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Occurrences, Physical and Biochemical Properties of Laccase

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Abstract:

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multicopper oxidases that are widely distributed among plants, insects, and fungi. They belong to a group of polyphenol oxidases containing copper atoms in the catalytic centre which are usually called multicopper oxidases. It utilizes molecular oxygen to oxidize various aromatic and non-aromatic compounds. They have been described in different genera of ascomycetes, some deuteromycetes, and mainly in basidiomycetes. The enzyme catalyze the one electron oxidation of a wide variety of organic and inorganic substrates, including mono-di- and polyphenols, amino-phenols, methoxyphenols, aromatic amines, and ascorbate, with the concamitant four electron reduction of oxygen to water. Laccases are found in Prokaryotes and Eukaryotes. Laccase is localized both intracellular and extracellular fraction. In addition to the general inhibitors a very wide range of compounds are known to inhibit laccase. Fungal laccases typically exhibit pH optima in the acidic pH range and stable at temperature between 30-50°C, and isoeletric points (pI) ranging between 3 to 7. Km ranges from 10s of mM for syringaldazine and ABTS to 100 s of mM for DMP and guaiacol. Their importance in the synthesis and biodegradation of lignin remains an intensively studied subject and the biotechnological aspects of these enzymes seems to be just beginning. This review helps to understand the properties of this important enzyme for efficient utilization for its biotechnological and environmental applications.

Keywords: Laccase, catalytic activity, substrates, inhibitors, isoenzymes

1.0 Introduction:

Laccase (p-diphenol: oxygen oxidoreductases) was first demonstrated in the exudates of *Rhus vernicifera*, the Japanese lacquer tree (Yoshida, 1883). Later it was demonstrated in fungi (Bertrand, 1896). It is a dimeric or tetrameric glycoprotein, which usually contains four copper atoms per molecule of enzyme (monomer) distributed in three different copper binding (redox) sites (Gianfreda et al., 1999). It is a glycosylated monomer or homodimer protein generally having fewer saccaride compounds (10-25%) in fungi and bacteria than in the plant enzymes. The carbohydrate compound contains monosaccarides such as hexoamines, glucose, mannose, galactose, fucose, and arabinose (Rogalaski and Leonowicz 2004). On SDS-PAGE, most laccases show mobilites corresponding to molecular weight of 60-100 KDa, of which 10-50 % may be attributed to glycosylation. Mannose is one of the major components of the carbohydrate attached to laccase. Glycosylation in laccase is responsible for secretion, proteolytic susceptibility, activity, copper retention, and thermal stability (xu 1999). These are crucial for numerous reactions in cell like pathogenicity, immunity and morphogenesis of organisms and in the metabolic turnover of complex organic substances such as lignin or humic matter. The copper atoms allows the proteins to perform electron transfer reactions, because copper atoms are able to switch their oxidation states between Cu^{1} and Cu^{n}. They were also able to oxidise non-phenolic structures (Bourbonnais and Paice, 1990; Call and Mücke, 1997), indicating that a range of compounds can be oxidised by these enzymes. Owing to their high non-specific oxidation capacity, laccases are useful biocatalysts in diverse biotechnological applications.

2.0 Occurences and Physiological Role of Laccase:

Until recently, laccases were only found in eukaryotes like higher plants (Huang et al, 1991), fungi (Thurston, 1994), insects (Thomas et al., 1989), wasp venom (Parkinson et al., 2001), but now there is strong evidence for their widespread distribution in prokaryotes (Claus, 2003).

2.1 Plants:

Laccases are widely distributed in eukaryotes like higher plants (Huang et al, 1991) Cell cultures of *Acer pseudoplatanus* have been shown to produce and secrete laccase (Tezuka et al., 1993), and *Pinus taeda* tissue contain eight laccases, all expressed

predominantly in xylem tissue (Sato et al., 2001). Other reports on the presence of laccase in leaves of *Aesculus parviflora* (Wosilait et al. 1954) and in green shoots of tea (Gregory and Bendall, 1966). Five distinct laccases have been shown to be present in the xylem tissue of *Populus euramericana* (Ranocha et al., 1999). Plant laccases participate in the radical-based mechanisms of lignin polymer formation (Hoopes and Dean, 2004). They are found in the xylem, where they presumably oxidize monolignols in the early stages of lignification (Gavnholt and Larsen, 2002). In addition, laccases have been shown to be involved in the first steps of healing in wounded leaves (De Marco and Roubelakis - Angelakis, 1997). Detection and purification of plant laccases is often difficult because crude plant extracts contain a large number of oxidative enzymes with broad substrate specificities (Ranocha et al., 1999), which could probably explain the lack of detailed information on the biochemical properties of plant laccases.

2.2 Fungi:

Laccase activity has been demonstrated in many fungal species and the enzyme has already been purified from many species of ascomycetes, deuteromycetes, and basidiomycetes fungi (Assavanig et al.,1992) . However, laccase production has never been demonstrated in lower fungi like Zygomycetes and Chytridiomycetes. Laccase was purified from phytopathogenic ascomycetes like *Magnaporthe grisea* (Iyer and Chattoo, 2003); *Mauginella* (Palonen et al., 2003), and *Melanocarpus albomyces* (Kiiskinen et al., 2002), laccase was also reported from some soil ascomycete species from the genera *Aspergillus*, *Curvularia* and *Penicillium* (Scherer and Fischer, 1998), as well as some freshwater ascomycetes (Junghanns et al., 2005). Majority of laccases characterized so far have been derived from fungi, especially from white-rot basidiomycetes that are efficient lignin degraders. Well-known laccase-producers include fungi such as *Agaricus bisporus* (Wood, 1980), *Botrytis cinerea* (Marbach et al., 1984), *Chaetomium thermophilum* (Chefetz et al., 1998), *Coprinus cinereus* (Schneider et al. ,1999), *Neurospora crassa* (Froehner and Eriksson, 1974), *Phlebia radiata* (Niku-Paavola et al., 1988), *Pleurotus ostreatus* (Sannia et al., 1986), *Pycnoporus cinnabarinus* (Eggert et al., 1996) and *Trametes versicolor, Coriolus polyporus* (Rogalski et al., 1991).

