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Fungal Laccases and Heavy Metal Polluted Soils Applications in Bioremediation

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Abstract

The monoelectronic oxidation of a wide range of substrates, such as ortho- and para-diphenols, polyphenols, aminophenols, and aromatic or aliphatic amines, is catalysed by laccases, blue multicopper oxidases, along with a complete, four-electron reduction of O2 to H2O. As a result, they can break down lignin and are widely distributed in various white-rot fungi. In the process of treating wastewater, laccases decolorize and detoxify industrial effluents. They can be utilised successfully in the paper and pulp industries, textile industries, xenobiotic degradation, bioremediation, and operate as biosensors. They act on both phenolic and nonphenolic lignin-related chemicals as well as very recalcitrant environmental contaminants. The scientific field of nanobiotechnology, which is expanding, has recently benefited from the use of laccase, which can catalyse electron transfer reactions without the need for additional cofactors. In order to immobilise biomolecules while preserving their enzymatic function, a number of approaches have been devised, including layer-by-layer, self-assembled monolayer, and micropatterning. In this paper, we discuss laccases' fungus of origin and how they can be used to safeguard the environment. Due to an increase in geologic and anthropogenic activities, heavy metal-polluted soils are now widespread throughout the world. These soils result in decreased plant performance, yield, and growth. Treatment of heavy metal-polluted soils via bioremediation is effective. It is a well known technique that is largely performed in situ, making it appropriate for the establishment or reestablishment of crops on treated soils. Different processes are used by microorganisms and plants for the bioremediation of contaminated soils. In the bioremediation of heavy metal-contaminated soils, using plants to treat polluted soils is a more popular method. A method of bioremediation that guarantees a more effective cleanup of soils contaminated with heavy metals combines both microorganisms and plants.

Keywords: Monoelectronic; Biotechnology; Heavy metal; Laccases

Introduction

Due in great part to the production of unique enzymes that can carry out challenging chemical reactions, fungi are able to thrive under unfavourable environmental conditions. Because laccases may be used to bioremediate phenolic chemicals and detoxify contaminants, interest in them has grown recently. Among other materials, these fungal enzymes may transform wood, plastic, paint, and jet fuel into nutrition [1]. In the manufacture of fine chemicals and the processing of pulp and paper, some of these enzymes have already been put to good use. Recent research has shown that lignin-degrading or "white-rot" fungus, such Phanerochaete chrysosporium and Trametes versicolor, could replace some of the chemical processes involved in the production of paper. Due to their great selectivity, high efficiency, and environmentally safe reactions, enzymes are being used more and more for the treatment and removal of industrial and environmental contaminants. Extracellular fungal peroxidases, including lignin peroxidase, manganese peroxidase, and fungal laccases, are two of the main families of enzymes that have been tested for the removal of hazardous phenolic compounds from industrial effluent and the breakdown of resistant xenobiotics. New fungal strains, altered growth conditions, the use of inducers, and the use of less expensive growth substrates including food and agricultural wastes have all been discussed in recent papers on how to enhance the production of these enzymes. There are a few enzymes that have been researched since the eighteenth century. Yoshida initially identified laccase in 1883 after removing it from the exudates of the Rhus vernicifera, a Japanese lacquer tree. The first time that laccase was shown to be a fungi enzyme was in 1896 by Bertrand and Laborde [2]. Due to their potential participation in the transformation of a wide range of phenolic compounds, including polymeric lignin and humic chemicals, laccases of fungi are attracting a lot of attention. The majority of lignolytic fungus species produce at least one laccase isoenzyme as a constitutive product, and laccases predominate among lignolytic enzymes in soil environments [3]. Additionally, laccase-mediated delignification enhances the nutritional value of agricultural byproducts for use as soil fertiliser or animal feed. They are appropriate for biotechnological applications such as the transformation or immobilisation of xenobiotic substances since their catalytic activity simply needs molecular oxygen. Many biotechnological applications, including the bioremediation of some toxic chemical wastes (such as chlorinated aromatic compounds, polycyclic aromatic hydrocarbons, nitroaromatics, and pesticides) and the development of biosensors, have evaluated the major role of laccases in the degradation of lignin and phenolic compounds [4]. Despite the fact that heavy metals are naturally present in soil, anthropogenic and geological processes have increased their concentration to levels that are detrimental to both plants and animals. Among these are the mining and smelting of metals, the burning of fossil fuels, the use of fertilisers and pesticides in agriculture, the manufacturing of batteries and other metal products in industries, the disposal of municipal garbage, and the use of fertilisers and pesticides in agriculture. It has been observed that plants growing in soils contaminated with heavy metals experience a loss in growth as a result of alterations in physiological and biochemical processes [5]. Plant growth continues to fall, which lowers production and eventually causes food insecurity. Soil cleanup for heavy metal contamination

