International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 1, November-December 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Fungal Laccase - A Review on Production and its Potential Application for Human Welfare

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ABSTRACT

Laccase belongs to the blue multi-copper oxidases, which are widely distributed in fungi and higher plants. Lignin degradation by several white-rot fungi, such as Phanerochaete chrysosporium, Pleurotus ostreatus, Coriolus versicolor, Cyathus stercoreus, and Ceriporiopsis subvermispora, have been studied. Laccase enzymes have attracted attention due to its wide use in textile, pulp and paper, and food industry. Recently, it is being used in developing biosensors for detection and removal of toxic pollutants, designing of biofuel cells and medical diagnostics tool. Laccase is also being used as a bioremediation agent as they have been found potent enough in cleaning up herbicides pesticides and certain explosives in soil. Because of having the ability to oxidize phenolic, non-phenolic lignin-related compounds and highly fractious environmental pollutants, laccases have drawn the attention of researchers in the last few decades. Commercially, laccases have been used to determine the difference between codeine and morphine, produce ethanol and are also being employed in de-lignify woody tissues. To sustain this trend widespread availability of laccase and efficient production systems have to be developed. The current review discuss major advances in application of fungal laccase in white biotechnology. It delineate the laccase production and various cultivation techniques that have been developed to efficiently produce laccase at the industrial scale. The role of laccase in different food industries, and significant recent advances in the use of laccases are discussed in this review.

KEYWORDS: *Bioremediation, biodegradation, biotechnology, Laccases, Phenols*

How to cite this paper: Sonal K. Makwana | Rakeshkumar R. Panchal | Kiran C. Deshmukh "Fungal Laccase - A Review on Production and its Potential Application for Human Welfare" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-1, December 2020, pp.1353-1358, URL: www.ijtsrd.com/papers/ijtsrd38221.pdf

IJTSRD38221

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INTRODUCTION

Laccase

Laccase is a type of copper-containing polyphenol oxidase that was discovered in the exudates of the Japanese lacquer tree *Rhus vernicifera*(Yoshida,1883) and subsequently was demonstrated as a fungal enzyme (Bertrand, 1895). To date there is only one bacterium, *Azospirillumlipoferum*in which a laccase type of phenol oxidase has been demonstrated (Givaudan *et al.,* 1993). Substrate oxidation by laccase is a one-electron reaction generating a free radical, which usually reacts further through non- enzymatic routes (Reinhammer and Malmstrom, 1981).

Bourbonnais and Paice (1992) have shown that the artificial laccase substrate ABTS (2,2'-azinobis-(3-ethylbenzthiazoline-6- sulphonate)) has the capacity to act as a mediator enabling the oxidation of non-phenolic lignin model compounds that are not laccase substrates on their own. The oxidation was the effect of a two-step process in which the enzyme first catalyzed the oxidation of primary substrate; the mediator and the oxidized mediator oxidized the secondary substrate, alkenes. The main reaction products of alkenes were the corresponding ketones or aldehydes. The reduction of oxygen to water is accompanied by the oxidation, typically of a phenolic substrate. Laccases are remarkably non-specific as to their reducing substrate and the range of substrates oxidized varies from one laccase to another. Simple diphenols like hydroquinone and catechol are good substrates for most laccases, but methoxy substituted monophenols like guaiacol and 2,6-dimethoxy phenol are often better, *p*-phenylenediamine (a diamine rather than a diphenol) (Assavaniget *al.,*1992) is a widely used substrate and syringaldazine is considered to be uniquely a laccase substrate (Assavaniget *al.,*1992; Chefetzet *al.,*1998).

Oxidation of lignin by fungal laccase has been studied intensively since the early 1970's. Oxidation of milled wood lignin, de-methylation and formation of carboxylation were observed. A series of studies revealed that laccase could take part in many of these actions required for lignonlysis; Bourbonnais and Paice, 1992, in an *in vitro* system using pure enzymes from *Rigidioporuslignosus*, laccase and manganese peroxidase were found to act synergistically to degrade radiolabelled lignin. Pure laccase from *Coriolus versicolor* can provide Mn (III) chelators from Mn (II) in the presence of phenolic accessory substrates (Aust 1990).Laccases are widely expressed in nature; they can be obtained from various fungi, plants, bacteria, lichen, and insects (Fig. 1), with laccases from each species exhibiting particular catalytic characteristics and sequences.

