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Review Article

A Brief Review on Laccase Enzyme From the Edible Mushroom *Lentinus edodes* and its Applications in Decontamination of Antibiotics from Wastewater

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Abstract

Recently, laccase has drawn the attention of researchers and manufacturers due to its various applications in different industries such as the pulp industry, bioremediation, food industry, and medical product industry. This eco-friendly enzyme is one of the leading enzymes of the multicopper oxidase family that has shown incredible ability to remove pollutants like antibiotics from wastewater and nature. This review aims to introduce the laccase enzyme and its history, explain its chemical features, and describe its constituent resources. Finally, this paper discusses its applications, especially the removal of antibiotics from wastewater with the help of syringaldehyde (SA) and hydroxybenzotriazole (HBT). In this research, basic and comprehensive information about laccase enzyme and their applications has been presented by reviewing numerous articles. These articles were searched using relevant keywords. Moreover, schematic diagrams, pictures, and tables were used to describe the data in the paper. Laccase treatment with SA in an enzymatic membrane reactor is one of the feasible solutions for removing antibiotics from wastewater, which showed the elimination of 32 different antibiotics. Laccase is a good choice for industrial applications and decontamination of antibiotics. This enzyme shows tolerance to industrial thermal conditions, hence its broad applications in the decolorization of textile dye and paper industries.

Keywords: Laccase, Biodegradation, Environmental, Fungi, Laccase applications, Removal of antibiotics

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1. Introduction

In general, enzymes are very important alternatives for many industrial applications due to their high specificity, low investment costs, low operating energy requirements, and natural origin. In particular, enzymes produced by white-rot fungi have become powerful tools in research related to waste treatment, environmental protection, degraded area restoration, and industrial wood processing. The use of enzymes in industrial processes can reduce or replace the use of aggressive compounds and allow processes at milder temperatures and pH values [1].

Laccase (EC 1.10.3.2, p-diphenol oxidase) has been studied since the 19th century. Laccase was discovered by Yoshida in the exudate of the lacquer tree *Rhus vernicifera* in 1883. However, Bertrand and Laborde demonstrated in 1896 that laccase is a fungal enzyme [2,3]. Laccases are copper-containing enzymes that catalyze the oxidation of various organic and inorganic substrates, such as monophenols, diphenols, polyphenols, aminophenols, methoxyphenols, aromatic amines, and ascorbate, with the concomitant four-electron reduction of oxygen to water [4]. Laccase is a member of the large blue copper proteins or blue copper oxidases. Other enzymes in this group include ascorbate oxidase in plants and the plasma protein ceruloplasmin in mammals. The ability of laccases to oxidize phenolic compounds and reduce molecular oxygen to water has led to intensive research on these enzymes [2].

Laccase enzymes are found primarily in fungi. They are also found in various organisms, including plants, bacteria, and a few insects [5,6]. Fungal laccase enzymes (e.g., laccase enzyme from the edible mushroom *Lentinus edodes*) are frequently used in the paper industry and are studied more frequently than other organisms due to their high ability to degrade wood [7,8]. Due to its high stability and specificity, it can be used in various industries and biotechnological applications to remove environmental pollutants such as antibiotics. The presence of these pollutants in wastewater leads to contamination of natural water sources [9]. Table 1 shows the general uses of laccase enzyme and its sources.

2. Chemical structure and properties of laccase

Laccase enzymes are glycoproteins with enormous diversity in mass, carbohydrate composition, and the number of protein chains. Most of the studied laccase enzymes of fungal origin have monomer, dimer, or

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Table 1. Production Sources and Uses of Laccase [10]

	Plant	Insects	Bacteria	Fungi
Source of laccase enzyme	Lacquer, Mango, Mung, Been, Sycamore	*Bombyx, Calliphora, Diploptera, Drosophila*, Lucillia, *Manduca, Musca, Orycetes, Papilio, Phormia, Rhodnius, Sarcophagi, Schistocerca, Tenebrio*	*Azospirillum lipoferum, Marinomosas mediterranea, Streptomyces griseus, Bacillus subtillis*	Ascomycetes, Basidiomycetes, Deuteromycetes
Application	Biosensor, immunochemical assays, biodegradation of wastes, organic compound, gold nanoparticle synthesis, DNA labeling, baking industry			

tetramer structures. The carbohydrate chain may be associated with the stability of enzymes [7]. The molecular weight of monomeric glycoproteins differs from 50 to 130 kDa. The carbohydrate moiety consists of mannose, acetylglucosamine, and galactose, which make up 10%-45% of the total mass of a monomeric enzyme [11].

