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Microbial Laccases: Occurrence, Properties and Applications

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SUMMARY

Laccase (benzenediol:oxygen-oxidoreductase: p-diphenol oxidase, urishiol oxidase, EC1.10.3.2) is a type of copper-containing polyphenol oxidase (PPO) that was discovered in the exudates of the Japanese lacquer tree *Rhus vernicifera* and was also demonstrated as a fungal enzyme. Laccase reduces dioxygen to two molecule of water and simultaneously performs oxidation of many aromatic substrates by removing an electron and a proton from one of their hydroxyl groups. The oxidizing reaction substrate range is fairly broad and includes polyphenols, methoxy-substituted monophenols, aromatic amines and other easily oxidized aromatic compounds. The initial reaction products are oxygen-centred radicles or cation radicles, which usually react further through non-enzymatic routes.

INTRODUCTION

Survival of living organisms on this planet is possible only through the continuous recycling of carbon in the biosphere made possible through the integrated activities of microorganisms, plants and animals. Capturing solar energy, green plants employ photosynthesis to reduce atmospheric carbon dioxide and generate the cellular materials collectively known as biomass. Existence of the rigid, three-dimensional lignin structure in higher plants, which is particularly resistant to biodegradation, allows for the highly efficient photosynthesis by these organisms. Although recalcitrant, lignin can be degraded through the action of specific oxidative microbial enzymes.

Enzymatic systems employed by microorganisms for oxidative transformation of various organic molecules include laccases, tyrosinases, monooxygenases and dioxygenases. Reactions

