Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6233

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (1):** 766-773 (2018)

Review Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Laccase - A Wonder Molecule : A Review of its Properties and Applications

Hiren V. Prajapati[1] and Farida P. Minocheherhomji[2]

Research Scholar[1], Associate Professor[2],

Microbiology Department, B.P. Baria Science Institute, Navsari, Gujarat, India

*Corresponding Author E-mail: hiren.prajapati91@gmail.com

Received: 16.01.2018 | Revised: 20.02.2018 | Accepted: 24.02.2018

ABSTRACT

Laccases are an interesting group of multi copper enzymes, which have received much attention of researchers in last few decades due to their ability to oxidize both phenolic and non-phenolic lignin based compounds as well as highly recalcitrant environmental pollutants. Laccase are found in many plants insects and microorganisms. Amongst various microorganisms laccase produced from fungus is of good quality as is reported by many researchers. They have been described in different genera of ascomycetes, some deuteromycetes, and mainly in basidiomycetes. This paper reviews the structure, important properties, occurrence in biological world, and applications of laccases within different industrial fields as well as their potential extension to the nanobiotechnology area.

Key words: *Laccase, Nanobiotechnology, Microorganisms, Enzymes*

INTRODUCTION

Laccase is one of the few enzymes that have been the subject of study since the end of the last century. This enzyme is a type of copper-containing polyphenol oxidase that was discovered in the exudates of the Japanese lacquer tree *Rhus verniczfera*[39] and subsequently was demonstrated as a fungal enzyme as well[3,19]. Laccases (EC 1.10.3.2) (*p*-diphenol: dioxygen oxidoreductases; benzenediol dioxygen oxidoreductases) are oxidative enzymes using molecular oxygen as the electron acceptor, which oxidize the phenolic units of lignin[7].Laccases have received attention of researchers in the last few decades due to their ability to oxidize both phenolic and nonphenolic lignin based compounds as well as highly recalcitrant environmental pollutants. Laccase participates in the cross-linking of monomers, degradation of polymers and ring cleavage of aromatic compounds. It is also used in the synthesis of organic substances, where typical substrates are amines and phenols, the reaction products are dimers and oligomers derived from the coupling of reactive radical intermediates.

Structural features of laccase

Laccases often occur as isoenzymes or monomers that oligomerize to form multimeric complexes[18].

Cite this article: Prajapati, H.V., Minocheherhomji, F.P., Laccase - a Wonder Molecule : A Review of its Properties and Applications, *Int. J. Pure App. Biosci.* **6(1):** 766-773 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6233

Each isoenzyme has four copper atoms and is able to individually carry on the catalytic mechanism of laccases; *Galerina* species. HC1 was able to produce 4 isoenzymes, whereas *Fusarium* species BOL35 laccase was seen as a single protein. The molecular mass of the laccase monomers ranges from 40 to 130 kDa with a covalently linked carbohydrate content of 10–25% in fungi and 20-45% in plants. The carbohydrate moiety typically consists of mannose, Nacetylglucosamine and galactose, which may contribute to the high stability of the enzymes[7,18] . Three-dimensional structural analysis of several fungal, bacterial and plant laccases reveal that all are composed of three sequentially arranged cuprodoxin-like domains; each with a greek key β-barrel topology, and highly related to small copper proteins such as azurin and plastocyanin[13]. The multiple alignment of primary sequences of laccases shows that the copper binding motifs are highly conserved in all sequences, which reflects a common mechanism for copper oxidation and oxygen reduction. However, putative binding pocket analysis reveals that bacterial laccases have larger binding cavities when compared to those from plants and fungi[10].Laccase contains four copper atoms which have been classified into three groups based on the absorption and Electronic Paramagnetic Resonance spectra. Type 1 (T1) paramagnetic "blue" copper has an intense absorption at 600-610 nm, which is caused by the covalent copper-cysteine bond and confers the typical blue color to the multicopper proteins. The T1 copper has a trigonal coordination with two histidines and one cysteine. In bacterial laccases the axial ligand is confirmed by methionine and in fungal laccase by leucine or phenylalanine[37]. Type 2 (T2) paramagnetic "non-blue" copper has no visible absorption spectrum and is coordinated by two histidines. Type 3 (T3) is diamagnetic coupled binuclear copper center, with an absorption band at 330 nm. It is coordinate by six histidines[7,37] . Nevertheless, it is possible to find non-blue laccases in nature the "white" laccases, as they are called, have been structurally characterized and atypically show the presence of one copper, one iron and two zinc atoms per molecule.

