



LACCASES: SOURCES AND THEIR ENVIRONMENTAL APPLICATION

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Abstract: Pollution increased day by day, laccase are an oxido reductases enzyme which play a significant role in remediation. Production of laccase has sink from plant, fungi, bacteria, and insect etc. Present study on their use in several industrial applications, includes dye decolourization, detoxification of environmental pollutants and revalorization of wastes and wastewaters etc. Here we provide a brief discussion of this interesting group of enzymes, increased knowledge of which will promote laccase based industrial processes in future.

Keywords: Laccase, Biodegradation and Bioremediation, Decolorization.

INTRODUCTION

Laccases (benzenediol: oxygen oxi-reductase, EC 1.10.3.2) exist widely in nature and belongs to small group of enzymes called the blue copper protein or copper oxidases (Sivakumar et al., 2010). These proteins are characterized by containing copper atoms. One copper is placed at the T1 site, where reducing substrate binds, and other three coppers are clustered in which molecular oxygen binds.

Laccases play an important role in several industry, paper and pulp industry, textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutant and removal of endocrine disruptors. Recently laccases have been efficiently applied to nano biotechnology due to their ability to catalyze electron transfer reactions without additional cofactor (Shraddha et al., 2011). In future, laccase are a useful enzyme for biotechnological application in decolorization and biodegradation of contaminating environmental pollutants. Laccases have many biotechnological applications because of their oxidation ability towards a broad range of phenolic and non-phenolic compounds (Mohammadian et al., 2010).

Laccases source:

In 1883, Yoshida first described laccase when he extracted it from the Japanese lacquer tree, *Rhus vernicifera*. In 1896 laccase was demonstrated to be present in fungi for the first time by both Bertrand and Laborde. Laccase are widely distributed in higher plants, bacteria, fungi, and insects which detail are present in below as:

Plant: Cabbages, Turnips, Beets, Apples, Asparagus, Potatoes, Pears, Peach, sycamore, tobacco and various other vegetables. Recently, laccase has been expressed in the embryo of maize (*Zea mays*)

seeds (Bailey et al., 2004; Arora and Sharma, 2010).

Fungi: Fungal laccases have higher redox potential than bacterial or plant. Fungi from the deuteromycetes, ascomycetes (Aisemberg et al., 1989) as well as basidiomycetes are the known producers of laccase (Sadhasivam et al., 2008) which name detail as:

(A) **Ascomycetes:** *Monocillium indicum* was first laccase to be characterised and *Polyporus versicolor*, *Neurospora crassa* and *Aspergillus nidulans* etc.

(B) **Basidiomycete:** *Schizophyllum commune*, *Phanerochaete chrysosporium*, *Theiophora terrestris*, *Lenzites*, *Betulina*, White-rot fungus (*Phlebia radiata*, *Pleurotus ostreatus*, *Trametes versicolor*), *Trametes fungy*, *Coriolopsis fulvocinerea* and *Cerrena unicolor* etc.

Other wood-rotting fungi include *T. hirsuta* (*C. hirsutus*), *T. villosa*, *T. gallica*, *Cerrena maxima*, *Lentinus tigrinus*, *T. ochracea*, *Pleurotuseryngii*, *Trametes (Coriolus) versicolor* and *Coriolopsis polyzona*, etc. (Morozova et al., 2007).

(C) **Deuteromycete:** Production of laccase by a newly isolated fungus was *Pestalotiopsis sp.* (Hao et al., 2007).

