Mycorrhiza (1998) 8:61–65
© Springer-Verlag 1998

REVIEW

J.W.G. Cairney · R.M. Burke

Do ecto- and ericoid mycorrhizal fungi produce peroxidase activity?

Accepted: 16 July 1998

Abstract Several reports attest to the apparent ability of some ectomycorrhizal (ECM) and ericoid (ERM) mycorrhizal fungi to produce peroxidase enzyme activities during growth in axenic culture. In critically reviewing these data, we highlight that apparent peroxidase activities have been observed during growth in media containing 60–70 μM Fe. ECM and ERM fungi are known to produce H_2O_2 via carbohydrate oxidase activity and conditions in common culture media are favourable to the production of hydroxyl radicals, superoxide radicals and ferryl ions via the Fenton reaction. Free radicals so produced can mediate oxidation of substrates commonly used in presumptive peroxidase assays, leading to false-positive results. We argue that there is currently no evidence to support production of peroxidase activity by ECM or ERM fungi, but highlight circumstances in which peroxidase expression might be observed in future work.

Key words Lignin degradation · Fenton reaction · *Hymenoscyphus ericae* · Lignin peroxidase · Ectomycorrhizal fungi

Introduction

The importance of ectomycorrhizal (ECM) and ericoid (ERM) mycorrhizal fungi in mineral nutrient cycling processes in many forest and heathland ecosystems is well established. Much of the total pools of these elements in forest soils, however, is tied up in organic forms and/or sequestered within the lignocellulose cell wall structures of dead plant material. In order to access mineral nutrients within moribund plant cells or to render complexed organic nitrogen and phosphorus substrates available for enzymatic attack by proteinases and phosphatases (see Smith and Read 1997), mycorrhizal fungi must operate in close association with saprotrophic organisms, or produce enzymes capable of hydrolysing and/or oxidising components of the plant cell wall. While we have little knowledge of the extent or nature of mycorrhizal fungus/saprotroph interactions, there is increasing evidence that some ECM and ERM fungi, are capable of producing plant cell wall-degrading enzymes (see Cairney and Burke 1994).

Lignified plant material comprises lignin, cellulose and hemicellulose, although the proportions differ between plant species (Fengel and Wegener 1983). Degradation of plant wall material thus requires the action of cellulases, hemicellulases and ligninases. Production of components of the cellulase enzyme complex and some hemicellulase activities have now been confirmed for some ECM fungi, indicating that they may be capable of at least partial degradation of some wall components (eg. Cao and Crawford 1993ab; Cairney and Burke 1996a). Observed degradation of ^{14}C-labelled cellulose and hemicellulose in the proximity of ECM extramatrical mycelium in a sterile system (Durall et al. 1994) further suggests expression of components of these enzyme groups during symbiosis with a host plant. Haselwandter et al. (1990) provided evidence that selected ECM and ERM fungi have the potential to degrade lignin or dehydrogenative polymerisates of lignin monomers in axenic culture. Such degradation, however, occurred to a lesser extent than in saprotrophic white rot fungi. At present the mechanism(s) by which ECM and ERM fungi mediate what appears to be partial lignin degradation are not clear. Understanding these mechanisms is fundamental to understanding how these fungi interact with dead plant material at the molecular level, and will allow investigation of expression and control of the enzyme systems, providing new

J.W. G. Cairney (✉)
Mycorrhiza Research Group, School of Science,
University of Western Sydney (Nepean),
P.O. Box 10,
Kingswood, NSW 2747, Australia
e-mail: j.cairney@nepean.uws.edu.au

R.M. Burke
Department of Biomolecular Sciences, UMIST, P.O. Box 88,
Manchester, M60 1QD, UK

insights into the ecological functions of ECM and ERM associations.

