Mycorrhiza (2002) 12:105–116
DOI 10.1007/s00572-002-0162-0

REVIEW ARTICLE

R. M. Burke · J. W. G. Cairney

Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi

Received: 23 August 2001 / Accepted: 12 February 2002 / Published online: 5 April 2002

Abstract Polyphenol oxidases are known to be produced by a range of ectomycorrhizal (ECM) and ericoid mycorrhizal fungi. These enzymes include laccase (EC 1.10.3.2), catechol oxidase (EC 1.10.3.1) and tyrosinase (EC 1.14.18.1), between which there exists considerable overlap in substrate affinities. In this review we consider the nature and function of these enzymes, along with the difficulties associated with assigning precise enzymatic descriptions. The evidence for production of laccase and other polyphenol oxidases by ECM and ericoid mycorrhizal fungi is critically assessed and their potential significance to the mycorrhizal symbioses discussed.

Keywords Laccase · Polyphenol oxidase · Catechol oxidase · Tyrosinase · Mycorrhizas

Introduction

Ectomycorrhizal (ECM) associations are thought to be important in tree nutrition and to represent a key component of mineral nutrient and carbon cycling processes in temperate, boreal and some tropical forest habitats (Smith and Read 1997). In addition to enhancing host access to inorganic forms of N and P, many ECM fungi are known to facilitate access to some organic forms of these elements in soil (Leake and Read 1997). Phylogenetic analyses of DNA sequences from extant fungal taxa suggest that the ECM condition evolved convergently from several ancestral lineages of saprotrophic fungi (Hibbett et al. 1997; Bruns et al. 1998; Kretzer and Bruns 1999). Although widely assumed to have diverged from their saprotrophic ancestors in their abilities to degrade dead plant cell wall polymers, it is increasingly clear that many ECM fungal taxa can partially degrade some plant wall components (reviewed in Cairney and Burke 1994; Leake and Read 1997). Such an ability may afford these fungi access to mineral nutrients either sequestered within dead plant cells or complexed with cell wall components such as lignins and their phenolic derivatives (Leake and Read 1997). The fact that evolutionary reversals from the ECM to saprotrophic condition also appear to have occurred in some basidiomycete taxa led Hibbett et al. (2000) to speculate that genes for hydrolytic and oxidative saprotrophic enzyme systems may be widespread in extant ECM fungi. Recent data support this hypothesis to some extent, and indicate that genes for lignin peroxidases and, to a lesser extent, manganese peroxidases (two key enzymes in lignin degradation by many white rot basidiomycetes) are present in a broad taxonomic range of extant ECM fungi (Chen et al. 2001). The extent to which the functional proteins encoded by these genes are expressed in the ECM fungi, however, remains unclear.

Partial mineralisation of lignin or dehydrogenative polymers of lignin monomers has been demonstrated for some ECM fungi growing in axenic culture (Trojanowski et al. 1984; Haselwandter et al. 1990). This has often been taken to indicate an ability to produce lignin peroxidase (EC 1.11.1.14) and manganese peroxidase (EC 1.11.1.13) activities (e.g. Griffiths and Caldwell 1992; Cairney and Burke 1994). We have argued elsewhere (Burke and Cairney 1998; Cairney and Burke 1998a) that this may not necessarily be the case; however, there is evidence that the ECM fungus *Tylospora fibrillosa* produces manganese peroxidase activity (Chambers et al. 1999). Furthermore, given the widespread occurrence of lignin peroxidase genes in ECM fungi (Chen et al. 2001), it seems likely that some taxa will also express these enzymes under some conditions.

In addition to lignin and manganese peroxidases, laccase is thought to contribute to lignin degradation by

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

J.W.G. Cairney (✉)
Mycorrhiza Research Group,
Centre for Horticulture and Plant Sciences, Parramatta Campus, University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW 1797, Australia
e-mail: j.cairney@uws.edu.au
Tel.: +61-2-96859903, Fax: +61-2-96859915

some white rot fungi (Hatakka 1994). Laccase, along with a range of related phenol-oxidising activities, has also been reported in several ECM fungi and the ericoid mycorrhizal fungal endophyte *Hymenoscyphus ericae*. These activities have been detected using a range of substrates, assay procedures and fungal tissues. In the following sections we review the nature of these enzymes, the evidence for their production by ECM and ericoid mycorrhizal fungi and consider their potential significance to the symbioses.

Laccases and other polyphenol oxidases

Characteristics of the enzymes

Laccase (systematic name: benzenediol:oxygen oxidoreductase; synonyms urishiol oxidase, *p*-diphenol oxidase. EC 1.10.3.2) is one of the blue copper proteins (sometimes called the blue copper oxidases) which catalyse the reduction of O_2 to H_2O using a range of phenolic compounds (though not tyrosine) as hydrogen donor (Thurston 1994). Unfortunately, laccase shares a number of hydrogen donors with monophenol monooxygenase (= tyrosinase) (systematic name: monophenol, L-DOPA (3, 4-dihydroxy-L-phenylalanine):oxygen oxidoreductase; synonyms phenolase, monophenol oxidase, cresolase. EC 1.14.18.1), making it difficult to assign unique descriptions to either enzyme. A further complication is the overlap in activity between monophenol monooxygenase and catechol oxidase (systematic name: 1,2-benzenediol:oxygen oxidoreductase; synonyms diphenol oxidase, *o*-diphenolase, phenolase, polyphenol oxidase, tyrosinase. EC 1.10.3.1).

Notwithstanding the broad range of substrates accepted by laccase as hydrogen donors, oxidation of syringaldazine [*N*, *N*′-bis(3, 5-dimethoxy-4-hydroxybenzylidene hydrazine], in combination with the inability to oxidise tyrosine (L-3-[4-hydroxyphenyl]alanine), has been taken to be an indicator of laccase activity (Harkin et al. 1974; Thurston 1994). The report of a "pluripotent" polyphenol oxidase from a marine bacterium (*Alteromonas* sp.) that is capable of oxidising syringaldazine along with monophenols and diphenols (Sanchez-Amat and Solano 1997), however, suggests that there may be exceptions to this definition of laccase activity. Unambiguous determination of laccase activity is best achieved by purification of the protein to electrophoretic homogeneity followed by determination of K_m or K_{cat} against multiple substrates. Ideally these should include substrates such as syringaldazine, ABTS (2, 2-azinobis-[3-ethylbenzothiazoline-6-sulphonic acid]) or catechol (1, 2 dihydroxybenzene), for which laccase has a high affinity, and some (e.g. tyrosine) for which laccase has little or no affinity (see e.g. Edens et al. 1999; Shin and Lee 2000). Where protein purification has not been undertaken, the use of selective inhibitors of tyrosinase activity [e.g. 4-hexylresorcinol or salycylhydroxamic acid (SHAM)] may facilitate estimation of laccase activity (Allen and Walker 1988; Dawley and Flurkey 1993).

In common with catechol oxidase and tyrosinase, laccase catalyses the four-electron reduction of O_2 to H_2O. In the case of laccase, at least, this is coupled to the single electron oxidation of the hydrogen-donating substrate (Reinhammar and Malmstrom 1981). Since four single-electron substrate oxidation steps are required for the four-electron reduction of water, the analogy of a four-electron "battery" has been proposed to explain this complex mechanism (Thurston 1994; Call and Mücke 1997). Though the exact catalytic mechanism has still to be elucidated, laccase is known to be highly oxidising [redox potential (E_o) 500–800 mV] (Xu 1996; Xu et al. 2000) and the presence of four cupric ions each co-ordinated to a single polypeptide chain is an absolute requirement for optimal activity (Ducros et al. 1998). In comparison to the E_o values for laccase (Xu 1996), there have been few measurements of the redox potentials of tyrosinase or catechol oxidase. However, Ghosh and Mukherjee (1998) estimated E_o of a tyrosinase model system to be 260 mV, considerably lower than that reported for laccase, suggesting that this class of enzyme is much less oxidising than laccase.