2 .1. Thermal studies on activity and stability of laccase

Laccase enzymes can show different stability and optimal activity depending on their substrate, pH condition, and temperature. It is reported that their optimal activity even changes in the presence and absence of light. A study has shown that laccase produced by *Tricholoma matsutake* reaches its optimal activity at 30 °C in the light; however, this temperature drops to 25 °C when the incubated fungus grows in the dark [12]. More research has recently been focused on the techniques that increase the stability and activity of laccase enzymes in higher temperatures. In 2020, Wang et al found that laccase immobilized onto zeolite imidazolate framework-67 (ZIF-67) indicates remarkable stability and higher reusability [13]. Moreover, pre-incubation of the laccases, extracted from *Marasmius quercophilus*, at 40 °C and 50 °C has been shown to increase the stability [7]. In general, the optimal temperature for the activity of laccase enzymes ranges from 30 °C to 50 °C [11], and pre-incubation and utilization of metal frameworks can help with the improvement of the enzyme.

2.2. Impacts of different pH conditions on laccase

It is believed that the initial pH for laccase enzyme production ranges from 4.5 to 6.0 [12]. This variation originates from the redox potential differences between the phenolic substrates and the copper oxidation sites, meaning that the enzyme works better at higher pH values with a difference in redox potential between the substrate and copper atom in the active site. Besides, the hydroxide, produced from the reaction, binds to the other 3 active sites, which results in the inhibition of the enzyme [7].

3. Sources and occurrence

According to the BRENDA database, more than 300 fungal laccases have been discovered that are responsible for fungi sporulation and formation of the fruit body, degradation of lignin, pigment production, and defense against stress [14]. The most famous species of these fungi, known for its ability to degrade lignin, is white-rot basidiomycete [7]. *Pycnoporus cinnabarinus, Pycnoporus sanguineus,* and *Neurospora crassa* have been reported to produce laccase [7]. Fungal laccases can be produced extracellularly, intracellularly, or even both, depending on fungal species.

As already mentioned, plant laccase was first extracted from *Rhusvernicifera* tree. Recently, *Gossypium spp.* [15], *Oryza sativa* [16], *Prunus avium* [17], *Pyrus bretschneideri* [18], *Amborella trichopoda, Glycine max, Physcomitrella patens, Ricinus communis, Triticum aestivum, Vitis vinifera* [16], *Setaria viridis* [19], and *Zea mays* [20] have been found to have genes to encode laccase enzyme [14]. Plant laccases undertake numerous physiological and biochemical actions such as lignin polymerization, defense mechanism, wound healing, maintenance of the structure, and the polymerization of phenolic compounds [14]. Although there has not been much research on plant-derived laccase enzymes, some studies have suggested that these kinds of laccase enzymes are good candidates for remodeling and improving the production of biofuels [14].

Laccase enzymes do also exist in some species of bacteria such as *Azospirillum lipoferum* [7], *Bacillus*, and *Streptomyces* genus [14]. Bacterial laccases have been shown to play some important biological roles, including pigmentation against UV light, degradation of lignin, and creation of antibiotics [14].

3.1. Fungal laccase

Fungal laccases are of great importance due to their widespread applications in agriculture, medicine, textiles, and the pulp industry. In Table 1, some fungal laccases with their specific applications in the industry are presented [10]. Ameri et al showed that by optimizing the growth conditions of mycelium and polysaccharides of *Lentinus edodes* on walnut shell by-products using response surface analysis, polysaccharides of *Lentinus edodes* can biologically decompose polyaromatic hydrocarbon by producing laccase enzyme [21].

4. The importance of enzymes

In the last 2 decades, climate change and human impacts on nature have urged scientists and researchers to innovate or discover bio-friendly solutions to reduce or remove industrial damage to the environment. It is considered one of the green enzymes to be replaced with various harmful industrial processes. Additionally, its

thermostability, versatility, and biocatalyst features have allowed laccase to be widely utilized in the paper and pulp industry, food processing industry, and even medical approaches and treatments [11].

5. Applications

5.1. Removal and degradation of antibiotics

One of the major global concerns of the current era is antimicrobial resistance. According to WHO, there are several reasons behind antimicrobial resistance including improper consumption of antibiotics by patients, overprescribing of antibiotics, overuse of antibiotics in livestock, and especially lack of access to clean and sanitized water. Unfortunately, the presence of antibiotics in wastewater of hotspots, including hospitals, wastewater treatment plants, animal feeding operations, and aquaculture operations, has accelerated the antimicrobial resistance [22]. These municipal sewage treatment plants and industrial wastewater treatment plants are major sources of antibiotic-resistance genes [23]. Therefore, there has been extensive research on the removal of antibiotics from the WWTP sewage. As mentioned earlier, laccase can also degrade nonphenolic compounds, including some sorts of antibiotics. As a result, it is proved that laccase can be a successful solution for clearing wastewater of treatment plants from antibiotics. Prieto et al showed that laccase produced by *Trametes versicolor* [8] could degrade more than 90% of ciprofloxacin and norfloxacin, which is in line with the results of the study by Becker et al [24]. However, it should be considered that intermediaries are essential for the oxidation of other nonphenolic substrates of laccase to take place.

