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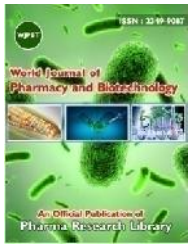

Review Article **Open Access**

Laccase–Production and It's Appliaction

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A B S T R A C T

Laccase belongs to the small group of enzymes called the blue multi copper oxidases. Laccase is widely distributed in higher plants, fungi, bacteri. By fermentation both submerged and solid state fermentation laccase can be produced by using various carbon and nitrogen sources. Laccases have been subject of intensive research in the last decades due to their broad substrate specificity. In the recent years, their uses span from the textile to the pulp and paper industries, and food applications to bioremediation processes. More recently, they have found applications in other field such as in the design of biosensors. In this review, the occurrence, structure, production, and application of laccases will be discussed.

Keywords: Laccase, sources, Production, structure, applications, Pulp and paper industry, Food industry, Bioremediation, Biosensors

A R T I C L E I N F O

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1. Introduction

Laccases (p-diphenol: dioxygen oxidoreductases; benzenediol dioxygen oxidoreductases; EC 1.10.3.2) are member of large blue copper proteins or the blue copper oxidases; other enzymes in this group are the plant ascorbate oxidase and the mammalian plasma protein ceruloplasmin [1,2]. Laccases are defined in the Enzyme Commission (EC) nomenclature as oxidoreductases which oxidize aromatic and nonaromatic compounds by a radical-

catalyzed reaction mechanism and use molecular oxygen as an electron acceptor. Laccases catalyze the oxidation of a variety of phenolic compounds, as well as diamines and aromatic amines, with concomitant reduction of molecular oxygen to water [1]. The syringaldazine [4-hydroxy-3,5-dimethoxy benzaldehyde azine] is considered the substrate oxidized only by laccase enzyme [1]. Thus, several organic compounds which contain hydroxil, acid, or amino groups can act like substrates [3]. Recently a novel polyphenol oxidase with laccase like activity was mined from a metagenome expression library from bovine rumen microflora [3].

2. Structure of Laccase

Laccases often occur as isoenzymes or monomers that oligomerize to form multimeric complexes. Each isoenzyme has four copper atoms and is able to individually carry on the catalytic mechanism of laccases. 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, N-acetylglucosamine and galactose, which may contribute to the high stability of the enzymes [23,24]. Laccases contain 4 copper atoms termed Cu T1 (where the reducing substrate binds) and trinuclear copper cluster T2/T3 (electron transfer from type I Cu to the type II Cu and type III Cu trinuclear cluster reduction of oxygen to water at the trinuclear cluster). These four copper ions are classified into three categories: Type 1 (T1), Type 2 (T2) and Type 3 (T3). These three types can be distinguished by using UV/visible absorption and electronic paramagnetic resonance (EPR) spectroscopy.

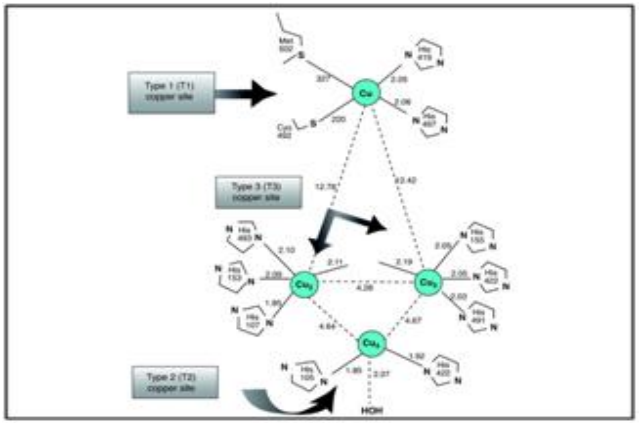

Figure 1: Scheme of T1 (Cu1) and T2/T3 (Cu4/Cu2-Cu3) copper sites of laccase

Laccase Production

Laccases occur as extracellular glyco-proteins, which allows for rapid removal from fungal or bacterial biomass [25,26]. One of the major limitations for the large-scale applications of fungal laccases is the low production rates by both wild type and recombinant fungal strains according to Galhaup et al. [27].

