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Characterization, Applications, and Industrial Potential of Bacterial Laccases: A Comprehensive Study

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ABSTRACT: Laccases, a type of copper oxidase belongs to the family of 1,4-benzenediol. Laccase, a glycoprotein, is found ubiquitously in various organisms ranging from fungi to larger plants. Over the last several decades, there has been an amazing increase in the application and consumption of bacterial laccases across a variety of sectors. Bacterial laccases offer several distinct advantages over fungal laccases from an industrial perspective. They have a variety of temperatures and pH tolerance ranges while staying extremely stable even in the presence of many hazardous chemicals. Laccases' cellular position in bacteria varies by species; most naturally produced laccases in bacteria, including *Bacillus subtilis* as well as *Sinorhizobium meliloti,* occur intracellularly, whereas a few, such as laccases from certain bacilli and actinomycetes, exist extracellularly. Laccase has evolved into an important commercially essential enzyme with a wide range of applications, including lignocellulosic material delignification, biomedical and pharmaceutical applications, waste detoxification, and textile dye decolorization. Bacterial laccases present several challenges, including the diversity of laccases, lack of standardization in characterization methods, difficulties in strain identification and isolation, optimization of production, and protein engineering. Scaling up for industrial applications, substrate specificity, stability, and regulatory considerations are also hurdles. The current study gives an in-depth look at laccase-producing bacteria, encompassing detailed information on enzyme properties, gene characterization, cloning techniques, and industrial applications.

Keywords: Flowering traits variation, heritability, genetic advance, multivariate cluster analysis.

INTRODUCTION

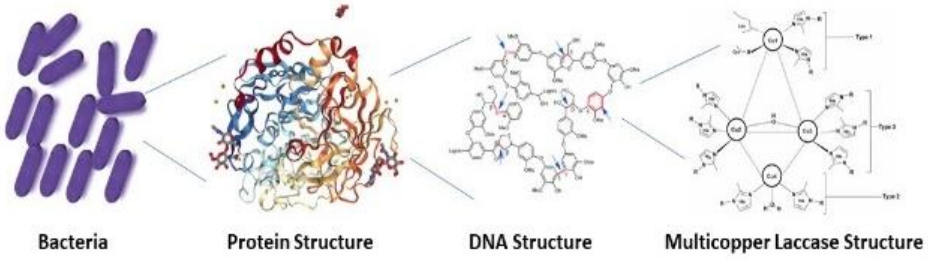
Laccases, a type of copper oxidase belongs to the family of 1,4-benzenediol: oxygen oxidoreductases that are present in microorganisms, larger plants, bacteria, and insects (Shraddha *et al*., 2011). Each molecule of these glycosylation polyphenol oxidases contains a $4Cu^{2+}$ ion which is responsible for the oxidative degradation of phenolic substances and non-phenolic substances, including aromatic amines, diphenols, aliphatic amines, and loss of electrons (Gianfreda *et al*., 2010; Rodríguez Couto & Toca Herrera 2006). Free electrons such as these contribute to the oxidative degradation of numerous aromatic and non-aromatic molecules having phenolic rings replaced with different functional chains, including methoxy, amino, diamino, and hydroxyindols. Furthermore, laccases may oxidize multiple additional complexes of metals such as $[Fe(CN)_6]_4$, and $[Os(CN)_6]_4$ including $[Mo(CN)_8]_4$ (Chandra & Chowdhary 2015a; Rezaei *et al*., 2017).

Laccase, a glycoprotein, is present in a wide range of species, from fungus to larger plants. Because of its high potential for redox reactions, it is mostly synthesized by white rot fungus and is widely used in industry. However, the commercial application of fungal laccase frequently faces obstacles by its long fermentation period, low production yield, and limited effectiveness primarily under acidic or mesophilic conditions. This poses a challenge as many industrial processes occur in harsh environments characterized by high temperatures, extreme pH levels, and elevated salt concentrations, where fungal laccase exhibits reduced activity (Du *et al*., 2015; Wang & Zhao 2008).