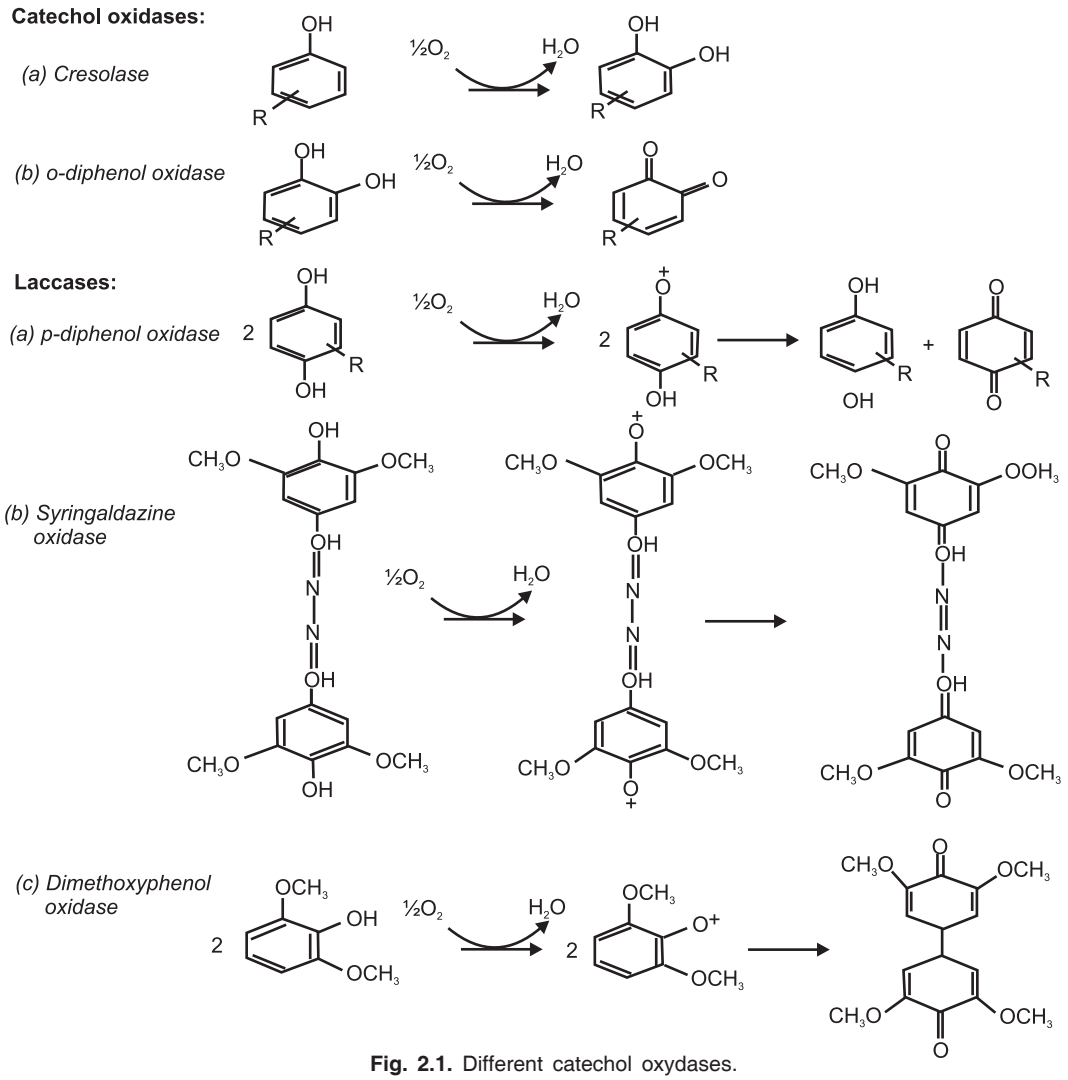

Fig. 2.1. Different catechol oxydases.

phenolic substrates, whereas animal melanins come from the phenolic amino acid L-tyrosine. The natural substrate of fungal tyrosinase is γ-L-glutaminyl-4-hydroxybenzene. Laccases are widely distributed in plants and fungi, but not in animals, where the related protein is ceruloplasmin (Messerschmidt and Huber, 1990; Adman, 1991). These enzymes act on p- and o-diphenols, showing more affinity for the former. The structural diagrams of fungal and bacterial laccase are shown in Fig. 2.2.

Laccases belong to the group of multicopper blue oxidases because they possess three spectroscopically different copper centres, types 1, 2 and 3. Copper type 3 is the only one common to tyrosinases and laccases. Although catechol oxidases and laccases are able to oxidize an overlapping range of compounds, these enzymes have been traditionally differentiated on the basis of substrate specificity and sensitivity to specific inhibitors. Concerning substrate specificity,

the most important differences between both types of enzymes resides in, that only tyrosinase seems to show cresolase activity, the capacity to oxidize L-tyrosine, and only laccase is able to oxidize activated methoxyphenols, such as syringaldazine.

DISTRIBUTION IN NATURE

Laccases are common enzymes found in nature. The first laccase was reported in 1883 from *Rhus vernicifera*, the Japanese lacquer tree, from which the designation laccase was derived. Subsequently, laccases have been discovered in numerous other plants, e.g., sycamore, poplar, tobacco and peach. The plant laccases are not characterized or used extensively despite their wide occurrence, because their detection and purification is often difficult as the crude plant extracts contain a large number of oxidative enzymes with broad substrate specificities. Among eukaryotes, laccases have been found in higher plants such as Chinese or Japanese *Rhus* trees (Mayer and Harel, 1979; Mayer, 1987) and insects. The sources of fungal and bacterial laccases are mentioned in Table 2.2.

Table 2.2: Sources of laccases.

