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REVIEW ARTICLE

Eminent Industrial and Biotechnological Applications of Laccases from Bacterial Source: a Current Overview

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Abstract

Laccases are blue multicopper oxidases that oxidize a wide range of phenolic as well as non-phenolic substrates in the presence or absence of mediators. They occur in various species of bacteria, fungi, insects, and plants; bacterial laccases show high substrate specificity. Bacteria produce these enzymes either extracellularly or intracellularly and exhibit stability to a wide range of pH and temperature. Therefore, they are suitable for various industrial processes such as food, textile, and paper and pulp industry. They are also valuable for producing biofuels, pharmaceuticals, biosensors, and degradation of various environmental pollutants and xenobiotics compounds. Since bacterial laccases are more versatile in the sense of nutritional needs and ecological factors, their use can provide a promising solution to various problems related to industry and the field of biotechnology. However, there is a need for a thorough understanding of the chemistry and activity of bacterial laccases to enable their full potential use in bioremediation and biofuel production.

Keywords *Bacillus* · Biofuel · Delignification · Laccases · Redox mediator · Stability

Introduction

Laccases are copper-containing 1,4-benzenediol:oxygen oxidoreductases (EC 1.10.3.2) present in microorganisms, higher plants, bacteria, and insects [1]. These are glycosylated polyphenol oxidases which contain four copper ions in each molecule. Copper ions carry the oxidation of phenolics and non-phenolic compounds such as aromatic amines, diphenols, and aliphatic amines, along with the reduction of oxygen to water [2–4]. The bacterial laccase activity was detected for the first time in *Azospirillum lipoferum* isolated from the rhizosphere of rice in 1993 [5]. Various genera of bacteria such as *Bacillus*, *Streptomyces*, *Klebsiella*, *Pseudomonas*, *Yersinia*, *Proteobacterium*, and *Marinomonas* contain laccases as part of their metabolic processes [6]. These enzymes have also been reported in some bacteria of domain Archea, such as *Haloferax volcanii* [7]. The molecular weight of

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bacterial laccases ranges from 50–70 kDa, with most of them existing in the form of monomeric extracellular proteins, and many few occur as intracellular proteins [8, 9]. They are involved in pigmentation, toxin oxidation, morphogenesis, protection against UV and oxidizing agents [10]. Cot A protein of *Bacillus subtilis* is copper-dependent laccase present in the spore coat of bacterium. It is the first-ever laccase reported to be present in endospore coat and is involved in the production of brown melanin-like pigment and helps in protecting the bacterium from UV light and H_2O_2 [11].

The extraordinary stability and remarkable range of substrate specificity make them suitable for several biotechnological and industrial applications [12]. The substrate range includes aromatic amines, polyphenols, methoxy phenols, and various other inorganic compounds [13]. Laccase can efficiently oxidize these substrates by accepting electrons from molecular oxygen and producing phenoxy radicals that spontaneously rearrange to open their aromatic rings and thus enhance polymerization. These phenolic compounds are considered to be the possible substrates for laccases due to their low redox potentials (0.5–1.0 V). Additionally, the spectrum of substrates that laccase can oxidize can be considerably expanded by the application of low molecular weight redox mediators [14]. Table 1 illustrates the activity and stability of bacterial laccases on substrates with and without redox mediators.

These oxidoreductase enzymes have proficiency and are helpful in the biosynthesis of fibers, bio-detection, and environmental protection. At the industrial level, they are used in a variety of processes such as food improvement, bio-pulping in the paper industry, denim washing in the textile industry, and cosmetics manufacturing [23, 24]. Due to the high demand for efficient oxidation technologies, laccases have gained importance as biological oxidative agents and are beneficial for oxidizing toxic as well as non-toxic substrates. Laccase mediator systems also play role in the degradation of lignin, biosensor systems, biofuel synthesis, nano-biotechnological applications, pharmaceutical applications, and the bioremediation of toxic waste [25].

Properties of Laccases

Laccases are associated with a small set of enzymes also known as multicopper oxidases and they have the ability to produce a variety of extracellular enzymes such as manganese peroxidase, lignin peroxidase, and versatile peroxidases [26]. Laccases are monomer or homodimers of glycosylated proteins; bacterial laccases contain 10–25% of sugar residues compared to plants laccases that have higher contents of saccharides. Laccases have a wide range of redox potentials, ranging from 400 mV for plant laccases to 790 mV for certain fungal laccases. Laccases hold four copper ions per molecule, and mediators such as electron shuttle help them in oxidizing non-phenolic compounds [3, 27]. In addition, mannose is one of the significant carbohydrate components of laccases [11].

