International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 07 Issue: 11 | Nov 2020 www.irjet.net p-ISSN: 2395-0072

A Review: Properties, Mode of Action and Applications of Fungal Laccase

Makwana Sonal[1], Pandya Nidhi[1], Kiransinh Rajput[2], Panchal Rakeshkumar[2*]

[1-2]*Department of Microbiology and Biotechnology, University School of Science, Gujarat University, Ahmedabad 380009, Gujarat, India.*

Abstract : Laccase belongs to the blue multi-copper oxidases, which are widely distributed in fungi and higher plants. It is present in Ascomycetes, Deuteromycetes, and Basidiomycetes and found abundantly in white-rot fungi. Laccase has its application in the area of textile, pulp and paper, and food industry because of their potent. It is used in biosensors for detection and removal of toxic pollutants, biofuel cells and medical diagnostics tool. Laccase is also being used as a bioremediation agent as they have been found in cleaning up herbicides pesticides. As they can oxidize phenolic, non-phenolic lignin-related compounds and highly fractious environmental pollutants, laccases have drawn the attention of researchers. Commercially, laccases have been used to determine the difference between codeine and morphine, produce ethanol and are also being employed in de-lignify woody tissues. To sustain this trend widespread availability of laccase and efficient production systems have to be developed. The present paper shows laccase properties and applications that have been developed to efficiently produce laccase at the industrial scale. The role of laccase in different food industries, particularly the recent developments in laccase application for food processing, is discussed.

1. Introduction

Lignin breakdown is thought to occur by concomitant action of ligninolytic enzymes. The main extracellular enzymes participating in lignin degradation are heme containing lignin peroxidase (LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13) and Copper-containing laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) reviewed by (Hatakka, 2001). A new group of ligninolytic heme-containing peroxidases, combining structural and functional properties of the lignin peroxidase (Lips) and manganese peroxidase (MNPs) was isolated from the white- rot fungi *Pleurotus eryngii*, *Bjerkandera adusta* (Heinfling *et al.,*1997, Wang *et al.,*2003) and *Bjerkandera*sp. BOS55 (Mester *et al.,*1996; Palma *et al.,*2000*) Pleurotus ostreatus* (Cohen *et al.,*2001).

2. Laccase

Laccase is a type of copper-containing polyphenol oxidase that was discovered in the exudates of the Japanese lacquer tree *Rhus vernicifera* (Yoshida,1883) and subsequently was demonstrated as a fungal enzyme (Bertrand, 1895). To date there is only one bacterium, *Azospirillum lipoferum* in which a laccase type of phenol oxidase has been demonstrated (Givaudan *et al.,*1993). Substrate oxidation by laccase is a one-electron reaction generating a free radical, which usually reacts further through non- enzymatic routes (Reinhammer and Malmstrom, 1981).

Bourbonnais and Paice (1992) have shown that the artificial laccase substrate ABTS (2,2'-azinobis-(3-ethylbenzthiazoline-6- sulphonate)) has the capacity to act as a mediator enabling the oxidation of non-phenolic lignin model compounds that are not laccase substrates on their own. The oxidation was the effect of a two-step process in which the enzyme first catalyzed the oxidation of primary substrate; the mediator and the oxidized mediator oxidized the secondary substrate, alkenes. The main reaction products of alkenes were the corresponding ketones or aldehydes. The reduction of oxygen to water is accompanied by the oxidation, typically of a phenolic substrate. Laccases are remarkably non-specific as to their reducing substrate and the range of substrates oxidized varies from one laccase to another. Simple diphenols like hydroquinone and catechol are good substrates for most laccases, but methoxy substituted monophenols like guaiacol and 2,6-dimethoxy phenol are often better, *p*-phenylenediamine (a diamine rather than a diphenol) (Assavanig *et al.,*1992) is a widely used substrate and syringaldazine is considered to be uniquely a laccase substrate (Assavanig *et al.,*1992; Chefetz *et al.,*1998).Oxidation of lignin by fungal laccase has been studied intensively since the early 1970's. Oxidation of milled wood lignin, de-methylation and formation of carboxylation were observed. A series of studies revealed that laccase could take part in many of these actions required for lignonlysis; Bourbonnais and Paice, 1992, in an *in vitro* system using pure enzymes from *Rigidioporus lignosus*, laccase and manganese peroxidase were found to act synergistically to degrade radiolabelled lignin. Pure laccase from *Coriolus versicolor* can provide Mn (III) chelators from Mn (II) in the presence of phenolic accessory substrates (Aust 1990).

