**Fungal Laccases: Production, Occurrence, and Application**

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**Abstract**

Laccases (EC 1.10.3.2) are enzymes from the family of oxidoreductases and it is a polyphenol oxidases (PPO) containing copper and is sometimes known as benzenediol: oxygen oxidoreductase. Oxidation of various compounds such as diamines, phenolic compounds, aromatic amines, cross-linking of monomer, degradation of the polymer, and ring cleavage of aromatic compounds is catalyzed by Laccase. So Laccases have great biotechnological significance. They also show low substrate specificity. It was first discovered in extracts of the Japanese lacquer tree *Rhus vernicifera*. Various organisms like bacteria, fungi, insects, and plants also produce this enzyme. Two molecules of oxygen are reduced to two molecules of water and simultaneously aromatic substrates are oxidized by removing electrons by Laccase. As an oxidase laccase is used in agricultural, medicinal, and industrial applications, and believed that Laccase plays an important role in morphogenesis, pathogenesis, and lignin degradation. Various harmful wastes such as dyes and chemicals released by many textile and dye industries can be degraded by Laccase. It is also helpful in paper and pulp bleaching, lignin degradation, food processing, and bioremediation.

**Keywords:** Laccases, *Rhus vernicifera****,*** Oxidoreductase, Ring cleavage

**I Introduction:**

In the era of urbanization and pollution where pollutants like chemicals, dyes, and plastics are becoming a threat to the environment there is a need to find natural degraders to treat these wastes irrespective of their structure and types. Many microbial enzymes play a significant role in degrading such pollutants and are environment-friendly. Laccase (benzenediol: oxygen oxidoreductase, EC1.10.3.2) belongs to a broad group of enzymes called polyphenol oxidases which contains copper atoms in their catalytic center and are usually called blue multicopper oxidases [2,5,8]. There are three types of copper atoms present in the Laccase enzyme, out of which one is responsible for their characteristic blue color [1, 2, 17, and 18]. Fungi, bacteria, and insects are known to produce the laccase enzyme fungal laccase is produced mainly by ascomycetes, basidiomycetes, and deuteromycete [2]. Four bacterial strains of *Streptomyces (S. cyaneus, S. ipomoea, S. griseus, S. psammoticus*) have been studied for their ability to produce active extracellular *laccases* enzyme in treated wastewater [20]. *Trichoderma muroiana IS 1037* is also found as a laccases producer [17].

Laccases are extracellular enzymes that catalyze the oxidation of a variety of phenolic compounds diamines and aromatic amines pigment it also catalyzes lignin degradation [1]. Laccase enzymes are currently used for bioremediation, delignification, insecticide degradation, biosensor, food processing, and bleaching of pulp [1, 6, 10, 17, 18]. Earlier studies have shown that bacterial strains degrade the low molecular lignin polymer, unlike fungi which secret extracellular enzymes called ligninases[19]. Due to impressive applications in the field of biotechnology, laccase production through fungi and optimization of enzymatic activity has been reinforced in recent years [2].

Laccases have been reviewed several times in recent years because of their increasing demand in industries and usefulness. In this article information available in the literature regarding laccase occurrence, production, isolation, screening, and eventually its uses in bioremediation has been briefly discussed.

**II Occurrence of Laccase in Fungal Systems**

In nature, laccase is one of the widespread enzymes. In the year 1883 the first laccase was reported from the Japanese lacquer tree, *Rhus vernicifera*, from which the term laccase was, derived [7]. Laccase activity has been demonstrated in several species of fungus leading to the fact that most all fungi produce laccase. Lower fungi such as Zygomycetes and Chytridiomycetes have never been reported for laccase production [9, 18]. *Ascomycetes* such as *Gaeumannomyces graminis*, *Magnaporthe grisea*, *Melanocarpus albomyces*, *Monocillium indicum*, *Neurospora crassa*, and *Podospora anserine* have been reported in several pieces of literature for the laccase production[18]. Some soil ascomycetes from the genera *Aspergillus, Curvularia, and Penicillium,* which are plant pathogenic species and in some freshwater ascomycetes are also known to produce Laccase [18]. The redox potential of fungal laccases ranges from 450 mV to 800 mV.

