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Review Article

Study of Laccase Producing Microorganisms and Their Applications

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ABSTRACT

A vast variety of bacteria, fungi, higher plants, and insects have laccases. Vegetables such as cabbage, turnips, potatoes, pears, and apples are examples of plants that contain laccases. It can be found in Ascomycetes, Deuteromycetes, and Basidiomycetes, and it is very common in white-rot fungi that degrade lignin. Hair coloring with natural phenols such gallic acid, catechol, syringaldehyde, etc. uses laccase enzyme. The dyes used in laccase-catalyzed dyes well penetrated the hair. The surface morphology of the colored hair was unaffected by the coloring process as well. The colored hair also displayed a variety of colors that met market demands and shown strong resistance to fading after shampooing and pH changes. In the production of lignocellulose-based composite materials, such as fiberboard, laccases can be employed to aid in the enzymatic attachment of fibers. Moreover, make improvements to the fiber products chemical or physical characteristics. Laccases are also used as cleaners in some water purification systems, as catalysts in the creation of anti-cancer drugs, and even as components in cosmetics.

INTRODUCTION

Since they are prevalent in nature, laccase is one of the first enzymes that scientists have studied [1]. Laccases are 1,4-benzenediol: oxygen oxidoreductases (EC 1.10.3.2) that contain copper and are present in higher plants and microorganisms [2]. They are part of a small class of enzymes known as blue copper proteins or blue copper oxidases that also includes, among others, plant ascorbate oxidase and the mammalian plasma protein ceruloplasmin [3]. The enzyme is a type of copper-containing poly-phenol oxidase that was discovered in the exudates of the Japanese lacquer tree Rhusvernicifera in 1883 [4] and successively was signified as a fungal enzyme [5, 6]. The most prevalent organic materials on earth, lignocelluloses, are becoming more valuable biomass resources because they can be easily transformed into a variety of energy-containing products and can be utilized as an alternative to fossil fuel resources [7]. Its potential to the four-electron reduction of oxygen to water is often combined with the one-electron oxidation of a

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wide variety of phenolic and non-phenolic substrates via laccase-driven catalysis [8]. Due to the fact that laccases (EC 1.10.3.2, benzenediol oxygen oxydoreductase) are multinuclear copper-containing enzymes, they are also known as "BLUE ENZYMES" [9]. The laccase enzyme (EC 1.10.3.2) is used in a variety of industrial fields, such as the food industry, to improve the organoleptic qualities of beverages, and to handle waste materials [10]. An enzyme called laccase catalyses the oxidation of phenolic substances while simultaneously reducing oxygen to water [11]. As a "green catalyst," this enzyme qualifies as having a significant industrial potential because it just needs "air" to function, releases water and the oxidised product, and uses oxygen as a co-substrate [12]. In general, bacteria are more tolerant of a wider range of habitats and develop more quickly than fungi [13]. Furthermore, some bacterial laccases can be much more robust and highly active at high temperatures, high pH, and high chloride concentrations in comparison to fungal laccases [14]. Both the *Bacillus atrophaeus* and *Bacillus pumilu* strains produced laccase enzymes that could break down or alter lignin and help liberate fermentable sugars from lignocellulose [15]. The manufacturing medium from Pseudomonas aeruginosa performed best under the following conditions: 72 hours of incubation, 40 °C temperature, pH-7, 2% glucose as the carbon source, and 2% peptone as the nitrogen source [16]. *Azospirillum lipoferum*, *Streptomyces lavendulae*, *Streptomyces cyaneus*, and *Bacillus subtilis* have all produced certain bacterial laccases that have been described. At high temperatures, high pH levels, high chloride concentrations, and other conditions, some bacterial laccases can be very active and much more stable [17].

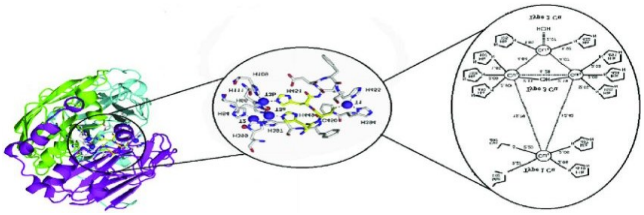

Fig. lignolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment – Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Molecular-structure-and-active-site-of-Laccase_fig1_339369606 [accessed 12 May, 2023]

Distribution in the Natural World

Currently, certain bacterial laccases from *Azospirilum lipoferum*[18], *Bacillus subtilis*[19], *Streptomyces lavendulae*[20], *Streptomyces* cyaneus[21], and *Marinomonas mediterranea*[22] have also been characterised. Other plant sources of laccase include: *Rhus succedanea*[23-a], *Acer pseudoplatanus*[23-b], *Pinus taeda*[23-c], *Populus euramericana*[23-d], *Liriodendron tulipifera*[23-e], *Nicotiana tobacum*[23-f], *Lolium perenne*[23-g], and *Zea mays* [23-h]. Laccases have diverse roles in fungi, including lignin degradation, morphogenesis, fungal-plant-pathogen/host interactions, and stress defense [24,25]. There are also some reports on laccase activity in bacteria [26]. Recently, proteins with characteristics typical of laccases have been identified in insects [27].