Besides, playing a role in delignification, fungal laccases appear to be involved innumerous physiological functions including fruit body development, detoxification of phenolic compounds via oxidative coupling and polymerization (Thurston,1994), pathogenesis (Bar-Nun *et al.,*1989), protection form

plant defence compounds (Pezet *et al.,*1991). Laccases have also been found to be associated with specific stages of fungal morphogenesis and protecting the conidiospores from the toxic phenolics (Andersson and Henrysson, 1996). The laccase enzyme is widely distributed among plants, where it is implicated in wound response and the synthesis of lignin, the complex polymer that constitute the main component of the plant cell wall. But in fungi, particularly laccases of basidiomycetes are capable of mineralizing lignin which is apparently a unique feature in this group of organisms (Thurston,1994).

2.1 Properties of Laccases

Current knowledge about the structure and physico-chemical properties of fungal proteins is based on the study of purified proteins. Up to now, more than 100 laccases have been purified from fungi and been more or less characterized. Glycosylation of fungal laccase is believed to play a role in secretion, susceptibility to proteolytic degradation, copper retention and thermal stability (K. Li, F 1999). Upon purification, laccase enzymes demonstrate considerable heterogeneity. Glycosylation content and composition of fungal glycoproteins can vary with growth medium composition (Pickard, M. A. 1982).

The molecular mass of the laccase monomers ranges from 40 to 130 Da with a covalently linked carbohydrate content of 10–25% in fungi and 20- 45% in plants.The sugar composition has been analysed in several examples, such as *Podospora ansenna* and *Botrytis cinerea*, *Trametes hirsuta, Trametes ochracea, Cerrena maxima* and *Coriolopsis fulvocinerea* (S.V. Shleev 2004) and *Melanocarpus albomyces* (K. Piontek 2003). An important feature is a covalently-linked carbohydrate moiety (10–45) % of total molecular mass, depending on the species or the heterologous host, due the high level of glycosylation, which may contribute to the high stability of the enzyme. The carbohydrate moiety typically consists of mannose, N-acetylglucosamine and galactose, which may contribute to the high stability of the enzymes (Kunamneni A 2002).

2.2 Catalytic Properties

The list of substrates oxidized by laccases has increased significantly in recent years. The methoxy-or amino-monophenols and several non-phenolic compounds such as aromatic diamines, ABTS, 1-naphthol, hydroxyindoles and syringaldazine are laccase substrates (Cai *et al.,* 1993). Moreover, in the presence of ABTS, laccases are able to oxidize several compounds, which are not laccase substrates on their own (Bourbonnais and Paice, 1992). Fungal and plant laccases have been extensively described by several substrates and inhibitors acting on them, but little data on bacterial laccase activity has been reported (Faure *et al.,*1995).

Laccase catalyses the reduction of O_2 to H_2O using a range of phenolic compounds (though not tyrosine) as hydrogen donors (Thurston, 1994; Solomon *et al.,* 1996).Unfortunately, laccase shares a number of hydrogen donors with tyrosinase, making it difficult to assign unique descriptions to enzyme. A further complication is the overlap inactivity between aminophenol monooxygenase and catechol oxidase (1, 2-benzenediol: oxygen oxidoreductase, EC 1.10.3.1). The broad range of substrates accepted by laccase as hydrogen donors notwithstanding, oxidation of syringaldazine in combination with the inability to oxidize tyrosine, has been taken to be an indicator of laccase activity (Harkin *et al.,*1974; Thurston, 1994). Ideally, these should include substrates such as syringaldazine, ABTS or catechol, for which laccase has a high affinity, and some (e.g. Tyrosine) for which laccase has little or no affinity (Edens *et al.,*1990). In common with catechol oxidase and tyrosinase, laccase catalyzes 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 'biofuel cell' has been proposed to explain this complex mechanism (Thurston, 1994; Barriere *et al.,*2000).

