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Occurrence and Applications of Fungal Laccases: A Comprehensive Biotechnological Review

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ABSTRACT: Laccases are enzymes that are typically excreted into the surrounding medium by filamentous fungi as part of their secondary metabolism. The production of laccases can be affected by various factors such as cultivation method, carbon availability, nitrogen source, and the concentration of microelements. The following sections of the paper will detail the impact that these different process parameters can have on laccase production. Fungal laccases are enzymes that belong to the oxidoreductase class of enzymes, which have a wide range of applications in various industries. Laccases are now being used more frequently in the food industry as a means of creating affordable and nutritious foods. However, to continue this trend, it is important to establish widespread access to laccase and improve production systems. The presence of laccase has been identified in multiple types of fungi, which suggests that it is possible for many fungi to produce laccase. To make laccases more accessible for industrial usage, cost-effective methods have been devised such as refining fermentation media, creating new fermentation techniques, and genetically modifying eukaryotic recombinant strains to produce laccases on a large scale. The low yield, heterogeneity, cost, and difficulties in extraction of fungal laccases are some of the hurdles in this area of research. This review paper provides a comprehensive overview of the sources, occurrence, and applications of fungal laccases. It highlights the potential of these enzymes for various industrial, medical, and environmental applications and provides insights into the future direction of laccase research.

Keywords: Enzymes, Sources, Laccases; Fungus, Applications.

INTRODUCTION

Laccases, which are enzymes found in plants, fungi, and bacteria, are a type of multicopper oxidase. They can oxidize various phenolic substrates and carry out one-electron oxidations, which can result in cross linking. For instance, laccases play a crucial role in the creation of lignin by promoting the oxidative coupling of monolignols, a group of natural phenols (Dwivedi *et al*., 2011). Some laccases, such as those produced by the fungus *Pleurotusostreatus*, help to break down lignin, and are therefore categorized as lignin-modifying enzymes. Certain laccases made by fungi can also aid in the biosynthesis of melanin pigments. Laccases are capable of catalyzing the ring cleavage of aromatic compounds (Pogni *et al*., 2015). Laccase was first investigated by Hikorokuro Yoshida in 1883, and subsequently studied by Gabriel Bertrand in 1894 in the sap of the Japanese lacquer tree, where it is involved in the formation of lacquer, hence the name "Laccase". Laccase is an enzyme that serves different functions in different organisms. In plants, it is involved in the process of lignification, while in fungi, it has been linked to delignification, pigment production, and sporulation. Fungal laccases are more common than plant laccases, and the first laccase-producing Ascomycetes member was *Monocilliumindicum*, which also exhibits peroxidase activity (Baldrian, 2006). Bacteria also utilize laccase for various functions such as copper homeostasis, pigment biosynthesis, morphogenesis, and protection of spores against hydrogen peroxide and ultraviolet light(Sharma *et al*., 2007). Insects also possess laccase, which is involved in the process of sclerotization. Fungal laccases have a higher redox potential than plant and bacterial laccases, and they can break down lignin and eliminate hazardous phenols(Dittmer *et al*., 2004). Fungal laccases also participate in morphogenesis by activating the synthesis of extracellular pigments or dihydroxy naphthalene melanin. The use of fungal laccases has grown significantly in biotechnological processes due to their stability and ability to function without hydrogen peroxide (Gochev and Krastanov 2007). Laccase has various applications in textile, food, paper and pulp industry, soil remediation, biodegradation of environmental pollutants, synthetic chemical, cosmetics, and the elimination of endocrine disruptors(Kudanga & Le Roes-Hill, 2014). The enzyme is utilized for delignification of pulp, breakdown of pesticides and insecticides, organic synthesis, waste detoxification, the transformation of textile dyes, food technology applications, and biosensor and analytical usage (Bagewadi *et al*., 2017). To effectively use laccases in food processing, it is necessary to produce large quantities of the enzyme