 Some species of ascomycete are closely related to wood-degrading fungi which help in the decay of dead plant biomass and have been shown to contain laccase genes and can oxidize syringaldazine [18]. *Trichoderma* and *Botryosphaeria* are wood-degrading ascomycetes and reported to have some laccase activity. Whereas Botryosphaeria sps. produces constitutively a dimethoxyphenol oxidizing enzyme that is probably a true laccase [18] Jaber *et al.*(2012) noticed that there are only some strains of *Trichoderma* that exhibit low-level production of Laccase and syringaldazine oxidizing enzyme [17]. While wood rotting *xylariaceous ascomycetes*, only two strains of this species and one strain of *Xylaria hypoxylon* showed oxidation of syringaldazine [18]. Appreciable titers of an ABTS (2, 2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) oxidizing enzyme have been produced by the fungi *X. hypoxylon and Xylaria polymorpha*, in the complex liquid media [18]. The production of laccase has not been reported in ascomycetous yeasts, but *Saccharomyces cerevisiae* contains plasma membrane-bound multi-copper oxidase Fet3p protein that shows structural and sequential similarity with fungal laccase [18].

*Cryptococcus neoformans* which is a *Basidiomycete* yeast produces a true laccase that is capable of oxidizing the phenols and amino phenols [18]. Wood rotting *Basidiomycetes* that cause white rot and a related group of saprotrophic fungi that decomposes litter are the best-known species for appreciable production of laccase. Almost all species of white rot fungi were reported to produce laccase to varying degree [18]. In the case of Pycnoporus cinnabarinus laccase was described as the only ligninolytic enzyme produced by this species that was capable of lignin degradation [18]. On the other hand, brown-rot fungi have not been reported for laccases production capabilities yet. Meanwhile, a DNA sequence with relatively high similarity to that of laccase was detected in Gloeophyllum trabeum(Basidiomycete) that was capable of oxidizing ABTS, and the oxidation of ABTS was also reported in *Laetiporus sulphureus* and syringaldazine oxidation has recently been detected in the brown-rot fungus *Coniophora puteana* [18]

Laccases have been discovered in numerous other plants also, e.g., sycamore, poplar, tobacco, and peach [7]. Despite the wide occurrence of plant laccases, they are not characterized or used, because of the difficulties in detection and purification as crude plant extracts. The main function of these plant oxidoreductases is the synthesis of lignins and regeneration of damaged tissues, which is aided by releasing the enzyme to the apoplast – a system formed by dead plant elements, used to transport water [5]. The applicability of plant laccases is very limited due to their low oxidoreductive potential (approx. 430 mV). Among eukaryotes, laccases have been found in higher plants such as Chinese or Japanese Rhus trees and insects.

**III Production**

Laccases are secreted by ﬁlamentous fungi as extracellular enzymes into the liquid medium as secondary metabolites [18]. Several factors can influence the production of laccases such as type of cultivation (submerged or solid-state), carbon limitation, nitrogen source, and concentration of micro-elements [18]. Among fungi, ascomycetes, basidiomycetes, and deuteromycetes can produce laccases, and white-rot basidiomycetes are the most eﬃcient lignin degraders and laccase producers [6, 9]. Laccases are secreted by white-rot fungi along with other ligninolytic enzymes including manganese peroxidase, lignin peroxidase, and many other peroxidase. Laccase was isolated and purified only from *Cryptococcus neoforman*s. This basidiomycetous yeast produces a true laccase that is capable of oxidation of phenols and aminophenols and unable to oxidize tyrosine [9]. Botryosphaeria produces a true laccase dimethoxyphenol oxidizing enzyme[10].

*Pleurotus ostreatus* and *Trametes versicolor* can be referred to as model organisms in basic and applied laccase research. Various other *Pleurotus* (e.g., *P. eryngii, P. ﬂorida, P. pulmonarius, and P. sajor-caju)* and *Trametes* (e.g., *T. hirsuta, T. pubescens, T. trogii, and T. villosa*) species are positive laccase producer[6]. The amount of secreted laccase activity in edible mushrooms and their growing cycles are closely related, and short growing cycles are accompanied by high laccase activity [6]. Laccase yields depend upon the species and strain, and most of the naturally-occurring species reported to date appear to be poor laccase producers so efforts are still being made for screening and isolation of naturally-occurring laccase producers with desired laccase yields and properties[6,10]. According to the previous studies, the genus *Cerrena* showed high laccase yields and potential application, so deserves attention, and the properties of its laccase can be even more desirable compared to the commercial ones. However, *Cerrena* species is a mushroom with medicinal properties and also shows antitumor activity but is less studied, as compared with *Trametes* species. *C.unicolor* [6]. Some Laccase-producing fungi are listed in table 1.