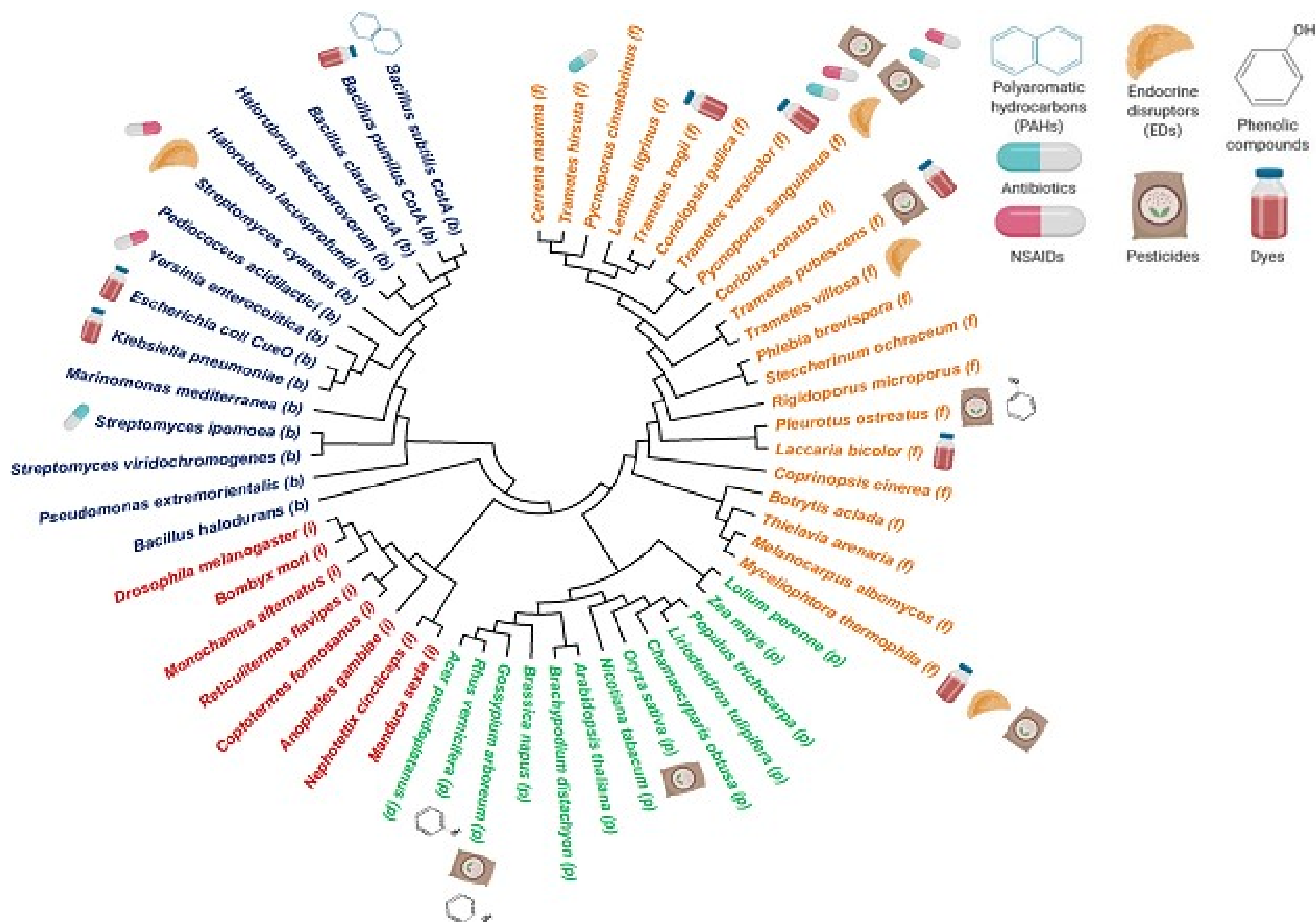

Fig. 1Phylogenetic tree constructed with some of the different organism sources of laccases, as well as some of their applications inbioremediation. According to their bacterial, insect, plant or fungal origin, they are colored with blue, red, green or orange, respectively. Thealignments and phylogenetic relationships were done using the MEGA X suite(Cited from- Arregui*et al. Microb Cell Fact (2019) 18:200*https://doi.org/10.1186/s12934-019-1248-0)