Bacteria: Bacterial laccase was first reported in *Azospirillum lipoferum* (Givaudan et al., 1993); it plays a role in cell pigmentation, oxidation of phenolic compounds (Faure et al., 1994, 1995) and/or electron transport (Alexandre et al., 1999). Other name as *E. coli*, *Bacillus subtilis*, *S. laven dulae*, *S. cyaneus*, *Marinomonas mediterranea*, *Aquifex aceolicus*, *Azospirillum lipoferum*, *Bacillus sp.*, *Bacillus halodurans*, *Leptothrix discophora* SS1, *Oceano bacillusihyensis (cotA)*, *Alpha-*

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proteobacterium SD21, Gama-proteobacterium JB, *Pseudomonas fluorescens* GB-1, *Pseudomonas maltophilia*, *Pseudomonas putida* GB1 (*cumA*), *Pseudomonas syringae* pv *tomato* (*copA*), *Pseudomonas aerophilum* (*pae1888*), *Streptomyces antibioticus*, *Streptomyces griseus* (*epoA*), *Thermus thermophilus* HB27, *Xanthomonas campestris* (*copA*) and *Streptomyces psammoticus* MTCC 7334 etc. (Sharma et al., 2007).

Insect: The insect laccase is a long amino-terminal sequence characterized by unique domain consisting of several conserved cysteine, aromatic, and charged residues. laccase enzyme has also been characterized in different insects, e.g., *Bombyx*, *Calliphora*, *Diptera*, *Drosophila*, *Lucilia*, *Manduca*, *Musca*, *Oryctes*, *Papilio*, *Phormia*, *Rhodnius*, *Sarcophaga*, *Schistocerca* and *Tenebrio* (Arora and Sharma, 2010). Recently, two isoforms of laccase 2 gene have been found to catalyse larval, pupal and adult cuticle tanning in *Tribolium castaneum* (Arakane et al., 2005; Sharma and Kuhad, 2008).

Laccases are classified into two groups in accordance with their source, i.e. plant and fungal. However, diphenol oxidases (Laccases) have also been identified in bacteria and insects (Shleev et al., 2004).

Role of laccase producing organism in environmental pollutant:

One of the goals of present work was to study and thoroughly compare the properties of laccase. Laccase is also used in bioremediation agent to clean up herbicides, pesticides and certain explosives in soil. Present time attention of researchers in the last few decades due to their ability to oxidize both phenolic and nonphenolic lignin-related compound as well as highly recalcitrant environmental pollutants (Shraddha et al., 2011).

Phanerochaete cryosporium (TL1), laccase would be a good target for the development of biotechnological tools because of its laccase in lignin degradation, phenol degradation, waste water treatment and others industrial application. 2, 6 dichlorophenol is one of the most dangerous environmental contaminants. Under the experimental condition purified laccase is able to convert 2, 6 dichlorophenol directly, this result investigated by Xiao. et al., 2003 (Prabu et al., 2006).

Dye decolorization: The treatment of industrial effluents containing aromatic compounds is necessary prior to final discharge to the environment (Khlifia et al., 2010). Nowadays, environmental regulations in most countries require that wastewater must be decolorized before its discharge (Moilanen et al., 2010) to reduce environmental problems related to the

effluent (Tavares et al., 2009). A wide range of physicochemical methods has been developed for the degradation of dye-containing wastewaters (Vandevivere et al., 1998; Tavares et al., 2009). These classical processes can cause a problem in environment and better treatment can be obtained using bioprocesses. *T. versicolour* to be treat a black liquors discharge for detoxifying and reducing the color, aromatic compound, COD etc. (Blanquez et al., 2004). *S. maltophilia* to be decolorize some synthetic dyes (methylene blue, methyl green, toluidine blue, congo red, methyl orange, and pink) as well as the industrial effluent (Romero et al., 2006). A newly isolate deuteromycete fungus *pestalotiopsis* sp. have high potential producer of industrially important laccase and decolorization of azo dye (Hao et al., 2007). Laccases from the white-rot fungi *Cerrena unicolor* and *Trametes hirsuta* for their ability to decolorize simulated textile dye baths (Moilanen et al., 2010).

