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MICROBIAL LACCASES: A MINI-REVIEW ON THEIR PRODUCTION, PURIFICATION AND APPLICATIONS

REVIEW ARTICLE

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ABSTRACTS

L*accases are chief ligninolytic enzyme which belongs to the blue multicopper oxidases and participates in cross-linking of monomers, degradation of polymers, and ring cleavage of complex aromatic compounds.There are diverse sources of laccase producing organisms like bacteria, fungi and plants. But they have been widely characterized in fungi than in higher plants. These have several biotechnological applications including food industry, paper and pulp industry textile industry, synthetic chemistry, cosmetics, soil bioremediation, removal of toxic pollutants agent like herbicides, pesticides, dye degradation and removal of endocrine disruptors. Due to broad range application there is a growing need for isolation and identification of new laccase producing organisms to be used in industries. These enzymes have received attention of researchers in the last few decades due to introduction of laccase mediator system which provide the ability to oxidize both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant environmental pollutants. This paper reviews the occurrence, mode of action, production and cultivation techniques, immobilization as well as potential applications of laccases within different industrial fields.*

Key Words: *Applications, Immobilization, Laccase Mediator system, Oxidation, Production.*

1. INTRODUCTION

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) belong to broad group of enzymes called polyphenol oxidases containing multi copper atoms in the catalytic center and are usually called multicopper oxidases [1-3]. Three types of copper atoms reside in these enzymes, one of which is responsible for their characteristic blue color. Typically laccase-mediated catalysis occurs with reduction of oxygen to water accompanied by the oxidation of substrate. Laccases are widely distributed in higher plants and fungi and have also been found in bacteria and insects [4]. Krishna Kant Sharma and Ramesh Chander Kuhad (2009) *et al.* [5] reported evidence of laccase in archaea too. In plants, laccases are found in pears, cabbages apples, potatoes and other vegetables. They have been isolated from Ascomyceteous, Deuteromycteous and Basidiomycetous fungi. Recently, most of the laccase studied are of fungal origin, especially from white-rot fungi, Anthracophyllum discolor, Pycnoporus sanguineus, Trichoderma harzianum etc [V. Sivakami *et al*. 2012, Alexandra M *et al.* 2004]. The white-rot Basidiomycetes fungi efficiently degrade the lignin in comparison to Ascomycetes and Deuteromycetes which oxidize phenolic compounds to give phenoxy radicals and quinines. Concerning their use in the biotechnology area, laccases have widespread applications, ranging from food industry, paper and pulp industry textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutant and removal of endocrine disruptors, many of which have been patented. Now a days biotechnological use of laccase has been expanded by the introduction of laccase-mediator systems, as they provide ablility to laccase to oxidise non-phenolic compounds that are otherwise hardly or not oxidised by the enzyme alone.

The successful application of laccases in mentioned areas would require production of high amounts at reduced costs [Muhammad I *et al.* 2012]. Several production strategies can be adopted along with media and process optimization to achieve better process economics. Concomitantly, overexpression of laccase in suitable host organisms would provide means to achieve high titers. Use of inducers could also enhance production capabilities.

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2. SOURCES OF LACCASES

Laccases are generally distributed in a wide range of higher plants and fungi [Savitha S. Desai 2011, Benfield *et al.* 1964]. Recently some bacterial species such as *S.lavendulae*, *S.cyaneus*, and *Marinomonas mediterranea* are identified showing laccase distribution [Diamantidis *et al.* 2000]. According to a report of Madhavi and Lele (2009) laccases in plants have been identified in trees, cabbages, turnips, apples, asparagus, potatoes and pears. In fungi, laccases appear more than the higher plants. Basidiomycetes such as *Phanerochaete chrysosporium*, *Theiophora terrestris*, and *Lenzites, betulina* [Viswanath *et al.* 2008] and white-rot fungi [Kiiskinen *et al.* 2004] such as *Ganoderma* sp, *Phlebia radiate*, Pleurotus *ostreatus* and *Trametes versicolour* [Krishna Kant Sharma *et al.* 2013, Shraddha *et al*. 2011] also produce laccase. Laccase from the *Monocillium indicum* was the first laccase to be characterized from Ascomycetes which shows peroxidase activity [Thakker GD *et al.* 1992].

