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Laccase Enzyme: As A Sustainable Catalyst For Bioremediation Strategies

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"Laccase, belonging to the blue multicopper oxidases enzyme category, exhibits notable oxidation capabilities. Despite its potential to generate reactive radicals, its commercial utilization has been largely underestimated. Nonetheless, laccase can be sourced from diverse origins, including bacteria(Bacillus, streptomyces etc), fungi like white rot fungi and plants for e.g. wheat, castor, white pear etc. It demonstrates efficacy in degrading both phenolic and non-phenolic compounds by converting molecular oxygen to water, offering a distinctive ability to detoxify environmental contaminants. Consequently, laccases have found extensive applications across industries such as paper, pulp, textiles, dye degradation and petrochemicals. Moreover, they are utilized in food processing, medical, and healthcare domains. Recent advancements have seen laccase employed in areas like biosensor development and nanotechnology. This review comprehensively examines laccase's biological functions, sources, mechanisms of action, and potential biotechnological applications."

Keywords: Biotechnological application; Detoxify; Environmental pollutants; Laccase; Phenolic compounds.

Laccase enzymes, also known as oxidoreductases (EC 1.10.3.2), are polyphenol oxidases that oxidise different phenolic compounds by using molecular oxygen as an electron acceptor. They have a carbohydrate content of 15-30% and a molecular mass of 60-90 kDa, making them one of the most ancient and thoroughly investigated enzyme systems[1]. Yoshida isolated laccase for the first time from the exudates of the Japanese lacquer tree, *Rhus vernicifera*, in 1883.[2] Laccase enzymes are members of the blue copper protein or blue copper oxidase class, which encompasses plant ascorbate oxidases and the mammalian plasma protein ceruloplasmin as well. Their capacity to convert molecular oxygen to water has sparked significant interest in these enzymes[2]. Laccases are found in a wide range of species, including plants, bacteria, fungus, and insects. They can be found in plants as vegetables such as cabbages, turnips, potatoes[3]. Insects belonging to genera such as *Calliphora, Bombyx, Diploptera, Lucilia, Drosophila*, and others also possess laccase enzyme[3]. Fungi, particularly wood-rotting fungi like *T. hirsuta, T. ochracea, T. versicolor, T. villosa, T. gallica, C. maxima, , P. eryngii,* and more, are known producers of laccase enzyme[4]. Laccases are involved in the breakdown and transformation of lignin, like other

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ligninolytic enzymes such as lignin peroxidase, manganese-dependent peroxidase, multifunctional peroxidase, and dye-decolorizing peroxidase[5]. Certain bacteria also exhibit laccase activity, including *Azospirillum lipoferum, Streptomyces griseus, and Bacillus subtilis*. Bacteria, known for their stability and adaptability to various environments, are often preferred for wastewater treatment compared to fungi. Their enhanced stability and impressive substrate specificity render them highly valuable biocatalysts across numerous biotechnological applications[6]. However there are some limitations of bacterial laccase also, like they have lower redox potential as compared with the fungal laccases. Laccase enzymes exhibits exceptional proficiency, encompassing attributes relevant to the advancement of fiber biosynthesis, efficient energy utilization, environmental preservation, bio-detection, and promising industrial implementations. These encompass enhancing food and cosmetics production, facilitating bio-pulping within the paper industry, driving innovations in textile manufacturing, and enabling the biodegradation of environmental contaminants. Laccase-mediator systems exhibit a pivotal role in various domains, excelling in tasks such as lignin degradation, biosensor utilization, biofuel production, organic synthesis, mitigation of hazardous chemical waste through bioremediation, contributions to the pharmaceutical field, and applications within nanobiotechnology[6].

Bibliometric analysis

Two databases were used to choose publications relevant to the work's development: ScienceDirect and Scopus. With the use of these databases, research may be carried out in a secure and dependable manner, guaranteeing increased consistency and accessibility to the published contents. Articles were searched on Scopus firstly by using the terms "laccase," "production," and "application" in the title, abstract, and keywords. The resulting research was analyzed using the "Bibliometrix" tool[7]. India ranks as the first highest country in publishing articles on this topic as shown in Fig.1 and Fig.2.