Laccases contain three types of copper prosthetic groups, which have been categorized on the basis of electron paramagnetic resonance signals and absorption spectra, namely type 1 copper (T1 Cu) is coordinated by two histidines, water molecules, and exhibit the first oxidation site, and shows absorption band at 600 nm in the UV–visible spectrum [28, 29]. T1 copper gives a blue shade due to the intense electronic absorbance of the cysteine-copper covalent bond [30]. Similar to T1 Cu, type 2 copper (T2 Cu) is also coordinated by two histidines and a water molecule. However, there does not exist any absorption band in the UV–visible spectrum but a parallel hyperfine coupling

Table 1 Catalysis of substrates by bacterial laccases with and without redox mediators

Organism	Enzyme symbol	Substrate	Optimal Temp./stability	Optimal pH	Molecular weight (kDa)	Industrial applications	References
Enzyme activity with redox mediators							
Bacillus subtilis	CotA	β-(10-phenothiazyl)-propionic acid	37 °C; 82% stability for 1 h	8	65	Decolorization of synthetic dyes containing indigoid, azo, anthraquinone, and triphenylmethane moieties	[15]
Streptomyces ipomoeae	SilA			6.8	39–40		
Stenotrophomonas maltophilia AAP56	SmLac	ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), ASGN (acetosyringone), SGA (syringaldehyde) and HOBT (1-Hydroxy-Benzotriazole)	40 °C; 99% stability for 1 h	7	66	Azo dye decolorization	[16]
Iodidimonas sp. Q-1	IOX	Iodide	60 °C; 46% stability for 1 h	5.5–6.5	-	Decolorization of orange G, indigo carmine, amido black, and Remazol Brilliant Blue R (RBBR)	[17]
Spirulina platensis CFTRI	-	2,2-Azino-bis-[3-ethyl benzothiazoline-6-sulphonic acid] (ABTS)	40 °C; 96% stability for 4 h	8.0	66	Decolorization of anthraquinonic dye and wastewater treatment	[18]
Enzyme activity without redox mediators							
Bacillus subtilis MTCC 1039	-	Guaiacol	30 °C; 100% stability for 60 h	5.0	-	-	[19]
Bacillus subtilis MTCC 2414	-	Agroresidue substrates	70 °C; -	9.0	52	Bioremediation, biopulping, textile, and food industries	[20]

Table 1 (continued)

Organism	Enzyme symbol	Substrate	Optimal Temp./stability	Optimal pH	Molecular weight (kDa)	Industrial applications	References
Bacillus cereus TSS1	-	Guaiacol	37 °C; -	7.0	-	Bioremediation and food industries	[21]
Alteromonas MMB-1	-	Tyrosine L-Dopa 2,4-Dimethoxy phenol	37 °C; -	5.0	46	Phenolic bio-transformations, food processing, or the cosmetic industry	[22]

(-) Not reported

is recorded in EPR ($A\| = (105–201) \times 10^{-4}$ cm^{-1}). Moreover, type 3 copper (T3 Cu) carries two copper atoms: T3α Cu and T3β Cu. Both of these molecules are coordinated by three histidine residues. The T3 Cu is characterized by a broad absorption band at 330 nm ensuing from the hydroxyl bridge-to-metal (HO^-–Cu) charge transition, but deficient in displaying EPR signals due to the anti-ferromagnetic coupling of two copper ions [14]. Usually, T2 and T3 copper sites are involved in the formulation of an oxygen-binding trinuclear cluster site (TNC) that is responsible for reducing oxygen to water. However, the T1 site functions by reducing substrate (electron donors) and transferring electrons to the T2–T3 copper clusters. In laccases, substrate oxidation at the T1 site is considered as a catalytic rate-limiting step, which is commonly controlled by the difference in the redox potential of this site and trinuclear site [31].

The entire laccase reaction entails a single electron ($1e^-$) sequential oxidation of four reducing substrate molecules, followed by two double electron reduction of oxygen atoms into their respective H_2O molecules. This reaction is usually catalyzed by the exchange of $4H^+$ equivalents. A laccase reaction is viewed structurally, mechanistically, and kinetically as two half-reactions that are joined by an internal electron transfer (IET) step and facilitated by the catalytic copper ions situated at the T1 Cu and T2 Cu/T3 Cu/T3 Cu trinuclear cluster (TNC) sites. Numerous sequence and metagenomic investigations have established that eleven (one Cys and ten His) residues composing the T1 copper and TNC laccase sites, and in general all multicopper oxidases (MCOs), explain their crucial involvement in the catalytic action of the enzyme. Similarly, other fully or highly conserved residues that play important roles in different catalytic steps involved in laccase action, such as the recognition and docking of reducing substrates, internal electron transfer (IET) from the T1 copper ion into the TNC site, and oxygen atom reduction at the TNC site have also been identified. These residues are typically found in close proximity to their respective areas of action, where they manifest as second sphere residues [32].

Three isozymes of laccases Lac I, Lac II, and Lac III have been discovered in bacteria, and studies show that laccase molecular weight varies in different organisms [11]. Bacterial laccases have a wider pH range as compared to fungal laccases, which make them efficient and more suitable for use in pulp processing in the paper industry and biobleaching in the textile industry [33]. Optimum temperature ranges from 30–50 °C, and they lose their activity completely at 60 °C [4]. The isoelectric point of laccases varies from 3 to 7, but the isoelectric point of plant laccase is as high as 9.0. The pH range for bacterial laccases is 3.0–9.0. However, *Bacillus tequilensis* SN4 laccase shows a maximum working temperature of 85 °C and a working pH of 8.0 [34–36]. They generate free radicals through the oxidation of substrate in a single electron response [4]. The major catalytic actions of laccase include reduction of type one copper by reducing substrate; electron transport among type 1, type 2, and type 3 copper; and reduction of final electron acceptor takes place which in this case is oxygen to form water at type 2 and type 3 copper sites. As mentioned earlier, T1 absorbs light in the range of 600 nm and is responsible for the blue color of the enzyme [4].