3. MODE OF ACTION AND MEDIATORS

Laccases are particularly abundant in white-rot fungi, which are the only organisms able to degrade the whole wood components. The use of molecular oxygen as the oxidant and the fact that water is the onlyby-product are very attractive catalytic features, rendering laccases as excellent catalysts [Reid and Paice 1994, S.Riva, 2006].The natural substrates of laccase include phenols like *ortho*- and *para*-diphenols, aminophenols, polyphenols, polyamines and aryl diamines. Substrate oxidation by laccase is a one-electron reaction generating a free radical. The initial product is typically unstable and may undergo a second enzyme-catalysed oxidation or otherwise a non-enzymatic reaction such as hydration, disproportionation or polymerization. These enzymes are polymeric and generally contain 1 each of type 1, type 2, and type 3copper centre/subunit where the type 2 and type 3 are close together forming a trinuclear copper cluster. The bonds of the natural substrate, lignin, that are cleaved by laccase include Cα- oxidation, Cα-Cβ cleavage and aryl-alkyl cleavage[Khushal B and Anne R *et al.* 2010**].** Laccase can oxidize only phenolic fragments of lignin due to the random polymer nature of lignin and to the laccase lower redox potential. Application of these enzymes in the presence of mediator compounds resulted in a high oxidation capability, leading to the oxidation of nonphenolic lignin model compounds.The first attempt at using laccase mediator couples for delignification in the pulp industry was the development of Lignozym process, which was described by some authors [H.P. Call and Mucke 1997, M. Kapoor, R.K. Kapoor, R.C. Kuhad 2007]. The diammonium salt 2,2'-azino*bis*(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1-hydroxybenzotriazole (HBT), N-hydroxyacetanilide (NHA), violuric acid (VLA), N-hydroxyphthalimide (HPT) and 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) (and its derivatives) are act as Synthetic mediators. In presence of ABTS oxygen uptake by laccase is faster than the HBT. The application of these mediators can be limited due to their high cost as well as their toxicity [S.Riva, 2006]

4. PRODUCTION OF LACCASE

Laccase activity was detected in the cultures of a wide range of fungi, from Ascomycetes to Basidiomycetes, and from wood- and litter-decomposing fungi to ectomycorrhizal fungi [J.M. Bollag and A. Leonowicz 1984]. White-rot fungi, such as *Coriolus versicolor* and *P. sanguineus*, *Murr.* are known producers of lignolytic enzymes that are involved in the natural delignification of wood [H.P. Call and Mucke, 1997]. Laccases are extracellular enzymes which are secreted out in medium by several fungi during the secondary metabolism. To satisfy the growing market for laccase in various biotechnological applications requires a huge amount of enzyme production. Gene expressions, the modification of culture conditions, or combinations of both strategies are utilized to cover the demand. Several factors including type of cultivation (submerged or solid state), carbon limitation, nitrogen source, and concentration of microelements can influence laccase production [Kusum Dhakar and Anita Pandey 2013, Gayazov R, Rodakiewicz-Nowak J 1996].

4.1 Influence of Carbon and Nitrogen Source

Glucose, maltose, sucrose, fructose, glycerol and lactose are the commonly used carbon sources. Fructose was shown to be a good carbon source for laccase production in *Pleurotus sajor-caju*, cellobiose in *T. pubescens*, and lactose or glycerol in *Pseudotrametes gibbosa*, *Coriolus versicolor* and *Fomes fomentarius*. The excessive concentrations of glucose are inhibitory to laccase production in various fungal strains. An excess of sucrose also reduced the production of laccase by blocking its induction and only allowed constitutive production of enzyme. This problem of production of enzyme can be improved by using polymeric substrates like cellulose as carbon source during cultivation. [Khushal B and Anne R *et al*. 2010, Shraddha *et al.* 2011, Lee KH *et al.* 2004]. Yeast extract, peptone, urea, $(NH_4)_2SO_4$, and NaNO3 are the commonly used nitrogen sources. Fungal laccases are mainly triggered by nitrogen depletion [Keyser P, Kirk TK, Zeikus JG. 1978], but it was also found that in some strains nitrogen had no effect on enzyme activity [Leatham GF, Kirk TK 1983]. High laccase activity was reported in some studies using low carbon to nitrogen ratio [Monteiro MC, De Carvalho MEA 1998], but other studies showed that higher laccase production was achieved at high carbon to nitrogen ratio [Buswell JA, Cai Y, Chang S-T 1995]. Laccase was also produced earlier when the fungus was cultivated in nitrogen rich media rather than nitrogen-limited media [Heinzkill *et al.* 1994].

4.2 Influence of pH and Temperature on Laccase Production

The optimal temperature of laccase differs greatly from one strain to another. 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[Kusum Dhakar and Anita Pandey 2013, C.F. Thurston 1994]. Farnet *et al.* (2000) found that preincubation 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°C–60°C, with maximum activity at 50°C. The information on effect of pH affects on laccase production is scarce, but most reports indicate initial pH between 4.5 and 6.0 that is suitable for enzyme production [Shraddha, Ravi Shekher and Ajay Kumar 2011]. Cordi *et al.* use syringaldazine as a substrate and determine the effect of pH on enzyme activity in the range of 3.0–8.0.

4.3 Influence of Agitator

Agitation is also an important factor which affects enzyme production. Hess *et al.* (2002) that mycelia of fungus are damaged when it is grown in the stirred tank reactor and laccase production by *Trametes multicolour* is considerably decreased. But in some other studies like Tavares *et al.* (2006) observed that agitation did not play any role in the production of laccase.