Properties of laccase

Laccases, a subset of multi-copper oxidases (MCOs), exhibit the capability to produce various extracellular enzymes, such as (MnPs) manganese peroxidase, (LiPs) lignin peroxidase and (VPLs) versatile peroxidase. Laccases are categorized into four enzyme families:(EC 1.10.3.2) laccases , (EC 1.10.3.3) ascorbate oxidases, (EC 1.16.3.1) ferroxidases, and (EC 1.16.3.1) ceruloplasmin[8]. Laccases typically function bas active holoenzymes, existing in monomeric, dimeric, or tetrameric glycoprotein forms. The molecular weight of laccases falls within the range of 50 to 130 kDa. These enzymes feature a carbohydrate component, comprising of acetylglucosamine , galactose and mannose, which constitutes 10 to 45% of the total protein content. The presence of this carbohydrate moiety significantly enhances their stability[9]. A wide range of substrates have the ability to interact with Laccases. Polyphenols, methoxy-substituted aromatic amines, and ascorbate are among the substances that fall into this category. Additionally, laccases demonstrate proficiency in catalyzing a four-electron reduction of O_2 to H_2O[10]. Laccases have ideal temperatures that normally range between 30 to 50 °C, however they exhibit a significant decrease in activity when exposed to temperatures above 60 °C. This temperature range tends to be influenced by the originating organism. Their isoelectric point varies across sources, spanning from pH 3 to pH 7, while in plants, it is relatively high at pH 9. Laccases play a pivotal role in various biological functions, including excretion, sensitivity to proteolytic degradation, and copper retention. Their thermal stability holds particular importance in biotechnological applications. These enzymes are related with cross-linking monomers, the disintegration of synthetic compounds, and the cleavage of aromatic compounds via ring cleavage[2].

Catalytic mechanism of laccase

Laccase structures commonly comprise four copper (Cu) atoms per monomer, strategically bound to three distinct redox potential sites known as Type-1, Type-2, and Type-3 Cu pairs, encircling the central copper core. These enzymes rely on copper(Cu) as a co-factor and molecular oxygen (O2) as a co-substrate for their activity. In the laccase derived from T. versicolor, three functional groups containing four copper atoms each are identifiable. Within these groups, a single copper center is present, referred to as T1 copper, which is triangularly coordinated by two histidine and one cysteine residue. Furthermore,

the trinucleated clusters feature a T2 copper atom, characterized by its non-blue hue, indicating the absence of absorption within the visible spectrum. In conjunction, a pair of T3 copper atoms complete this trinucleated configuration. The T2 copper is coordinated by two histidine residues, while the T3 copper atoms are intricately coordinated by six histidine residues, contributing to the overall structural and functional intricacies of laccase[11].

The catalytic mechanism of laccase involves:

(i) The reducing substrate facilitates the reduction of the Type-1 copper.

(ii) Internal electrons were transported from Type-1 Cu to the Type-2 and Type-3 Cu sites.

(iii) The conversion of O2 into H2O occurs through the r eduction process at the Type-2 and Type-3 copper site.

The T1 Cu site is accountable for the distinctive blue coloration of laccase, signifying its potent oxido reduction capabilities. This attribute is linked to a noteworthy electronic absorption peak around 600 nm. In the realm of biotechnology, the EPR (Electron Paramagnetic Resonance) technique is particularly pertinent. It holds significance for applications such as efficient bioremediation and bio-bleaching treatments. On the other hand, the T2 Cu site lacks a discernible color and cannot be identified spectrophotometrically. Despite this, it generates a distinctive EPR signal that stands apart[12]. The binuclear T3 Cu site is diamagnetic, exhibiting a spectral absorbance peak within the range of 330 nm. Additionally, it presents a distinct fluorescence spectrum that is characteristic of this site[13].

Sources of laccase

Fungal laccase

As reflected in the BRENDA database—The Comprehensive Enzyme Information System, the repository now encompasses descriptions of over 300 laccases, predominantly sourced from fungal species (Fig.5). This underscores the significance of laccases in fungal biology and their relevance in diverse applications. When the amount of carbon and nitrogen become scarce in the growth medium, numerous fungi engage in the production of laccases through secondary metabolism. These enzymes play a crucial role in various physiological and ecological processes[14]. Various fungi have been documented as producers of laccases, with White-Rot Fungi (WRF) being the most widely acknowledged among them[15]. *Pleurotus ostreatus* and *Trametes versicolor* are often reported fungus in the context of laccases and can be considered model organisms in basic and applied research for many objectives[16].

