Significance, Production, Physiochmical Characterization, Mode of Action and Applications of Microbial Laccase

Tariq A.L
Department of Biotechnology, Government Degree College Beerwah-193411, Kashmir, India

Abstract

Laccase (Benzenediol: Oxygen oxidoreductase, EC 1.10.3.2) is a multi-copper-bearing lignolytic enzyme, which catalyzes the one-electron oxidation of many phenolic compounds with concomitant reduction of oxygen to water. The laccase catalysis occurs due to the reduction of one oxygen molecule to water accompanied with the oxidation of one electron with a wide range of aromatic compounds which includes polyphenol, methoxy-substituted monophenols, and aromatic amines. It is widely distributed in the higher plants, some insects, a few bacteria, and fungi. Most of the known laccases are of fungal origin, in particular from the white rot fungi. These fungi secrete lignolytic enzymes, which generate radical species that cause the complete biodegradation of lignin polymers. Because of the complex structure of lignin, the biodegradation system is highly non-specific; therefore lignolytic enzymes can be used in the degradation of environmental pollutants that differ structurally. The ability to oxidize priority pollutants with somewhat low substrate specificity has attracted interests in its possible use in wastewater treatment and bioremediation. The laccases have been found in Ascomycetes, Deuteromycetes and Basidiomycetes; being particularly abundant in many white-rot fungi that are involved in lignin metabolism. Fungal laccases have higher redox potential than bacterial or plant laccases (up to +800 mV), and their action seems to be relevant in nature. Regarding their use in the biotechnology area, fungal laccases have prevalent applications, extending from effluent discoloration, detoxification to pulp bleaching, removal of phenolics from wines, organic synthesis, biosensors, synthesis of complex medical compounds and dye transfer blocking functions in detergents and washing powders, many of which have been patented.

KEYWORDS: Laccases, Lignolytic Enzyme, Degradation of Aromatic Compounds.

1. Introduction

Laccase is widely distributed in higher plants, fungi and has been found also in insects and bacteria. Recently a novel polyphenol oxidase with laccase like activity was mined from a metagenome expression library from bovine rumen microflora (Beloqui et al., 2006). The fungal laccases are involved in the degradation of lignin or in the removal of potentially toxic phenols arising during lignin degradation (Zilly, et al., 2012). Concerning their use in the biotechnology area, fungal laccases have widespread applications in effluent decolouration, detoxification, pulp bleaching, removal of phenolics from wines, organic synthesis, biosensors, synthesis of complex medical compounds, dye transfer blocking functions in detergents, washing powders and many of which have been patented (Yaver et al., 2001). The biotechnological use of laccase has been expanded by the introduction of laccase-mediator systems, which are able to oxidize non-phenolic compounds that are otherwise hardly or not oxidized by the enzyme alone.

The ability to oxidize priority pollutants with somewhat low substrate specificity has attracted interests in its possible use in wastewater treatment and bioremediation (Ullah et al., 2000).

2. General properties of laccase enzymes

Most fungal laccases are monomeric, dimeric or tetrameric glycoproteins. Glycosylation of fungal laccase is believed to play a role in secretion, susceptibility to proteolytic degradation, copper retention and thermal stability. Glycosylation content and composition of fungal glycoproteins can vary with growth medium composition. An important feature is a covalently linked carbohydrate moiety (10–45% of total molecular mass), which may contribute to the high stability of the enzyme. The sugar composition has been analyzed in Podospora ansenna, Botrytis cinerea, Trametes hirsuta, Trametes ochracea, Cerrena maxima, Coriolopsis fulvocinerea and Melanocarpus albomyces (Shleev et al., 2004).

3. Isozymes

The isozymes have been found to originate from the same or different genes encoding for the laccase enzyme (Piontek et al., 2002). The number of isozymes present differs between species and within species depending on whether they are induced or non-induced. They can differ markedly in their stability, optimal pH, temperature and affinity for different substrates (Michniewicz et al., 2006). Furthermore, these different isozymes can modulate different roles in the physiology of different species or in the same species under different conditions (Bulter et al., 2003). Native production of laccase by fungi or bacteria usually needs to be induced by the addition of toxic aromatic compounds such as O-toluidine, 3, 5-dihydroxytoluene, 2, 4-trichlorophenol, guaiacol and heavy metal ions including copper ion (Xiao et al. 2003). Moreover the enzymes obtained by recombinant expression of laccase genes are also relatively low for the demands in industrial applications (Hong et al., 2006; Li et al., 2007). Therefore, it is of high priority and urgently needed for scientists to develop substitutive approaches for the production of laccase with high efficiency, environmental friendliness and cost effectiveness.

4. Occurrence and location of laccases

Laccase is the most widely distributed of all the large blue copper-containing proteins, as it is found in a wide range of higher plants, fungi and recently some bacterial laccases have also been characterized from Azospirillum lipoferum, Bacillus subtilis, Streptomyces lavendulae, Streptomyces cyaneus and Marinomonas mediterranea (Diamantidis et al., 2000; Martins et al., 2002; Suzuki et al., 2003). The occurrence of laccases in higher plants appears to be far more limited than in fungi. Laccases in plants have been identified in trees, cabbages, turnips, beets, apples, asparagus, potatoes, pears and various other vegetables. The classical demonstration of laccase in R. vernicifera is well documented. In addition the lacquer tree is member of the Anacardiaceae family appear to contain laccase in the resin ducts and in the secreted resin. Cell cultures of Acer pseudoplatanus have been shown to contain eight laccases, all expressed predominantly in xylem tissue. The presence of a laccase found in leaves of Aesculus parviflora and in green shoots of tea. Laccase from Monocillium indicum was the first laccase to be characterized from an Ascomycete showing peroxidative activity (Thakker et al., 1992).