4.4 Induction of Laccase

Laccases were generally produced in low concentrations by laccase producing fungi but higher concentrations were obtainable with the addition of various supplements to media [I.Y Lee *et al.* 2000]. There are various response element sites in the promoter regions of laccase genes that can be induced by certain xenobiotic compounds, heavy metals or heatshock treatment [V. Faraco, P. Giardina, G. Palmieri and G. Sannia 2002]. The addition of xenobiotic compounds such as xylidine, lignin, and veratryl alcohol increased and induced laccase activity [Xavier *et al.* 2001]. In one study by Lu *et al.* (1996) it was observed that addition of cellobiose can induce appreciable laccase activity in some species of *Trametes*. Low concentration of copper was also shown to exhibit inducible effect on laccase activity [Palmieri G *et al.* 1997]. Palmieri et al. found that the addition of 150 µM copper sulphate to the cultivation media can result in a fifty-fold increase in laccase activity compared to a basal medium. Employing copper sulphate as laccase inducer or supplementing the culture medium with veratryl alcohol, led to maximum values of laccase activity [Dominguez, J. Gomez, M. Lorenzo and A. Sanroman 2007].

4.5 Overexpression of Laccase

Due to increased interest in the use of these enzymes for various industrial applications the demand in the market is growing greatly up during these years [Couto SR, Toca-Herrera JL. 2007]. To fulfill these demands a large scale production is needed. Recombinant technology can make this possible. Heterologous expression of the genes encoding the laccases is carried out in order to increase their production and to be able to apply them in large-scale processes/applications. Laccase genes have been successfully cloned and heterologously expressed in the filamentous fungi *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei* [Shraddha, Ravi Shekher and Ajay Kumar 2011**,** Couto SR, Toca-Herrera JL. 2007]. In spite of the fact that laccase production levels have often been improved significantly by expression in heterologous hosts, the reported levels are still rather low for industrial applications. These days' most commercial laccases are produced in *Aspergillus* hosts.Another efficient expression system was developed for the basidiomycete *P. cinnabarinus* and this was used to transform a laccase-deficient monokaryotic strain with the homologous laccase gene. The yield was above 1.2 g of laccase per litre and represents the best laccase production reported for recombinant fungal strains.

5. TYPE OF CULTIVATION

Submerged and solid-state modes of fermentation are used mainly for the production of laccase. Wild-type filamentous fungi are used for large-scale production of laccase in different cultivation techniques**.**

5.1 Submerged Cultivation

Submerged cultivation process involves the growth of microorganisms in a liquid medium provided with nutrients under aerobic conditions. In order to achieve high production, the first step is focused on the optimization of nutritional and operational conditions. Waste and cheap materials which are produced in large amounts can be utilized in submerged cultivation. These materials can contain considerable concentrations of soluble carbohydrates, nitrogen, minerals and vitamins, and even inducers for enzyme production **[**Khushal B *et al.* 2010] (Table 1). The industrial production of enzymes is mainly achieved by submerged cultivation. One of the major challenges in fungal submerged fermentations is viscosity of broth. Mycelium formation during growth of fungal cells can also impede impeller action causing blockades resulting in oxygen and mass transfer limitations; however, this has been overcome by immobilization. Sedarati *et al.* (2003) compared the free cell cultures of *T. versicolor* with immobilized cultures using nylon mesh for the bioremediation of pentachlorophenol (PCP) and 2,4-dichlorophenol (2,4 DCP). Authors observed that immobilized cultures led to efficient removal. Couto *et al.* (2004) investigated different synthetic materials as carriers for the immobilization of the white rot fungus *Trametes hirsuta* in fixed bed bioreactors operated in batch. They found that among the different materials tested, stainless steel sponge led to the highest laccase activities. Process controlling and monitoring in submerged cultivation is easy and microbial growth and product formations are fast and more efficient than solid state.

Table 1: Production of laccase in submerged cultivation using agricultural and synthetic materials

Source	Support	Reactor	Reference
Galerina sp. HCl	Orange peels, Bagasse	Batch	Laura Mendoza et al. 2014
Ganodermaadspersum	Corn bran, soy bran, chicken feathers, wheat bran, kiwi	Batch	Songulashvili et al. 2006
Lentinus edodes	Malted barley (brewing process)	Batch	Hatvani & Mécs 2001
Neurospora crassa	Capillary membrane, supports	Batch	Rodríguez Couto & Herrera 2007
Phellinus robustus	fruits, banana peels, mandarin peels, ethanol production residue and cotton stalks	Batch	Songulashvili et al. 2006
Pleurotus eryngii	Dried ground, mandarin peels	Batch	Staji et al. 2006
Pleurotus ostreatus	Immobilized in polyurethane foam	Packed bed reactor	Rodríguez Couto & Herrera 2007
Pycnoporus cinnabarinus	Cubes of sponge	Packed bed reactor	Rodríguez Couto & Herrera 2007
Trametes hirsuta	Alginate beads	Air lift reactor	Rodríguez Couto & Herrera 2007
Trametes versicolor(CBS100.29)	Grape seeds, grape stalks and barley bran	Batch	Lorenzo et al. 2002