The consumption and application of bacterial laccase in numerous sectors have increased rapidly in the past few decades. Bacterial laccases offer several distinct advantages over fungal laccases from an industrial perspective. They have a variety of temperatures and pH tolerance range while staying extremely stable even in the presence of many hazardous chemicals (Guan *et al*., 2015). Furthermore, bacterial laccases are economically advantageous for industrial use due to their broad substrate specificity, rapid enzyme production, and efficient expression with the help of cloning in host organisms under relevant control (Prins *et al*., 2015). These features make bacterial laccases highly valuable in diverse applications include paper and pulp bio-bleaching, in textile dye as decolorising agent, (Mathews *et al.*, 2016) even in research of biosensors.

Various laccase produced by bacteria have been identified, expressed in appropriate host species, and chemically investigated extensively. This includes laccases derived from various bacterial species, which have been investigated for their enzyme properties, gene data, cloning methods, and practical applications (Chandra & Chowdhary 2015b; Narayanan *et al.*, 2015; Sharma *et al*., n.d.; G. Singh *et al*., 2011). The current study gives a complete look at laccase-producing bacteria, encompassing detailed information on enzyme properties, gene characterization, cloning techniques, and industrial applications.

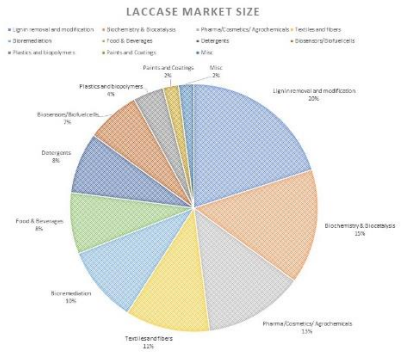
Occurrence of Bacterial Laccase: Bacterial laccases, although less common and less extensively studied than fungal laccases, have been reported in certain bacterial species. They are present in either in Gram-positive or in Gram-negative bacteria that live in water as well as in soil. Bacteria, that produce laccase are found in the phyla Gemma Proteobacteria, Alpha, Firmicutes, Aquificae, Deinococcus, Cyanobacteria, Thermus, include a few Archaea members (Janusz *et al*., 2020). Laccases' cellular site in bacteria can differ by species; most natively showed laccases in bacteria, like those from *Sinorhizobium meliloti* and *Bacillus subtilis* (S. Singh *et al*., 2021) occur inside the cell, whereas a few occur outside the cell, with the value as laccases present in certain *actinomycetes* and bacteria (Dubé *et al.*, 2008). Because the laccase process creates toxic chemicals, inside of cells bacterial laccases have ways to deal with the toxicity. (Janusz *et al*., 2020). For example, laccase production has been observed in *B. subtilis* and *Bacillus licheniformis* (Janusz *et al*., 2020).



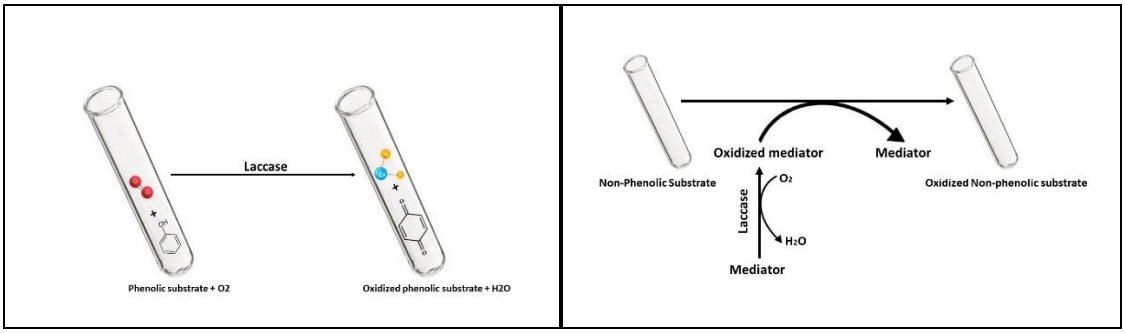
Some strains of *Pseudomonas aeruginosa*, including *Pseudomonas putida* and *Pseudomonas fluorescens*, have also been found to produce laccase enzymes (Unuofin *et al.*, 2019). In addition, certain species of Streptomyces, such as *Streptomyces cyaneus* and *Streptomyces ipomoeae*, have demonstrated laccase activity (Lisov *et al.*, 2019; Suryadi *et al*., 2022). Furthermore, *Thermus thermophilus*, a thermophilic bacterium, has been reported to produce a heat-stable laccase (Adekunle *et al.*, 2017). Although these studies highlight the presence of bacterial laccases, it's important to note that the occurrence and diversity of bacterial laccases are still areas of ongoing research.