Yeasts are a physiologically specific group of both ascomycetes and basidiomycetes. Until now, laccase was only purified from the human pathogen *Cryptococcus (Filobasidiella neoformans)*. This basidiomycete yeast produces a true laccase capable of oxidation of phenols and aminophenols and unable to oxidize tyrosine (Williamson, 1994). The enzyme is tightly bound to the cell wall and contributes to the resistance to fungicides (Zhu *et al*., 2001). Several attempts have been undertaken to detect ligninolytic enzymes, including laccases in ectomycorrhizal (ECM) fungi (Burke & Cairney, 2002). Gene fragments with a high similarity to laccase from wood-rotting fungi have been found in several isolates of ectomycorrhizal (ECM) fungal species including *Amanita, Cortinarius, Hebeloma, Lactar-ius, Paxillus, Piloderma, Russula, Tylospora* and *Xerocomus* (Chen *et al,* 2004). Fungal laccases have enormous physiological roles. For example, Laccases from white-rot fungi, *Trametes versicolor* and *Pycnoporus cinnabarinus* participate in lignin biodegradation, where in they mainly oxidize the phenolic subunits of lignin (Thurston 1994; Hatakka 2001). In plant-pathogenic fungi, laccases are important virulence factor in many diseases caused by fungi. Laccase can protect the fungal pathogen from the toxic phytoalexins and tannins in the host environement. The grapevine grey mould, *Botrytis cinerea,* produces a laccase that is necessary for pathogenesis, and the role of this laccase is presumably related to detoxification of toxic defence metabolites produced by the plant (Bar-Nun et al., 1988). Laccases have also been shown to be important for pathogenesis in the chestnut blight fungus *Cryphonectria parasitica* (Rigling and van Alfen, 1991; Choi et al., 1992; Mayer and Staples, 2002). In *Cryptococcus neoformans* infection to immunonocompromised patients with HIV virus, laccase is present as a tightly associated cell wall enzyme that is readily accessible for interactions with host immune cells (Zhu et al., 2001). Laccase activity in *Aspergillus nidulans,* is related to pigment production, and deletion of the laccase gene *yA* abolishes the green color of conidial spores (Adams et al. ,1998). The fungal and plant laccase enzyme differ in their function. Fungal enzyme is responsible for removing toxic phenols from the substrate in which these fungi grow under natural conditions, while the plant enzymes are involved in synthetic process such as lignin formation (Benfield et al.1964)

2.3 Insects:

Laccase is also found to be present in insects of genera that include Bombyx, calliphora, Diploptera, Drosophila, Lucilia, Manduca, Musca, Oryctes, Papilio, Phormia, Rhodnius, Sarcophaga, Schistocerca and Tenebrio (xu 1999). In insects,

the laccase-catalysed oxidative coupling of catechols with proteins may be involved in cuticle sclerotization (Dittmer et al., 2004).

2.4 Prokaryotes:

The first convincing data for a prokaryotic laccase activity were presented for *Azospirillum lipoferum* (Givaudan et al., 1993). It is composed of a catalytic subunit and one or two larger chains. Three bands in SDS-PAGE are present with molecular masses of 48.9, 97.8 and 179.3 kDa (Diamantidis et al., 2000). Similar genes are found in gram-negative and gram-positive bacteria, including species living in extreme habitats, e.g. in *Oceanobacillus iheyensis* or *Aquifex aeolicus* and in the archaebacterium, *Pyrobaculum aerophilum.* Reports of laccases in actinomycetes were based on rather non-specific substrate reactions, but have been verified for *Streptomyces griseus* (Freeman et al., 1993), *S. lavendulae* (Suzuki et al., 2003). *Marinomonas mediterranea* is a melanogenic marine bacterium expressing both an SDS-activated tyrosinase and a laccase (Sanchez-Amat and Solano, 1997). Spores of *Bacillus sphaericus* strain showed Laccase-like activity (Claus and Filip, 1997). The spore protein CotA of *Bacillus subtilis* has been recognized to be a laccase (Hullo et al., 2001). CotA Mutants lost ability to produce a brownish spore pigment. CotA protect the spores against stress factors such as UV radiation or hydrogen peroxide. The protein, which was over expressed in *E. coli*, had a molecular mass of 65 kDa, an iso-electric point of 7.7 and is highly thermostable (Martins et al., 2002). Cross-linking of proteins residues in microorganisms, eg. tyrosine to dityrosine, has been discussed as the function of laccases and in the assembly of the heat & UV-resistant Bacillus spores. Thus, it can be summarized that laccases are exceptionally versatile and ubiquitous enzymes. More in depth investigations are necessary for a better understanding of their physiological importance and to further enhance their biotechnological potential.

3.0 Cellular localization of Laccase:

While, most laccases purified so far are extracellular, Laccase is localized both intracellular and extracellular fraction. The laccases of wood-rotting fungi are usually also found intracellularly. When *Trametes versicolor* was grown on glucose, wheat straw and beech leaves, it produced laccases both in extracellular and intracellular fractions (Schlosser et al., 1997). The intra- and extracellular presence of laccase activity was also detected in *Phanerochaete chrysosporium* (Dittmer et al., 1997) and *Suillus granulatus* (Günther et al., 1998). A fraction of laccase activity in *Neurospora crassa*, *Rigidoporus lignosus* and one of the laccase isoenzymes of *Pleurotus ostreatus* is also probably localized intracellularly or on the cell wall (Palmieri et al., 2000). Laccase activity is almost exclusively associated with cell walls in the white-rot basidiomycete *Irpex lacteus* (Svobodová, 2005). The localization of laccase is probably connected with its physiological function and determines the range of substrates available to the enzyme. The cell wall and spore - associated laccases were linked to the possible formation of melanin and other protective cell wall compounds (Galhaup and Haltrich, 2001).