Laccase production by fungi

White-rot fungi

White-rot fungi are a physiological group of fungi capable of biodegrading lignin. The name white-rot derives from the white appearance of the wood when is attacked by these fungi, where the wood gets this bleached appearance due to lignin removal (Pointing, 2001). Though some white-rot fungi are Ascomycetes, most of them are Basidiomycetes (Eaton and Hale, 1993).Lignin degradation by several white-rot fungi, such as *Phanerochaetechrysosporium, Pleurotusostreatus, Coriolus versicolor, Cyathusstercoreus,* and*Ceriporiopsissubvermispora*, have been studied (Martinez *et al.,* 2004; Ruttimann-Johnson, Salas, Vicuna, & Kirk, 1993; Wan & Li, 2012. Some white-rot fungi such as *C. subvermispora, Phellinus pini, Phlebia spp.,* and*Pleurotus spp*. delignify wood by preferentially attacking lignin more readily than hemicellulose and cellulose. However, other white-rot fungi such as *Trametes versicolor, Heterobasidionannosum,* and *Irpexlacteus* degrade the cell wall components simultaneously (Wong, 2009).

White-rot basidiomycetes are a group of fungi capable of polymerizing and mineralizing lignin with their extracellular and nonspecific ligninolytic enzymes. In the 1980s, this fact stimulated research on the ability of ligninolytic fungi to degrade organic pollutants (Pointing, 2001; Gao *et al.,* 2010).

They can grow in a wide range of temperatures and withstand a wide range of pH (Verma and Madamwar, 2002), but no growth has been observed below 10 ºC. White-rot fungi are the only organisms able to degrade lignin efficiently (Heinzkill*et al.,* 1998) metabolizing it to CO_2 and H_2O (Tien and Kirk 1983, Kirk and Farrell 1987, Kaal *et al.,* 1995). This ability is correlated to the capacity of these organisms to synthesize extracellular lignin-degrading enzymes. The non-specific nature of such enzymes allows them to degrade a wide variety of persistent environmental pollutants (Barr and Aust*et al.,* 1994), including dyes (Robinson *et al.,* 2001, Wesenberg*et al.,* 2003).

Lignin is an heterogeneous, complex and stable polymer composed of various aromatic monomers connected to each other by various bonds carbon-carbon and ethers (Gellerstedt and Northy (1989) .The most common bond is the -aryl ether (-O-4) linkage, which may represent 50% of the inter-monomeric bonds in softwood lignin and 60% in hardwood lignin (Sjöström 1993). Due to the similarity between the lignin structure and the chemical structure of several dyes, the use of white-rot fungi and their enzymes for the degradation of dyes had been considered (Paszczynski*et al.,* 1991, Zhou and Zimmermann 1993).

The extracellular enzymatic system of white-rot fungi generally consists of lignin peroxidase (LiP, E.C. 1.11.1.14), manganese-dependent peroxidase (MnP, E.C. 1.11.1.13) and laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2I). LiPcatalyzes the oxidation of non-phenolic aromatic compounds such as veratryl alcohol (VA). MnP preferably

oxidizes Mn^{2+} to Mn^{3+} which is able to oxidize many phenolic compounds. Laccase is a copper-containing enzyme that catalyzes the oxidation of phenolic substrates with the reduction of oxygen to water (McMullan *et al.,* 2001, Wesenberg*et al.,* 2003). A third group of peroxidases, versatile peroxidase (VP, E.C.1.11.1.16), has been further recognized in species of *Pleurotus*and*Bjerkandera (*Heinfling*et al.,* 1998). Also, accessory enzymes such as H_2O_2-forming glycoxal oxidase (GLOX), aryl alcohol oxidase (AAO), oxalate producing oxalate decarboxylase (ODC), NAD-dependent formate dehydrogenase (FDH) and P450 monooxygenase have been isolated from many white-rot fungi strains (Wesenberg*et al.,* 2003, Aguiar *et al*., 2006).