Mechanisms of lignin degradation

Lignin degradation by saprotrophic fungi has been extensively studied and it is now clear that degradation proceeds via oxidative processes. In the case of white-rot fungi, oxidative combustion of the polymer is initiated largely by lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.1.1.13) and laccase (EC 1.10.3.2), with different combinations of the three enzymes operating in different fungal taxa (Hatakka 1994). Lignin peroxidase can cleave non-phenolic methoxyl-substituted lignin subunits (and so facilitate complete lignin degradation), while manganese peroxidase and laccase oxidise only phenolic residues (Kuhad et al. 1997). Lignin peroxidase and manganese peroxidase both use H_2O_2, derived by the action of carbohydrate oxidases, to produce reactive intermediates which cause single electron oxidations of aromatic nuclei within the lignin polymer. Lignin peroxidase oxidises aromatic reducing substrates, yielding aryl cation radicals, while manganese peroxidase oxidises Mn^{2+} yielding Mn^{3+} (Renganathan and Gold 1986; Wariishi et al. 1989). Laccase catalyses the one-electron oxidation of phenols to phenoxy radicals. It acts in a similar way to manganese peroxidase, catalysing alkyl-phenyl and C_α–C_β cleavage of phenolic dimers (Higuchi 1990). In an alternative process, brown-rot fungi can mediate partial lignin degradation by producing hydroxyl radicals from H_2O_2 in the presence of Fe^{2+} (Backa et al. 1992). The hydroxyl radical, however, is unable to catalyse cleavage of β-1 and β-O-4 bonds in lignin, degradation being confined to fragmentation via demethoxylation, ring hydroxylation and side chain oxidation (Gierer et al. 1992).

Evidence for peroxidase activities in ECM and ERM fungi

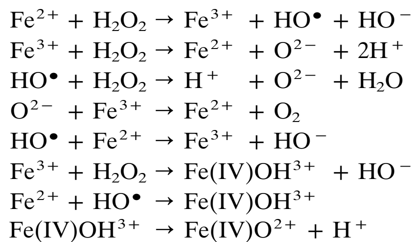
Studies of ligninolytic activities in ECM and ERM fungi have so far largely focused on assays for peroxidase and laccase activities in axenic culture. At first glance, there appears to be conflicting evidence regarding their abilities to produce peroxidative enzyme activities, with some studies reporting peroxidase activities in at least some isolates, while others report no detectable activity (Table 1). Differences in the fungal taxa investigated by the various authors notwithstanding, closer inspection of these data reveals an interesting trend. Positive evidence for putative peroxidase production has only been obtained from agar plate assays [decoluorisation of Remazol Brilliant Blue (RBB) or Poly R-478, or oxidation of pyrogallol]. The two studies which assayed for activity in culture filtrates of ECM fungi were unable to detect activity (Bending and Read 1996; Burke and Cairney 1998). It is also noteworthy that, while Bending and Read (1997) observed putative peroxidase activity (pyrogallol oxidation) in several ECM fungi and the ERM endophyte *Hymenoscyphus ericae* using agar plate assays, no activity was detected in filtrates from liquid cultures of *H. ericae.* No attempt was made to assay for peroxidase activity in ECM fungi. The authors reconciled the inconsistency between agar plate and liquid culture assays in *H. ericae* with the plausible hypothesis that peroxidase activity must be associated with the hyphal surface. Recent data, however, suggest an alternative interpretation.

Several ECM fungi and *H. ericae* have been shown by direct assay to produce cellobiose oxidase (EC 1.1.3.25) and glucose oxidase (EC 1.1.3.4) activities during axenic growth (Burke and Cairney 1998). Release of H_2O_2 from mycelia of these fungi (presumed to result from activity of the carbohydrate oxidases) has also been demonstrated (Burke and Cairney 1998). As outlined above, H_2O_2 produced by saprotrophic brown rots in the presence of Fe^{2+} is known to generate many free radicals according to the Fenton reaction and related reactions (Fig. 1) (Fenton 1894; Halliwell and Gutteridge 1985; Wood 1994). These include hydroxyl radicals, superoxide radicals, and ferryl ions (Fe^{4+}) which, by virtue of their having unpaired electrons in the 3-d orbital, behave in solution as free radicals. The free radicals so produced can then catalyse a number of further reactions. Hydroxyl radicals catalyse the formation of phenols from aromatic compounds. Further attack by the hydroxyl radical on phenols results in a

Table 1 Studies in which peroxidase activities in ectomycorrhizal and ericoid mycorrhizal fungi were assessed by either indirect agar plate assays or direct assay of culture filtrates (± apparent/no apparent peroxidase activity, respectively)

Assay	Medium	Result	Reference
RBB decolouration	MMN agar	+	Griffiths and Caldwell 1992
RBB decolouration	MMN agar	+	Bending and Read 1997
Poly R-478 decolouration	MMN agar	+	Griffiths and Caldwell 1992
Pyrogallol oxidation	MMN agar	+	Bending and Read 1992
Pyrogallol oxidation	MMN agar	+	Gramss et al. 1998
catechol → *p*-quinone	liquid MMN[a]	−	Bending and Read 1996
2,4-DCP → quinoneimine	liqhid MMN (low Fe)	−	Burke and Cairney 1998