Pleurotus ostreatus, for instance, belongs to a subclass of lignin degrading microorganisms that produce laccase, manganese peroxidase and veratryl alcohol oxidase but no lignin peroxidase (Stajic, et al., 2006). Pycnoporus cinnabarinus has been shown to produce laccase as the only ligninolytic enzyme and Pycnoporus sanguineus produces laccase as the sole phenol oxidase. In plants, laccase plays a role in lignification, where as in fungi laccases have been implicated in many cellular processes, including delignification, sporulation, pigment production, fruiting body formation and plant pathogenesis. Only a few of these functions have been experimentally demonstrated.

4.1. Occurrence of laccases in bacteria

The first report of prokaryotic laccase is from the rhizospheric bacterium Azospirillum lipoferum (Givaudan et al., 1993). A marine bacterium Marinomonas mediterranea producing two different polyphenol oxidases (PPO), an unusual multi-potent PPO able to oxidize substrates characteristic of both tyrosinase and laccase (Solano et al., 1997). Laccase like activity has also been found in other bacteria e.g., CopA protein from Pseudomonas syringae (Mellano and Cooksey, 1988) and PcoA protein from Escherichia coli (Brown et al., 1995).

5. Mechanism of Laccases

The laccase catalysis occurs due to the reduction of one oxygen molecule to water accompanied with the oxidation of one electron with a wide range of aromatic compounds which includes polyphenol, methoxy substituted monophenols, and aromatic amines. Laccases contain 4 copper atoms termed Cu T1 and trinuclear copper cluster T2/T3. These four copper ions are classified into three categories: Type 1 (T1), Type 2 (T2) and Type 3 (T3). These three types can be distinguished by using UV/visible and electronic paramagnetic resonance (EPR) spectroscopy. At oxidizing state, the Type 1 Cu gives blue colour to the protein at an absorbance of 610nm which is EPR detectable, Type 2 Cu does not give colour but is EPR detectable, and Type 3 Cu contains a pair of atoms in a binuclear conformation that give a weak absorbance in the near UV region but not detected by EPR signal. The Type 2 copper and Type 3 copper form a trinuclear center which is involved in the enzyme catalytic mechanism. The O_2 molecule binds to the trinuclear cluster for asymmetric activation, and it is postulated that the O_2 binding compartment appears to restrict the access of oxidizing agents. During steady state, laccase catalysis indicates that O_2 reduction takes place. Laccase operates as a battery and stores electrons from individual oxidation reactions to reduce molecular oxygen. Hence, the oxidation of four reducing substrate molecules is necessary for the complete reduction of molecular oxygen to water. When laccase oxidizes the substrate, free radicals are generated. The lignin degradation proceeded by phenoxy radical leads to oxidation at α-carbon or cleavage of bond between α-carbon and β-carbon. This oxidation results in an oxygen-centered free radical, which can be converted into a second enzyme catalyzed reaction to quinone. The quinine and the free radicals can then undergo polymerization. The organization of the copper sites in laccase is explained by the spectroscopic studies which reveal that Type 2 copper coordinates two His-N and one oxygen atom as OH−while each copper of Type 3 coordinates three His residues. Further both T2 and T3 copper sites have open coordination positions towards the center of trinuclear cluster with the negative protein compartment (Zoppellaro et al., 2000). The laccase mediated

catalysis can be extended to nonphenolic substrates by the insertion of mediators. Mediators are low molecular weight organic compounds that are oxidized by laccase. The highly active cation radicals oxidize the non-phenolic compounds that laccase alone cannot oxidize. The most common synthetic mediators are 1-hydroxy benzotriazole (HOBT), N-hydroxyphthalimide (NHPI), 2, 2-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) and 3-hydroxyanthranilic acid (Gochev and Krastanov, 2007). In presence of ABTS oxygen uptake by laccase is faster than the HOBT.

6. Production of Laccase

Laccases are the enzymes which are secreted out in the medium extracellularey by several fungi during the secondary metabolism such as Zygomycetes and Chytridiomycetes (Morozova et al., 2007). Ligninolytic enzyme production by wood rotting fungi is a phenomenon involving the interaction between fungal physiology and composition of the essential media used for cultivation (Bakkiyaraj et al. 2013).The literature describes the production of laccase by soil as well as some freshwater Ascomycetes species (Abdel-Raheem and Shearer, 2002). Basidiomycetes and Saprotrophic fungi are the most widely known species that produce substantial amount of laccase in changeable quantity (Dos Santos, et al., 2015). In case of Pycnoporus cinnabarinus, laccase was the only ligninolytic enzyme which degrades lignin. The brown-rot fungus Coniophora puteana oxidizes the syringaldazine and supports the oxidation of ABTS in Laetiporus sulphureus (Schlosser and Hofer, 2002). The robustness of a high-redox potential laccase has been enhanced by swapping its second cupredoxin domain with that from another fungal laccase, which introduced a pool of neutral mutations in the protein sequence without affecting enzyme functionality. The new laccase showed outstanding stability to temperature, pH (2–9) and to organic solvents, while maintaining the ability to oxidize high-redox potential substrates. By engineering the signal peptide, enzyme secretion levels in Saccharomyces cerevisiae were increased, which allowed to purify the engineered enzyme for further characterization. The purified domain-swap laccase presented higher activity in the presence of ethanol or methanol, superior half-lives at 50–70 °C, improved stability at acidic pH, and similar catalytic efficiency for DMP albeit a lower one for ABTS (due to a shift in optimum pH). A new N-glycosylation site and a putative new surface salt bridge were evaluated as possible determinants for the improved stability by site-directed mutagenesis. Although neither seemed to be strictly responsible for the improved thermostability, the new salt bridge was found to notably contribute to the high stability of the swapped enzyme in a broad pH range. Finally, the application potential of the new laccase was demonstrated with the enzymatic treatment of kraft lignin, an industrially relevant lignin stream, at high temperature, neutral pH and short incubation times (Pardo, et al., (2018).