Industrial production of Bacterial Laccase: Laccase scaling up gives more realistic information on energy and material consumption, allowing for an entire cost assessment of those products. Batch fermentation took place in a 10-L bench-top bioreactor with a working capacity of 4 L, which was outfitted using three 6-bladed disc-turbine impellers & four baffles and was connected to a digital control device. The fermenter vessel containing the in statistical terms analyzed medium was autoclaved for 20 minutes at 121 °C, while the $CuSO_45H_2O$ and $MnSO_4H_2O$ solutions were sterilized separately and then added aseptically to the bioreactor, as well as sucrose with vanillin acid solutions were sterilized separately by syringe filter. After that, the bioreactor was injected with prepared inoculum. The temperature, agitation acceleration, airflow rate, and beginning pH included every one set to 30°C, 200 RPM, 1.0 VVM, and 7.0, respectively. To prevent foam growth, antifoam agent needs to be added at a ratio of 1:100 (v/v) in distilled water at the start of the operation. Fermentation takes place under unregulated circumstances. To determine biomass, amount of protein, total carbohydrates intake, and laccase synthesis, samples must be taken at regular intervals (Abdelgalil *et al*., 2022).

Applications of Bacterial Laccase: Laccase has grown into a significant industrially useful enzyme with a broad spectrum of applications, including lignocellulosic material delignification, biomedical and pharmaceutical applications, bioremediation applications such as trash detoxing, & fabric dye decolorization. The majority of significant industrial applications are described as.



Laccase's role in the biomedical and pharmaceutical industry: Laccases have catalysed the metabolic processes for several phenolic compounds and non-phenolic substances. Because of their extensive substrate range, laccases can be utilized in several uses in biotechnology (Tabassum *et al*., 2022; Althobaiti *et al*., 2022). The current evaluation was done to give academic and industry researchers a comprehensive background in laccase-related pharmacological and biological applications. First, an outline of laccases' biological roles was provided. Laccase-mediated techniques for imparting antibacterial and antioxidant characteristics to various surfaces were also explored (Mohit *et al.*, 2020).



Laccase catalysis phenolic and non- phenolic substrate activity.

Laccase's role in degradation of dye: The usage of dyes has grown to the point that around 10,000 different dyestuffs are utilized and manufactured each year. The globe creates around 7×10^5-1×10^8 tons of dyestuff yearly; on average, one-tenth of this dyestuff enters the environment via industrial effluent. Some of these dyes develop resistance to environmental (temperature, light, pH) and biological in origin (microorganisms) variables (Adekunle *et al*., 2017; Ba & Vinoth Kumar 2017). Color may be removed from wastewater using different physical and chemical processes such as oxidation as well as flocculation, while these procedures have drawbacks such as expense and the formation of chemical sludge. As a result, the creation of a biological process capable of acting on a diverse spectrum of waste is in high ultimatum (Narayanan *et al.*, 2015) Bacterial laccase has several advantages over fungal laccases, including the ability to function in severe settings and tolerate salt (Jiang *et al.*, 2022; Rovaletti *et al.*, 2023).

Kumar *et al*. (2022) revealed that *B. subtilis* WD23 enzyme removed the color of difficult anthraquinone dyes (owing to an aromatic ring) such as Remazol Brilliant Blue R, methyl orange, Congo red, and Alizarin red at alkaline pH in the absence of nutrients and mediators. Furthermore, except for reactive yellow, thermoactivated laccase generated by *B. subtilis* efficiently destroyed azo and anthraquinone Sudan orange G (SOG) dye.

Laccase's role in paper and pulp industry: Production, include recycling of paper firms confront several challenges to eliminate phenolic compounds such as lignin or for the creation of visible pulp characteristics. This may be accomplished by a variety of chemical-based procedures, but they are extremely dangerous and contribute to increasing environmental contamination. This results in the utilization of ligninolytic together with hemicellulolytic enzymes, that finish the process (Wani *et al.*, 2022a-c). Enzymatic deinking improves intensity, colour, ink that remain, and other properties while reducing energy and overall costs (Saxena & Singh Chauhan 2017; Virk *et al*., 2013 and Dhanjal *et al*., 2022). Enzyme-mediated bleaching of aged newspaper pulp improves whiteness or clarity by eliminating the lignin ingredient (Garajová *et al.*, 2021; Kumar *et al*., 2019).