Fungal laccases	Putative bacterial laccases (By Alexander and Zulin)	Known bacterial laccases	Laccases like proteins
Phanerochaete chrysosporium	*Mycobacterium tuberculosum*	*Azospirillum lipoferum*	*Aquifex aeolicus (sufI)*
Pycnoporus cinnabarinus	*Escherichia coli*	*Bacillus subtilis (cotA)*	*Bacillus* sp. *(mnx G)*
Trametes versicolor	*Caulobacter crescentus*	*Bacillus halodurans C-125 (lbh 2082)*	*Bacillus sphaericus*
Agaricus bisporus	*Pseudomonas syringae*	*Marinomonas mediterranea(ppoA)*	*Escherichia coli (yacK)*
Sporotrichum pulverulentum	*Bordetella pertusis*	*Y. proteobacterium JB*	*Leptothix discophora SS1*
Pleurotus ostraetus	*Xanthomonas campestris*		*Oceanobacillus iheyensis (cotA)*
Rhizoctonia praticola	*Pseudomonas aeruginosa*		*Á. proteobacterium SD 21*
Neurospora crassa	*Mycobacterium avium*		*Pseudomonas fluorescens GB-1*
Lentinus edodes	*Pseudomonas putida*		*Pseudomonas maltophila*
Aspergillus nidulans	*Rhodobacter capsulatus*		*Pseudomonas putida (CumA)*
Podospora	*Yersinia pestis*		*Pseudomonas* spp. *(CumA)*
Botrytis cinerea	*Campylobacter jejuni*		*Pseudomonas syringae (copA)*
Fomes annosus	*Escherichia coli*		*Pyrobaculum aerophilum (pae1888)*
Polyporus anceps	*Aquifex aeolicus*		*Streptomyces antibioticus*
Pholiota mutabilis			*Streptomyces griseus (epoA)*
Schizophyllum commune			*Thermus thermophillus HB27*
Monocillium indicum			*Xanthomonas campesteris (copA)*
Cryptococcus neoformans			
Trametes villosa			
Chaetomium thermophilum			
Gaeumannomyces graminis			
Coprinus cinereus			
Marasmius quercophilus			

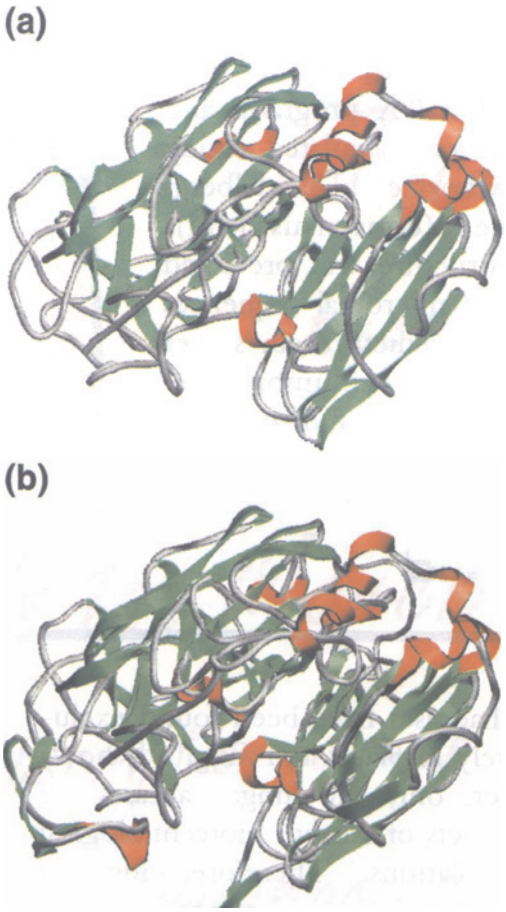

Fig. 2.2. Structural diagram of fungal (a) and bacterial (b) laccase.

THE REACTION MECHANISM OF LACCASE

In contrast to most enzymes, which are generally very substrate specific, laccases act on a surprisingly broad range of substrates, including diphenols, polyphenols, different substituted phenols, diamines, aromatic amines, benzenethiols, and even some inorganic compounds such as iodine (Xu, 1996). When oxidized by a laccase, the reducing substrate loses a single electron and usually forms a free radical. The unstable radical may undergo further laccase-catalyzed oxidation or non-enzymatic reactions including hydration, disproportionation and polymerization. When oxidised by laccase, the substrate donates an electron to the T1 copper. The reduction of oxygen takes place in the trinuclear copper centre, which is located about 12 Å away from T1. One catalytic cycle involves the transfer of altogether four electrons, which are carried from T1 to T2/T3 cluster presumably through a conserved His-Cys-His tripeptide (Messerschmidt *et al.,* 1990). The reaction mechanism of laccase has been studied intensively by monitoring the coordination states of the coppers during the reaction cycle by spectroscopical methods such as

Table 2.3: Properties of laccases.

