LACCASE: AN EVER LASTING STORY

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Summary

Laccases have received much attention from researchers in last decade due to their ability to oxidize both phenolic and non-phenolic lignin related compounds which makes them very useful for their application to several processes. Laccases have been found to be applicable in textile, food, pulp and paper industries. These can be used in immunoassay, as biosensors as well as biolinkers, degradation of xinobiotics and bio-remediation, organic synthesises, cosmetics and antimicrobials. The present review describes the properties and applications if laccases.

Introduction

Current research in the modern microbiology is focused on separation, synthesis or application of various useful enzymes. Eventually, there are very few enzymes which have got potential biotechnical and industrial applications such as pectinases, chitinases lipases, laccases etc. Laccases (EC 1.10.3.2, p-diphenol:dioxygen oxidoreductase) belong to the so-called blue-copper family of oxidases. Laccases are glycoproteins, which are abundunt in nature – they have been reported in higher plants and virtually every fungus that has been examined for them. Yoshida described the enzyme of this group for the first time at the end of the 19th century as a component of the resin ducts of the lacquer tree *Rhus vernicifera* (1); more recently, proteins with features typical of laccases have been identified in insects and prokaryotes and algae (2, 3). Though the laccases from different origin are different from each other, they all catalyse polymerization or depolymerization processes. They are an important virulence factor in many fungal diseases as these enzymes can protect fungal pathogens from toxic phytoalexins and tannins (4).

The reactions catalysed by laccases go on by the monoelectronic oxidation of a suitable substrate molecule (phenols and aromatic or aliphatic amines) to the corresponding reactive radical. The redox process takes place with the assistance of a cluster of four copper atoms that form the catalytic core of the enzyme; they also confer the typical blue colour to these enzymes because of the intense electronic absorption of the Cu–Cu linkages (5). The overall outcome of the catalytic cycle is the reduction of one molecule of oxygen to two molecules of water and the concomitant oxidation of four substrate molecules to produce four radicals (6). These reactive intermediates can then produce dimers, oligomers and polymers.

The simplest case is the one in which the substrate molecules are oxidized to the corresponding radicals by direct interaction with the copper cluster.

The high redox potential of substrate or their large size to penetrate into the enzyme active site makes it difficult to be oxidized directly by the laccases. "Chemical mediators" can be added that act as intermediate substrates for the laccase to overcome this limitation, whose oxidized radical forms are able to interact with the bulky or high redox-potential substrate targets.

