Biotecnología Aplicada

Elfos scientiae
http://elfosscientiae.cigb.edu.cu/

REVIEW

Fungal laccase mediator and its biocatalytic potential applications: A review

Ram Sahay

Department of Chemistry, Maharana Pratap P.G. College Jungle Dhusan, Gorakhpur 273014, India

rs_chemistry@rediffmail.com

ABSTRACT

Laccases are dioxygen oxidoreductase belonging to the family of multicopper proteins, first isolated from the sap of Japanese lacquer tree Rhus vernicifera. Due to their broad substrate specificity, they are considered as a promising candidate in various industrial and biotechnological sectors. Detailed structure of laccases in their four different copper catalytic forms per protein unit have been delt with PDB database. Mechanism of reaction catalysed by lacasses involves direct and indirect oxidation of the substrate, and it have been studied with the help of e-sources available. Yellow and white category of lacasses have been studied using EPR technique. The efficiency of substrate oxidation by a laccase have been studied using mediators like 2,2′-azino-bis(3- ethylbenzothiazoline -6-sulphonicacid) diammonium salt (ABTS), 1-hydroxybenzotriazole (HBT) , TEMPO, Vilouric acid, syringaldehyde and acetosyringone. Laccases are immobilized for recycling, operational stability, and resistance to application conditions. Laccases have high catalytic efficiency and are used for technical applications in various industrial and biotechnological domains. The present review deals with the application of lacasses in biodegradation of persistent organic pollutants (POPs), pharmaceuticals and personal care products (PPCPs), polycyclic aromatic hydrocarbon (PAHs), dyestuffs, pesticides, antibiotics and endocrine disrupters. Thus, this review would help in understanding laccases along with the areas, which has not been focused and requires attention. Since past, immense work has been carried out on laccases; yet, new discoveries and application are ever increasing which includes biofuel, biosensor, fiber board synthesis, bioremediation, clinical, textile industry, food, cosmetics, and many more.

Keywords: Multi-copper, Laccase, Bioremediation, Biofuel, Biocatalytic potentials, Mediator, PPCPs

RESUMEN

La lacasa fúngica mediadora y sus aplicaciones biocatalíticas potenciales: una revisión. Las lacasas son enzimas dioxígeno oxidoreductasas pertenecientes a la familia de las proteínas multi-cobre, primeramente aisladas de la savia del árbol japonés de la laca Rhus vernicifera. Debido a su amplia especificidad de sustratos, se le ha considerado un candidato prometedor en varios sectores industriales y biotecnológicos. La estructura detallada de las cuatro formas catalíticas del cobre en la lacasa ha sido resuelta en la base de datos PDB. El mecanismo de reacción catalítico involucra a la oxidación directa o indirecta del sustrato, y han sido estudiadas con la ayuda de los recursos electrónicos disponibles. Las categorías amarilla y blanca de las lacasas se ha investigado mediante la técnica de EPR. En el caso de la eficiencia de la oxidación del sustrato, esta ha sido estudiada usando mediadores de sales diamonio de 2,2-azino-bis(3etilbenzotiazolino-6-acido sulfónico)(ABTS), 1-hidroxibenzotiazol (HBT), TEMPO, ácido vilourico, siringaldehido y acetosiringona. Las lacasas se inmovilizan para los fines de reciclaje, estabilidad operacional y la resistencia a las condiciones de aplicación. Estas enzimas tienen alta eficiencia catalítica y se usan para operaciones técnicas en varios dominios industriales y biotecnológicos. La presente revisión trata sobre la aplicación de las lacasas en la biodegradación de contaminantes orgánicos persistentes (POP), productos farmacéuticos y de higiene personal (PPCP), hidrocarbonos aromáticos policíclicos (PAH), tintes, pesticidas, antibióticos y disruptores endorinos. Por lo tanto, esta revisión ayuda a comprender las lacasas y sus áreas de aplicación. Estas enzimas han sido ampliamente estudiadas; sin embargo, continúan los hallazgos y las nuevas aplicaciones en áreas como los biocombustibles, los biosensores, la síntesis de paneles de fibras, la bioremediación, la clínica, la industria textil, los alimentos, los cosméticos y muchas más..

Palabras clave: Multi-cobre, lacasa, biorremediación, biocombustible, potenciales biocatalizadores, mediadores, PPCP

How to cite (Vancouver style):
Sahay R. Fungal laccase mediator and its biocatalytic potential applications: A review. Biotecnol Apl. 2020;38(1):1101-9.

Introduction

Laccases are p-diphenol: dioxygen oxidoreductase belonging to the family of multicopper proteins. It was first isolated from the sap of Japanese lacquer tree *Rhus vernicifera* [1]. They are widely distributed in nature being found in plants, fungi [2-4] insects and bacteria. The first bacterial laccase was discovered in *Azospirillum lipoferum*. Since bacterial laccases have low redox potential [5], fungal laccases [6] are preferred, owing to their high redox potential [7-9]. Laccases have the ability to oxidize a wide range of aromatic and non-aromatic compounds which includes substituted phenols, some inorganic ions, and a variety of non phenolic compounds [10]. Due to its low substrate specificity it can act on a broad range of substrates and has attracted considerable attention in different environmental, industrial and biotechnological sectors [11-12]. Laccases have been regarded as a Green Tool, because they require molecular oxygen (O_2) as the only co-substrate for bio-catalysis and not hydrogen peroxide H_2O_2. Laccases have the capabil-

1. Yoshida H. Chemistry of lacquer (Urishi) part-1. J Chem Soc (Tokyo). 1883;43,472-86.

2. Sahay R, Yadav RSS, Yadav KDS. Purification and Characterization of extracellular laccase secreted by *Pleurotus sajor-caju* MTCC-141.C. J Biotechnol. 2008;24(12):2068-73.

3. Dwivedi UN, Singh P, Pandey VP, Kumar A. Structure function relationship among bacterial, fungal and plant laccases, J Mol Cat B: Enzyme. 2011;68:117-28.

Publicación libre de costo para el autor
No article processing charges

ity to reduce dioxygen to water by one electron oxidation of substrate [13] which are mainly substituted phenolic compounds.

This type of enzymes have high catalytic efficiency and are used for technical applications in various industrial and biotechnological domains [14-15], including: improving properties of fibers, biosynthesis, energy exploitation, environmental protection, biodetection, degradation of synthetic dyes, printing and dyeing industry, biopulping in paper industry, conversion of aromatic compounds [10], and removal of phenols which causes cancer and teratogenicity when present in waste water [16]. Moreover, it is also used in fast moving consumer goods as toothpaste, mouthwash, detergent, soap, and diapers, in cosmetics as deodorants; in beverage and food industry for wine and juice stabilization [17, 18], in dough or baked products to increase strength of gluten structures; in pharmaceutical industries as anesthetics, anti-inflammatory drugs, antibiotics, and sedatives and in nanobiotechnology as nanoparticle-based biosensors. However, laccases are not able to exhibit full efficiency under harsh conditions. Therefore, novel strains which can tolerate harsh conditions and give maximum enzyme production with minimum energy consumption are in huge demand. The present review is an attempt to provide cumulative information on various aspects of fungal laccases, which includes information pertaining to the structure, reaction mechanism, categories, and industrial and biotechnological application of laccase.

Structure and reaction mechanism of laccase

Laccases are known to exist in a variety of forms; they can be monomeric, homotetrameric, heterodimeric, and multimeric. Their molecular weight ranges from 50 to 130 kDa depending on the isolation organism [19]. Plant laccases approximately contains 45 % carbohydrate content, whereas for fungal laccases, it is 10-30 % of its molecular weight [20]. It is assumed that the carbohydrate portion of laccase ensures the conformational stability of the protein part and protects the enzyme from proteolysis and inactivation by radicals. The primary structure of laccases consists of Greek key β barrel topology constituted of approximately 500 amino acid residues organized in three consecutive domains, distributed in 150, 150 and 200, respectively.