5.2. Elimination of antibiotics by laccase and syringaldehyde

Becker et al reported that a combination of immobilized laccase (*Trametes versicolor)* and SA mediator in an enzymatic membrane reactor eliminated a mixture of 38 antibiotics at an environmentally relevant concentration (10 $\mu g \cdot L^{-1}$) [24]. SA is the main mediator, mostly occurring in plants, that helps with the degradation of lignin. This organic compound has lower redox potential compared to laccase. Therefore, when it comes to the reaction, SA gets oxidized faster and the reaction releases radicals that oxidize nonphenolic compounds, which is exactly how mediators help laccase with degrading other nonphenolic substrates.

Becker et al showed that the addition of SA enhances the degradation of antibiotics. Of the 38 antibiotics, 32 were degraded by more than 50% within 24 hours. Amoxicillin and ampicillin had the highest removal percentage (up to 90%) among the other penicillins, which were mostly stable. The treatment had no significant impact on the removal of quinolones, metronidazole, and trimethoprim (less than 30%). However, 60% of pipemidic acid was removed [24].

Degradation of antibiotics was intensified at higher concentrations of SA (1000 $\mu mol \cdot L^{-1}$). Incredibly, 17 out of 38 antibiotics were cleared more than 90% after 24 hours. Sulfonamides, except for sulfanitran, were observed to have the highest removal rate (>97% removal after 2 hours). Moreover, the degradation of quinolones was improved, except for cinoxacin (15% removal), reaching more than 70% after 24 hours. Fluoroquinolones, except for difloxacin (49%), orbifloxacin (33%), and flumequine (42%), also showed the same pattern of removal. However, SYR1000 could not enhance the removal of all 38 antibiotics. Comparing the removal of tetracyclines with SYR1000 to its lower concentration, it was seen that this class of antibiotics indicated better decomposition (60%–90% after 24 hours) with SYR1000 [24].

In conclusion, 32 out of 38 antibiotics at environmentally relevant concentrations can be cleared (more than 50%) in an enzymatic membrane reactor containing laccase immobilized on ceramic membranes and SA. Whereas there was much removal in the experiment without SA as a mediator [24].

5.3. Laccase treatment in the presence of HBT compared to manganese peroxidase

Many studies have emphasized the importance of the accompaniment of mediators to laccase enzymes in accelerating the catalyzation of antibiotics. Not only SA but also many other chemical compounds like hydroxybenzotriazole (HBT) [25] can boost oxidation reactions with laccase enzymes. It has been reported that the laccase–HBT system could clear tetracycline, chlortetracycline, doxycycline, and oxytetracycline from reaction mixtures [25]. Furthermore, it has been reported that ligninolytic enzymes (manganese peroxidase, lignin peroxidase, and laccase) can degrade antibiotics [26]. Manganese peroxidase is an organic compound that is produced by white rot fungi, especially *Trametes versicolor*. It consists of a heme that functions as its peroxidase site, which converts Mn (II) to Mn (III) [26].

Suda et al observed that laccase–HBT system was more effective in the degradation of antibiotics than manganese peroxidase and laccase alone [25]. In addition, the removal rates of tetracycline, chlortetracycline, doxycycline, and oxytetracycline were increased by 16%, 48%, 34%, and 14%, respectively [24].

6. Biotechnological purposes

One of the characteristics of laccase enzymes is that they can catalyze substrate, needing high reduction potential to get oxidized. Furthermore, laccases produced by white-rot basidiomycete fungi have shown a high redox potential and the ability to remove Bisphenol A in applications associated with bioremediation [14]. Laccase

enzyme has a high ability to catalyze various biological compounds and can remove complex structures such as antibiotics in the bioremediation process.

6.1. Potential for the decolorization of textile dyes

Due to their oxidative characteristics, laccases have captured the attention of manufacturers as they can be applied in industrial processes such as decolorization of textile dyes, Malachite Green decolorization, and fiber confining [12].

6.2. Medical treatments

Pathogenic yeast *Cryptococcus neoformans* encodes the genes of laccase, which plays an important role in the pigmentation of melanin and the production of immunomodulatory agents. Therefore, it can be considered a remarkable virulence [12]. Moreover, laccase extracted from oyster mushroom (*Pleurotus ostreatus*) is used as herbal medicine to inhibit hepatitis C virus replication [12].

7. Conclusion

Laccase is one of the leading future bioremediation solutions for the degradation of industrial pollutants and antibiotics. One of the chief functions of the laccase enzyme is the removal of antibiotic pollutants, which have become one of the biggest challenges of this decade. Maintaining the stability of laccase at high temperatures in renewable industrial processes such as textiles, pulp industry, medical treatments, and bioremediation processes is a great challenge that scientists are facing. Despite the decontamination ability, laccase treatment reactions can generate some other pollutants. Recent studies have been working out solutions to minimize the production of these pollutants.

The elimination of antibiotic pollutants in relevant environmental conditions using laccase and SRY as a mediator is a significant breakthrough.

Authors' Contribution

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Competing Interests

The authors declared no conflict of interest.

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