6.1. Type of Cultivation

Submerged and solid-state modes of fermentation are used intensely for the production of laccase. Wild-type filamentous fungi are used for large-scale production of laccase in different cultivation techniques.

6.1.1. Submerged Fermentation.

Submerged fermentation (SmF) involves the growth of microorganisms in a liquid medium rich in nutrients and with a high oxygen concentration (Rosales et al., 2002). The industrial production of enzymes is mainly performed by SmF. Growth patterns in submerged cultures usually result into uncontrolled growth of mycelium. The extension of the bacterial biomass has profound effects on mass transfer, metabolic rate and product secretion. In this Trametes versicolour is employed which decolorizes the synthetic dye, and for this purpose pulsed system has been developed (Romero et al., 2006).

6.1.2. Solid-State Fermentation.

SSF is suitable for the production of enzymes by using natural substrates such as agricultural residues because they mimic the conditions under which the fungi grow naturally (Pandey et al., 199, Imran, et al., 2016). The lignin, cellulose and hemicelluloses are rich in sugar and promote fungal growth in fermenter and make the process more economical. Different bioreactor configurations have been studied for laccase production such as immersion configuration, expanded bed, tray, inert (nylon) and non-inert support (barley bran) in which tray configuration gave the best response. A tray and immersion configuration is compared for laccase production by using grape seeds and orange peel as substrate (Couto and Toca-Herrera, 2007). Laccase production by both solid-state and submerged fermentation is higher in case of rice bran than other substrates (Akpiner and Ozturk Urek, 2017). The rice bran inductive capability is based on the phenolic compounds such as ferulic acid and vanillic acid which induce the laccase production (Souza et al., 2002). Vantamuri and Kaliwal (2016) stated that rice bran was the best substrate for production under SSF conditions when compared to other lignocellulosic substrates, rice straw, sugarcane bagasse, saw dust and pigeon pea waste. Many agricultural wastes such as grape seeds, grape stalks, barley bran, cotton stalk, molasses waste water and wheat bran are also used as substrate for laccase production (Xin and Geng, 2011). However, laccase production in both solid-state and submerged fermentation did not reach up to the maximum level; that is why prolonged cultivation is required (Lorenzo et al., 2002).

7. Purification of Laccase

Ammonium sulphate precipitation, anion exchange chromatography, desalt/buffer exchange of protein and gel filtration chromatography is being commonly used for the enzyme purification. Single step laccase purification from Neurospora crassa takes place by using celite chromatography and 54 fold purification was obtained with specific activity of 333 U mg^{-1} (Grotewold et al., 1998). Laccase from LLP13 was first purified with column chromatography and then purified with gel filtration. Laccase from T. versicolour is purified by using ethanol precipitation, DEAE-Sepharose, Phenyl-Sepharose and Sephadex G-100 chromatography which is a single monomeric laccase with a specific activity of 91,443Umg−1 (Hess et al., 2002). Laccase from T. versicolour is purified with Ion Exchange chromatography followed by gel filtration with specific activity of 101UmL−1 and 34.8-fold purification (Cordi et al., 2007). Laccase from Stereum ostrea is purified with ammonium sulphate precipitation followed by Sephadex G-100 column chromatography with 70-fold purification (Viswanath et al., 2008). Laccase from fruiting bodies is purified with ammonium sulphate precipitation with 40–

70% saturation and DEAE cellulose chromatography then 1.34 and 3.07 fold purification is obtained respectively (Khammuang et al., 2009).

8. Physiochemical characters of Laccase

Several factors influence laccase production such as type of cultivation (submerged or solid state), carbon limitation and nitrogen source (Gayazov and Rodakiewicz-Nowak, 1996).

8.1. Influence of Carbon and Nitrogen Source

The organism grown in the defined medium contains 0.1%w/v yeast extract and 1% (w/v) different carbon sources as well as nitrogen sources. Glucose, mannose, maltose, fructose, and lactose are the commonly used carbon sources. The excess glucose and sucrose reduce the production of laccase by obstructing the initiation. This problem of production of enzyme is improved by using polymeric substrates like cellulose (Lee et al., 2004). Yeast extract, peptone, urea, $(NH4)_2SO_4$, and $NaNO_3$ are the commonly used nitrogen sources. Laccase production is triggered by nitrogen depletion but some nitrogen strains do not affect the enzyme activity. Some studies show that the elevated laccase activity was achieved by using low carbon-to-nitrogen ratio while others show that it was achieved at high carbon-to-nitrogen ratio (Buswel et al., 1995; Patel et al, 2009).

