, the focus is on the present knowledge on lignin degradation under natural or nearly natural conditions, i.e., on wood or straw, and in the enzymology of lignin biodegradation, including mineralization of lignin by ligninolytic enzymes *in vitro*. In addition, the possible involvement of nonenzymatic agents in the fungal lignin biodegradation process is discussed.

2 Historical Outline

Lignin degradation is in a central position in the earth's carbon cycle, because most renewable carbon is either in lignin or in compounds protected by lignin from enzymatic degradation (cellulose and hemicellulose) (Kirk, 1983). Lignin biodegradation is also responsible for much of the natural destruction of wood in use, and it may have an important role in plant pathogenesis. On the other hand, potential applications utilizing lignin-degrading organisms and their enzymes have become attractive, because they may provide environmentally friendly technologies for the pulp and paper industry and for the treatment of many xenobiotic compounds, stains, and dyes. Despite its signifi-

cance, lignin biodegradation has only slowly been defined chemically and biochemically. One of the main reasons for that was the poor knowledge of the chemical structure of lignin until the late 1960s when it became better known (Kirk, 1971, 1983; Adler, 1977; Eriksson et al., 1990). The scheme for structural features of conifer lignin presented by Adler (1977) has been commonly used in the literature, and only recently new structures have been discovered (Argyropoulos and Menachem, 1997; Chapter 3, this volume). Successful studies on the biodegradation of lignin require a good cooperation between microbiologists, biochemists, and chemists, and relatively expensive equipment and materials, e.g., ^{14}C -labeled lignins or lignin model compounds that have not been commercially available. Lignin biodegradation was also considered an unusual biological process involving extracellular oxidations.

Prior to the 1920s, little research was conducted on lignin biodegradation (Kirk, 1983). Some findings, summarized in the 1930s (for reviews, see Kirk, 1983), are still today valid:

- 1) lignin is among the plant cell wall polymers the most resistant to biological degradation, although it is degraded,
- 2) white-rot fungi degrade lignin in wood, and
- 3) completely selective removal of lignin (without concomitant removal of wood carbohydrates) had not been observed.

Waksman et al. (1939) had studied lignin degradation, e.g., in compost and soil environment (reviewed by Tuomela et al., 2000). Gottlieb and Pelczar (1951) in their review reported that the white-rot fungus *Polyporus* (syn. *Trametes*) *versicolor* used Brauns' native lignin" as the growth substrate. Although the lignin structure is unaltered due to the mild

procedure, the preparation has a relatively low molecular weight. This finding, indicating that lignin could be used as a sole carbon and energy source for white-rot fungi, has not been verified. Also, many other faulty techniques had been used in other studies. In the 1950s in addition to white-rot fungi, other groups of fungi were found to degrade lignin, at least partially, namely basidiomycetous litter-decomposing and brown-rot fungi as well as soft-rot fungi (reviewed by Kirk, 1971, 1983). The first lignin model compound studies were published in the 1960s (Russell et al., 1961; Ishikawa et al., 1963; Kirk et al., 1968; Fukuzumi et al., 1969).

Biodegradation assays based on ^{14}C -lignins were developed in the 1970s (Haider and Trojanowski, 1975; Kirk et al., 1975, 1978), and using this techniques, called radiorespirometry, it was revealed how lignin was optimally degraded under laboratory conditions by white-rot fungi. The white-rot fungus *Phanerochaete chrysosporium* was used as the main experimental organism in USA, while in some other laboratories, the anamorph of the same fungus, *Sporotrichum pulverulentum*, had been chosen for lignin biodegradation studies (Ander and Eriksson, 1976; Ander et al., 1980). Before that, *Trametes versicolor* was a popular experimental fungus (Cowling, 1961; Russell et al., 1961).

In the late 1970s and in the beginning of the 1980s many important findings in the physiology of lignin degradation by *P. chrysosporium* were made. The experiments were carried out almost only in synthetic liquid media and using ^{14}C -labeled synthetic lignin (DHP) (Kirk et al., 1975, 1978). The most important discoveries can be listed as follows:

- 1) the effect of nutrient nitrogen, showing that low nitrogen was required for lignin degradation, and indicating that the min-

- eralization of lignin occurred during secondary metabolism,
- 2) the effect of atmosphere, 100% oxygen giving the highest mineralization, thus demonstrating that lignin degradation is oxidative,
 - 3) the detrimental effect of agitation in lignin mineralization, and
 - 4) the concomitant production of veratryl alcohol during lignin degradation.

Some other fungi, e.g., *Phlebia radiata* (Hatakka and Uusi-Rauva, 1983; Hatakka et al., 1983, 1991) were also found to readily degrade lignin and lignin model compounds in a similar way.

Radical chemistry is an essential part of lignin biodegradation. Before the finding of lignin peroxidases, it was assumed that the generation of H_2O_2 and other easily diffusible activated oxygen species, such as hydroxyl radicals (OH^\bullet), superoxide anion radical ($O_2^{\bullet-}$), and singlet oxygen (1O_2) might be responsible for fungal decay of lignin and lignocellulose (Hall, 1980). The involvement of hydroxyl radicals and singlet oxygen was discounted later (Kirk and Farrell, 1987).

A breakthrough in the enzymology of lignin biodegradation occurred in 1983–1984 when the first extracellular enzymes involved in the degradation of lignin were discovered (Tien and Kirk, 1983; Glenn et al., 1983; Kuwahara et al., 1984). Since that large amounts of studies on the biochemistry and molecular biology of these enzymes have been published, but they were again strongly concentrated on the enzymes of one fungus, *P. chrysosporium*. The catalytic mechanism of lignin peroxidase, based on initial one-electron oxidation of the lignin model compounds followed by subsequent breakdown reactions via radical cation intermediates was proposed and experimentally verified (Kersten et al., 1985; Schoemaker et al., 1985; Kirk et al., 1986). Numerous

publications describing the effect of ligninolytic enzymes on lignin model compounds also appeared and were frequently reviewed (Higuchi, 1985; Buswell and Odier, 1987; Kirk and Farrell, 1987).

In the 1990s, in addition to detailed studies on catalytic and enzymatic properties of the lignin-modifying peroxidases as well as their molecular biology, major lines of research have dealt with the potential applications of white-rot fungi and their enzymes in biopulping (biomechanical pulping) and pulp bleaching. The most promising fungi for biopulping are so-called selective lignin degraders, i.e., fungi that degrade larger amounts of lignin relative to carbohydrates (Eriksson et al., 1990).