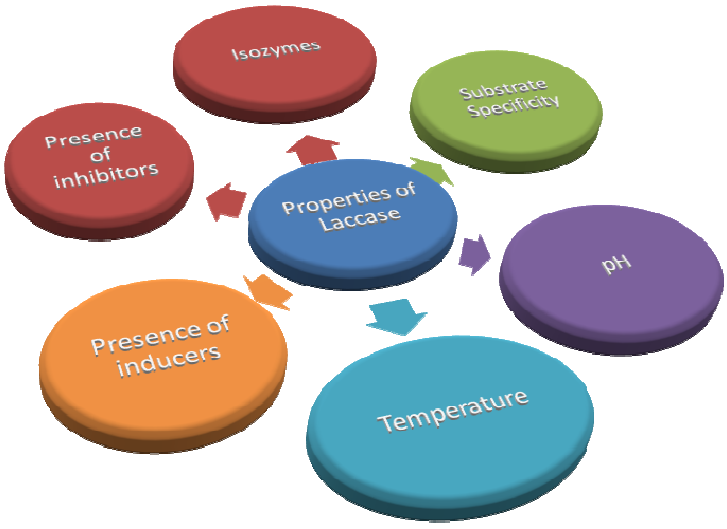
Occurrence and location of laccases

Laccases are found in a wide range of higher plants and fungi (7) and previously some bacterial laccases have also been characterized from *Azospirillum lipoferum* (8), *Bacillus subtilis* (9), *Streptomyces lavendulae* (10), *S. cyaneus* (11) and *Marinomonas mediterranea* (12) and recently some soil algae (3). Laccases in plants have been identified in trees, cabbages, turnips, beets, apples, asparagus, potatoes, pears, and various other vegetables (13). The occurrence of laccases in higher plants appears to be far more limited than in fungi. The classical demonstration of laccase in *R. vernicifera* is well documented. In addition, the lacquer tree is a member of the Anacardiaceae family, appear to contain laccase in the resin ducts and in the secreted resin (14). Further, eight types of laccases have been found to be expressed in xylem tissue in cell cultures of *Acer pseudoplatanus* (15). Other reports on the presence of a laccase in leaves of *Aesculus parviflora* and in green shoots of tea (16). Laccases have been isolated from Ascomyceteous, Deuteromyceteous and Basidiomyceteous fungi (17). The peroxidative activity of laccases has been first laccase to be characterized from *Monocillium indicum* (18). The white-rot basidiomycetes are the most efficient degraders of lignin and also the most widely studied. The enzymes implicated in lignin degradation are: lignin peroxidase (catalyses the oxidation of both phenolic and non-phenolic units), manganese-dependent peroxidase, (iii) laccase (which oxidises phenolic compounds to give phenoxy radicals and quinines) glucose oxidase and glyoxal oxidase (for H_2O_2 production) and cellobiose-quinone oxidoreductase (for quinone reduction) are the enzymes implicated in lignin degradation (18). The veratryl alcohol oxidase and some esterases may also play roles in the complex process of natural wood decay.

The different degrees of lignin degradation with respect to other wood components depend on the environmental conditions and the fungal species involved. There is no exclusive mechanism to achieve the process of lignin degradation and that the enzymatic machinery of the various microorganisms differs. For instance, *Pleurotus ostreatus,* belongs to a subclass of lignin–degrading microorganisms that produce laccase, manganese peroxidase and veratryl alcohol oxidase but no lignin peroxidase (19). Ligninolytic enzymes have mostly been reported to be extracellular but there is evidence that intracellular laccases are present in white–rot fungi (20). Froehner and Eriksson identified intracellular as well as extracellular laccases for *Neurospora crassa*, and suggested that the intracellular laccase function as a precursor for extracellular laccase as there were no differences between the two laccases other than their occurrence (21).

Properties of the laccase

Figure 1



Factors influencing properties of enzymes

Factors influencing the properties of laccase enzymes

Laccases from the fungal origin are monomeric, dimeric or tetrameric glycoproteins. Glycosylation of fungal laccase is believed to play a pivotal role in secretion, susceptibility to proteolytic degradation, copper retention and thermal stability (22). Laccase enzymes demonstrate considerable heterogeneity, even in purified form. Glycosylation content and composition of fungal glycoproteins can vary with growth medium composition. The hetrogenity in the data is because of the above reasons. The molecular mass of the monomer ranges from about 50 to 100 kDa. An important feature is a covalently-linked carbohydrate moiety (10–45% of total molecular mass), which may contribute to the high stability of the enzyme (23)..

Isozymes

Many laccase producing fungi secrete isoforms of the same enzyme (24) which have been found to originate from the same or different genes encoding for the laccase enzyme (25). The number of isozymes can be distinguished between species and also within species depending on whether they are induced or non-induced (17). They can differ markedly in their stability, optimal pH and temperature and affinity for different substrates (26). Furthermore, these different isozymes can modulate various roles in the physiology of different species or in the same species under different conditions (17). Various laccase encoding gene sequences have been identified from a range of ligninolytic fungi; these sequences encode for proteins between 515 and 619 amino acid residues and close phylogenetic proximity between them. (27).

Substrate specificity of laccase

Laccases are remarkably non-specific as to their reducing substrates, and the range of substrates oxidised varies from one laccase to another. These enzymes catalyse the one-electron

oxidation of a wide variety of organic and inorganic substrates, including polyphenols, methoxy-substituted phenols, aromatic amines and ascorbate with the concomitant four-electron reduction of oxygen to water (28). Laccase has broad substrate specificity towards aromatic compounds containing hydroxyl and amine groups, and as such, the ability to react with the phenolic hydroxyl groups found in lignin. Kinetic data of laccases from different sources have been reported (29). The source of enzyme and substrate/type of reaction are responsible for heterogenous turn over.. The kinetic constants differ in their dependence on pH. K_m is pH-independent for both substrate and co-substrate, while K_{cat} is pH-dependent.