Property	Range for laccases
Molecular mass (kDa)	60 – 390
pH optimum	3.0 – 7.5
Temp optimum (°C)	40°C – 80°C
No. of polypeptide chains	11
No. of disulphide bridges	3
Carbohydrate content (%)	1 – 45
Copper content (atoms per molecule), PQQ	2 – 16
Redox potential (mV)	180 – 800
Sedimentation coefficient: $S^{20}w$	6.0 – 6.1
Isoelectric points	2.6 – 7.6
No. of isozymes	Up to 5
V_{max} (Ms^{-1})	O_2: 50 – 300
K_{cat} (pH dependence for O_2 substrate)	100 – 2300
K_M (M)	O_2: 10^{-5}

THE REACTIONS CATALYZED BY LACCASE

The reactions catalyzed include demethylation, demethoxylation, decarboxylation, Cα-Cβ cleavage, alkylaryl cleavage, Cα-oxidation (in β-1-lignin model substrate) and formation of phenoxy radicals.

(a) The reaction mechanism: Laccase reduces dioxygen to two molecules of water and simultaneously performs oxidation of many aromatic substrates by removing an electron and a proton from one of their hydroxyl groups. The oxidizing reaction substrate range is fairly broad and includes polyphenols, methoxy-substituted monophenols, aromatic amines and other easily oxidized aromatic compounds. The initial reaction products are oxygen-centred radicals or cation radicals, which usually react further through non-enzymatic routes.

The initial product is typically unstable and may undergo a second enzyme-catalyzed oxidation or non-enzymatic reactions such as hydration or disproportionation and/or may take part in a polymerization reaction giving amorphous insoluble melanin-like products. As one-electron substrate oxidation is coupled to four-electron reduction of oxygen, the reaction mechanism cannot be entirely straightforward and has, therefore, been the subject of much investigation. In a sense, laccase must operate as a battery, storing electrons from individual oxidation reaction in order to reduce molecular oxygen.

(b) Substrates of laccase: Laccases are remarkably nonspecific as to oxidizing their substrate and the range of substrates oxidized varies from one laccase to another (Wood, 1980). Simple diphenols like hydroquinone, pyrogallol and catechol are good substrates (for most laccases, but not all), but guaiacol and 2,6-dimethoxyphenol (2,6-DMP) (both are methoxy-substituted monophenols) are often better. *p*-phenylenediamine (a diamine rather than a diphenol) is a widely used substrate, and syringaldazine [N,N- bis (3,5-dimethoxy-4-hydroxybenzlidene hydrazine)] is considered to be uniquely a laccase substrate as long as hydrogen peroxide is rigorously excluded since this compound is also oxidized by the manganese-dependent peroxidases produced by many lignolytic basidiomycetes. To sum up, laccase is an oxidase that oxidizes polyphenols, methoxy-substituted phenols, diamines and a considerable range of other compounds, but does not oxidize tyrosine (as tyrosinases do).

Despite their different biological functions, the substrate specificity of laccase is close to that of ceruloplasmin. The one or two magnitude higher catalytic rates indicate the better adaptation of laccase to their oxidative function compared to ceruloplasmin, which had been used in determination of aromatic diamines and aminophenols.

(i) Non-phenolics as substrates: In the degradation of lignin by white-rot fungi, the redox potential of the lignin-degrading enzymes has long been believed to play a crucial role because non-phenolic subunits, the most predominant lignin structures in wood, have high redox potentials. The well-studied lignin peroxidase (LiP) is able to oxidize non-phenolic aromatic compounds with very high ionization potentials such as 1,2-dimethoxybenzene ($E_{1/2}$ = 1500 mV) and veratryl alcohol. LiP was thus once believed to be a key enzyme for fungal degradation of lignin, whereas laccase was thought to be less important because it could not oxidize veratryl alcohol (a typical model compound for non-phenolic lignin). The highest redox potential of a laccase reported so far does not exceed 800 mV, which is believed not to be high enough to oxidize a non-phenolic lignin structure. However, it has been demonstrated that laccase is able to oxidize some compounds (redox mediators) with a higher redox potential than itself, although the mechanism by which this happens is not known. In the presence of such redox mediators, laccase is also able to oxidize non-phenolic lignin model compounds.

For a long time it has been recognized that laccase-catalyzed reactions can be modulated. For example, the oxidation of a worse laccase substrate (2,4-dichlorophenol) can be prevented by addition of a good substrate (catechol). In contrast, syringic acid results in a threefold enhancement of the conversion, i.e., "cross-coupling" of substrate can alter the reaction of this chlorinated substrate.