The stabilization of laccase structure is due to the presence of disulphide bonds between domains I and II and between domains I and III [21]. However, in *Melanocarpus albomyces*, three disulfide bridges are present of which one is inside domain I, another between domains I and III and the last one between domains II and III [22]. Laccases are known to exist in four different Cu catalytic forms per protein unit. These four Cu ions are divided into three types of structures, name type 1 to type 1 to type 3, corresponding to paramagnetic blue cooper, paramagnetic normal/non-blue cooper and diamagnetic spin coupled cooper-cooper pair, respectively.

Type-1: Paramagnetic blue copper

Type-1 copper confers blue color to multicopper proteins, which is due to the intense electronic absorption caused by the covalent copper cysteine bond. Due to its high redox potential of +790 mV, substrate oxidation takes place at the type-1 copper site and has an absorbance at 610 nm. Type-1 copper has a trigonal coordination, with two histine and a cysteine as conserved equatorial ligands, and one position usually variable, and in case of fungal laccases, the axial ligand is leucine or phenylalanine. It has even been argued that the axial position ligand influences the oxidation potential of the enzyme, which possibly provides the mechanism for regulating its activity [23].

Type-2: Paramagnetic 'normal/non-blue copper

Type-2 or normal Cu site is characterized by the lack of strong absorption features in the visible region and reveals usual electron paramagnetic resonance (EPR) spectra. Type-2 copper is coordinated by two histidines residues and is strategically positioned close to type-3 copper.

Type-3: Diamagnetic spin coupled copper-copper pair

It is a binuclear center regulated by six histidines and spectroscopically characterized by an electron adsorption at 330 nm oxidized form and absence of an EPR signal due to the strong antiferromagnetical coupling between the two type-3 copper atoms which is related to the presence of a hydroxyl bridge. The type-2 copper and type-3 copper form a trinuclear cluster where molecular oxygen is reduced and release of water takes place. An example of one electron oxidation of phenolic hydroxyl groups, while reducing oxygen and forming phenoxy radicals along with water are represented below [24]:

The Type-3 copper centers also have common feature of another protein super family which includes the tyrosinases and haemocyanins [25] (Figure 1).

Reaction mechanism of laccase: direct and indirect oxidation

The basic reactions catalyzed by laccase can be of two types: direct oxidation and indirect oxidation. The direct oxidation involves the oxidation of substrate to the corresponding radical as a result of direct interaction that occurs with copper cluster. However, in certain reactions, direct oxidation is not feasible as laccase can only oxidize those compounds whose ionization potential does not exceed redox potential of T1 copper ion. Nevertheless, the limitation can be overcome by the use of mediator which is a two step process: first enzyme catalyzes the oxidation of the mediator and then the oxidized mediator oxidizes the substrate. However, for the reaction to occur without any obstruction, certain features should be exhibited by the mediator: (a) the reaction should occur without any hindrance it must be good substrate for laccase both in its oxidized and reduced forms, should be stable; it must not inhibit enzymatic reaction, and conversion must be cyclic in nature [26] shown in figure 2.

4. Buddolla V, Bandi R, Avilala J, Arthala PK, and Golla N. Fungal laccases and their applications in Bioremediation. Enzyme Research. 2014;2014:163242.

5. Surwase SV, Patil SA, Srinivas S, Jadhav JP. Interaction of small molecules with fungal laccase: a surface plasmon resonance based study. Enzyme Microb Technol. 2016;82:110-4.

6. Akpinar M, Ozturk UR. Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization, Biotech. 2017:7:98.

7. Sahay R, Yadav RSS, Yadav, KDS. Purification and characterization of laccase secreted by *L. lividus*. Appl Biochem Biotechnol. 2009;157:311-20.

8. Songulashvili G, Flahaut S, Demarez M, Trico TC, Bauvois C, Debaste F, et al. High yield production in seven days of *Coriolopsis gallica*-1184 laccase at 50 L scale; enzyme purification and molecular characterization. Fungal Biol. 2016;120(4):481-8.

9. Feng, Wang, Ling Xu, Liting Zhao, Zhongyang, Ding, Haile Ma, and Norman T. Fungal laccase production from lignocellulosic agricultural wastes by solid-state fermentation. Microorganisms. 2019;7:665.

10. Zheng F, Cui BK, Wu XJ, Meng G, Liu HX, Si J. Immobilization of laccase on to chitosan beads to enhance its capability to degrade synthetic dyes. Int Biodeterior Biodegrad. 2016;110:69-78.

11. Afreen S, Anwer R, Singh RK, Fatma T. Extracellular laccase production and its optimization from *Arthrospira maxima* catalyzed decolorization of synthetic dyes. Saudi J Biol Sci. 2018;25(7):1446-53.

12. Chenthamarakshan A, Parambayil N, Miziriya N, Soumya PS, Lakshmi MSK, Ramgopal A, *et al*. Optimization of laccase production from *Marasmiellus palmivorus* LA1 by Taguchi method of design of experiments. BMC Biotechnol. 2017;17:12.

13. Sahay R, Yadav RSS, Yadava S, Yadav KDS. A Laccase of *Fomes durissimus* MTCC-1173 and its role in the conversion of methylbenzene to benzaldehyde. Appl Biochem Biotechnol. 2012;166: 563-75.

14. Xenakis A, Zoumpanioti M, Stamatis H. Enzymatic reactions in structured surfactant free microemulsions. Curr Opin Colloid Interface Sci. 2016;22:41-5.

15. Nguyen KA, Wikee S, Lumyong S. Brief review: Lignocellulolytic enzymes from polypores for efficient utilization of biomass. Mycosphere. 2018;9:1073-88.

16. Pang S, Wu Y, Zhang X, Li B, Ouyang J, Ding M. Immobilization of laccase via adsorption onto bimodal mesoporous Zr-MOF. Process Biochem. 2016;51(2): 229-39.

17. Piacquadio P, De Stefano G, Sammartino M, Sciancalepore V. Apple juice stabilization by laccase immobilized on metal chelate regenerable carriers. Industrie Delle Bevande. 1998;27:378-83.

18. Alper N, Acar J. Removal of phenolic compounds in pomegranate juices using ultrafiltration and laccase ultrafiltration combinations. Food/Nahrung. 2004;48(3):184-7.

Besides mediators, the use of inducer to enhance laccase production has been widely practiced in fungi especially in the white rots where metals, aromatic compounds, and phenolic compounds [27] have been used as inducers. Conversely, there are certain substances which can inhibit the production of laccase and are known as the inhibitors such as sodium azide and DTT.

Yellow laccase

Yellow laccase is artificially reduced blue laccase as it does not have absorption at 600 nm and EPR spectrum [28]. Alteration of yellow to blue laccase can occur by the reduction of type I copper site by aromatic product of lignin degradation or binding of specific amino acid of enzyme polypeptide to a molecule of modified product produced by lignin degradation; it can also be due to heterogeneity induced by glycosylation. The modified molecule bound to the apoenzyme performs the function of electron transfer mediator analogous to the role of 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) or other compounds in the reaction of blue laccase, hence having high redox potential which allows them to oxidize nonphenolic compounds without any mediators and having greater industrial potential [29]. The change in protein conformation may explain the sensitivity of yellow laccase to CO and other inhibitors, e.g., *P. tigrinus*. At present, information about the purification and characterization of yellow laccase is extremely limited and their catalytic properties are still seldom investigated. Much work has not been done on the yellow laccase, but a few strains which are reported for the production of yellow laccase are as follows: *P. tigrinus* [28, 29], *S. aeruginosa*, *G. fornicatum* [29], *Pleurotus ostreatus* [30], *Phlebia radiate*, *Phlebia tremellosa*, *Pleurotus ostreatus* D1 (YLPO) and *Sclerotinia sclerotiorum*.