8.2. Influence of Temperature

The effect of temperature is limited in case of laccase production. The optimal temperature of laccase differs greatly from one strain to another. It has been found that 25 ºC is the optimal temperature for laccase production in presence of light, but in case of dark, the optimal temperature is 30 ºC. The optimum temperature range for laccase production is between 25 ºC to 30 ºC. The pre-incubation of enzymes at 40 ºC and 50 ºC greatly increased laccase activity (Farnet et al. 2000). The laccase from P. ostreatus is almost fully active in the temperature range of 40 ºC –60 ºC with maximum activity at 50 ºC. The activity remains unaltered after prolonged incubation at 40 ºC for more than 4hours (Palmieri et al., 1993). The laccase produced by T. modesta was fully active at 50 ºC and was very stable at 40 ºC but half-life decreased to 120min at higher temperature at 60 ºC (Nyanhongo et al., 2002).

8.3. Influence of pH

The effect of pH is limited in case of laccase production. The optimum value of pH varies according to the substrate because different substrate causes different reaction for laccases. Many reports suggested that the bell-shaped profile occurs in case of laccase activity. At high pH value, the potential difference between the phenolic substrate and the T1 copper can increase the substrate oxidation while the hydroxide anion (OH^-) binds to the T2/T3 copper centre. These effects help us in determining the optimum value of pH for laccase enzyme. The use syringaldazine as a substrate and determine the effect of pH on enzyme activity in the range of 3.0–8.0. The optimum pH for L1 (isozyme of laccase) was 4.0 whereas the optimum pH for L2 was 5.0 extracted laccase from Trametes versicolour which showed high enzyme activity at broad range of pH and temperature ranges but the optimum activity was found on pH 3.0 at 50 ºC temperature (Han et al. 2005). Laccase extracted from Stereum ostrea showed the highest activity on pH 6.0 at 40

ºC temperature (Valeriano et al., 2009).When fungi are grown in the medium of pH 5.0, the laccase will produce in excess but most studies show that pH between 4.5 and 6.0 is suitable for enzyme production.

8.4. Influence of Inducer

Laccase production has been seen to be highly dependent on fungus cultivation. During secondary metabolic phase, ligninolytic systems are activated and triggered by nitrogen concentration. Laccases are generally produced in low concentrations by laccase-producing fungi, but higher concentrations were obtained with the addition of various supplements such as xenobiotic compound to media (Piscitelli, et al., 2011). The addition of aromatic compounds such as 2,5- xylidine, lignin, veratryl alcohol is known to increase and induce laccase activity. Veratryl alcohol is an aromatic compound; its addition to cultivation media results in an increase of laccase production (Bertrand et al., 2013. The addition of 2,5-xylidine after 24 h of cultivation gave the highest induction of laccase activity and increased laccase activity nine fold. At higher concentrations the 2,5-xylidine had a reducing effect due to toxicity (El-Bakry et al., 2015). The promoter region encoding for laccase contains various recognition sites that are specific for xenobiotics and heavy metals; they bind to the recognition sites and induce laccase production. The addition of inducer increases the concentration of a specific laccase enzyme (Robene-Soustrade and Lung-Escarmant, 1997). An alcohol enhanced laccase activity more in comparison to xylidine and this is a very economical way to enhance laccase production (Lee et al., 1999). Cellobiose increase laccase activity by profusing branch in certain Trametes species. A low concentration of Cu^{+2} to the cultivation media increases the laccase production 50 times in comparison to basal medium (Assavanig et al., 1992). A new basidiomycete, Trametes sp. 420, produced laccase in glucose medium and in cellobiose medium with induction by 0.5mM and 6mM o-toluidine (Tong et al., 2007).

9. Mode of action of the laccase enzyme

Laccase only attacks the phenolic subunits of lignin, leading to Cα oxidation, Cα-Cβ cleavage and aryl-alkyl cleavage. Laccases are able to reduce one molecule of dioxygen to two molecules of water while performing one-electron oxidation of a wide range of aromatic compounds, which includes polyphenols, methoxy-substituted monophenols and aromatic amines. This oxidation results in an oxygen-centred free radical, which can then be converted in a second enzyme-catalyzed reaction to quinone. The quinone and the free radicals can then undergo polymerization. Laccases are similar to other phenol-oxidizing enzymes, which preferably polymerise lignin by coupling of the phenoxy radicals produced from oxidation of lignin phenolic groups. Due to this specificity for phenolic subunits in lignin and its restricted access to lignin in the fibre wall, laccase has a limited effect on pulp bleaching. The substrate range of laccase can be extended to non-phenolic subunits of lignin by the inclusion of a mediator such as 2, 2-azinobis-(3-ethylbenzthiazoline-6-sulfonate).

10. Applications of Laccase

Laccase is important because it oxidizes both the toxic and nontoxic substrates. It is utilized in textile industry, food processing industry, wood processing industry,

pharmaceutical industry, and chemical industry (Upadhyay et al., 2016). This enzyme is very specific, ecologically sustainable and a proficient catalyst. Applications of laccase are as follows.