5.2 Solid-State Fermentation is a cultivation technique occurring in absence or near absence of free liquid, employing an inert substrate (synthetic materials) or a natural substrate (organic materials) as a solid support [Pandey A, Selvakumar P, Soccol CR, Nigam P 1999]. SSF is suitable for the production of enzymes by using natural substrates such as agricultural residues because they mimic the conditions under which the fungi grow naturally. Laccase production by both solid-state and submerged fermentation is higher in case of rice bran than other substrates. Many agricultural wastes such as grape seeds, grape stalks, barley bran, cotton stalk, molasses waste water [R.Bourbonnais 1997] and wheat bran are also used as substrate for laccase production [Table 2]. Solid substrate cultivation is cost effective than submerged as low cost substrates are being used.

The principal disadvantages for growing microorganisms in SSF are the low transfer of oxygen, nutrients, moisture, temperature and regulation of pH due to lack of any established bioreactor designs.

Table 2: Production of laccase under solid state fermentation

Source	Support	Reactor	Reference
Galerina sp. HCl	Orange peels	Cotton plugged Erlenmeyer flasks	Laura Mendoza et al. 2010
Pycnoporus cinnabarinus	Sugarcane bagasse	Packed reactor	Meza et al. 2006
Trametes hirsuta	Grape seeds	Tray	Rodríguez Couto et al. 2006
Trametes hirsuta	Kiwi fruits	Cotton plugged Erlenmeyer flasks	Rosales et al. 2005
Trametes hirsuta	Nylon sponge	Tray	Rodríguez Couto et al. 2006
Trametes hirsuta	Orange peels	Tray	Rosales et al. 2007
Trametes pubescens	Banana skin	Cotton plugged Erlenmeyer flasks	Osma et al. 2007
Trametes versicolor	Barley bran	Immersion	Rodríguez Couto et al. 2003
Trametes versicolor	Nylon sponge	Expanded bed	Rodríguez Couto et al. 2003
Trametes versicolor	Nylon sponge	Tray	Rodríguez Couto et al. 2003

6. DOWNSTREAMING PROCESSING OR PURIFICATION OF LACCASE– *'a step after the cultivation process'*

In general, plant laccases are purified from sap or tissue extracts. But in case where white rot fungi are used as the source for laccase production, the enzyme is extracellular produced in the medium. Then fungal laccases are purified from culture medium (fermentation broth) of the selected organism. A complete down-streaming process can carried out in two operations, in primary operation solid/liquid separation by filtration and centrifugation. Dewatering can be done by salting out precipitation method using ammonium sulphate salt. In secondary operation enzyme purification can be achieved through ion exchange and gel filtration chromatographic techniques. Purification may be single or multi-step process. E.Grotewold et al. performed single-step laccase purification from *Neurospora crassa* by using celite chromatography and 54 fold purification with specific activity of 333 U mg−1. 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 [J.Hess, C.Leitner, C.Galhaup et al., 2002]. 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 [L.Cordi *et al.* 2007]. After separation, enzyme processing is carried out through immobilization techniques.

7. APPLICATIONS OF LACCASE

7.1 Food Industry

Laccase application in the food industry is based on its ability to polymerize molecules. Laccases can be applied to certain processes that enhance or modify the colour appearance of food or beverage for the elimination of undesirable phenolics, responsible for the browning, haze formation and turbidity in clear fruit juice, beer and wine [Shraddha *et al.* 2011**,** Rodríguez and Toca, 2006]. Laccase is also employed to ascorbic acid determination, sugar beet pectin gelation, baking and in the treatment of olive mill wastewater. Selinheimo *et al.* (2006) showed that 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 [Khushal B et al. 2010, E.Selinheimo et al. 2006]

7.2 Pulp and paper industry

Laccases are able to depolymerize lignin and delignify wood pulps, kraft pulp fibers and chlorine-free in the biopolpation process. Laccases are more easily able to delignify pulp when they are used together with mediators. Mediators may be used to oxidize the non-phenolic residues from the oxygen delignification. The mediator is oxidized by laccase and the oxidized mediator molecule further oxidizes subunits of lignin that otherwise would not be laccase substrates.Laccase mediator systems can also be applied to remove pitch and dyes from wood-based materials. Laccases can be used for binding fiber-, particle- and paper-boards [Khushal B *et al.* 2010].

7.3 Textile industry

The chemical reagents used in textile industry are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products. Different chemicals are used and most of them are difficult to decolourise due to their synthetic origin. Conventional processes to treat dye wastewater are ineffective and not economical. Therefore, the development of processes based on laccases seems an attractive solution due to their potential in degrading dyes of diverse chemical structure, including synthetic dyes currently employed in the industry [Khushal B *et al.* 2010]. It is reported that laccases-mediator system finds potential application in enzymatic modification of dye bleaching in the textile and dyes industries.