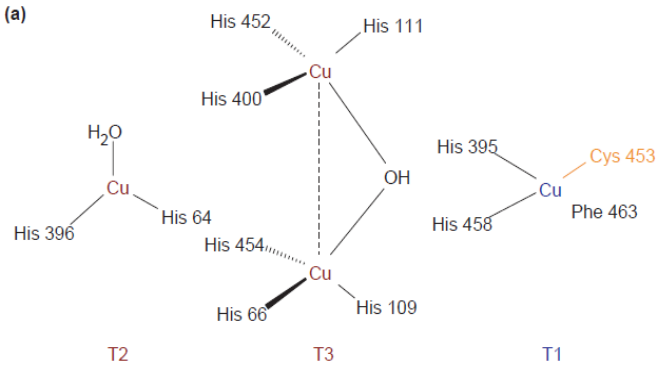

Fig. 1: Active site of laccase enzyme

Catalytic mechanism of laccase enzyme

In the catalytic mechanism of laccase, molecular oxygen is reduce to water. Firstly the substrate reduces the T1 site, after that it transfers the electron to the trinuclear cluster T2 / T3. Here, two possible mechanisms for reduction of the trinuclear cluster are conceivable: either T1 and T2 sites together reduce T3, or each copper on the cluster is sequentially reduced by electron transfer starting from T1. Once the enzyme is completely reduced, one oxygen atom is bound with the T2 and T3 copper ions, and the other oxygen atom is bound with the other copper ion of T3, forming the peroxide intermediate. Subsequently, the peroxide bond (O ─ O) is broken to produce a fully oxidized form of native intermediate, which will end the catalytic cycle with the reduction of oxygen to water.