Laccases belong to the group of blue multi copper oxidases that catalyze a one electron oxidation concomitantly with the four-electron reduction of molecular oxygen to water (Solomon *et al.,*2001). The substrates are oxidized by the T1 copper and the extracted electrons are transferred, probably through a strongly conserved His-Cys-Histripeptide motif, to the T2/ T3 site, where molecular oxygen is reduced to water (Messerschmidt, 1997). Some enzymes lack the T1 copper and some authors hesitate to call them true laccases. Others use the term 'yellow laccases' because these enzymes lack the characteristic absorption band around 600nm (Leontievsky *et al.,* 1997). Until recently, the three-dimensional structure of five fungal laccases has been reported: *Coprinus cinereus* (in a copper type-2-depleted form), *Trametes versicolor* (Bertrand *et al.,* 2009), *P. cinnabarinus* (Antorini *et al.,* 2002) and *R. lignosus* (Garavaglia *et al.,*2004), the latter four enzymes with a full complement of copper ions. Moreover, the three dimensional structure of the CoA laccase from *Bacillus subtilis* endospore has also recently been published (Enguita *et al.,* 2004).

2.3 Biochemical Properties of Laccase

The catalytic performance of laccases can be described by their activity and stability in different pH and temperature conditions. The pH activity profiles of laccases are often bell shaped with optima around 4-6 when measured with phenolic substrates (Chefetz *et*

*al.,*1998; Garzillo *et al.,*1998). The decrease in laccase activity in neutral or alkaline pH values is affected by increasing hydroxide anion inhibition because as a small anion, hydroxide ion is also a laccase inhibitor (Xu, 1997). The increasing pH decreases the redox potential of the phenolic substrate, which makes the substrate more susceptible to oxidation by laccase (Xu, 1997). In contrast to their activity stability of laccases is generally highest at pH values around 8-9 (Nizhizawa *et al.,*1995; Xu *et al.,*1996; Chefetz *et al.,*1998).Temperature stabilities of laccases vary considerably, depending on the source organism. In general, laccases are stable at 30-50°C and rapidly lose activity at temperatures above 60°C (Heinzkill *et al.,*1998; Schneider, 1999; Jung *et al.,*2002). The most thermostable laccases have been isolated from bacteria. The half-life of *Streptomyces lavendulae* Laccase was 100 minutes at 70°C and that of *Bacillus subtilis* CoA was 112 minutes at 80°C. The typical half-lives of fungal laccases are clearly below 1 hour at 70°C and below 10 minutes at 80°C (Wood *et al.,*1980; Jung *et al.,*2002; Palonen *et al.,*2003).

2.4 Structure of Catalytic Mechanism of Laccase

Laccases are defined in the enzyme commission nomenclature as oxido reductase, which oxidize diphenols and related substances and use molecular oxygen as an electron acceptor. The overall fold of laccase comprises of three cuperdoxin like domains A, B, and C that are of equal size. The cupredoxin fold is common among copper containing 40 proteins and it has also been found in the simple copper proteins plant plastocyanin and bacterial azurin as well as more complex multicopper oxidases as ascorbate oxidase ceruloplasmin. All three domains are important for the catalytic activity of laccases. A substrate-binding site is located in a cleft between domains B and C, a mononuclear copper center is located in domain C and a trinuclear copper center is located at the interface between domains A and C (Thurston, 1994).

In contrast to most enzymes which are generally very substrate specific, laccases act on a surprisingly broad range of substrates including diphenols, polyphenols, different substituted phenols, diamines, aromatic amines, benzenethiols and even some in organic compounds such as iodine (Xu, 1996). 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, dis proporatanatin and polymerization. Major substrates of laccase a rep-diphenol and the non-phenolic substrate ABTS (2, 2'-azinobis-(3-ethylbenz thiazoline-6-sulphonate)). Other well - known substrates are syringaldazine (3, 5-dimethoxy-4-hydroxybenzaldehyde azine), 1-naphthol, p-cresol (1-hydroxy-4- ethybenzene), 2, 6-dimethoxyphenol, and guaiacol (2- methoxyphenol).