4.0 Catalytic Properties of Laccase:

Laccase nomenclature is defined in the Enzyme Commission (EC 1.10.3.2) 1 indicates oxidoreductases EC 1.10 acting on diphenols and related substances as donors E.C.1.10.3 with oxygen as acceptor, E.C.1.10.3.2. Laccases, (benzenediol: oxygen oxidoreductase) are either mono or multimeric copper-containing oxidizes that catalyze the reduction of oxygen to water accompanied by the oxidation of a phenolic substrates. Molecular oxygen serves as the terminal electron acceptor and is thus reduced to two molecules of water (i)

$$O_2 + 4\,\bar{e} + 4\,H^+ \longrightarrow 2\,H_2O \qquad \text{(i)}$$

As one electron oxidation of a substrate is coupled to a four-electron reduction of oxygen the reaction mechanism cannot be straight forward (Thurston, 1994). When oxidized by a laccase, the reducing substrate loses a single electron and usually forms a free radical (Thurston 1994). The unstable radical may undergo further laccase-catalyzed oxidation or non-enzymatic reactions including hydration, disproportionation, and polymerization (Thurston 1994). Laccase-catalyzed oxidation of phenolic hydroxyl groups to phenoxy radicals is given in equation (ii)

$$4\,PhOH + O_2 \xrightleftharpoons{\text{Laccase}} 4\,PhO^+ + 2\,H_2O \qquad \text{(ii)}$$

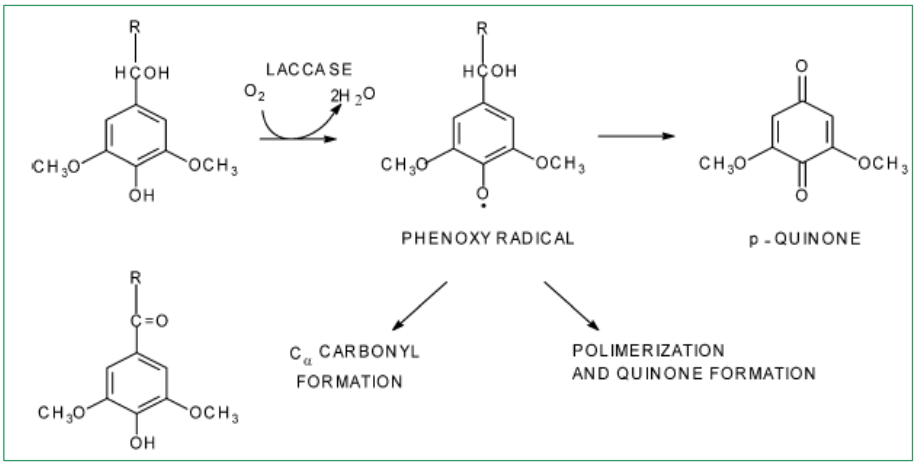


Fig: 1. Phenolic subunits catalysed by laccase

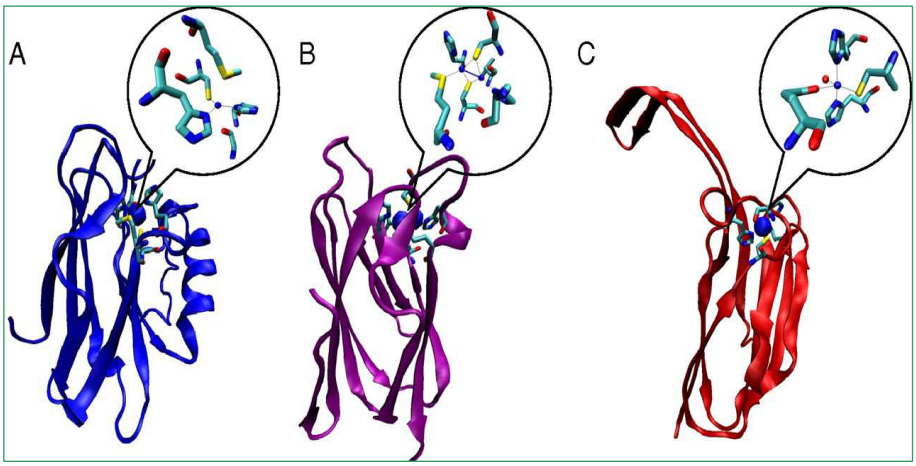


Fig: 2. Cupredoxin folds (Source: Masha G.Savelieff etal (2008)

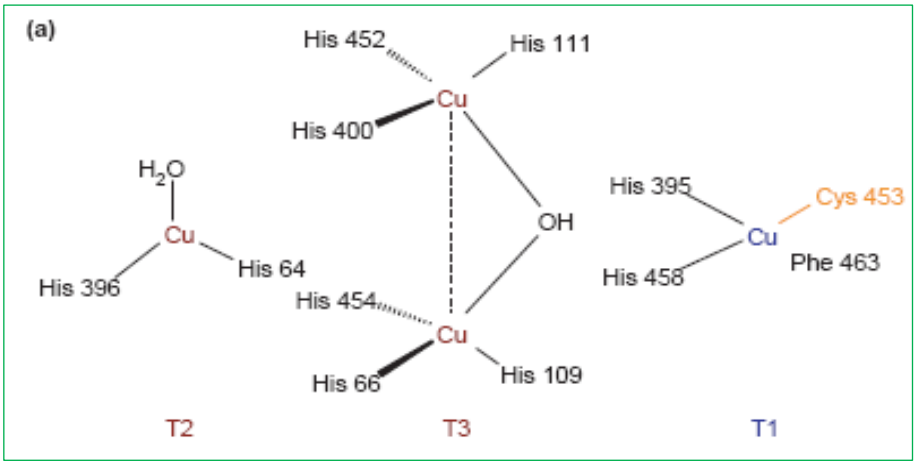


Fig 3: Model of the catalytic cluster of Laccase from *Trametes versicolor* made of four copper atoms (Source: Sergio Riva, 2006)