Table: Application of several intriguing fungi laccases that break down various substances

Laccases source	Application	Reference
Trametes trogii in *Pichia pastoris*	Decolorization dyes (amaranth, carmoisine, cochineal red, sunset yellow, blue indigo and alizarin red S	[28]
Aspergilus expressing a laccase from *Myceliophth*	Decolorization of synthetic dyes	[29, 30, 31]

ora thermophila Trametes trogii BAFC 463 *Trametes trogii* BAFC 463 in *Pichia pastoris*		
Trametes versicolor	Biodegradation of triphenylmethane dyes	[32]
Recombinant laccase (Lcc IIIb) from *Trametes versicolor* expressed in *Yarrowia lipolytica*	Decolorization of pollutant dyes: bromocresol purple, safranin, malachite green, bromothymol blue, nigrosine and phenol red	[33]
P. pastoris or A. thaliana expressing Lcc9 from *Laccaria bicolor*	Decolorization of triphenylmethane dyes, employed in industrial dyeing processes	[34]
Oudemansiella canarii	Decolorization of congo red	[35]
Ganoderma lucidum E47 strain	Decolorizing xant,hene, azo and triarylmethane dyes	[36]
Pleurotus ostreatus URM 4809	Decolorization dyes used in the textile industry	[37]
Neosartorya fischeri	Asphaltene oxidation and mineralization (refractory petroleum fraction)	[38]
Anthracophyllum discolor	Degradation of polycyclic aromatic hydrocarbons (PAH)	[39]
Nicotiana tabacum expressing a laccase from *Pleurotus ostreatus*	Phytoremediation of phenol content from olive mill wastewaters	[40]
Trametes versicolor BAFC 2234	In vitro oxidation of phenol	[41,42]
Recombinant laccase from *Trametes sanguineus* in *Trichoderma atroviride*	Degradation of xenobiotic compounds (phenanthrene and benzo[α]pyrene)	
Trametes villosa	Bisphenol A (BPA) degradation	[43]
Coriolopsis rigid LPSC 232	Detoxification of water soluble fraction from ‘‘alpeorujo” (WSFA)	[44]
Pycnoporus sanguineus CCT-4518	Laccase removal of 17-alpha-ethynilestradiol (EE2)	[45]
Pycnoporus sanguineus (CS43)	Degradation of emerging endocrine disruptor (bisphenol A)	[46]
Trametes hirsuta	Degradation of chloramphenicol (CAP)	[47]
Trametes versicolor	Degradation of PhAC: diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole Chlortetracycline (CTC) degradation	[48, 49]
Pycnoporus sanguineus	Degradation of estrogens tested	[50]
Pleurotus ostreatus	Degradation of ciprofloxacin (CIP)	[51]
Pycnoporus sanguineus CS43f	Degradation of endocrine disrupting chemicals (EDCs): nonylphenol and triclosan (a biocide)	[52]

APPLICATIONS OF THE LACCASE ENZYME IN DIFFERENT INDUSTRY

Paper & Pulp Industry

Replacement of traditional, harmful, chlorine-based delignification/bleaching techniques is urged by environmental concerns. Using ligninolytic enzymes to pre-treat wood pulp may result in delignification methods that are more gentle, cleaner, and considerate of cellulose integrity [53]. Laccases have been proposed as a way to activate the fiberbound lignin during composites manufacturing, producing boards with good mechanical properties without the use of hazardous synthetic adhesives [54]. According to preliminary findings, laccases can graft several phenolic acid derivatives onto the fibres of kraft pulp [55,56].

Cosmetics

Application of laccase in the beauty industry has not gone unnoticed; for instance, laccase-based hair colours are less irritating and easier to use than current hair dyes because laccases take the role of H2O2 as an oxidising ingredient in the dye formulation [57,58,59]. Nowadays, Proteins found in cosmetic and medical products for skin lightening [60].

Food Industry

The capacity of laccases to cross-link biopolymers is currently of interest in baking. Thus, the laccase from the white-rot fungus *Trametes hirsuta* increased the maximum resistance of dough and decreased its extensibility in both flour and gluten dough [61]. Applications of laccase include bioremediation, beverage processing, ascorbic acid measurement, sugar beet pectin gelation, baking, and as a biosensor in the food sector. However, they recommended greater research into laccase production and low-cost immobilisation strategies to enhance the industrial application of this enzyme [62].