Bacterial laccases are easy to produce at the commercial level, and they show high resistance to inhibitory agents. Furthermore, they are more tolerant to a wide range of temperature and pH and offer a broad range of substrate specificity. The short generation time of bacteria makes it compatible to be used as a source of laccases at a commercial scale [9]. They are easy to clone and can be expressed in a variety of suitable hosts with suitable manipulation [37, 38].

Catalytic Properties of Bacterial Laccases

Fungal and bacterial laccases have been widely distinguished by numerous identifying approaches; most desirable is by employing substrates as well as inhibitors acting on them. Only brief data is present on bacterial laccase activity and their catalytic characteristics [39]. Marine *Alteromonas* sp. possessed both catalytic capacities of tyrosinase and laccase. The tyrosinase-related activities, cresolase, and catechol oxidase were selectively stimulated in the presence of small quantities of SDS in the assay mixture, and however, detergent did not enhance the oxidation of laccase substrates [40]. The presence of these two leading types of PPOs has been documented in certain fungi like *Neurospora crassa* and *Agaricus bisporus* [41] but not in prokaryotic cells, where laccases are uncommon [5]. The demarcation between tyrosinases and laccases in the bacterial kingdom may not be as obvious as suggested for PPOs derived from eukaryotic cells. The low molecular weight external fungal laccases have been described from *Volvariella volvacea* (58 kDa) [42] and *Marasmius quercophilus* (63 kDa) [43] compared to the internal bacterial laccases which have large molecular weight. Widely, fungal laccases have an acidic pH optimum, while most bacterial laccases are able to activate and are stable across larger pH (6.0–8.5) range [44, 45]. Bacterial laccase from γ-proteobacterium was stable in the pH range 4.0–9.0, but 70% stable at pH 3.0 and 10.6 even after 48 h of incubation at 37 °C. The enzyme was 100, 60, and 49% stable at pH 9.0 (Tris–HCl, 0.1 M), 10.6 (glycine–NaOH, 0.1 M), and 4.0 (citrate, 0.1 M), respectively after 60 days at 4 °C incubation; this stability of the enzyme might be because the organism did not create protease [44]. Metagenome-derived laccase showed optimal pH 9.0 for syringaldazine. Alkaline bacterial laccase (Lbh1) from *B. halodurans* C-125 showed a pH optimum of 7.5–8.0 using syringaldazine as a substrate [46]. The activity of bacterial laccases across a broader pH range might make them better as compared to fungal ones in industrial applications like biobleaching of paper pulp as well as dyestuffs processing [33].

Structural Classification of Bacterial Laccases

Laccases belong to the cupredoxin family, which contains many other oxidases such as manganese oxidase and ascorbate oxidase [47]. Cuperdoxin fold is the common signatory fold of this family. This fold is also known as the Greek-key motif, which consists of four beta-sheets arranged opposite to each other and are joined through hairpin loops. The first sheet is also directly connected with the fourth sheet through a long connection [48]. Depending upon the structure, bacterial laccases can be divided into two types: two-domain laccases and three-domain laccases [9].

Three-Domain Laccases

Endospore cotA laccase of *Bacillus subtilis* is a well-characterized three-domain laccase; therefore, it can be taken as a reference for understanding the structure of three-domain laccases. Cot A is a monomeric protein with a molecular weight of 65 kDa [49] and contains three cuperdoxin domains, as shown in Fig. 1. The primary domain (blue color) makes the N terminal of the protein and consists of 8 beta barrels which are connected with the first and second domain. Hydrogen bonding stabilizes the linkage among domains [48].