The structure of laccases comprises of three cupredoxin-like domains A, B & C of equal size (Enguita et al., 2003). It is common among copper-containing proteins, and is found in simple copper proteins plant plastocyanin (Inoue et al., 1999), bacterial azurin (Norris et al., 1983), multicopper oxidases ascorbate oxidase (Messerschmidt et al. 1992) and ceruloplasmin (Murphy et al. 1997). A, B & C domains are important for the catalytic activity of laccases: between domains B and C the substrate-binding site is located in cleft, in domain C a mononuclear copper center is located, at the interface between domains A and C a trinuclear copper center is located.

Trametes versicolor has three catalytic clusters made of four copper atoms. The mononuclear copper center contains one type-1 (Tl) copper atom that is trigonally coordinated to two histidines and a cysteine. The trinuclear cluster contains one type-2 (T2) copper atom which is c 'non blue' copper and shows no absorption in the visible spectrum, and a pair of type-3 (T3) coppers (Messerschmidt 1997). The T2 copper is coordinated by two and the T3 copper atoms by six conserved histidines (Piontek et al., 2002).

Laccase catalysis involves
(i) Reduction of the type 1 copper by reducing substrate
(ii) Internal electron transfer from the type 1 to the type 2 and type 3 copper
(iii) Reduction of oxygen to water at the type 2 and type 3 copper site

A characteristic blue appearance is exhibited with purified laccases from electronic absorption around 600nm. UV-visible spectra of laccase (at resting state) typically show two maxima around 280 nm to that at 600nm are generally 14 to 30, and the ratio of the absorbance at 330nm to that at 600nm is 0.5 to 2 (Xu 1999). Most laccases have four copper atoms per monomer in holoenzyme form (Coll et al., 1993), the prosthetic group laccase from phlebia was reported to have two copper and one pyrroloquinoline quinone. White laccase was described in one of the laccases from *pleurotus ostreatus* (Palmieri et al., 1997). Using UV/visible and electron paramagnetic resonance (EPR) spectroscopy the copper atoms are classified in three groups (Leontievsky et al., 1997). Blue colour of the enzyme is due to Type I copper (T1) , and is the site for oxidation with standard redox potential. It has a strong electronic absorption around 600nm, EPR is detectable. The type II copper (T2) is colourless, but EPR is detectable, and the type 3 copper (3) give a weak absorbance near the UV spectrum in the near 330 nm and consists of a pair of copper atoms but no EPR signal. Copper sites of T2 and T3 are close together and form a trinuclear centre (Leontievsky et al.,1997), place where binding of dioxygen and four - electron reduction to water occur (Piontek et al., 2002). For some laccases that T1 is the primary center at which electrons are accepted from reducing substrates [F. Xu., 1996]. High redox potential T1 site are of special interest in biotechnology e.g., bioremediation processes (Mayer et al., 2002), for different bleaching (Balakshin et al., 2001)

Polyphenol oxidases are associated with three types of activities (Mayer 1987)

- catechol oxidase or o- diphenol: oxygen oxidoreductase (EC 1.10.3.1)
- Laccase or p- diphenol: oxygen oxidoreductase (EC 1.10.3.2)
- Cresolase or monophenol monooxygenase (EC 1.18.14.1)

It is difficult to define laccase by its reducing substrate due to its very broad substrate range, which varies from one laccase to another and overlaps with the substrate range of another enzyme the monophenol mono-oxygenase tyrosinase (EC 1.14.18.1). Although laccase was also called diphenol oxidase, monophenols like 2,6-dimethoxyphenol or guaiacol are often better substrates than diphenols, e.g. catechol or hydroquinone. Laccases can oxidize a wide range of molecules as their reducing substrates as described in Table 1. Thurston (1994) stated in a review that simple diphenols like hydroquinone and catechol are substrates (for most laccases, but not for all), but that guaiacol and 2,6 - dimethoxyphenol (DMP) are often better. Para - phenylenediamine is widely used substrate, and syringaldazine (N,N'-bis 3,5 dimethoxy -4 hydroxy benzylidene hydrazine) is a unique substrate for laccase only(Harkin et al., 1974) as long as hydrogen peroxide is avoided in the reaction, as this compound is also oxidized by peroxidases. Many of the compounds occur naturally and/or can be important industrial precursors, products, or byproducts. Their transformability by laccases might allow various viable applications of these enzymes.