Because *P. chrysosporium* typically produces lignin peroxidases (LiPs) along with manganese peroxidases (MnPs), LiP was considered the most important lignin-degrading enzyme. However, the optimal conditions for lignin degradation and expression of LiP did not seem to be appropriate for many other fungi, and especially, the most efficient selective lignin degraders apparently did not degrade lignin under these artificial conditions. Moreover, some of these fungi did not even produce LiP at all, e.g., the most promising fungus for biopulping, *Ceriporiopsis subvermispora*, only produces MnP and laccase. When many different fungi had been studied in detail, it became clear that MnP is the most commonly occurring peroxidase while it was difficult to demonstrate the expression of LiP (Hatakka, 1994).

The role of laccase, a phenol-oxidizing enzyme typically produced by almost all lignin-degrading basidiomycetous white-rot fungi (Käärik, 1965), but importantly not so by the model fungus *P. chrysosporium*, was almost totally ignored until the beginning of the 1990s when the efficiency of so-called “mediator” compounds was recognized. These compounds, e.g., 2,2'-azinobis(3-

ethylbenzthiazoline-6-sulfonate) (ABTS) or 1-hydroxybenzotriazole (HBT) (Bourbonnais and Paice, 1990; Call and Mücke, 1997), being readily oxidizable primary substrates, extend laccase substrate range to the most recalcitrant nonphenolic subunits of lignin. Recombinant laccases can be relatively easily produced in common industrial host organisms, e.g., in *Trichoderma reesei* (Saloheimo and Niku-Paavola, 1991) or in *Aspergillus oryzae* (Stewart et al., 1996), and therefore, the prospects of the use of laccase in bleaching of pulp, textiles, and dyes, and in bioremediation of environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) are realistic. Shortly after the discovery of these synthetic mediators, a report on a natural mediator, 3-hydroxyanthranilate (3-HAA), was published by Eggert et al. (1996a). These findings can be considered another breakthrough in the enzymology of lignin degradation. Due to the low appreciation of laccase as a lignin-degrading enzyme, the first 3-D structure of this enzyme, although the enzyme was first described as early as in the 1880s (Yoshida, 1883; Thurston, 1994) was solved only very recently (Ducros et al., 1998).

The developments before 1983, i.e., before “ligninase” was found, have been summarized by Kirk (1983). After that some of the main events and achievements may be listed as follows:

- ligninase (lignin peroxidase, LiP) discovered (Tien and Kirk, 1983; Glenn et al., 1983)
- manganese peroxidase (MnP) discovered (Kuwahara et al., 1984)
- one-electron oxidation mechanism and cation radical formation by LiP discovered (Kersten et al., 1985; Schoemaker et al., 1985)
- glyoxal oxidase (GLOX) discovered (Kersten and Kirk, 1987)
- the concept of “enzymatic combustion” (Kirk and Farrell, 1987)
- molecular biological studies (earlier studies reviewed by Gold and Alic, 1993)
 - cloning and sequencing of LiP gene (Tien and Tu, 1987)
 - cloning and sequencing of MnP gene (Pribnow et al., 1989; Mayfield et al., 1994a)
 - heterologous expression of laccase (Saloheimo and Niku-Paavola, 1991)
 - homologous expression of peroxidases (Mayfield et al., 1994b; Sollewijn Gelpke et al., 1999)
 - heterologous expression of peroxidases (Doyle and Smith, 1996; Stewart et al., 1996)
 - 3-D structure of LiP (Piontek et al., 1993; Poulos et al. 1993)
 - 3-D structure of MnP (Sundaramoorthy et al., 1994)
 - 3-D structure of laccase (Ducros et al., 1998)
 - detailed structure of MnP–LiP hybrid enzyme (Ruiz-Dueñas et al., 1999a)
- different ligninolytic systems in fungi recognized (reviewed by Hatakka, 1994)
- the laccase-mediator system discovered (Bourbonnais and Paice, 1990; Call and Mücke, 1997)
- a natural laccase mediator discovered (Eggert et al., 1996a)
- the separation of LiP from lignocellulose medium (Vares et al., 1995)
- the involvement of lipid peroxidation in lignin degradation proposed (Kapich and Shishkina, 1995; Jensen et al., 1996; Kapich et al., 1999a, b)
- cell-free mineralization of ^{14}C -labeled synthetic and natural lignins by MnP (Hofrichter et al., 1999a, b, c).

3

Bacteria and Microfungi

To the wood-rotting microorganisms, wood may be considered as a series of conveniently orientated holes surrounded by food. The shape, size, and orientation of the holes depend directly on the anatomy of wood species, and the chemical composition of the cell walls or the cell contents. These have all effects on the particular microorganism to use them as a nutrient source (Blanchette, 1995). Softwood (gymnosperms) is formed largely by tracheids, the cell lumen being completely enclosed by a cell wall. Therefore microorganisms must pass through the cell wall or pit membrane. Hardwood (angiosperms) consists of vessels, fibers, and parenchyma cells as well as some tracheids, and here the vessels are formed from open-ended vessel elements. The fungal hyphae or bacteria may thus move also without passing through the cell wall or pit membrane. The hemicellulose as well as lignin components differ markedly in softwood and hardwood species. Softwood contains mainly glucmannans as hemicellulose and guaiacyl (G) type lignin, while hardwood contains glucuronoxylans and guaiacyl-syringyl (GS) type lignin. Sapwood contains accumulated nutrients such as starch or lipids stored by the ray parenchyma cells in addition to extractives, while heartwood contains more and different extractives (pitch) (Sjöström, 1993).

Chemically and morphologically distinct types of decay result from attack of different microorganisms. Wood decay fungi are usually separated into three main groups, causing white, soft, or brown rot. Bacterial degradation of wood has also been reported including erosion, tunneling, and cavity formation (Eriksson et al., 1990; Blanchette, 1995; Daniel and Nilsson, 1998).

3.1

Actinomycetes

Certain bacteria can degrade lignified cell walls of wood. Filamentous bacteria belonging to the genus *Streptomyces* are well-known degraders of lignin and can mineralize up to 15% of labeled lignins but usually much less (Crawford and Sutherland, 1980; Vicuña, 1988; Zimmerman, 1990; Godden et al., 1992; Berrocal et al., 1997). Typically, *Streptomyces* spp. solubilize part of lignin, and the end product is water-soluble acid-precipitable polymeric lignin (Crawford et al., 1983). Berrocal et al. (1997) compared mineralization of ¹⁴C-(lignocellulose)-wheat straw by 9 *Streptomyces* spp. *Streptomyces cyaneus* CECT 3335 was one of the most efficient new isolates in solubilizing 45% of the ¹⁴C-(lignin) fraction and mineralizing 3% of the label in 21 d. However, the reference species *S. viridosporus* solubilized 59% and mineralized 4.5% of the same substrate. The presence of phenol oxidase and peroxidase was found in *S. cyaneus*, the former activity being 100 times greater as determined by the oxidation of ABTS and 2,4-dichlorophenol, respectively. *S. cyaneus* degraded a nonphenolic arylglycerol β -aryl ether by a mechanism indicating the cleavage of C $_{\alpha}$ -C $_{\beta}$ bond in lignin (Zimmerman et al., 1988).