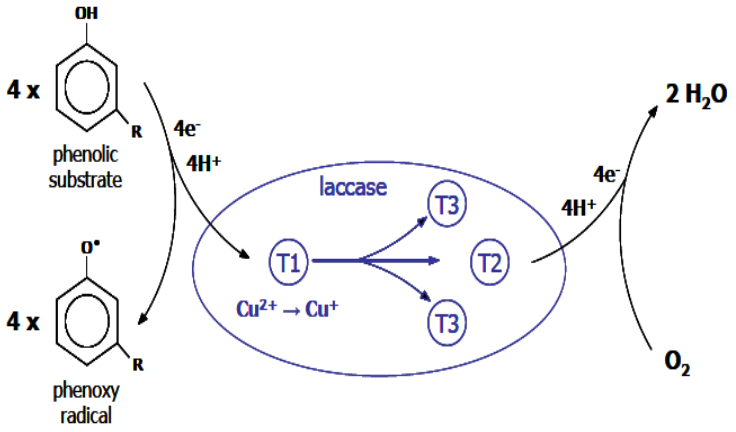

Fig. 2: Catalytic Mechanism of Laccase enzyme

OCCURANCE

Over the years, with the development of newer techniques, the structural analysis of plants have confirmed that plant laccases participate in the early steps of lignification. Some examples of plant sources from which laccases have been isolated include *Acer pseudoplatanus*, *Pinus taeda*[25], *Rhus vernicifera* and *Populus euramericana*[27]. Fungal laccases are involved in delignification of lignocellulosic material, protection against toxic compounds, formation of fruiting body, fungal morphogenesis and sporulation[10] White rot fungi present on decay wood and soil have been reported as best laccase producer fungi in the studies carried out by various researchers. The fungi such as *Phanerochaete chrysosporium, Theiopkora terrestris, Lenzites betulina, Phlebia radiata, Pleurotus ostreatus,* and *Trametes versicolor* belonging to the basidiomycetes group are well known laccase producing organisms[1]. Among the basidiomycetes, *Trametes versicolor*,formerly known as *Coriolus versicolor* or *Polyporus versicolor*, is the most characterized and studied fungus for the production of laccase. It is one of the principal white-rot decaying fungi as it is able to produce other oxidoreductases besides laccase. Fungal laccase have huge applications in different industries as well as in bioremedation which makes the fungi very important laccase producers. Laccases has been characterized from many bacterial species such as *Bacillus subtilis, Streptomyces lavendulae,* and *Marinomonas mediterranea*[34]. It was first reported in the melanin producing *Azospirillum lipoferum*[14]. Bacterial laccase may be involved in various functions like manganese oxidation, cell pigmentation, melanin production and morphogenesis[34]. Characterization of bacterial laccases has revealed that they have a low redox potential (0.45-0.54 V) but they are active and stable at high temperatures, pH and salt concentrations[9]. Laccases or laccase-like activities have been found in some insects in addition to plants, fungi and bacteria, where they have been suggested to be active in cuticle sclerotization[8].

Laccase production

Laccase is a biotechnologically important enzyme. Laccases are used in various biotechnological applications like bioremediation, textile processing, dye degradation and biotransformation. The immense demand of laccase in different applications requires the production of the enzyme in large amounts. Laccases are extracellular enzyme secreted by various fungi during secondary metabolism. Laccase production can be achieved by various modes of fermentation like Submerged and Solid State Fermentation.

Submerged cultivation

The process of submerged cultivation involves the growth of microorganisms in a liquid medium, rich in nutrients under aerobic conditions. The submerged fermentation can be carried out using cheap material source considered as "waste". This material contains considerable amount of soluble carbohydrates, nitrogen, minerals and vitamins, and inducers

for enzyme production. The main disadvantage of this technique is the excessive growth of mycelium, which affects the production yield due to mass transfer and metabolic rate limitation.

Solid state fermentation

Solid state fermentation (SSF) is defined as a fermentation process in which microorganisms grow on solid materials without the presence of free liquid. During the cultivation, synthetic or natural substrates can be used as the solid support for the production of variety of enzymes, including laccases from fungal origin. Various agricultural waste like rice straw, rice bran, wheat straw, wheat bran and sorgum straw have been used as cheap raw substrate for the production of laccase enzyme. By using agricultural waste as solid substrate a high production yield of laccases have been reported by various researchers. A possible reason for the high level of enzyme production obtained by SSF is that it stimulates the conditions of natural growth for fungi[15]. In addition to it, the lower cost of the substrates, coupled with the reduced risk of bacterial contamination and low energy requirements as well as downstream processing of the enzyme, are considered as the principal advantages of implementing this type of cultivation[29].

Biotechnological and industrial applications of laccases

Wide range of natural substrates of laccase enzymes makes them a promising tool in important biotechnological and industrial applications. As an important biocatalyst, Laccase have replaced conventional chemical processes in several industries such as pulp and paper, textile, food, synthetic, pharmaceutical and nanobiotechnology[24].

Laccase in pulp and paper industry

In paper and pulp industry during pulping, the raw materials are reduced to the fibrous state using mechanical and chemical methods. In the chemical method, chlorine- or oxygen-based chemical oxidants are used for the separation and degradation of lignin present in wood pulp. This conventional method for lignin degradation results in the formation of chlorinated aliphatic and aromatic compounds that could be acutely toxic, mutagenic and carcinogenic[2]. The use of laccases have been shown to possess the feasibility to substitute chlorine-containing reagents, thus reducing the pollution load caused by chloroorganics.

Laccases from white-rot fungi can be applied as biopulping agents to wood chips before pulping for the partial degradation of lignin, and for reduction of kappa number in pulp which is a measure of residual lignin[5,38] Use of laccase saves electrical energy, improves strength properties, and is environmentally compatible due to reduced effluent toxicity[31].