Regulation of laccase expression in white rot fungi

From what is known, regulation of laccase production in white rot fungi is complex and may vary in different taxa. In *Pycnoporus cinnabarinus*, for example, an increase in the C:N ratio increases laccase activity (Eggert et al. 1996b). In *Phanerochaete chrysosporium*, readily available C in the form of glucose represses laccase production irrespective of the amount of N present; however, in the presence of the more recalcitrant microcrystalline cellulose, increasing N availability increases laccase activity (Srinivasan et al. 1995). In *Trametes versicolor*, increasing availability of Cu or N resulted in increased levels of laccase mRNA transcripts and laccase activity (Collins and Dobson 1997). A variety of aromatic compounds have further been shown to induce laccase production in a variety of white rot basidiomycetes (Collins and Dobson 1997; Koroljova-Skorobogat'ko et al. 1998).

Intracellular laccase activities?

Although most reports of laccase activities in white rot fungi relate to extracellular enzymes, several suggest the existence of intracellular laccases in, for example, the white rot basidiomycete *T. versicolor* and the chestnut blight fungus *Cryphonectria parasitica* (e.g. Roy-Arcand and Archibald 1991; Rigling and Van Alfen 1993; Schlosser et al. 1997). Such intracellular laccases have been separated from their extracellular counterparts on the basis of pH optimum, isoelectric point and/or molecular mass, but all appear to have similar substrate ranges. Whether the intracellular activities represent actual intracellular laccases, extracellular laccases in the process of being exported, or a combination of the two remains to

be determined (Schlosser et al. 1997). The functional significance (if any) of the intracellular activities thus remains unclear. However, a number of potential functions have been proposed for extracellular laccases.

Functions proposed for laccases in non-mycorrhizal fungi

Laccases in lignin degradation

Given the oxidising characteristics (high redox potential) of laccase, and the fact that it is produced during lignin degradation by some white rot fungi, it has been suggested to contribute to lignin degradation by oxidising free phenolic groups to phenoxy cation radicals (e.g. Hatakka 1994; Youn et al. 1995). Laccase has also been shown to degrade non-phenolic lignin model compounds in systems incorporating naturally occurring or synthetic redox mediators (e.g. Call and Mücke 1997; Johannes and Majcherczyk 2000; Srebotnik and Hammel 2000). Redox mediators are oxidisable, low molecular weight, laccase substrates. These substrates yield radicals that are capable of diffusing away from the reactive site and acting as oxidants for other compounds. Importantly, some mediators can oxidise compounds such as lignin, which would not be oxidisable by laccase alone (Johannes and Majcherczyk 2000). *P. cinnabarinus*, which produces no other lignin-degrading enzymes but completely degrades lignin, produces a metabolite (3-hydroxyanthranilate) that acts as a redox mediator for the depolymerisation of non-phenolic lignin by laccase (Eggert et al. 1996a, 1997). For most fungi, however, the precise role(s) of laccase in ligninolysis remain poorly understood (Thurston 1994). In contrast to laccase, the low redox potential of tyrosinase (Ghosh and Mukherjee 1998) suggests that this enzyme does not participate in degradative processes.

Laccases in growth and development

Laccase activity has been reported to be associated with normal mycelial growth in a range of fungi (Das et al. 1997), and in the development of rhizomorphs and sclerotia in certain basidiomycete and ascomycete taxa (Worrell et al. 1986; Rehman and Thurston 1992; Willetts and Bullock 1992; Griffith et al. 1994). It has also been detected in association with a change from coenocytic to septate mycelial growth of *Phlebia* spp. (White and Boddy 1992a), and in zones of mycelial contact between competing basidiomycetes (White and Boddy 1992b; Iakovlev and Stenlid 2000). Several workers have noted associations between laccase activity and basidiome production. In *Schizophyllum commune*, basidiome-forming dikaryons, but not non-fruiting monokaryons, produce abundant extracellular laccase activity (Leonard 1971; De Vries et al. 1986), while in *Lentinula edodes* the enzyme has been implicated in both basidiome initiation and further development (Leatham and Stahmann 1981; Zhao and Kwan 1999). In some saprotrophic basidiomycetes, expression of laccase genes and/or accumulation of laccase occurs during vegetative growth, but both expression and activity decline following basidiome initiation (e.g. Wood 1980a, b; Ross 1982; Bonnen et al. 1994; Ohga et al. 1999). While indicative of putative relationships between laccase activity and basidiome production, these observations provide no confirmation of a direct developmental role for laccase. The respective conditions required for maximal laccase synthesis and for basidiome production in *S. commune* are, for example, quite different (De Vries et al. 1986). In *Agaricus bisporus*, oscillations in laccase activity may relate to cycles of ligninolytic activity, and basidiome production may simply be coincident with these (Bonnen et al. 1994; Thurston 1994). Indeed, in *Pleurotus sajor-caju*, similar oscillations in laccase activity occur in the absence of basidiome initiation (Tan and Wahab 1997).

Relationships between laccase activities and fruiting have been investigated in more detail in ascomycetes and there is evidence of regulation both spatially and temporally at the tissue level during differentiation of conidia and ascomata in *Aspergillus* and *Sordaria* spp. (Clutterbuck 1972; Hermann et al. 1983; Broxholme et al. 1991). In *Aspergillus nidulans*, different laccase isozymes are expressed during asexual and sexual reproductive phases. Laccase I is expressed during asexual development and is required for formation of a green pigment from a yellow polyketide-derived precursor (Aramayo and Timberlake 1993), while laccase II is specifically expressed during cleistothecial development and may be involved in hyphal aggregation or pigment conversion (Scherer and Fischer 1998).

With the exception of the *A. nidulans* system, the roles played by laccases in fungal growth and differentiation are largely unclear. Laccase has, however, been variously implicated in chemical cross-linking of hyphae via oxidative polymerisation of phenols with hyphal surface carbohydrates and proteins in rhizomorphs, sclerotia and fruiting bodies (e.g. Bu'Lock 1967; Leatham et al. 1980; Broxholme et al. 1991; Zhao and Kwan 1999). In addition, laccases have been suggested to be involved in melanin production (Iakovlev and Stenlid 2000), detoxification of toxic phenolics (e.g. Haars et al. 1981) and/or promotion of hyphal insulation via polymerisation of secondary metabolites and proteins (Griffith et al. 1994). While such functional roles remain the subject of discussion, it is increasingly clear that many fungi produce multiple laccase isozymes, encoded by differentially expressed genes, that may fulfil different functions (e.g. Wahleithner et al. 1996; Yaver et al. 1996; Mansur et al. 1998; Scherer and Fischer 1998; Zhao and Kwan 1999; Chakraborty et al. 2000; Shin and Lee 2000).

Laccases in pathogen-host interactions

Laccase is thought to be important in pathogenicity of the human pathogen *Cryptococcus neoformans*

(Williamson 1994). Its role in pathogenicity was regarded as oxidation of aromatic substrates to products that subsequently polymerise to form melanin, a compound thought to protect the pathogen against host oxidative immune responses or to bind microbicidal proteins (Williamson 1994; Wang et al. 1995; Salas et al. 1996; Doering et al. 1999). Recent observations, however, cast some doubt on the postenzymatic polymerisation of melanin from oxidation products produced by laccase (Liu et al. 1999b). Rather, laccase may protect *C. neoformans* from hydroxyl radical-mediated attack by macrophages via oxidation of phagosomal iron to Fe^{3+} and a concomitant reduction in hydroxyl radical formation (Liu et al. 1999a). Laccases have further been implicated in pathogenicity of a range of plant pathogenic fungi that includes the soft rot fungus *Botrytis cinerea*, the chestnut blight fungus *Cryphonectria parasitica*, the Dutch elm disease fungus *Ophiostoma novo-ulmi* and the take-all pathogen *Gaeumannomyces graminis* (Bar-Nun et al. 1988; Rigling and Van Alfen 1993; Binz and Canevascini 1996; Edens et al. 1999). The roles of laccases in plant:pathogen interactions remain poorly understood, but a role in melanin synthesis has, again, been proposed in some plant pathogenic fungi (Edens et al. 1999). Other proposed roles of plant pathogen laccases include detoxification of phytoalexins and other plant phenolics (Rigling and Van Alfen 1993; Anderson and Nicholson 1996; Binz and Canevascini 1996; Breuil et al. 1999) and protection against host oxidative responses (Edens et al. 1999). In *Armillaria mellea*, oxidation of Fe^{2+} by laccase within host tissue may enhance fungal competition for the element (Curir et al. 1997), while a potential role for laccase in lignin degradation has also been proposed for *G. graminis* during penetration of host defence-related lignotubers (Edens et al. 1999).