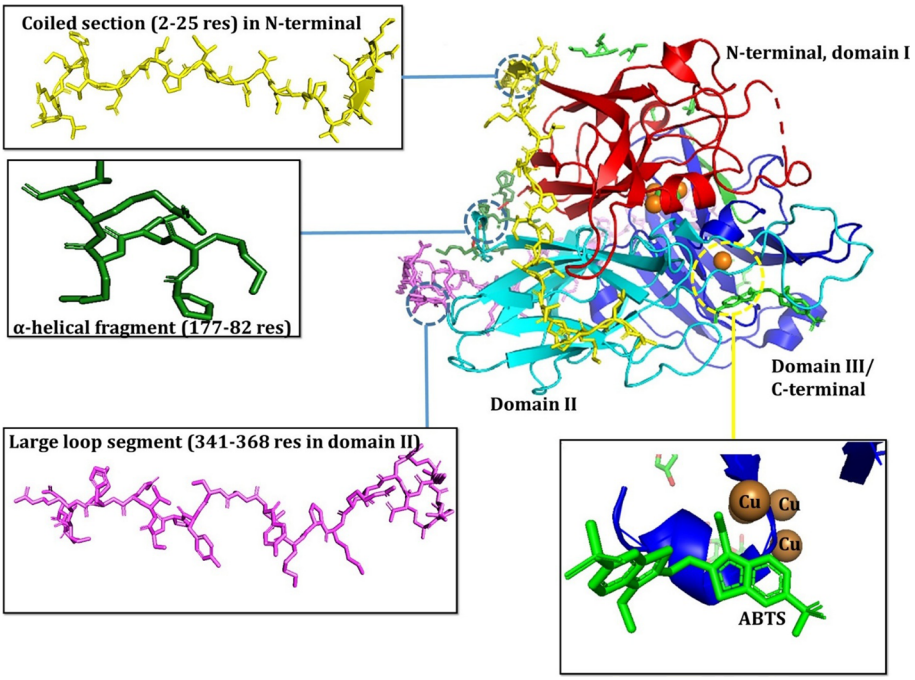

Fig. 1 Three-dimensional structure of CotA laccase from *Bacillus subtilis* retrieved from PDB: 3ZDW, it contains three domains. The first (N-terminal, domain I; in red color) cupredoxin-like domain of CotA (2–176 residues) appears in a somewhat distorted configuration. This N-terminal domain comprises of eight strands organized in β-barrel form and start with a coiled section (in yellow-colored sticks on the left side). The coiled section connects domains I and II. The second cupredoxin-like domain (domain II; in cyan color) with 183–340 residues carries α-helical fragment (in green colored sticks) connects domains I and II. Another large loop segment in domain II (in violet-colored sticks) connects domains II and III. The last C-terminal, domain III (369–501 residues) is represented in blue color. The copper atoms within the CotA structure are represented as light brown spheres and the mediator ABTS molecule close to the mononuclear copper center is shown in green color (modified from Enguita et al. [50])

Domain two is located between domains 1 and 3; it consists of a comprehensive loop of a beta-barrel which is made up of 12 filaments. The small alpha-helical region connects domains 1 and 2, while domains 2 and three are connected with the help of a portion of the loop [50]. C terminal domain represented in the form of red color (Fig. 1) comprises of mono-nuclear copper center and helps to provide attachment point at T3 copper site [9]. It also provides an attachment site for the substrate near the T1 copper center, which is important for single-electron oxidation of the substrate. The highly compact structure and high amount of hydrophobic bonds of Cot A laccase make it thermostable [9].

Two-Domain Laccases

This class of laccases can be explained by using *Streptomyces coelicolor* laccase as a reference. These laccases consist of only two domains (Fig. 2) and lack a domain 2, which was responsible for connecting domain one with domain three in two-domain laccases [51].

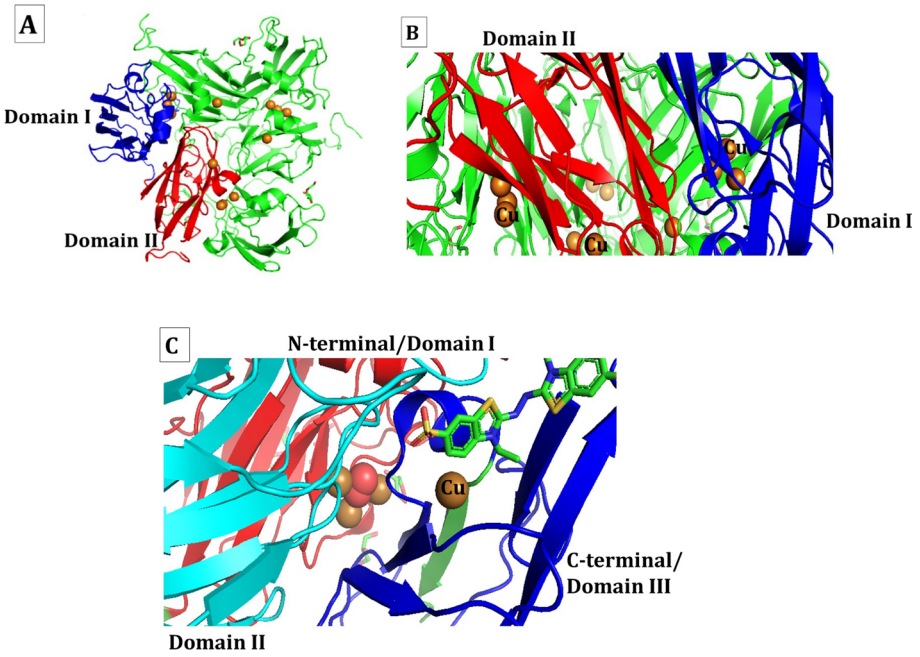

Fig. 2 (**a**) Structure of two domains laccase from *Streptomyces coelicolor* PDB: 3CG8, where domain-I with 45–185 residues is shown in blue color and domain-II with 186–316 residues are shown in red color, while three trinuclear copper clusters (shown in brown spheres) are placed between domains I and II. (**b**) Enlarged image of two domains laccase from *Streptomyces coelicolor* is shown the additional helices and loops, and the major difference between two domain laccase, and (**c**) three-domain laccase from *Bacillus subtilis* (PDB ID: 3ZDW) is that mono-nuclear copper exist in domain III of three domain laccases as shown in figure (modified from Skálová et al. [51])

Formation of trinuclear center becomes difficult in the absence of a domain; oligomerization is required in this case to form catalytic sites [9]. The formation of homotrimers takes place for the creation of a trinuclear center which is very important for the proper activity of laccases [52, 53]. Generally, laccases from different sources are divided into low redox potential and high redox potential laccases. Bacteria, plants, and insects contain low redox potential laccases, while high redox potential laccases commonly occur in fungi [54].