It was only in 1990 when the study showed for the first time that in the presence of recognized primary substrates such as Remazol Brilliant Blue (RBB) and ABTS [2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid)], laccase from *Trametes versicolor* could oxidatively cleave the Cα-Cβ linkage in a non-phenolic β-1 lignin model dimer, and oxidize veratryl alcohol to veratraldehyde. It was proposed that since phenols are likely to be present during wood degradation, laccases play an important role in lignin depolymerization, and that this role is not limited to phenolic subunits. However, laccase could effectively demethylate and delignify hardwood kraft pulp when the mediator ABTS was present.

Studies confirmed that laccase, like other phenol-oxidizing enzymes such as peroxidases, preferentially polymerizes lignin by coupling of the phenoxy radicals produced by oxidation of lignin phenolic groups. It was suggested that laccase possesses both polymerization and depolymerization activities on some preparations of lignosulphonates. However, the results showed that when laccase is used alone, the only reaction that can be observed on kraft lignin is polymerization. The presence of ABTS prevented and even reversed kraft lignin polymerization and promoted delignification of kraft pulp by laccase from *T. versicolor*. They concluded that on the basis of earlier model compound studies, delignifications of pulp and kraft lignin depolymerization with laccase-ABTS are likely to proceed by a combination of two actions: Cα-Cβ cleavage of non-phenolic sites in lignin, and second, solubilization of the lignin fragments by formation of hydrophilic lignin-ABTS complexes.

Laccase mediator systems, commonly referred to as LMS, have been applied to a wide range of applications, such as pulp delignification, textile dye bleaching PAH (polycyclic aromatic

Studies were conducted to compare natural mediators with synthetic mediators in the oxidation of polycyclic aromatic hydrocarbons (PAH) by LMSs. It was found that best natural mediators such as phenol, aniline, 4-hydroxybenzoic acid and 4-hydroxybenzylalcohol were as efficient as ABTS and HBT.

(c) Inhibitors of laccase: Independent of the source, laccase can be inhibited by many anions, which are able to interact with the copper sites, e.g., azide, cyanide, thiocyanide and fluoride. Complexing agents remove copper from the active site, thus inhibiting the activity reversibly. The impact of chelating agents on multicopper oxidases reveals differences among these enzymes. In the past, an influence on activity by EDTA has been reported for several fungal laccases. About 50% inhibition of syringaldazine oxidation was reported for seven fungal laccases from different sources using 3 mM EDTA. One reason for the sensitivity towards chelating agents might be that type 2 copper is exposed to solvent molecule in all blue oxidases. The selective removal by chelating agents [EDTA (ethylenediamine tetra-acetic acid), dimethyl glyoxime (DMG), N, N-diethyldithiocarbamate (DDC) and nitrilotriacetic acid (NTA)] leads to a loss of catalytic activity. But the sensitivity of the copper site is different for laccases and ascorbate oxidases.

For inhibition studies, the compounds usually used include L-cysteine, sodium azide, potassium cyanide, DDC, DMG, tropolone, sodium fluoride, DTT (dithiothreitol) and *p*-coumaric acid.

(d) Inducers of laccase: In fungi, laccase production can be considerably stimulated in the presence of inducing substances like ethanol, vertryl alcohol, 2,5-xylidine, ferulic acid and guaiacol. Copper has been reported as strong inducer in fungi. Its effect on bacterial laccase (γ-proteobacterium JB) showed that laccase levels were readily detectable in cultures supplemented with 0-0.5 mM $CuSO_4$, but highest enzyme titres (13-fold) were recorded with 0.1 mM $CuSO_4$. Ethidium bromide is the only dye reported as inducer of laccase production in *Cyathus bulleri*. Enhanced production of bacterial laccase was observed on addition of different dyes such as Malachite Green (triphenylmethane), ethidium bromide (phenanthridinium), Thymol Blue (triphenylmethane) and Phenol Red (triphenylmethane).