Evidence for laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi

Phenol oxidising activities, including laccase, have been reported for a number of ECM fungi and for the ericoid mycorrhizal fungus *H. ericae* (Table 1). These activities have been detected using axenic mycelia, non-sterile mycelia, sporocarp tissue or ECM root tips, and encompass unspecified polyphenol oxidases, along with apparent catechol oxidase, tyrosinase and laccase activities. In most cases, the identity of laccase or other phenol oxidising enzymes has been assigned on the basis of their activities against single hydrogen donating substrates.

That extracellular oxidation of polyphenolic compounds occurs in axenic cultures of some ECM fungi and *H. ericae* has been demonstrated repeatedly via observation of the "Bavendamm reaction" during growth in agar plates (Lindeberg 1948; Giltrap 1982; Griffiths and Caldwell 1992; Bending and Read 1997; Gramms et al. 1998). This method involves incorporation of polyphenolic substrates, usually gallic or tannic acids, into the agar, with darkening of the substrate indicating that oxidation has occurred. Oxidation of these substrates can, however, be mediated by a number of phenol-oxidising enzymes, including catechol oxidase, laccase and tyrosinase (Gramms et al. 1998) and, as such, provides no indication as to the enzyme activities involved.

Günther et al. (1998) investigated the disappearance of a range of phenols and phenolic acids from solutions in which axenically-grown mycelia of *Suillus granulatus* or *Paxillus involutus* were suspended. The presence of either fungus resulted in significant reductions in the concentrations of the substrates, particularly for compounds that bore two hydroxyl groups in the *ortho*-position (catechol and 3, 4-dihydroxybenzoic acid) and *para*-hydroxylated compounds (vanillic and ferulic acids). These observations are consistent with activity of either tyrosinase or catechol oxidase (rather than laccase), for which considerable overlap in substrate range exists.

Activities associated with non-sterile mycelia and basidiome material

Several studies have investigated activities associated with extramatrical mycelia of ECM fungi in non-sterile field soil or partly humified litter (Bending and Read 1995; Colpaert and Van Laere 1996; Gramms 1997; Timonen and Sen 1998). While providing ecologically relevant information on enzymatic activities in the mycorrhizosphere, most of these studies were of enzyme activities in extracts of ECM mycelia and the surrounding soil matrix (Bending and Read 1995; Colpaert and Van Laere 1996; Gramms 1997). For this reason, they do not necessarily demonstrate involvement of ECM fungi, as opposed to other components of the soil microflora, in producing the activities reported. Culture fluids from axenic ECM *Pinus sylvestris-S. granulatus* seedlings, however, showed ABTS-oxidising activity (Günther et al. 1998). In isolation, oxidation of ABTS, while indicative of polyphenol oxidase activity is not diagnostic of a particular enzyme. However, in combination with the observed disappearance of *p*-cresol (4-methylphenol) from the culture fluid, Günther et al. (1998) were able to conclude that the activity was probably tyrosinase-like.

Timonen and Sen (1998) adopted a slightly different approach, removing fragments of ECM fungal mycelium from several regions of a microcosm system and assaying for associated enzyme activities. Oxidation of ABTS was observed in the fungal components of the system, indicating polyphenol oxidase activity. Differences were found in L-DOPA-oxidising activities in different parts of extramatrical mycelia within the microcosm. This was taken by the authors to indicate spatial variation in catechol oxidase activity; however, laccase and tyrosinase can also oxidise this substrate (Allen and Walker 1988; Chefetz et al. 1998). No oxidation of guiacol (2-methoxyphenol) or syringaldazine was observed, allowing Timonen and Sen (1998) to conclude that ABTS-oxidation was not due to laccase activity. Similar activities were also found to be associated with both axenic and

Table 1 Phenol oxidising activities reported for ectomycorrhizal (ECM) fungi and the ericoid mycorrhizal endophyte *Hymenoscyphus ericae*. *ABTS* 2, 2-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); *Bavendamm* gallic and/or tannic acid; *catechol* 1,2-dihydroxybenzene; *p-cresol* 4-methylphenol; *L-DOPA* 3,4-dihydroxy-L-phenylalanine; *DMP* 2,6-dimethoxyphenol; *DPD N,N*-dimethyl-*p*-phenylenediamine; *guaiacol* 2-methoxyphenol; *gum guaiac* a natural mixture of ca. 70% α and β guaiaconic acids, 11% guaicic and guaiaretic acids, 15% vanillin, 4% guaiacin; *pyrogallol* 1,2,3-trihydroxybenzene; *SHAM* salicylhydroxamic acid; *syringaldazine* 4-hydroxy-3,5-dimethoxybenzaldehyde azine; *TMPD=N, N, N′, N′*-tetramethyl-*p*-phenylenediamine; *L-tyrosine* L-3-(4-hydroxyphenyl)alanine

Activity	Tissue	Assay	Substrate	Fungi	+/–	Reference
Polyphenol oxidase	Sterile cultured mycelium	Agar plate assay	Bavendamm	ECM fungi	+/–	Lindeberg (1948)
Polyphenol oxidase	Sterile cultured mycelium	Agar plate assay	Bavendamm	ECM fungi	+/–	Giltrap (1982)
Polyphenol oxidase	Sterile cultured mycelium	Agar plate assay	Bavendamm	ECM fungi + *Hymenoscyphus ericae*	+/–	Bending and Read (1997)
Polyphenol oxidase	Sterile cultured mycelium	Agar plate assay	Bavendamm	ECM fungi	+/–	Gramms et al. (1998)
Polyphenol oxidase	Sterile cultured mycelium	Agar plate assay	Bavendamm	ECM fungi	+/–	Griffiths and Caldwell (1992)
Polyphenol oxidase	Sterile cultured mycelium	Spot test in 95% ethanol	Gum guaiac	ECM fungi	+/–	Gramms et al. (1998)
Polyphenol oxidase	Sterile cultured mycelium	Spot test in 95% ethanol	Gum guaiac	*Sphaerosporella brunnea*	+	Egger (1986)
Polyphenol oxidase	Sterile cultured mycelium	Spot test in H_20	Pyrogallol	ECM fungi + *H.* ericae	–	Bending and Read (1997)
Polyphenol oxidase	Sterile cultured mycelium	Crude culture filtrate	Catechol	ECM fungi + *H. ericae*	+/–	Bending and Read (1996a, b)
Polyphenol oxidase	Sterile cultured mycelium	Crude culture filtrate	Catechol	*H. ericae*	+	Bending and Read (1997)
Polyphenol oxidase	Sterile cultured mycelium	Extract from macerated tissue	ABTS	*Suillus bovinus*	–	Timonen and Sen (1998)
Polyphenol oxidase	Sterile cultured mycelium	Extract from macerated tissue	L-DOPA	*Pisolithus tinctorius*	+	Sword and Garrett (1994)
Polyphenol oxidase	Sterile cultured mycelium	Extract from macerated tissue	L-DOPA	ECM fungi	+	Ramstedt and Söderhäll (1983)
Polyphenol oxidase	Sterile cultured mycelium	Extract from macerated tissue	L-DOPA	*Pisolithus tinctorius*	+	Sword and Garrett (1994)
Polyphenol oxidase	Sterile ECM root tips	Extract from macerated tissue	ABTS	ECM roots	+/–	Günther et al. (1998)
Polyphenol oxidase	Intact sterile ECM system	Crude culture solution	ABTS	ECM fungi	+/–	Günther et al. (1998)
Polyphenol oxidase	Sporocarps	Tissue explants	Gum guaiac	ECM fungi	+/–	Agerer et al. (2000)
Polyphenol oxidase	Non-sterile ECM mycelium	Crude extract from colonised soil	Catechol	*Paxillus involutus*	+	Bending and Read (1995)
Polyphenol oxidase	Non-sterile ECM mycelium	Crude extract from colonised soil	L-DOPA	ECM fungi	+/–	Colpaert and Van Laere (1996)
Catechol oxidase (1.10.3.1)	Sterile cultured mycelium	Extract from macerated tissue	L-DOPA	*Suillus bovinus*	–	Timonen and Sen (1998)
Catechol oxidase (1.10.3.1)	Non-sterile ECM mycelium	Extract from macerated tissue	L-DOPA	ECM fungi	+	Timonen and Sen (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Spot test in 95% ethanol	α-Naphthol	*Hebeloma* spp	+	Soponsathien (1998)
Laccase	Sterile cultured mycelium	Spot test in 95% ethanol	α-Naphthol	*Sphaerosporella brunnea*	+	Egger (1986)
Laccase (1.10.3.2)	Sterile cultured mycelium	Spot test in 95% ethanol	1-Naphthol	ECM fungi	+/–	Hutchison (1990)
Laccase (1.10.3.2)	Sterile cultured mycelium	Spot test in 95% ethanol	1-Naphthol	ECM fungi	+/–	Gramms et al. (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Spot test in 95% ethanol	Guaiacol	*Hebeloma* spp	+/–	Soponsathien (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Spot test in 95% ethanol	Syringaldazine	*Hebeloma* spp	+/–	Soponsathien (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Crude culture filtrate	ABTS	ECM fungi	–	Peláez et al. (1995)