The first step of catalytic cycle of laccases involves the formation of fully reduced laccase in which all four coppers are in a reduced state (Solomon *et al.,*1996); Molecular oxygen then oxidises the fully reduced laccase, presumably via a per oxy intermediate and is reduced to water. Oxidation of the peroxy intermediate generates oxygen activated native intermediate laccase in which all four coppers are in oxidized form and three trinuclear copper atoms are all bridged by hydroxide or oxo group (Lee *et al.,* 2004). The bridging makes the native intermediate prone to reduction and it can quickly enter another catalytic cycle (Fu and Viraraghavan, 2001).The most effective inhibitors of laccase are small anions especially azide, cyanide and fluoride ions, which bind to the electron flow (Solomon *et al.,* 1996; Xu, 1996. Other laccase inhibitors include EDTA, Fatty acids, coumaric acid, and (Bollag, 1992; Faure *et al.,* 1995; Sethuraman *et al.,* 1999; Xu, 2000; Jung *et al.,* 2002).

3. Mode of action

Laccase only attacks the phenolic subunits of lignin, leading to carbon oxidation, C- C cleavage and aryl-alkyl cleavage. Laccases are able to reduce one molecule of dioxygen to two molecules of water while performing one-electron oxidation of a wide range of aromatic compounds, which includes polyphenols (Bourbonnais, *et al.,* 1998), methoxy-substituted monophenols and aromatic amines (Archibald *et al.,* 1997; Bourbonnais *et al.,* 1995). This oxidation results in an oxygen-centred free radical, which can then be converted in a second enzyme-catalysed reaction to quinone. The quinone and the free radicals can then undergo polymerisation (Thurston, 1994). Laccases are similar to other phenol-oxidising enzymes which preferably polymerise lignin by coupling of the phenoxy radicals produced from oxidation of lignin phenolic groups. Owing to this specificity for phenolic subunits in lignin and its restricted access to lignin in the fibre wall, laccase has a limited effect on pulp bleaching. The substrate range of laccase can be extended to non-phenolic subunits of lignin by the inclusion of a mediator such as ABTS (2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (Bollagg *et al.,* 1988).In some fungi, the reactions of laccase are unrelated to ligninolysis. Laccase plays role in the morphogenesis and differentiation of sporulation and resting structures in basidiomycetes as well as lignin biodegradation of wood in white- rot fungi. It is responsible for pigment formation in mycelia and fruiting bodies, improves cell-to-cell adhesion, assists in the formation of rhizomorphs and is also responsible for the formation of polyphenolic glue that binds hyphae together. Various plant pathogens also produce extracellular laccases that enable the fungus to overcome the immune response of the host (Thurston, 1994). The laccase also facilitates the detoxification of the plant tissue via the oxidation of antifungal phenols or deactivation of phytoalexins (Assavanig *et al.,*1992; Robene-Soustrade and Lung-Escarmant, 1997).

3.1 The Laccase Mediator system

Non-chlorine bleaching of pulp with laccase was first patented in 1994 using an enzyme treatment to obtain a brighter pulp with low lignin content. Studies on bio bleaching with lignin modifying enzymes were, however, not successful until the discovery of mediators. The laccase was only successful in reducing the lignin content of pulps in the presence of the living fungus, which indicated that the enzyme alone is not responsible for delignification. According to the enzyme required an unknown substance present in the culture broth, which is probably some form of mediator (Call and Mucke, 1997). Although its mechanism is not yet fully understood, it is known that kraft pulp is delignified by laccase only in the presence of a mediator such as 2, 2-azino bis (3-ethylbenzthiazoline-6-sulphonate) (ABTS), but never by the laccase enzyme alone. The substrate range of laccase could be extended to non-phenolic subunits with the inclusion of primary mediators such as 1-hydroxybenzotriazole (HBT) (Chandrakant *et al.,*2011). The ABTS has the ability to act as a mediator for laccase, thereby enabling the oxidation of non-phenolic lignin compounds that are not laccase substrates. This mediator was found to prevent and even reverse polymerisation of kraft lignin and promotes the delignification of kraft pulp by laccase (Chen *et al.,*2004). Laccase-ABTS treatment can de lignify kraft pulp up to 40% under similar conditions to those currently used in kraft bleaching. Although ABTS is an effective mediator it was originally developed for analytical purposes (Bourbonnais *et al.,*1998) and its implementation as a mediator does not seem feasible, as it is estimated that the price would be too high, even if manufactured in bulk. It is postulated that the mediator molecules are converted to a reduced state in the presence of laccase. The mediator functions as an electron carrier that is able to diffuse into the secondary wall of wood fibres and react directly with the lignin, while the relatively large size of the laccase prevents it from diffusing into the cell walls. This enables laccase to oxidise veratryl alcohol to veratryl aldehyde and non-phenolic compounds to be cleaved or oxidized at the carbon position. (Lee, 1997).

