FUNGAL LACCASES: PRODUCTION,OCCURRENCE AND APPLICATION

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. Compounds such as diamines, phenolic compounds, aromatic amines gets oxidised by laccase, monomer cross-linking, polymer degradation, and ring cleavage of aromatic compounds is also 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. While aromatic substrates are being oxidised by Laccase by withdrawing electrons, two molecules of oxygen are simultaneously reduced to two molecules of water. 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

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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 multi-copper 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]. The laccase enzyme is known to be produced by fungi, bacteria, and insects [2]. Ascomycetes, basidiomycetes and deuteromycetes are the principal producers of fungal laccase. The capacity of four Streptomyces bacterial strains (*S. cyaneus, S. ipomoea, S. griseus, and S. psammoticus*) to produce active extracellular laccases enzyme in treated wastewater has been investigated [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

Laccase is one of the numerous enzymes found in nature. The term laccase was derived from Japanese lacquer tree, *Rhus vernicifera* and also in 1883 laccase was discovered from this tree [7]. Since multiple different fungus species have shown to make laccase, the majority of fungi are thought to do so. Laccase synthesis has never been observed in lower fungi like Zygomycetes and Chytridiomycetes [9, 18]. Ascomycetes with a history of producing laccase include *Gaeumannomyces graminis, Magnaporthe grisea, Melanocarpus albomyces, Monocillium indicum, Neurospora crassa,* and *Podospora anserine* [18]. Certain soil ascomycetes that are plant pathogenic species from the genera Aspergillus, Curvularia, and Penicillium, as well as some freshwater ascomycetes are also known to produce laccase [18]. Fungal laccase has redox potential that ranges from 450 mV to 800 mV.

Some ascomycete species have been shown to have laccase genes and the ability to oxidise syringaldazine, and they are closely related to wood degrading fungi that aid in the breakdown of dead plant material [18]. Ascomycetes that degrade wood, include

Trichoderma and Botryosphaeria, both of which have been found to exhibit some laccase activity. While a dimethoxyphenol oxidising enzyme produced by *Botryosphaeria sp.* is likely a real laccase [18]. Jaber et al., (2012) observed that only a small subset of Trichoderma strains produce laccase and the enzyme that oxidises syringaldazine at low levels (17). Only two strains of this species and one strain of *Xylaria hypoxylon*, which are *xylariaceus* ascomycetes that cause wood rot, displayed syringaldazine oxidation.The fungus *X.hypoxylon* and Xylaria polymorpha have been found to produce significant levels oxidising enzyme in the artificial liquid media which can degrade ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Ascomycetous yeasts have not been found to produce laccase although *Saccharomyces cerevisiae* does possess the multicopper oxidase Fet3p protein, which is plasma membrane-bound and exhibits structural and sequential similarities to fungal laccase [18].

A genuine laccase that can oxidise phenols and amino phenols is produced by basidiomycetes yeast *Cryptococcus neoformans* [18]. The best- known species for appreciable laccase synthesis are the wood-rotting Basidiomycetes that cause white rot and a related genus of saprotrophic fungi that decomposes litter. According to reports almost all species of white rot fungi produce laccase to variable degrees [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 laccase 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].

Other plants which are known to produce laccase include sycamore, poplar, tobacco, and peach [7]. Despite their widespread distribution, plant-based laccases are not classified because they are difficult to identify and purify as crude plant extracts. This plant oxidoreductases primary job is to produce lignins and aid in the regeneration of injured tissues by releasing the enzyme into the apoplast, a system made of dead plant a component that carries water [5]. Due to their low oxidoreductive potential, plat laccases have limited range of applications (approx. 430 mV). Eukaryotes like insects and Japanese or Chinese Rhus trees have been found to produce laccase.

III. PRODUCTION

Many of the filamentous fungi produce laccase as extracellular enzyme into the medium as secondary metabolite [18]. Several physical, cultural and chemical factors like temperature, pH, state of fermentation (solid or submerged) limitation of nutrients like carbon and concentration of micro-element effect the laccase production in the laboratory [18]. White-rot basidiomycetes are most efficient lignin degraders and laccase producers among other laccase producing fungi like ascomycetes, basidiomycetes, and deuteromycetes [6, 9]. Along with other ligninolytic enzymes including manganese peroxidase, lignin peroxidase, and numerous other peroxidise laccases are secreted by white-rot fungi. Laccase was isolated and purified only from *Cryptococcus neoformans*. A true laccase that can oxidise phenols and

aminophenols but not tyrosine, is produced by basidiomycetous yeast [9]. A true laccase dimethoxyphenol oxidizing enzyme is produced by Botryosphaeria [10].