Influence of pH

The optimum pH for laccases are highly dependable on the substrate. In acidic medium the pH optima are found in the range 3.0-5.0 (26). Mostly, optimal pH that varies considerably has got a bell shaped profile, The substrate, oxygen or the enzyme itself are the main causes of these variations(30). The difference in redox potential between the phenolic substrate and the T1 copper could increase oxidation of the substrate at high pH values, but the hydroxide anion (OH^-) binding to the T2/T3 coppers results in an inhibition of the laccase activity due to a disruption of the internal electron transfer between the T1 and T2/T3 centres. These two opposing effects can play an important role in determining the optimal pH of the bi-phasic laccase enzymes (30). This could be understood with the example that laccase produced by *Trametes modesta* was fully active at pH 4.0 and very stable at pH 4.5 but its half-life decreased to 125 min at pH 3.0 (31).

Influence of temperature

The optimal temperature of laccase can differ greatly strain by strain. The laccases isolated from a strain of *Marasmius quercophilus* (32) were found to be stable for 1 h at 60°C. Farnet *et al*. (32) further found that pre-incubation of enzymes at 40°C and 50°C greatly increased laccase activity.

The laccase from *P. ostreatus* is almost fully active in the temperature range of 40-60°C, with maximum activity at 50°C. The activity remains unaltered after prolonged incubation at 40°C for more than 4 h (33). Nyanhongo *et al*. showed that laccase produced by *T. modesta* was fully active at 50°C and was very stable at 40°C but half-life decreased to 120 min at higher temperature (60°C) (31). Immobilisation of the enzyme on the glass powder can be used to increase the stability of laccase which can be used in specific biotechnological applications where the stability is essentially required (34).

Influence of Inducers

Laccase production can be remarkably increased and induced by the addition of various compounds like copper sulphate, ethanol, cellobiose and by the addition of xenobiotics, for example, 2,5-xylidine, veratryl alcohol, lignin etc. Some of these compounds affect the growth rate or metabolism, while others, like ethanol trigger laccase production indirectly. The promoter regions of the genes encoding for laccase contain various recognition sites that are specific for xenobiotics and heavy metals (35). These can bind to the recognition sites when present in the substrate and induce laccase production. Certain inducers, when added, can increase the concentration of a specific laccase or induce the production of new isoforms of the enzyme. Rest others can interact variably with different fungal strains.

Influence of Inhibitors

Many ions such as azide, halides, cyanide, thiocyanide, fluoride and hydroxide bind to the type 2 and type 3 Cu, resulting in the interruption of internal electron transfer and accordingly therefore inhibition of activity. In general, laccases respond to several inhibitors of enzyme activity in similar fashion(36). Other inhibitors include metal ions (e.g. Hg^{2+}), fatty acids, sulfhydryl reagents, hydroxyglycine, kojic acid, desferal and cationic quaternary ammonium detergents, the reactions with

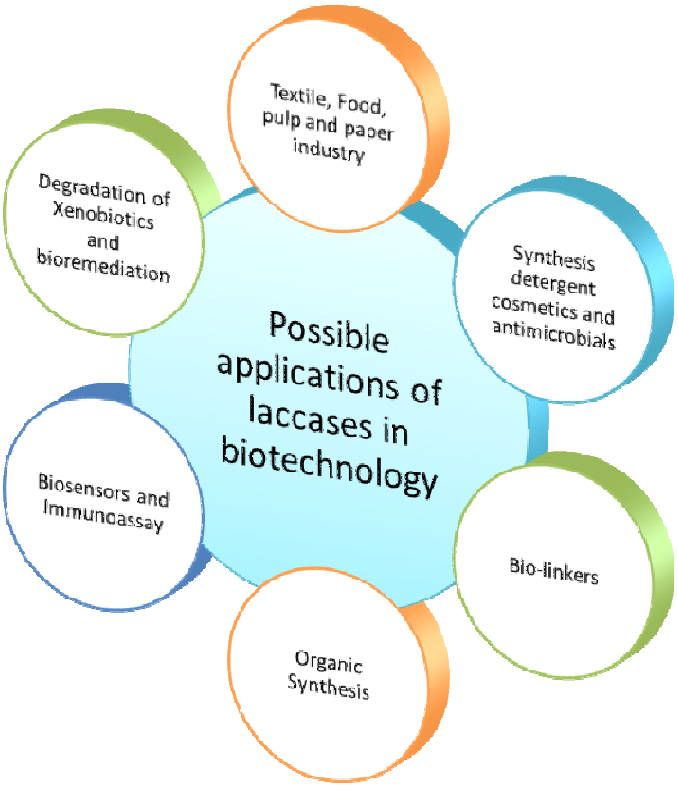
which may involve amino acid residue modifications, confirmational changes or Cu chelation (37). The state of oxidation of the copper atoms is the key factor for conformational changes which makes it sensitive towards chelating agents. The selective removal of Cu by chelating agents (EDTA, dimethyl glyoxime, N,N'-dimethyldithiocarbamate, NTA) leads to a loss of catalytic activity.