White laccase

The white laccase exhibits neutral pH and has anomalous metal content which is responsible for its unique characteristic [31]. It exhibits absorption peak at 400 nm but absence of peak at 605 nm T1 copper site and 330 nm (T3 binuclear copper). White laccase does not exhibit EPR spectra as well as T1 and T2 signals [32]. The reason for the colorlessness iron, but it was conferred that the lack of absorbance at 605 nm can be due to the incomplete oxidation state of copper which has fully occupied electron configuration of d^{10} and no d-d transition [32] and may be responsible for extra high activity of protein. White laccase has been considered under laccase family, because the primary structure of the white laccase is identical to those of known laccase and it uses oxygen (O_2) as an oxidative substrate. There is the absence of the formation of hydrogen peroxide (H_2O_2) as the product of catalyzed reaction and substrate specificity exhibited is also the same as that of known laccase [31], e.g., *Lepista nuda* molecular mass of 56 kDa [33]. Few strains reported for white laccase production include *P. ostreatus* [31] and *Myrothecium verrucaria* NF-05 [32]. These strains besides single copper atom consist of various.

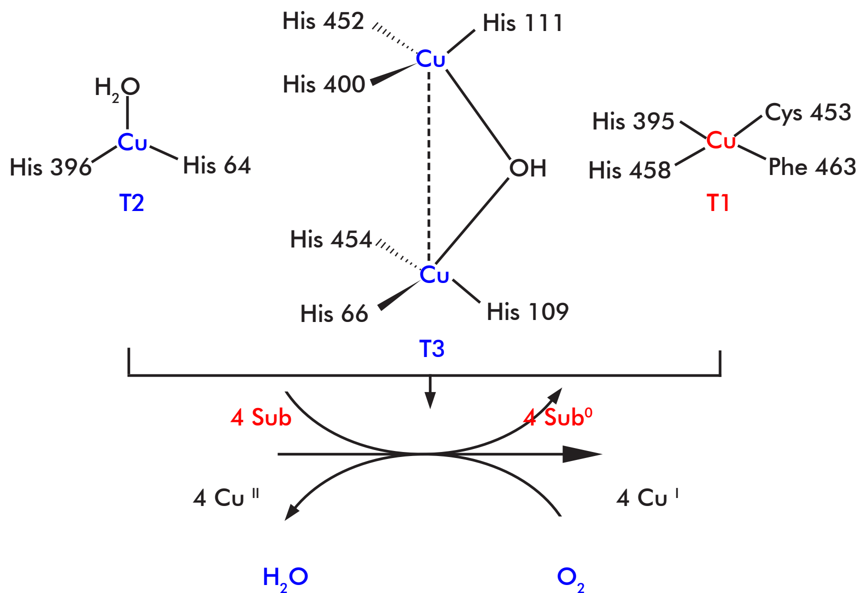

Figure 1. Schematic representation of the active site of the laccase from Trametes versicolor and of the reactions catalyzed in a redox cycle.

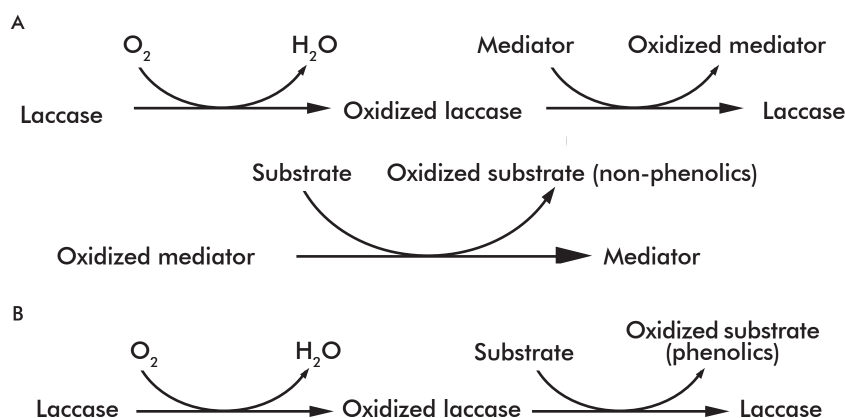

Figure 2. A schematic representation of reaction mechanism of blue as well as yellow laccases for different substrates. A) Oxidation of non-phenolic substrates with mediators, generally done by blue laccases B) Oxidation of phenolic substrates without mediators, generally done by yellow laccases.

Laccase mediator

The efficiency of substrate oxidation by a laccase depends on the difference between the redox potentials [34] of the substrate and the T1 Cu. Due to the lower redox potentials of laccases (≤ 0.8 V) compared to ligninolytic peroxidases (> 1 V) [35] laccases are originally thought to be able to oxidize only the phenolic lignin moiety, with the majority of lignin being nonphenolic and with higher redox potentials. Low molecular- weight redox mediators are used to expand the laccase substrate range or increase the reaction rate, especially for substrates with higher redox potentials or too large to fit in the enzyme's active site. Commonly used laccase mediators include synthetic mediators such as 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and 1-hydroxybenzotriazole (HBT) as well

19. Jaiswal N, Pandey VP, Dwivedi UN. Purification of a thermostable alkaline laccase from papaya (*Carica papaya*) using affinity chromatography, Int. J. Biol Macromol. 2015;72:326-32.

20. Baldrian P. Fungal laccase occurrence and properties. FEMS Microbiol Rev. 2006;30(2):215-42.

21. Plácido J, Capareda S. Ligninolytic enzymes: a biotechnological alternative for bioethanol production. Bioresour Bioprocess. 2015;2(1):23.

22. Hakulinen N, Kiiskinen L, Kruus K, Saloheimo M, Paananen A, Koivula A, Rouvinen J. Crystal structure of a laccase from *Melanocarpus albomyces* with an intact trinuclear copper site. Nat Struct Mol Biol. 2002;9:601-5.

as natural phenolic mediators such as syringaldehyde and acetosyringone. Despite the proven efficiency of artificial mediators, natural mediators (believed to be true mediators of fungal laccases in nature) are considered to be alternatives to the artificial ones because they are more economically feasible and environmentally friendly. Laccase oxidation of the substrate may proceed differently with a mediator. However, it is not always the case. Malachite green degradation products in the presence and absence of ABTS have been shown to be identical or different, depending on the enzyme.

Different types of mediators have different catalytic mechanisms. ABTS mediated substrate oxidation proceeds via an electron transfer route. ABTS is first oxidized to its radical cation (ABTS•+) and then to the dication (ABTS2+) with redox potentials of 472 and 885 mV, respectively. Unlike ABTS, an N-OH type mediator (such as HBT and violuric acid) forms the N-oxy radical upon laccase oxidation and subsequent deprotonation of the radical in turn abstracts the benzylic hydrogen atom from the substrate. Similarly, phenolic mediators also follow a radical hydrogen abstraction mechanism, but with the intermediate being a phenoxy radical. The effect of a mediator on laccase oxidation varies with the laccase and substrate and depends on the radicals formed, recyclability of the mediator and stability of the laccase in the presence of the mediator [36]. Regardless of the reaction mechanism, mediators incur additional costs, and can cause toxicity [37] and laccase inactivation [38]. Although, laccases without the requirement for facilitating mediators, the laccase/mediator system is regarded as a feasible industrial solution, ideal mediators that are cheap, green, effective, stable, recyclable, not toxic, or enzyme-inactivating should be ascertained [39] (Figure 3).