Actinomycetes produce extracellular peroxidases (Pasti et al., 1991; Mercer et al., 1996), e.g., lignin peroxidase-type enzyme (Ramachandra et al., 1988; Adhi et al., 1989). *Streptomyces* sp. EC1 produces peroxidase and cell-bound demethylase requiring H₂O₂ and Mn²⁺, both of which are produced at relatively high levels in the presence of Kraft lignin or wheat straw, while protochaetocate 3,4-dioxygenase and β -carboxymucronate decarboxylase activity were less induced (Godden et al., 1992). Extracellular peroxidase and catalase activity was found in all six actinomycetes strains studied (Godden

et al., 1992). Lignin model compound studies demonstrated that monomeric compounds, i.e., vanillic acid and protocatechuic acid were formed, indicating the cleavage of C_α-C_β bonds, as well as demethylation and oxidation of C_α in the side chain to carbonyl group.

3.2

Other Bacteria

Nonfilamentous bacteria usually mineralize less than 10% of lignin preparations and can degrade only the low-molecular weight part of lignin as well as degradation products of lignin (Rüttimann et al., 1991; Vicuña et al., 1993). Thus, they may play some role in final mineralization of lignin. Among these eubacteria, *Pseudomonas* spp. are the most efficient degraders (Vicuña, 1988; Zimmermann, 1990). However, since these bacteria do not produce extracellular oxidoreductases, and large molecules apparently cannot be taken up into the cell, they are obviously unable to attack polymeric lignin.

3.3

Soft-Rot Fungi and Other Microfungi

Ascomycetes and deuteromycetes (fungi imperfecti) generally cause soft-rot decay of wood (Blanchette, 1995; Daniel and Nilsson, 1998). The decayed wood has a brown, soft appearance that is cracked and checked when dry. Two forms of soft rot have been described, type I consisting of biconical or cylindrical cavities that are formed within secondary walls while type II refers to an erosion form of degradation (Blanchette, 1995). In contrast to nonselective white-rot fungi, the middle lamella is not attacked by type II soft-rot fungi. Xylariaceous ascomycetes from genera such as *Daldinia*, *Hypoxylon*, and *Xylaria* have earlier often been regarded as white-rot fungi, but nowadays these fungi are

grouped to soft-rot fungi since they cause typical type II soft rot. They primarily occur on hardwood, and weight losses up to 53% of birch wood were found within 2 months by the most efficient fungus of this group, *Daldinia concentrica* (Nilsson et al., 1989). The highest lignin loss observed was 44% at the stage when weight loss was 77% after 4 months incubation. Pine wood was degraded, however, very little, only showing 2.5% weight loss (Nilsson et al., 1989). The high concentration of guaiacyl units in the middle lamella of coniferous wood may cause the resistance to the decay by soft-rot fungi. Ligninolytic peroxidases or laccases of soft-rot fungi may not have the oxidative potential to attack the recalcitrant guaiacyl lignin. On the other hand, syringyl lignin apparently is readily oxidized and mineralized by the enzymes of soft-rot fungi (Nilsson et al., 1989). Unfortunately, ligninolytic enzymes of xylariaceous ascomycetes are not well known.

Microfungi or molds, i.e., deuteromycetes and certain ascomycetes that are usually thought to degrade mainly carbohydrates in soil, forest litter, and compost, can also degrade lignin in these environments (Rodriguez et al., 1996a; Regalado et al., 1997; Tuomela et al., 2000). Thus, some microfungi are able to mineralize grass lignins up to 27% (Haider and Trojanowski, 1980). Although actinomycetes were predominant among 82 strains selected in a screening for ligninolytic microorganisms in a forest soil, also some microfungi were identified, e.g., *Penicillium chrysogenum*, *Fusarium oxysporum*, and *Fusarium solani* (Rodriguez et al., 1996a). In 28 d, these fungi mineralized 27.4%, 23.5%, and 22.6% of a ¹⁴C-labeled lignin prepared from milled wheat straw. However, lignin prepared from pine was much less degraded, and mineralization rate of less than 3% was obtained. Another soil-inhabiting mold, *Fusarium proliferatum*, min-

eralized 3.5% of a ^{14}C -(ring)-labeled DHP and 10% of $^{14}\text{C}_\beta$ -labeled DHP in 30 d. The degradation was maximal during primary metabolism (Regaldo et al., 1997). Laccase, aryl alcohol oxidase, and superoxide radicals were detected in liquid cultures of *F. proliferatum*, but neither MnP nor LiP were present (Regaldo et al., 1999). Superoxide radicals that may generate highly reactive hydroxyl radicals were found between days 3 and 7, i.e., during the highest mineralization of lignin. In contrast, activities of extracellular laccase and aryl alcohol oxidase reached their maximum relatively late (Regaldo et al., 1999), and their role may be not so important in the mineralization of lignin. Laccase activities have also been described in *P. chrysogenum* (Rodriguez et al., 1996b). The fungus mineralized 7.9% of $^{14}\text{C}_\beta$ -DHP in 29 d.

The degradation of lignin has been studied in the ascomycete *Chrysonilia sitophila*, “red mold of bread”, which is the anamorph stage, while its teleomorph stage is *Neurospora sitophila* (Rodriguez et al., 1997). Three isoforms of LiP were purified and characterized from this fungus but the production appeared not to be reproducible. Phenol oxidase activity, probably laccase, was found to be stable, and the fungus produces this activity on media containing pine wood or lignosulfonate. MnP has not been found in this fungus. *C. sitophila* caused 20% weight loss of pine wood in 3 months, with the losses of carbohydrate and lignin being 18% and 25%, respectively. Analysis of the decayed lignin suggested that oxidative C_α - C_β and β -O-aryl cleavages occurred during lignin degradation.