Laccase enzymes differ considerably in their catalytic preferences. Laccases can be grouped according to their preference for ortho-, meta- or para- substituted phenols. Ortho-substituted compounds (guaiacol, o-phenylenediamine, caffeic acid, catechol, dihydroxyphenylalanine,

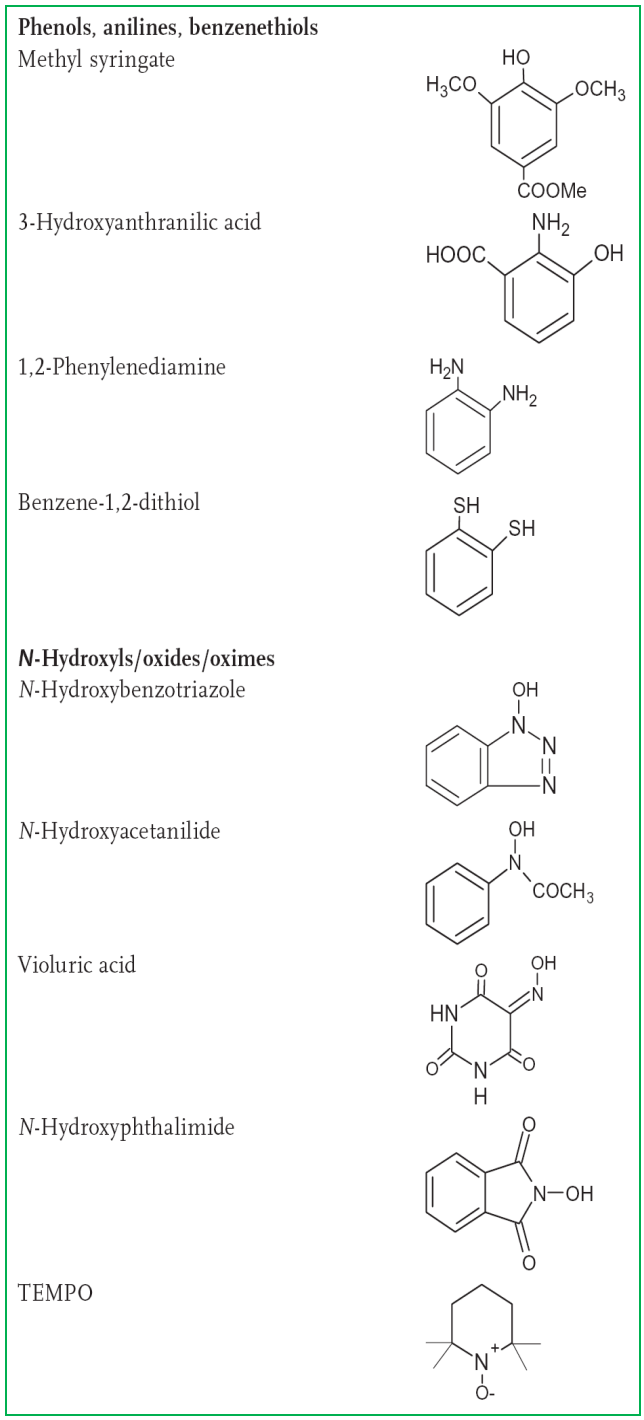
protocatechuic acid, gallic acid and pyrogallol) were better substrates than para-substituted compounds (p-phenylenediamine, p-cresol, hydroquinone) and the lowest rates were obtained with meta-substituted compounds (m-phenylenediamine, orcinol, resorcinol and phloroglucinol) with crude laccase preparations from *L. litschaueri* and *P. brumalis* (Blaich & Esser, 1975). Similar results were also obtained with *T. versicolor* and the ascomycetes *P. anserina* and *Pyricularia oryzae*, whereas laccase from *Ganoderma lucidum* catalyzed the oxidation of only ortho and para dihydroxyphenyl compounds, p-phenylenediamine and polyphenols, not the meta hydroxymethyl compounds or ascorbic acid (Fahraeus, 1961; Fahraeus & Ljunggren, 1961; Sch´aneˇl & Esser, 1971; Lalitha Kumari & Sirsi, 1972). More than 70% oxidation of o-substituted compounds was obtained with laccase from *M. indicum*, whereas p-compounds and the m-phenol phloroglucinol were oxidized at a relatively low rate (Thakker et al., 1992).

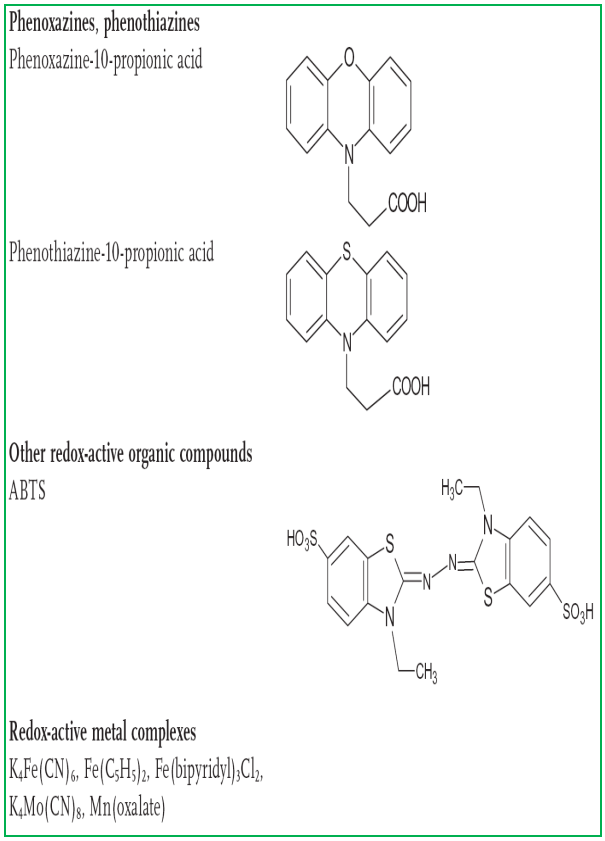
Laccase substrate range varies from one organism to another. *Neurospora crassa* laccase (Germann et al., 1988) only effectively oxidizes para and ortho-diphenols with the exception of phloroglucinol, while laccase from *pyricularia oryzae* preferred phlorogulcinol as a substrate above other substituted monophenols. Laccase from *Cerrena unicolor* and *Trametes versicolor* oxidizes ortho-substituted phenols to the greatest extent (Jolivalt et al. 1999). An immobilized commmercial laccase was able to degrade meta, ortho, and para -substituted methoxyphenols, chlorophenols, and cresols. The substituted phenols from these three types of phenols are oxidized in different orders and to different extents (Lante et al., 2000). Phenolic compounds that were oxidized very slowly by laccase have recently been used to increase the storage stability of laccase activity for *Trametes versicolor* (Mai et al., 2000). The increased stability of laccase could have technological importance, as there are so many potential applications for laccase.