[a] The initial absorbance of tubes containing a mixture of substrate, buffer, H_2O_2 and culture filtrate was measured before incubation for 1 h. Peroxidase activity was assessed by subtracting initial absorbance from final absorbance



$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-}$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O^{2-} + 2H^{+}$$
$$HO^{\bullet} + H_2O_2 \rightarrow H^{+} + O^{2-} + H_2O$$
$$O^{2-} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$
$$HO^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + HO^{-}$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe(IV)OH^{3+} + HO^{-}$$
$$Fe^{2+} + HO^{\bullet} \rightarrow Fe(IV)OH^{3+}$$
$$Fe(IV)OH^{3+} \rightarrow Fe(IV)O^{2+} + H^{+}$$

Fig 1 Reactions of Fe in the presence of H_2O_2 that will generate hydroxyl radicals ($HO^{\bullet}$), superoxide radicals (O^{2-}) and/or ferryl ions [$Fe(IV)OH^{3+}$, $Fe(IV)O^{2+}$].

mixture of polyhydroxylated phenolics. The superoxide and ferryl radicals both catalyse the formation of quinones from polyphenolics such as pyrogallol, catechol and guaiacol. The latter two compounds are widely used substrates in presumptive peroxidase assays, but will give false (chemical) positives for peroxidase activity in Fenton's reagent (Halliwell and Gutteridge 1985). The mechanism of RBB dye decolorisation in agar plate assays proceeds via an initial electron abstraction (Glenn and Gould 1985) and consequently can be catalysed by the superoxide radical.

Each of the studies outlined in Table 1 utilised a form of Modified Melin Norkrans (MMN) medium (Marx 1969; Marx and Bryan 1975) as the basal medium for ECM and ERM fungal growth. MMN contains ca. 70 μM Fe, generally added as $FeCl_3$, and has recently been shown to contain sufficient Fe^{2+} to generate significant quantities of hydroxyl radicals when H_2O_2 is added to fresh medium (Burke and Cairney 1998). In contrast, in MMN from which $FeCl_3$ is excluded, addition of H_2O_2 fails to generate hydroxyl radicals (Burke and Cairney 1998). Conditions favourable to the Fenton reaction thus clearly occur in the MMN basal solution used in the studies outlined in Table 1. Furthermore, in the presence of cellobiose oxidase, Fe^{3+} is preferentially reduced to Fe^{2+} (Wood 1994), while Fe^{3+} can also be chelated by oxalic acid and will spontaneously decompose, yielding Fe^{2+} (Schmidt et al. 1981). Production of cellobiose oxidase by ECM fungi and chelation with oxalates produced during ECM fungal growth will facilitate considerable reduction to Fe^{2+} and are likely to enhance free radical formation in MMN (see Burke and Cairney 1998). Thus reports of apparent peroxidase activity in ECM fungi growing on MMN probably reflect free radical- rather than peroxidase-mediated oxidation of the substrate. This argument is strengthened considerably by the failure of Burke and Cairney (1998) to detect peroxidase activity in culture filtrates from low Fe (6–7 μM Fe) MMN cultures.

The failure of Bending and Read (1996, 1997) to detect oxidation of catechol in MMN (high Fe) culture filtrates of ECM fungi and *H. ericae* may seem at odds with this. Activities in the former, however, were determined by subtracting the initial absorbance of a mixture of substrate, buffer, H_2O_2 and culture filtrate, from the final absorbance of the same mixture following incubation at 25 °C (Bending and Read 1996). Oxidation of catechol by the superoxide radical and/or Fe^{4+} in the initial mixture would have been rapid and the subtractive assay would effectively mask this background absorbance. The second order rate constant (k_2) for the reaction of the superoxide radical with catechol is 1×10^{-9} $M^{-1}.s^{-1}$ (Halliwell and Gutteridge 1985); thus when [catechol] is 90.8 mM (Bending and Read 1996), the lifetime of the superoxide radical is 11×10^{-9} s. A similar method was used to measure activity in *H. ericae*, but the absorbance of the mixture containing boiled culture filtrate was subtracted from the absorbance of the same mixture containing fresh culture filtrate (Bending and Read 1997). Since [Fe] is unaffected by boiling, the amount of catechol oxidised after the addition of H_2O_2 would be the same in the assay and the control and the absorbance values of both mixtures would have been identical. The subtractive assay would again have indicated no substrate oxidation (at least in the absence of peroxidase). Indeed, browning of MMN containing ca. 70 μM Fe can be demonstrated by spotting 1% catechol and 150 μM H_2O_2 onto agar medium (RM Burke and JWG Cairney, unpublished results). Such chemical browning must also be accounted for if plate assays are employed.