Extracellular peroxidases and oxidases, e.g., laccase (Hofrichter and Fritsche, 1996; Chefetz et al., 1998; Li et al., 1999) are produced also by other microfungi. They may not be so efficient in oxidizing lignin as those of white-rot fungi, but they may have special properties. *Thermoascus aurantiacus*

is a thermophilic ascomycete that commonly grows in the heated parts of wood chip piles in Scandinavia (Bergman and Nilsson, 1971). A strain of *T. aurantiacus* isolated from eucalyptus wood in Brazil (Machuca et al., 1995) shows unusual characteristics, i.e., it degrades extractives of *Eucalyptus grandis* and bleaches eucalyptus Kraft pulp. This strain produces high levels of phenol oxidase that has optimal pH values between 2.6 and 3.0, and optimal temperature up to 70–80 °C. The authors could not study higher temperatures than 80 °C, and it is possible that the true optimum temperature is even higher (Machuca et al., 1998).

4

Brown-Rot Basidiomycetes

The largest group of fungi that degrades wood is the basidiomycetes. It has been calculated that in North America there are 1600–1700 species of wood-degrading basidiomycetes (Gilbertson, 1980). Reproduction of basidiomycetes usually occurs by haploid basidiospores and thus the primary mycelium developed after spore germination is also haploid. Dikaryotic secondary mycelium arises as the result of hyphal fusion between different primary mycelia. Dikaryotic mycelium can be identified by the presence of clamp connections at the septum (Alexopoulos et al., 1996). Wood-rotting basidiomycetous fungi are usually divided into white-rot and brown-rot fungi. They are taxonomically closely related, and white-rot and brown-rot fungi can be found in the same genera. Most wood rotters belong to the orders Agaricales and Aphyllophorales (phylum Basidiomycota) (Alexopoulos et al., 1996).

Brown-rot fungi mainly decompose the cellulose and hemicellulose components in wood, but they can also modify the lignin to a

limited extent (Eriksson et al., 1990). They have been much less investigated than white-rot fungi in spite of their enormous economic importance in the destruction of wood. Brown-rotted wood is dark, shrink, and typically broken into brick-shaped or cubical fragments that easily break down into brown powder (Blanchette, 1995). The brown color indicates the presence of modified lignin in wood. Many brown-rot fungi such as *Serpula lacrymans*, *Coniophora puteana*, *Meruliporia incrassata*, and *Gloeophyllum trabeum* are destructive to wood used in buildings and other structures (Blanchette, 1995). *C. puteana* and the so-called dry-rot fungus *S. lacrymans*, two of the most harmful fungi occurring in wood in temperate regions, prefer softwood to hardwood as substrates.

The fungal hyphae penetrate from one cell to another through existing pores in wood cell walls early in the decay process. The penetration starts from the cell lumen where the hyphae are in close connection with the S_3 layer. In brown rot the decay process is thought to affect the S_2 layer of the wood cell wall first (Eriksson et al., 1990). Brown-rot fungi have a unique mechanism to break down wood polysaccharides. In contrast to white-rot fungi that successively depolymerize cell wall carbohydrates only to the extent that they utilize hydrolysis products in fungal metabolism, brown-rot fungi rapidly depolymerize cellulose and hemicellulose, and degradation products accumulate since the fungus does not use all the products in the metabolism (Cowling, 1961).

Potential biotechnical applications of brown-rot fungi have been studied occasionally, e.g., solid-state fermentation of pine sawdust for the production of cattle feed (Agosin et al., 1989), or the use of brown-rotted lignin for adhesives, e.g., to replace phenol-formaldehyde flakeboard resin (Jin et al., 1991). Brown-rotted lignin is more reactive than native lignin due to the in-

creased content of phenolic hydroxyl groups (Jin et al., 1990b). *G. trabeum* caused a significant release of alkali-soluble lignin especially during the first week of the growth on pine sawdust (Agosin et al., 1989).

To some extent, brown-rot fungi have similar degradative capabilities and pathways as white-rot fungi. Both wood decay mechanisms rely on radical formation, low pH, and the production of organic acids. They cause increased alkali solubility of lignin, and the decay is enhanced by high oxygen tension, all of which indicate a crucial involvement of radicals, especially in the early stages of decay (Kirk, 1975; Jin et al., 1990a). The production of lignin peroxidase and manganese peroxidase has been described in the brown-rot fungus *Polyporus ostreiformis* that removed 18.6% of the lignin from rice straw within 3 weeks (Dey et al., 1994). Laccase gene-specific sequences have been detected in brown-rot fungi (D'Souza et al., 1996).

Usually, it has been assumed that all brown-rot fungi use the same mechanism in wood decay, and that the decay involves a Fenton-type catalytic system producing hydroxyl radicals that attack wood components. However, analogously to different groups of white-rot fungi using different mechanisms in lignin degradation, brown-rot fungi also seem to possess clearly different wood decay mechanisms. The first group could be *Gloeophyllum trabeum* (syn. *Lenzites trabea*)-type fungi, and the second group includes *Coniophora puteana* and *Poria* (*Postia*) *placenta*-type fungi. Among brown-rot fungi, *G. trabeum* has been mostly studied.

The initiators of both cellulose and lignin breakdown are suggested to be small molecular weight compounds that can readily diffuse from the hyphae and penetrate into the wood cell and start decay (Evans et al., 1994; Wood, 1994; Goodell et al., 1997; Shimada et al., 1997). All models suggested to explain brown-rot decay involve hydroxyl

radical generation. However, all the proposed models are not fully experimentally verified. Many of these low-molecular mass agents have been isolated from cultures of both white-rot and brown-rot fungi which makes it difficult to understand their specific role in brown-rot decay. These compounds may be, e.g., phenolates or other types of iron-chelating compounds (siderophores), oxalate, and simple aromatic compounds (Koenigs, 1974; Fekete et al., 1989; Espejo and Agosin, 1991; Jellison et al., 1991; Goodell et al., 1997; Enoki et al., 1997; Shimada et al., 1997).

G. trabeum showed an unusual ability to rapidly degrade an aliphatic polyether via extracellular one-electron oxidation (Kerem et al., 1998). Recently, Paszczynski et al. (1999) found that *G. trabeum* produces simple aromatic compounds, 4,5-dimethoxycatechol and 2,5-dimethoxyhydroquinone. The authors suggested that these compounds may serve as ferric chelators, oxygen-reducing agents, and redox-cycling compounds. Kerem et al. (1999) have also reported that cultures of *G. trabeum* produced 2,5-dimethoxy-1,4-benzoquinone.

Certain brown-rot fungi accumulate oxalic acid causing a noticeable decrease of pH, but not *G. trabeum*, probably because of oxalate decomposing enzymes (Shimada et al., 1997). It has been suggested that these brown-rot fungi may use oxalic acid as a proton donor for enzymatic and nonenzymatic hydrolysis of polysaccharides and as a chelator for a Fe(II)-H₂O₂ system generating hydroxyl radicals (Shimada et al., 1997). In the model of Hyde and Wood (1997), who studied *Coniophora puteana*, it is suggested that Fe(III) is reduced by cellobiose dehydrogenase (CDH) within the fungal cells, and that then the Fe(II) diffuses at some distance from the hyphae, where a Fe(II)-oxalate complex is formed and again, Fenton reaction-based hydroxyl radical formation occurs.