while keeping costs low. Various production strategies can be employed, including optimizing the media and process, to improve the economic feasibility of the process (Bagewadi *et al*., 2017; Frazao *et al*., 2014). In addition, overexpressing laccase in appropriate host organisms could help achieve high levels of the enzyme. Inducers can also be utilized to enhance the production capacity of laccase. Laccase has recently found effective use in nanobiotechnology because of its unique ability to catalyze electron transfer reactions without the need for additional cofactors (Mukhopadhyay *et al*., 2015). The first cDNA sequence and gene for laccase were identified from the fungi *Neurospora crassa* and *Aspergillus nidulans*. The number of identified laccase genes has grown significantly, and protein and gene sequence databases now contain hundreds of laccase gene sequences (Hoshida *et al*., 2001). However, many of these sequences are only partial fragments of putative laccase genes discovered through genome-wide sequencing projects and annotated based on their similarity to known laccases (Ryu *et al*., 2008). The review article discusses fungal laccases and their applications in various fields such as biotechnology, food processing, and environmental remediation. Fungal laccases are enzymes that can break down lignin and eliminate hazardous phenols. They also participate in morphogenesis and pigment production in fungi. Compared to plant and bacterial laccases, fungal laccases have a higher redox potential and do not require hydrogen peroxide in their catalytic activity. This makes them useful in biotechnological processes. Various strategies can be employed to produce large quantities of laccases at low costs. The use of laccase in delignification of pulp, breakdown of pesticides and insecticides, waste detoxification, and elimination of environmental pollutants has significant potential. Laccase also has applications in food processing, cosmetics, and the transformation of textile dyes.

Occurrence of laccase in fungal system. Microorganisms are present everywhere due to their diversity in adaptability (Cockell 2021; Wani *et al*., 2022). Numerous fungal species exhibit laccase activity, and this enzyme has been identified in various species. Most fungal species have an extracellular form of laccase, but certain taxonomic and physiological groups of fungi tend not to produce significant amounts of the enzyme (Thakker *et al*., 1992). Laccase is only produced by a small number of species, and lower fungi such as Zygomycetes and Chytridiomycetes never produce it (Faure *et al*., 1994). White-rot basidiomycetes are the most extensively researched and effective lignin degraders, and several enzymes are involved in the breakdown of lignin, including cellobiose-quinone oxidoreductase, lignin peroxidase, manganese-dependent peroxidase, laccase, glucose oxidase, and glyoxal oxidase (Elisashvili and Kachlishvili 2009). Laccase plays a role in lignification in plants, while in fungi, it has been linked to a range of cellular processes, including delignification, sporulation, pigment generation, fruiting body formation, and plant disease.

Although literature indicates that ligninolytic enzymes are mainly extracellular and intracellular laccase occurrence is only found in white rot fungi, Froehner and Eriksson discovered both intracellular and extracellular laccase in *Neurospora crassa*, and they suggested that there is no difference in the occurrence of any two laccases (Froehner and Eriksson 1974). Intracellular laccase serves as a precursor for extracellular laccase. Additionally, ascomycete species closely related to wood-degrading fungi have been shown to participate in the breakdown of dead plant material in salt marshes to oxidize syringaldazine and have laccase genes. Basidiomycete yeast such as C*ryptococcus neoformans* produces a genuine laccase that can oxidize phenols and aminophenol but not tyrosine (Viswanath *et al*., 2014). Despite not being able to produce laccase, the plasma membrane-bound multicopper oxidase Fet3p from *Saccharomyces cerevisiae* displays structural and sequence similarities with fungal laccase and exhibits spectroscopic characteristics similar to fungal compounds. Laccase and laccase-like enzymes share a similarity in the way their type-1 Cu sites are arranged, which enables them to oxidize Cu1. Many ECM species, such as *Amanita, Cortinarius, Hebeloma, Lactarius, Paxillus, Piloderma, Russula, Tylospora*, and *Xerocomus*, have gene fragments that closely resemble laccase found in fungi that cause wood decay(Kiiskinen and Saloheimo 2004). Using RT-PCR, the transcription of the presumed laccase sequence in *Pilodermabyssinum* was confirmed. However, the production of an enzyme is not always dictated by a specific gene sequence(Chen *et al*., 2003).

Structural diversity of fungal laccase. Fungi-derived laccases are commonly found as isoenzymes that combine to form multimeric complexes. The individual monomer has a molecular mass ranging between 50 and 100 kDa and an attached carbohydrate moiety that can make a significant contribution to its high stability. The laccases from fungi belong to the blue multicopper oxidase (BMCO) family and catalyze the oxidation of one electron and the reduction of four electrons of molecular oxygen to water(Solomon *et al*., 1996). The enzyme includes different copper centers that ensure its catalytic activity. BMCOs are characterized by at least one type-1 (T1) copper ion and three additional copper ions grouped in a trinuclear cluster comprising one type-2 (T2) and two type-3 (T3) copper ions. Spectroscopic characteristics distinguish between the various copper centers. The T1 copper ion has significant absorption at approximately 600 nm, while T2 copper has very little visible absorption. The T2 site is EPR-active, and the T3 site is EPR-silent due to antiferromagnetic coupling mediated by a bridging ligand (Wu *et al*., 2010). The T1 copper oxidizes substrates and extracts electrons, which are then transferred to the T2/T3 site, where molecular oxygen is reduced to water, probably passing through a highly conserved His-Cys-His tripeptide motif. Some researchers are hesitant to classify certain enzymes as true laccases because they lack the T1 copper. Currently, the study of purified protein is based on the structure and physiochemical properties of fungal

laccase proteins, with over 100 laccases purified and characterized from fungi to varying degrees (Piontek *et al*., 2002).