Synthetic chemistry

They have been suggested to have applications in the synthesis of complex polymers, pharmaceuticals [63,64,65,66,67], and oxidative deprotection [68]. Using Suberase® (Novo Nordisk A/S, Bagsvaerdt, Denmark), an industrial laccase, they recently created phenolic colourants [69].

Soil bioremediation

In order to detoxify the munitions residue, laccases were able to mediate the coupling of reduced 2,4,6-trinitrotoluene (TNT) metabolites to an organic soil matrix [70]. It was also discovered that laccases may breakdown PAHs, which are produced by natural oil deposits and the use of fossil fuels [71]. Additionally, *Trametes modesta* laccase contributed to the immobilisation of TNT breakdown products [72].

Nanobiotechnology

Through controlled deposition and targeted adsorption of biomolecules on various surfaces, reaching micro and nanoscale order, nanotechnology aids in the development of smaller and more effective biosensors [73]. Analytical applications have included advances in bioelectrochemistry, such as biosensors that serve as detectors in environmental and clinical analysis [74]**.** Additionally, plant flavonoids [75], electroimmunoassay [76], and biosensors for the detection of morphine and codeine [77], catecholamines [78,79,80] have also been created. The creation of extracellular matrix islands that are only a few micrometres across, the arrangement of which might dictate where the endothelium and bovine cells were located [81]. Investigations into the non-specific protein adsorption have been successful when controlling the nature and density of the groups (such as alkys, amides, and alcohols) on surfaces constructed with assembled monolayers [82]. Laccase from *Trametes versicolor* cross-linked enzyme crystals (CLEC) have significant advantages over the soluble enzyme for usage in biosensor applications [83].

Coriolus versicolor laccase immobilised on self-assembled monolayers of N-hydroxysuccinimide on gold. *T. versicolor* on glassy carbon electrodes was used to achieve nanomolar detection limits for the catecholamin neurotransmitters dopamine, adrenaline, and norepinephrine using ultrasensitive amperometric detection [84]. The layer-by-layer method enables the management of macromolecular structures down to the nanoscale level, producing surfaces with well-defined thicknesses [85]. After removing the core, the LbL approach has also been utilised to create hollow polyelectrolyte capsules [86]. Additionally, laccase can be immobilised on the cathode of biofuel cells, which could supply power for small transmitter systems, for example [87,88]. Recently, it has been feasible to recrystallize bacterial proteins using flat polyelectrolyte multilayers created by the alternate adsorption of oppositely charged polyelectrolytes, enabling the construction of artificial cell walls [89]. The relationship between salt content and pH in hollow polyelectrolyte multilayer capsule permeability properties [90]. Additionally, the rubella virus has been employed to host and activate colloidal particles covered with polyelectrolytes and phospholipids [91].

Textile Industry

Since laccase is utilised not only to decolorize textile effluents as previously mentioned, but also to bleach fabrics and even synthesise colours, its application in the textile industry is expanding quickly [92]. Government regulations governing the removal of dyes from industrial effluents are getting stricter and stricter, especially in the more industrialised countries [93]. Many dyes are manufactured from substances that are known to cause cancer, like benzidine and other aromatic compounds, raising concerns [94]. The majority of currently used methods for treating wastewater containing dyes are inefficient and expensive [95,96]. Because laccase-based procedures have the potential to degrade colours with a variety of chemical structures [97,98,99], including synthetic dyes currently used in industry, they appear to be an appealing solution [100]. Two-thirds of the market for dyestuffs goes to the textile industry, which also uses a significant amount of water and chemicals for the wet processing of textiles [101]. The chemical makeup of the chemical reagents utilised is quite varied, ranging from inorganic chemicals to polymers and organic products [102,103,104]. With more than 7*10^5 t of dyestuff produced annually, there are more than 100,000 commercially accessible dyes [105,106]. Dyes are tough to decolorize because of their synthetic origins, which makes them resistant to fading when exposed to light, water, and various chemicals [107,108].

CONCLUSION & FUTURE PERSPECTIVE

Reversing the human-caused contamination of the world's water resources is today urgently necessary. Laccases have the capacity to oxidise these molecules and produce less damaging and toxic inactive chemicals, making them appear to be an efficient biocatalytic instrument. Engineering laccases using contemporary methods like in vitro evolution and site-directed mutagenesis, strengthened by theoretical tools like molecular modelling and dynamic simulations, among others, can overcome the complex composition of contaminated water (high pH levels or salt concentrations). For a variety of substrates, laccase or laccase-mediator systems do offer an alternative to conventional chemical oxidants that are also more environmentally friendly. One area that needs more research in the future is testing the laccase enzyme using different hybrid methods. (2) Creation of ideal laccase application circumstances, such as pH, temperature, matrix composition, and immobilised cell size (3) Research on numerous by-products made possible by the use of laccase in diverse sectors.

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