Application of laccase

Non-specific and undesirable side-reactions and use of environmentally hazardous chemicals are the major drawbacks of most of the conventional oxidation technologies to be used for the insepaerable oxidation reactions in several industries.. Necessity of a new technology made the inventions and has further impelled the search for new oxidation technologies based on biological systems such as enzymatic oxidation. Enzymes are specific and biodegradable catalysts and enzymes reactions are carried out in mild conditions are some of the many advantages of the biological system based oxidation technologies over the chemical ones. A diagrammatic presentation of applications of laccases in different fields has been summarized in figure 2.

Enzymatic oxidation techniques have potential within a great variety of industrial fields including the pulp and paper, textile and food industries. Enzymes recycling on

Figure 2



Possible applications of laccases in Biotechnology

molecular oxygen as an electron acceptor are the most interesting ones. Thus, laccase is in particular is the enzyme of choice for the above-mentioned purposes. . This enzyme catalyses the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins and aryl diamines as well as some inorganic ions coupled to the reduction of molecular dioxygen to water (29). The availabilty of small molecules capable to act as electron transfer mediators, laccases were also able to oxides even those molecules with more redox potentials than that of laccases, for example,laccases were also able to oxidise non-phenolic structures , expanding, thus, the range of compounds that can be oxidised by these enzymes (38). In many of the industrial processes such as pulp delignification (22) oxidation of organic pollutants (39) and the development of biosensors or biofuel cells (40) laccase-mediated systems (LMS) have been imployed . Claus et al. found that the LMS enhanced dye decolourization

and some dyes resistant to laccase degradation were decolourised (41). Lu and Xia have recently reviewed the applications of the LMS, which comprise pulp bleaching, textile biofinishing and environmental protection processes (42). However, despite that LMS has been studied extensively there are still unsolved problems concerned with mediator recycling, cost and toxicity.

Food industry

In the food industry, laccases can be applied to certain processes that enhance or modify the colour appearance of food or beverage. Along the linea significant application of laccase in the food industry is the elimination of undesirable phenolics, responsible for the browning, haze formation and turbidity development in clear fruit juice, beer and wine. In baking industry the ability of laccases to cross-link biopolymers has been exploited. A laccase from the white-rot fungus *Trametes hirsuta* increased the maximum resistance of dough and decreased the dough extensibility in both flour and gluten dough (43) is one of those examples where this property has been revealed. Minussi et al. have described the potential applications of laccase in different aspects of the food industry such as bioremediation, beverage processing, ascorbic acid determination, sugar beet pectin gelation, baking and as a biosensor (44). Above all the further studies of laccase production and immobilization technique should be focused at lower costs to improve the industrial application of this enzyme.

Pulp and paper industry

The separation and degradation of lignin in wood pulp is required for the industrial preparation of paper. Environmental concerns advocate to replace conventional and polluting chlorine-based delignification/bleaching procedures. Oxygen delignification processes have been industrially introduced, but pre-treatments of wood pulp with ligninolytic enzymes might provide milder and cleaner strategies of delignification however oxygen delignification process is industrially accepted (45).

Although extensive studies have been performed to develop alternative bio-bleaching systems, few enzymatic treatments exhibit the delignification/brightening capabilities of modern chemical bleaching technologies. Laccase is more readily available and easier to manipulate than both lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) and LMS has already found practical applications such as the Lignozym®-process (38). Several authors applied the LMS to pulp biobleaching. In this sense, Camarero et al. explored the potential of LMS to remove lignin-derived products responsible for colour from a high-quality flax pulp. They showed the feasibility that chlorine-containing reagents can be substituted with LMS in manufacturing of these high-price paper pulps (46).

The capability of laccases to form reactive radicals in lignin can also be used in targeted modification of wood fibers. The enzymatic adhesion of fibers in the manufacturing of lignocellulose based composite materials such as fiberboards can be done by laccases. Laccases have been proposed to activate the fiberbound lignin during manufacturing of the composites, thus, resulting in boards with good mechanical properties without toxic synthetic adhesives (14). Another possibility is to functionalise lignocellulosic fibers by laccases in order to improve the chemical or physical properties of the fiber products. Preliminary results have shown that laccases are able to graft various phenolics acid derivatives onto kraft pulp fibers (47).