Laccase in fibre modifications

Laccase have been proposed to activate the fibre bound lignin and enzymatic cross-linking of lignin-based materials to produce medium density fibre boards (MDF) with good mechanical properties which are now a days employed in the construction and production of furniture devoid of any use of formaldehyde based toxic synthetic adhesives[11,36]. Another possibility is to activate the lignocellulosic fibres by its adhesion with laccases which ultimately improves the chemical or physical properties of the fibres. Attachment of guaiacol sulfonate using laccase makes lignin more water soluble. The attachment of 4-hydroxyphenylacetic acid was also demonstrated, but no differences in lignin solubility could be distinguished[20]. High kappa pulps were modified with 4-hydroxyphenylacetic acid in the presence of laccase. This modification increased the carboxylic acid groups, water retention, tensile strength and burst strength of the resulting paper[6]. Laccase in the presence of specific peroxides also grafted acrylamide onto lignin[22]. Incubation of lignin with wood-based fibres in the presence of laccase formed covalent attachments with carbohydrates[16].

Laccase in Textile industry

Laccase is used in commercial textile applications to improve the whiteness in conventional bleaching of cotton and also recently in finishing applications like biostoning for denim fabrics. Laccase can also be used *in situ* to convert dye precursors for

better and more efficient fabric dyeing. Laccases find potential applications for cleansing such as cloth and dishwashing. Laccase may be included in a cleansing formulation to eliminate the odor on fabrics, including tapestry cloth used on sofa surface and curtains, or in detergents to eliminate the odor generated during cloth washing. A patent application about the use of LMS to increase the shrink resistance of wool has been published. It has been also found that wool fibers can be activated with LMS. Therefore, the use of laccase for anti-shrink treatment of wool seems to be a very attractive proposition[21].

Laccase in Bioremedation

Bioremediation is a process that removes recalcitrant compounds from the environment. The process of bioremediation employs microorganisms to remove the contaminating organic compounds by metabolizing them to carbon source. In recent years, many researchers have deduced that laccase from fungus is a promising tool in wastewater treatment for removal of these contaminants which cause pollution in the environment and are difficult to remove.

Degradation of Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are aromatic compounds made up of three or more fused benzene rings. PAHs can persist in the environment for longer periods due to its complex structure and they are well known for their toxicity, carcinogenicity and mutagenicity. The redox potential of these compounds is too high for laccase to directly oxidize them via electron transfer. PAH degradation was checked with laccase from different strain of *Pycnoporus sanguineus* where it was found that degradation rate of PAH was high in a shorter time period. Even degradation of PAHs by laccase did not lead to formation of new toxicants as there was zero mortality of *Artemia* exposed to the PAH sample that was treated for 24 hours[33].

Degradation of Polymers / Plastics

Abundance of plastic on biosphere has increased with the period of time. As plastics are very difficult to remove from the nature they are the cause distress to human as well as animal health and decrement of soil and water quality. Microbial degradation is one of the ways to remove this toxic compound from the soil and water with the combination of other mechanical methods. As laccase has broad substrate specificity it can be a good option in removal of these plastics. Oxidation of the hydro-carbon backbone of polyethylene can be carried out by laccase. It is reported that Cell-free laccase incubated with polyethylene causes reduction of average molecular weight and molecular number of polyethylene by 20% and 15 % respectively[4].

Decolorization of Dyes present in effluent

Decolorization of dye waste water is a challenging process to the textile industry, where the great potential of microbial decolorizing can be adopted as an effective tool. Laccase producing Fungus *Aspergillus oryzae*, *Aspergillus niger* can be a good tool in removal of textile dye with great efficiency when used as individually or in a consortium[26]. Crude laccase obtained from *Funalia trogii* has great potential in degradation of Reactive Black 5 and Reactive Blue 171 dye. Laccase from *Funalia trogii* was also able to degrade Reactive Black 5 and Reactive Blue 171 dyes either individually or in combination[39]. Azo dyes are highly carcinogenic and cause various types of cancer in humans exposed to it in a short time period. Thus removal of these toxic carcinogens from the nature is of utmost importance. Laccases from white rot fungi have the capacity to remove these dye and its product laccase from strain *Ganoderma cupreum* AG-1 (Genbank accession no. HQ328947) isolated from the decayed wood has the ability to decolorize Reactive Violet 1 and various other azo based dyes.