The radicals formed by brown-rot fungi can remove methoxyl groups from lignin and produce methanol, and thus they leave a residue that consists mainly of modified lignin (Eriksson et al., 1990; Jin et al., 1990a). Demethoxylation of methoxyl groups and aromatic hydroxylation result in increased phenolic hydroxyl groups, which makes lignin more reactive. The presence of carboxyl and carbonyl groups is also evidenced by an increased oxygen content (Jin et al., 1990b). Davis et al. (1994) used ¹³C-CP-MAS NMR spectrometry and found that *P. placenta* mainly removed carbohydrate components from spruce wood, and hemicelluloses were removed faster than cellulose, resembling thus the results obtained with *G. trabeum* (Agosin et al., 1989). *P. placenta* further caused demethoxylation of spruce lignin, an increase in vanillin-like structures and α -carbonyl moieties, but there was no evidence for ring opening (Davis et al., 1994). Birch wood was degraded in a different way, and the cleavage of lignin β -O-4 linkages was the most prominent reaction occurring, rather than demethoxylation of lignin (Davis et al., 1994).

The presence of a wood environment may have a significant effect on the level of demethoxylation of lignin and lignin model compounds by brown-rot fungi. When *G. trabeum* was cultivated under optimized conditions on pine wood flakes, the evolved ¹⁴CO₂ from ¹⁴C-methylated lignin was about 30% in 52 d (Jin et al., 1990a). The presence of wood stimulated the evolution of ¹⁴CO₂ from a nonphenolic (4-O¹⁴CH₃)-labeled β -O-4 dimer by *G. trabeum*, and 30–60% of the applied radioactivity was released as ¹⁴CO₂ within 8 weeks (Niemenmaa et al., 1992). *P. placenta* produced ¹⁴CO₂ from O¹⁴CH₃-labeled vanillic acid rather well, but poorly from the β -O-4 dimer, only 3% as ¹⁴CO₂ within 4 weeks under similar conditions (Niemenmaa et al., 1992).

5

White-Rot Basidiomycetes

The only organisms capable of mineralizing lignin efficiently are basidiomycetous white-rot fungi and related litter-decomposing fungi (Kirk and Cullen, 1998). White-rot fungi are a heterogeneous group of fungi classified in the Basidiomycota. Different white-rot fungi vary considerably in the relative rates at which they attack lignin and carbohydrates in woody tissues. Some remove lignin more readily than carbohydrates, relative to the original amount of each (Blanchette, 1995). Many white-rot fungi colonize cell lumina and cause cell wall erosion. Eroded zones coalesce as decay progresses and large voids filled with mycelium are formed. This type of rot is referred to as nonselective or simultaneous rot (Blanchette, 1995). *Trametes* (syn. *Coriolus*, *Polyporus*) *versicolor* is a typical simultaneous-rot fungus (Cowling, 1961; Eriksson et al., 1990).

Some white-rot fungi preferentially remove lignin without a substantial loss of cellulose, and cause white-pocket or white-mottled type of rot, e.g., *Phellinus nigrolimitatus* (Blanchette, 1984b, 1995; Otjen and Blanchette, 1987). There are also fungi that are able to produce both types of attack in the same wood (Eriksson et al., 1990). Typical examples of such fungi are *Ganoderma applanatum* and *Heterobasidion annosum*. Because fungi selectively degrading lignin are considered the most promising fungi for applications in the pulp and paper industry, the search among these fungi has attained a considerable interest. However, the ratio lignin-hemicellulose-cellulose decayed by a selected fungus can differ enormously, and even different strains of the same species, e.g., of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermisporea*, may behave differently on the same kind of wood. Variations

within a substrate are also found in one fungal strain (Eriksson et al., 1990; Blanchette et al., 1992). Several screening studies to find suitable fungi for biopulping of wood or straw, have revealed fungi that, under certain conditions, degrade lignin preferentially to cellulose. Such lignin-selective fungi are, e.g., *P. chrysosporium*, *C. subvermisporea* (Otjen and Blanchette, 1987; Eriksson et al., 1990), *Pycnoporus cinnabarinus* (Ander and Eriksson, 1977), *Pleurotus ostreatus* (Martínez et al., 1994), *Pleurotus eryngii* (Martínez et al., 1994; Dorado et al., 1999), *Phlebia radiata* (Ander and Eriksson, 1977), *Phlebia tremellosus* (syn. *Merulius tremellosa*) (Ander and Eriksson, 1977; Eriksson et al., 1990), *Phlebia subserialis* (Akhtar et al., 1998), *Phellinus pini* (Eriksson et al., 1990), and *Dichomitus squalens* (Eriksson et al., 1990). The lignin-degrading systems of these fungi are important to study since they are very efficient. *C. subvermisporea* may be considered as a model fungus for selective lignin degradation. Calcium oxalate and MnO₂ accumulate when the decay proceeds (Blanchette, 1984a, 1995; Eriksson et al., 1990). Manganese peroxidase (MnP) is the most important ligninolytic enzyme produced by *C. subvermisporea* and many other lignin-selective fungi and will be discussed later.

White-rot fungi are more commonly found on angiosperm than on gymnosperm wood species in nature (Gilbertson, 1980). Usually syringyl (S) units of lignin are preferentially degraded whereas guaiacyl (G) units are more resistant to degradation. When grown on straw, transmission electron microscopy revealed that *C. subvermisporea* and *P. eryngii* partially removed the middle lamella while *Phlebia radiata* apparently removed lignin from secondary cell walls (Burlat et al., 1998). In fibers, the middle lamella contains a high concentration of G lignin while secondary walls contain a high proportion of S lignin.

Basic research on lignin degradation, e.g., its mechanisms, physiology, enzymology, and molecular biology, has been mainly carried out with the corticioid fungus *P. chrysosporium* (Kirk and Farrell, 1987; Eriksson et al., 1990; Gold and Alic, 1993). After more and taxonomically different fungi had been studied in more detail, it was revealed that both the physiological conditions for lignin degradation and the enzyme systems expressed are fungus-specific and differ from those found in *P. chrysosporium*. Differences may be connected to the taxonomic position and/or ecology of the fungi, e.g., substrate specialization (hardwood, softwood, or certain wood species, heartwood or sapwood), the stage of degradation, etc. For example, the production of MnP in poplar wood was significant by *Ganoderma lucidum* but the fungus did not produce MnP in pine wood medium (D'Souza et al., 1999). The fungus obviously has the genetic potential to produce LiP since *lip*-like sequences were found in Southern hybridization, but LiP activity could not be detected.