Textile industry

The textile industry accounts for two-thirds of the total dyestuff market (48) and consumes large volumes of water and chemicals for wet processing of textiles. The chemical reagents used are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products (49). The chemical structure of dyes made them resistant to fading on exposure to light, water and different chemicals and most of them are difficult to decolourise due to their synthetic

origin (50). As several dyes are made from known carcinogens such as benzidine and other aromatic compounds government legislation is becoming more and more stringent, especially in the more developed countries, regarding the removal of dyes from industrial effluents (51). . Most currently existing processes to treat dye wastewater are ineffective and not economical (52, 53). Due to the potential in degrading dyes of diverse chemical structure (54), including synthetic dyes currently employed in the industry, laccases has been the hot cake in the development of such processes (55).. The use of laccase in the textile industry is growing very fast, since besides to decolourise textile effluents as commented above, laccase is used to bleach textiles and even to synthetize dyes (56). In 1996 Novozyme (Novo ordisk, Denmark) launched a new industrial application of laccase enzyme, related to textile bleaching, in denim finishing: DeniLite®, the first industrial laccase and the first bleaching enzyme acting with the help of a mediator molecule. In 2001 the company Zytex (Zytex Pvt. Ltd., Mumbai, India) has also been developed a formulation based on LMS capable of degrading indigo in a very specific way. The trade name of the product is Zylite.

Nanobiotechnology

Bioelectochemistry has been the talk of the day during the past two dacades. Progress on bioelectrochemistry has been integrated into analytical applications, e.g. in biosensors working as detectors in clinical and environmental analysis (57). As laccases cancatalyse electron transfer reactions without additional cofactors, their use has also been studied in biosensors to detect various phenolic compounds, oxygen or azides. furthermore, biosensors for detection of morphine and codeine, catecholamines, plant flavonoids and also for electroimmunoassay have been developed (58, 59). Nanotechnology aims to the development of smaller and more efficient biosensors achieving micro and nanometer order, through controlled deposition and specific adsorption of biomolecules on different types of surfaces. . on the subject of laccases, the immobilisation has an important influence

on the biosensor sensitivity (60). Micropatterning had been an efficient method for the immobilization of laccases in order to develop a multi-functional biosensor (61). The cross linked enzyme crystals (CLEC) of the laccases could be used in biosensor applications with great advantage over the soluble enzyme (62). immobilization of laccases from *Coriolus versicolor* on N-Hydroxysuccinimide-terminated self-assembled monolayers on gold (63)could be useful for the further development of biosensors. Additionally ultrasensitive amperometric detection of the catecholamine neurotransmitters dopamine, epinephrine and norepinephrine is made possible by, an enzyme electrode based on the co-immobilisation of an osmium redox polymer and a laccase from *T. versicolor* on glassy carbon electrodes, attaining nanomolar detection limits (64). Laccase can also be immobilized on the cathode of biofuel cells that could provide power, for example, for small transmitter systems. Besides, biofuel cells are extremely attractive from an environmental point of view because electrical energy is generated without combusting fuel, thus, providing a cleaner source of energy. Surfaces of well defined thikness can be made possible by The layer-by-layer technique (LbL).

Other laccase applications

Soil bioremediation

The catalytic properties of the laccase can be attributed to the degradation of many compounds like polycyclic aromatic hydrocarbons (PAHs) together with other xenobiotics which are a major source of contamination in soil and whose degradation is of great importance for the environment. . Detoxification of the munition residue by laccase is because of the laccases mediate coupling of reduced 2,4,6-trinitrotoluene (TNT) metabolites to an organic soil matrix.(65). Furthermore, PAHs, which arise from natural oil deposits and utilisation of fossil fuels, were also found to be degraded by laccases (66). The application of laccases in the immobilization of TNT degradation products has also been revealed(31).

Synthetic chemistry

Laccases may also be of great interest in synthetic chemistry in future, where they have been proposed to be applicable for oxidative deprotection (67) and production of complex polymers and medical agents (68, 69). Suberase® (Novo Nordisk A/S, Bagsvaerdt, Denmark) is an industrial analogue of laccase has been used in the synthesis of phenolic colourants(70).

Cosmetics

Laccases are second to none in the application in cosmetic world.Laccase-based hair dyes is one of the example which as compare to other commercially available hair dyes are less irritant and easier to handle. The science behind is just the replacement of irritant H_2O_2 used as an oxidising agent in the dye formulation with laccase (71). More recently, cosmetic and dermatological preparations containing proteins for skin lightening have also been developed (72).