During the last decades, the scientific interest in the utilization of white-rot fungi and their ligninolytic enzymes for bleaching processes, environmental applications, synthesis of complex compounds and biofuel production has largely increased (Luisa *et al.,* 1996, Asgher*et al.,*2008). This interest is justified by the presence of the above-mentioned microorganisms in a good number of natural and contaminated environments.

Production of fungal laccases

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 Ecto-mycorrhizal fungi (J. M. Bollag*et al.,* 1984). White-rot fungi have been studied extensively for application in biological pulping and bleaching (M. Luisa, F.C. *et al.,* 1996.) because they are of the only organisms that are able to degrade lignin efficiently. White-rot fungi, such as *Coriolus versicolor* and *P. sanguineus*, are known producers of lignolytic enzymes that are involved in the natural delignification of wood. This group of fungi has evolved complex enzymatic systems that enable them to degrade lignin (A.M.V. Garzillo, 1998). In general, laccases occur as extracellular glyco-proteins, which allows for rapid removal from fungal biomass. One of the major limitations for the large-scale applications of fungal laccases is the low production rates by wild type and recombinant fungal strains according to Galhaup*et al.,* White-rot fungi constitutively produce low concentrations of various laccases (Galhaup, C and D. Haltrich, 2001) when they are cultivated in submerged culture or on wood. Higher concentrations can be induced by the addition of various aromatic compounds such as 2, 5-xylidine and ferulic acid. High concentrations of laccase have also been observed in old non-induced cultures. The mechanisms of metabolism in microorganisms are used and controlled by its environmental conditions and medium composition .There are various response element sites in the promoter regions of laccase genes that can be induced by certain xenobiotic compounds, heavy metals or heat shock treatment (V. Faraco*et al.,*.2001).

Ligninolytic enzymes have mostly been reported to be extracellular but there is evidence in literature of the occurrence of intracellular laccases in white–rot fungi. Intracellular as well as extracellular laccases were identified for *Neurospora crassa*by Froehner and Eriksson (S. C. Froehner*et al.*2002,), who suggested that the intracellular laccase functioned as a precursor for extracellular laccase as there were no differences between the two laccases other than their occurrence. Solid state fermentation (SSF) processes have shown to be particularly suitable for the production of enzymes by filamentous fungi (Pandey A., Selvakumar P. *et al.,* 1999) since they reproduce the natural living conditions of such microorganisms.

One of the advantages often cited for SSF processes is that enzyme titres are higher than in SmF, when comparing the same strain and fermentation broth (Viniegra-González *et al.,2003*) compared the productivity of three fungal enzymes, invertase, pectinase and tannase, using SSF and submerged fermentation (SmF) techniques. They reported that the higher titres found in SSF than in SmF were due to SSF cultivation works as a fed batch culture with fast oxygenation but slow sugar supply. Castilho *et al.,* performed a comparative economic analysis of solid-state and submerged processes for the production of lipases by *Penicillium restrictum*. They found that for a plant producing $100m^3$ lipase concentrate per year, the process based on SmF needed a total capital investment 78% higher than the one based on SSF and its product had a unitary cost 68% higher than the product market price. These results showed the great advantage of the SSF due to its low cost. (Castilho L.R., *et al.,* 2000).

Methods for Laccase Production

Solid State Fermentation (SSF) or Solid State Cultivation (SSC) is generally defined as a growth of micro-organisms on solid materials in the absence or near absence of free water (Winquist, E. *et al.,* 2008). SSF processes have shown to be particularly suitable for the production of enzymes by filamentous fungi since they reproduce the natural living conditions of such fungi. In recent years, there has been an increasing trend towards the utilization of organic wastes such as residues from the agricultural, forestry and alimentary industries as raw materials to produce value-added products by SSF technique (Osma, J.F *et al.,*2007). Furthermore, most of these wastes contain lignin or/and cellulose and hemicellulose, which act as inducers of the ligninolytic activities. Moreover, most of them are rich in sugars, which make the whole process much more economical. The application of laccase enzyme in industrial and environmental technologies, including the modern concept of integrated bio refineries requires significant amounts of these enzymes at low cost. Laccase has also been produced under SSF, especially during the last decades. The use of natural solid substrates, especially lignocellulosic agricultural residues, as growth substrates for fungi has been studied for laccase production in recent years (Rodriguez Couto S. *et al.,* 2005). Furthermore, such residues contain cellulose, which act as an inducer of laccase activity.