Production of Bacterial Laccases

Laccases are found in Gram-positive and Gram-negative bacteria inhabiting soil and aquatic environment. Laccase-producing bacteria belong to Phyla Alpha, Gemma Proteobacteria, Firmicutes, Cyanobacteria, Aquificae, Dinococcus, Thermus, and some members of domain Archea [49]. The occurrence of laccases have been reported in the following species, *Bacillus subtilis* [55], *B. licheniformes* [56], *B. pumilus* [57], *Sinorhizobium meliloti* [58], *S. lavendulae* [59], *P. syringae* [60], *S. griseus* [61], *Escherichia coli* [62], *Thermus thermophilus* [63], *Oscillatoria boryana* [64], *Alteromonas* sp. [40], and *Haloferax volcanii* [7]. The cellular location of laccases in bacteria varies from specie to specie; most of the natively expressed laccases in bacteria such as *Bacillus subtilis* [65] and

Sinorhizobium meliloti [58] occurs intracellularly while few occurs extracellularly such as laccases of some bacilli and actinomycetes [66]. As the reaction of laccases produces toxic products, bacteria containing intracellular laccases have mechanisms to cope up with the toxicity [49]. Table 2 represents the applications of bacterial laccases.

Purification of Laccase from Different Sources

Laccase produced from *S. psammoticus* was partially purified by ammonium sulfate precipitation and immobilized in alginate beads by entrapment method using calcium and copper. Copper alginate beads retained 61% of laccase activity, compared to calcium alginate beads, which retained only 42.5% of laccase activity [74]. Without induction, Zhang et al. [75] produced laccase from *Panus rudis* in a specified shaken liquid culture; purified laccase enzyme had a molecular weight of 58 kDa with an isoelectric point of 3.5. McMahon et al. [34] purified laccase from cell extracts of soil bacterium *P. putida* F6 using a combination of anion exchange chromatography and gel filtration, and found laccase activity of 518 U mg^{-1} with a molecular mass of about 59 kDa. Suzuki et al. [59] identified a laccase from cell extracts of *Streptomyces lavendulae* REN (ST SL). On SDS-PAGE, the isolated enzyme appeared as a single protein band with a molecular mass of about 30 kDa. Da Cunha et al. [76] determined laccase activity spectrophotometrically using syringaldazine and observed an increase in absorbance when substrates were oxidized at room temperature. According to Jhadav et al. [77], purified laccase obtained from glucose and guaiacol medium demonstrated lower activity than its crude counterpart, and the efficiency of the purified extract was determined using SDS-PAGE. Diamantidis et al. [78] purified *Azospirillum lipoferum* laccase using dialysis and ammonium sulfate precipitation of the proteins from the supernatant. Laccase activity was detected in 30–60% saturated fractions with a molecular mass of approximately 60–70 kDa and an acidic isoelectric point (pI) of approximately pH 4.0 since laccases have been isolated and purified from various sources including fungi and plants [79]. Therefore, bacterial laccases carry several unique properties that are not characteristics of fungal and plant laccases such as stability at high temperature and pH.

Table 2 Shows the applications of laccases from different bacterial species

Bacterial Species	Applications	References
Azospirillum lipoferum	Oxidation of phenolic compounds	[5]
Bacillus licheniformis	Dimerization of phenolic acids	[56]
Pseudomonas stutzeri	Biobleaching of Eucalyptus Kraft pulp	[67]
Streptomyces cyaneus	Biobleaching of Eucalyptus Kraft pulp	[68]
Trametes versicolor	Decolorization of synthetic dyes	[69]
Streptomyces psammoticus	Phenol removal and decolorization of synthetic dyes	[70]
Streptomyces spp.	Degradation of azo dyes	[71]
Phanerochaete chrysosporium	Degradation of azo dyes	[71]
Bacillus SF strain	Degradation of textile dyes	[72]
Gamma proteobacterium JB	Oxidation of industrial effluents	[73]
Bacillus endospores	Phenols degradation	[74]

Biotechnological and Industrial Applications

Although oxidation reactions are the biggest need of the industry, most of the conventional oxidation technologies are non-specific, produce undesirable side reactions, and involve the use of toxic compounds which are a potential hazard for the environment. These drawbacks of conventional chemical oxidation technologies have compelled industrialists and researchers to look for the use of enzymes in oxidation technologies. Enzymes are specific in their reaction and highly biodegradable, and require mild conditions for the reaction, which render them appropriate for use in industry as well as a research field. Due to the wide range of substrate specificity and no potential threat to the environment, laccases are seen as the best replacement for various chemical treatments [3]. Figure 3 elucidates the application of laccases. The following are the application of bacterial laccases in biotechnology and industry.