10.1. Bioremediation and Biodegradation.

Due to rapid industrialization and extensive use of pesticides for better agricultural productivity, contamination of soil, water and air take place which is a serious environmental problem of today. Polychlorinated biphenyls, benzene, toluene, ethyl benzene, xylene, polycyclic aromatic hydrocarbons, pentachlorophenol, 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane and trinitrotoluene are the substances which are known for their carcinogenic as well as mutagenic effect and are persistent in the environment. Fungi renovate a wide variety of hazardous chemicals; that is why the researcher's interest is generated in them (Riva, 2006). The 2,4-dichlorophenoxyacetic acid degradation pathway is one of the best understood and provides a good model for such investigations. This herbicide has been released into the environment for more than 40 years and is degraded in soils fairly rapidly. Many bacteria of different genera are able to completely degrade 2,4-Dichlorophenoxyacetic acid (Haggblom, 1992). Degradation of 2,4-Dichlorophenoxyacetic acid via oxidative cleavage of the ether bond with subsequent chlorophenol hydroxylation followed by the modified ortho-cleavage pathway of chlorocatechols has been demonstrated for most of these isolates. This pathway has been most extensively studied using Alcaligenes eutrophus strain JMP134 (pJP4) (Don and Pemberton, 1981). The enzymes participating in the 2,4-Dichlorophenoxyacetic degradation pathway have been purified and characterized, and genes that code for these have been sequenced (Anwar et al., 2014). Many 2,4-Dichlorophenoxyacetic degrading microorganisms have been isolated from agricultural, urban, industrial soils, sediments and the catabolic pathway of 2,4- Dichlorophenoxyacetic mineralization in Ralstonia eutropha JMP134 (pJP4) is probably the best investigated. In this bacterial strain, the catabolic pathway involve initial ether bond cleavage to form 2,4-dichlorophenol followed by hydroxylation to form 3,5-dichlorocatechol, before intradiol ring cleavage (Leveau et al., 1999). Some raw information about kinetics of cell growth and biodegradation of 2,4-Dichlorophenoxyacetic of microorganisms growing on this herbicide exists, however, to understand the microbial response to environmental variables such as toxic compound concentration, a thorough kinetic analysis of raw data obtained from batch or continuous cultures is required. In addition to water contamination caused by the agricultural use of pesticides, effluents from wastewater treatment plants greatly contribute to the contamination of surface waters with these compounds (Nitscke and Schussler, 1998). Due to rainfall, pesticides applied in urban areas are transported to the sewer system and subsequently to wastewater (Schueler, 1995). In some regions, urban uses of pesticides exceed those in agriculture and frequently a conventional wastewater treatment process is not enough to degrade it, so effluents from treatment plants have the potential to contribute to the contamination of surface waters by non-degraded toxic compounds (Gerecke et al., 2002). Toxic and bio-refractory compounds could affect waste water treatment plants when streams of industrial and municipal wastewater are mixed together as occurs with 2,4-dichlorophenoxyacetic acid in the waste water treatment plant of Ecatepec, Mexico (Buenrostro-Zagal and Ramırez-Oliva, 2000). T. versicolour is used for the

bioremediation of atrazine in soil with low moisture and organic contents that are normally found in semiarid and Mediterranean-like ecosystems (Bastos and Magan, 2009). The laccase from T. versicolour and Pleurotus ostreatus for the degradation of PCBs as well as phenol found as chlorination increases, degradation rate decreases and concluded that 3-hydroxy biphenyl was more resistant to laccase degradation than 2- or 4-hydroxy analogues (Keum and Li, 2004). After five days of incubation, when glucose and fructose were used as a co-substrate than 71% of p-hydroxy benzoic acid and 56% of protocatechuic acid were degraded (Udayasoorian and Prabu, 2005).

10.2. Paper and Pulp Industry.

Chlorine and oxygen-based chemical oxidants are used for the separation and degradation of lignin which is required for the preparation of paper at industrial level (Knezevic et al., 2013). But some problems such as recycling, cost, and toxicity remain unsolved. However, in the existing bleaching process, LMS could be easily implemented because it leads to a partial replacement of ClO_2 in pulp mills (Kunamneni et al., 2007).

10.3. Food Processing Industry.

In food industry, laccase is used for the elimination of undesirable phenolic compound in baking, juice processing, wine stabilization and bioremediation of waste water. Laccase improves not only the functionality but also the sensory properties. In beer industry, laccase not only provides stability but also increases the shelf life of beer. In beer, haze formation takes place which is stimulated by the naturally present proanthocyanidins polyphenol and is referred to as chill haze. At room temperature or above, warming of beer can redissolve the complex. After certain periods of time, phenolic rings are replaced by the sulfhydryl group and permanent haze is formed which cannot be redissolved. For polyphenol oxidation, laccase has been used which is capable of removing the excess oxygen and also due to which the shelf life of beer increases. For making a fruit juice stable, laccase is commonly used. Phenol compounds and their oxidative products present naturally in the fruit juice give colour and taste to the juice. Colour and aroma change when polymerization and oxidation of phenolic and polyphenol take place. These changes are due to the high concentration of polyphenol and referred to as enzymatic darkening (Ribeiro et al., 2010). Laccase treatment removes phenol as well as substrate-enzyme complex by the help of membrane filtration, and colour stability is achieved, although turbidity is present. Laccase treatment is more effective in comparison to conventional methods. For improving the texture, volume, flavor and freshness of bread, wide range of enzymes are used. When laccase is added to the dough, strength of gluten structures in dough and baked products is improved: product volume increases, crumb structure improves, and softness of baked products takes place. Due to the laccase addition, stickiness decreases, strength and stability increase and the ability of machine are also improved which can also seen by using low-quality flour. At crushing and pressing stage, the high concentration of phenolic and polyphenolic compound plays an important role in the wine production. The high concentration of polyphenol obtained from the stems, seeds and skins which depends on the grape variety and vinification conditions contributes to of colour and astringency. Due to the complex event, polyphenol oxidation occurs in musts and wines resulting in the increase in colour and flavor change which is referred to as maderization. Catalytic factors, polyphenol removal,

clarification, polyvinyl poly pyrrolidone (PVPP), and high doses of sulfur dioxide are utilized to prevent maderization. Polyphenol removal is selective and results in undesirable organoleptic characteristics and concluded that laccase treatment is feasible, increasing storability and reducing processing costs (Minussi et al., 2007).