Cloning and overexpression of fungal laccase. Fungal laccases are enzymes that are widely used in industrial processes such as bioremediation, pulp and paper production, and dye decolorization (Bollag and Leonowicz 1984; Safari Sinegani *et al*., 2007). Cloning and overexpression of fungal laccases can provide a large amount of the enzyme for these applications. Fungal laccases are attracting attention for their ability to oxidize aromatic chemicals, both phenolic and non-phenolic, which makes them useful for various industrial applications such as food, pulping, textiles, wastewater treatment, and bioremediation (Wu *et al*., 2010). However, in order to make laccase available for industrial use, cost-cutting measures such as optimizing fermentation media, using novel fermentation methods, and genetic modification for large-scale production using eukaryotic recombinant strains are employed. Fermentation media optimization is easily determined, but industrial scale growth can increase costs due to the use of inducer and cofactor compounds. Genetic modification can provide a promising method of laccase overexpression for specific uses, but novel fermentation methods may cause unfavorable conditions and cost (Kudanga and Le Roes-Hill 2014).

Only a few bacterial laccases have been studied extensively, revealing that they have industrial advantages over fungal laccases. Bacterial laccases are more active and stable at higher temperatures and have a higher pH value than fungal laccases. Research has shown that a previously identified fungal strain from the genus *Gandoderma*, WR-1, can produce maximum laccase under media conditions with multiple micronutrients. This white-rot fungus strain was isolated using a tissue culture technique, and it was discovered that tree bark produced a large amount of laccase during fermentation(Abyanova *et al*., 2010).To increase laccase production, genes from the basidiomycete *Tramete hirsuta* were successfully transferred to a heterologous expression into the ascomycete *Penicillium canescens*. Penicillium was chosen for its ability to secrete enzymes into culture media and the synthesized enzymes were found to be safe for human consumption. After successful transformation, 98% of the target enzyme activity was detectable in the liquid culture medium, and the recombinant enzyme's molecular weight matched that of *T. hirsuta's* native laccase (Abyanova *et al*., 2010).

Production of fungal laccase. Laccase activity was discovered in various types of fungi, ranging from Ascomycetes to Basidiomycetes, including wood and litter-decomposing fungi and ectomycorrhizal fungi. Laccases are glycoproteins that are extracellular and can be easily extracted from fungal biomass. The main limitation of fungal laccases is their low production rates, which applies to both wild-type and recombinant fungal strains(Couto & Toca-Herrera, 2007). The addition of aromatic compounds such as 2,5-xylidine and ferulic acid can increase laccase concentrations. Laccase concentrations were also found to be high in old non-induced cultures. Microorganisms are regulated by metabolic mechanisms, and certain compounds such as xenobiotics, heavy metals, or heat shock treatments can induce response element sites in the promoter regions of laccase genes (Micallef *et al*., 2015). White rot fungi produce nonspecific extracellular enzymes such as LiP, MnP, and laccase, which can break down lignin, cellulose, and hemicellulose. These fungi are known for their ability to efficiently degrade whole wood in a short fermentation time. Screening fungal strains suitable for laccase production in solid-state fermentation (SSF) is becoming increasingly important in research. Filamentous fungi have been shown to produce enzymes more efficiently in SSF, which is a fed-batch culture with fast oxygenation but slow sugar supply, compared to submerged fermentation (SmF). In a study comparing the productivity of three fungal enzymes, invertase, pectinase, and tannase, SSF was found to have higher titers due to its cultivation method (Arregui *et al*., 2019).