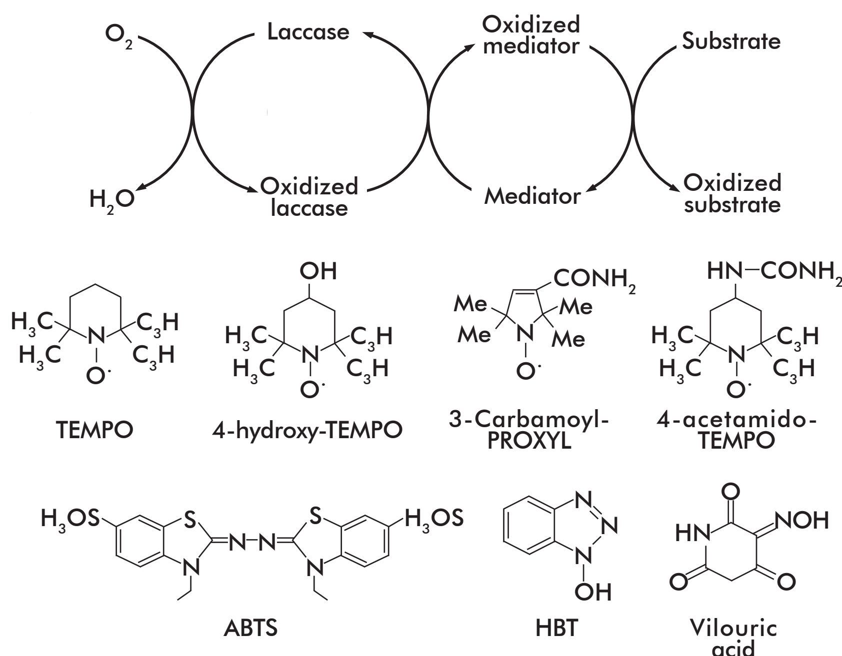

Figure 3. Catalytic cycle and common mediators of laccases.

Laccase immobilization

Laccases are immobilized for recycling, operational stability, and resistance to application conditions. Immobilization techniques include entrapment, adsorption, covalent binding, self immobilization as well as combinations of the aforementioned techniques. Activity recovery varies based on the enzyme, the immobilization method of choice, and preparation parameters [40]. Compared with their free counterparts, immobilized laccases are more tolerant to high temperatures and storage and can be reused multiple times [41, 42]. They are also more resistant to inhibitors such as NaCl. Immobilization sometimes improves the catalytic activity of laccases [43, 44] despite the common concern of reduced enzyme flexibility, steric hindrance and diffusion limitations [45]. Readers can refer to reviews on preparation and applications of immobilized laccases [41, 42].

Laccase applications in biodegradation of PPCPs

The value of fungi as well as fungal enzymes in pollution control and environment management has been recognized. Examples of environmentally important enzymes comprise hydrolases, laccases, lyases, peroxidases, tyrosinases, and P450 cytochrome monooxidases [46-49]. The ability of laccases to effectively degrade and detoxify a variety of persistent organic pollutants (POPs) has received considerable attention in the field of bioremediation [4, 50, 51] and laccases can also be used in enzymatic biosensors for environmental pollution monitoring [47]. A summary of environmental contaminants as laccase substrates is provided in (Table 1). The contaminants investigated include dyestuffs [52, 61], polycyclic aromatic hydrocarbons (PAHs) [60], endocrine disrupters [50, 62] and pesticides [58] shown in figure 4.

Pharmaceuticals and personal care products (PPCPs) are becoming ubiquitous in the environment and are recognized as emerging trace organic contaminants [55, 63, 64]. Laccases can be employed for their removal [50]. Laccases have been used in PPCPs as an ingredient; many products generated by laccases have antimicrobial, anticancer, antioxidant, detoxifying, or other activities [65]. Specifically, laccases can be used to synthesize novel antibiotics and laccase based antimicrobial formulations are considered a safe and green alternative to chemical decontamination. Nonetheless, the focus of this review lies in the degradation and detoxification of PPCP contaminants with laccases.

Degradation of antibiotics

Antibiotics constitute one of the most used classes of drugs in the world; they are used in human and veterinary medicine as well as livestock farming. Antibiotics that are not metabolized enter the environment. Conventional water treatment processes cannot effectively remove antibiotics [55], while more efficient advanced treatment methods have disadvantages such as high costs and secondary

23. Enguita FJ. Structural biology of fungal multicopper oxidases. In: Leitao AL (ed). Mycofactories. Emirate of Sharjah, United Arab Emirates: Bentham Science Publishers; 2011. p. 57-72.

24. Felby C, Thygesen LG, Sanadi A, Barsberg S. Native lignin for bonding of fiber boards evaluation of bonding mechanisms in boards made from laccase-treated fibers of beech (*Fagus sylvatica*). Ind Crops Prod. 2004;20:(2):181-1.

25. Decker H, Terwilliger N. Cops and robbers: putative evolution of copper oxygen binding proteins, J Exp Biol. 2000;203:1777-82.

26. Johannes C, Majcherczyk A. Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl Environ Microbiol. 2000;66(2):524-8.

27. Terrón MC, González T, Carbajo JM, Yagüe S, Arana-Cuenca A, Téllez A, *et al*. Structural close-related aromatic compounds have different effects on laccase activity and on *lcc* gene expression in the ligninolytic fungus *Trametes* sp. I-62. Fungal Genet Biol. 2004;41:(10):954-62.

28. Leontievsky A, Myasoedova N, Pozdnyakova N, Golovleva L. Yellow laccase of Panus tigrinusoxidizes non-phenolic substrates without electron transfer mediators. FEBS Lett. 1997;413(3):446-8.

29. Iracheta-Cárdenas MM, Rocha-Peña MA, Galán-Wong LJ, Arévalo-Niño K, Tovar-Herrera OE. A *Pycnoporus sanguineus* laccase for denim bleaching and its comparison with an enzymatic commercial formulation. J Environ Manage. 2016;177:93-100.

30. Pozdnyakova NN, Rodakiewicz-Nowak J, Turkovskaya OV. Catalytic properties of yellow laccase from *Pleurotus ostreatus* D1. J Mol Catal B: Enzym. 2004;30(1):19-24.