APPLICATIONS OF LACCASES

Laccases harbour great biotechnological potential because of their broad substrate specificity. Current applications for these enzymes include pulp delignification, textile dye bleaching, effluent detoxification, washing powder components, removal of phenolics from wines and transformation of antibiotics and steroids. The catalytic properties of laccases have had a great impact on the development of biosensors for both environmentally important pollutants, phenolics in tea and clinically relevant metabolites. New studies have widened the variety of xenobiotics that can be degraded by laccases in the presence of redox mediators. These range from simple phenols, anilines and benzenethiols (Xu, 1996) to polycyclic aromatic hydrocarbons, organophosphorus insecticides and even nerve agents. The applications of laccases may be broadly divided into industrial, environmental, food, technical and pharmaceutical fields.

Industrial applications: Laccases can be applied as advantageous biocatalysts to replace hazardous/expensive chemicals and save on energy/resources consumption, create novel functionalities, or reduce detrimental impacts on the environment.

could be used to replace these chemicals and serve as a bioadhesive. To initiate or enhance the cross-linking efficiency, laccase could be used in three ways: directly oxidizing wood particles/ pulp to generate radicals for cross-linking, functionalizing wood particles or pulp with small compounds (such as aromatic, carboxyl, isocyanate, or acrylamide substances) which act as cross-linking agents or by transforming isolated lignin (often a by-product from pulping), starch, phenolic polysaccharide, or protein into radical-rich and non-toxic adhesives. Such applications of laccase could not only replace toxic or expensive chemical adhesives, but also transform wastes such as lignin from paper industry into value-added product.

Enhancing ethanol production: The phenolic compounds are important inhibitors of fermentation, and there is a definite advantage of using laccase expressing yeast strains for producing ethanol from lignocellulose (Mayers and Staples, 2002). Laccase from white-rot fungus, *Trametes versicolor*, was expressed under the control of the PGK 1 promoter in *S. cerevisiae* to improve ethnol production from lignocellulosic hydrolysates.

ENVIRONMENTAL APPLICATIONS

Biodegradation: Oxidoreductases may be applied to degrade various substances such as undesirable contaminants, by-products, or discarded materials. Recently, laccase has been shown to be capable of oxidizing and degrading lipids such as trilinolein and methyl linoleate. These unsaturated fatty compounds are not typical laccase substrates. The products include hydroperoxides and epoxides. Laccase probably promoted the initial pentadienyl and subsequent peroxy radical formation. The reaction is of interest because of the occurrence of the fatty compounds in wood and food, which may get involved in laccase catalyzed delignification and food modification, respectively. Laccase may be applied to degrade plastic waste having olefin units. The oxidation of the olefin units by the enzyme, preferably in the presence of small redox mediators, could initiate a radical chain reaction, leading to the disintegration of the plastic. Laccase, peroxidase and oxygenase are being studied as biocatalysts for degrading hazardous coal substances, particularly the sulphur-containing components. The study is of interest in terms of reducing the pollution around coal mines and emission of acid rain causing agents from power plants.

Biodetoxification and bioremediation: Many pesticides, xenobiotics, coal substances, and industrial products derived from polycyclic, aromatic, halogenated hydrocarbons and other organic compounds are hazardous environment pollutants. Use of oxidoreductases to detoxify and remove them is attracting active research efforts. Laccase and peroxidase have been used to transform (often in the presence of redox mediators) various xenobiotics, polycyclic aromatic hydrocarbons and other pollutants found in industrial waste and contaminated soil or water. In general, the redox potential of these compounds is too high for laccase to directly oxidize them via electron transfer. The use of redox mediator allows other reactions, such as H^+ extraction, to take place. The laccase catalysis could result in either direct degradation or polymerization/immobilization. The processes include polymerization among pollutants themselves or copolymerization with other non-toxic substances (such as humic materials). Polymerized pollutants often become insoluble or immobilized, thus facilitating easy removal by means such as adsorption, sedimentation, or filtration. Reported examples of biodegradation by laccase include:

(i) Dechlorination of 2,4,6 trichlorophenol to 2,6-dichloro-1,4-hydroquinol and 2,6-dichloro-1,4-benzoquinone by intact fungal cultures of *Panus tigrinus* and *Coriolus versicolor* and by the purified ligninolytic enzymes including Mn-peroxidases and laccases of both fungi.