Food Industry

The quantity of phenolic content present in fruit juices greatly affects the quality of juices. High contents of phenols can change the color and taste of the juices, thus affecting the value. Darkening of these products takes place due to polyphenols; the use of laccases to remove phenol contents and avoiding chemical treatment such as the one which involves the use of activated carbon can help in maintaining color stability [80, 81]. Laccases can

Fig. 3 Various applications of laccases, such as in paper and pulp industry, textile industry, beverages, bioremediation, biofuel production, and in biosensors

be beneficial for wine stabilization through controlling phenol contents in the wine [82]. White wine is also treated with laccases, and due to the excellent stability of bacterial laccases at low pH, inhibition of laccases with sulfites can be easily reversed. Wine treatment with laccases increases its shelf life and reduces the production cost [82, 83]. The presence of phenolic compounds such as proanthocyanidines causes the precipitation of protein and results in the formation of haze in beer; therefore, beer stabilization is also a very necessary step for enhancing shelf life [81]. Laccases are added at the end of the production process in order to increase the half-life of beer [82].

Laccases have also been reported to be used in baking due to their texture-improving properties. Their usage is beneficial for improving the consistency of the dough and enhancing the tensile strength of gluten structure [84]. A noticeable change in volume and softness of dough and transition in the structure of crumbs has been observed after adding laccases [81]. Laccases are also valuable for producing sugar beet pectin gelatin which is a gel formed by the oxidative cross-linking of ferulic acid. The oxidative process during the production of sugar beet pectin gelatin is carried out using oxidoreductases such as laccases and peroxidases. Laccases and peroxidases differ on the base of their electron acceptors; laccases require molecular oxygen as their terminal electron acceptor, while peroxidases require hydrogen peroxide (H_2O_2) as the final electron acceptor [84]. The gel which is obtained after the treatment with laccases is thermo-irreversible and can be used in luncheon meat and many other food products [83, 85].

Textile Industry

The textile industry makes a total of one-third of the dyestuff market, and it consumes a large volume of water and chemicals for the wet processing of textile [3]. The chemicals used in textiles are very diverse in nature and composition, ranging from inorganic compounds, polymers to organic compounds [86–88]. These dyes released from textile mills create a large volume of colored wastewater, and their inability to fade in the presence of water, light, and other chemicals makes them resistant to degradation [89]. Due to increased awareness about the environmental issues, new legislation requires textile industries to treat colored water before discharge [90].

The carcinogenic nature of several dyes such as benzidine raises concern about the environmental impact of these chemicals [91]. The wastewater from textile mills is usually treated through chemical or physical methods, which include irradiation, precipitation, ozonation, electrokinetic coagulation, use of activated carbon, and mixture of various gases [92]. Currently available methods for treating textile waste and dyes are ineffective and non-economical; therefore, the treatment method based upon the use of laccases provides promising solution due to their ability to degrade dyes of diverse chemical nature as well as synthetic dyes [93, 94]. Laccase-based hair dyes have also been introduced, which are less irritant and are easier to handle as compared to conventional dyes due to the replacement of H_2O_2 with laccases [95]. Laccases are also capable of enhancing dechlorination activity which causes the decrease in dissolved oxygen concentration [96].

Laccases are used in denim finishing for the removal of indigos in order to create abrasion effects on fabrics. However, fungal laccases such as the laccase from *Trametes versicolor* have proved to be more effective for this purpose [97]. Laccases are useful for the fixation of dyes on wool; this process is very economical at a large scale as less dye concentration is required and deep color fixation takes place [98]. Roving treatment of yarn

under mild conditions enhances the regularity of yarn; use of laccases ensures desirable results with no impact on the integrity of the environment [23, 99]. Conventional anti-shrinking treatment for wool involves chlorination which has a hazardous impact on the eco-system; however, laccases use along with a mediator is a suitable method for preventing fabric shrinking [100]. Azo dyes make about half of the total synthetic dyes; laccases from bacterial sources such as *Streptomyces* have been reported to be used for the removal of azo dyes [101]. Bacterial laccases originated from *Trametes versicolor* [69], *Stenotrophomonas maltophilia*, and *Streptomyces psammoticus* are competent for the removal of a wide range of synthetic dyes [70].

Paper and Pulp Industry

Paper industries use the chlorination process for the delignification of wood and bleaching of paper, which is a pollution-generating process [102]. From the past few years, conventional chemical delignification and bleaching methods have been replaced by a safer method which includes the use of laccases from microbial sources. These enzymes not only help in effective delignification but also help in maintaining the integrity of cellulose [103] to get a better quality paper. As laccases cannot degrade lignin directly, various mediators are required for the proper activity of the enzymes. Therefore, during the process of delignification, the oxidation of the mediator is carried out first, and then, the oxidized mediator oxidizes the lignin in the wood [104]. Pre-bleaching of pulp with laccase reduces the need for hypochlorite by 10% and has been proved helpful in achieving as much brightness of resultant paper as achieved in the case of chemically treated pulped [105].