5.0 Laccase Assays:

One unit of laccase activity was defined as the enzyme amount oxidising one µmol substrate per min. Suitable amounts of enzyme necessary to obtain extinction of 0.4–1 after approximately 1.5 min was used in all photometric measurements applying common test conditions. The ABTS (ϵ_{420} = 36 000 M^{-1} cm^{-1}; Childs and Bardsley, 1975) test. It is performed in 100 mM acetate buffer pH 5.0 with 5 mM final concentration of the substrate. DMP (2 mM; ϵ_{468} = 49600 M^{-1} cm^{-1}; Wariishi et al., 1992) is used in 10% ethanol in 100 mM acetate buffer pH 5.0, guaiacol (5 mM; ϵ_{436} = 6400 M^{-1} cm^{-1}; Eggert et al., 1996) in 50 mM sodium tartrate buffer pH 4.0, and syringaldazine (0.12 mM; ϵ_{526} = 65 000 M^{-1} cm^{-1}.

Fig.4: Types and examples of Molecules directly oxidized by Laccase





(Source : Rolf D. et al (2007)

6.0 Inhibitors:

A very wide range of compounds has been described to inhibit laccase (Table.1). In addition to the general inhibitors of metal-containing oxidases like cyanide, sodium azide or fluoride, there are some selective inhibitors for individual oxidases. They may facilitate estimation of laccase activity when protein purification is not successful. Inhibition by diethyl dithiocarbamate and thioglycolic acid could be supposed to be due to the presence of copper in the catalytic centre of the enzyme, and several sulfhydryl organic compounds have been described as laccase inhibitors: e.g. dithiothreitol, thioglycolic acid, cysteine and diethyldithiocarbamic acid. However, experiments with *T. versicolor* laccase showed that the inhibitory effect found with these compounds is probably due to the methodology using ABTS as the enzyme substrate (Johannes & Majcherczyk, 2000) and that these compounds, contrary to sodium azide, do not decrease the oxygen consumption by laccase during the catalysis.

6.1 Heavy Metal Inhibitors:

Given the natural occurrence of laccases in soil, the inhibition by heavy metals and humic substances must be taken into account (Zavarzina et al., 2004). While some laccases exhibit a sensitivity towards heavy metals (Table.1), others, e.g. the enzyme from *G. lucidum*, are completely insensitive (Lalitha Kumari & Sirsi., 1972). In the complex environment of soil or decaying lignocellulosic material, many different substrates of laccase are usually present that can compete for the oxidation and thus competitively inhibit the transformation of other compounds (Itoh et al., 2000). Thus it is difficult to estimate the transformation rates of laccase substrates in soils based on laboratory results and these rates can significantly differ in different soils.

7.0 Molecular Properties:

The optimum pH range of fungal laccases is in the acidic pH range. Highest activities at pH of 2-3 was observed by Oxidation of non-phenolic substrates, such as ABTS (Xu 1997; Garzillo et al., 2001).Phenolic compounds like Dimethoxy phenol (DMP), guaiacol and syringaldazine exhibit often bell- shaped higher values between 4.0 and 7.0 (Garzillo et al., 2001). Laccases from *Rhizoctonia praticola* and *T. versicolor* formed different products from syringic and vanillic acids at different pH values, but both enzymes generated the same products at a particular pH (Leonowicz et al., 1984; Xu, 1997). Fungal laccases are more stable at higher acidic pH (Leonowicz et al., 1984), although exceptions exist (Baldrian, 2004). The fungal laccases have their pH optima between pH 3.6 and 5.2, while laccase from plants like *Rhus vernicifera* have pH optima between 6.8 and 7.4. Fungal laccase have low pH optima may be because they are adapted to grow under acidic condition, while the plant laccase being intracellular have their pH optima nearer to the physiological range. Fungal laccases have isoeletric points(pI) ranging between 3 to 7, whereas plant laccase pI range to 9.

The optimum temperature for laccase usually depend upon the source of organism. In general, laccases have optimum temperatures at 30-50°C and rapidly lose activity at temperatures above 60°C (Galhaup et al., 2002; Jung et al., 2002; Palonen et al., 2003). Few enzymes with optima below 35°C have been described, e.g. the laccase from *Gonoderma lucidum* with the highest activity at 25°C (Ko et al., 2001). The temperature stability varies considerably. For example the half life at 50°C ranges from minutes in *B. cinnerea* (Slomczynski et al., 1995), to over 2–3 h in *Lentinula edodes* and *A. bisporus* (D'Annibale et al., 1996), to up to 50–70 h in *Trametes sp.* (Smirnov et al., 2001).