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cannot be overstated. There are numerous techniques for cleaning up metal-polluted soils, ranging from physical and chemical techniques to biological techniques. The majority of physical and chemical techniques (such as soil washing and flushing, encapsulation, solidification, stabilisation, electrokinetics, vitrification, and vapour extraction) are expensive and do not make the soil appropriate for plant growth. On the other hand, the biological technique (bioremediation) promotes the establishment of plants on contaminated soils [6]. Because it is accomplished by natural processes, it is an environmentally friendly strategy. When compared to other cleanup procedures, bioremediation is also a more affordable option. The nature and characteristics of soils contaminated with heavy metals are covered in this essay. On these soils, plant growth and performance were studied. The use of biological techniques in the treatment of heavy metal-polluted soils was also highlighted [7].

Functions of Laccases

In addition to some bacteria and insects, laccases are a typical enzyme that is extensively present in plants, fungi, and other living things. These biocatalysts, which can be secreted or found inside cells, have distinct physiological roles in the different organisms, but they all catalyse the processes of polymerization and depolymerization. According to what was previously stated, the first laccase was discovered in 1883 and was described as a metal-containing oxidase. It came from the Japanese lacquer tree Rhus vernicifera. As a result, it ranks among the earliest enzymes to ever be described. Later, laccases were found in a wide variety of plants, although it can be challenging to identify and purify these laccases since they are frequently mixed in with a lot of oxidising enzymes in crude plant extracts. The exception is Rhus vernicifera laccase, which has undergone substantial research, particularly in relation to its spectroscopic characteristics. The laccase from R. vernicifera has also been extensively employed in studies of the general reaction mechanism of laccases. Plant laccases are present in the xylem, where they likely oxidise monolignols during the early stages of lignification and take part in the radical-based mechanisms of lignin polymer synthesis. Laccases have also been demonstrated to play a role in the initial stages of recovery in injured leaves. But compared to fungus, higher plants appear to have far less of an abundance of laccases. There have only been a few bacterial laccases described so far. The "Azospirillum lipoferum" plant root-associated bacterium was found to contain the first bacterial laccase.

Functions of Laccases [8]

For the purpose of choosing appropriate laccase-producing organisms, screening of laccase-producing fungal species and their variations is crucial. For this reason, one typically relies on the utilisation of quick, easy, and affordable testing techniques. The goal of the screening technique must be to find fungal strains and enzymes that are suitable for use in industrial settings. For industrial applications, it is crucial to find novel laccases with various substrate specificities and enhanced stabilities. Both liquid cultivations and solid medium containing coloured indicator compounds that make it easier to visually detect the formation of laccase have been used to screen for laccase-producing fungi. Because no sample handling or measurement is necessary, using coloured indicators is typically easier. Laccases oxidise a range of substrate types.

Laccase Production

The expression of enzymes is greatly influenced by the culture conditions and medium characteristics. Many fermentation characteristics, including time of cultivation, stationary or submerged cultures, organic or inorganic compound concentrations, inducer concentration, aeration, and breakdown or activation by protease, have a significant impact on the generation of laccase by fungi. Laccases are typically formed by white-rot fungi growing on natural substrate or in submerged culture during the secondary metabolism process. White-rot fungus have different physiological requirements, and extensive research has been done on the effects of agitation, pH, temperature, carbon and nitrogen sources, as well as microelements and their concentrations. When grown on low or high nitrogen medium with cellulose as the carbon source, Phanerochaete chrysosporium produced laccases, but neither low nor high nitrogen medium with glucose did. In contrast, the white-rot fungus Ganoderma lucidum produced higher levels of laccases in high nitrogen medium with glucose as the carbon source. White-rot fungi's ligninolytic systems were typically active during the secondary metabolic phase and were frequently set off by nitrogen concentrations or when carbon or sulphur was scarce. It was previously established that the addition of xenobiotic substances including xylidine, lignin, and veratryl alcohol might enhance and promote laccase activity.