In the case of laccase activity, where 1-naphthol oxidation in MMN agar medium (in the absence of H_2O_2) has been used as a presumptive laccase assay in ECM fungi (Gramss et al. 1998), release of H_2O_2 into the medium in the presence of Fe may also result in a false-positive result. The 1-naphthol method is regarded as a presumptive peroxidase assay when conducted in the presence of H_2O_2 (Harkin and Obst 1973), meaning that chemically mediated oxidation would proceed as outlined above. It is pertinent in this context that Bending and Read (1997) did not detect laccase activity in culture filtrates of *H. ericae* using the boiling-subtractive method and syringaldazine as substrate.

Do ECM and ERM fungi produce peroxidase activities?

We argue that there is currently no convincing evidence to support the production of peroxidative enzyme activities in either ECM or ERM fungi. The conditions under which assays have been conducted, especially the presence of Fe in media and assay mixtures, mean that chemical oxidation by free radicals (mediated by fungal carbohydrate oxidases) can readily account for all apparent peroxidase activities. In this context, it may be significant that $Fe(IV)O^{2+}$ (Fig. 1) is in fact the so-called 'compound II' of the peroxidase catalytic cycle. Thus Fenton's reagent ($Fe + H_2O_2$) contains a non-enzymatic pseudoperoxidase activity. In fact, where lignin model substrate degradation by ECM and ERM fungi has been demonstrated in vitro (Trojanowski et al. 1984; Haselwandter et al. 1990), either Moser medium

(Moser 1958) or MMN was used as the basal medium. Both media contain 60–70 μm Fe, meaning that the Fenton reaction would be favoured as H_2O_2 was released by carbohydrate oxidase activity. This argument is strongly supported by the fact that the extent of substrate degradation (<24% over 60) is similar to that observed in hydroxyl radical-mediated degradation of similar substrates by brown rot saprotrophs (Kirk et al. 1975; Haider and Trojanowski 1980).

Whether or not conditions favourable to the production of hydroxyl radicals prevail in soil or decomposing litter is not clear. While Fe availability in most soils is low, proton extrusion at soil microsites can certainly increase Fe^{3+} availability (Römheld and Marschner 1986). Along with the presence of Fe-chelating siderophores and/or oxalate, which are known to be produced by ECM fungi (Szaniszlo et al. 1981; Griffiths et al. 1994), this may, in turn, lead to increased Fe^{2+} availability in the presence of carbohydrate oxidase activity in the manner described above. The extent to which this might occur, however, remains highly speculative.

Although at present there is no convincing evidence for expression of peroxidase activity in ECM or ERM fungi, we cannot discount the possibility that the enzymes are expressed by some taxa under some circumstances. Assays for peroxidase activity have been undertaken solely using fungi growing in MMN-based media. While Fe in these media can clearly interfere with assays for peroxidase activity, other media characteristics may influence peroxidase expression. In particular, the high concentrations of carbon (10–20 g l^{-1} glucose) and nitrogen (ca. 50 mg l^{-1}) may repress expression. In saprotrophic white-rot basidiomycetes, the major requirement for expression of peroxidative activities is the switch from primary to secondary metabolism. In *Phanerochaete chrysosporium*, for example, the onset of idiophase is triggered mainly by nitrogen and/or carbon limitation (Keyser et al. 1978; Jeffries et al. 1981). Where direct assay of culture filtrates has been used to investigate peroxidase activities in ECM fungi and *H. ericae*, the 7 (Bending and Read 1997) and 21 (Burke and Cairney 1998) growth periods may not have been sufficient for depletion of nutrients in the media and triggering of peroxidase expression. In studies where agar-based assays have been used, although in at least one case (Bending and Read 1997) fungi were grown for 7 weeks prior to substrate addition, the presence of Fe would prevent positive identification of peroxidase activity.