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References

1. Yoshida H: Chemistry of lacquer (urushi). J Chem Soc 1883; 43:472-486.

2. Claus H: Laccases and their occurrence in prokaryotes. Arch Microbiol 2003; 179:145-150.

3. Otto B, Schlosser D, Reisser W: First description of a laccase-like enzyme in soil algae. Arch Microbiol 2010; 192:759-768.

4. Zhu X, Williamson PR: Role of laccase in the biology and virulence of cryptococcus neoformans. FEMS Yeast Res 2004; 5:1-10.

5. Piontek K, Antorini M, Choinowski T: Crystal structure of a laccase from the fungus trametes versicolor at 1.90-a resolution containing a full complement of coppers. J Biol Chem 2002; 277:37663-37669.

6. Claus H: Laccases: Structure, reactions, distribution. Micron 2004; 35:93-96.

7. Kiiskinen LL, Palonen H, Linder M et al.: Laccase from melanocarpus albomyces binds effectively to cellulose. FEBS Lett 2004; 576:251-255.

8. Diamantidis G, Effosse A, Potier P et al.: Purification and characterization of the first bacterial laccase in the rhizospheric bacterium azospirillum lipoferum. Soil Biology and Biochemistry 2000; 32:919-927.

9. Martins LO, Soares CM, Pereira MM et al.: Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the bacillus subtilis endospore coat. J Biol Chem 2002; 277:18849-18859.

10. Suzuki T, Endo K, Ito M et al.: A thermostable laccase from streptomyces lavendulae ren-7: Purification, characterization, nucleotide sequence, and expression. Biosci Biotechnol Biochem 2003; 67:2167-2175.

11. Arias ME, Arenas M, Rodriguez J et al.: Kraft pulp biobleaching and mediated oxidation of a nonphenolic substrate by laccase from streptomyces cyaneus cect 3335. Appl Environ Microbiol 2003; 69:1953-1958.

12. Jimenez-Juarez N, Roman-Miranda R, Baeza A et al.: Alkali and halide-resistant catalysis by the multipotent oxidase from marinomonas mediterranea. J Biotechnol 2005; 117:73-82.

13. Levine WG: Laccase: A review. The biochemistry of copper: proceedings, September 8-10, 1965 1966:371.

14. Huttermann A, Mai C, Kharazipour A: Modification of lignin for the production of new compounded materials. Appl Microbiol Biotechnol 2001; 55:387-394.

15. Sato Y, Wuli B, Sederoff R et al.: Molecular cloning and expression of eight laccase cdnas in loblolly pine (pinus taeda)*. J Plant Res 2001; 114:147-155.

16. Gregory RP, Bendall DS: The purification and some properties of the polyphenol oxidase from tea (camellia sinensis l.). Biochem J 1966; 101:569-581.

17. Assavanig A, Amornikitticharoen B, Ekpaisal N et al.: Isolation, characterization and function of laccase from trichoderma. Appl Microbiol Biotechnol 1992; 38:198-202.

18. Thakker GD, Evans CS, Rao KK: Purification and characterization of laccase from monocillium indicum saxena. Appl Microbiol Biotechnol 1992; 37:321-323.

19. Palmieri G, Giardina P, Bianco C et al.: A novel white laccase from pleurotus ostreatus. J Biol Chem 1997; 272:31301-31307.

20. Schlosser D, Grey R, Fritsche W: 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 1997; 47:412-418.

21. Froehner SC, Eriksson KE: Purification and properties of neurospora crassa laccase. J Bacteriol 1974; 120:458-465.

22. Li K, Xu F, Eriksson KE: Comparison of fungal laccases and redox mediators in oxidation of a nonphenolic lignin model compound. Appl Environ Microbiol 1999; 65:2654-2660.

23. Durán N, Rosa MA, D'Annibale A et al.: Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: A review. Enzyme Microb Technol 2002; 31:907-931.

24. Leontievsky A, Myasoedova N, Pozdnyakova N et al.: 'yellow' laccase of panus tigrinus oxidizes non-phenolic substrates without electron-transfer mediators. FEBS Lett 1997; 413:446-448.

25. Archibald FS, Bourbonnais R, Jurasek L et al.: Kraft pulp bleaching and delignification by trametes versicolor. J Biotechnol 1997; 53:215-236.