7.4 Bioremediation

Due its catalytic properties laccases are involved in green biodegradation. The xenobiotic compound is a major source of contamination in soil and laccase degrade it. Moreover, polycyclic aromatic hydrocarbons (PAHs), which arise from natural oil deposits and utilisation of fossil fuels, are also degraded. They could be used for decolorizing dye house effluents that are hardly decolorized by conventional sewage treatment plants. 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 laccases [Khushal Brijwani, Anne Rigdon, and Praveen V. Vadlani 2010].

7.5 Pharmaceutical Sector

Due to their specificity and bio-based nature, potential applications of laccases in the field of pharma sector are inducing the research activities. Pharma companies are using these enzymes in the synthesis of complex medical compounds such as anesthetics, anti-inflammatory, antibiotics, sedatives, etc., including triazolo(benzo) cycloalkyl thiadiazines, vinblastine, mitomycin, penicillin X dimer, cephalosporins, and dimerized vindoline.

One potential application is laccase-based *in situ* generation of iodine, a reagent widely used as disinfectant. Also, in one report laccase has been found to possess significant HIV-1 reverse transcriptase inhibitory activity [Alpeshkumar J.Shiroya, H.X.Wang, T.B.Ng 2004].

7.6 Cosmetic sector:
More recently laccases are being used in cosmetics also. Cosmetic and dermatological preparations containing proteins for skin lightening have been developed. Recently developed laccase-based hair dyes could be less irritant and easier to handle than current hair dyes. Laccases may find use as deodorants for personal-hygiene products, including toothpaste, mouthwash, detergent, soap, and diapers. Protein engineered laccase may be used to reduce allergenicity [H.X.Wang, T.B.Ng, 2004].

8. LACCASE IMMOBILIZATION

Enzymes exhibit a number of features that make their use advantageous as compared to conventional chemical catalysts. But effective applications of enzymes may be hampered by undesirable properties of the enzymatic proteins such as their non-reusability, high sensitivity to several denaturizing agents and presence of adverse sensory or toxicological effects. Many of these undesirable limitations may be overcome by the use of immobilized enzymes [Khushal B *et al.* 2010]. Immobilization is achieved by fixing enzymes to or within solid supports. There are many procedures which are available for enzyme immobilization. Immobilization procedures greatly influences the properties of the resulting biocatalyst. They include several parameters such as overall catalytic activity, effectiveness of catalyst utilization, deactivation and regeneration of kinetics, cost. Also, toxicity of immobilization reagentsshould be considered in connection with the immobilization process, waste disposal and final application of theimmobilized enzyme catalyst [Bailey JE, Ollis DF 1986, Sun WQ, Payne GF 1996, Munoz C 1996].

Enzymes may be immobilized by a variety of methods (adsorption, entrapment, crosslinking and covalent bonding) mainly based on chemical and/or physical mechanisms. Enzyme immobilization by physical entrapment has the benefit of a wide applicability and may provide relatively small perturbation of the enzyme native structure and function. The most widely used system for enzyme entrapment in a polymer lattice is the immobilization within a poly acrylamide gel, obtained by polymerization/cross-linking of acrylamide in the presence of the enzyme. The most commonly employed water-insoluble supports currently used for enzyme immobilization are cyanogens bromide-activated sepharose and sephadex. The chemical activated method for sepharose CL-6B with an epoxide is quite efficient to obtain an aldehyde activating group in the support able to react with the enzyme [Munoz C *et al.* 1996]. A proper choice between chemical and physical methods depends on several factors

9. FUTURE TRENDS AND PERSPECTIVES

This review mainly summarizes the existed or recent importance about the properties, heterologous production, media optimization 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. More research needs to be carried out to improve laccase production with reducing cost, which includes further medium optimisation considering variety of cheap biological waste material such as lignocellulosic wastes and other agricultural waste. Due to the relatively low yield of laccases obtained by the existing purification process, further small scale experiments shall be carried out to optimise the procedures and parameters at different purification steps in order to achieve high yield, and a robust scalable purification process.

On the other hand, the development of an effective system for laccase immobilisation also deserves great attention to achieve the general goal of obtaining stable catalysts with long life times and low cost. Immobilisation could be achieved by chemical modification of the substrates.

10. CONCLUSION

Laccases are the versatile enzymes which catalyze oxidation reactions coupled to four-electron reduction of molecular oxygen to water. They are multicopper enzymes which are widely distributed in higher plants, fungi and also reported in some bacteria and archaea. With introduction of the laccase-mediator system laccases can 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. They can be effectively used in paper and pulp industries, textile industries, xenobiotic degradation, and bioremediation and act as biosensor. Laccase has been applied to nanobiotechnology which is an increasing research field and catalyzes electron transfer reactions without additional cofactors. The biotechnological significance of these enzymes has led to a drastic increase in the demand for these enzymes in the recent time. To satisfy the demands large scale production of laccases can be achieved through overexpression of laccases in heterologous host, media optimization and use of appropriate inducers. Recently several chemical and physical techniques have been developed for the immobilization which immobilize laccase and preserve their enzymatic activity.