Laccase enzyme has the molecular weight renges from 38 to 150 kD and its isoelectric

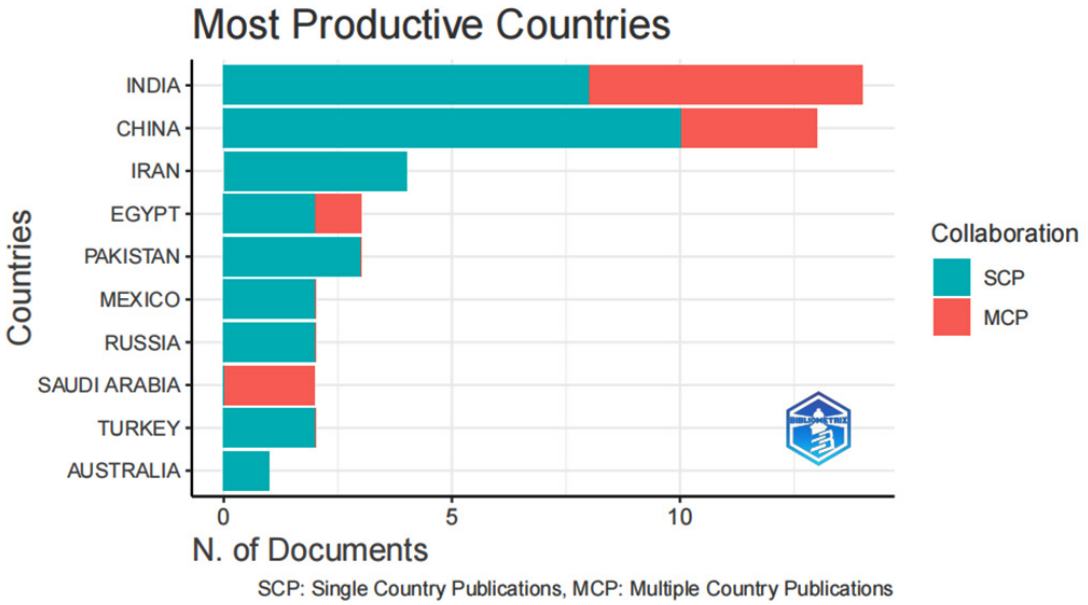

Fig. 1. Most productive countries published laccase related papers

point is near about pH 4. As a consequence of this isoelectric point, they manifest heightened effectiveness in acidic reactions, particularly within the pH range of 3.5 to 5.5. Certain fungal laccases display enhanced resistance to temperature variations, showcasing their ability to maintain effective catalytic performance across a broader temperature range, spanning from 25 to 60 °C, in contrast to most enzymes which perform optimally between 30 and 55 °C[17].