Induction of laccase production

Laccase production has been found to be highly dependent on the conditions for the cultivation [26] and media supporting high biomass did not necessarily support high laccase yields [27]. Laccase production often triggered by nitrogen concentration [28] or when carbon or sulfur became limiting [26]. The addition of aromatic compounds such as 2,5-xylidine, lignin, and veratryl alcohol is known to increase and induce laccase activity [27]. The promoter regions of the genes encoding for laccase contain various recognition sites that are specific for xenobiotics and heavy metals [29]. These can bind to the recognition sites when present in the substrate and induce laccase production.The addition of certain inducers can increase the concentration of a specific laccase or induce the production of new isoforms of the enzyme [30]. The addition of ethanol as an indirect inducer of laccase activity offers a very economical way to enhance laccase production[31].

Influence of carbon sources on laccase production

The carbon sources in the medium play an important role in ligninolytic enzyme production[30]. Hatvani et al. showed that fructose induced 100-fold increase in laccase production of Basidiomycete sp. I-62. *T. versicolor* is an excellent producer of laccase in fermentation of mandarin peels [32]. Glucose and cellobiose were efficiently and rapidly utilized by *Trametes pubescens* with high laccase activity [33]. Similarly, the replacement of crystalline cellulose or xylan by cellobiose increased laccase activity of *C. unicolor* by 21- and 70-fold, respectively [34].

Influence of nitrogen sources on laccase production

Monteiro and De Carvalho [34] reported high laccase activity with semi-continuous production in shake-flasks using a low carbon to nitrogen ratio (7.8 g/g). Buswell et al. [28] found that laccases were produced at high nitrogen concentrations, although it is generally accepted that a high carbon to nitrogen ratio is required for laccase production. Laccase was also produced earlier when the fungus was cultivated in a substrate with a high nitrogen concentration and these changes did not reflect differences in biomass.

Influence of pH on laccase production

Most reports indicated initial pH levels set between pH 4.5 and pH 6.0 prior to inoculation, but the levels are not controlled during most cultivation [32]. It is reported that an initial pH of 7.0 was the best for optimal growth and laccase production.

Influence of temperature on laccase production

It has been found that the optimal temperature for fruiting body formation and laccase production is 25°C in the presence of light, but 30°C for laccase production when the cultures are incubated in the dark [6]. In general the fungi were cultivated at temperatures between 25°C and 30°C for optimal laccase production [39].

Inhibition of laccase production

It seems as if the use of excessive concentrations of glucose as carbon source in cultivation of laccase producing strains has an inhibitory effect on laccase production [37]. An excess of sucrose or glucose in the cultivation media can reduce the production of laccase, as these components allow constitutive production of the enzyme, but repress its induction when applicable [40]. A simple but effective way to overcome this problem is the use of cellulose as carbon source during cultivation [37].

3. Purification of Laccase

In general, plant laccases are purified from sap or tissue extract, whereas extracellular laccases are purified from culture medium (fermentation broth) of the selected organism. Various protein purification techniques are frequently used in purifying laccase. Typical purification protocol involves ultrafiltration, ion exchange, gel filtration, hydrophobic interaction, or other electrophoretic and chromatographic techniques. Affinity chromatography using a phenolic group as ligand can increased purification efficiency. The purity of laccase preparation is often measured by SDS-PAGE and by the ratio of the absorbance at 280 nm to that at 600 nm. Ammonium sulphate is being commonly used for the enzyme purification for many years. But researchers have found much more efficient methodologies such as protein precipitation by ammonium sulphate, anion exchange chromatography, desalt/buffer exchange of protein, and gel filtration chromatography. Single-step laccase purification from Neurospora crassa takes place by using celite chromatography and 54 fold purification was obtained with specific activity of 333 U mg−1 [17]. Laccase from LLP13 was first purified with column chromatography and then purified with gel filtration. Laccase from T. versicolour is purified by using ethanol precipitation, DEAE-Sepharose, Phenyl- Sepharose and Sephadex G-100 chromatography which is a single monomeric laccase with a specific activity of 91,443Umg−1 [41]. Laccase from T. versicolour is purified with Ion Exchange chromatography followed by gel filtration with specific activity of 101UmL−1 and 34.8-fold purification. Laccase from Stereum ostrea is purified with ammonium sulphate followed by Sephadex G-100 column chromatography with 70-fold purification. Laccase from fruiting bodies is purified with ammonium sulphate precipitation with 40–70% saturation and DEAE cellulose chromatography then 1.34 and 3.07 fold purification is obtained respectively [42].