In basic and applied laccase study *Pleurotus ostreatus* and *Trametes versicolor* are referred as model organisms. There are other species of 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*) are also known producer of laccase [6]. In most of the edible mushrooms laccse productivity and their growing cycles are closely related, high laccase activity is represented by short growing cycle [6]. Depending on the species and strain, laccase yields also vary. Most of the naturally-occurring fungal species are known to be poor producers of laccase therefore researchers are still screening the naturally-occurring laccase producers which can give higher yields of laccase [6,10]. According to research findings, genus high laccase yields has been reported from *Cerrena* so deserves attention, and the properties of its laccase enzyme can be even more desirable and useful as compared to the commercial ones. However, as compared to *Trametes* species Cerrena species are less studied. One species of Cerrena which is also a laccase producer, *C. unicolor,* is a medicinal mushroom with antitumor activities[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. INfIUENCE OF CARBON AND NITROGEN SOURCE IN PRODUCTION OF LACCASE

Production of ligninolytic enzymes is influenced by the carbon and nitrogen. Commonly used carbon sources are glucose, mannose, maltose, fructose, and lactose. A higher laccase production can be achieved by lower concentration of glucose. But sometimes laccse production is affected in negative way due to overconsumption of glucose and sucrose.

Use of polymeric compound as substrate such as cellulose can be the solution of this problem.

Yeast extract, peptone, casein, urea, $(NH_4)_2SO_4$, and $NaNO_3$ as source of nitrogen for laccase production. Laccase production is triggered by nitrogen draining but some fungal strains produce much more efficient Lacasse without getting affected by deficiency of nitrogen. Contradictory evidence has been reported in many studies regarding the effect of nitrogen sources on laccase production. Some studies show that the elevated laccase activity was achieved by using high nitrogen concentration in the medium others show that increased concentration of nitrogen results in lower production of laccase also a low carbon-to-nitrogen ratio produces high laccase, 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, to be best suitable for *Aspergillus* sp. for production and isolation of laccase [2].

V. INFLUENCE OF TEMPERATURE AND PH ON THE PRODUCTION OF LACCASE

Temperature plays an important role in the metabolism of fungi. Productions of many of the secondary metabolites are greatly affected by a slight change in temperature. The optimum temperature for the biomass and enzyme production varies with the fungal species and a slight variation in temperature effects the laccase production. In many cases production of enzyme and biomass is found to show parallel relation suggesting that laccase production is associated with growth.

According to research, the ideal temperature for laccase formation in the presence of light is 25°C, while in the absence of light, the ideal temperature is 30°C[1,3,10]. According to Shraddha et al. (2016), temperature has a minimal impact on laccase production[10]. According to Rajesh Kumar et al. (2016), Aspergillus sp. performs best at 25°C and pH7 for the synthesis and separation of fungal laccase [2]. T. harzianum could be regarded as one of the most significant sources of Laccase synthesis at 35°C and pH 5[3,] according to Rehman A. Abd EL Monssef et al. (2016). The laccase generated by T. modesta was fully active at 50°C, as demonstrated by Nyan Hongo et al. in 2002.

VI. APPLICATIONS

1. **Industrial applications:** To replace these harmful and expensive chemicals and conserve resources, generate novel functions, or lessen negative environmental effects, laccases can be employed as favourable biocatalysts. They can also be used as a bioadhesive. Three methods of using laccase to start or improve the cross-linking efficiency are as follows: By directly oxidising wood pulp to produce radicals for cross-linking, functionalizing wood pulp with tiny compounds that act as cross-linking agents (such as aromatic, carboxyl, isocyanate, or acrylamide substances), or by converting isolated lignin (by-product), starch, phenolic polysaccharide, or protein into radical-rich and non-toxic adhesives. Such laccase uses could not only replace harmful or pricey chemical adhesives but also turn wastes like lignin from the paper industry into goods with added value [7].

2. **Enhancing ethanol production**: Phenolic molecules are substantial fermentation inhibitors; hence employing laccase to produce ethanol from lignocellulose has a fixed advantage. To increase the production of ethanol from lignocellulosic hydrolysates, laccase from Trametes Versicolor, a white-rot fungus, was expressed in S. cerevisiae under the regulation of the PGK 1 promoter. [7].

3. **Biodegradation**: Oxidoreductases can be used to decompose a variety of compounds, including unwanted pollutants, byproducts, and waste items [7]. Recently discovered lipids like trilinolein and methyl linoleate can be oxidised and degraded by laccase[7]. These unsaturated fatty acids are not the usual substrates for laccase. Epoxides and hydroperoxides are present in the products. The existence of fatty molecules in food and wood, which may participate in food modification and laccase-catalyzed delignification, respectively, makes the reaction important [7]. As biocatalysts for the degradation of dangerous coal compounds, notably sulfur-containing components, laccase, peroxidase, and oxygenase are being investigated. Olefin-containing plastic waste can be broken down using laccase. 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].

4. **Bioremediation:** For the degradation of pollutants including pesticides, xenobiotics, coal substances, and industrial products, biocompatible and cost-effective procedures are needed. These are dangerous environmental contaminants that are formed from polycyclic, aromatic, halogenated hydrocarbons and certain other organic molecules. Along with laccase and peroxide, the enzyme oxidoreductases are known to detoxify these substances. A synthetic dye known as azo-dye can be broken down by laccase into less harmful chemicals. Laccase can also be used to decolorize a wide variety of textile dyes, including reactive red, brilliant blue, and reactive orange [7].

5. **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 a conventional dye solution that oxidatively polymerizes various phenolics (such as oleuropein) in olive, resulting in color darkening and debittering[7].

6. **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].

7. **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 can this but the binary system 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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