Laccase production under SmF and SSF

There are many contributions to the field of laccase production under SmF using different microorganisms, at different scales and with the possible use of immobilization supports and the addition of inducers. Some of the most remarkable results in terms of laccase activity were obtained by the *Trametes*genus: *T. pubescens T. versicolor* and *T. hirsuta*(Box GEP *et al.,*1957). In almost all cases, the cultures were supplemented with laccase-inducing compounds. Galhaup*et al.,* obtained a maximum laccase activity of 740, 000 U/L by *T. pubescens*cultured in a 20-L stirred-tank reactor (STR) with a stirring speed of 100 *rpm* and with 2 mM Cu^{+2}. Font *et al.,* 2003) It obtained a maximum laccase activity of 16,000 U/L by free pellets of *T.versicolor*in a 0.5-L pulsed-bed reactor. Tavares *et al.,* reported a maximum

laccase activity of 11,403 U/L when cultured the same fungus on a STR of 1 liter, supplementing the medium with 30 μM of xylidine. Rodríguez Couto *et al.,* 2001reported a maximum laccase activity of 19,400 U/L by culturing *T. hirsuta*in a 6-L airlift reactor (ALR) and supplementing the medium with glycerol and Cu^{+2}. (Box GEP *et al.,* 1957)

Utilizing the rice straw in a solid-state fermentation process using *Pleurotussajor-caju*, laccase was produced and subjected to isolation and purification processes. The optimum laccase production was studied from 0 to 21 days. The highest laccase activity of 224.93 U/mg was obtained from the crude extract after 9 days of fermentation with *Pleurotussajor-caju*. It was concentrated by ultrafiltration and purified using DEAE-Sepharose anion exchange chromatography. The peak fraction obtained was then loaded into Sephacryl S200-HR gel filtration chromatography. Laccase was purified by 43-fold to a specific activity of 19335 U/mg, with an overall protein recovery of 18.6 %. It appeared as a single band on SDS-PAGE with an apparent molecular weight of 53 kDa.

Several studies on laccase production in SSF using agro-industrial wastes have already been reported. Gómez *et al.,* found that barley bran is the best lignocellulosic waste to produce laccase by solid state cultures of *C.rigida*. The higher roughness and porosity of barley bran makes easier the attachment of the fungus to the support. Oil palm frond parenchyma tissue was used as a solid substrate for the production of laccase via solid-state Fermentation using the white rot fungus *Pycnoporussanguineus*. The laccase activity reached its maximum on day 8 (950 U/m^3) of the SSF.(Annuar, M.S.M *et al.,*2010) Laccase activity of 1570 U/L (on day 20) was resulted from SSF of *Trametespubescens*and banana skin as support substrate. The most important characteristics that influence adhesive behaviour of filamentous fungi to the support are hydrophobicity and surface charge. The higher hydrophobicity causes the attachment of the fungus to the carrier easily and also together with its high content in carbohydrates). The use of rice straw as one of agricultural wastes was suitable for laccase production since it might contain substances acting as inducers for laccase. (Couto and Sanroman*et al.,* 2003) stated that employing organic wastes rich in cellulose stimulated laccase production.