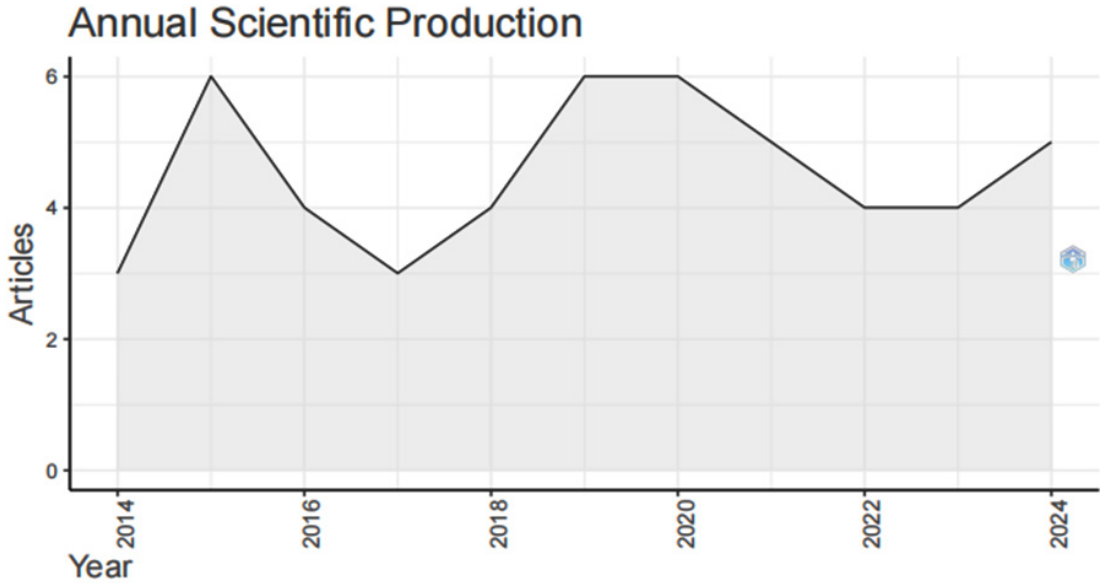

Fig. 2. Annual scientific production of articles on laccase and its application

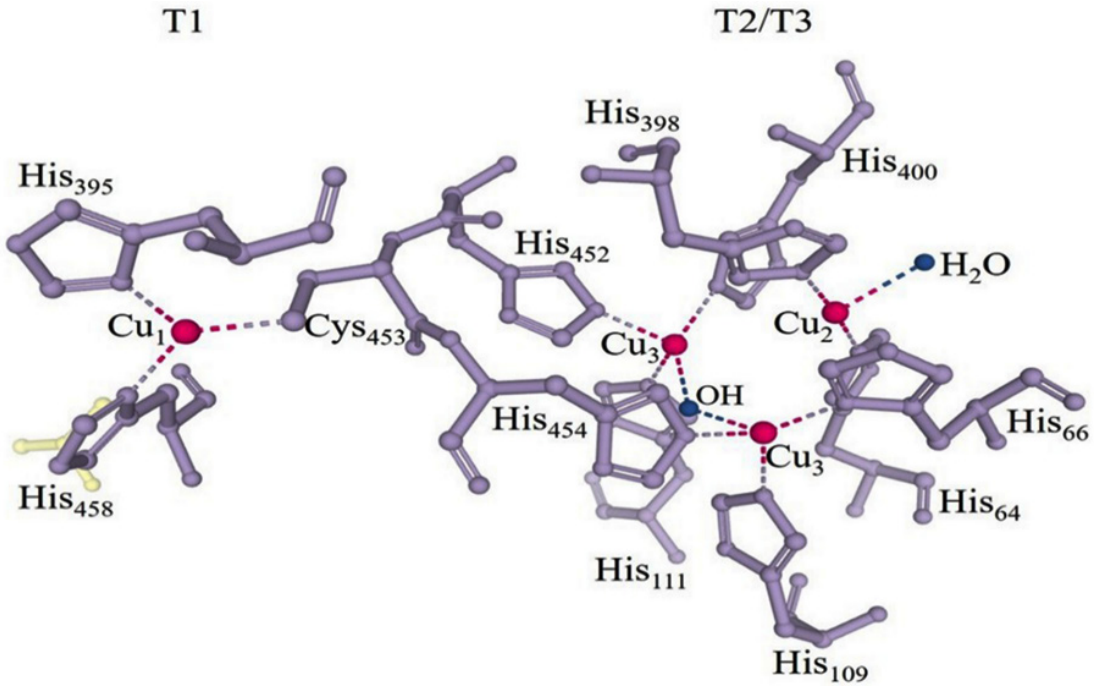

Fig. 3. Illustration of the *Trametes versicolor* laccases' Cu catalytic site (RCBS; Protein Data Bank code 1GYC) in relation to various amino acids; acronyms stand for His for histidines and Cys for cysteine. The pink spheres are the atoms of copper.[12]