Table 1: Laccases sensitivity towards heavy metals

Inhibitor	Species	Reference
Ca^{2+}	*Lentinula edodes*	Nagai et al., 2002
Cd^{2+}	*Daedalea quercina* *Lentinula edodes*	Baldrian, 2004 ; Nagai et al., 2002
Co^{2+}	*Daedalea quercina*	Baldrian, 2004 ;
Fe^{2+}	*Chaetomium termophilum* *Pleurotus ostreatus*	Chefetz et al., 1998; Pozdnyakova et al., 2004;
Hg^{2+}	*Lentinula edodes* *Pleurotus pulmonarius*	(D'Annibale et al., 1996; Nagai et al., 2002) ; De Souza & Peralta, 2003
Mn^{2+}	*Daedalea quercina* *Pleurotus pulmonarius*	Baldrian, 2004 ; De Souza & Peralta, 2003
Rb^{+}	*Lentinula edodes*	Nagai et al., 2002
Sn^{2+}	*Lentinula edodes*	Nagai et al., 2002
Zn^{2+}	*Lentinula edodes* *Pleurotus ostreatus*	Nagai et al., 2002 Das et al., 2000
CN^{-}	*Botrytis cinerea* *Ganoderma lucidum* *Lactarius piperatus* *Melanocarpus albomyces* *Pleurotus ostreatus* *Rigidoporus lignosus* *Trametes gallica* *Trametes sanguinea*	Zouari et al., 2002 (Lalitha Kumari & Sirsi, 1972; Ko et al., 2001) ; Iwasaki et al., 1967 ; Kiiskinen et al., 2002 ; Giardina et al., 1999 ; Cambria et al., 2000; Dong & Zhang, 2004; Nishizawa et al., 1995
2-Mercaptoethanol	*Pleurotus pulmonarius* *Thelephora terrestris*	De Souza & Peralta, 2003 Kanunfre & Zancan, 1998
Ascorbic acid	*Chaetomium termophilum* *Trichoderma sp.*	(Chefetz et al., 1998; Ishigami et al., 1998) ; Assavanig et al., 1992
Cetyltriammonium bromide	*Agaricus bisporus*	Wood, 1980 ;
Cysteine	*Coriolus hirsutus* *Marasmius quercophilus* *Pycnoporus cinnabarinus* *Pycnoporus coccineus* *Sclerotium rolfsii* *Volvariella volvacea*	Eggert et al., 1996 ; (Dedeyan et al., 2000; Farnet et al., 2004) Eggert et al., 1996 ; Oda et al., 1991 ; Ryan et al., 2003 ; Chen et al., 2004;
Diethyldithiocarbamic acid	*Botrytis cinerea* *Coriolus hirsutus* *Ganoderma lucidum* *Monocillium indicum* *Panaeolus papilionaceus* *Pycnoporus cinnabarinus* *Panaeolus sphinctricus* *Sclerotium rolfsii*	Zouari et al., 2002; Eggert et al., 1996 ; (Lalitha Kumari & Sirsi, 1972; Ko et al., 2001); Thakker et al., 1992; Heinzkill et al., 1998 ; Eggert et al., 1996, 1995 Heinzkill et al., 1998 Ryan et al., 2003
Dithiothreitol	*Coriolus hirsutus* *Daedalea quercina* *Lentinula edodes* *Pycnoporus cinnabarinus*	Eggert et al., 1996 ; Baldrian, 2004 ; D'Annibale et al., 1996; Nagai et al., 2002 ; Eggert et al., 1996, 1995 ;

	Pycnoporus coccineus *Volvariella volvacea*	Oda et al., 1991 ; Chen et al., 2004 ;
EDTA	*Chaetomium termophilum* *Marasmius quercophilus* *Volvariella volvacea*	Chefetz et al., 1998; (Dedeyan et al., 2000; Farnet et al., 2004); Chen et al., 2004 ;
Kojic acid	*Daedalea quercina* *Lentinula edodes* *Pleurotus ostreatus*	Baldrian, 2004; (D'Annibale et al., 1996; Nagai et al., 2002); (Palmieri et al., 1997; Giardina et al., 1999; Pozdnyakova et al., 2004; Das et al., 2000)
NaF	*Dichomitus squalens* *Melanocarpus albomyces* *Sclerotium rolfsii* *Trametes trogii*	Perie et al., 1998; Kiiskinen et al., 2002; Ryan et al., 2003; Garzillo et al., 1998
NaN_3	*Botrytis cinerea* *Coriolus hirsutus* *Chaetomium termophilum* *Daedalea quercina* *Dichomitus squalens* *Ganoderma lucidum* *Lentinula edodes* *Melanocarpus albomyces* *Pleurotus ostreatus* *Phellinus ribis* *Pleurotus pulmonarius* *Sclerotium rolfsii* *Trametes sp. AH28-2* *Volvariella volvacea*	Zouari et al., 2002 ; Eggert et al., 1996; Ishigami et al., 1998 ; Baldrian, 2004; Perie et al., 1998; (Lalitha Kumari & Sirsi, 1972; Ko et al., 2001); (D'Annibale et al., 1996; Nagai et al., 2002); Kiiskinen et al., 2002; (Palmieri et al., 1997; Giardina et al., 1999; Das et al., 2000; Pozdnyakova et al., 2004;) Min et al., 2001; De Souza & Peralta, 2003; Ryan et al., 2003; Xiao et al., 2003; Chen et al., 2004 ;
Thiogylcolic acid	*Chaetomium termophilum* *Monocillium indicum* *Pleurotus ostreatus* *Phellinus ribis* *Sclerotium rolfsii* *Volvariella volvacea*	Ishigami et al., 1998 ; Thakker et al., 1992; (Palmieri et al., 1997; Giardina et al., 1999; Pozdnyakova et al., 2004; Das et al., 2000) ; Min et al., 2001; Ryan et al., 2003; Chen et al., 2004 ;

A very wide range of substrates has been shown to be oxidized by fungal laccases (Fig.1) but the catalytic constants have been reported mostly for a small group of substrates – e.g. the non-natural test substrate ABTS and the phenolic compounds 2,6-dimethoxyphenol (DMP), guaiacol and syringaldazine. Km ranges from 10s of mM for syringaldazine and ABTS to 100s of mM for DMP and guaiacol. The catalytic performance expressed as kcat spans several orders of magnitude for different substrates and is usually characteristic for a specific protein. Laccases in general combine high affinity for ABTS and syringaldazine with high catalytic constant, whereas the oxidation of guaiacol and DMP is considerably slower and the respective Km constants higher. Low Km values are typical for sinapic acid, hydroquinone and syringic acid, whereas relatively high values were found for para-substituted phenols, vanillic acid or its aldehyde. For the species capable of oxidizing polycyclic aromatic hydrocarbons or pentachlorophenol, only very low catalytic constants were detected for these xenobiotic compounds; the Km value is also high for pentachlorophenol with *T. versicolor* laccase. Valeriano *et al.,* (2009) reported that for laccase from *Stereum ostrea*, Km and Vmax values for the substrate guaiacol were found to be 13.25 mM and 255 nkat mg-1 of protein, respectively.