(ii) Oxidation of alkenes by laccase from the white-rot fungus, *Trametes hirsuta*. The oxidation is the effect of a two-step process in which the enzyme first catalyzed the oxidation of mediator (added to the reaction), and then the oxidized mediator oxidizes the alkene to the corresponding ketone or aldehyde. The best results were obtained by using hydroxybenzotriazole as the mediator. Aliphatic polyunsaturated and aromatic allyl alcohols were completely oxidized within 2 h at 20ºC.

(iii) Isoxafutole is an herbicide activated in soils and plants to its diketonitrile derivative, the active form of the herbicide. The diketonitrile derivative undergoes cleavage to the inactive benzoic acid analogue. Laccase enzymes from two fungi, *Phanerochaete chrysosporium* and *T. versicolor,* were able to convert the diketonitrile to the acid. Report is present on the rapid oxidation of degradation of O, O-diethyl S-(N, N- Diisopropylaminoethyl) phosphorothiolate (Dipr-Amiton), and the nerve agents (VX) and O-isobutyl S-(N, N-diethylaminoethyl)- methylphosphonothiolate (RVX) by laccase purified from *Pleurotus ostreatus* using ABTS as a mediator. This approach offered an important advantage over existing biocatalyst that require precise protein engineering of the catalytic site and also overcomes the problem of low binding affinity of certain organophosphorus substrates.

Wastewater treatment: Laccase could be used for decolorizing dye house effluents that are hardly decolorized by conventional sewage treatment plants (Abadulla *et al*., 2000). Crude laccase from *Pleurotus ostreatus* immobilized to Eupergit was found to be useful to remove phenolic pollutants. Continuous elimination of 2,6-dimethoxyphenol by immobilized laccase was carried out in a packed-bed reactor followed by filtration of the formed precipitate.

Laccase could be useful for the treatment of agro-industrial effluents containing polyphenols. Lentinula edodes laccase immobilized on Oxirane has been proved to be efficiently stable in removing olive mill wastewater phenolics.

Food applications: Laccase may be applied to certain processings that enhance or modify the color and appearance of food or beverages. In the ripe-olive processing, laccase can replace conventional lye solution and oxidatively polymerize various phenolics (such as oleuropein) in olive, resulting in colour darkening and debittering.

Clarification of juices and wine: Browning, haze formation, and turbidity development during the processing or storage of clear fruit juice, beer, and wine is a major problem for the industry. It is believed that phenolic compounds are involved in this process. Conventionally, undesirable phenolics are adsorbed and removed by various fining agents (e.g., gelatin, bentonite) that usually have low specificity, may affect colour or aroma, and can pose disposal problems. Laccase and other oxidases may be used to remove or modify problematic phenolic saccharides and improve the clarity, colour appearance, flavour, aroma, taste, or stability in fruit juice or fermented alcohol beverages. Following treatment by laccase, oxidized and polymerized (or precipitated) unwanted phenolic substances could be removed by silicate fining or filtration.

Bio-reporters: Oxidoreductase catalysis can be used to assay other enzymes. In these assays, either the enzyme of interest catalyzes the production of a compound whose subsequent transformation by an oxidoreductase generates detectable physical change, or a product from the oxidoreductase catalysis (whose production is accompanied by a detectable physical change) is quenched by the activity of the enzyme of interest. This strategy has been applied using laccase to assay various enzymes including amylase, alanine-, cysteine- or leucine-specific aminopeptidases, alkaline phosphatase, angiotensin I converting enzyme, arylamidase, cellobiose oxidase, chymotrypsin, glucosidase, γ-glutamyl transpeptidase, kallikrein, plasmin, thrombin and coagulant X factor.

The covalently conjugated enzyme to a bio-binding molecule can be used as a reporter for immunochemical (ELISA, Western blotting), histochemical, cytochemical, or nucleic acid-detection assays .The bio-binding molecules include antibody, antigen, DNA, RNA, biotin or streptavidin. Binding of the antibody to its antigen target can be detected by localized laccase activity on a gel or a blot membrane, much like the conventional peroxides or phosphatase assisted immunochemical assays. Under certain conditions, the antibody-antigen binding impairs the function of conjugated laccase, thus allowing immunochemical detection through modulation of laccase activity. The bioreporter applications are of interest for the high-sensitivity required in diagnostic field.