We recommend, therefore, that future investigations of peroxidase activities be conducted following growth of ECM or ERM fungi in media containing Fe at low concentrations (we find 6–7 μM is suitable), coupled with sufficient carbon and nitrogen to allow for reasonable mycelial biomass yield. The homogeneous nature of the liquid and agar media routinely adopted for fungal culture may also influence expression of enzyme systems. As we have noted elsewhere, gradients of carbon and nutrient availability in a symbiotic mycorrhizal mycelium growing in soil may promote physiological heterogeneity within the mycelium (Cairney and Burke 1996b). Thus peroxidases and other cell wall-degrading enzymes may be expressed only in discrete regions of a mycelium wherein localised nitrogen depletion or cessation of carbon flow from the host might trigger expression. A possible means of generating such heterogeneity in axenic culture may be the use of solid-state fermentation media. Solid-state media are by nature heterogeneous and have been shown to enhance expression/extracellular protein production in a range of saprotrophic fungal taxa (Tuohy et al. 1989). We have successfully measured expression of a number of wall-degrading enzymes in *H. ericae* using a solid-state fermentation system (Burke and Cairney 1997). While our attempts to grow selected ECM fungi using the same system have so far been unsuccessful, it should be possible to develop a solid-state medium which will promote mycelial heterogeneity in ECM fungi.

Furthermore a combined molecular and biochemical approach has revealed that genes for peroxidative activities in *P. chrysosporium* may be expressed in the absence of the expected extracellular enzyme activities (Broda et al. 1995). Some peroxidative activities may thus have a periplasmic or cell-surface location and may only be released into growth media during hyphal senescence. Finally, in targeting ECM fungi which are potential producers of ligninolytic peroxidases, the systematic affinities of the fungi should be considered. There may be some merit in targeting taxa most closely related to known white rots. In this respect, the Stereales (*sensu* Hawksworth et al. 1995), which includes the ECM genera *Amphinema*, *Piloderma* and *Tylospora*, along with many white-rot saprotrophs, may be worthy of investigation.

References

Backa S, Gierer J, Reitberger T, Nilsson T (1992) Hydroxyl radical activity in brown-rot fungi studied by a new chemiluminescence method. Holzforschung 46:61–67

Bending GD, Read DJ (1996) Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. Soil Biol Biochem 28:1995–1602

Bending GD, Read DJ (1997) Lignin and soluble-phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycol Res 101:1348–1354

Broda P, Birch PRJ, Brooks PR, Sims PFG (1995) PCR-mediated analysis of lignocellulolytic gene transcription by *Phanerochaete chrysosporium* –substrate-dependent expression within gene families. Appl Environ Microbiol 61:2358–2364

Burke RM, Cairney JWG (1997) Carbohydrolase production by the ericoid mycorrhizal fungus *Hymenoscyphus ericae* under solid-state fermentation conditions. Mycol Res 101:1135–1139

Burke RM, Cairney JWG (1998) Carbohydrate oxidases in ericoid mycorrhizal fungi: a possible source of Fenton radicals during the degradation of lignocellulose. New Phytol (in press)

Cairney JWG, Burke RM (1994) Fungal enzymes degrading plant cell walls: their possible significance in the ectomycorrhizal symbiosis. Mycol Res 98:1345–1346

Cairney JWG, Burke RM (1996a) Plant cell wall-degrading enzymes in ericoid and ectomycorrhizal fungi. In: Azcon-Aguilar C, Barea JM (eds) Mycorrhizas in integrated systems. European Commission: Brussels, pp 218–221

Cairney JWG, Burke RM (1996b) Physiological heterogeneity within fungal mycelia: an important concept for a functional understanding of the ectomycorrhizal symbiosis. New Phytol 98:1345–1346

Cao W, Crawford DL (1993a) Carbon nutrition and hydrolytic and cellulolytic activities in the ectomycorrhizal fungus *Pisolithus tinctorius*. Can J Microbiol 39:529–535

Cao W, Crawford DL (1993b) Purification and some properties of β-glucosidase from the ectomycorrhizal fungus *Pisolithus tinctorius* strain SMF. Can J Microbiol 39:125–129

Durall DM, Todd AW, Trappe JM (1994) Decomposition of ^{14}C-labelled substrates by ectomycorrhizal fungi in association with Douglas fir. New Phytol 127:725–729

Fengel D, Wegener G (1983) Wood: chemistry, ultrastructure, reactions. de Gruyter, Berlin

Fenton HJH (1894) Oxidation of tartaric acid in the presence of iron. J Chem Soc 65:899–910