Bacterial laccase

The bacterial laccases was discovered in *Azospirillum lipoferum*, which was isolated from the *oryza sativa* rhizosphere[18]. Until now, a substantial number of identified laccases have been found in the *Bacillus* and *Streptomyces* genera. In the *Bacillus* genus, laccases play a role in coloring the endospore coat brown, which serves as a protective shield for the bacteria, offering defense against external stressors and UV radiation. Laccases from the *Streptomyces* genus perform a wide range of tasks, including morphogenesis, sporulation, coloration, bacterial interactions, antibiotic synthesis, and, most significantly, lignin breakdown[19]. Over the past decade, numerous bacterial species have been documented for their ability to produce laccases like as *Pseudomonas sp*.[20], *B. safensis*[21], *B. tequilensis*[22], *Geobacillus sp*.[23], *P. putida*[24] and *M. mediterrânea*[25]. Most laccases produced by bacteria are intracellular as in *M. mediterranea, B. subtilis, Thermus thermophilus*[19]. On the other hand, extracellular laccases were found in some species of *Bacillus*[26], *Streptomyces*[19]. The optimum temperature for bacterial enzyme production is near about 45°C and the optimal pH can vary according to the substrate. The maximum activities was shown by laccase enzyme at neutral or alkaline pH[19].

Plant laccase

Plant laccases were initially detected in concentrated sap from the lacquer tree, Rhus vernicifera.[27] Laccase plays vitral roles in plants, encompassing activities such as lignin polymerization, responses to environmental stress, defense mechanisms, the healing of wounds, the maintenance of structural integrity, and the polymerization of phenolic compounds[19]. Laccase enzyme were recently found in various plants including *Pyrus breschneideri* (White pear)[28], *Prunus avium* (Cherry)[29], *Ricinus communis* (castor), Triticum aestivum (Wheat) [30], *Setaria viridis* (Grass)[31] and *Zea mays* (Corn)[32]. Proteins typically comprise approximately 500 to 600 amino acids and exhibit a higher molecular weight compared to laccases found in fungi and bacteria, with a size range spanning from 60 to 130 kDa[33]. They have an (P_i) isoelectric point of 5.0 to 9.0 and values of optimum ph is 5 to 7[19] resulting in increased activity at neutral or alkaline pH[15].

Insects laccase

Ohnishi (1954) was the initial scientist to document laccase-like activity in the pharate pupal

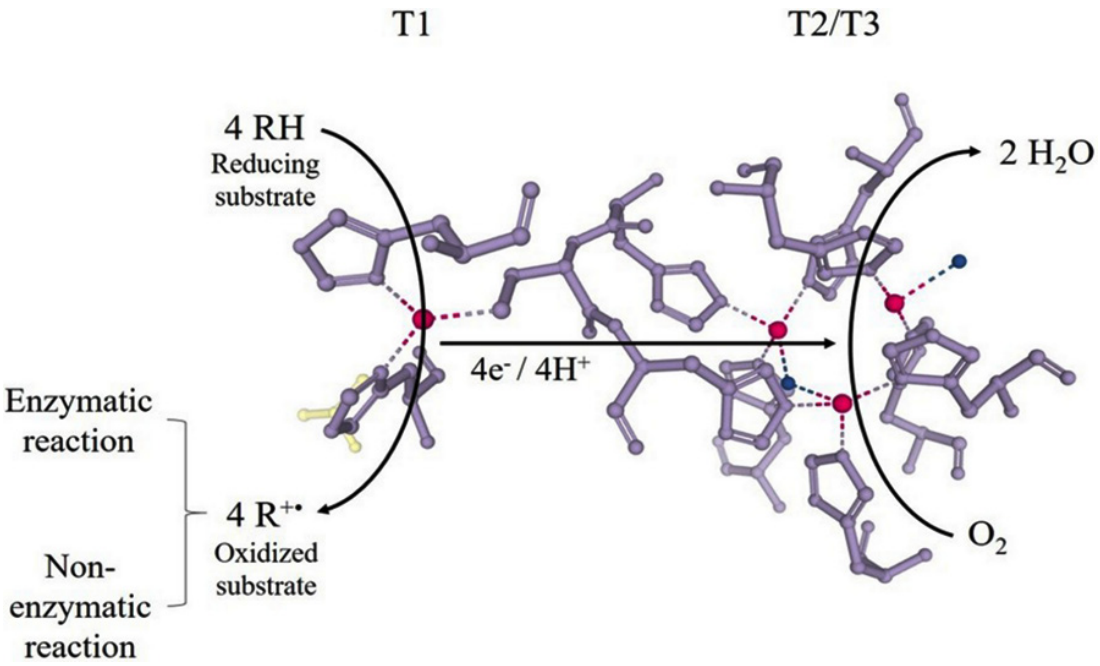

Fig. 4. Diagram illustrating the laccase mechanism of action in *Trametes versicolor's* catalytic center (RCBS—Protein Data Bank code PDB 1GYC)[12]