4. Biotechnological applications of ligninolytic enzymes

Lignin-degrading enzymes have significant potential in industrial and biotechnological applications. The use of lignin-degrading enzymes in the pre-treatment of recalcitrant lignocellulosic biomass would provide an environmentally friendly alternative to biofuel production compared to the thermal and chemical pre-treatment techniques for the biofuel production (Wan & Li, 2012; Weng, Li, Bonawitz, & Chapple 2008). LiP, MnP, VP, and laccase can be used in the delignification and bioleaching of wood pulp to replace the traditional non-environmentally friendly chlorine-based delignification. They can also be applied in the decolorization of the dye wastewater from the textile industry. Research on the biotechnological applications of laccase and laccase/mediator system has attracted much attention recently due to their eco-friendly nature as they use oxygen as electron donor and produce water as the only reaction by-product (Riva, 2006). They have wide applications in the paper, textile, and food industries (Canas & Camarero, 2010).

Delignification of Lignocellulose

Production of ethanol as alternative fuel using lignocellulosic substrates as raw materials is one of the most desirable goals to overcome the fossil fuel crisis. The transformation of lignocellulose into ethanol is achieved in three steps: (a) delignification to release cellulose and hemicellulose from their complex with lignin, (b) depolymerisation of the carbohydrate polymers to produce free sugars, and (c) fermentation to ethanol using the liberated sugars. Biological treatment using white-rot fungi or other ligninolytic microorganisms including *Streptomyces* has been proposed, to replace the physicochemical treatments. Biological treatment can also be used for the removal of inhibitors prior to the fermentation. The advantages of using biological treatment include (i) mild reaction conditions, (ii) higher product yields, (iii) fewer side reactions, and (iv) less energy demand (Lee, 1997).

Biopulping and Biobleaching

Lignin removal is important in the pulping and paper industry. Bio pulping is the treatment of wood chips with lignin-degrading microorganisms to alter the lignin in the cell walls of wood, making the wood chips softer. This treatment not only improves paper strength and remove wood extractives but also reduces the energy consumption in the process of pulping. The production of pulp uses mechanical or chemical processes or a combination of the two processes. Pre-treatment of wood chips for mechanical and chemical pulping with white-rot fungi has been developed (Mendonça, Jara, González, Elissetche, & Freer, 2008). Laccases from white-rot fungi can be applied in bio pulping to partially degrade the lignin and therefore loosen lignin structures (Mendonça *et al.,* 2008). *Ceriporiopsis subvermispora* and *Pleurotus sp.* are fungi used in bio pulping (Pérez, Muñoz-Dorado, De La Rubia, & Martínez, 2002).

Bio bleaching is the bleaching of pulps using enzymes or ligninolytic fungi that reduce the amount of chemical bleach required to obtain a desirable brightness of pulps. Laccase-mediator system has been shown to possess the potential to substitute for chlorine-containing reagents. Laccases can also be applied as bio bleaching agents as they degrade lignin and decolorize the pulp (Call & Call, 2005). Laccase produced by *T. versicolor* has been studied for bioleaching of paper pulp and other industrial applications (Wesenberg, Kyriakides, Agathos,