10.4. 2, 4-dichlorophenoxyacetic acid (2, 4-D) degradation

Chlorinated aromatic compounds are a major group of chemicals responsible for environmental pollution. They are highly toxic and resistant to degradation, and thus they accumulate in the environment. Many of these compounds are widely used as herbicides (Chaudhry and Chapalamadugu, 1991). Residual amounts of herbicide can persist for a significant period after application. This may have detrimental effects on the ecosystem. The degradation of herbicides in the soil environment, like that of any hydrocarbon, is affected by environmental conditions. Two chlorinated aromatic herbicides commonly used in both agriculture and forestry for vegetation control are 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) and 2,4-dichlorophenoxyacetic acid. The aromatic compound 2, 4- Dichlorophenoxyacetic acid is widely used as a systemic herbicide for controlling broadleaf vegetation. It is usually applied as a postemergence herbicide. The salt forms of 2,4-Dichlorophenoxyacetic acid is readily absorbed by plant roots and move upward in the transpiration system. The ester forms of 2,4- Dichlorophenoxyacetic acid is readily absorbed by the leaves and is converted to the acid form by the plant. The acid is then translocated within the phloem. The herbicide accumulates mainly at the meristematic regions of the roots and shoots. Because it is an extremely active auxin, 2, 4- Dichlorophenoxyacetic acid causes the plant to grow too fast, changing the normal growth patterns and resulting in death of the plant (Harborne, 1988). Microbial degradation of 2,4- Dichlorophenoxyacetic acid is known to occur in warm, moist soil; however, the rate is very dependent on environmental conditions and soil characteristics. Microbial degradation of 2, 4- Dichlorophenoxyacetic acid has been shown with both pure cultures and mixed cultures. The critical role of micro-organisms in the degradation of organic pollutants is well known. Although the microorganisms capable of degradation of organic pollutants and their catabolic pathways have been investigated intensively, information on the microbial degradation of xenobiotic in environments of high salinity and alkalinity is still very limited (Oren et al., 1992, Kaur, et al., 2016). Comparison of analogous catabolic enzymes and pathways of phylogenetically diverse bacteria isolated from extreme and moderate environments should provide information regarding their metabolic diversity. The study of xenobiotic-degrading extremophiles may also help in evaluating their use in bioremediation of contaminated saline and/or alkaline environments.

Reference

1. Abdel-Raheem, A., and Shearer, C.A. (2002). Extracellular enzyme production by freshwater ascomycetes. Fungal Diversity.11, pp. 1–19.

2. Akpinar, M., and Ozturk Urek, R., (2017). Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization. 3 Biotech. 7, 2, pp.98-107.

3. Anwar, Z., Gulfraz, M., and Irshad, M. (2014) Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. Journal of Radiat Res Appl Sci. 7, pp.163–173

4. Assavanig A., Amornkitticharoen, B., Ekpaisal, N., Meevootisom, V., and Flegel, T.W., (1992). Isolation, characterization and function of laccase from Trichoderma. Applied Microbiology and Biotechnology. 38, 2, pp. 198–202.

5. Bakkiyaraj, S., Aravindan, R., Arrivukkarasan, S. and Viruthagiri, T. (2013). Enhanced laccase production by Trametes hirusta using studies of extracellular fungal laccases. Applied and Environmental Microbiology. 48, pp.849-854.

6. Bastos, A.C., and Magan, N., (2009). Trametes versicolor: potential for atrazine bioremediation in calcareous clay soil, under low water availability conditions. International Biodeterioration and Biodegradation. 63, 4, pp.389–394.

7. Beloqui, A., Pita, M., Polaina, J., Martinez-Arias, O.V., Golyshina, A., Zumarraga, M., Yakimov, M.M., García-Arellano, H., Alcalde, M., Fernández, V.M., Elborough, K., Andreu, J.M., Ballesteros, A., Plou, F.J., Timmis, K.N., Ferrer, M., and Golyshin, P.N. (2006). Novel polyphenol oxidase mined from a metagenome expression library of bovine rumen: biochemical properties, structural analysis and phylogenetic relationship. Journal of Biol Chem. 281, pp. 22933-22942.

8. Bertrand, B., Martınez-Morales, F., Trejo-Hernandez, M.R. (2013). Fungal laccases: induction and production. Rev Mex Ing Quim. 12, pp.473–488

9. Brown, N.L., Barrett, S.R., Camakaris, J., Lee, B.T., and Rouch, D.A. (1995). Molecular gene and transport analysis of the copper resistance determinant (pco) from Escherichia coli plasmid pRJ 1004. Mol Microbiol. 17, pp.1153–1166.

10. Buenrostro-Zagal, J.F., Ramırez-Oliva, A., Caffarel-Mendez, S., Schettino-Bermu dez, B., Poggi-Varaldo, H.M. (2000). Treatment of a 2,4-dichlorophenoxyacetic acid (2,4-D) contaminated wastewater in a membrane bioreactor. Water Sci Technol. 42, (5–6), pp.185–192.

11. Bulter, T., Alcalde, M., Sieber, V., Meinhold, P., Schlachtbauer, C., Arnold, F.H. (2003). Functional expression of a fungal laccase in Saccharomyces cerevisiae by directed evolution. Applied Environmental Microbiology. 69, pp.987-995.