Isolation of laccases by metagenomics. Laccases are copper-containing enzymes that have garnered significant attention in recent years due to their wide range of applications in various fields, including bioremediation, pulp and paper production, and food processing (Akhtar *et al*., 2021; Mir *et al*., 2022; Suresh *et al*., 2008). Traditionally, laccases have been isolated from specific organisms, such as fungi and bacteria, through traditional cultivation-based methods. However, these methods have limitations in identifying the full diversity of laccases in the environment (More *et al*., 2011; Wani *et al*., 2022). Metagenomics is a powerful tool that allows for the isolation and study of genetic material from environmental samples without the need for individual organism cultivation. Metagenomics involves the sequencing and analysis of DNA extracted from environmental samples, providing access to the genetic material of entire microbial communities. The approach can identify the presence and diversity of laccase genes, as well as their function and expression levels (Handelsman 2004; Wani *et al*., 2022; Wani *et al*., 2022). By using metagenomics to analyze environmental samples, researchers have discovered novel laccase genes from various sources, including soil, sediment, and wastewater (Azli *et al*., 2022; Wani *et al*., 2021; Wani *et al*., 2022).

Isolating laccases through traditional methods is challenging, as the enzyme is often present at low concentrations and can be difficult to purify. Moreover, laccase genes may not be expressed under laboratory conditions or may be expressed at low levels, making their isolation even more difficult. Metagenomics can circumvent these issues by identifying the full complement of laccase genes in a sample, regardless of their expression level.

Factors affecting production of laccases, Laccase is produced by several microorganisms, including fungi, bacteria, and plants. The production of laccase is affected by various factors, including environmental factors, nutritional factors, and genetic factors.

Environmental factors. The production of laccase is significantly influenced by environmental factors, such

as pH, temperature, and oxygen. The optimal pH range for laccase production varies depending on the microorganism producing the enzyme. Fungal laccases typically have an optimal pH range between 4.0 and 6.0, while bacterial laccases have a higher optimal pH range of 7.0 to 9.0. Temperature is another critical factor affecting laccase production, and different microorganisms have different optimal temperature ranges (Patel *et al*., 2014). For example, fungal laccases have optimal temperature ranges between 25°C and 40°C, while bacterial laccases have a broader optimal temperature range of 20°C to 60°C. Oxygen is also essential for laccase production, and aeration is necessary to maintain the oxygen levels required for laccase production (Singh *et al*., 2015).

Nutritional factors. The production of laccase is influenced by various nutritional factors, including carbon and nitrogen sources. Different carbon sources, such as glucose, sucrose, and lactose, affect laccase production differently, depending on the microorganism producing the enzyme. Nitrogen sources, such as ammonium sulfate, peptone, and yeast extract, also affect laccase production. The optimal carbon and nitrogen sources for laccase production vary depending on the microorganism (Durán-Sequeda *et al*., 2021).

Genetic factors. The production of laccase is also influenced by genetic factors. The genetic makeup of the microorganism producing laccase affects the quantity and quality of laccase produced. Several genes are involved in laccase production, and the expression of these genes is regulated by various factors, including environmental factors and nutritional factors (Lu *et al*., 2013).

Applications of laccases. Laccases are multicopper oxidases that have received increasing attention in recent years due to their broad range of applications in various fields. Laccases can oxidize a wide variety of organic compounds, including phenols, polyphenols, aromatic amines, and lignin, using molecular oxygen as a co-substrate. The broad substrate specificity of laccases has made them attractive for applications in many different industries, including bioremediation, pulp and paper, textile, food, and pharmaceuticals (Fig. 1).

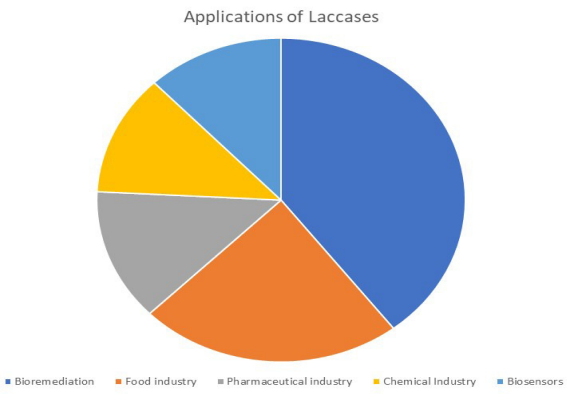

Fig. 1. Explored applications of laccases in different fields.

Bioremediation. Laccases have been widely used in bioremediation of various environmental pollutants. They can degrade a range of pollutants, such as polycyclic aromatic hydrocarbons (PAHs), pesticides, and dyes. Laccases can also detoxify toxic compounds, such as chlorinated phenols, and convert them into less toxic compounds (Singh *et al.* 2015; Viswanath *et al*., 2014; Wani *et al*., 2022, 2022).