cuticle of *Drosophila virilis*. Since then, scientists have investigated the properties of laccases in a variety of other insect species. These investigations have examined laccases in insects like the silkworm *Bombyx mori,* termites (*Reticulitermes flavipes*), *Riptortus pedestris, Nysius lebeius*, and *Megacopta punctatissima*[34]. Among all identified laccases, those present in insects are the least explored and comprehended. These insect laccases play a crucial role in insect physiology, specifically in processes like the hardening of the cuticle (sclerotization) and the synthesis of melanin (melanization)[35].

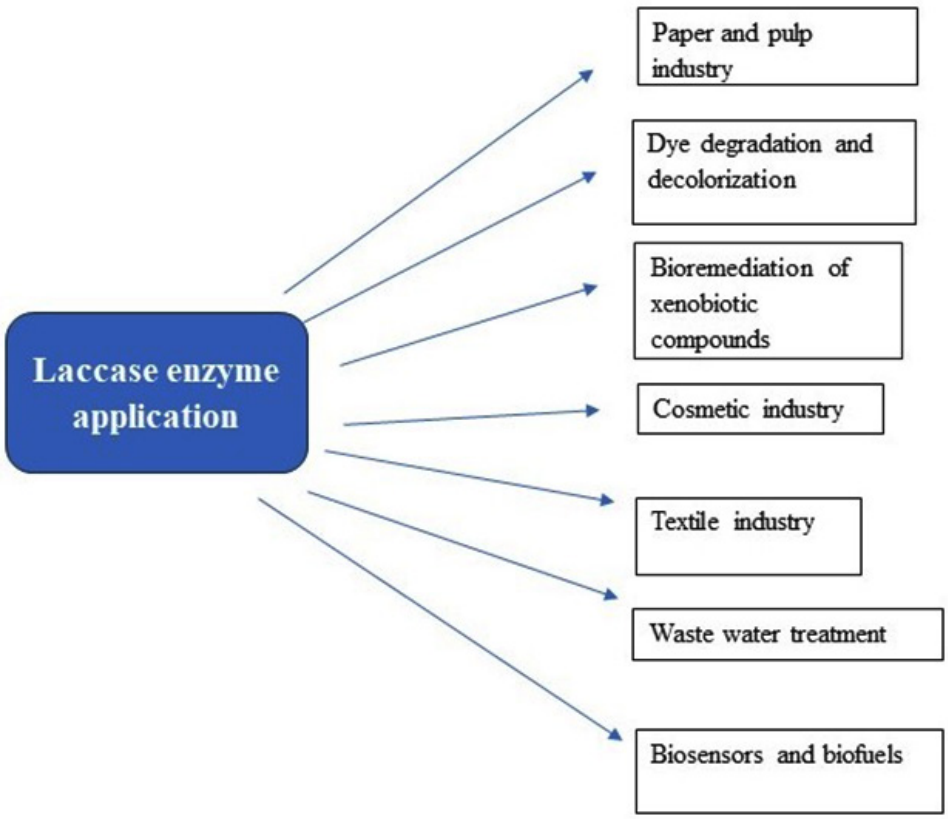

Fig. 5. Various application of Laccase Enzyme in various industries

Table 1. Table showing Different sources of laccase production and their applications

Sources	Strains	Properties	Application	References
Fungus	*Trametes versicolor*	Improved thermal stability	Elimination of micropollutants from wastewater.	[16]
	White rot fungi	reduction of O2 to H2O	Decolorization of synthetic dyes	[17]
Bacteria	*Streptomyces coelicolor*		Decolorization of indigo dyes	[26]
	Bacillus tequilensis	Reduction in kappa numberhighly thermostable	Bioremediation	[22]
Plants	*Prunus avium L.*	bioactive moleculesproduction, highly perishable	browning and the preservation of the appealing red color	[29]
	Zea mays	polar and hydrophobic surfaces	Catalytical application stuides	[32]
Insects	*Acrobasis nuxvorella*	antiproliferative activity	eco-friendly biocatalysts.	[35]
	Bombyx mori	Cuticle sclerotization	genetic engineering	[34]