12. Buswel, J.A.I, Cai, Y., and Chang, S.T. (1995). Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by Lentinula (Lentinus) edodes. FEMS Microbiology Letters.128,1, pp. 81–88.

13. Chaudhry, G.R., and Chapalamadugu, S. (1991). Biodegradation of halogenated organic compounds. Microbiol Rev. 55, pp.59-79.

14. Cordi L., Minussi, R.C., Freire, R.S. and Duran, N. (2007). Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. African Journal of Biotechnology. 6, 10, pp.1255–1259.

15. Couto, S.R., and Toca-Herrera, J.L. (2007). Laccase production at reactor scale by filamentous fungi. Biotechnology Advances. 25, 6, pp.558–569.

16. Diamantidis, G., Effosse, A., Potier, P., and Bally, R. (2000). Purification and characterization of the first bacterial laccase in the rhizospheric bacterium Azospirillum lipoferum. Soil Biol Biochem. 32, pp.919-927.

17. Don, R.H., and Pemberton, J.M. (1981). Properties of six pesticide degradation plasmids isolated from Alcaligenes eutrophtls and Alcaligenes paradoxus. Journal of Bacteriol.45, pp.681-686.

18. Dos Santos, Y.V.S., Freire, D.A., Pinheiro, S., Fontao, L., De Souza, J.V.B., and Cavallazzi, J.R.P. (2015). Production of laccase from a white rot fungi isolated from the Amazon forest for oxidation of Remazol Brilliant Blue-R. Sci Res Essays. 10, pp.132–136.

19. El-Bakry, M., Abraham, J., Cerda, A., Barrena, R., Ponsa, S., Gea, T., and Sanchez, A. (2015) From wastes to high value added products: novel aspects of SSF in the production of enzymes. Crit Rev Env Sci Technol. 45,pp.1999–2042

20. Farnet, A.M., Criquet, S., Tagger, S., Gil, G., and Le Petit, J. (2000). Purification, partial characterization, and reactivity with aromatic compounds of two laccases from Marasmius quercophilus strain 17. Canadian Journal of Microbiology. 46, 3, pp.189–194.

21. Gayazov, R., and Rodakiewicz-Nowak, J. (1996). Semi-continuous production of laccase by Phlebia radiata in different culture media. Folia Microbiologica. 41, 6, pp. 480–484.

22. Gerecke, A.C., Scharer, M., Singer, H.P., and Muller, S. (2002). Sources of pesticides in surface waters in Switzerland: pesticide load trough waste water treatment plants—current situation and reduction potential. Chemosphere. 48, 3, pp.307–315.

23. Givaudan, A., Effosse, A., Faure, D., Potier, P., Bouillant, M.L., and Bally, R. (1993) Polyphenol oxidase in Azospirillum lipoferum isolated from rice rhizosphere :evidence for laccase activity in nonmotile strains of Azospirillum lipoferum. FEMS Microbiol Lett. 108, pp.205–210.

24. Gochev, V.K., and Krastanov, A.I. (2007). Fungal laccases. Bulgarian Journal of Agricultural Science.13, pp.75–83.

25. Grotewold, E., Taccioli, G.E., Aisemberg, G.O., and Judewicz, N.D. (1998). Purification of an extracellular fungal laccase. Mircen Journal of Applied Microbiology and Biotechnology. 4, pp. 357–363.

26. Haggblom, M.M. (1992). Microbial breakdown of halogenated aromatic pesticides and related compounds. FEMS Microbiol Rev. 103,pp. 29-72.

27. Han, M.J., Choi, H.T., and Song, H.G. (2005). Purification and characterization of laccase from the white rot fungus Trametes versicolor. Journal of Microbiology. 43, 6, pp.555–560.

28. Harborne, J.B. 1988. Introduction to ecological biochemistry, 3rd ed., pp.356. Academic Press, Inc., San Diego, Calif.

29. Hess, J., Leitner, C., Galhaup, C., Kulbe, K.D., Hinterstoisser, B., Steinwender, M., and Haltrich, D. (2002). Enhanced formation of extracellular laccase activity by the white-rot fungus Trametes multicolor. Applied Biochemistry and Biotechnology. 98,100, pp. 229–241.

30. Hong, Y., Xiao, Y., Zhou, H., Fang, W., Zhang, M., Wang, J., Wu, L., and Yu, Z. (2006) Expression of a laccase cDNA from Trametes sp. AH28-2 in Pichia pastoris and mutagenesis of transformants by nitrogen ion implantation. FEMS Microbiol Lett. 258, 1, pp.96–101.

31. Hou, H., Zhou, J., Wang, J., Du, C., and Yan, B. (2004). Enhancement of laccase production by Pleurotus ostreatus and its use for the decolorization of anthraquinone dye. Process Biochemistry. 39,11, pp.1415–1419.

32. Imran, S.G., Aziz, G.M., and Razuqi, N.S. (2016) Optimization of ligninolytic enzymes production from Aspergillus terreus SG-777 by solid state fermentation. Iraqi Journal of Vet Med. 40, 1, pp.116–124.

33. Kaur, H., Kapoor, S., and Kaur, G. (2016). Application of ligninolytic potentials of a white-rot fungus Ganoderma lucidum for degradation of lindane. Environ Monit Assess. 188, pp.588–589.

34. Keum, Y.S., and Li, Q.X. (2004). Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls, Chemosphere. 56, 1, pp.23–30.