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REFERENCES

1. DESAI, S. S., TENNALI, G. B., CHANNUR, N., ANUP, A. C., DESHPANDE, G., AZHAR MURTUZA, B. P. 2011. Isolation of laccase producing fungi and partial characterization of laccase. *Biotechnology, Bioinformatics and Bioengineering*, 1, 543-549.
2. IMRAN, M., ASAD, M. J., HADRI, S. H., MEHMOOD, S. 2012. Production and industrial applications of laccase enzyme. *Journal of Cell and Molecular Biology*, 10, 1-11.
3. BENFIELD, G., BOCKS, S. M., BROMLEY, K., BROWN, B. R. 1964. Studies in fungal and plant laccases. *Phytochemistry*, 3, 79-88.
4. SHARMA, K. K., KUHAD, R. C. 2008. Laccase: enzyme revisited and function redefined. *Indian Journal of Microbiology*, 48, 309-316.
5. SHARMA, K. K., KUHAD, R. C. 2009. An evidence of laccases in archaea. *Indian Journal of Microbiology*, 49, 142-150.'
6. SIVAKAMI, V., RAMACHANDRAN, B., SRIVATHSAN, J., KESAVAPERUMAL, G., SMILY, B., KUMAR, D. J. M. 2012. Production and optimization of laccase and lignin peroxidase by newly isolated *Pleurotus ostreatus LIG 19*. *Journal of Microbiology and Biotechnology*, 2, 875-881.
7. ALVES, A. M. C. R., RECORD, E., LOMASCOLO, A., SCHOLTMEIJER, K., ASTHER, M., WESSELS, J. G. H., WOSTEN, H. A. B. 2004. Highly Efficient Production of Laccase by the Basidiomycete *Pycnoporus cinnabarinus.Applied and Environmental Microbiology*, 70, 6379-6384.
8. DIAMANTIDIS, G., EFFOSSE, A., POTIER, P., BALLY, R. 2000. Purification and characterization of the first bacterial laccase in rhizospheric bacterium *Azospirillum lipoferum*. *Soil Biology and Biochemistry*, 32, 919-927.
9. MADHAVI, V., LELE, S. S. 2009. Laccase: properties and applications. *BioResources.* 4, 1694-1717.
10. VISWANATH, B., VISWANATH, B., M SUBHOSH CHANDRA, PALLAVI H, RAJASEKHAR REDDY B. 2008. Screening and assessment of laccase producing fungi isolated from different environmental samples. *African Journal of Biotechnology*, 7, 1129-1133.
11. KIISKINEN, L. L., KRUUS, K., BAILEY, M., YLÖSMÄKI, E., SIIKA-AHO, M., SALOHEIMO, M. 2004. Expression of *Melanocarpus albomyces* laccase in *Trichoderma reesei* and characterization of the purified enzyme. *Microbiology*, 150, 3065-3074.
12. SHARMA, K. K., SHRIVASTAVA, B., SASTRY, V. R. B., SEHGAL, N., KUHAD, R. C. 2013. Middle-redox potential laccase from *Ganoderma* sp.: its application in improvement of feed for monogastric animals. *Science Reports*, 3, 1299.
13. SHRADDHA., SHEKHER, R., SEHGAL, S., KAMTHANIA, M., KUMAR, A. 2011. Laccase: Microbial Sources, Production, Purification, and Potential Biotechnological Applications. *Enzyme research*, 2011:217861.
14. THAKKER, G. D., EVANS, C. S., KOTESWARA, R. K. 1992. Purification and characterization of laccase from Monocillium indicum Saxena. *Applied Microbiology and Biotechnology*, 37, 321-323.
15. RIVA, S. 2006. Laccases: blue enzymes for green chemistry. *Trends in Biotechnology*, 24, 219-226.
16. BRIJWANI, K., RIGDON, A., VADLANI, P. V. 2010. Fungal Laccases: Production, Function and Applications in Food Processing. *Enzyme Research*, 2010:149748.
17. CALL, H. P., MUCKE, I. 1997. History, overview and applications of mediated lignolytic system, specially laccase mediator systems (lignozym-process). *Journal of Biotechnology*, 53, 163-202.
18. KAPOOR, M., KAPOOR,R. K., KUHADR. C. 2007. Differential and synergistic effects of xylanase and laccase mediator system (LMS) in bleaching of soda and waste pulps. *Journal of Applied Microbiology*,103, 305-317.
19. BOLLAG, J. M., LEONOWICZ, A. 1984. Comparative studies of extracellular fungal laccase. *Applied and Environmental Microbiology*, 48, 849-854.
20. DHAKAR, K., PANDEY, A. 2013. Laccase Production from a Temperature and pH Tolerant Fungal Strain of *Trametes hirsuta* (MTCC 11397).*Enzyme Research*, 2013:869062.
21. GAYAZOV, R., RODAKIEWICZ-NOWAK, J. 1996. Semi-continuous production of laccase by *Phlebia radiata* in different culture media. *Folia Microbiologica*, 41,480-484.
22. LEE, K. H., WI, S. G., SINGH, A. P., KIM, Y. S. 2004. Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus *Coniophora puteana*. *Journal of Wood Science*, 50, 281-284.
23. KEYSER, P., KIRK, T.K., ZEIKUS, J. G. 1978. Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. *Journal of Bacteriology*, 135, 790-797.
24. LEATHAM, G. F., KIRK, T. K. 1983. Regulation of ligninolytic activity by nutrient nitrogen in white-rot basidiomycetes. *Microbiology Letters*, 16, 65-67.
25. MONTEIRO, M. C., DE CARVALHO, M. E. A. 1998. Pulp bleaching using laccase from *Trametes versacolor* under high temperature and alkaline conditions. *Applied Biochemistry and Biotechnology*, 70-72, 983-993.
26. BUSWELL, J. A., CAI, Y., CHANG, S. T. 1995. Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production *by Lentinula (Lentinus) edodes*. *Microbiology Letters*, 128, 81-88.