Gierer J, Yang E, Reitberger T (1992) The reactions of hydroxyl radicals with aromatic rings in lignins, studied with creosol and 4-methylveratrol. Holzforschung 46:495–504

Glenn JK, Gould MH (1985) Purification and characterisation of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Arch Biochem Biophys 242:329–341

Gramss G, Günther T, Fritsche W (1998) Spot tests for oxidative enzymes in ectomycorrhizal, wood-, and litter decaying fungi. Mycol Res 102:67–72

Griffiths RP, Caldwell BA (1992) Mycorrhizal mat communities in forest soil. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB, Wallingford, pp 98–105

Griffiths RP, Baham JE, Caldwell BA (1994) Soil solution chemistry of ectomycorrhizal mats in forest soil. Soil Biol Biochem 26:331–337

Haider K, Trojanowski J (1980) A comparison of the degradation of ^{14}C-labelled DHP and corn stalk lignins by micro- and macrofungi and by bacteria. In: Kirk TK, Higuchi T, Chang HM (eds) Lignin biodegradation: microbiology, chemistry and potential applications, vol 1. CRC, Boca Raton, FIa, pp. 111–134

Halliwell B, Gutteridge JMC (1985) Free radicals in biology and medicine. Clarendon, Oxford

Harkin JM, Obst JR (1973) Syringaldazine, an effective reagent for detecting laccase and peroxidase in fungi. Experientia 29:381–387

Haselwandter K, Bobleter O, Read DJ (1990) Degradation of ^{14}C-labelled lignin and dehydropolymer of coniferyl alcohol by ericoid and ectomycorrhizal fungi. Arch Microbiol 153:352–354

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

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) Ainsworth and Bisby's Dictionary of the Fungi. CAB, London

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

Jeffries TW, Choi S, Kirk TK (1981) Nutritional regulation of lignin degradation by *Phanerochaete chrysosporium*. Appl Environ Microbiol 42:290–296

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

Kirk TK, Connors WJ, Bleam RD, Hackett WF, Zeikus JG (1975) Preparation and microbial decomposition of synthetic (^{14}C) lignins. Proc Natl Acad Sci USA 72:2515–2519

Kuhad RC, Singh A, Eriksson K-EL (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. In: Eriksson K-EL (ed) Biotechnology in the pulp and paper industry. Springer, Berlin, pp 45–125

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic fungi. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Marx DH, Bryan WC (1975) Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. For Sci 21:245–254

Moser M (1958) Die künstliche Mykorrhizaimpfung an Forstpflanzen. Forst Centralblatt 77:32–40

Reganathan V, Gold MH (1986) Spectral characterization of the oxidised states of lignin peroxidase, an extracellular heme enzyme from the white-rot basidiomycete *Phanerochaete chrysosporium*. Biochemistry 25:1626–1631

Römheld V, Marschner H (1986) Mobilization of iron in the rhizosphere of different plant species. In: Tinker B, Läuchli A (eds) Advances in plant nutrition vol 2. Praeger, New York, pp 155–204

Schmidt CJ, Whitten BK, Nicholas DD (1981) A proposed role for oxalic acid in non-enzymatic wood decay by brown-rot fungi. Proc Am Wood-Preserv Assoc 77:157–164

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, London

Szaniszlo PJ, Powell PE, Reid CPP, Cline GR (1981) Production of hydroxamate siderophore iron chelators by ectomycorrhizal fungi. Mycologia 73:1158–1174

Trojanowski J, Haider K, Hütterman A (1984) Decomposition of ^{14}C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. Arch Microbiol 139:202–206

Tuohy MG, Buckley RJ, Griffin IC, Connelly IC, Shanley NA, Ximenes E, Filho F, Hughes P, Grogan P, Coughlan MP (1989) Enzyme production by solid-state cultures of aerobic fungi on lignocellulosic substrates. In: Coughlan MP (ed) Enzyme systems for lignocellulose degradation. Elsevier, London, pp 293–312

Wariishi H, Dunford HB, Macdonald ID, Gold MH (1989) Manganese peroxidase from the lignin-degrading basidiomycete *Phanerochaete chrysosporium*: transient state kinetics and reaction mechanism. J Biol Chem 264:3335–3340

Wood PM (1994) Pathways for production of Fenton's reagent by wood-rotting fungi. FEMS Microbiol Rev 13:313–320