Laccase in Food Processing Industry

Laccase application in the food industry is based on its ability to polymerize and oxidize molecules. Wine grapes possess a very high phenolic content compared to many other fruits and crops. The phenolic compounds

significantly affect the color, odor and taste of the wine. Treatment of must with laccase promotes wine stabilization by removal of phenols and avoidance of reactions leading to the development of brown color which occurs due to the presence of phenols in wine.Further, waste water released from wine production units have an hazardous effect which can be detoxified by the use of laccases. Enzymatic oxidation of polyphenols in vitro yields oligomeric products with enhanced pharmacological properties[7]. Laccase is also commonly used to stabilize fruit juices. Many fruit juices contain naturally occurring phenolics and their oxidation products, which contribute to obnoxious color and taste. The natural polymerization and cooxidation reactions of phenolics and polyphenols over time, results in undesirable changes in color and aroma. The color change, referred to enzymatic darkening, increases due to a higher concentration of polyphenols naturally present in fruit juices[28].

Laccase in nanobiotechnology

Nanotechnology contributes to the development of smaller and efficient particle through controlled deposition and specific adsorption of biomolecules on different types of surfaces, achieving micro and nanometer order called as nanoparticle.

Laccase based nanoparticles have been reported by various researchers for phenolic compound oxidation and degradation[12,30] . Laccase can also be immobilized on the cathode of biofuel cells that can provide power for small transmitter systems. Biofuel cells are extremely attractive from an environmental point of view because electrical energy is generated without combustion of fuel, thus providing a cleaner source of energy. Zinc tetraaminophthalocyanine-Fe3 O4 nanoparticle composites were prepared with laccase. This kind of immobilized laccase has good thermal, storage and operation stability, and could be used as the sensing biocomponent for the fiber optic biosensor based on enzyme catalysis[17].

CONCLUSION

The goal of this review was to offer a brief knowledge on laccase a wonder molecule. Different type of plants, fungi and also bacteria are able to produce laccase. The functions of the enzyme varies from organism to organism and typify the diversity of laccase in nature. Laccase have a lot of potential applications in the biotechnology industry, such as in food, textile, bioremediation, pulp and paper industries and others. It can be foreseen that the number of laccase-based industrial oxidation processes will increase significantly and that, at the same time, there will be an increasing interest in their synthetic exploitation. In future, application of genomics may be very useful in hyperproduction of laccase by characterising laccase producing gene and cloning it in various organisms. The gene level study will focus light on the potential of laccase and its biotechnological and industrial applications.

REFERENCES

1. Baldrian, P. (2006). Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews.* **30:** 215-242 (2006).
2. Bermek, H., Li, K. and Eriksson, K.E.L. Studies on mediators of manganese peroxidase for bleaching of wood pulps. *Bioresour Technol.* **85(3):** 249-252 (2002).
3. Bertrand, G. Sur la presence simultanee de la laccase et de la tyrosinase dans le suc de quelques champignons. *C* R *Hebd Seances Acad Sci* **123:** 463-465 (1896).
4. Bhardwaj H, Gupta R, Tiwari A Microbial Population Associated With Plastic Degradation**.** **1:** 272. doi:10.4172/scientificreports.272 (2012).
5. Camarero, S., Ibarra, D., Martinez, A.T., Romerob, J., Gutierrez, A. and del Rio J.C. Paper pulp delignification using laccase and natural mediators. *Enzyme Microbial Technol.* **40:** 1264-1271 (2007).
6. Chandra, R.P. and Ragauskas, A J. Evaluating laccase-facilitated coupling of phenolic acids to high-yield kraft pulps. *Enzyme Microbial Technol.* **30:** 855- 861 (2002).
7. Claus H. Laccases and their occurrence in prokaryotes. *Arch Microbiol*. **179:** 145–150, (2003)