Bioremediation and Biodegradation: Keum and Li obtained laccase from *T. versicolour* and *Pleurotus ostreatus* for degradation of PCB as well as phenol. *T. versicolour* is used for the bioremediation of atrazine in soil (Shraddha et al., 2011). *T. villosa* remediates the soil by degrading 2, 4-DCP (2, 4- dichlorophenol). *Cerrena unicolor* has the capability of reducing lignin content from sugarcane bagasse (D'sauza et al., 2009). Decolorization and detoxification of a textile industry effluent by laccase from *trametes trogii* (Imran et al., 2012). Large amount of polyphenol is present in the beer factory wastewater which is dark brown in colour and degraded by the white-rot fungus *Coriolopsis gallica* (Yague et al., 2000). Laccase produced from *Trametes* sp. bioremediate the distillery wastewater generated from the sugarcane molasses fermentation with high content of organic matter (Gonzalez et al., 2000).

Laccase application in insects: Epidermis of the tobacco hornworm, *manduca sexta*, and may oxidize toxic compounds ingested by insect (Diltmer, et al., 2004). Recently, a laccase in the salivary glands of *N. cincticeps* was identified. Salivary laccase rapid oxidization of potentially toxic monolignols to nontoxic polymers during feeding (Hattori et al., 2005).

CONCLUSION

Laccases are produced by various sources like fungi, bacteria, plant, insects etc. They have many industrial applications because of their innate ability of oxidation of phenolic and nonphenolic compounds. Laccase enzyme has the property to act on a range of substrates and to detoxify a range of pollutants. They decolorize and detoxify the industrial effluents and help in wastewater treatment. They act on both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant environmental pollutants

which help researchers to put them in various biotechnological applications.