Pulp and Paper. Laccases have been widely used in the pulp and paper industry to improve the quality of pulp and paper products. Laccases can improve the delignification process of wood pulp, which is necessary to produce high-quality paper products. Laccases can also improve the brightness and strength of paper products, which results in a better product (Adarme *et al*., 2019; Singh *et al*., 2015).

Textile. Laccases have been used in the textile industry for various applications. They can be used to bleach and dye fabrics, remove excess dye from wastewater, and improve the color fastness of textile products. Laccases have also been used to degrade textile dyes, which can reduce the amount of dye released into the environment (Aamr and Cuiling 2012).

Challenges and future perspectives. Fungal laccases are copper-containing enzymes that are widely distributed in the fungal kingdom. They are known to have a wide range of applications in biotechnology, including bioremediation, paper pulp bleaching, textile dye decolorization, and biosensor development. However, the extraction and application of fungal laccases face several challenges. The yield of laccases obtained from fungal cultures is often low, which makes the process of extraction and purification expensive and time-consuming. Fungal laccases are a diverse group of enzymes that differ in their molecular weight, pH, and temperature optima, and substrate specificity. This heterogeneity makes it difficult to develop universal protocols for their extraction and purification(Akhtar *et al*., 2022; Arregui *et al*., 2019; Forough *et al*., 2014; Sadeghi *et al*., 2014; Stasi *et al*., 2023). Fungal laccases are often unstable and lose activity under certain conditions, such as high temperatures, extremes of pH, and exposure to organic solvents. This limits their use in certain applications. Fungal laccases have a narrow substrate specificity, which limits their use in certain applications. However, this can be overcome by genetic engineering to improve their substrate specificity. The cost of producing fungal laccases at an industrial scale is often high due to the high cost of production media and the low yields obtained. The use of fungal laccases in industrial applications is subject to regulation, which may limit their use in certain countries or industries. Overall, the challenges in extraction and application of fungal laccases can be overcome through research and development aimed at improving yields, stability, and substrate specificity, and reducing the cost of production(Mohammadi *et al*., 2019; Thakkar, 2014; Zahra *et al*., 2017).

The future scope of laccases is vast and promising, with potential applications in diverse fields ranging from biotechnology and bioremediation to the food industry

and nanotechnology. With continued research and development, laccases could become an important tool in the development of sustainable technologies that benefit both human society and the environment. The laccases have potential applications in diverse fields ranging from biotechnology and bioremediation to the food industry and nanotechnology (Bhat *et al*., 2021; Bilal Ahmad *et al*., 2020). Genome editing of laccases could potentially enhance their activity, specificity, stability, or other properties, leading to improvements in their performance and wider application. Another approach to genome editing of laccases is through directed evolution, which involves random mutagenesis of the laccase gene followed by screening for improved enzyme activity (Mir *et al*., 2022; Rahayu *et al*., 2022; Wani *et al*., 2022). This approach has been successful in improving the activity and stability of laccases, and it can be combined with computational modeling to guide the design of mutations and optimize enzyme performance. Artificial intelligence (AI) can be used to enhance the performance and efficiency of laccases by predicting enzyme properties, designing experiments, and optimizing conditions for enzyme activity. AI can also be used to identify new laccase enzymes with novel functions and applications. In addition, AI can be used to identify new laccase enzymes with novel functions and applications. By analyzing large datasets of enzyme sequences, AI can identify new laccase enzymes with unique structural features or activity profiles, opening up new possibilities for enzyme engineering and biotechnological applications (Althobaiti *et al*., 2022; Bao and Xie 2022; Raza *et al*., 2022; Raza *et al*., 2022).

CONCLUSIONS

The review paper provides an in-depth analysis of fungal laccases and their biotechnological applications. The paper discusses the various sources of fungal laccases, their properties, and their potential applications in various industries such as bioremediation, pulp and paper, textile, food, and pharmaceuticals. The paper highlights the importance of fungal laccases in bioremediation, where they can degrade and detoxify various pollutants such as PAHs, pesticides, and dyes. Fungal laccases also have applications in the pulp and paper industry, where they can improve the quality of paper products by improving the delignification process, brightness, and strength. Moreover, fungal laccases can be used in the textile industry for bleaching and dyeing fabrics, and for removing excess dye from wastewater. They also have potential applications in the food industry to improve the shelf life of food products and enhance the aroma and flavor of wine and beer products. Finally, the paper discusses the potential applications of fungal laccases in the pharmaceutical industry, where they can be used for the synthesis of pharmaceutical compounds and the detection of pharmaceuticals in environmental samples.

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