**Table 1. Laccase-producing Fungi.**

|  |  |  |
| --- | --- | --- |
| S.No. | Name of Fungi | References |
| 1. | ***White-rot fungi****Agaricus bisporus, Cryptococcus neoforman, Ganoderma austral, Lentinula edodes, Pleurotus florida, Polyporus versicolor, Sclerotium rolfsii, Trametes gibbosa, Pleurotus erygnii, Cerrena unicolor, Coprinopsis cinerea, Coriolopsis gallica, Polyporus brumalis, Ganoderma lucidum* | (6), (9), and (12) |
| 2. | ***Ascomycetes****Cryphonectria parasitica, Glomerella sp., Melanocarpus albomyces, Neurospora sp., Podospora anserine, Xylaria polymorpha* | (6), (7), (8), and (19) |
| 3. | ***Imperfect fungi****Aspergillus nidulans, Botrytis cinerea, Cantharellus cibarius, Gaeumannomyces graminis, Monocillium indicum, Ophiostoma ulmi, Penicillium chrysogenum, Trichoderma atroviride, Trichoderma giganteum.* | (2), (12), (13), (17) and (18) |

**IV Inﬂuence of Carbon and Nitrogen Source in production of laccase**

The fungi were grown in the defined medium which contains 0.1% (w/v) yeast extract and 1% (w/v) diﬀerent carbon sources as well as nitrogen sources. Commonly used carbon sources are glucose, mannose, maltose, fructose, and lactose. But according to studies the overconsumption of glucose and sucrose reduce the production of laccase by making hurdle in the initiation, so this problem can be resolved by using polymeric substrates like cellulose.

Common nitrogen sources that are being used for laccase production are yeast extract, peptone, urea, (NH4)2SO4, and NaNO3. Laccase production is triggered by nitrogen draining but some nitrogen strains produce much more efficient Lacasse without getting affected by deficiency of nitrogen. Some studies show that the elevated laccase activity was achieved by using a low carbon-to-nitrogen ratio, while others show that it was achieved at a high carbon-to-nitrogen ratio[1]. Rajesh Kumar et al.(2016) reported 8% cellulose, and 2% nitrogen, for *Aspergillus* sp. Production and isolation[2].

**V Influence of Temperature and pH on the production of Laccase**

Laccase production is not immensely affected by temperature but the optimal temperature of laccase production diﬀers greatly from one strain to another. It has been found that 25°C is the optimal temperature for laccase production in presence of light, but, in the case of dark, the optimal temperature is 30◦C[1,3,10]. Shraddha et al.(2016) reported that the effect of temperature is limited in the production of Laccase[10]. Rajesh Kumar et al.(2016) reported 25°C temperature and pH7 best for *Aspergillus* sp. Production and isolation[2]. According to Rehman A. Abd EL Monssef et al. (2016), T. harzianum could be considered one of the most important sources of Laccase production at 35°C and pH 5[3]. Nyan Hongo et al. (2002) showed that laccase produced by T. modesta was fully active at 50°C.

**VI Application**

**a. Industrial applications**: Hazardous and expensive chemicals can be replaced by laccases as advantageous biocatalysts and this can save resource consumption, create unique functionalities, or reduce damaging impacts on the environment Laccase can also serve as a bio adhesive. The cross-linking efficiency can be increased or enhanced, laccases can be used in different ways: by directly oxidizing wood pulp, functionalizing wood pulp with small compounds (such as aromatic, carboxyl, isocyanate, or acrylamide substances) which act as cross-linking agents for generating radicals for cross-linking or by transforming isolated lignin (by-product), starch, phenolic polysaccharide, or protein into radical-rich and non-toxic adhesives. Such applications of laccase could not only replace toxic or expensive chemical adhesives but also transform wastes such as lignin from the paper industry into value-added products [7].

