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

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**Abstract**

Laccases (EC 1.10.3.2) are enzymes from oxidoreductases family and a type of copper-containing polyphenol oxidase (PPO) and also known as benzenediol: oxygen oxidoreductase. Laccases catalyze the oxidation of various phenolic compounds, diamines, aromatic amines, cross-linking of monomer, degradation of polymer and ring cleavage of aromatic compounds. So Laccases have great biotechnological importance. They have low substrate specificity. It was first discovered in extracts of the Japanese lacquer tree *Rhus vernicifera*. It has been detected in various organisms like bacteria, fungi, plants, and insects. Laccase reduces two molecules of oxygen to two molecules of water and simultaneously oxidizes aromatic substrates by removing electrons. 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. Laccase can degrade a range of harmful wastes that are released by many textile and dye industries. 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 pollution and so many waste products scientists intended to find natural degraders of wastes irrespective of their structure and types. Many microorganisms are found to produce enzymes that are capable of degrading such pollutants and also not harmful. Laccase (benzenediol: oxygen oxidoreductase, EC1.10.3.2) is a part of the broad group of enzymes called polyphenol oxidases containing copper atoms in the catalytic center and are usually called blue multicopper oxidases[2,5,8]. Laccases contain three types of copper atoms, one of which is responsible for their characteristic blue color[1,2,17,18]. Laccases are produced by many fungi, bacteria, and insects in which ascomycetes, basidiomycetes, and duteromycetes (fungi) are determined as efficiently Laccases producers [2]. Four strains of the bacterial genus *Streptomyces (S. cyaneus, S. ipomoea, S. griseus, S. psammoticus*) were studied for their ability to produce active extracellular *Laccases* in treated wastewater[20]. Trichoderma muroiana IS 1037 is also found as a Laccases producer [17].

Laccases are extracellular enzymes and catalyze the oxidation of a variety of phenolic compounds diamines and aromatic amines pigment formation, lignin degradation, and detoxification [1]. With increasing concern for an eco-friendly environment, research has been focused on the use of enzymes rather than chemicals, these include Laccases, which are currently very widely used in 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 biotechnological applications, Laccase production through fungi and optimization of an enzyme 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. The main objective of this article is to summarize the wealth of information available in the literature regarding Laccase occurrence, production, isolation, screening, and eventually its uses in bioremediation.

**II Occurrence of Laccase in Fungal Systems**

Laccases are widespread enzymes found in nature. The first laccase was reported in 1883 from Rhus vernicifera, the Japanese lacquer tree, from which the designation laccase was derived[7]. Laccase activity has been demonstrated in several fungal species leading to the fact that most all fungi produce laccase. Laccase production has never been demonstrated in lower fungi, that is, Zygomycetes and Chytridiomycetes [9,18]. By referring to several literature reports, laccase can be produced through *ascomycetes* such as *Gaeumannomyces graminis*, *Magnaporthe grisea*, *Melanocarpus albomyces*, *Monocillium indicum*, *Neurospora crassa*, and *Podospora anserine*[18]. Laccase production was also reported from some soil ascomycete from the genera *Aspergillus, Curvularia, and Penicillium, which are plant pathogenic species* [18], and in some freshwater ascomycetes [18]. The redox potential of fungal laccases ranges from 450 mV to 800 mV.

Ascomycete species 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 oxidize syringaldazine [18]. *Trichoderma* and *Botryosphaeria* are wood degrading ascomycetes and reported to have some laccase activity. While Botryosphaeria produces constitutively a dimethoxyphenol oxidizing enzyme that is probably true laccase[18]. S.M. 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 these species and one of *Xylaria hypoxylon* exhibited syringaldazine oxidation[18]. In complex liquid media, the fungi *X. hypoxylon and Xylaria polymorpha* produced appreciable titers of an ABTS oxidizing enzyme [18]. The production of Laccase was not reported in ascomycetous yeasts, but *Saccharomyces cerevisiae* has a plasma membrane-bound multicopper oxidase Fet3p protein that shows structural and sequence similarity with fungal laccase [18].

*Basidiomycete* yeast like *Cryptococcus neoformans* produces a true laccase that is capable of oxidizing the phenols and amino phenols[18]. Wood rotting *Basidiomycetes* causing 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 the 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 extracellular enzymes secreted into the medium by ﬁlamentous fungi [18]. Laccases are generally produced by the fungi during their secondary metabolism. Several factors can influence the production of laccase such as type of cultivation (submerged or solid-state), carbon limitation, nitrogen source, and concentration of micro-element[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 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 as the 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 levels 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 are variable depending on the species and strain, but most naturally-occurring species appear to be poor laccase producers so eﬀorts are still being made to screen naturally-occurring laccase producers with desired laccase yields and properties[6,10]. Studies have shown that the genus *Cerrena* has high laccase yields and application potentials, so deserves attention, and the properties of its laccase can be even more desirable compared to the commercial ones. However, Cerrena species are less studied, as compared with *Trametes* species. *C. unicolor*, is a medicinal mushroom with antitumor activities[6]. Some Laccase producing fungi are listed in table 1.