8. Dittmer, N.T., Suderman, R.J., Jiang, H., Zhu, Y.C., Gorman, MJ., Kramer, K.J., Kanost, M.R. Characterization of cDNAs encoding putative laccase like multicopper oxidases and developmental expression in the tobacco homworm, *Manduca sexta*, and the malaria mosquito, *Anopheles gambiae. Insect Biochemistry and Molecular Biology*. **34:** 29-41 (2004).
9. Durao, P., Bento, I., Fernandes, A., Melo, E., Lindley, P., Martins, L. Perturbations of the T1 copper site in the Cot A laccase *from Bacillus subtilis:* structural, biochemical, enzymatic and stability studies. *Journal of Biological Inorganic Chemistry*. **11:** 514-526 (2006).
10. Dwivedi, P., Vivekanand, V., Ganguly, R., Singh, R.P.: *Parthenium* sp. as a plant biomass for the production of alkalitolerant xylanase from mutant *Penicillium oxalicum* SAUE-3.510 in submerged fermentation. *Biomass Bioenerg*. **33:** 581–588 (2009)
11. Felby, C, Hassingboe, J. and Lund, M. Pilot-scale production of fiberboards made by laccase oxidized wood fibers: board properties and evidence for cross-linking of lignin. *Enzyme Microbial Technol.* **31:** 736-741 (2002).
12. Galliker, P., Hommes, G., Schlosser, D., Corvini, P.F.X., Shahgaldiana, P. Laccase-modified silica nanoparticles efficiently catalyze the transformation of phenolic compounds. *Journal of Colloid and Interface Science.* **349:** 98-105 (2010).
13. Giardina P, Faraco V, Pezzella C, Piscitelli A,Vanhulle S, Sannia G. Laccases: a never-ending story. *Cell Mol Life Sci*. **67:** 369–385, (2010).
14. Givaudan, P., Effose, A., Faure, D., Potier, P., Bouillant, M.L., Bally, R. Polyphenol oxidase in *Azospirillium lipoferum* isolated from rice rhizosphere: evidence for laccase activity in non motile strain of *Azospirillium lipoferum. FEMS Microbiology Letters.* **108:** 205-210 (1993).
15. Hatvani N, and Mecs I Production of laccase and manganese peroxidase by Lentinusedodes on malt containing by product of the brewing process. Process Biochem., **37:** 491– 496 (2001).
16. Huttermann, A., Majcherczyk, A., Braun-LuUemann, A., Mai, C, Fastenrath, M., Kharazipour, A., Huttermann, J. and Huttermann, A.H. Enzymatic activation of lignin leads to an unexpected copolymerization with carbohydrates. *Naturwissenschaften.* **87:** 539-541 (2000).
17. Jun Huang, Cheng Liu, Haiyan Xiao, Juntao Wang, Desheng Jiang, and Erdan GU Zinc tetraaminophthalocyanine-Fe_3O_4 nanoparticle composite for laccase immobilization Int J Nanomedicine. 2007 Dec; **2(4):** 775–784 (2007).
18. Kunamneni, A., Ghazi, I., Camarero, S., Ballesteros, A., Plou, F.J., Alcalde, M.Decolorization of synthetic dyes by laccase immobilized on epoxy-activated carriers. *Process Biochemistry*. **43:** 169-178 (2008).
19. Laborde, 1. Sur la casse des vins. *C* R *Hebd Seances Acad Sci* **223:** 1074-1075 (1896).
20. Lund, M. and Ragauskas, A.J. Enzymatic modification of kraft lignin through oxidative coupling with water-soluble phenols. *Appl Microbiol Biotechnol.* **55:** 699-703 (2001).
21. Madhavi, V., Lele, S.S. Laccase: Properties and applications. *BioResources.* **4:** 1694-1717 (2009).
22. Mai, C, Milstein, O. and Huttermann, A. Fungal laccase grafts acrylamide onto lignin in presence of peroxides. *Appl Microbiol Biotechnol.* **51:** 527-531 (1999).
23. Mayur Gahlout, Shilpa Gupte, Akshaya Gupte Optimization of culture condition for enhanced decolorization and degradation of azo dye reactive violet 1 with concomitant production of ligninolytic enzymes by Ganoderma cupreum A*G-1 3 Biotech* **3:** 143–152 (2013).
24. Nyanhongo, G.S., Gomes, J., Gubitz, G., Zvauya, R., Read, J.S. and Steiner, W. Production of laccase by a newly isolated

strain of *Trametes modesta. Bioresour Technol.* **84:** 259-263 (2002).