Heavy Metal Polluted Soils

The metallic qualities of ductility, malleability, conductivity, cation stability, and ligand specificity are characteristics of heavy metals. They are distinguished by having an atomic number more than 20, a comparatively high density, and a high relative atomic weight. Organisms need a little amount of some heavy metals as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn. These metals can, however, become toxic to organisms in large quantities. Since they are extremely damaging to both plants and animals, other heavy metals including Pb, Cd, Hg, and As are considered to be the "primary dangers" because they have no good effects on organisms. Metals can be found on their own or in conjunction with other elements in the soil. Inorganic solids' surfaces may have exchangeable ions sorbed on them, as well as nonexchangeable ions and insoluble inorganic metal compounds like carbonates and phosphates, soluble metal compounds or free metal ions in the soil solution, metal complexes of organic materials, and metals bound to silicate minerals. In contrast to metals that exist as distinct entities or those present in high concentration in the other 4 components, metals bound to silicate minerals constitute the background soil metal concentration and do not pose contamination or pollution issues. The density and type of charge in soil colloids, the level of complexation with ligands, and the relative surface area of the soil are all factors that affect the availability of metals in soil. Soil colloids contribute to a large interface and particular surface areas that aid in regulating the concentration of heavy metals in natural soils. Additionally, soil particles having a large specific surface area may be able to lower the quantities of metals that are soluble in contaminated soils, but this may be metal-specific. For instance, Mcbride and Martnez reported that while Ni and Zn's solubility remained unchanged, the solubility of As, Cd, Cu, Mo, and Pb reduced as a result of the addition of an amendment made up of hydroxides with high reactive surface area. Microbial activity, mineral composition, and soil aeration.

Growth Effects of Heavy Metal Polluted Soil

The heavy metals that are easily soluble by root exudates or present as soluble components in soil solutions are those that are available for plant uptake. Even while some heavy metals are necessary for the growth and maintenance of plants, too much of these metals can be hazardous to them. Plants can acquire other non-necessary metals because to their capacity to accumulate essential metals. Metals cannot be broken down, thus when concentrations inside the plant surpass ideal levels; they have a negative direct and indirect impact on the plant. High metal concentrations have a number of direct harmful consequences, such as cytoplasmic enzyme inhibition and oxidative stress-related cell structural destruction.

Bioremediation of Soils Contaminated with Heavy Metals

The process of bioremediation involves using living things to clean up contaminated soil. Due to the perception that it results from natural processes, this type of soil remediation is widely accepted. When bioremediation was utilised to treat 1 acre of Pb-polluted soil, compared to the case when a traditional approach was employed for the same reason, there was a 50% to 65% cost savings. This makes it an equally cost-effective form of soil remediation. The utilisation of bioremediation for the treatment of soils contaminated with heavy metals is occasionally influenced by the climatic and geological characteristics of the site to be remedied, despite the fact that it is a nondisruptive approach of soil remediation.

Phytoremediation of Heavy Metal Polluted Soils

Phytoextraction

This type of phytoremediation is the most prevalent. The roots and shoots of phytoremediation plants accumulate heavy metals in this process. The later harvest and burning of these plants. Rapid growth rate, high biomass, wide root system, and capacity to tolerate high concentrations of heavy metals are typically found in plants utilised for phytoextraction. These plants' capacity to withstand large concentrations of heavy metals may result in metal accumulation in the harvestable section, which could be problematic due to contamination of the food chain.

Phytoextraction can be done in one of two ways, depending on the properties of the plants used. The first strategy uses naturally occurring hyperaccumulators, or plants with extremely high metal-accumulating capacity, whereas the second strategy uses high biomass plants whose potential to accumulate metals is stimulated by the application of chelates, or soil additives with metal mobilising activity. They are ideal for phytoremediation since hyperaccumulators accumulate 10 to 500 times more metals than other plants. The ability of these plants to tolerate rising amounts of toxic metals is a crucial trait that makes hyperaccumulation conceivable.

Phytostabilization

Metals can be immobilised by plants, which lowers their bioavailability by preventing leaching and erosion. When phytoextraction is neither desirable nor even feasible, it is typically utilised. The method of phytoremediation is best used when the soil is so extensively polluted that employing plants to extract metal would take a long time to achieve and would therefore not be adequate. When the concentration of heavy metal in the soil was high, the growth of plants was negatively impacted.