Table 1. Laccase applications in biodegradation and bioremediation

Type of compound	Compound	Laccase	Enzyme source	Mediator	Reference
Dyestuffs	Bromophenol Blue, Congo Red, Coomassie Blue, Tripan Blue	*T. sanguineus* laccase expressed in *T. atroviride*	Free	–	52
	Acid Black 172, Congo Red, Crystal Violet, Direct Fast Blue FBL, Indigo Blue, Naphthol Green B, Methylene Blue, Neutral Red, Reactive Brilliant Blue X-BR, Remazol Brilliant Blue Reactive (RBBR)	*T. pubescens*	Chitosan beads	–	10
	Acid Orange 67, Basic Red 18, Basic Yellow 28, Direct Black 166, Direct Yellow 107, Disperse Yellow 79	*Paraconiothyrium variabile*	Free	HBT	53
	Brilliant Blue G, Brilliant Blue R, Bromophenol Blue, Coomassie Blue R250, Crystal Violet, Malachite Green, Methylene Blue, Xylene Cyanol, RBBR	*P. sanguineus*	Free	VA	29
	RBBR	*Cerrena sp. HYB07*	Cross-linked enzyme aggregates	–	42
	Coomassie Blue R250	*Cerrena sp. HYB07*	Cross-linked enzyme aggregates	ABTS, AS, HBT, SA, SYA	54
Endocrine disrupters	Bisphenol A (BPA)	*Coriolopsis gallica*, *Bjerkandera adusta*, *T. versicolor*	HBT	–	37
		T. sanguineus laccase expressed in *T. atroviride*	In culture	–	52
		T. versicolor laccase expressed in *S. cerevisiae*	Surface display	ABTS	55
		M. thermophila laccase expressed in *A. oryzae* (Novozyme)	On granular activated carbon, continuous flow packed-bed reactor	–	56
	BPA, 17α-ethinylestradiol	*T. versicolor*	Polyamide 6/chitosan nanofibers	–	56
		Phoma sp. UHH 5-1-03	Free	SA	57
	BPA, 17α-ethinylestradiol, 17α-estradiol, 17α-estradiol 17–acetate, estriol, estrone	*M. thermophila* laccase expressed in *A. oryzae* (Novozyme)	Enzymatic membrane reactor	SA	56
Pesticides	Atrazine	*P. ostreatus*	Free	ABTS, HBT, HPI, TEMPO, SA, VA, VAN	29
	Atrazine, isoproturon	*O. sativa* laccases expressed in *P. pastoris*	In culture	–	58
	Ametryn, atrazine, clofibric acid, fenoprop, pentachlorophenol, propoxur	*M. thermophila* laccase expressed in *A. oryzae* (Novozyme)	Enzymatic membrane reactor	SA	56
Phenols	Chlorophenols, cresols, nitrophenols	*Trametes sanguineus* laccase expressed in *Trichoderma atroviride*	In culture	–	52
	Technical nonylphenol	*Phoma sp.* UHH 5-1-03	Free	SA	59
	Oxybenzone, pentachlorophenol	*P. ostreatus*	Free	ABTS, HBT, HPI, TEMPO, SA VA, VAN	29
	4-tert-butylphenol, 4-tert-octylphenol	*Myceliophthora thermophila* laccase expressed in *Aspergillus oryzae* (Novozyme)	Enzymatic membrane reactor	SA	56
	2,4-dichlorophenol	*Pycnoporus sanguineus* CS43	Free	–	36
Polycyclic aromatic hydrocarbons (PAHs)	All 15 US EPA priority PAHs	*B. subtilis* CotA expressed in *E. coli*	Free	ABTS	60
	Naphthalene, phenanthrene	*T. versicolor* (Sigma-Aldrich)	Nonionic surfactant-modified clay	–	60
	Benzo[a]pyrene, phenanthrene	*T. sanguineus laccase* expressed in *T. atroviride*	Free	–	52

pollution. Antibiotics pose health risks by selecting for antibiotic resistant bacteria (ARB). Antibiotics, ARB, and antibiotic resistant genes have been detected in soil, sediments, and water bodies including wastewater drinking water and marine water. There has been a fast growth in the literature describing laccase utilization in antibiotic removal, especially since 2018 on, but this topic has not been properly reviewed.

Target antibiotics under investigation include penicillins, tetracyclines, sulfonamides, quinolones, trimethoprim. Sulfamethoxazole and tetracycline are two most studied (Table 2). The removal time ranges from minutes to hours, depending on the laccase, antibiotic and treatment parameters. Mediators such as HBT, ABTS, and SA are often used to enable or accelerate antibiotic conversion by laccases. In fact, significant antibiotic removal within 1 h usually requires involvement of an appropriate mediator [77]. Manganese peroxidase was more efficient in tetracycline conversion than laccase, but the addition of HBT can promote laccase catalysis to a rate higher than that of manganese peroxidase, although still slower than that of lignin peroxidase (95% degradation efficiency in 5 min). Interestingly, mediators, i.e., ABTS, SA, and AS, are consumed without ob-

31. Palmieri G. Giardina P, Bianco, C Scaloni A, Capasso A, Sannia G. A novel white laccase from *Pleurotus ostreatus*. J Biol Chem. 1997;272(50):31301-7.

32. Zhao D, Zhang X, Cui D, Zhao M. Characterisation of a novel white laccase from the deuteromycete fungus *Myrothecium verrucaria* NF-05 and its decolourisation of dyes. PLoS ONE. 2012;7(6):38817.

33. Zhu M, Zhang G, Meng L, Wang H, Gao K, Ng T. Purification and characterization of a white laccase with pronounced dye decolorizing ability and HIV-1 reverse transcriptase inhibitory activity from *Lepista nuda*. Molecules. 2016;21(4):415.

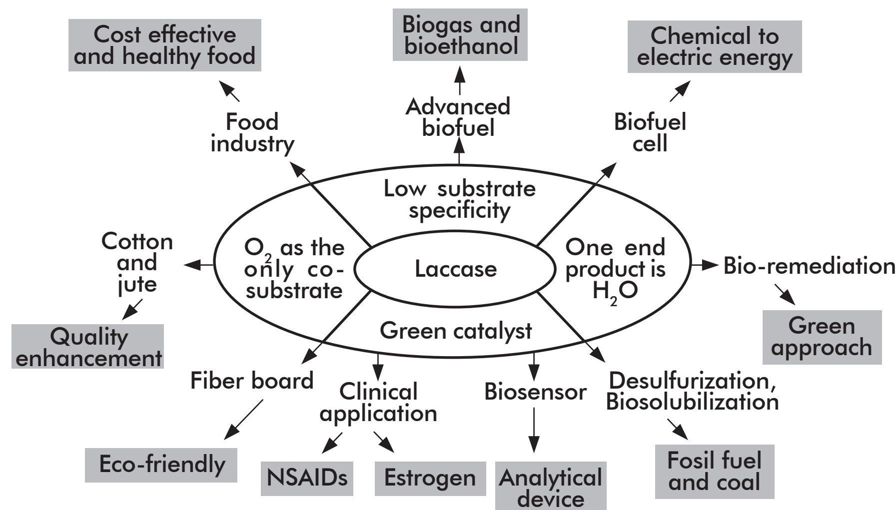

Figure 4. Applications of laccase in various industries and biotechnological sectors. The inner ring stand for laccases' properties, elements in white represent application sectors and gray boxes stand for endpoint applications and products.

served catalytic activity during degradation of sulfamethoxazole [53]. Sulfonamides and tetracyclines are more easily attacked by laccase compared with quinolones [67].

This is presumably due to the strong electron donating aromatic amine group in sulfonamides and the phenol group in tetracyclines, which are not found in quinolones. However, identified tetracycline transformation intermediates suggest that the phenol group is not the primary target for laccase oxidation, and that oxygen addition, demethylation, water elimination reactions occur during laccase treatment [75]. For sulfonamides, increasing electronegativity of the substituents is accompanied by decreased degradation. Two sulfonamides, namely sulfapyridine and sulfathiazole, are desulfonated by laccase. Covalent cross coupling of sulfonamides is observed with laccase and mediator SA or AS [68], but not ABTS [72]. Trimethoprim has 2 amine groups and 3 methoxy groups and is usually administered in combination with sulfamethoxazole. Little [66] to over 60% [71] degradation of this antibiotic without a mediator have been reported. Furthermore, SA at 1,000 µM, but not 10 µM, increases trimethoprim removal from 27 to 67 %; nearly complete elimination of sulfamethoxazole is achieved under the same conditions [76].

Some antibiotics (e.g., penicillins) are unstable in aqueous solutions, and attention should be paid to sample preservation and quantification [76]. Laccase from *T. versicolor*, especially the product sold by Sigma-Aldrich, is most frequently used in biodegradation studies of antibiotics as well as other trace organic contaminants. Other laccases include laccases from basidiomycetes *Cerrena* sp. HYB07, *Echinodontium taxodii*, *Perenniporia* strain TFRI 707 and *P. sanguineus*, from ascomycetes *Phoma* sp. and *Myceliophthora thermophila* (recombinantly expressed in *Aspergillus oryzae*) and from actinobacteria *Streptomyces ipomoeae* (expressed in *E. coli*). Laccases immobilized by different methods have been used for antibiotic degradation; including enzymatic membrane reactors [54, 56, 73, 74], granular activated carbon [59], silica beads oriented immobilization [68], magnetic cross linked enzyme aggregates and cell surface display [67]. In particular, enzymatic membrane reactors (gelatin ceramic membranes grafted with commercial *T. versicolor* laccase) in tetracycline degradation have been evaluated in depth with respect to membrane preparation, efficiency, kinetics, and economics [76]. Mathematical cost estimation indicates that the enzymatic process is still economically uncompetitive. Improvements should be made in terms of enzyme kinetics, reactor effective lifetime and regeneration costs. For example, a pore diameter of 1.4 µm, in contrast to 0.2 µm, increases enzyme loading of the membrane reactor, avoids extensive membrane area, and facilitates tetracycline degradation.