Table 2: Physical and biochemical Properties of Laccase

Species from which Laccase isolated	Substrate	MW (kDa)	(pI)	Km (µM)	pH	Temp (°C)	Reference
Coprinus cinereus	ABTS	58	4.0	26	4.0	60-70	Schneider *et al.*(1999)
Lentinula edodes Lcc1	ABTS	72	3.0	108	4.0	40	Nagai *et al.* (2002)
Marasmius quercophilus	ABTS	65		8	2.6	80	Farnet *et al.* (2004)
Melanocarpus albomyces	ABTS	80	4.0		3.5	65	Kiiskinen *et al.* (2002)
Pleurotus Ostreatus POXA 3b	ABTS	83-85	4.3	74	3.6	35	Palmieri *et al.* (2003)
Trametes gallica Lac 1	ABTS	60	3.1	12	2.2	70	Dong & Zhang (2004)
Volvariella volvacea	ABTS	58	3.7	30	3.0	45	Chen *et al.* (2004)
Botrytis cinerea	DMP	74	4.0	100	3.5	57	Slomczynski *et al.* (1995)
Coniothyrium minitans	DMP	74	4.0	100	3.5	60	Dahiya *Etal.*(1998)
Trametes gallica Lac II	DMP	60	3.0	410	3.0	70	Dong & Zhang (2004)
Pleurotus ostreatus POXA3a	DMP	83-85	4.1	14000	5.5	35	Palmieri *et al.*(2003)
Pleurotus ostreatus POXA2	DMP	67	4.0	740	6.5	25-35	Palmieri *et al.*(1997)
Cerrena maxima	Guaiacol	57-67	3.5	160 - 300		50	Shleev *et al.*(2004)
Pleurotus ostreatus POXC	Guaiacol	59	2.9	1200	6.0	50-60	Palmieri *et al.*(1997)
Thelephora terrestris	Guaiacol	66		121	4.8	45	Kanunfre & Zancan (1998)
Daedalea quercina	Syringaldazine	69	3.0	131	7.0	55	Baldrain (2004)
Magnaporthe grisea	Syringaldazine	70		118	6.0	30	Iyer & Chattoo (2003)
Marasmius quercophilus	Syringaldazine	60		4.2	4.5	80	Farnet *et al.(* 2004)
Pleurotus ostreatus POXA1w	Syringaldazine	61	6.7	130	6.0	45-65	Palmieri *et al.*(1997)

Table.3: Microorganisms with Isoenzymes and Molecular Mass

Organism	Isoenzymes	Molecular Mass (kDa)	References
Trametes multicolor	5	63	Leitner et al. 2002
Ganoderma lucidum	3	65-68	Ko et al. 2001
Podospora anserine	3	70 80 390	Thurston 1994
Ceriporiopsis subvermispora	2	71 68	Fukushima and Kirk 1995
Ganoderma lucidum	2	40 66	D'Souza et al.1999
Polyporus versicolor	2	60 ~65	Mosbach 1963
Pycnoporus cinnabarinus	1	81	Eggert et al.1996
Coriolus hirsutus	1	73	Shin and Lee 2000
Coriolus hirsutus	1	55	Koroljova –Skorobogat' Ko et al.1998
Chaetomium thermophilum	1	77	Chefetz et al. 1998
Pleurotus ostreatus	1	59	Sannia et al. 1986
Monocillium indicum	1	72	Thakker et al. 1992

8.0 Isoenzymes of Laccase:

Many laccase-producing fungi secrete isoforms of the same enzyme (Leontievsky et al.,1997). These isozymes have been found to originate from the same or different genes encoding for the laccase enzyme (Archibald et al., 1997). Some fungi produce isoenzymes with similar KM and kcat values. In wood-rotting basidiomycetes that are usually dikaryotic this fact probably indicates that allelic variability is responsible for the production of isoenzymes rather than the evolution of enzymes adapted to the special needs of the fungus. In the case of *P. ostreatus*, however, the isoenzymes show the KM and kcat values for 2,6-dimethoxyphenol or guaiacol differing by several orders of magnitude and the POXA1 isoenzyme is not active with guaiacol at all. The number of isoenzymes present differs between species and also within species depending on whether they are induced or non-induced. Isoenzymes can differ markedly in their stability, optimal pH and temperature, and affinity for different substrates (Assavanig et al., 1992); Heinzkill et al., 1998). Various laccase encoding gene sequences have been reported from a range of ligninolytic fungi. These sequences encode for proteins between 515 and 619 amino acid residues and close phylogenetic proximity between them is indicated by sequence comparisons (Bourbonnias et al., 1995).

9.0 Conclusions:

Laccases are ubiquitous in nature, being produced by wide variety of plants, fungi, and also bacteria. The functions of enzyme differ from organism to organism. Laccase plays an important role in carbon cycle and could help in degrading a wide range of xeno-aromatics. The problem with laccase is its low substrate specificity and a very wide range of reactions that it can potentially catalyze. Despite many efforts to address the involvement of laccase in the transformation of lignocellulose, it is still not completely clear how important a role laccase plays in lignin degradation and if it contributes more to the formation or decomposition of humic substances in soils. Hopefully, these questions will attract more attention of researchers in the future. Therefore, it is not surprising that this enzyme has been studied intensively and yet remains a topic of research today in environmental Biotechnology.

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