27. HEINZKILL, M., BECH, L., HALKIER, T., SCHNEIDER, P., ANKE, T. 1998. Characterization of laccases and peroxidases from wood-rotting fungi (family Coprinaceae). *Applied and* Environmental Microbiology, 64, 1601-1606.
28. THURSTON, C. F. 1994. The structure and function of fungal laccases. Microbiology, 140, 19-26.
29. FARNET, M., CRIQUET, S., TAGGER, S., GIL, G., LE PETIT. J. 2000. Purification, partial characterization, and reactivity with aromatic compounds of two laccases from Marasmius quercophilus strain 17. *Canadian Journal of Microbiology*, 46, 189-194.
30. CORDI, L., MINUSSI, R. C., FREIRE, R. S., DURAN, N. 2007. Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. African Journal of Biotechnology, 6, 1255-1259.
31. HESS, J., LEITNER, C., GALHAUP, C., KULBE, K. D., HINTERSTOISSER B., STEINWENDER, M., HALTRICH, D. 2002. Enhanced formation of extracellular laccase activity by the white-rot fungus *Trametes multicolor*. *Applied Biochemistry and Biotechnology*, 98-100, 229-241.
32. TAVARES, A. P., COELHO, M. A., AGAPITO, M. S., COUTINHO, J. A., XAVIER, A. M. 2006. Optimization and modeling of laccase production by *Trametes versicolor* in a bioreactor using statistical experimental design. *Applied Biochemistry and Biotechnology*, 134, 233-248.
33. LEE, I. Y., JUNG, K. H., LEE, C. H., PARK, Y. H. 1999. Enhanced production of laccase in *Trametes vesicolor* by the addition of ethanol. *Biotechnology Letters*, 21, 965-968.
34. FARACO, V., GIARDINA, P., PALMIERI, G., SANNIA, G. 2002. Metal-activated laccase promoters. *Progress in Biotechnology*, 21, 105-111.
35. XAVIER, A. M. R. B., EVTUGUIN, D.V., FERREIRA, R. M.P., AMADO, F. L. 2001. Laccase production for lignin oxidase activity. In: Proceedings of 8th International Conference on Biotechnology; Helsinki, Finland.
36. LU, S. X. F., JONES, C. L., LONERGAN, G. T. 1996. Correlation between fungal morphology and laccase expression under the influence of cellobiose. In: Proceedings of the 10th International Biotechnology Symposium; Sydney, Australia.
37. PALMIERI, G., GIARDINA, P., BIANCO, C., FONTALLELLA, B., SANNINA, G. 2000. Copper induction of laccase isoenzyme in the lignolytic fungus *Pleurotus ostreatus*. *Applied Microbiology and Biotechnology*, 66, 920-924.
38. DOMINGUEZ, A., GOMEZ, J., LORENZO. M., SANROMAN, A. 2007. Enhanced production of laccase activity by *Trametes versicolor* immobilized into alginate beads by addition of different inducers. *World Journal of Microbial Biotechnology*, 23, 367-373.
39. COUTO, S. R., TOCA-HERRERA, J. L. 2007. Laccase production at reactor scale by filamentous fungi. *Biotechnology Advances*, 25, 558-569.
40. SEDARATI, M. R., KESHAVARZ, T., LEONTIEVSKY, A. A., EVANS, C. S. 2003. Transformation of high concentrations of chlorophenols by the white-rot basidiomycete *Trametes versicolor* immobilized on nylon mesh. *Electronic Journal of Biotechnology*, 6, 27-37.
41. RODRIGUEZ, C. S., SANROMAN, M. A., HOFER, D., GUBITZ, G. M. 2004. Stainless steel sponge: a novel carrier for the immobilisation of the white-rot fungus *Trametes hirsuta* for decolourisation of textile dyes. *Bioresource Technology*, 95, 67-72.
42. SONGULASHVILI, G., ELISASHVILI, V., WASSER, S., NEVO, E., HADAR, Y. 2006. Laccase and manganese peroxidase activities of *Phellinus robustus* and *Ganoderma adspersum* grown on food industry wastes in submerged fermentation. *Biotechnology Letters*, 28, 1425-1429.
43. Hatvani, N., Mecs, I. 2001.Production of Laccase and Manganese Peroxidase by *Lentinus edodes* on Malt-Containing by-Product of the Brewing Process. *Process Biochemistry*, 37, 491-496.
44. STAJI, M., PERSKY, L., FRIESEM, D., HADAR, Y., WASSER, S. P., NEVO, E., VUKOJEVI J. 2006. Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. *Enzyme and Microbial Technology*, 38, 65-73.
45. LORENZO, M., MOLDES, D., COUTO, S. R., SANROMÁN, A. 2002. Improving laccase production by employing diffrent lignocellulosic wastes in submerged cultures of *Trametes versicolor*. *Bioresource Technolology*, 82, 109-113.
46. PANDEY, A., SELVAKUMAR, P., SOCCOL, C. R., NIGAM, P. 1999. Solid state fermentation for the production of industrial enzymes. *Current Science*, 77, 149-162.
47. BOURBONNAIS, R., PAICE, M. G., FREIERMUTH, B., BODIE, E., BORNEMAN, S. 1997. Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. *Applied and Environmental Microbiology*, 12, 4627-4632.
48. MEZA, J. C., SIGOILLOT, J. C., LOMASCOLO, A., NAVARRO, D., AURIA, R. 2006.New Process for Fungal Delignification of Sugar-Cane Bagasse and Simultaneous Production of Laccase in a Vapor Phase Bioreactor. *Journal of Agricultural and Food Chemistry*, 54, 3852–3858.
49. COUTO, S. R., HERRERA, J. H 2006. Industrial and biotechnological applications of laccases: a review.*Biotechnology Advances, 24,500-513.*
50. ROSALES, E., COUTO, S. R., SANROMÁN, M. 2005. Reutilisation of food processing wastes for production of relevant metabolites: application to laccase production by Trametes hirsuta. *Journal of Food Engineering, 66, 419–423.*