Table 1 (continued)

Activity	Tissue	Assay	Substrate	Fungi	+/–	Reference
Laccase (1.10.3.2)	Sterile cultured mycelium	Crude culture filtrate	ABTS	ECM fungi	+/–	Günther et al. (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Crude culture filtrate	Syringaldazine	*H. ericae*	–	Bending and Read (1997)
Laccase (1.10.3.2)	Sterile cultured mycelium	Extract from macerated tissue	ABTS	ECM fungi	+	Günther et al. (1998)
Laccase (1.10.3.1)	Sterile cultured mycelium	Extract from macerated tissue	ABTS	*Laccaria amethystea*	+	Münzenberger et al. (1997)
Laccase (1.10.3.2)	Sterile cultured mycelium	Purified enzyme extract	ABTS	*Thelephora terrestris*	+	Kanunfre and Zancan (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Purified enzyme extract	Guaiacol	*T. terrestris*	+	Kanunfre and Zancan (1998)
Laccase (1.10.3.2)	Sterile cultured mycelium	Purified enzyme extract	Syringaldazine	*T. terrestris*	+	Kanunfre and Zancan (1998)
Laccase (1.10.3.1)	Sterile ECM root tips	Extract from macerated tissue	ABTS	ECM roots	+	Münzenberger et al. (1997)
Laccase (1.10.3.2)	Sporocarps	Tissue explants	Syringaldazine	ECM fungi	+/–	Marr et al. (1986)
Laccase (1.10.3.2)	Sporocarps	Extract from macerated tissue	DPD	*Tuber* spp	–	Miranda et al. (1996)
Laccase (1.10.3.2)	Non-sterile ECM mycelium	Extract from macerated tissue	Guaiacol	ECM fungi	–	Timonen and Sen (1998)
Laccase (1.10.3.2)	Non-sterile ECM mycelium	Extract from macerated tissue	Syringaldazine	ECM fungi	–	Timonen and Sen (1998)
Laccase (1.10.3.2)	Non-sterile m ECM myceliu	Crude extract from colonised soil	ABTS	*Hebeloma crustuliniforme*	+	Gramms (1997)
Laccase (1.10.3.2)	Non-sterile ECM mycelium	Crude extract from colonised soil	DMP	*H. crustuliniforme*	+	Gramms (1997)
Laccase (1.10.3.2)	Non-sterile ECM mycelium	Crude extract from colonised soil	Guaiacol	*H. crustuliniforme*	+	Gramms (1997)
Laccase (1.10.3.2)	Non-sterile m ECM myceliu	Crude extract from colonised soil	TMPD	*H. crustuliniforme*	+	Gramms (1997)
Laccase (1.10.3.2)	Non-sterile ECM mycelium	Crude extract from colonised soil	Pyrogallol	*H. crustuliniforme*	–	Gramms (1997)
Laccase (1.10.3.2)	Non-sterile ECM mycelium	Crude extract from colonised soil	Syringaldazine	*H. crustuliniforme*	–	Gramms (1997)
Tyrosinase	Sterile cultured mycelium	Spot test in 95% ethanol	*p*-Cresol	*Sphaerosporella brunnea*	+	Egger (1986)
Tyrosinase	Sterile cultured mycelium	Spot test in 95% ethanol	*p*-Cresol	ECM fungi	+/–	Hutchison (1990)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Spot test in 95% ethanol	*p*-Cresol	ECM fungi	+/–	Gramms et al. (1998)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Spot test in H_2O	*p*-Cresol	ECM fungi	+/–	Gruhn and Miller (1991)
Tyrosinase (1.10.3.2 or 1.14.18.1)	Sterile cultured mycelium	Spot test in 95% ethanol	*p*-Cresol	Hebeloma spp	+/–	Soponsathien (1998)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Incubated intact axenic mycelia	*p*-Cresol	ECM fungi	+/–	Günther et al. (1998)
Tyrosinase (1.10.3.1)	Sterile cultured mycelium	Crude culture filtrate	Catechol (– syringaldazine)	*H. ericae*	+	Bending and Read (1997)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Crude culture filtrate	L-DOPA (+ sham)	ECM fungi	–	Günther et al. (1998)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Crude culture filtrate	*p*-Cresol	ECM fungi	+/–	Günther et al. (1998)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Extract from macerated tissue	L-DOPA	ECM fungi	+/–	Gruhn and Miller (1991)
Tyrosinase (1.14.18.1)	Sterile cultured mycelium	Extract from macerated tissue	L-DOPA (+ sham)	ECM fungi	+/–	Günther et al. (1998)
Tyrosinase (1.14.18.1)	Intact sterile ECM system	Crude culture solution	*p*-Cresol	ECM fungi	+/–	Günther et al. (1998)

Table 1 (continued)

Activity	Tissue	Assay	Substrate	Fungi	+/–	Reference
Tyrosinase (1.14.18.1)	Sporocarps	Tissue explants	*p*-Cresol	ECM fungi	+/–	Marr et al. (1986)
Tyrosinase (1.14.18.1)	Sporocarps	Tissue explants	L-Tyrosine	ECM fungi	+/–	Marr et al. (1986)
Tyrosinase (1.14.18.1)	Sporocarps	Extract from macerated tissue	L-Tyrosine	Tuber spp	+	Miranda et al. (1996)
Tyrosinase (1.14.18.1)	Non-sterile ECM mycelium	Crude extract from colonised soil	Chlorogenic acid	*H. crustuliniforme*	+	Gramms (1997)
Tyrosinase (1.14.18.1)	Non-sterile ECM mycelium	Crude extract from colonised soil	L-DOPA	*H. crustuliniforme*	+	Gramms (1997)

non-sterile mycelia of *P. involutus*, suggesting that this activity was probably of fungal origin. The assay used by Timonen and Sen (1998), however, involved maceration of fungal tissue prior to testing for substrate oxidation, making it impossible to determine if the measured activities were intracellular or cell-surface-bound. Thus, although a convincing demonstration that physiological heterogeneity exists within ECM mycelia in soil, the functional relevance of the measured activities in terms of interactions between the fungi and soil organic matter is difficult to assess. Similar arguments apply to the ABTS-oxidising activity (suggested by the authors to be laccase, but see above) measured in association with macerated non-sterile ECM root tips by Münzenberger et al. (1997).