4. Laccase Application

A few laccases are at present in the market for textile, food and other industries (Table 3), and more candidates are being actively developed for future commercialization [16]. Laccase is important because it oxidizes both the toxic and nontoxic substrates. Laccases have been used in a variety of industrial and environmental applications since they can oxidize a number of natural and synthetic substrates using oxygen, producing water as the only by-product. Laccases are versatile enzymes able to oxidize recalcitrant compounds like lignin, which makes them attractive for use in various biotechnological processes. A vast amount of industrial applications for laccases have been proposed. This enzyme is very specific, ecologically sustainable and a proficient catalyst. Applications of laccases are as follows.

Nanobiotechnology

Since laccases are able to catalyse electron transfer reactions without additional cofactors, their use has also been studied in biosensors or bioreporters to detect various phenolic compounds, oxygen or azides. Moreover, biosensors for detection of morphine and codeine, catecholamines, plant flavonoids, for determination of glucose, aromatic amines and phenolic compounds and also for electroimmunoassay have been developed. The Biotechnological application of such micropatterned surfaces: the production of islands of micrometer size of extracellular matrix, where the pattern of these islands could determine the position and distribution of bovine and endothelial cells [42]. Laccase covalently conjugated to a bio-binding molecule can be used as a reporter for immunochemical (ELISA, Western blotting), histochemical, cytochemical, or nucleic acid-detection assays.

Bioremediation and Biodegradation

Laccases have many possible applications in bioremediation. Laccase may be applied to degrade plastic waste having olefin units. Likely, an oxidation of the olefin units by the LMS, could initiate a radical chain reaction, leading to the disintegration of the plastic. Also this LMS can be used to degrade polyurethanes. LMS facilitated the degradation of phenolic compounds (environmental hormones) from biphenol and alkylphenol derivatives and also the decomposition of fluorescent brighteners [17].

Laccase may also be used to eliminate odor emitted from places such as garbage disposal sites, livestock farms, or pulp mills. Also, they could be used for decolorizing dye house effluents that are hardly decolorized by conventional sewage treatment plants. In addition to dye house effluents, laccases can decolorize waste waters from olive oil mills and pulp mills by removing colored phenolic compounds. Another potential environmental application for laccases is the bioremediation of contaminated soils, as laccases and LMS are able to oxidize toxic organic pollutants, such as various xenobiotics, polycyclic aromatic hydrocarbons (PAHs), chlorophenols, and other contaminants.

The catalytic properties of laccases can be used to degrade such compounds. Phenolic compounds are present in wastes from several industrial processes, as coal conversion, petroleum refining, production of organic chemicals and olive oil production among others. Immobilized laccase was found to be useful to remove phenolic and chlorinated phenolic pollutants. Laccase was found to be responsible for the transformation of 2,4,6- trichlorophenol to 2,6-dichloro-1,4-hydroquinol and 2,6-dichloro-1,4-benzoquinone. LMSs have been also used to oxidize alkenes, carbazole, N-ethylcarbazole, fluorene, and dibenzothiophene. Isoxaflutole is an herbicide activated in soils and plants to its diketonitrile derivative, the active form of the herbicide: laccases are able to convert the diketonitrile into the acid. Laccase can be also used to reduce the concentration of synthetic heterocyclic compound such as halogenated organic pesticides in the soil. LMS has been extensively study in the oxidation of recalcitrant PAHs, main components of several ship spills. In this sense, LMS is being included in several enzymatic bioremediation programs. *Cerrena unicolosr* produces laccase in the low nitrogen medium which has thecapability of reducing lignin content from sugarcane bagasseup to 36% within 24 h at 30°C.

Future Trends and Perspectives

This review summarizes the available recent important about the properties, production of laccases and possible industrial and biotechnological use. Laccases are promising enzymes to replace the conventional chemical processes of several industries such as the pulp and paper, textile, pharmaceutical, and nanobiotechnology. However, one of the limitations to the large-scale application of laccases is the lack of capacity to produce large volumes of highly active enzyme at an affordable cost. The use of inexpensive sources for laccase production is being explored in recent times. Thus, efforts have to be made in order to achieve cheap over production of laccase from hosts, and also their modification by chemical means or protein engineering, to obtain more robust, active and less expensive enzymes. Another additional problem is the cost and toxicity of redox mediators. Further investigations should consider different and less polluting mediators such as the natural mediators produced by laccase in a bio-environment during lignin degradation. The current development in laccase catalysis research and the design of mediators along with the research on its heterologous expression opens a wide spectrum of possible applications in the near future. Moreover, laccase can also offer a simple and convenient alternative to using peroxidases with H_2O_2, because laccases are available on an economically feasible scale.On the other hand, the development of an effective system for laccase immobilisation also deserves great attention. Immobilization could be achieved by chemical modification of the substrates. Hence, micropatterning, SAMs and LbL techniques can be used to functionalise flat and curved surfaces in order to have specific adsorption. Laccase encapsulation with polyelectrolytes will be used as a microreactor for catalytic reactions by changing the permeability properties of the capsule wall. Since the general goal is to obtain stable catalysts with long life times and low cost, the combination of these techniques will enhance:

a. The adsorption of laccase on a suitable substrate,
b. The lifetime of the laccase activity and
c. Reutilisation of the substrate/laccase product.