Occasionally, laccases do not participate in antibiotic removal by white rot fungi; for instance, laccase was not responsible for oxytetracycline degradation by *P. ostreatus* or *T. versicolor* or sulfamethoxazole degradation by aquatic ascomycete *Phoma* sp. UHH 5-1-03. In these cases, other enzymes, such as cytochrome P450, may be responsible for biodegradation. It should still be pointed out that even when extracellular laccase is not able to directly oxidize sulfamethoxazole, when a mediator is added, significant removal is achieved.

Laccases are also applied in combination with other processes in antibiotic treatment, such as ultrasound [57] and soil adsorption. The involvement of other processes facilitates degradation of antibiotics, e.g., quinolone antibiotics, which are recalcitrant to laccase oxidation. Laccase can also improve efficiency and stability of antibiotic removal by other organisms. When sulfamethoxazole is the transformed by non-laccase producing bacterium *Alcaligenes faecalis*, the efficiency drops when some metabolites such

34. Ashe B, Nguyen L N, Hai FI, Lee DJ, Vande Merwe JP, Leusch FDL et al. Impacts of redox mediator type on trace organic contaminants degradation by laccase: degradation efficiency, laccase stability and effluent toxicity. Int Biodeterior Biodegradation. 2016;113:169-76.

35. Sitarz AK, Mikkelsen JD, Meyer AS. Structure, functionality and tuning up of laccases for lignocellulose and other industrial applications. Crit Rev Biotechnol. 2016;36:70-86.

36. Rodríguez-Delgado M, Orona-Navar C, García-Morales R, Hernandez-Luna C, Parra R, Mahlknecht J, et al. Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from *Pycnoporus sanguineus* CS43 fungi. Int Biodeter Biodegr. 2016;108:34-41.

37. Daâssi D, Prieto A, Zouari-Mechichi H, Martínez MJ, Nasri M, Mechichi T. Degradation of bisphenol A by different fungal laccases and identification of its degradation products. Int Biodeterior Biodegradation. 2016;110:181-8.

38. Becker D, Varela Della Giustina S, Rodriguez-Mozaz S, Schoevaart R, Barcelo D, de Cazes M, *et al*. Removal of antibiotics in wastewater by enzymatic treatment with fungal laccase. Degradation of compounds does not always eliminate toxicity. Bioresour Technol. 2016;219:500-9.

39. Weng SS, Liu SM, and Lai HT. Application parameters of laccase-mediator systems for treatment of sulfonamide antibiotics. Bioresour Technol. 2013;141:152-9.

40. Fatemeh T, Davood A. Urease covalently immobilized on cotton-derived nanocellulose-dialdehyde for urea detection and urea-based multicomponent synthesis of tetrahydro-pyrazolopyridines in water. RSC Adv. 2019;9:41893.

41. Fernández-Fernández M, Sanromán MÁ, Moldes D. Recent developments and applications of immobilized laccase, Biotechnol Adv. 2013;31:1808-25.

Table 2. Laccase treatment of antibiotics

Type of antibiotic	Antibiotics	Lacasse	Enzyme source	Reaction parameters	Efficiency	Toxicity after treatment	Reference
Dihydrofolate Reductase Inhibitor	Trimethoprim	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 μg/L antibiotic, 1 mM SA, starting pH 6, 25 °C, 0.07 m/s flow, tangential configuration	66.8% after 24 h	Increased (*B. subtilis* and *V. fischeri*)	38
		T. versicolor (Fluka)	Magnetic cross-linked enzyme aggregates	100 μg/L antibiotic, 1 U/mL laccase, 0.1 mM ABTS, pH 7, 20 °C, 125 rpm	47% after 6 h; 60% after 12 h	NR	71
Nitroimidazole	Metronidazole	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 μg/L antibiotic, 10 μM SA, starting pH 6, 25 °C, 0.07 m/s flow, tangential configuration	25.9% after 24 h	Increased (*B. subtilis* and *V. fischeri*)	38
Peniclins	Amoxicillin, ampicillin, cloxacillin, penicillin G, penicillin V, oxacillin	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 μg/L antibiotics, 1 mM SA, starting pH 6, 25 °C, 0.07 m/s flow, tangential configuration	54-100% after 24 h	Increased (*B. subtilis* and *V. fischeri*)	38
Quinolones	Ciprofloxacin	*A. oryzae*	Free	10 mg/L antibiotic, 0.02% (w/v) laccase, pH 6, 60 °C, 200 rpm ultrasound (75 W, 22 kHz, 50% duty cycle)	51% after 5 h	NR	59
	Cinoxacin, ciprofloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, flumequine, marbofloxacin, nalidixic acid, norfloxacin, ofloxacin, orbifloxacin, oxolinic acid, pipemidic acid	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 μg/L antibiotics, 10 or 1,000 μM SA, starting pH 6, 25 °C, 0.07 m/s flow, tangential configuration	0-84% after 24 h with 10 μM SA; 15–93% after 24 h with 1,000 μM SA	Increased (*B. subtilis* and *V. ischeri*)	38
	Flumequine	*T. versicolor* (Sigma-Aldrich)	Free	90 mg/L antibiotic, 6 U/mL laccase, 1.35 mM ABTS, pH 4, 39 °C, 150 rpm	98% after 2 h	NR	67
Sulfonamides	Sulfadiazine, sulfamethazine, sulfamethoxazole	*Echinodontium taxodii*	Oriented immobilization on Fe3O4 nanoparticles	50 mg/L antibiotic, 0.2 U/mL laccase, 1 mM AS, SA or SYA, pH 5	>95% after 30 min	Reduced (*E. coli* and *S. aureus*)	69
	Sulfadimethoxine	*T. versicolor*	Free	Per gram soil: 2 μg antibiotic, 10 U laccase, 8 μmol ABTS or HBT, 1 mg peat; room temperature	>90% after 72 h	NR	76
	Sulfadimethoxine, sulfamonomethoxine	*Perenniporia* strain TFRI 707	Free	50 mg/L antibiotic, 6 U/mL, 1 mM ABTS or VA, pH 4.1, 30 °C, 8 % glycerol	t(1/2) (min): 1.8–4.1	NR	77
		Perenniporia strain TFRI 707	Free	50 mg/L antibiotic, 6 U/mL laccase, 8 % glycerol; 1 mM ABTS, pH 4, 50-60 °C; 1 mM VA, pH 4, 40-60 °C; 2 mM SA, pH 6, 50 °C	100% after 30 min with ABTS; 100% after 15 min with VA; >95% after 60 min with SA	Reduced (*V. fischeri*) with VA and HBA; increased with ABTS and SA	39
	Sulfamethoxazole	*M. thermophila* laccase expressed in *A. oryzae* (Novozyme)	Enzymatic membrane reactor	830 μg/L antibiotic, 70–100 μM/min laccase, 5 μM SA, 3 g/L granular activated carbon	65 %	Increased (Tox Screen assay with *Photobacterium leiognathi*), which can be reduced by granular activated carbon addition	72
		T. versicolor	Free	1,100 μg/L antibiotic, 1 mM HBT, 25 °C, 70 rpm	41 % after 22 h	NR	47
		T. versicolor (Sigma-Aldrich)	Free	73-93 μM antibiotic, mediator/laccase ratio: 1.1 (ABTS), 1.7 (SA) or 2.4 (AS), pH 6, 25 °C, static	100 %	Reduced (algae *Pseudokirchneriella subcapitata*)	70
		T. versicolor Lac3 expressed in *S. cerevisiae*	Surface display	30 μM antibiotic, 0.25 U/mL laccase, pH 5, 37 °C, 250 rpm	44 % after 30 h	NR	55
	Sulfamethoxazole, sulfathiazole	*T. versicolor*	On porous silica beads	50 mg/L antibiotic, 1 U/mL laccase, 1 mM HBT, pH 5, 40 °C, 50 rpm	76-85 % after 1 h	Reduced (*E. coli*, *P. aeruginosa*, *H. influenza*, *S. enterica*, *S. aureus*, *S. pneumoniae*)	75
	Sulfapyridine, sulfathiazole	*T. versicolor* (Sigma-Aldrich)	Free	16–20 mg/L antibiotic, 50–55 U/L laccase, 0.8 mM VA, pH 4.5, 25 °C, 135 rpm	100 % after 8 h	NR	74