2003). The role of lignin-degrading enzymes from *Streptomyces* in bio pulping and bioleaching has also been studied. Bio bleaching of eucalyptus Kraft pulp with *S.albus* culture supernatant in the presence of H_2O_2 resulted in a significant reduction of kappa number with no change in viscosity suggesting a potential application of *S.albus* in bioleaching (Antonopoulos, Hernandez, *et al.,*2001). *Streptomyces cyaneus* laccase was able to delignify kraft pulp with ABTS as a mediator, indicating the potential application of the laccases from *Streptomycetes* in bioleaching of Kraft lignin in the presence of synthetic mediators (Arias *et al.,*2003). Bio bleaching experiments carried out on *Eucalyptus globulus* Kraft pulps with *S. ipomoea* laccase in the presence of acetosyringone as a natural mediator also showed reduction in kappa number and increase of brightness without decreasing the viscosity values significantly (Eugenio *et al.,* 2011). These results suggest significant promise for the use of Streptomycetes lignin-degrading enzymes in industrial application. (Arias *et al.,*2003)

Textile Dye Transformation

The textile industry uses water as a medium for removing impurities, application of dyes and finishing agents. There is a significant water pollution associated with these processes due to the highly toxic dyes, bleaching agents, salt acids, and the alkali employed. LiP and MnP from the white-rot fungus *P. chrysosporium* have been investigated for their dye decolorization with the results showing the capacity to mineralize a variety of recalcitrant aromatic pollutants (Mehta, 2012). Decolorization of 23 industrial dyes by 16 white-rot fungi has also been investigated. The crude extracts of the cultures showed laccase, LiP, and aryl alcohol oxidase activities. However, only laccase activity was correlated with color removal (Rodríguez, Pickard, & Vazquez-Duhalt, 1999). Although some dyes are not degradable by laccases, many are oxidized by the enzyme and therefore initiating the destruction of the dyes (Schliephake, Mainwaring, Lonergan, Jones, & Baker, 2000).

An extensive review on the role of peroxidases in the treatment and decolorization of wide spectrum of aromatic dyes from polluted water can be found in the literature (Husain, 2010). Kirby, Marchant, and Mcmullan (2000) reported that laccase from *P. tremellosa* decolorized synthetic textile dyes. Also, laccases used in combination with mediators and cellobiose dehydrogenase were shown to be an ecofriendly alternative for chemical treatment of textile dye wastes (Ciullini, Tilli, Scozzafava, & Briganti, 2008).

Decolorization of Distillery Effluent and Waste Effluent Treatment

The characteristic of the dark brown appearance of distillery wastewater is mainly due to the high molecular weight organic compounds called melanoidin, a product of the Mallard reaction of sugars with proteins. The brown color is also due to the presence of phenolics from the feedstock, caramels from overheated sugars, and furfurals from acid hydrolysis (Kort, 1979). The detoxification and decolorization of this industrial waste is performed using oxidative enzymes (laccases and peroxidases) from bacteria, fungi, and yeast (Rajasundari & Murugesan, 2011). *Coriolus versicolor* was the first fungal strain shown to decolorize this type of waste (Watanabe, Sugi, & Tanaka, 1982). *P. chrisosporium* JAG-40 decolorized synthetic and natural melanoidin (Dahiya, Singh, & Nigam, 2001). Part of the treatment of these wastes includes the use of laccases and peroxidases that oxidize phenolic compounds to aryl-oxyradicals creating complexes that are insoluble. Other mechanisms carried out by these enzymes include the polymerization of the contaminants themselves or the copolymerization with other non-toxic substrates to facilitate their removal by sedimentation, adsorption, or filtration (Gianfreda, Iamarino, Scelza, & Rao, 2006; Rabinovich, Bolobova, & Vasil'chenko, 2004).