25. O´Malley D.M.,whetten, R.,Bao, W., Chen, C.L., Sederoff R R. The role of laccase in lignification. The plant journal **4:** 751-757 (1993).
26. Rahna, K., Steny, R., Mary Anto, M., ,Lilly Rajan, L., ,Sreedevi, E.S., Ambili, M. and Balasaravanan, T., Comparative studies of Decolorization of Toxic Dye with Laccase Enzymes producing Mono and Mixed cultures of Fungi. **1:** 21-24 (2013)
27. Ranocha, P., McDougall, G., Hawkins, S., Steijiades, R., Borderies, G., Stewart, D., Cabanes M. M., Boudet, A.M., Goffaer, D. Biochemical characterization, molecular cloning and expression of laccases-a divergent gene family-in poplar. *European Journal of Biochemistry.* **259:** 485-495 (1999).
28. Ribeiro, D.S. Henrique, S.M.B., Oliveira, L.S., Macedo, G.A., Fleuri, L.F. Enzymes in juice processing: a review. *International Journal of Food Science and Technology.* **45:** 635-641 (2010).
29. Robinson, T., McMullan, G., Marchant, R Nigam, P. Remediation of dyes in textile effluent, a critical review on current treatment technologies with a proposed alternative. *Bioresource Technology*. **77:** 247-255 (2001).
30. Salis, A., Pisano, M., Monduzzi, M., Solinas, V., Sanjust, E. (2009). Laccase from *Pleurotus sajur-caju* on functionalized SBA-15 mesoporous silica immobilization and use for the oxidation of phenoloic compounds. *Journal of Molecular Catalysis.* **58:** 175-180 (2009).
31. Scott, G.M., Lentz, M., Akhtar, M., Sykes, M. and Abubakr, S. Environmental aspects of biosulfite pulping. *Proceedings of 1995 international environmental conference Atlanta: TAPPI.* 1155-1161 (1995).
32. Soares, A., K. Jonasson, E. Terrazas, B. Guieysse, and B. Mattiasson. The ability of white-rot fungi to degrade the endocrine - disrupting compound nonylphenol. *Appl. Microbiol. Biotechnol.* **66:** 719–725 (2005).
33. Vikineswary, S.; Abdullah, N.; Renuvathani, M.; Sekaran, M.; Pandey, A. and Jones, G.E.B. Productivity of laccase in solid substrate fermentation of selected agro-residues by*Pycnoporus sanguineus*. *Bioresource Technology*, **97(1):** 171-177 (2006).
34. Viswanath, B., Chandra, M.S., Kumar, K.P., Rajasekhar-Rqddy, B. Production and purification of laccase from *Stereum ostrea* and its ability to decolorize textile dyes. Dyn. Biochem. *Process Biotechnology and Molecular Biology.* **2:** 19-25 (2008).
35. Wang C, Zhao M, Li D, Cui D, Lu L, Wei XD Isolation and characterization of a novel Bacillus subtilis WD23 exhibiting laccase activity from forest soil. *Afr. J. Biotech*., **9(34):** 5496-5502 (2010).
36. Widsten, P. and Kandelbauer, A. Laccase applications in the forest products industry: A review. *Enzyme Microbial Tech.* **42:** 293-307 (2008).
37. Witayakran & Ragauskas A.J Modification of High –lignin softwood kraft pulp with laccase and amino acid. Enzyme and microbial technology **44(3):** 176-181 (2009).
38. Wong, D.W.S. Structure and action mechanism of ligninolytic enzymes. *Applied Biochemistry and Biotechnology.* **157:** 174-209 (2009).
39. Yoshida, H. Chemistry of lacquer (urushi). *Journal of The Chemical Society.* **43:** 472-486 (1883).