Laccases from bacterial sources such as *P. stutzeri* (soil bacterium) [67] and S. *cyaneus* [68] are used in the bleaching of eucalyptus pulp along with the use of HOBt (hydrobenzotriazole) and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) as mediators [72]. Lignocellulolytic based composite materials can be manufactured by using laccases for the adhesion of lignin fibers. The composite material prepared by enzymatic adhesion has good mechanical properties and does not require the use of toxic synthetic adhesives [106]. Recent studies show that the laccases can also be used to graft the various phenolic acids on the surface of eucalyptus kraft pulp fiber [107]. This ability of laccases can be helpful in the future for attaching versatile materials on the surface of fibers or composite materials to achieve hybrid materials with desirable properties such as hydrophobicity and charge [3]. The combination of xylanase and laccase can be used as effective tools for minimizing lignin and xylose contents from the pulp [108]. Both laccases and xylanases have a synergistic effect in enhancing the pulp quality [9]. Old newspapers and used papers can be recycled by using the combination of physical as well as enzymatic methods, while physical methods include sonication and microwaving [109]. Figure 4 represents the paper preparation process in which laccases were used.

Pollution Degradation

The nature and catalytic activity of laccases make them compatible to be used in the biodegradation of various pollutants. Laccases from *Klebsiella pneumonia* are thermostable and pH stable; they can be used for degrading a wide range of dyes (such as brilliant blue X-BR, Congo red, malachite blue, bromophenol blue, etc.) produced during industrial processes under diverse pH ranges and temperature of 70 °C in a very short time of 90 min [6]. *Bacillus pumilus* Cot A laccase expressed in *E. coli* has been

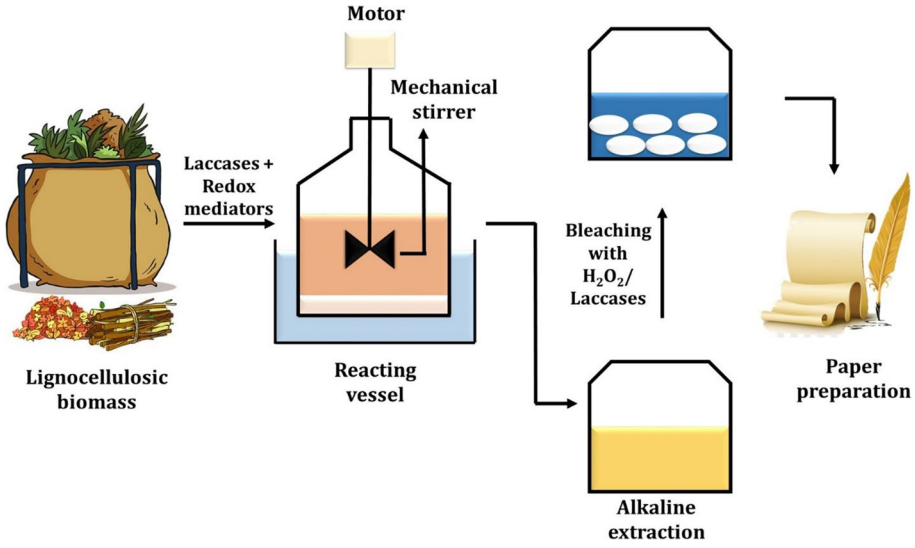

Fig. 4 Use of laccases in paper and pulp industry [105]

implemented for the degradation of a wide range of dyes, and results predict the higher discoloration yield for anthraquinonic and triphenylmethane dyes as compared to aromatic heterocyclic dyes [32]. Moreover, *Cerrena* sp. HYB07, a white-rot fungal strain is known to give a high yield of laccases with high specific activity and decolorizing ability under short production cycles. In this regard, Yang et al. [110] have investigated the application of a purified HYB07 laccase, in decolorization and detoxification of malachite green.

Laccases have also been reported to be helpful in aromatic xenobiotics and other pollutants found in industrial wastewater, contaminated soil, or water [31]. Laccases can inactivate contaminants either by complete degradation or by polymerization. Polymerization can be carried out among pollutants themselves or the copolymerization of pollutants with non-toxic substances. After polymerization, pollutants become insoluble in the water and can be easily removed through adsorption, sedimentation, and filtration [111]. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously present in the environment and are responsible for the contamination of air, soil, and water [112]. The low solubility and recalcitrant nature of PAHs render them resistant to degradation [31]. Therefore, the knowledge of microorganisms that exhibit a high potential to degrade PAHs is essential. Lignocellulolytic bacteria and fungi have the ability to degrade PAHs to achieve a safe level of PAHs in the environment. However, white-rot fungi have more advantages over lignocellulolytic bacteria due to their non-specific nature. Hence, white-rot fungi can help degrade a wide range of pollutants, including PAHs, to an undetectable level [31]. Wastewater from distilleries, beer mills, and olive mills contains an adequate amount of phenolic and non-phenolic compounds, which can pose harm to the environment; this waste can also be treated by using laccases from various microbial sources [31]. Laccases have also been proved useful in oxidizing the estrogenic hormones from sewage water [113]. Studies show that the laccases can mediate the coupling of 2,4,6-trinitrotoluene (TNT) to an organic soil matrix which can help in detoxifying ammunition residues [114].