Industrial application of laccase enzyme

Laccases are intriguing enzymes that have received a lot of attention due to their numerous uses in biotechnology. These enzymes are members of the multicopper oxidase family and may be found in a variety of species such as bacteria, fungus, plants, and insects. Laccases catalyse the oxidation of a wide range of substrates by transferring electrons to molecular oxygen, resulting in the conversion of oxygen into water[36]. Laccases have shown exceptional performance as biocatalysts in a variety of industrial and environmental environments. They have been used successfully for wastewater treatment in a variety of industries, including textiles, paper & pulp, and petrochemicals. Furthermore, its uses include the food sector and medical diagnostics. Laccases have also been used in the modification of lignin and as agents for bioremediation against herbicides and insecticides[37].

Laccases are well-known enzymes that hold great promise for dealing with phenol-contaminated environments as well as a wide range of biotechnological applications (Fig.5). Extensive research has been conducted to assess their potential in detoxifying harmful substances in both aquatic and terrestrial ecosystems production treatment of beverages, analytical tools and biosensors . Lignosulfates undergo industrial polymerization for subsequent use as surfactants, dispersants, and plasticizers within the cement and concrete industry[15].

Waste water treatment

The wastewater treatment industry is progressively emphasizing enzymatic bioconversion technologies because of their capacity to effectively remove harmful substances under mild conditions, while also minimizing the generation of undesirable by-products associated with biodegradation. Utilizing enzymes in wastewater treatment comes with significant benefits, thanks to their precise targeting of specific substrates, resilience in handling sudden increases in pollutant loads, fast reaction rates, efficient use of oxidants, and effective treatment even when dealing with low substrate concentrations [38]. The capacity of laccase to efficiently break down diverse phenolic compounds without inducing harmful side effects has sparked interest in its potential application for detoxifying contaminated wastewater. Laccase enzyme also degrade other water pollutants such as pharmaceuticals, plyhyrdoxyalkanoates (PHAs), polypropylene copolymer (PPCPs). The enzyme functions without the need for unusual co-substrates, instead using easily available oxygen as an electron acceptor. Furthermore, the radicals produced during laccase oxidation bypass the phases involved in the formation of carcinogenic amines[39]. Degradation of pharmaceutically active compounds such as Tetracycline[14], Sulfamethoxazole[40], Chlortetracycline[41] from the environment using laccase has been reported. Laccase regularly outperforms medicines in decomposing pesticides and other organic compounds, regardless of immobilization support or reaction time. This increased efficiency can be ascribed to the phenolic properties of these molecules, which provide ideal substrates for the enzyme's catalytic activities[37].

Pulp and paper industry

Laccase is employed in several aspects, such as breaking down lignin, assisting in deinking processes, addressing pitch-related problems, detoxifying wastewater, enabling bio-pulping, and enhancing paper quality through fiber grafting. These applications encompass enhancements in the physical, chemical, and mechanical properties of paper products[42]. To attain high-quality paper, it's crucial to remove the inherent lignin responsible for its dark color. Traditionally, this has been achieved through bleaching processes involving chlorine-based chemicals. Laccase offers an eco-friendly alternative to the traditional and environmentally harmful chlorine and chlorine-based bleaching techniques in the biobleaching of pulp. Unlike other enzymes such as xylanases and mannanases, laccase does not have a detrimental impact on the final pulp yield through its reduction process[21]. Laccase has also been used to remove lignin from biomass such as kraft pulp[43], olive pomace[44] to aid in recycling and increase the efficiency of cellulose extraction and hydrolysis.