Table 1: Sources of Laccase Enyme Andi'ts Physiological role

sources		Physiological role	References
Plant	*Rhus vernicifera,* cabbages, turnips, beets, apples, asparagus, potatoes, pears, sycamore maple poplar tobacco , peach loblolly pine	• oxidize monolignols in the early stages of lignifications • involved in the first steps of healing in wounded leaves • in defense mechanism against external conditions	[6,1,7,8,9,10,11,12]
Fungi	*Monocillium indicum* *Phanerochaete chrysosporium,* *Theiophora terrestris,* *Lenzites, betulina,* *Phlebia radiate,* *Pleurotus ostreatus,* *Trametes versicolour* (formerly known as *Coriolus versicolor or Polyporus versicolor*) *Trametes versicolor* *Tramete. pubescens,* *T. hirsuta* *T. gallica* *Trichoderma atroviride,* *T. harzianum,* *T. longibrachiatum* *Pleurotus ostreatus,* *Pycnoporus sanguineus*	• delignification of lignocellulosic material, • sporulation, • pigment production, • fungal morphogenesis, • protection against toxic compounds, • fruiting body formation • plant pathogenesis	[1,13,14,15,16,17,18,7,19,20]
Bacteria	*Azospirillum lipoferum* *Azospirillum lipoferum,* *Bacillus subtilis,* *Streptomyces lavendulae,* *S. cyaneus*	• melanin production for cell pigmentation • spore coat resistance against hydrogen peroxide and UV	[21,18,14,22]

	Marinomonas mediterranea. *Bacillus subtilis* *Streptomyces cyaneus,*	• involvement in morphogenesis • involved in the solubilization and mineralization of lignin	

Table 2: Production of laccase under solid state fermentation

Source	Support	Reactor	Production (U/l)	Reference
Galerina sp. HCl	Orange peels	Cotton plugged Erlenmeyer flasks	720	Laura Mendoza et al. 2010
Pycnoporus cinnabarinus	Sugarcane bagasse	Packed reactor	10000	Meza et al. 2006
Trametes hirsuta	Grape seeds	Tray	18715	Rodríguez Couto et al. 2006
Trametes hirsuta	Kiwi fruits	Cotton plugged Erlenmeyer flasks	5399	Rosales et al. 2005
Trametes hirsuta	Nylon sponge	Tray	6898	Rodríguez Couto et al. 2006
Trametes hirsuta	Orange peels	Tray	12000	Rosales et al. 2007
Trametes pubescens	Banana skin	Cotton plugged Erlenmeyer flasks	1570	Osma et al. 2007
Trametes versicolor	Barley bran	Immersion	600	Rodríguez Couto et al. 2003
Trametes versicolor	Barley bran	Tray	3500	Rodríguez Couto et al. 2003
Trametes versicolor	Nylon sponge	Expanded bed	229	Rodríguez Couto et al. 2003
Trametes versicolor	Nylon sponge	Immersion	229	Rodríguez Couto et al. 2003
Trametes versicolor	Nylon sponge	Tray	343	Rodríguez Couto et al. 2003

Table 3 : Commercial preparations based on laccases for industrial processes.

Industry	Main application	Brand name	Manufacturer
Food industry	Brewing	Flavourstar	Advanced Enzyme Technologies Ltd. (India)
	Colour enhancement in tea, etc.	LACCASE Y120	Amano Enzyme USA Co. Ltd.
	Cork modification	Suberase	Novozymes (Denmark)
Paper industry	Pulp bleaching	Lignozym-process	Lignozym GmbH (Germany)
	Paper pulp delignification	Novozym 51003	Novozymes (Denmark)
Textile Industry	Denim bleaching	Bleach Cut 3-S	Season Chemicals (China)
		DeniLite	Novozymes (Denmark)
		ZyLite	Zytex Pvt. Ltd. (India)
	Denim finishing	Cololacc BB	Colotex Biotechnology Co. Ltd. (Hong Kong)
		Ecostone LC10	AB Enzymes GmbH (Germany)
		IndiStar	Genencor Inc. (Rochester,