REFERENCE

- Alexandre G, Bally R, Taylor BL, Zhulin IB. Loss of cytochrome oxidase activity and acquisition of resistance to quinine analogs in a laccase-positive variant of *Azospirillum lipoferum*. *J. Bacteriol.*, 1999, 181: 6730-6738.
- Arakane Y, Muthukrishnan S, Beeman RW, Kanost MR, Kramer KJ. Laccase 2 is the phenoloxidase gene required for beetle cuticle tanning. *PNAS.*, 2005, 102: 11337-11342.
- Arora DS, Sharma RK. Ligninolytic Fungal Laccases and Their Biotechnological Applications. *Appl. Biochem. Biotechnol.*, 2010, 160:1760-1788.
- Bailey MR, Woodard SL, Callaway E, Beifuss K, Lundback MM, Lane J. Improved recovery of active recombinant laccase from maize seed. *Appl Microbiol Biotechnol.*, 2004, 63: 390-397.
- Dittmer NT, Suderman RJ, Jiang H, Zhu YC, Gorman MJ, Kramer KJ, Kanost MR. Characterization of cDNA encoding putative laccase-like multicopper oxidases and developmental expression in the tobacco hornworm, *Manduca sexta*, and the malaria mosquito, *Anopheles gambiae*. *Insect Biochem Mol Biol.*, 2004, 34: 29-41.
- D'Souza-Ticlo, Sharma D., Raghukumar C. A Thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus. *Marine biotechnology.*, 2009, Vol.11, no.6, pp.725-737.
- Faure D, Bouillant ML, Bally R. Isolation of *Azospirillum lipoferum* 4T Tn5 mutants affected in melanization and laccase activity. *Appl Environ Microbiol.*, 1994, 60, 3413-3415.
- Givaudan A, Effosse A, Faure D, Potier P, Bouillant ML, Bally R. Polyphenol oxidase in *Azospirillum lipoferum* isolated from rice rhizosphere: evidence for laccase activity in non-motile strains of *Azospirillum lipoferum*. *FEMS Microbiol. Lett.*, 1993, 108: 205-210.
- Gonzalez T., Terron M.C., Yague S., Zapico E., Galletti G.C., Gonzalez A.E. Pyrolysis/gas chromatography/mass spectrometry monitoring of fungal-biotreated distillery waste water using *Trametes* sp. I-62 (CECT20197). *Rapid communications in mass spectrometry.*, 2000, Vol.14, no.15, pp.1417-1424.
- Hao J., Song F., Huang F., Yang C., Zhanng Z., Zheng Y., Tian X. Production of laccase by a newly isolated deuteromycete fungus *Pestalotiopsis* sp. and its decolorization of azo dye. *J. Ind. microbial biotechnol.*, 2007, 34 (3):233-4017171552 cit: 4.
- Imran M., Asad J.M., Hadri H.S. and Mehmood S. Production and industrial applications of laccase enzyme. *Journal of cell and molecular biology*, 2012, 10(1): 1-11, 2012.
- Keum Y.S., Li Q.X. Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls. *Chemosphere.*, 2004, Vol. 56, no.1, pp 23-30.
- Khlifia R, Belbahria L, Woodwarda S, Ellouza M, Dhouiha A, Sayadia S, Mechichia T. Decolorization and detoxification of textile industry wastewater by the laccase-mediator system. *J Hazard Mater.*, 2010, 175: 802-80.
- Mohammadian M, Roudsari MF, Mollania N, Dalfard AB, Khajeh K. Enhanced expression of a recombinant bacterial laccase at low temperature and microaerobic conditions: purification and biochemical characterization. *J Ind Microbiol Biotechnol.*, 2010, 5: 41-45.
- Moilanen U, Osma JF, Winquist E, Leisola M, Couto SR. Decolorization of simulated textile dye baths by crude laccases from *Trametes hirsute* and *Cerrena unicolor*. *Eng Life Sci.*, 2010, 10 (3): 1-6.
- Mongkoltharuk W., Tongbopit S. and Bhoonbong A. Independent behavior of bacterial laccases to inducers and metal ions during production and activity. *African Journal of biotechnology.*, 2012, Vol.11 (39), pp.9391-9398.
- Morozova OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI. "Blue" Laccases. *J. Biochem.*, 2007, 72(10): 1136-1150.
- Prabu P.C., Udayasoorian C. and Balasubramanian. Isolation, Molecular characterization and Reactivity with 2,6 Dichlorophenol of a laccase and isolation of laccase gene specific sequences from lignin degrading basidiomycete *Phanerochaete chrysosporium* (TL1). *Biotechnology*, 2006, 5(4):522-529.
- Sadhasivam S, Savitha S, Swaminathan K, Lin FH. Production, purification and characterization of mid-redox potential laccase from a newly isolated *Trichoderma harzianum* WL1. *Process biochem.*, 2008, 43: 736-742.
- Sharma KK and Kuhad RC. Laccase: enzyme revisited and function redefined. *Ind J. Microbiol.*, 2008, 48: 309-316.
- Sharma P, Goel R, Caplash N. Bacterial laccases. *World J Microbiol Biotechnol.*, 2007, 23: 823-832.
- Shleev S.V., Morozova O.V., Nikitina O.V., Gorshina E.S., Rusinova T.V., Serezhnikov V.A., Burbayev D.S., Gazaryan I.G., Yaropolov A.I. Comparison of physico-chemical characteristics of four laccase from different basidiomycetes. *Biochimie*, 2004, 86:693-703.
- Shraddha, Shekher R., Sehgal S., Kamthania M., Kumar A. Laccase: Microbial sources, Production, and potential Biotechnological applications. *Enzyme Research*, 2011, Vol.2011:11 page.
- Sivakumar R., Rajendran R., Balakumar C., Tamilvendan M. Isolation, Screening and Optimization of production medium for thermostable Laccase Production from *Ganoderma* sp. *International journal of engineering science and technology*, 2010, vol.2 (12), 7133-7141.
- Tavares APM, Cristovao RO, Gamelas JAF, Loureiro JM, Boaventuraa RAR, Macedo EA. Sequential decolorization of reactive textile dyes by laccase mediator system. *J Chem Technol Biotechnol.*, 2009, 84: 442-446, 2009.
- Vandevivere PC, Bianchi R, Verstraete W. Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies. *J Chem Technol Biotechnol*, 1998, 72: 289-302.
- Yague S., Terron M.C., Gonzalez T. et al. Biotreatment of tannin-rich beer-factory wastewater with white-rot basidiomycete *Coriolopsis gallica* monitored by pyrolysis/gas chromatography/mass spectrometry. *Rapid communications in mass spectrometry*, 2000, Vol.14, no.10, pp.905-910.

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