26. Heinzkill M, Bech L, Halkier T et al.: Characterization of laccases and peroxidases from wood-rotting fungi (family coprinaceae). Appl Environ Microbiol 1998; 64:1601-1606.

27. Bourbonnais R, Paice MG, Reid ID et al.: Lignin oxidation by laccase isozymes from trametes versicolor and role of the mediator 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization. Appl Environ Microbiol 1995; 61:1876-1880.

28. Thurston CF: The structure and function of fungal laccases. Microbiology 1994; 140:19.

29. Yaropolov AI, Skorobogat'Ko OV, Vartanov SS et al.: Laccase: Properties, catalytic mechanism, and applicability. Appl Biochem Biotechnol 1994; 49:257-280.

30. Xu F: Effects of redox potential and hydroxide inhibition on the ph activity profile of fungal laccases. J Biol Chem 1997; 272:924-928.

31. Nyanhongo GS, Gomes J, Gubitz G et al.: Production of laccase by a newly isolated strain of trametes modesta. Bioresour Technol 2002; 84:259-263.

32. Farnet AM, Criquet S, Tagger S et al.: Purification, partial characterization, and reactivity with aromatic compounds of two laccases from marasmius quercophilus strain 17. Can J Microbiol 2000; 46:189-194.

33. Palmeiri G, Giardina P, Marzullo L et al.: Stability and activity of a phenol oxidase from the ligninolytic fungus pleurotus ostreatus. Appl Microbiol Biotechnol 1993; 39:632-636.

34. Rasera K, Ferla J, Dillon AJP et al.: Immobilization of laccase from pleurotus sajor-caju in polyamide membranes. Desalination 2009; 245:657-661.

35. Demir G: Degradation of toluene and benzene by trametes versicolor. J Environ Biol 2004; 25:19-25.

36. Bollag JM, Leonowicz A: Comparative studies of extracellular fungal laccases. Appl Environ Microbiol 1984; 48:849-854.

37. Gianfreda L, Xu F, Bollag JM: Laccases: A useful group of oxidoreductive enzymes. Bioremediation Journal 1999; 3:1-26.

38. Call HP, Mücke I: History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems (lignozym®-process). J Biotechnol 1997; 53:163-202.

39. Collins PJ, Kotterman M, Field JA et al.: Oxidation of anthracene and benzo[a]pyrene by laccases from trametes versicolor. Appl Environ Microbiol 1996; 62:4563-4567.

40. Palmore GTR, Kim HH: Electro-enzymatic reduction of dioxygen to water in the cathode compartment of a biofuel cell. J Electroanal Chem 1999; 464:110-117.

41. Claus H, Faber G, Konig H: Redox-mediated decolorization of synthetic dyes by fungal laccases. Appl Microbiol Biotechnol 2002; 59:672-678.

42. Lu R, Xia L-M: Advances in research and application of laccase mediator system [j]. J Cellulose Sci Tech 2004; 12:37-44.

43. Selinheimo E, Kruus K, Buchert J et al.: Effects of laccase, xylanase and their combination on the rheological properties of wheat doughs. J Cereal Sci 2006; 43:152-159.

44. Minussi RC, Pastore GM, Duran N: Potential applications of laccase in the food industry. Trends Food Sci Tech 2002; 13:205-216.

45. Kuhad RC, Singh A, Eriksson KEL: Microorganisms and enzymes involved in the degradation of plant fiber cell wall. In: Eriksson kel, editor. Biotechnology in the pulp and paper industry. Advances in biochemical engineering biotechnology, Berlin: Springer Verlag,Chapter 2, 1997.

46. Camarero S, Garcia O, Vidal T et al.: Efficient bleaching of non-wood high-quality paper pulp using laccase-mediator system. Enzyme Microb Technol 2004; 35:113-120.

47. Chandra RP, Ragauskas AJ: Evaluating laccase-facilitated coupling of phenolic acids to high-yield kraft pulps. Enzyme Microb Technol 2002; 30:855-861.

48. Riu J, Schönsee I, Barceló D: Determination of sulfonated azo dyes in groundwater and industrial effluents by automated solid-phase extraction followed by capillary electrophoresis/mass spectrometry. J Mass Spectrometry 1998; 33:653-663.

49. Ramalho PA, Cardoso MH, Cavaco-Paulo A et al.: Characterization of azo reduction activity in a novel ascomycete yeast strain. Appl Environ Microbiol 2004; 70:2279.