Phenol-oxidising activities have also been detected in sporocarp tissue of a large number of ECM fungal taxa (Table 1). These have been detected using complex natural substrates, such as gum guaiac (a general indicator of polyphenol oxidase activity) (Agerer et al. 2000) and more specific indicators of catechol oxidase and/or tyrosinase activities (Marr et al. 1986; Miranda et al. 1996). Syringaldazine-oxidising activity (probably indicating laccase) has also been observed in sporocarp tissue of a range of ECM fungal taxa (Marr et al. 1986). These assays were conducted by incubating sporocarp explants, from which intracellular enzymes can readily leak, with substrates (Marr et al. 1986; Agerer et al. 2000) or using macerated tissue preparations (Miranda et al. 1996), making it impossible to determine the extent to which the activities were intra- or extra-cellular. Similarly, some assays have been carried out on extracts of macerated mycelia and ECM root tips grown in axenic culture (Table 1). Four of these studies (Ramstedt and Söderhäll 1983; Gruhn and Miller 1991; Sword and Garrett 1994; Münzenberger et al. 1997), used only L-DOPA or ABTS as substrate, neither of which discriminates laccase from other phenol-oxidising activities. By including the tyrosinase inhibitor SHAM, Günther et al. (1998) were able to conclude that L-DOPA- and ABTS-oxidising activities in mycelial extracts from *P. involutus* and *S. granulatus* were attributable to laccase, and that tyrosinase/catechol oxidase activities were also present. In contrast, lack of activity against syringaldazine, coupled with oxidation of L-DOPA, indicated no laccase activity in extracts from *Suillus bovinus* mycelium (Timonen and Sen 1998).

Activities associated with sterile mycelia in axenic culture

In other studies, assays were conducted as "spot tests" whereby drops of substrate were applied to the surface of intact mycelia, or to wells cut in mycelia, on agar plates in ca. 95% ethanol. Use of *p*-cresol in this way has revealed the presence of tyrosinase/catechol oxidase activities in a broad taxonomic range of ECM fungi (Egger 1986; Hutchison 1990; Gramms et al. 1998; Soponsathien 1998). Similarly, 1-naphthol has been used as a presumptive indicator of laccase activity in a range of taxa; however, the specificity of this substrate for laccase does not appear to have been established unequivocally (Gramms et al. 1998). As highlighted by Gramms et al. (1998), ethanol treatment is likely to compromise the integrity of hyphal membranes, again blurring the distinction between intracellular and extracellular activities observed in "spot tests".

Gruhn and Miller (1991) observed oxidation of aqueous solutions of *p*-cresol applied to the uncut surface of *Boletinellus meruloides*, *Pisolithus tinctorius* and *Suillus pictus*, indicating the presence of an extracellular, or at least a cell-surface-bound, tyrosinase/catechol oxidase activity. Crude culture filtrates from a number of taxa have also been assayed in attempts to identify extracellular enzyme activities. Peláez et al. (1995) found no evidence of ABTS-oxidising activity in filtrates from cultures of a number of ECM fungi. As indicated by the authors this implies a lack of extracellular laccase activity. Given the lack of specificity of ABTS as a laccase indicator, however, their data further suggest a lack of any extracellular polyphenol oxidase activity under the conditions adopted.

Although Bending and Read (1996a, b, 1997) assessed oxidation of catechol in culture filtrates of a range of fungi, significant activity (indicative of polyphenol oxidase) was demonstrated for only *Lactarius controver-*

sus and the ericoid mycorrhizal fungus *H. ericae*. When similar culture filtrates of *H. ericae* were tested for their ability to oxidise syringaldazine (a presumptive laccase indicator) no activity was detected (Bending and Read 1997). The observed polyphenol oxidase activity thus seems likely to be of the catechol oxidase (possibly tyrosinase) type. Günther et al. (1998) observed oxidation of both L-DOPA and ABTS in culture filtrates of *S. granulatus*. Oxidation of L-DOPA by *S. granulatus* filtrate was inhibited by the tyrosinase inhibitor SHAM, suggesting that this activity was largely tyrosinase, rather than a laccase. The ABTS-oxidising activity was largely unaffected by SHAM, suggesting the presence of laccase activity. Interestingly, neither activity was detected in culture filtrates of *P. involutus*.

The most convincing demonstration of extracellular laccase production by an ECM fungus is that of Kanunfre and Zancan (1998). These authors detected a 61 kDa protein (similar in size to other purified laccases) in concentrated, dialysed culture filtrates of *Thelephora terrestris* that oxidised syringaldazine, ABTS and guaiacol. Determination of K_m values against these substrates revealed that the enzyme had highest affinity for syringaldazine, consistent with the description of the protein as a laccase. Indeed, the K_m value for this enzyme was of a similar order of magnitude to those reported for other purified fungal laccases (Chefetz et al. 1998; Shin and Lee 2000).

Discussion

From the literature reviewed herein, it is clear that considerable polyphenol oxidase activities are associated with mycelia of many ECM fungi and the ericoid mycorrhizal endophyte *H. ericae* under some circumstances. It is further evident that the overlapping substrate ranges of polyphenol oxidases means that ascribing a unique enzymatic description to a given activity is often problematic. Differences exist between laccases and other polyphenol oxidases in their oxidative potential, and so their relative abilities to participate in degradative processes. For this reason, if the potential ecological role of an enzyme is to be extrapolated from an enzyme assay, it is important to adopt methods that discriminate between laccase and other polyphenol oxidases.

Discrimination between laccase and other polyphenol oxidases requires determination of relative K_m values against a range of substrates including at least one for which laccase has high affinity (e.g. syringaldazine) and another for which affinity is low or non-existent (e.g. tyrosine). In the context of mycorrhizal fungi, such an approach allowed Kanunfre and Zancan (1998) to demonstrate extracellular laccase activity in *T. terrestris*. Miranda et al. (1996) used a similar approach to identify the main polyphenol oxidase activity in extracts from *Tuber* spp. sporocarps as tyrosinase/catechol oxidase rather than laccase. Likewise, Bending and Read (1997) used relative affinities for catechol and syringaldazine to demonstrate tyrosinase/catechol oxidase, but not laccase, in culture filtrates of *H. ericae*.

It is worth noting that determination of K_m yields two important characteristics for a given enzyme. First, comparison of relative K_m values against a range of substrates reveals the substrate for which the enzyme has greatest affinity. Secondly, calculation of K_{cat} (V_{max}/K_m, U s^{-1}) or K_{cat}/K_m (M^{-1} s^{-1}), permits comparison of the catalytic efficiency of several enzymes with the same substrate affinity. This may be useful in predicting the likely relative contributions to polymer breakdown of, for example, laccases produced by mycorrhizal and other fungi that might be competing for the same substrates in soil.

Several authors have utilised various substrate combinations, but without kinetic data, to infer the most probable identity of polyphenol oxidase activities in crude enzyme preparations from ECM or ericoid mycorrhizal fungi. Using a range of substrates in combination with a selective inhibitor of tyrosinase/catechol oxidase activity, Günther et al. (1998) concluded that the predominant phenol-oxidising activities in culture filtrates of *S. granulatus* and in mycelial extracts of *S. granulatus* and *P. involutus* were most probably laccases. Although syringaldazine oxidation data were not presented in all cases, no oxidation of this substrate was observed for the *P. involutus* mycelial extract. Given the widespread acceptance of high affinity for syringaldazine as a key indicator of laccase activity (Thurston 1994), unequivocal identification of these activities as laccases requires further characterisation. Indeed, Bending and Read (1997) and Timonen and Sen (1998) interpreted oxidation of catechol or L-DOPA in the absence of activity against syringaldazine as indicating an absence of laccase activity in culture filtrate of *H. ericae* and mycelial extracts of *S. bovinus*, respectively.

Many studies have relied upon qualitative colorimetric "spot tests" using multiple substrates to differentiate between laccase and tyrosinase/catechol oxidase activities in ECM fungi (Marr et al. 1986; Hutchison 1990; Saponsathien 1998). While these methods may be useful as preliminary indicators of laccase activity, they do not permit comparison of relative kinetic parameters and are of limited value in unambiguously separating laccases from other polyphenol oxidases. Furthermore, these spot tests involve application of the chromogenic substrates in 95% ethanol and cannot discriminate between intra- and extra-cellular enzyme activities. There is no question that data of this nature are of value as diagnostic characteristics in fungal taxonomy. However, extrapolation from the presence of an activity to a potential ecological role is probably inappropriate.