**b. Enhancing ethanol production**: It is known that phenolic compounds act as important inhibitors of the fermentation process, but the use of laccase was found advantageous in ethanol production from lignocellulosic compounds. Improved ethanol production from lignocellulosic hydrolysate was observed as Laccase from white-rot fungus, *Trametes Versicolor*, was expressed under the control of the PGK 1 promoter in *S. cerevisiae* [7].

**c. Biodegradation**: Many the enzymes such as oxidoreductases can be applied to degrade various harmful substances such as undesirable contaminants, by-products, or discarded materials [7]. Recently, laccase is capable of oxidizing and degrading lipids like methyl linoleate and trilinolein but these are not the typical laccase substrates which are generally unsaturated fatty compounds. The products formed generally comprise hydroperoxides and epoxides. Because of the presence of the fatty compounds in wood and food, the reaction is important because it is involved in the laccase catalyzed process namely delignification and food modification, respectively [7]. Enzymes like laccase, peroxidase, and oxygenase have been studied as biocatalysts for degradation of hazardous coal substances, particularly sulfur-containing components, and hence laccase can be utilized as an important tool in reducing acid rain around coal mines caused by by-products of power plants. Plastic pollution has also become a threat to the environment nowadays. Laccase is known to degrade some low molecular weight Polyvinyl chloride (PVC) it can also degrade plastic waste containing olefin units.

**d.** **Bioremediation and Bio detoxification:** Bio-friendly and economic processes are required for the degradation of compounds like pesticides, xenobiotics, coal substances, and industrial products. These are derived from polycyclic, aromatic, halogenated hydrocarbons and some other organic compounds and they are hazardous environmental pollutants. The enzyme oxidoreductases are known to detoxification of these compounds along with laccase and peroxidise. Laccase is also known to degrade synthetic dye namely azo-dyes into less toxic compounds. Many other textile dyes such as reactive red, brilliant blue reactive orange can also be decolorized by using laccase.

**e. Food applications**: Laccase is used in many food processing industries such as beverage processing industries, wine stabilization, baking, etc. In food industries, laccase is used for the determination of lignin compounds. Laccase is used in beverage industries for color modification, used for selective polyphenol removal in the wine industry; it is used for oxygen removal from the final process of beer preparation. It is used in the baking industry also for increasing the strength of the dough. It is used in the olive ripening process, when conventional dye solutions are used they generally cause darkening and debittering of olives as a result of oxidatively polymerizing various phenolics (such as oleuropein) in olive. This can be avoided by using the laccase enzyme hence the quality of the product can be improved. (7)

**f. Biofuel cells**: Biofuels are an alternative source of energy. There is a modification of biofuels known as enzymatic biofuels cell (EBC) in which precious metals are replaced by enzymes that oxidise the fuel as catalysts. These EBCs are portable and eco-friendly. Laccase electrodes are in use today for enzymatic biofuel cells. Due to the higher redox potential activity of laccase the activity of the fuel cell is enhanced when used as a bio-cathode. Laccase is generally electro-polymerized on an electrode along with compounds like chitosan. This enzyme provides direct electron transfer and results in higher energy output [7].

**g. Disinfection/antifungal agent**: Iodine is known as one of the potent disinfectants and laccase is known to oxidize the unreactive iodide to a reactive form of iodine. Laccase-iodide combination has been reported to show antimicrobial activity. The binary system formed by the combination of laccase and iodide has resulted in the generation of a potent sterilization system when compared with only iodine. This combination can be used for wound disinfection also. This system has various applications various industrial, medical, domestic, and personal care (deodorants, toothpaste, mouthwash, chewing gum, detergent, soap, and diapers), etc. Not only this but the binary system can be used in water treatment also like sterilization of drinking water and swimming pools. [7]. Laccase enzyme is also known to show some antifungal activity also. Leaf spot disease of sugar beet is caused by the fungus *Cercospora.* This fungus produces a toxin known as cercosporin which damages the plant by the formation of superoxides. This cercospora toxin can be degraded by laccase [7].

As the laccase enzyme has a specific nature for its substrate it is gaining the attention of researchers all over the world. Due to its effective catalytic properties, laccase has been proven to be better than the conventional chemicals used in various industries. There is a need for the bulk production and purification of the laccase enzyme. Some new approaches have to be searched for the production of this enzyme from waste material and to study some new areas of application.

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