50. Mohanty K, Naidu JT, Meikap BC et al.: Removal of crystal violet from wastewater by activated carbons prepared from rice husk. Ind Eng Chem Res 2006; 45:5165-5171.

51. O'Neill C, Hawkes FR, Hawkes DL et al.: Colour in textile effluents-sources, measurement, discharge consents and simulation: A review. J Chem Tech Biotech 1999; 74:1009-1018.

52. Allen SJ, Khader KYH, Bino M: Electrooxidation of dyestuffs in waste waters. J Chem Tech Biotech 1995; 62:111-117.

53. Cooper P: Removing colour from dyehouse waste waters—a critical review of technology available. J Soc Dyers Colour 1993; 109:97-100.

54. Blanquez P, Casas N, Font X et al.: Mechanism of textile metal dye biotransformation by trametes versicolor. Water Res 2004; 38:2166-2172.

55. Rodriguez Couto S, Sanromán MA: Application of solid-state fermentation to ligninolytic enzyme production. Biochemical Engineering Journal 2005; 22:211-219.

56. Setti L, Giuliani S, Spinozzi G et al.: Laccase catalyzed-oxidative coupling of 3-methyl 2-benzothiazolinone hydrazone and methoxyphenols. Enzyme Microb Technol 1999; 25:285-289.

57. Haghighi B, Gorton L, Ruzgas T et al.: Characterization of graphite electrodes modified with laccase from trametes versicolor and their use for bioelectrochemical monitoring of phenolic compounds in flow injection analysis. Anal Chim Acta 2003; 487:3-14.

58. Jarosz-Wilkoazka A, Ruzgas T, Gorton L: Use of laccase-modified electrode for amperometric detection of plant flavonoids. Enzyme Microb Technol 2004; 35:238-241.

59. Kuznetsov, Shumakovich GP, Koroleva OV et al.: On applicability of laccase as label in the mediated and mediatorless electroimmunoassay: Effect of distance on the direct electron transfer between laccase and electrode. Biosens Bioelectron 2001; 16:73-84.

60. Freire RS, Duran N, Kubota LT: Effects of fungal laccase immobilization procedures for the development of a biosensor for phenol compounds. Talanta 2001; 54:681-686.

61. Martelé Y, Callewaert K, Naessens K et al.: Controlled patterning of biomolecules on solid surfaces. Mater Sci Eng: C 2003; 23:341-345.

62. Roy JJ, Abraham TE, Abhijith KS et al.: Biosensor for the determination of phenols based on cross-linked enzyme crystals (clec) of laccase. Biosens Bioelectron 2005; 21:206-211.

63. Cabrita JF, Abrantes LM, Viana AS: N-hydroxysuccinimide-terminated self-assembled monolayers on gold for biomolecules immobilisation. Electrochimica Acta 2005; 50:2117-2124.

64. Ferry Y, Leech D: Amperometric detection of catecholamine neurotransmitters using electrocatalytic substrate recycling at a laccase electrode. Electroanalysis 2005; 17:113-119.

65. Duran N, Esposito E: Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: A review. Applied Catalysis B: Environmental 2000; 28:83-99.

66. Pointing S: Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 2001; 57:20-33.

67. Semenov AN, Lomonsova IV, Berezin VI et al.: Peroxidase and laccase as catalysts for removal of the phenylhydrazide protecting group under mild conditions. Biotechnol Bioeng 1993; 42:1137-1141.

68. Nicotra S, Cramarossa MR, Mucci A et al.: Biotransformation of resveratrol: Synthesis of trans-dehydrodimers catalyzed by laccases from myceliophtora thermophyla and from trametes pubescens. Tetrahedron 2004; 60:595-600.

69. Kurisawa M, Chung JE, Uyama H et al.: Enzymatic synthesis and antioxidant properties of poly(rutin). Biomacromolecules 2003; 4:1394-1399.

70. Mustafa R, Muniglia L, Rovel B et al.: Phenolic colorants obtained by enzymatic synthesis using a fungal laccase in a hydro-organic biphasic system. Food Res International 2005; 38:995-1000.

71. Lang G, Cotteret J: Hair dye composition containing a laccase. 1999; Int Pat Appl WO9936036.

72. Golz-Berner K, Walzel B, Zastrow L et al.: Cosmetic and dermatological preparation containing copper-binding proteins for skin lightening. 2004; Int Pat Appl WO2004017931.