Table 2. Laccase treatment of antibiotics (continued)

Type of antibiotic	Antibiotics	Lacasse	Enzyme source	Reaction parameters	Efficiency	Toxicity after treatment	Reference
Tetracyclins	Chlortetracycline, doxycycline, oxytetracycline, tetracycline	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	10 μg/L antibiotics, 10 μM SA, starting pH 6, 25 °C, 0.07 m/s flow, tangential configuration	85-98 % after 24 h	Increased (*B. subtilis* and *V. fischeri)*	38
	Oxytetracycline, tetracycline	*Cerrena* sp. *HYB07*	Magnetic cross-linked enzyme aggregates	100 μg/mL antibiotic, 40 U/mL laccase, pH 6, 25 °C	80 % after 12 h	Reduced (*E. coli* and *B. licheniformis*)	68
	Tetracycline	*T. versicolor* (Sigma-Aldrich)	Enzymatic membrane reactor	20 mg/L antibiotic, 0.002 g/L laccase, pH 6, 25 °C, batch	0.34 mg/h for 10 d	NR	73
				20 mg/L antibiotic, 10 g/L laccase, 1.4 μm pore size, 25 cm length, tangential (10 L/h), 25 °C, 8 L/h/m2 permeation	> 200 mg/h/m² for 24 h	NR	73
		T. versicolor	Free	100 μg/mL antibiotic, 17.5 μg/mL laccase, pH 7, 20 °C	78% after 18 h	Reduced (B. subtilis)	68

NR: Not reported.

as N4 acetyl sulfamethoxazole are transformed back to the parent compound. The removal efficiency does not decrease when the coculture of *A. faecalis* with laccase producing *P. sanguineus* is used or when cell free laccase was added to *A. faecalis* culture [69]. Toxicity of antibiotics after laccase treatment is commonly accessed via growth inhibition assay or bioluminescence inhibition test. Antibiotic degradation by laccase mostly leads to reduced toxicity. A good example comes from the comparison of the sulfamethoxazole transformation products and their toxicity by *A. faecalis* with or without exogenous laccase. N-hydroxy sulfamethoxazole (HO-SMX), a toxic and recalcitrant intermediate of sulfamethoxazole, is formed upon A. faecalis treatment. Additional laccase, on the other hand, eliminates HO-SMX along with the toxicity. However, sometimes laccase/mediator catalyzed antibiotic transformation results in even higher toxicity and this seems to frequently associate with the mediator SA [69]. It is postulated that the enhanced toxicity can be derived from oxidation of aromatic structures, especially phenols, to quinonoids.

The majority of studies on antibiotic degradation were carried out in aqueous environments, but there have been a few studies on remediation of soil, river sediment and sludge [70]. A laccase-containing extract from spent mushroom compost of *Pleurotus eryngii*, and extract containing microcapsules, enhanced degradation of three tetracyclines in river sediments, as well as degradation of three sulfonamides in sewage sludge. Sulfonamide antibiotics can form stable covalent bonds with humic constituents, and laccase can catalyze unreactive hydroquinone moieties in humic acid to reactive, electrophilic quionone moieties which in turn react with the antibiotic. This will affect the fate, bioactivity, and extractability of sulfonamides in soils.

Conclusions

Laccases have been regarded as a green catalyst which have high catalytic efficiency and are used for technical applications in various industrial and biotechnological domains. Laccases exist in a variety of forms; monomeric, homotetrameric, heterodimeric, and multimeric and posses four copper catalytic forms per protein unit. Their molecular weight ranges from 50 to 130 kDa depending upon the organism. Laccases undergo direct and indirect oxidation in the presence of substrate. Laccases posses low redox potential as compared to other ligninolytic enzymes and thus less oxidative. Limitations of the reactions catalysed is overcome in the presence of mediators. Commonly used laccase mediators include synthetic mediators such as 2,2'-azino-bis(3- ethylbenzothiazoline -6-sulphonicacid) diammonium salt (ABTS),1-hydroxybenzotriazole (HBT) , TEMPO, Vilouric acid as well as natural phenolic mediators such as syringaldehyde and acetosyringone. Yellow laccases are often called blue laccasses as it does not possess absorption band at 600nm and EPR spectrum. White laccase lacks absorption at 605nm and does not exhibit EPR spectra as well as T1 and T2 signals which is a peculiar character for white laccases. Immobilization improves the catalytic activity of laccases, more tolerant to high temperatures and storage and can be reused multiple times.

The ability of laccases to effectively degrade and detoxify a variety of persistent organic pollutants (POPs) is tabulated in the manuscript. Laccases have been used in PPCPs as an ingredient; many products generated by laccases have antimicrobial, anticancer, antioxidant, detoxifying, or other activities. These enzymes, immobilized by different methods, have been used for antibiotic degradation; including enzymatic membrane reactors, granular activated carbon silica beads, and oriented immobilization magnetic cross linked enzyme aggregates and cell surface display. Laccases are also applied in combination with other processes which facilitates degradation of antibiotics which are recalcitrant to laccase oxidation in antibiotic treatment, such as ultrasound and and soil adsorption. This review clearly professed that laccase is one of the outstanding enzymes being used in peculiar areas of biotechnology such as medicine, bioremediation, mediator which includes improving properties of fibers, biosynthesis, energy exploitation, environmental protection, biodetection,

42. Asgher M, Shahid M, Kamal S, Iqbal HMN. Recent trends and valorization of immobilization strategies and ligninolytic enzymes by industrial biotechnology, J Mol Catal B Enzym. 2014;101:56-66.

43. Yang J, Xu X, Yang X, Ye X, Lin J. Cross linked enzyme aggregates of *Cerrena* laccase: preparation, enhanced NaCl tolerance and decolorization of Remazol Brilliant Blue Reactive. J Taiwan Inst Chem Eng. 2016;65:1-7.

44. Sinirlioglu ZA, Sinirlioglu D, Akbas F. Preparation and characterization of stable cross-linked enzyme aggregates of novel laccase enzyme from *Shewanella putrefaciens* and using malachite green decolorization. Bioresour Technol. 2013;146:807-11.

45. Kumar VV, Sivanesan S, Cabana H. Magnetic cross-linked laccase aggregates bioremediation tool for decolorization of distinct classes of recalcitrant dyes. Sci Total Environ. 2014;487:830-9.

46. Talekar S, Joshi A, Joshi G, Kamat P, Haripurkar R, Kambale S. Parameters in preparation and characterization of cross linked enzyme aggregates (CLEAs). RSC Adv. 2013;3:12485-511.