Ligninolytic activities and enzymes of litter-decomposing basidiomycetous fungi have been only very little studied. In a study with some fungi, e.g., from the genera *Stropharia* and *Agrocybe*, the mineralization of ^{14}C -(ring)-labeled synthetic lignin (DHP) was about half of the level obtained with wood-inhabiting white-rot fungi (Steffen et al., 1999). The main ligninolytic enzymes in litter-decomposing fungi such as *Agaricus bisporus* (Bonnen et al., 1994) and *Stropharia rugosoannulata* (Steffen et al., 1999) are laccase and MnP. In a recent study, laccase was found to be the only ligninolytic enzyme in *Marasmius quercophilus* (Dedeyan et al., 2000).

5.1

Mineralization of ^{14}C -Labeled Lignins

The determination of $^{14}\text{CO}_2$ evolution from ^{14}C -DHP (dehydrogenation polymer of coniferyl alcohol or other lignin precursors) or other ^{14}C -(lignin)-lignocelluloses has been the most often adopted method to determine lignin-degrading ability (Haider and Trojanowski, 1975; Kirk et al., 1975, 1978). The method has been widely used for more than 20 years (Buswell and Odier, 1987; Kirk and Farrell, 1987; Eriksson et al., 1990). Preparations from different laboratories have given comparable results (Hatakka and Uusi-Rauva, 1983; Hatakka et al., 1983). Buswell and Odier (1987) compared different nonradioactive and ^{14}C -labeled lignin preparations for microbiological studies and concluded that all lignin preparations have disadvantages in reproducibility in preparation, or altered structure and molecular weight compared to natural lignin. Nevertheless, the use of ^{14}C -labeled preparations can be considered the most reliable method. In addition, methods to study the degradation of polymeric lignin by, e.g., NMR spectroscopy have been developed (Davis et al., 1994; Gamble et al., 1994), but they are not easily amenable for detailed physiological studies with microorganisms or biochemical studies with enzymes. Dimeric lignin model compounds attached to a polymer backbone, e.g., polystyrene (Lundell et al., 1992) or to more soluble polymers, e.g., polyethylene glycol (Kawai et al., 1995), have allowed to use efficient analytical tools (e.g., NMR) for more defined structures.

Solubilization (formation of water-soluble lignin fragments) and mineralization (evolution of $^{14}\text{CO}_2$) of ^{14}C -labeled natural and synthetic lignins have been demonstrated for various white-rot fungi (Kirk et al., 1975, 1978; Hatakka and Uusi-Rauva, 1983; Hatakka et al., 1983; Boyle et al., 1992; Hatakka,

1994; Vares et al., 1994; Vares and Hatakka, 1997; Hofrichter et al., 1999b). A high mineralization of ^{14}C -(ring)-DHP was observed in the case of *Phlebia radiata* that released up to 71% $^{14}\text{CO}_2$ from ^{14}C -DHP when grown in a liquid medium (Hatakka et al., 1983). Typically, only very low amounts, in the range of 3% of the ^{14}C -label from DHP, were associated with the fungal biomass after 40 d of cultivation. Similar results were obtained using natural ^{14}C -labeled lignins, e.g., from fir or oak (58–61% mineralization, 12–13% ^{14}C in the mycelium) (Leatham, 1986) or different lignocelluloses (Hatakka et al., 1983). *Nematoloma frowardii* caused even higher mineralization (75%) of ^{14}C -DHP during growth on wheat straw while again only a small percentage of the initial radioactivity (6%) was incorporated into the residual straw and the fungal biomass (Hofrichter et al., 1999b).

Usually only the end product, $^{14}\text{CO}_2$, is determined. This may give misleading results, since in some fungi or under some conditions, the rate-limiting steps may be the reactions after the initial attack on polymeric lignin. Water-soluble degradation products may accumulate, e.g., under conditions of low oxygen tension (Hatakka and Uusi-Rauva, 1983). The array of different ligninolytic enzymes may also influence the production of $^{14}\text{CO}_2$.

5.2

Ligninolytic Enzymes

Enzyme systems for the degradation of macromolecular lignin face several challenges (Kirk and Cullen, 1998). The substrate is a large heterogeneous polymer that necessitates attack by extracellular enzymes or agents. Lignin does not contain hydrolyzable linkages, which means that the enzymes must be oxidative. Lignin is stereoirregular,

which also points to more nonspecific attack compared to many other natural polymers.

Extracellular enzymes involved in lignin degradation are lignin peroxidases (LiPs, “ligninases”, EC 1.11.1.14) and manganese peroxidases (MnPs, “Mn-dependent peroxidases”, EC 1.11.1.13), as well as laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2). In addition, some accessory enzymes are involved in hydrogen peroxide production. Glyoxal oxidase (GLOX) and aryl alcohol oxidase (AAO) (EC 1.1.3.7) belong to this group. Table 1 shows a general overview of the main cofactors or substrates and the principal effects or reactions of each enzyme.

LiPs and MnPs are heme-containing glycoproteins which require hydrogen peroxide as an oxidant. Most fungi secrete several isoenzymes into their cultivation medium (Table 2). LiP oxidizes nonphenolic lignin substructures by abstracting one electron and generating cation radicals that are then decomposed chemically (Kirk et al., 1986; Kirk and Farrell, 1987) (Figure 1). Reactions of LiP using a variety of lignin model compounds and synthetic lignin have thoroughly been studied, the catalytic mechanisms elucidated, and its capability for C_α – C_β bond cleavage, ring opening, and other reactions has been demonstrated (Kirk and Farrell, 1987). MnP oxidizes Mn(II) to Mn(III) which then oxidizes phenolic rings to phenoxyl radicals which lead to decomposition of compounds (Gold et al., 1989) (Figure 2).