			USA)
		Novoprime Base 268	Novozymes (Denmark)
	Denim bleaching and shading	Primagreen Ecofade LT100	Genencor Inc. (Rochester, USA)

5. Conclusion

Laccases are widespread in nature, being produced by a wide variety of plants, fungi and also bacteria. The functions of the enzyme differ from organism to organism to organisms. Laccases catalyse the oxidation of phenolic compounds whilst simultaneously reducing molecular oxygen to water. The catalytic ability of laccases has, not surprisingly, led to diverse biotechnological applications of this enzyme. The introduction of the laccase-mediator system provides a biological alternative to traditional bleaching processes. The laccase enzyme has a wide field of application including food industries, the pulp and paper industries, the treatment of various industrial effluents, enzymatic decolouring of material and bioremediation of soils. It is therefore not surprising that this enzyme has been studied intensively since the nineteenth century and yet remains a topic of intense research today.

6. Reference

1. Sudhir Kumar and Abha Mishra, (2011) Optimization of laccase production from WRF-1 on groundnut shell and cyanobacterial biomass: By application of Box-Behnken experimental design, J. Microbiol. Biotech. Res., 2011, 1 (2): 33-53, ISSN : 2231 –3168.
2. Alpeshkumar J.Shiroya, recent advances of laccase enzyme in industrial biotechnology: a review, pharmatutor-art-1419pharmatutor-art-1419
3. A.E.Gardiol, R.J.Hernandez, B.R.Harte, (1998). Device for detecting oxygen with oxidase. United States Patent 5,804,401.
4. Y.Arakane, S.Muthukrishnan, R.W.Beeman, M.R.Kanost, K.J.Kramer, (2005). “Laccase 2 is the phenoloxidase gene required for beetle cuticle tanning,” Proceedings of The National Academy of Sciences of the United States of America, vol. 102, no. 32, pp. 11337–11342.
5. A.De Marco, K.A.Roubelakis-Angelakis, (1997). Laccase activity could contribute to cell-wall reconstitution in regenerating protoplasts. Phytochemistry, 46:421.425.
6. D.M.O’Malley, R.Whetten, W.Bao, C.L.Chen, R.R.Sederoff, (1993). The role of laccase in lignification. Plant J., 4:751.757.
7. E.Lehman, E.Harel, A.M.Mayer, (1974). Copper content and other characteristics of purified peach laccase. Phytochemistry, 13:1713.1717.
8. P.Ranocha, G.McDougall, S.Hawkins, R.Sterjiades, G.Borderies, D.Stewart, M.Cabanes-Macheteau, A.M.Boudet, D.Goffner, (1999). Biochemical characterization, molecular cloning and expression of laccases a divergent gene family - in poplar. Eur. J. Biochem, 259:485.495.
9. R.Bligny, R.Douce, (1983). Excretion of laccase by sycamore (Acer pseudoplatanus L.) cells. Purification and properties of the enzyme. Biochem. J, 209:489.496.
10. U.Dwivedi, P.Singh, V.Pandey, A.Kumar, (2011).J Mol Catal B- Enzym, 68: 117-128.
11. W.G.Levine, (1965). Laccase: a review, In: The biochemistry of copper (Academic Press Inc., New York), pp. 371-385.
12. C.Galhaup, H.Wagner, B.Hinterstoisser, D.Haltrich, (2002). Increased production of laccase by the wood-degrading basidiomycete Trametes pubescens. Enz. Microb. Technol., 30:529.536.
13. C.Held, A.Kandelbauer, M.Schroeder, A.Cavaco-Paulo G.Guebitz, (2005). Environ Chem Lett, 3: 74–77.
14. C.S. Evans and J.N. Hedger, Degradation of plant cell wall polymers, Fungi in bioremediation. (Ed. G.M. Gadd, British Mycological Society. Cambridge Univ. Press. UK 2001), pp. 1-20.
15. C.S.Chen, M.Mrkisch, S.Huang, G.M.Whitesides, D.E.Ingber, (1998). Micropatterned surfaces for control of cell shape, position, and function. Biotechnol Prog, 14:356–63.
16. D.D’Souza-Ticlo, D.Sharma, C.Raghukumar, (2009). “A thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus,” Marine Biotechnology, vol. 11, no. 6, pp. 725–737.
17. D.Faure, M.L.Bouillant, R.Bally, (1994). Isolation of Azospirillum lipoferum 4T Tn5 mutants affected in melanization and laccase activity. Appl. Environ. Microbiol., 60:3413.3415.
18. D.Rigling, N.K.van Alfen, (1991). Regulation of laccase biosynthesis in the plant-pathogenic fungus Cryphonectria parasitica by double-stranded RNA. J. Bacteriol, 173:8000.8003.
19. D.S.Yaver, R.M.Berka, S.H.Brown, F.Xu, (2001). The presymposium on recent advances in lignin biodegradation and biosynthesis, Vikki Biocentre, University of Helsinki, Finland, pp.40.
20. A.Givaudan, A.Effose, D.Faure, P.Potier, M.L.Bouillant, R.Bally, (1993). Polyphenol oxidase in Azospirillum lipoferum isolated from rice rhizosphere: evidence for laccase activity in non-motile strains of Azospirillum lipoferum. FEMS Microbiol. Lett., 108:205.210.
21. P.Sharma, R.Goel N.Capalash, (2007). World J Microbiol Biotechnol, 23:823–832.
22. A.Kunamneni, F.Plou, A.Ballesteros, M.Alcalde, (2008). Recent Patents on Biotechnology 2: 10-24.