Biofuel cells: Oxidoreductase may be applied as a biocatalyst for the electrode reactions. Laccase may be adsorbed, entrapped, or wired onto the cathode to catalyze the O_2 reduction. A biological fuel cell comprising two carbon fibres coated with the enzymes, glucose oxidase and laccase has been developed and has the ability to produce electricity from glucose and oxygen in the bloodstream (newscientist.com).

PHARMACEUTICAL APPLICATIONS

Organic synthesis: Laccase can be used to synthesize several complex medicinal agents including triazolo (benzo) cycloalkyl thiadiazines, vinblastine, penicillin X dimer, cephalosporin antibiotics, and dimerized vindoline. Laccase can also be used to synthesize various functional organic compounds including polymers with specific mechanical/electrical/optical properties, textile dyes, cosmetic pigments, flavour agents, and pesticides.

Disinfection

One potential application is laccase-based *in situ* generation of iodine, a reagent widely used as a disinfectant. A laccase-iodide salt binary iodine-generating system (for sterilization) may have several advantages over the direct iodine application. First, the iodide salt is more stable and much safer than I_2 in terms of storage, transport, and handling. Second, the release of iodine from a laccase-iodide system can be easily controlled (by means such as adjusting laccase concentration). The system may be used in various industrial, medical, domestic and personal-care applications such as sterilization of drinking water and swimming pools, as well as disinfection of minor wounds.

Antifungal Agent

Leaf spot disease caused by the fungus *Cercospora* is most noted for its economic impact on sugar beet production. The pathogenesis of *Cercospora* can be restricted by degrading its cercosporin toxin with the enzyme laccase. Cercosporin toxin damages the plant by inducing oxygen radical and superoxide production in the presence of oxygen and sunlight. These compounds can degrade cell membrane fatty acids and consequently disrupt the plant cells. Using laccase to prevent cell death inhibits fungal growth by diminishing the food source of fungi. It also reduces secondary infections by pathogenic bacteria that take advantage of the fungal damage.

Medicinal and Other Applications

A low-molecular-mass laccase purified from the mushroom *Tricholoma giganteumis* has been reported to possess significant HIV-1 reverse transcriptase inhibitory activity.

Another laccase has been shown to be capable of fighting aceruloplasminemia (a medical condition of lacking ceruloplasmin, a multi-Cu serum oxidase whose ferroxidase activity regulates iron homeostasis). These promising applications provide a great hope for the utilization of laccases to combat fatal diseases.

Personal-care applications: Oxidoreductases may find use as deodorants for personal-hygiene products, including toothpaste, mouthwash, chewing gum, detergent, soap, and diapers. For instance, laccase can oxidize many thiol, sulphide, ammonia, and amine compounds that cause the malodour in halitosis, bromhidrosis, and hyperhidrosis. Rather than simply masking the malodour with fragrances as conventional deodorants do, an enzymatic system can degrade the offensive molecules, or even kill the microbes that generate them.

Skin care: Poison ivy dermatitis is caused mainly by urushiol, a catechol-derived toxin. Oxidized urushiol (an o-quinone derivative), however, is non-toxic. Laccase has been shown to oxidize, polymerize, and detoxify urushiol, thus reducing the effect of poison ivy dermatitis.

Laccase in denim processing: Several steps are involved in the manufacture of denim garments between dyeing and the final stone washing where excessive amounts of indigo are removed from the fabrics and discharged with the wastewater.

Advantages of Enzyme Washing

(i) Soft handle and attractive clean appearance is obtained without severe damage to the surface of yarn.
(ii) Inexpensive, low-grade fabric quality can be finished to a top quality product by the removal of hairiness fluff and pills, etc.
(iii) Simple process handling and minimum effluent problem.
(iv) Better feel to touch and increased gloss or lustre.
(v) Prevents tendency of pilling after relatively short period of wear.
(vi) Can be applied on cellulose and its blend.
(vii) Mild condition of treatment process is less corrosive.

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