Studies with different white-rot fungi have shown that MnP appears to be more common than LiP (Orth et al., 1993; Hatakka, 1994; Vares and Hatakka, 1997), and during the recent years the characteristics and role of MnP have been extensively studied. MnP has an essential role in the depolymerization of lignin (Wariishi et al., 1991) and chlorolignin (Lackner et al., 1991), as well as in

Tab. 1 Enzymes involved in the degradation of lignin and their main reactions

Enzyme Activity, Abbreviation	Cofactor or Substrate, "Mediator"	Main Effect or Reaction
Lignin peroxidase, LiP	H ₂ O ₂ , veratryl alcohol	aromatic ring oxidized to cation radical
Manganese peroxidase, MnP	H ₂ O ₂ , Mn, organic acid as chelator, thiols, unsaturated lipids	Mn(II) oxidized to Mn(III); chelated Mn(III) oxidizes phenolic compounds to phenoxy radicals; other reactions in the presence of additional compounds
Laccase, Lacc	O ₂ ; mediators, e.g., hydroxybenzotriazole or ABTS	phenols are oxidized to phenoxy radicals; other reactions in the presence of mediators
Glyoxal oxidase, GLOX	glyoxal, methyl glyoxal	glyoxal oxidized to glyoxylic acid; H ₂ O ₂ production
Aryl alcohol oxidase, AAO	aromatic alcohols (anisyl, veratryl alcohol)	aromatic alcohols oxidized to aldehydes; H ₂ O ₂ production
Other H ₂ O ₂ producing enzymes	many organic compounds	O ₂ reduced to H ₂ O ₂

the demethylation of lignin and bleaching of pulp (Paice et al., 1993). Moreover, the enzyme mediates initial steps in the degradation of high-molecular weight lignin (Pérez and Jeffries, 1992). Recently, it has been shown that MnP in the presence of suitable organic acids is even able to mineralize lignin and lignin model compounds to considerable amounts (Hofrichter et al., 1999a). Table 3 summarizes conditions and results of the main published *in vitro* experiments, where LiPs and MnPs have been used in the mixture with different accessory compounds.

Laccase is a copper-containing oxidase that utilizes molecular oxygen as oxidant and also oxidizes phenolic rings to phenoxy radicals (Thurston, 1994) (Figure 2). It has also a capacity to oxidize nonphenolic compounds under certain conditions, e.g., if the reaction mixture is supplemented with ABTS (Bourbonnais and Paice, 1990) or other mediating molecules (Li et al., 1999). Most white-rot fungi typically produce laccase (Käärik, 1965; Bollag and Leonowicz, 1984).

Phylogenetic analysis of wood-rotting and other homobasidiomycetes fungi, based on ribosomal DNA sequences (Hibbett et al., 1997), may give new insights into the relationships of different ligninolytic systems. Production of LiP may be typical for wood-rotting fungi, phylogenetically separated from "euagarics" such as *Pleurotus ostreatus*, *Agaricus bisporus*, and related fungi. "Euagaric" fungi typically produce MnPs and laccase but in most cases truly lack LiP. However, the system of *C. subvermispora*, most probably belonging to the group of wood-rotting fungi, although not included in the analysis by Hibbett et al. (1997), obviously contains *lip*-like genes (Rajakumar et al., 1996). The system used by the white-rot fungus *P. cinnabarinus* is possibly unique since this fungus does not produce any peroxidases (Eggert et al., 1996b).

Almost all white-rot fungi produce MnPs and laccase, but only some fungi produce LiP, the only enzyme capable of attacking non-phenolic lignin substructures. Production of

Tab. 2 Molecular masses and isoelectric points of ligninolytic enzymes from selected white-rot and litter-decomposing fungi (the fungi may also produce other ligninolytic enzymes, not listed here)

Fungus	Molecular Masses <i>M</i> , [kDa]	Isoelectric Points [pIs]	Reference
<i>Abortiporus biennis</i>	MnPs: 38–45 (many isoforms)	MnPs: 3.9–6.5 (many isoforms)	Vares and Hatakka (1997)
<i>Agaricus bisporus</i>	MnPs: ND laccase: 65, 66 (straw)	MnPs: 3.35, 3.25, 3.3 (straw compost) laccase: ND	Bonnen et al. (1994), Perry et al. (1993), Leontievsky et al. (1997b)
<i>Armillaria mellea</i>	laccase I: 60, II: 60–62	laccase I: 4, II: 3.3	Billal and Thurston (1996)
<i>Bjerkandera</i> sp. BOS55	LiPs: (2 isozymes) 40–42 MnPs: 44, 45	ND MnPs: 3.45, 3.40	ten Have et al. (1998), Palma et al. (2000)
<i>Bjerkandera adusta</i>	LiPs (2 isoenzymes): 48, 48 MnPs (2 isoenzymes): 44, 44	LiPs: 3.1, 3.2 MnPs: 3.45, 3.35	Kimura et al. (1990), Heinfling et al. (1998a)
<i>Ceriporiopsis subvermispora</i>	MnPs: ND laccase: 71, 68	MnPs: 4.1–4.6 (liquid), 3.2, 3.3, 3.4, 3.5 (wood) laccase: 3.4, 4.8	Lobos et al. (1994), Salas et al. (1995), Fukushima and Kirk (1995)
<i>Coprinus cinereus</i>	laccase: 58 (no MnP, no LiP)	laccase: 4	Heinzkill et al. (1998), Schneider et al. (1999)
<i>Cyathus stercoreus</i>	MnPs: ND laccase: 70 (no LiP)	MnPs: ND laccase: 3.5, 4.8	Sethuraman et al. (1999)
<i>Dichomitus squalens</i>	MnPs: 48, 48.9 laccase: 66	MnPs: 3.9, 4.15 laccase: 3.5, 3.6	Périé et al. (1996, 1998)
<i>Ganoderma lucidum</i>	laccases: 40, 66	laccases: 3.0, 4.25, 4.5, 4.8, 5.1	D'Souza et al. (1999)
<i>Heterobasidion annosum</i>	MnPs: ND	MnPs: 3.3, 3.5, 3.7	Maijala et al. (2000)
<i>Junghuhnia separabilima</i>	LiPs: 43–47 laccase: 58–62	LiPs: 3.4–3.5 laccases: 3.4–3.6	Vares et al. (1992)
<i>Lentinula edodes</i>	MnPs: ND laccase: 66	MnPs: 3.2 laccase: ND	Forrester et al. (1990)
<i>Marasmius quercophilus</i>	laccase: 63 (no MnP, no LiP)	laccase: 3.6	Dedeyan et al. (2000)
<i>Nematoloma frowardii</i>	MnPs: 42–44 (liquid) 50 (straw)	MnPs: 3.1–4.0 (liquid) 3.2 (straw)	Schneegeß et al. (1997), Hofrichter et al. (1999b)
<i>Panus tigrinus</i>	MnPs: 43 laccase: 64	MnPs: 2.95, 3.2 laccase: 2.9–3.0	Maltseva et al. (1991)
<i>Phanerochaete chrysosporium</i> BKM-F-1767 (= ATCC 24725) ME446 OGC101	LiPs: 38–43 MnPs: 46	LiPs: 3.3–4.7 MnPs: 4.2–4.9 MnP: 4.9 (aspen pulp)	Farrell et al. (1989), Gold et al. (1989), Pease and Tien (1992), Datta et al. (1991)
<i>Phanerochaete chrysosporium</i> ME446	laccase: 100		Dittmer et al. (1997)
<i>Phanerochaete sordida</i>	MnPs: 45 (no LiP, no laccase)	MnPs: 3.3, 4.2, 5.3	Rüttimann-Johnson et al. (1994)
<i>Phanerochaete flavido-alba</i>	LiPs: 45 MnPs: 45 laccase: 94	LiPs: < 3.55 MnPs: 3.55–5.85 laccase: < 3.55	Ben Hamman et al. (1999), Pérez et al. (1996)
<i>Phlebia ochraceofulva</i>	LiPs: 38–46 (no MnP)	ND	Vares et al. (1993)
<i>Phlebia radiata</i> 79 (ATCC 64658)	LiPs: 39–44 MnPs: 44–48 laccase: 64	LiPs: 3.2–4.1 MnPs: 3.6, 3.85, 4.85 (liquid culture), 3.3–5.9 (straw) laccase: 3.5	Lundell and Hatakka (1994), Moilanen et al. (1996), Vares et al. (1995), Leontievsky et al. (1997b)
<i>Phlebia tremellosa</i> 2845 (ATCC 48754)	LiPs: 35–40 MnPs: activity found, not puri- fied laccase: 64	LiPs: 3.1–4.0 MnPs: ND laccase: ND	Vares et al. (1994), Leontiev- sky et al. (1997b)
<i>Pleurotus eryngii</i>	laccase: 61, 65 MnPs: 43 (2 isoenzymes)	laccase: 4.2, 4.1 MnPs: 3.65, 3.75 (2 isoen- zymes)	Muñoz et al. (1997), Martí- nez et al. (1996)
<i>Pleurotus ostreatus</i>	MnPs: 42–45	MnPs: 3.5, 3.7, 4.3 (liquid) 3.5, 3.7 (wood)	Becker and Sinitzyn (1993), Sarkar et al. (1997)
<i>Pycnoporus cinnabarinus</i>	laccase: 81 (no MnP, no LiP)	laccase: 3.7	Eggert et al. (1996b)
<i>Rigidoporus lignosus</i>	MnPs: 42 laccase: 53	MnPs: 3.5, 3.7 laccase: 3.8	Galliano et al. (1991)
<i>Trametes hirsuta</i>	laccase: 64–68 (2–3 isoforms)	laccase: 3.7–4.0 (2–3 isoforms)	Vares and Hatakka (1997)
<i>Trametes trogii</i>	LiPs: 41–44 (2 isoforms)	LiPs: 3.7–3.8 (2 isoforms)	Vares and Hatakka (1997)