**Table 1. Laccase producing Fungi.**

|  |  |  |
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| 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) |
| 4. |  |  |

**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, 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**: Laccases can be applied as advantageous biocatalysts to replace hazardous and expensive chemicals and save on resource consumption, create unique functionalities, or reduce damaging impacts on the environment could be used to replace these chemicals and serve as a bioadhesive. To initiate or enhance the cross-linking efficiency, laccase could be used in three ways: directly oxidizing wood pulp to generate radicals for cross-linking, functionalizing wood pulp with small compounds (such as aromatic, carboxyl, isocyanate, or acrylamide substances) which act as cross-linking agents 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**: The phenolic compounds are important inhibitors of fermentation, and there is a fixed advantage of using laccase for producing ethanol from lignocellulose. Laccase from white-rot fungus, *Trametes versicolor*, was expressed under the control of the PGK 1 promoter in *S. cerevisiae* to improve ethanol production from lignocellulosic hydrolysates[7].

**c. Biodegradation**: Oxidoreductases may be applied to degrade various substances such as undesirable contaminants, by-products, or discarded materials[7]. Recently, laccase is capable of oxidizing and degrading lipids such as trilinolein and methyl linoleate[7]. These unsaturated fatty compounds are not typical laccase substrates. The products contain hydroperoxides and epoxides. The reaction is important because of the presence of the fatty compounds in wood and food, which may get involved in laccase catalyzed delignification and food modification, respectively[7]. Laccase, peroxidase, and oxygenase are being studied as biocatalysts for degrading hazardous coal substances, particularly the sulfur-containing components. Laccase may be applied to degrade plastic waste containing olefin units. The study is interesting in terms of reducing the pollution around coal mines and the emission of acid rain-causing agents from power plants[7].

**d.** **Bioremediation and Biodetoxification:** Many pesticides, xenobiotics, coal substances, and industrial products derived from polycyclic, aromatic, halogenated hydrocarbons and other organic compounds, are hazardous environmental pollutants. The use of oxidoreductases to detoxify and remove them is attractive to research efforts. Laccase and peroxidase have been used to transform (often in the presence of redox mediators) various xenobiotics, polycyclic aromatic hydrocarbons, and other pollutants found in industrial waste and contaminated soil or water[7]. In general, the redox potential of these compounds is too high for laccase to directly oxidize them via electron transfer. By using redox mediators, other reactions like H+ extraction can be taken place so that these compounds get oxidized easily. The processes include polymerization among pollutants themselves or copolymerization with other non-toxic substances. Polymerized pollutants often become insoluble or immobilized, thus facilitating easy removal by means such as adsorption, sedimentation, or filtration [7].

**e. Food applications**: Laccase may be applied to certain processes that enhance or modify the color and appearance of food or beverages. In ripe-olive processing, laccase can be used in place of conventional dye solution that oxidatively polymerizes various phenolics (such as oleuropein) in olive, resulting in color darkening and debittering[7].

**f. Biofuel cells**: Oxidoreductase may be applied as a biocatalyst for electrode reactions. Laccase may be adsorbed, entrapped, or wired onto the cathode to catalyze the O2 reduction. A biological fuel cell comprising two carbon fibers coated with the enzymes, glucose oxidase, and laccase has been developed and can produce electricity from glucose and oxygen in the bloodstream[7].

**g. Disinfection/antifungal agent**: One potential application is laccase-based in situ generations of iodine, a reagent widely used as a disinfectant. A laccase-iodide salt binary iodine-generating system (for sterilization) may have several advantages over the direct iodine application[7]. This system may be used in various industrial, medical, domestic, and personal care(deodorants, toothpaste, mouthwash, chewing gum, detergent, soap, and diapers) applications such as sterilization of drinking water and swimming pools, as well as disinfection of minor wounds[7].

A fungus *Cercospora which* is a causative agent of leaf spot disease is most noted for its economic impact on sugar beet production. The pathogenesis of *Cercospora* can be restricted by degrading its cercosporin toxin with the enzyme laccase. Cercosporin toxin induces oxygen radical and superoxide production in the presence of oxygen and sunlight that damages the plant[7].

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