References

1. Archibald, F. S., Bourbonnais, R., Jurasek, L., Paice, M. G., & Reid, I. D. (1997). Kraft pulp bleaching and delignification by Trametes versicolor. Journal of biotechnology, 53(2-3), 215-236.
2. Arias, M. E., Arenas, M., Rodríguez, J., Soliveri, J., Ball, A. S., & Hernández, M. (2003). Kraft pulp biobleaching and mediated oxidation of a nonphenolic substrate by laccase from Streptomyces cyaneus CECT 3335. Applied and Environmental Microbiology, 69(4), 1953-1958.
3. Bertrand, Thomas, Claude Jolivalt, Pierre Briozzo, Eliane Caminade, Nathalie Joly, Catherine Madzak, and Christian Mougin. "Crystal structure of a four-copper laccase complexed with an arylamine: insights into substrate recognition and correlation with kinetics." Biochemistry 41, no. 23 (2002): 7325-7333
4. Bollag, J. M., Shuttleworth, K. L., & Anderson, D. H. (1988). Laccase-mediated detoxification of phenolic compounds. Applied and Environmental Microbiology, 54(12), 3086-3091.
5. Chandrakant, S, Rarigar & Shwetha Rao 2011, 'Role of Microbial Enzymes in the bioremediation of pollutants', Enzyme Research, vol.11, pp.1-11.
6. Chefetz, B., Chen, Y., & Hadar, Y. (1998). Purification and characterization of laccase from Chaetomium thermophilium and its role in

humification. Applied and Environmental Microbiology, 64(9), 3175-3179.
7. Chen, S., Ge, W., & Buswell, J. A. (2004). Biochemical and molecular characterization of a laccase from the edible straw mushroom, Volvariella volvacea. European Journal of Biochemistry, 271(2), 318-328.
8. Enguita, F. J., Marçal, D., Martins, L. O., Grenha, R., Henriques, A. O., Lindley, P. F., & Carrondo, M. A. (2004). Substrate and dioxygen binding to the endospore coat laccase from Bacillus subtilis. Journal of Biological Chemistry, 279(22), 23472-23476.
9. Enguita, F. J., Marçal, D., Martins, L. O., Grenha, R., Henriques, A. O., Lindley, P. F., & Carrondo, M. A. (2004). Substrate and dioxygen binding to the endospore coat laccase from Bacillus subtilis. Journal of Biological Chemistry, 279(22), 23472-23476.
10. Faure, D., Bouillant, M., & Bally, R. (1995). Comparative Study of Substrates and Inhibitors of Azospirillum lipoferum and Pyricularia oryzae Laccases. Applied and environmental microbiology, 61(3), 1144-1146.
11. Givaudan, A., Effosse, A., Faure, D., Potier, P., Bouillant, M. L., & Bally, R. (1993). Polyphenol oxidase in Azospirillum lipoferum isolated from rice rhizosphere: evidence for laccase activity in non-motile strains of Azospirillum lipoferum. FEMS Microbiology Letters, 108(2), 205-210.
12. Heinfling, A., Martínez, M. J., Martínez, A. T., Bergbauer, M., & Szewzyk, U. (1998). Purification and characterization of peroxidases from the dye-decolorizing fungus Bjerkandera adusta. FEMS Microbiology Letters, 165(1), 43-50.
13. Kunamneni, A., Ghazi, I., Camarero, S., Ballesteros, A., Plou, F. J., & Alcalde, M. (2008). Decolorization of synthetic dyes by laccase immobilized on epoxy-activated carriers. Process Biochemistry, 43(2), 169-178.
14. Lee, J. S., Lim, M. O., Cho, K. Y., Cho, J. H., Chang, S. Y., & Nam, D. H. (2006). Identification of medicinal mushroom species based on nuclear large subunit rDNA sequences. JOURNAL OF MICROBIOLOGY-SEOUL-, 44(1), 29.
15. Nishizawa, Y., Nakabayashi, K., & Shinagawa, E. (1995). Purification and characterization of laccase from white rot fungus Trametes sanguinea M85-2. Journal of fermentation and bioengineering, 80(1), 91-93
16. Thurston, C. F. (1994). The structure and function of fungal laccases. Microbiology, 140(1), 19-26.
17. Wesenberg, D., Kyriakides, I., & Agathos, S. N. (2003). White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnology advances, 22(1-2), 161-187.
18. Xu, F., Kulys, J. J., Duke, K., Li, K., Krikstopaitis, K., Deussen, H. J. W., & Schneider, P. (2000). Redox chemistry in laccase-catalyzed oxidation of N-hydroxy compounds. Applied and environmental microbiology, 66(5), 2052-2056.