Laccase production in white rot fungi is regulated by a range of factors including the relative availability of C and N and/or concentration of Cu. Clearly, the molecular structure of laccase requires that Cu is available for incorporation at the active site. Of the investigations of laccase activity in ECM and ericoid mycorrhizal fungi we have cited, Cu was specifically added to the growth

medium in only two (Münzenberger et al. 1997; Kanunfre and Zancan 1998). However, in the remaining cases it is likely that other media components contained sufficient trace amounts of Cu for its concentration not to be limiting. In each case, the medium used was sufficient in available C and N, a factor known to repress laccase production in at least some white rot fungi. Manipulation of culture media by reducing the concentration of C and/or N, or their inclusion in more recalcitrant forms, may result in conditions more favourable for laccase production. Because expression may be influenced by culture conditions, screening for genes that encode laccases and other polyphenol oxidases may represent a more reliable means of identifying potential enzymatic activities in ECM fungi. Furthermore, many ECM taxa that are important components of below-ground communities are difficult to culture, rendering biochemical assays of laccase activities impossible. Such approaches have been used successfully in the context of other lignin-degrading enzymes in white rot and ECM fungi (Chambers et al. 1999; Varela et al. 2000; Chen et al. 2001). DNA sequences for laccase genes from a number of fungi are now available (e.g. D'Souza et al. 1996; Yaver et al. 1996; Collins and Dobson 1997; Zhao and Kwan 1999) and offer the opportunity to design molecular probes or primers for identification of similar genes and/or laccase mRNA transcripts in mycorrhizal fungi.

It has been suggested that laccases produced by ECM and ericoid mycorrhizal fungi may be involved in a number of processes that relate to functioning of the symbioses. Broadly speaking, these encompass depolymerisation and polymerisation reactions. Suggested roles in depolymerisation reactions include lignin and polyphenol degradation, release of N from insoluble protein-tannin complexes and degradation of polycyclic aromatic hydrocarbon pollutants (Leake and Read 1989; Hutchison 1990; Bending and Read 1996a, b, 1997; Braun-Lüllemann et al. 1999). Durall et al. (1994) found little evidence of degradation of ^{14}C-labelled mixed humic polymers by four ECM fungi, suggesting that their abilities to depolymerise these substrates were low. Unless laccase and a suitable redox mediator are present together, such depolymerisation reactions are unlikely to occur. Based on information available for white rot fungi, candidates for such mediators would include simple aromatic compounds and amino acids containing sulphydryl groups (Johannes and Majcherczyk 2000). For example, 4-hydroxybenzoic acid and 4-hydroxybenzyl alcohol are efficient mediators of laccase activity, and are produced either as plant secondary metabolites by the action of actinomycetes on lignin or, as is the case for white rot fungi, by the fungi themselves (Shunming et al. 1985; Pometto and Crawford 1986; Johannes and Majcherczyk 2000). However, no studies of redox mediator production by mycorrhizal fungi, or the potential for compounds of plant or microbial origin to act as mediators of mycorrhizal laccases have yet been undertaken. In the absence of this information, it is difficult to predict the potential contribution of laccases to depolymerisation processes in soil.

Proposed roles in polymerisation of phenolic compounds include pigment production, neutralisation of host defence compounds, humus formation and detoxification of the soil environment (e.g. Hutchison 1990; Sword and Garrett 1994; Bending and Read 1996a, b, 1997; Günther et al. 1998; Kanunfre and Zancan 1998). None of these processes requires the presence of a redox mediator.

When laccase acts on a phenol, a reactive semiquinone is produced that, depending upon the degree to which it becomes autoxidised, can dismutate to yield quinones which, in turn, polymerise to form insoluble complexes (see Guillén et al. 2000). Alternatively, the reactive semiquinone may react with O_2 to yield a superoxide radical ($O_2^{-}\bullet$) that may contribute to depolymerising processes (Guillén et al. 2000). This contrasts with other polyphenoloxidases, the products of which are quinones rather than semiquinones, which polymerise directly to form insoluble complexes (Bending and Read 1996a; Cairney and Burke 1998b). Not only is formation of such humic compounds likely to be important in detoxification of the mycorrhizosphere soil environment (Bending and Read 1996a), it may also be important in minimising ecosystem N losses, reducing toxic metal availability and improving soil physical and chemical conditions for root growth (Northup et al. 1998).

From the information we have reviewed here, it is evident that considerable gaps exist in our understanding of polyphenol oxidase, in particular laccase, enzymology in the context of ECM and ericoid mycorrhizal fungi. Given the potentially important roles that these enzymes might play in nutrient cycling and other soil processes, more detailed investigation of their activities is clearly warranted.

References

Agerer R, Schloter M, Hahn C (2000) Fungal enzymatic activity in fruitbodies. Nova Hedwigia 71:315–336

Allen AC, Walker JRL (1988) The selective inhibition of catechol oxidase by salicylhydroxamic acid. Phytochemistry 27:3075–3076

Anderson DW, Nicholson RL (1996) Characterization of laccase in the conidial mucilage of *Colletotrichum graminicola*. Mycologia 88:996–1002

Aramayo R, Timberlake WE (1993) The *Aspergillus nidulans yA* gene is regulated by abaA. EMBO J 12:2039–2048

Bar-Nun N, Tal Lev A, Harel E, Mayer AM (1988) Repression of laccase formation in *Botrytis cinerea* and its possible relation to phytopathogenicity. Phytochemistry 27:2505–2509

Bending GD, Read DJ (1995) The structure and function of the vegetative mycelium of ectomycorrhizal plants. VI. Activities of nutrient mobilising enzymes in birch litter colonised by *Paxillus involutus* (Fr.) Fr. New Phytol 130:411–417

Bending GD, Read DJ (1996a) Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. Soil Biol Biochem 28:1595–1602

Bending GD, Read DJ (1996b) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil Biol Biochem 28:1603–1612

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

Binz T, Canevascini G (1996) Differential production of extracellular laccase in the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi*. Mycol Res 100:1060–1064

Bonnen AM, Anton LH, Orth AB (1994) Lignin-degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. Appl Environ Microbiol 60:960–965

Braun-Lüllemann A, Hüttermann A, Majcherczyk A (1999) Screening of ectomycorrhizal fungi for degradation of polycyclic aromatic hydrocarbons. Appl Microbiol Biotechnol 53:127–132

Breuil AC, Jeandet P, Adrian M, Chopin F, Pirio N, Meunier P, Bessis R (1999) Characterization of a pterostilbene dehydropolymer produced by laccase of *Botrytis cinerea*. Phytopathology 89:298–302

Broxholme SJ, Read ND, Bond DJ (1991) Developmental regulation of proteins during fruit-body morphogenesis in *Sordaria brevicollis*. Mycol Res 95:958–969

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Li Y (1998) A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272

Bu'Lock JD (1967) Fungal metabolites with structural function. In: Essays in biosynthesis and microbial development. ER Squibb lectures on chemistry and microbial products. Wiley, New York, pp 1–18

Burke RM, Cairney JWG (1998) Carbohydrate oxidases in ericoid and ectomycorrhizal fungi: a possible source of Fenton radicals during the degradation of lignocellulose. New Phytol 139:637–465

Cairney JWG, Burke RM (1994) Fungal enzymes degrading plant cell walls: their possible significance in the ectomycorrhizal symbiosis. Mycol Res 98:1345–1356

Cairney JWG, Burke RM (1998a) Do ecto- and ericoid mycorrhizal fungi produce peroxidase activity? Mycorrhiza 8:61–65

Cairney JWG, Burke RM (1998b) Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil. Plant Soil 205:181–192

Call HP, Mücke I (1997) History, overview and application of mediated ligninolytic systems, especially laccase-mediator systems (Lignozyme®-process). J Biotechnol 53:163–202