Tab. 2 (cont.)

Fungus	Molecular Masses M_r [kDa]	Isoelectric Points [pIs]	Reference
<i>Trametes versicolor</i>	LiPs: 43–45 MnPs: 49 laccase: 64	LiPs: 3.2–3.4 MnPs: 2.9–3.2 laccase: ND	Johansson and Nyman (1993), Leontievsky et al. (1997b)
<i>Trametes villosa</i> (syn. <i>Trametes pinsitus</i> , <i>C. pinsitus</i>)	laccase: dimeric, 2 subunits of 63	laccase: 3.5, 6–6.5, 5–6 (3 isoforms)	Yaver et al. (1996)

ND = not determined or reported; LiP, lignin peroxidase; MnP, manganese peroxidase

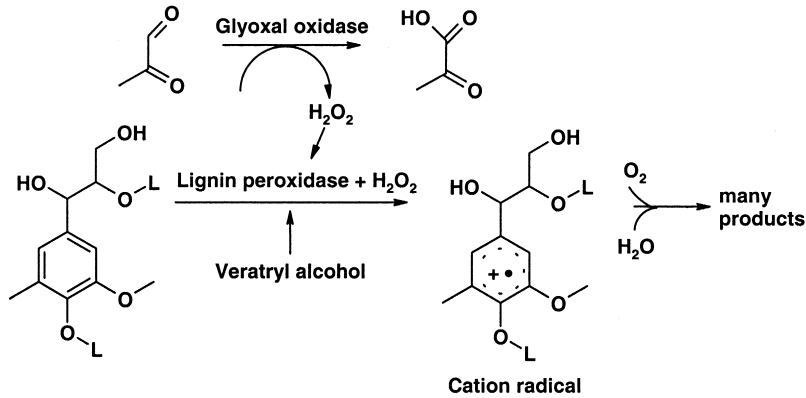


Fig. 1 Simplified reactions of lignin peroxidase and glyoxal oxidase (redrawn according to Kirk and Cullen, 1998).

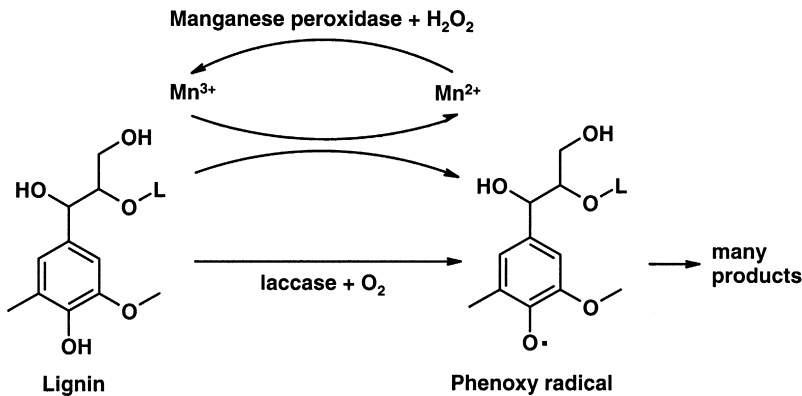


Fig. 2 Simplified reactions of manganese peroxidase and laccase (redrawn according to Kirk and Cullen, 1998).

ligninolytic enzymes in many different fungi, belonging to the family of Corticiaceae (e.g., *Phanerochaete* spp., *Phlebia* spp.) and Polyporaceae (e.g., *Trametes* spp. and many other species) and the degradation of ^{14}C -labeled lignin by the same fungi, show great

variability (Hatakka, 1994; Vares and Hatakka, 1997). Based on their main expressed extracellular ligninolytic enzymes, white-rot fungi can be classified into three or four groups (Hatakka, 1994). Fungi from the first group produce LiPs, MnPs, and laccase, and