Laccase's role in the textile industry: Laccases have the ability to decompose phenolic as well as aromatic amines, along with their substituted byproducts with different chemical groups (Sondhi *et al*., 2015). Due to this property of these enzymes, they can be employed in textile manufacturing to remove textile colors as well phenols, as well as for sewage purification. Due to their higher redox potential, laccases generated by fungus have been frequently used for the decomposition of colors found in textile effluents (Nguyen & Juang 2013; Plácido & Capareda, 2015; Wandhwa *et al*., 2023). Furthermore, due of their fascinating properties like quick production, low-cost medium, and stable nature, bacterial laccases can be applied to textile dye degradation.

Laccase's role in degradation of pollutants: Polycyclic aromatic hydrocarbons (PAHs) having most common contaminant found in natural environments such as soil, air, and water. They are composed of a benzene ring that has been organised in a linear in nature, sharp, or clustered shape (Bhandari *et al*., 2021; Leong *et al.*, 2022). The majority of these pollutants and intermediate steps are harmful to humans and carcinogenic to living beings. Because of their low affinity to water and slow breakdown rate, these aromatic hydrocarbons are harmful in nature (Ihssen *et al.*, 2015 and Rahayu *et al.*, 2022). Few data exist to date that demonstrates the potential of bacterial sp. to digest xenobiotic substances (Debnath & Saha 2020; Zeng *et al*., 2011). Laccase enzyme is thought to convert hydrocarbons that are polycyclic aromatic to quinines and then to carbon dioxide. When acenaphthylene and acenapthylene are coupled with the mediator HBT, an enzyme converts them to 1,2-acenapthalenedione and 1,8-napthelic acid (Madhavi & Lele 2009).

Laccase and biosensor technology: Laccase may react with phenolic compounds and can function on a

variety of substrates, making it valuable in biosensor technology. When paired with a distinct physical device that works as a biosensor, oxygen, as well as other oxidizing substrates (particularly anilines, phenols etc.) catalyzed by laccase are quickly detected.

Laccase-based biosensors are generally classified into two types: those that assess enzyme spectrum variations (at an absorption intensity of 600 nm) and those that monitor voltage fluctuations from a oxygen sensor that is modified (Madhavi & Lele 2009; Mir *et al*., 2022). Immobilized alkali-toluene laccase on the nitrocellulose cell membrane, which reacted linearly even at low concentrations to a range of substrates, including that include catechin, catechol, syringaldazine, and L-DOPA.

CONCLUSIONS

Finally, the current study includes thorough information on the occurrence, molecular cloning, structural features of various bacterial laccases, as well as uses with laccase industrial production. Laccase enhancement strategies include genetic modification and cloning in appropriate heterologous hosts for enzyme excessive production. Laccase enzyme may function on a number of substrates, purify a range of pollutants, and oxidize dangerous substances, making it useful in the paper production, Fiber production, pulp as well as in textile industries, among others. Usage of cheap resources for laccase manufacture has recently been examined. A novel concept in the manufacturing sector wastewater handling in this context is harnessing its nutritive ability to manufacture laccase. Aside from solid trash, drainage from the agriculture and food processing sector is of especially significant importance. Second, laccase is essential for the breakdown of wide range of pollutants and phenols. Major problem with this enzyme is due to its limited specificity for substrates and can potentially catalyse a wide variety of reactions. As a result, more research regarding this topic is extremely important. In addition, despite several attempts to find out the function of laccases in lignocellulose transformation, the subject is still undetermined concerning the role of laccases in lignin decomposition in plant biomass, it can be utilized as an enzymatic treatment method for cellulosic production of ethanol. In the future, researchers should pay greater attention to these issues. As a result, it will not be surprising that such an enzyme is being thoroughly explored and is going to continue to do so in the future.

FUTURE SCOPE

The future scope of research on bacterial laccases is promising, with opportunities in expanding biotechnological applications, customizing enzyme properties through genetic engineering, advancing green chemistry, integrating with nanotechnology, exploring synergistic enzyme combinations, contributing to sustainability initiatives, developing regulatory frameworks, and enhancing economic viability. Global collaboration, public education, and exploration of emerging markets will also shape the future of bacterial laccase research, offering innovative and sustainable solutions across various industries.

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Conflict of Interest. None.

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