23. F.S. Archibald, R. Bourbonnais, L. Jurasek, M.G. Paice, and I.D. Reid, Journal of Biotechnology, 53, 215 (1997).
24. M. Heinzkill, L. Bech, T. Halkier, P. Schneider and T. Anke, Applied and Environmental Microbiology 64, 1601 (1998).
25. A.M.R.B. Xavier, D.V. Evtuguin, R.M.P. Ferreira and F.L. Amado, Laccase production for lignin oxidative activity. Proceedings of the 8th International Conference on Biotechnology in the Pulp and Paper Industry, 4-8 June, Helsinki, Finland, 2001.
26. J.A. Buswell, Y. Cai and S. Chang, FEMS Microbiology Letters 128, 81 (1995).H. Park, Biotechnology Letters 21, 965 (1999).
27. V. Faraco, P. Giardina, G. Palmieri and G. Sannia, Progress in Biotechnology 21, 105 (2002).
28. G.Songulashvili, V.Elisashvili, S.Wasser, E.Nevo, Y.Hadar, (2006). Biotechnol Lett, 28:1425–1429.
29. Robene-Soustrade and B. Lung-Escarmant, European Journal of Forest Pathology 27, 105 (1997).
30. N.Hatvani, I.Mécs, (2001). Process Biochem, 37: 491-496.
31. M.Staji, L.Persky, D.Friesem, Y.Hadar, S.Wasser, E.Nevo, et al., (2006). Enzyme Microb Technol, 38: 65-73.
32. M.C. Monteiro and M.E.A. De Carvalho, Applied Biochemistry and Biotechnology 70-72, 983 (1998).
33. C. Galhaup and D. Haltrich, Applied Microbiology and Biotechnology 56, 225 (2001).
34. A.F. Vasconcelos, A.M. Barbosa, R.F.H. Dekker, I.S. Scarminio and M.I. Rezende, Process Biochemistry 35, 1131 (2000).
35. E.Selinheimo, K.Kruus, J.Buchert, A.Hopia, K.Autio, (2006). Effects of laccase, xylanase and their combination on the rheological properties of wheat doughs. J Cereal Sci, 43:152–9.
36. S.Khammuang, R.Sarnthima, (2009). "Laccase activity from fresh fruiting bodies of Ganoderma sp. MK05: purification and remazol brilliant blue R decolorization," Journal of Biological Sciences, vol. 9, no. 1, pp. 83–87.
37. R.F.Taylor, (1991). Protein immobilization: Fundamental and applications (Marcel Dekker Inc., New York, USA).
38. R.C.Minussi, G.M.Pastore, N.Durán, (2002). Potential applications of laccase in the food industry. Trends Food Sci Technol, 13:205–16.
39. A.I. Yaropolov, O.V. Skorobogat'ko, S.S. Vartanov and S.D. Varfolomeyev, Applied Biochemistry and Biotechnology 49, 257 (1994).
40. P.T.Hammond, G.M.Whitesides, (1995). Formation of polymer microstructures by selective deposition of polyion multilayers using patterned selfassembled monolayers as a template. Macromolecules, 28: 7569–71.