47. Demarche P, Junghanns C, Nair RR, Agathos SN. Harnessing the power of enzymes for environmental stewardship. Biotechnol Adv. 2012;30:933-53.

48. Yang S, Hai FI, Nghiem LD, Roddick F, Price WE. Removal of trace organic contaminants by nitrifying activated sludge and whole cell and crude enzyme extract of *Trametes versicolor*. Water Sci Technol. 2013; 67:1216-23.

49. Yadav M, Yadav HS. Applications of ligninolytic enzymes to pollutants, wastewater, dyes, soil, coal, paper and polymers. Environ Chem Lett. 2015;13:309-18.

50. Martinkova L, Kotik M, Markova E, Homolka L. Biodegradation of phenolic compounds by *Basidiomycota* and its phenol oxidases: a review, Chemosphere. 2016;149:373-82.

51. Gasser CA, Ammann EM, Shahgaldian P, Corvini PFX. Laccases to take on the challenge of emerging organic contaminants in wastewater. Appl Microbiol Biotechnol. 2014;98:9931-52.

degradation of synthetic dyes, printing and dyeing industry, biopulping in paper industry, conversion of aromatic compounds and removal of phenols which causes cancer and teratogenicity when present in waste water. In addition, it is also used in fast moving consumer goods (FMCG) as tooth-paste, mouthwash, detergent, soap, and diapers in cosmetics as deodorants; in beverage and food industry for wine and juice stabilization.

Acknowledgements

The author R. Sahay thankfully acknowledges the support of Department of Chemistry, Maharana Pratap P. G. College J. D. Gorakhpur, India.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

52. Catherine H, Penninckx M, Frederic D. Product formation from phenolic compounds removal by laccases: a review, Environ Technol Innov. 2016;5:250-66.

53. Mikolasch A, Manda K, Schluter R, Lalk M, Witt S, Sefeldt S. Comparative analyses of laccase catalyzed amination reactions for production of novel α-lactam antibiotics. Biotechnol Appl Biochem. 2012;59:295-306.

54. Yang J, Yang X, Ye X, Lin J. Destaining of Coomassie Brilliant Blue R-250-stained polyacrylamide gels with fungal laccase. Anal Biochem. 2016;493:27-9.

55. Onesios KM, Yu JT, Bouwer EJ. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation. 2009;20:441-66.

56. Nguyen LN, Vande Merwe JP, Hai FI, Leusch FD, Kang J, Price WE. Laccase-syringaldehyde-mediateddegradation of trace organic contaminants in an enzymatic membrane reactor: removal efficiency and effluent toxicity. Bioresour Technol. 2016;200:477-84.

57. Hofmann U, Schlosser D. Biochemical and physicochemical processes contributing to the removal of endocrine disrupting chemicals and pharmaceuticals by the aquatic ascomycete *Phoma* sp. UHH 5-1-03. Appl Microbiol Biotechnol. 2016;100:2381-99.

58. Husain Q, Qayyum S. Biological and enzymatic treatment of bisphenol A and other endocrine disrupting compounds: a review. Crit Rev Biotechnol. 2012;33:260-92.

59. Sutar RS, Rathod VK. Ultrasound assisted Laccase catalyzed degradation of Ciprofloxacin hydrochloride. J Ind Eng Chem. 2015;31:276-82.

60. Senthivelan T, Kanagara J, Panda RC. Recent trends in fungal laccase for various industrial applications: an ecofriendly approach a review. Biotechnol Bioprocess Eng. 2016;21:19-38.

61. Balcazar-Lopez E, Mendez-Lorenzo LH, Batista-Garcia RA, Esquivel-Naranjo U, Ayala M, Kumar VV, *et al*. Xenobiotic compounds degradation by heterologous expression of a *Trametes sanguineus* laccase in *Trichoderma atroviride*. PLoS ONE. 2016;11:14-29.

62. Zeng J, Zhu Q, Wu Y, Lin X. Oxidation of polycyclic aromatic hydrocarbons using *Bacillus subtilis* CotA with high laccase activity and copper independence. Chemosphere. 2016;148:1-7.

63. Huang MT, Lu YC, Zhang S, Luo F, Yang H. Rice (*Oryza sativa*) laccases involved in modification and detoxification of herbicides atrazine and isoproturon residues in plants. J Agric Food. 2016;64:6397-6406.

64. Yang J, Li W, Ng TB, Deng X, Lin J, Ye X. Laccases production, expression, regulation and applications in pharmaceutical biodegradation. Front Microbiol. 2017; doi:10.3389/fmicb.2017.00832.

65. Chen Y, Stemple B, Kumar M, and Wei N. Cell surface display fungal laccase as a renewable biocatalyst for degradation of persistent micropollutants bisphenol A and sulfamethoxazole. Environ Sci Technol. 2016;50:8799-808.

66. Margot J, Copin PJ, Von-Gunten U, Barry DA, Holliger C. Sulfamethoxazole and isoproturon degradation and detoxification by a laccase-mediator system: influence of treatment conditions and mechanistic aspects. Biochem Eng J. 2015;103:47-59.

67. Forootanfar H, Faramarzi MA. Insights into laccase producing organisms, fermentation states, purification strategies, and biotechnological applications. Biotechnol Prog. 2015;31:1443-63.

68. Llorca M, Rodríguez-Mozaz S, Couillerot O, Panigoni K, de Gunzburg J, Bayer S, *et al*. Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by an on-line turbulent flow liquid-chromatography coupled to a high resolution mass spectrometer LTQ-Orbitrap. Chemosphere. 2015; 119:90-8.

69. Weng SS, Ku KL, Lai HT. The implication of mediators for enhancement of laccase oxidation of sulfonamide antibiotics. Bioresour Technol. 2012;113:259-64.

70. Singh G, Kaur K, Puri S, Sharma P. Critical factors affecting laccase-mediated biobleaching of pulp in paper industry. Appl Microbiol Biotechnol. 2015;99:155-64.

71. Nguyen LN, Hai FI, Price WE, Leusch FD, Roddick F, Ngo HH, *et al*. The effects of mediator and granular activated carbon addition on degradation of trace organic contaminants by an enzymatic membrane reactor. Bioresour Technol. 2014;167:169-77.

72. Shi L, Ma F, Han Y, Zhang X, Yu H. Removal of sulfonamide antibiotics by oriented immobilized laccase on Fe3O4 nanoparticles with natural mediators. J Hazard Mater. 2014;279:203-11.

73. Rahmani K, Faramarzi MA, Mahvi AH, Gholami M, Esrafili A, Forootanfar H. Elimination and detoxification of sulfathiazole and sulfamethoxazole assisted by laccase immobilized on porous silica beads. Int Biodeterior Biodegradation. 2015;97:107-14

74. Rodriguez-Rodriguez CE, Garcia-Galan MA, Blanquez P, Diaz-Cruz MS, Barcelo D, Caminal G. Continuous degradation of a mixture of sulfonamides by *Trametes versicolor* and identification of metabolites from sulfapyridine and sulfathiazole. J Hazard Mater. 2012;213:347-54.

75. Becker D, Rodriguez MS, Insa S, Schoevaart R, Barcelo D, Cazes M, et al. Removal of endocrine disrupting chemicals in wastewater by enzymatic treatment with fungal laccases. Org Process Res Dev. 2017;21:(4):480-91.

76. De Cazes M, Belleville MP, Mougel M, Kellner H, Sanchez-Marcano J. Characterization of laccase-grafted ceramic membranes for pharmaceuticals degradation. J Membr Sci. 2015;476:384-93.

77. Upadhyay P, Shrivastava R, Agrawal PK. Bioprospecting and biotechnological applications of fungal laccase. Biotech. 2016;6:15.

Received in January, 2021.
Accepted in February, 2021.