Bioremediation of pollutants

Laccase catalyzes the oxidation of a diverse range of compounds, including phenolic and diphenolic compounds, as well as a wide variety of aromatic substances, such as benzenthiols and Polycyclic Aromatic Hydrocarbons (PAHs)[45]. Organopollutants, including chlorinated phenols, polychlorinated biphenyls, and similar compounds,

have been employed as models for xenobiotic degradation. Because of their widespread use in industries such as plastics, paper, and agriculture, xenobiotic chemicals are extremely hazardous, mutagenic, and carcinogenic pollutants that are routinely found in the environment. Therefore, it is imperative to remove these chemicals from the environment[19].

Biosensors and biofuel cells

Laccase is now being studied for its potential applications in the creation of biosensors and biofuel cells[47]. Laccase's ability to detect numerous phenolic chemicals on a wide range of substrates makes it ideal for applications in biosensor technology. The applications of laccase go beyond biosensors to include fuel cell production. One of the main reasons for laccase's interest in this application is its capacity to use oxygen as a substrate, which is transformed into water during the process[48]. Laccase-based biosensors have been developed for a variety of applications in addition to medical applications, extend beyond biosensors to include the formation of fuel cells. In addition to medical applications, laccase-based biosensors have been developed for various uses. For example, a zinc-laccase biofuel cell has been created and operated under open ambient conditions[46].There are some limitation of using laccase in biofuels cells, such as enzyme immobilization, cost and scalability, mediator related problems etc[11].

Dye degradtion and decolorization

Laccases find extensive application in various segments of the textile industry. However, bacterial laccases are particularly utilized for their dye degradation capabilities. Dyes are pigmented substances that adhere to fibers, imparting permanent color and resistance to fading when exposed to factors such as sweat, light, water, and chemicals. The worldwide yearly release of textile dyes into the environment totals around 50,000 tons. These dyes are inherently toxic and pose a threat to aquatic and other forms of wildlife. Research has highlighted the involvement of laccase from *Brevibacillus sp*. in the degradation of dyes such as reactive black 5, fuchsine, allure red, and acid red 37 in wastewater, offering a potential solution to address this environmental concern[47]. Similarly, laccase from *Bacillus vallismortis* can degrade malachite green completely in 48 h[49]. There are reports indicating the impressive dye degradation capabilities of a two-domain laccase sourced from *Streptomyces griseorubens*. This enzyme has the remarkable ability to decolorize up to 90% of indigo dye in a mere 20 minutes, showcasing its rapid and efficient dye degradation potential[50]. Both fungal and bacterial laccases have previously been extensively studied for their capacity to breakdown various azo dyes. Anaerobic, facultative aerobic, and aerobic bacteria can decolorize azo dyes. Laccases are able to use a diverse spectrum of phenolic and non-phenolic chemicals due to their low substrate specificity. Laccases' versatility enables them to effectively act on a wide range of xenobiotic chemicals, including those prevalent in industrial effluents and wastewater dyes[5]. A variety of variables impact dye degradation, including the chemical composition of the dye, ambient conditions (pH, temperature), the presence of catalysts or enzymes, dye concentration, and the specific treatment procedure used. Optimising these parameters is critical for increasing degrading efficiency, lowering environmental impact, and making treatment methods economically feasible[13].

CONCLUSION

Ligninolytic enzymes play a pivotal role in the conversion of persistent compounds, with laccases, in particular, demonstrating significant potential for cost-effective treatment of wastewater containing phenolic compounds, PHAs, chemical pesticides, synthetic dyes, and emerging pollutants. In specific cases, the enzymatic oxidation of phenolic compounds can generate by-products that transform blue laccases into yellow laccases (YL). Importantly, YLs exhibit the ability to degrade pollutants without the need for any mediator, distinguishing them from blue laccases, as emphasized by some researchers. Consequently, there is an urgent call for heightened research attention in this field. Laccases display notable adaptability and find applications in detoxifying wastewater, transforming textile dyes, enhancing food technology, contributing to personal and medical care, as well as being utilized in biosensors and analytical applications. The increasing biotechnological importance of bacterial laccases has led to a growing demand for them in recent years.

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Conflict of Interest

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Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author Contribution

Kamaljit Panchal : All data analysis and writing original draft preparation, Ritika Gera : Discussion and editing, Ramika Garg : Disscussion and editing, Rajesh Kumar : supervision and reviewand editing.

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