The scarcity of bioreactor designs to perform solid-state processes together with the advantages offered by such processes promote the necessity of developing new bioreactor configurations or modifying the designs that already exist. These bioreactor designs should be able to operate in continuous mode with high enzyme productivity for prolonged periods of time without operational problems as well as permit the scale-up of the process. Thus, Rivela*et al.,* developed a new bioreactor design for the production of ligninolytic enzymes under SSF conditions named immersion bioreactor. They attained high ligninolytic activities and, in addition to this, the bioreactor was able to operate in continuous mode. (Dominguez *et al.,* 2001) developed a rotating drum reactor (RDR) for the production of ligninolytic enzymes under SSF conditions. This bioreactor was able to operate in batch and continuous mode. Also, (Böhmer*et al.,* 2003) reported the advantages of adapting the temporary immersion RITA®-System (Récipient à Immersion TemporaireAutomatique) as a bioreactor for laccase production by white-rot fungi and its application to synthetic dye discoloration.

Rodríguez Couto *et al.,*2006tested three bioreactor configurations (immersion, expanded bed and tray) with different agitation systems (mechanical, pneumatic and static, respectively) for laccase production by *T.versicolor*under SSF conditions using an inert (nylon sponge) and a non-inert (barley bran) support. They found that the tray configuration with barley bran as support-substrate led to the highest laccase activities. More recently ,Rodríguez Couto *et al.,*compared two bioreactor configurations(immersion and tray) for laccase production by *T. hirsuta*using grape seeds as support-substrate and found that much higher laccase activities were attained in the tray bioreactor. Also, they reported much higher laccase activities in a tray bioreactor than in a fixed-bed one for *T. hirsuta*grown on ground orange peelings (Rosales E.,*et al.,* 1997).The process of submerged cultivation involves the growth of microorganisms in a liquid medium rich in nutrients under aerobic conditions. In order to achieve high production, the studies are first focused on the optimization of nutritional and operational conditions. *Galerina*sp. HC1 produced high laccase activity under optimized conditions in batch submerged fermentation. Submerged cultivation can be carried out by utilizing cheap materials considered as "waste" and which are produced in large amounts. These materials can contain considerable concentrations of soluble carbohydrates, nitrogen, minerals, vitamins and even inducers for enzyme production. The industrial production of enzymes is mainly achieved by submerged cultivation. One of the disadvantages of this method is the excessive growth of mycelium, which affects the production yield due to mass transfer and metabolic rate limitation. The excessive growth can also affect the mechanical set up of the used reactor. In other words, the mycelia can wraparound the impellers, cause blockage of the system and increase viscosity; however, this has been overcome by immobilization of the cells in various supports or by using fed-batch cultivation for controlling the fungal growth. (Galhaup C *et al.,* 2002).

Biotechnological applications of ligninolytic enzymes

Lignin-degrading enzymes have significant potential in industrial and biotechnological applications. The use of lignin-degrading enzymes in the pre-treatment of recalcitrant lignocellulosic biomass would provide an environmentally friendly alternative to biofuel production compared to the thermal and chemical pre-treatment techniques for the biofuel production (Wan & Li, 2012; Weng, Li, Bonawitz, & Chapple 2008. They can also be applied in the decolorization of the dye wastewater from the textile industry. Research on the biotechnological applications of laccase and laccase/mediator system has attracted much attention recently due to their eco-friendly nature as they use oxygen as electron donor and produce water as the only reaction by-product (Riva, 2006). They have wide applications in the paper, textile, and food industries (Canas&Camarero, 2010). We discuss some of these applications briefly in (fig-2) showing Laccase as multi-purpose biocatalyst at the forefront of biotechnology.

Delignification of Lignocellulose

Production of ethanol as alternative fuel using lignocellulosic substrates as raw materials is one of the most desirable

goals to overcome the fossil fuel crisis. The transformation of lignocellulose into ethanol is achieved in three steps: (a) delignification to release cellulose and hemicellulose from their complex with lignin, (b) depolymerisation of the carbohydrate polymers to produce free sugars, and (c) fermentation to ethanol using the liberated sugars. Biological treatment using white-rot fungi or other ligninolytic microorganisms including *Streptomyces* has been proposed, to replace the physicochemical treatments. Biological treatment can also be used for the removal of inhibitors prior to the fermentation. The advantages of using biological treatment include (i) mild reaction conditions, (ii) higher product yields, (iii) fewer side reactions, and (iv) less energy demand (Lee, 1997).