Chakraborty TK, Das N, Sengupta S, Mukherjee M (2000) Accumulation of a natural substrate of laccase in gills of *Pleurotus florida* during sporulation. Curr Microbiol 41:167–171

Chambers SM, Burke RM, Brooks PR, Cairney JWG (1999) Molecular and biochemical evidence for manganese-dependent peroxidase activity in *Tylospora fibrillosa*. Mycol Res 103:1098–1102

Chefetz B, Chen Y, Hadar Y (1998) Purification and characterization of laccase from *Chaetomium thermophilum* and its role in humification. Appl Environ Microbiol 64:3175–3179

Chen DM, Taylor AFS, Burke RM, Cairney JWG (2001) Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi. New Phytol 152:151–158

Clutterbuck AJ (1972) Absence of laccase from yellow spored mutants of *Aspergillus nidulans*. J Gen Microbiol 70:423–435

Collins PJ, Dobson ADW (1997) Regulation of laccase gene transcription in *Trametes versicolor*. Appl Environ Microbiol 63:3444–3450

Colpaert JV, Van Laere A (1996) A comparison of the extracellular enzyme activities of two ectomycorrhizal and a leaf-saprotrophic basidiomycete colonizing beech leaf litter. New Phytol 133:133–141

Curir P, Thurston CF, Daquila F, Pasini C, Marchesini A (1997) Characterisation of a laccase secreted by *Armillaria mellea* pathogenic for *Genista*. Plant Physiol Biochem 35:147–153

Das N, Sengupta S, Mukherjee M (1997) Importance of laccase in vegetative growth of *Pleurotus florida*. Appl Environ Microbiol 63:4120–4122

Dawley RM, Flurkey WH (1993) Differentiation of tyrosinase and laccase using 4-hexylresorcinol, a tyrosinase inhibitor. Phytochemistry 33:281–284

De Vries OMH, Kooistra WHCF, Wessels GH (1986) Formation of an extracellular laccase by *Schizophyllum commune* dikaryon. J Gen Microbiol 132:2187–2826

Doering TL, Nosanchuk JD, Roberts WK, Casadevall A (1999) Melanin as a potential cryptococcal defence against microbicidal proteins. Med Mycol 37:157–181

D'Souza TM, Boominathan K, Reddy CA (1996) Isolation of laccase gene-specific sequences from white rot and brown rot fungi by PCR. Appl Environ Microbiol 62:3739–3744

Ducros V, Brzozowski AM, Wilson KS, Brown SH, Østergaard P, Schneider P, Yaver DS, Pedersen AH, Davies GJ (1998) Crystal structure of the type-2 Cu depleted laccase from *Coprinus cinereus* at 2.2 angstrom resolution. Nat Struct Biol 5:310–316

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

Edens WA, Goins TQ, Dooley D, Henson JM (1999) Purification and characterization of a secreted laccase of *Gaeumannomyces graminis* var. *tritici*. Appl Environ Microbiol 65:3071–3074

Egger KN (1986) Substrate hydrolysis patterns of post-fire ascomycetes (Pezizales). Mycologia 78:771–780

Eggert C, Temp U, Dean JFD, Eriksson K-EL (1996a) A fungal metabolite mediates oxidation of non-phenolic lignin model compounds and synthetic lignin by laccase. FEBS Lett 391:144–148

Eggert C, Temp U, Eriksson K-EL (1996b) The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. Appl Environ Microbiol 62:1151–1158

Eggert C, Temp U, Eriksson K-EL (1997) Laccase is essential for lignin degradation by the white rot fungus *Pycnoporus cinnabarinus*. FEBS Lett 407:89–92

Ghosh D, Mukherjee R (1998) Modeling tyrosinase monooxygenase activity. Spectroscopic and magnetic investigations of products due to reactions between copper(I) complexes of xylyl-based dinucleating ligands and dioxygen: aromatic ring hydroxylation and irreversible oxidation products. Inorg Chem 37:6597–6605

Giltrap NJ (1982) Production of polyphenol oxidases by ectomycorrhizal fungi with special reference to *Lactarius* spp. Trans Br Mycol Soc 78:75–81

Gramms G (1997) Activity of oxidative enzymes in fungal mycelia from grasslands and forest soils. J Basic Microbiol 6:407–423

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

Griffith GS, Rayner ADM, Wildman HG (1994) Interspecific interactions and mycelial morphogenesis of *Hypholoma fasciculare* (Agaricaceae) Nova Hedwigia 59:47–75

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 International, Wallingford, pp 98–105

Gruhn CM, Miller OK (1991) Effect of copper on tyrosinase activity and polyamine content of some ectomycorrhizal fungi. Mycol Res 95:268–272

Guillén F, Muñoz C, Gómez-Toribio V, Martínez AT, Martínez MJ (2000) Oxygen activation during oxidation of methoxyhydroquinones by laccase from *Pleurotus eryngii*. Appl Environ Microbiol 66:170–175

Günther T, Perner B, Gramms G (1998) Activities of phenol oxidising enzymes of ectomycorrhizal fungi in axenic culture and in symbiosis with Scots pine (*Pinus sylvestris* L.). J Basic Microbiol 38:197–206

Haars A, Chet I, Huttermann A (1981) Effect of phenolic compounds and tannin on growth and laccase activity of *Fomes annosus*. Eur J For Pathol 11:67–76

Harkin JM, Larsen MJ, Obst JR (1974) Use of syringaldazine for detection of laccase in sporophores of wood rotting fungi. Mycologia 66:469–476

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

Hermann TE, Kurtz MB, Champe SP (1983) Laccase located in hulle cells and cleistothecial primordia of *Aspergillus nidulans*. J Bacteriol 154:955–964

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997) Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc Natl Acad Sci USA 94:12002–12006

Hibbett DS, Gilbert L-B, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature 407:506–508

Hutchison LJ (1990) Studies on the systematics of ectomycorrhizal fungi in axenic culture. III. Patterns of polyphenol oxidase activity. Mycologia 82:424–435

Iakovlev A, Stenlid J (2000) Spatiotemporal patterns of laccase activity in interacting mycelia of wood-decaying basidiomycete fungi. Microb Ecol 39:236-245

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

Kanunfre CC, Zancan GT (1998) Physiology of exolaccase production by *Thelephora terrestris*. FEMS Microbiol Lett 161:151–156

Koroljova-Skorobogat'ko OV, Stepanova EV, Gavrilova VP, Morozova OV, Lubimova NV, Dzchafarova AN, Jaropolov AI, Makower A (1998) Purification and characterization of the constitutive form of laccase from the basidiomycete *Coriolus hirsutus* and effect of inducers on laccase synthesis. Biotechnol Appl Biochem 28:47–54

Kretzer AM, Bruns TD (1999) Use of *atp*6 in fungal phylogenetics: an example from the Boletales. Mol Phylogenet Evol 13:483–492

Leake JR, Read DJ (1997) Mycorrhizal fungi in terrestrial habitats. In: Wicklow D, Söderström B (eds) The Mycota IV. Environmental and microbial relationships. Springer, Berlin Heidelberg New York, pp 281–301

Leake JR, Read DJ (1989) The effect of phenolic compounds on nitrogen mobilisation by ericoid mycorrhizal systems. Agric Ecosyst Environ 29:225–236

Leatham GF, Stahmann MA (1981) Studies on the laccase of *Lentinus edodes*: specificity, localization and association with developing fruiting bodies. J Gen Microbiol 125:147–157

Leatham GF, King V, Stahmann MA (1980) In vitro protein polymerisation by quinones or free radicals generated by plant or fungal oxidative enzymes. Phytopathology 70:1134–1140

Leonard TJ (1971) Phenoloxidase activity and fruiting body formation in *Schizophyllum commune*. J Bacteriol 106:162–167

Lindeberg G (1948) On the occurrence of polyphenol oxidases in soil-inhabiting basidiomycetes. Physiol Plant 1:196–205

Liu L, Tewari RP, Williamson PR (1999a) Laccase protects *Cryptococcus neoformans* from antifungal activity of alveolar macrophages. Infect Immun 67:6034–6039