Precipitation, sorption, metal valence reduction, or complexation all result in the phytostabilization of heavy metals. Depending on the plant and soil amendment utilised, phytostabilization can be more or less effective. Through their root systems, plants contribute to soil stabilisation and hence reduce soil erosion. Plant metal uptake is prevented and biological activity is decreased by soil amendments employed in phytostabilization because they assist in inactivating heavy metals. The most common organic materials employed in phytostabilization are soil amendments. After applying manure or compost to contaminated soils where Solanum nigrum was planted, the amount of Zn percolating through the soil was reduced by 80%.

Phytovolatilization

Polluting substances are absorbed by plants from the soil, converted to volatile forms, and then transpired into the atmosphere. The main use of phytovolatilization is the cleaning up of mercury-polluted soils. Hg changes from its poisonous form to one that is less hazardous. This process has a flaw in that the new product created, elemental Hg, may be recycled by precipitation and then redeposited into lakes and rivers, which would then trigger anaerobic bacteria to produce more methyl-Hg.

Food and Beverage Industry

The beverage industry is also anticipated to gain from laccase. By eliminating phenols such coumaric acids, flavans, and anthocyanins, laccase extends the shelf life of beverages by preventing undesired changes like discoloration, clouding, haze, or flavour alterations in fruit juices, wine, and beer. Due to the usefulness of laccases, researchers have been looking for sources of the enzyme from white-rot fungi and using mediators, which aid or expedite enzyme function. The information that is currently accessible regarding the occurrence, biological characteristics, and biotechnological uses of fungal laccases is compiled in this review.

Discussion

This interest is mostly caused by the utilisation of oxygen as a substrate, which is then changed into water. Since oxygen can be scavenged from the bloodstream and the byproduct (water) is benign, this has the obvious advantage of having potential medicinal uses in nanotechnology for living creatures. By modifying the zinc-air cell architecture, they created a zinc-laccase biofuel cell. In contrast to other biofuel cells, this zinc-laccase cell worked in an open environment. A membraneless cell design was used in this single chamber, and the laccase biocatalyst was allowed to float freely (as opposed to being immobilised) in a pH-6.5 quasineutral potassium dihydrogen phosphate buffer electrolyte. PGPR have generally been employed in phytoremediation research to lessen plant stress related to heavy metal polluted soils. When the plants were inoculated with Bacillus sp., increased hyperaccumulator accumulation of heavy metals like Cd and Ni was seen. However, when the tomato plant was inoculated with Methylobacterium oryzae and Burkholderia spp., it was observed that plant growth was increased since there was less Cd and Ni accumulating in the shoot and root tissues. Accordingly, this suggests that the PGPR mechanisms used in the phytoremediation of heavy metal-polluted soils may vary depending on the type of PGRP and plant used in the procedure. PGPR and mycorrhizal fungus use together in studies is not widespread, though.

Conclusion

Laccases are attracting a lot of attention from academics all over the world due to their unique characteristics. Since these enzymes were found in white-rot fungi, there has been a sharp rise in interest in using laccases for biotechnological purposes. Emerging technologies include selective delignification for cellulosic production in pulp bleaching, the conversion of lignocellulosics into feed and biofuel, and the treatment of toxicants and pollutants produced by various industrial processes. The functionalization of lignocellulosic materials, the alteration of wood fibre, the remediation of contaminated soil and effluents, as well as the use of laccases in biosensors, have all been extensively explored as a result. This analysis demonstrates that laccase has a wide range of possible uses in environmental protection. Changes in the physiological and biochemical activity of plants growing in heavy metal-polluted soils result in a reduction in growth. This is particularly true when the heavy metal in question does not contribute in any way to the growth and development of plants. For the successful treatment of soil contaminated with heavy metals, bioremediation can be used. Due to the fact that it is a nondisruptive form of soil remediation, it is ideal when the remediated site is used for crop production. As opposed to using microorganisms, using plants for bioremediation (phytoremediation) is a more popular method for bioremediation of heavy metals. Different techniques are used by plants to clean up soils that have been contaminated with heavy metals. The most popular technique for treating heavy metal-polluted soils using phytoremediation is phytoextraction. It guarantees the contaminant is entirely removed. The bioremediation process works more effectively when microbes and plants are used together. Numerous phytoremediation initiatives have successfully incorporated mycorrhizal fungi as well as other PGPR. The types of microbes and plants used, as well as the amount of the heavy metal in the soil, all play a role in how successfully these organisms can be used in combination.

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Conflict of Interest

The author has no known conflict of interest associated with this paper.

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