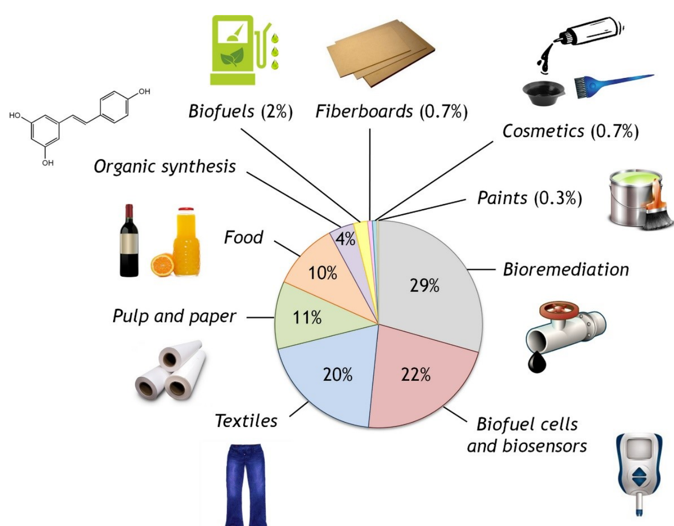

Fig2-Breakdown of the biotechnological applications of laccases. Data extracted from Scopus database search for articles that included the following keywords: (i) 'laccase' and 'bioremediation'; (ii) 'laccase' and 'biofuel cell' or 'biosensor'; (iii) 'laccase' and 'textiles' or 'textiles industry'; (iv) 'laccase' and 'pulp and paper' or 'pulp and paper industry'; (v) 'laccase' and 'food' or 'food industry'; (vi) 'laccase' and 'organic synthesis'; (vii) 'laccase' and 'biofuel production'; (viii) 'laccase' and 'fibreboards'; (ix) 'laccase' and 'cosmetics'; (x) and 'laccase' and 'paints'.(Microbial Biotechnology, Volume: 10, Issue: 6, Pages: 1457-1467, First published: 03 October 2016, DOI: (10.1111/1751-7915.12422)

Biopulping and Biobleaching

Lignin removal is important in the pulping and paper industry. Bio pulping is the treatment of wood chips with lignin-degrading microorganisms to alter the lignin in the cell walls of wood, making the wood chips softer. This treatment not only improves paper strength and remove wood extractives but also reduces the energy consumption in the process of pulping. The production of pulp uses mechanical or chemical processes or a combination of the two processes. Pre-treatment of wood chips for mechanical and chemical pulping with white-rot fungi has been developed (Mendonça, Jara, González, Elissetche, & Freer, 2008). Laccases from white-rot fungi can be applied in bio pulping to partially degrade the lignin and therefore loosen lignin structures (Mendonça*et al.,* 2008). *Ceriporiopsissubvermispora*and *Pleurotus sp.* are fungi used in bio pulping (Pérez, Muñoz-Dorado, De La Rubia, & Martínez, 2002).

Bio bleaching is the bleaching of pulps using enzymes or ligninolytic fungi that reduce the amount of chemical bleach required to obtain a desirable brightness of pulps. Laccase-mediator system has been shown to possess the potential to substitute for chlorine-containing reagents. Laccases can also be applied as bio bleaching agents as they degrade lignin and decolorize the pulp (Call & Call, 2005). Laccase produced by *T. versicolor* has been studied for bioleaching of paper pulp and other industrial applications (Wesenberg, Kyriakides, Agathos, 2003). The role of lignin-degrading enzymes from *Streptomyces* in bio pulping and bioleaching has also been studied. Bio bleaching of eucalyptus Kraft pulp with *S.albus*culture supernatant in the presence of H_2O_2 resulted in a significant reduction of kappa number with no change in viscosity suggesting a potential application of *S.albus*in bioleaching (Antonopoulos, Hernandez, *et al.,*2001). *Streptomyces cyaneus*laccase was able to delignify kraft pulp with ABTS as a mediator, indicating the potential application of the laccases from *Streptomycetes* in bioleaching of Kraft lignin in the presence of synthetic mediators (Arias *et al.,*2003). Bio bleaching experiments carried out on *Eucalyptus globulus* Kraft pulps with *S. ipomoea* laccase in the presence of acetosyringone as a natural mediator also showed reduction in kappa number and increase of brightness without decreasing the viscosity values significantly (Eugenio *et al.,* 2011). These results suggest significant promise for the use of Streptomycetes lignin-degrading enzymes in industrial application.