Biofuels

Lignocellulolytic biomass is one of the most abundant biomass on earth, and recent studies have invented a way to produce bioethanol from lignocelluloses. However, the efficiency of this method depends upon the ease of hydrolysis of polysaccharides which depends upon the cost-effective treatment of lignocellulosic biomass to remove lignin. Lignin removal is an important step to make sugars freely available for the action of hydrolytic enzymes [115]. The use of laccases in degrading lignin is an important breakthrough in the biofuel industry, and they have been proved useful for not only removing lignin from lignocellulolytic biomass but also as biocatalysts for removing yeast growth inhibitors for further enzymatic action [116]. The use of laccases in corn stover hydrolysate has been reported to remove 84% of the phenolic content, and the addition of laccase before cellulose hydrolysis has been helpful in increasing the yield of bioethanol by 10% [117].

Cosmetics

Cosmetic and dermatological preparations containing laccases can be used for skin lightening purposes [118]. Furthermore, they also have application in hair bleaching and dying. The use of H_2O_2 in bleach and hair colors can have a damaging impact on the scalp as well as hair, so laccases are a safe replacement of H_2O_2 [119] as an oxidizing agent in hair dyes and bleaches. Laccases from bacterial sources such as actinomycetes (*Thermobifida fusca*) and basidiomycetes (*Flammulina velutipes*) have been tested for their oxidative potential and are widely used in hair colors [120, 121].

Biomedicines and Pharmaceuticals

Laccases can be used in biosensor systems due to their ability to reduce oxygen to water; consumption of oxygen during analyte oxidation can be a quantitative indicator for the laccase-based biosensors [115]. Laccase-based biosensors can be used in the food industry to detect the presence of polyphenols in fruits juices and beverages in order to monitor the quality of these products [122]. In the biomedical field, laccase biosensors have been developed to monitor the concentration of insulin, morphine, and codeine [123]. The incorporation of blood-tolerant laccases in self-powered and wireless medical devices and environmental sensors can provide a promising edge in creating high-tech devices for enhancing monitoring efficiencies [124]. In the pharmaceutical industry, laccases find applications in the development of antimicrobial and detoxifying agents. Anti-inflammatory agents, sedatives, anesthetic, and antibiotics are being synthesized by using laccases [125]. Anticancer drug actinocin has been made at the expense of laccases. This drug creates a hindrance in the growth of the tumor by interrupting the DNA transcription of tumor cells [126]. Laccases have also been proved efficient against reverse transcriptase of the human immunodeficiency virus (HIV) [127, 128] which have reported the anti-allergic activity of laccases.

Future Prospects and Conclusion

In conclusion, the current study summarizes the occurrence and molecular, biochemical, and structural features of the many bacterial laccases described to date. Use of homologous and heterologous expression systems, expression regulation, laccase mediators,

immobilized enzyme for operational stability, and mutagenesis are the efficient ways that can be optimized and implemented in the future to get higher enzyme yield without increasing production cost to improve enzyme activity and stability. Laccase enzymes have the ability to work on a wide variety of substrates and detoxify a wide variety of contaminants, as well as the oxidation of hazardous compounds, making them valuable in the paper, pulp, and textile industries. However, one of the barriers to broad-scale laccase utilization is the inability to create huge quantities of the highly active enzyme at a cheap price. Recently, the utilization of low-cost sources for laccase manufacturing has been investigated. In this context, an emerging field in industrial wastewater treatment is harnessing its nutritional potential for laccase production. Apart from solid wastes, wastewater from the food processing sector has great potential. Secondly, laccase is involved in the carbon cycle and may aid in the degradation of a broad variety of xenobiotic or phenolic substances. The issue with laccase is that the enzyme has a poor substrate specificity and may possibly catalyze a large variety of reactions. According to some authors, the enzymatic oxidation of aromatic compounds may create by-products that convert blue laccase to yellow laccase (YL), which, unlike blue laccase, does not need a mediator to breakdown pollutants. As a result, further study in this area is required in the near future. Furthermore, despite numerous efforts to understand the role of laccase in the transformation of lignocelluloses, it is also unclear how significant a role laccase plays in lignin degradation, because in plant biomass it could be utilized as an enzymatic pretreatment method in cellulosic ethanol production.

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Declarations

Ethical Statement We assure the integrity and quality of our research work. It is also stated that there is no plagiarism in this work and all points taken from other authors are well cited in the text. This study is completely independent and impartial.

Research Involving Human Participants and/or Animals N/A. This research did not involve human participants and/or animals.

Informed Consent N/A. This research did not involve human participants.

Conflict of Interest The authors declare no competing interests.

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