51. ROSALES, E., COUTO, S. R., SANROMÁN, M. A. 2007. Increased laccase production by *Trametes hirsuta* grown on ground orange peelings. *Enzyme and Microbial Technology*, 40, 1286–1290.
52. OSMA, J. F., SARAVIA, V., HERRERA, J. L. T., COUTO, S. R. 2007.Mandarin peelings: the best carbon source to produce laccase by static cultures of Trametespubescens. *Chemosphere*, *67*, *1677*.
53. COUTO, S. R., MOLDES, D., LIÉBANAS, A., SANROMÁN, A. 2003. Laccase activity from the fungus *Trametes hirsuta* using an air-lift bioreactor. *Biochemical Engineering Journal*, *15, 21-26.*
54. GROTEWOLD, E., TACCIOLI, G.E., AISEMBERG, G.O., JUDEWICZ, N.D. 1998. Purification of an extracellular fungal laccase. *Mircen Journal of Applied Microbiology and Biotechnology*, 4, 357-363.
55. HESS, J., LEITNER, C., GALHAUP, C. 2002. Enhanced formation of extracellular laccase activity by the white-rot fungus *Trametes multicolor*. *Applied Biochemistry and Biotechnology*, 98-100, 229-241.
56. SELINHEIMO, E., KRUUS, K., BUCHERT, J., HOPIA, A., AUTIO, K. 2006. Effects of laccase, xylanase and their combination on the rheological properties of wheat doughs. Journal of Cereal Science, 43,152–159
57. KUMAR, A., SHIROYA, J**.** Recent advances of laccase enzyme in industrial biotechnology: a review. Pharma tutor pharmacy infopedia web. articles@pharmatutor.org
58. WANG, H. X., NG, T. B. 2004. A novel laccase with fair thermostability from the edible wild mushroom (Albatrella dispansus). *Biochemical Biophysics Res Communication*, *315, 450-454*
59. BAILEY, J. E., OLLIS, D. F. 1986. Biochemical engineering fundamentals. *2nd ed. New York: McGaw Hill; p. 157*
60. SUN, W. Q., PAYNE, G. F. 1996. Tyrosinase-containing chitosan gels: a combined catalyst and sorbent for selective phenol removal. *Biotechnol Bioengineering*, *51*, *79–86.*
61. MUNOZ, C., MARTINEZ, A. T., BLANCO, R. M., GUISAN, J. M., GALLETTI, G. C., MARTINEZ, M. J. 1996. Studies on physically and chemically-immobilized laccase from *Pleurotus eryngii*. *In: Proceedings of the International Conference on Biotechnol Pulp Pap Ind*, *43–6.*
62. MENDOZA, L., IBRAHIM, V., ÁLVAREZ, M. T., HATTI-KAUL, R., MAMO, G. 2014. Laccase production from Galerina sp.: and its application in dye decolourization. Journal of Yeast and Fungal Research, 5, 13-22.

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