Liu L, Wakamatsu K, Ito S, Williamson PR (1999b) Catecholamine oxidative products, but not melanin, are produced by *Cryptococcus neoformans* during neuropathogenesis in mice. Infect Immun 67:108–112

Mansur M, Suárez T, González AE (1998) Differential gene expression in the laccase gene family from basidiomycete I-62 (CECT 20197). Appl Environ Microbiol 64:771–774

Marr CD, Grund DW, Harrison KA (1986) The taxonomic potential of laccase and tyrosinase spot tests. Mycologia 78:169–184

Miranda M, Zarivi O, Bonfigli A, Porretta R, Aimola P, Pacioni G, Ragnelli AM (1996) White truffles, like black ones, are tyrosinase positive. Plant Sci 120:29–36

Münzenberger B, Otter T, Wüstrich D, Polle A (1997) Peroxidase and laccase activities in mycorrhizal and non-mycorrhizal fine roots of Norway spruce (*Picea abies*) and larch (*Larix decidua*). Can J Bot 75:932–938

Northup RR, Dahlgren RA, McColl JG (1998) Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: a positive feedback? Biogeochemistry 42:189–220

Ohga S, Smith M, Thurston CF, Wood DA (1999) Transcriptional regulation of laccase and cellulase genes in the mycelium of *Agaricus bisporus* during fruit body development on a solid substrate. Mycol Res 103:1557–1560

Peláez F, Martínez MJ, Martínez AT (1995) Screening of 68 species of basidiomycetes for enzymes involved in lignin degradation. Mycol Res 99:37–42

Pometto AL, Crawford DL (1986) Catabolic fate of *Streptomyces viridosporus* T7A-produced, acid-precipitable polymeric lignin upon incubation with ligninolytic *Streptomyces* species and *Phanerochaete chrysosporium*. Appl Environ Microbiol 51:171–179

Ramstedt M, Söderhäll K (1983) Protease, phenoloxidase and pectinase activities in mycorrhizal fungi. Trans Br Mycol Soc 81:157–161

Rehman AU, Thurston CF (1992) Purification of laccase I from *Armillaria mellea*. J Gen Microbiol 138:1251–1257

Reinhammar B, Malmstrom BG (1981) "Blue copper-containing oxidases. In: Spiro TG (ed) Copper proteins (Metal ions in biology, vol 3) Wiley, New York, pp 109–149

Rigling D, Van Alfen NK (1993) Extra- and intracellular laccases of the chestnut blight fungus *Cryphonectria parasitica*. Appl Environ Microbiol 59:3634–3639

Ross IK (1982) The role of laccase in carpophore initiation in *Coprinus congregatus*. J Gen Microbiol 128:2763–2770

Roy-Arcand L, Archibald FS (1991) Direct dechlorination of chlorophenolic compounds by laccases from *Trametes* (*Coriolus*) *versicolor*. Enzyme Microb Technol 13:194–203

Salas SD, Bennett JE, Kwonchung KJ, Perfect JR, Williamson PR (1996) Effect of the laccase gene, CNLAC1, on virulence of *Cryptococcus neoformans*. J Exp Med 184:377–386

Sanchez-Amat A, Solano F (1997) A pluripotent polyphenol oxidase from the melanogenic marine *Alteromonas* sp. shares capabilities of tyrosinases and laccases. Biochem Biophys Res Commun 240:787–792

Scherer M, Fischer R (1998) Purification and characterization of laccase II of *Aspergillus nidulans*. Arch Microbiol 170:78–84

Schlosser D, Grey R, Fritsche W (1997) Patterns of ligninolytic enzymes in *Trametes versicolor*. Distribution of extra- and intracellular enzyme activities during cultivation on glucose, wheat straw and beech wood. Appl Microbiol Biotechnol 47:412–418

Shin K-S, Lee Y-J (2000) Purification and characterization of a new member of the laccase family from the white-rot basidiomycete *Coriolus hirsutus*. Arch Biochem Biophys 384:109–115

Shunming W, Yinlian Z, Yingpe C (1985) A preliminary study on the phenolic glucosides and phenolic acids from the bark of several species of poplar. Chem Ind For Prod 5:1–8

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

Soponsathien S (1998) Some characteristics of ammonia fungi. 1. In relation to their ligninolytic enzyme activities. J Gen Appl Microbiol 44:337–345

Srebotnik E, Hammel KE (2000) Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. J Biotechnol 81:179–188

Srinivasan C, D'Souza TM, Boominathan K, Reddy CA (1995) Demonstration of laccase in the white rot basidiomycete *Phanerochaete chrysosporium* BKM-F1767. Appl Environ Microbiol 61:4274–4277

Sword MA, Garrett HE (1994) Boric acid-phenolic relationships within the *Pinus echinata-Pisolithus tinctorius* ectomycorrhizal association. Tree Physiol 14:1121–1130

Tan YH, Wahab MN (1997) Extracellular enzyme production during anamorphic growth of the edible mushroom, *Pleurotus sajor-caju*. World J Microbiol Biotechnol 13:613–617

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

Timonen S, Sen R (1998) Heterogeneity of fungal and plant enzyme expression in intact Scots pine-*Suillus bovinus* and -*Paxillus involutus* mycorrhizospheres developed in natural forest humus. New Phytol 138:355–366

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

Varela E, Martínez AT, Martínez MJ (2000) Southern blot screening for lignin peroxidase and aryl-alcohol oxidase genes in 30 fungal species. J Biotechnol 83:245–251

Wahleithner JA, Xu F, Brown KM, Brown SH, Golightly EJ, Halkier T, Kauppinen S, Pederson A, Schneider P (1996) The identification and characterization of four laccases from the plant pathogenic fungus *Rhizoctonia solani*. Curr Genet 29:395–403

Wang Y, Aisen P, Casadevall A (1995) *Cryptococcus neoformans* melanin and virulence: mechanisms of action. Infect Immun 63:3131–3136

White NA, Boddy L (1992a) Differential extracellular enzyme production in colonies of *Coriolus versicolor*, *Phlebia radiata* and *Phlebia rufa*: effect of gaseous regime. J Gen Microbiol 138:2589–2598

White NA, Boddy L (1992b) Extracellular enzyme localization during interspecific fungal interactions. FEMS Microbiol Lett 98:75–80

Willetts HJ, Bullock S (1992) Developmental biology of sclerotia. Mycol Res 96:801–816

Williamson PR (1994) Biochemical and molecular characterization of the diphenol oxidase of *Cryptococcus neoformans* – identification as a laccase. J Bacteriol 176:656–664

Wood DA (1980a) Production, purification and properties of extracellular laccase of *Agaricus bisporus*. J Gen Microbiol 117:327–338

Wood DA (1980b) Inactivation of extracellular laccase during fruiting of *Agaricus bisporus*. J Gen Microbiol 117:339–345

Worrell JJ, Chet I, Hüttermann A (1986) Association of rhizomorph formation with laccase activity in *Armillaria* spp. J Gen Microbiol 132:2527–2533

Xu F (1996) Oxidation of phenols, anilines and benzenethiols by fungal laccases: correlation between activity and redox potentials as well as halide inhibition. Biochemistry 35:7608–7614

Xu F, Kulys JJ, Duke K, Li KC, Krikstopaitis K, Deussen H-JW, Abbate E, Galinyte V, Scheider P (2000) Redox chemistry in laccase-catalysed oxidation of *N*-hydroxy compounds. Appl Environ Microbiol 66:2052–2056

Yaver DS, Xu F, Golightly EJ, Brown KM, Brown SH, Rey MW, Schneider P, Halkier T, Mondorf K, Dalbøge H (1996) Purification, characterization, molecular cloning, and expression of two laccase genes from the white rot basidiomycete *Trametes villosa*. Appl Environ Microbiol 62:834–841

Youn H-D, Hah YC, Kang SO (1995) Role of laccase in lignin degradation by white-rot fungi. FEMS Microbiol Lett 132:183–188

Zhao J, Kwan HS (1999) Characterization, molecular cloning, and differential expression analysis of laccase genes from the edible mushroom *Lentinula edodes*. Appl Environ Microbiol 65:4908–4913