Textile Dye Transformation

The textile industry uses water as a medium for removing impurities, application of dyes and finishing agents. There is a significant water pollution associated with these processes due to the highly toxic dyes, bleaching agents, salt acids, and the alkali employed. LiP and MnP from the white-rot fungus *P. chrysosporium*have been investigated for their dye decolorization with the results showing the capacity to mineralize a variety of recalcitrant aromatic pollutants (Mehta, 2012). Decolorization of 23 industrial dyes by 16 white-rot fungi has also been investigated. The crude extracts of the cultures showed laccase, LiP, and aryl alcohol oxidase activities. However, only laccase activity was correlated with color removal (Rodríguez, Pickard, & Vazquez-Duhalt, 1999). Although some dyes are not degradable by laccases, many are oxidized by the enzyme and therefore initiating the destruction of the dyes (Schliephake, Mainwaring, Lonergan, Jones, & Baker, 2000).

An extensive review on the role of peroxidases in the treatment and decolorization of wide spectrum of aromatic dyes from polluted water can be found in the literature (Husain, 2010). Kirby, Marchant, and Mcmullan (2000) reported that laccase from *P. tremellosa*decolorized synthetic textile dyes. Also, laccases used in combination with mediators and cellobiose dehydrogenase were shown to be an ecofriendly alternative for chemical treatment of textile dye wastes (Ciullini, Tilli, Scozzafava, & Briganti, 2008).

Decolorization of Distillery Effluent and Waste Effluent Treatment

The characteristic of the dark brown appearance of distillery wastewater is mainly due to the high molecular weight organic compounds called melanoidin, a product of the Mallard reaction of sugars with proteins. The brown color is also due to the presence of phenolics from the feedstock,

caramels from overheated sugars, and furfurals from acid hydrolysis (Kort, 1979). The detoxification and decolorization of this industrial waste is performed using oxidative enzymes (laccases and peroxidases) from bacteria, fungi, and yeast (Rajasundari& Murugesan, 2011). *Coriolus versicolor* was the first fungal strain shown to decolorize this type of waste (Watanabe, Sugi, & Tanaka, 1982). *P. chrisosporium*JAG-40 decolorized synthetic and natural melanoidin (Dahiya, Singh, & Nigam, 2001). Part of the treatment of these wastes includes the use of laccases and peroxidases that oxidize phenolic compounds to aryl-oxyradicals creating complexes that are insoluble. Other mechanisms carried out by these enzymes include the polymerization of the contaminants themselves or the copolymerization with other non-toxic substrates to facilitate their removal by sedimentation, adsorption, or filtration (Gianfreda, Iamarino, Scelza, & Rao, 2006; Rabinovich, Bolobova, &Vasil'chenko, 2004).

Concluding remarks and future prospects

Laccase is currently considered a 'trendy' enzyme and by many, the ideal green catalyst. However, although several companies offer laccases in their catalogues to the food, textile, pulp and paper, pharma, cosmetic, paint or furniture industries, to fully realize the potential of laccases to compete in the biotechnology race, some hurdles must still be overcome.The diversity of structures as well as biological targets of laccase and its application in synthetically designed analogues thereof is immense. Because of their versatile biochemical properties, high protein stability, broad substrate spectrum, and their wide range of applications, laccases are very impressive and useful biocatalysts in fungal biotechnology. The activity of laccases can be potentially increase by the immobilisation of fungal laccases with carriers such as chitosan which can be an emerging application in low cost removal of environmental effluents. Also, the metagenomics techniques in mining enzymes from microbial communities which has become more feasible due to advances in sequencers technologies should be considered in the search for novel laccases. The increased application of gene technology and protein engineering as well as the improvement of immobilization and scale-up techniques for optimization of product yields and avoidance of side reactions remain as important tasks for the future.

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