35. Khammuang, S., and Sarnthima, R. (2009). Laccase activity from fresh fruiting bodies of Ganoderma sp. MK05: purification and remazol brilliant blue R decolorization. Journal of Biological Sciences. 9, 1, pp.83–87.

36. Knezevic, A, Milovanovic, I., Stajic, M., Loncar, N., Brceski, I., Vukojevic, J.C., and Cilerdzic, J. (2013). Lignin degradation by selected fungal species. Bioresour Technol. 138, pp.117–123.

37. Kunamneni, A., Ballesteros, A., Plou, F.J., and Alcalde, M. (2007). Fungal laccase-a versatile enzyme for biotechnological applications. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. A. Mendez-Vilas, Ed., 1, pp. 233–245.

38. Lee, I.Y., Jung, K.H., Lee, C.H., and Park, Y.H. (1999). Enhanced production of laccase in Trametes vesicolor by the addition of ethanol. Biotechnology Letters. 21,11, pp.965–968.

39. Lee K.H., Wi, S.G., Singh, A.P., and Kim, Y.S. (2004). Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus Coniophora puteana. Journal of Wood Science. 50, 3, pp.281–284.

40. Leveau, J.H., Konig, F., Fuchslin, H., Werlen, C., and Van der Meer, J.R. (1999). Dynamics of multigene expression during catabolic adaptation of Ralstonia eutropha JMP134 (pJP4) to the herbicide 2,4-dichlorophenoxyacetate. Mol Microbiol. 33, pp.396–406.

41. Li, J.F., Hong, Y.Z., Xiao, Y.Z., Xu, Y.H., and Fang, W. (2007). High production of laccase B from Trametes sp. in Pichia pastoris. World Journal of Microbiol Biotechnol 23, pp.741–745.

42. Lorenzo, M., Moldes, D., Couto, S.R., and Sanroman, A. (2002). Improving laccase production by employing different lignocellulosic wastes in submerged cultures of Trametes versicolor. Bioresource Technology. 82, 2, pp.109–113.

43. Martins LO, Soares CM, Pereira MM, Teixeira M, Costa T, Jones GH,Henriques AO (2002). Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the Bacillus subtilis endospore coat. J. Biol. Chem. 277: 18849-18859.

44. Mathur, G., Mathur, A., Sharma, B.M. and Chauhan, R.S. (2013). Enhanced production of laccase from Coriolus sp. using Plackett- Burman design. Journal of Pharmacy Research. 6, pp. 151-154.

45. Mellano, M.A., and Cooksey, D.A (1988). Nucleotide sequence and organization of copper resistance genes from Pseudomonas syringae pv.tomato. Journal of Bacteriol. 170,pp.2879–2883.

46. Michniewicz, A., Ullrich R., Ledakowicz, S., and Hofrichter, M. (2006). The white-rot fungus Cerrena unicolor strain 137 produces two laccase isoforms with

different physico-chemical and catalytic properties. Applied Microbiology and Biotechnology. 69,6, pp. 682-688.

47. Minussi R. C., M. Rossi, L. Bologna,D. Rotilio, G.M. Pastore, and N. Duran, (2007).. "Phenols removal in musts: strategy for wine stabilization by laccase. Journal of Molecular Catalysis B: Enzymatic. 45, 3-4, pp.102–107.

48. Morozova, O.V., Shumakovich, G. P., Gorbacheva, M. A., Shleev S. V., and Yaropolov A.I. (2007). Blue laccases. Biochemistry (Moscow).72, 10, pp.1136–1150.

49. Nitscke, L., and Schussler, W. (1998). Surface pollution by herbicides from effluents of waste water treatment plants. Chemosphere.36,1, pp.35–41.

50. Nyanhongo, G.S., Gomes, J., Gubitz, G., Zvauya, R., Read, J.S., and Steiner, W. (2002). Production of laccase by a newly isolated strain of Trametes modesta. Bioresource Technology. 84, 3, pp.259–263.

51. Oren, A., Gurevich, P., Azachi, M., and Henis, Y. (1992). Microbial degradation of pollutants at high salt concentration. Biodegradation. 3, pp.387-398.

52. Palmieri, G., Giardina, P., and Marzullo, L., Desiderio, B., Nitti, G., Cannio, R., Sannia, G. (1993). Stability and activity of a phenol oxidase from the ligninolytic fungus Pleurotus ostreatus. Applied Microbiology and Biotechnology.39, 4-5, pp.632–636.

53. Pandey, A., Selvakumar, P., Soccol, C.R., and Nigam, P. (1999). Solid state fermentation for the production of industrial enzymes. Current Science. 77, 1, pp.149–162.

54. Pardo, I., Rodriguez-Escribano, D., Aza, P., De Salas, F., Martinez, A.T., and Camarero., S. (2018). A highly stable laccase obtained by swapping the second cupredoxin domain. Scientific Reports. 8, pp.15669-15678.

55. Patel, H., Gupte, A., and Gupte, S. (2009). Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal isolates Pleurotus ostreatus HP-1 under solid state fermentation. Bioresources. 4, pp.268–284.

56. Piontek, K., Antorini, M., and Choinowski, T. (2002). Crystal structure of a laccase from the fungus Trametes versicolor at 1.90-Å resolution containing a full complement of coppers. Journal of Biological Chemistry. 277, 40, pp.37663–37669.

57. Piscitelli, A., Giardina, P., Lettera, V., Pezzella, C., Sannia, G., and Faraco, V. (2011) Induction and transcriptional regulation of laccases in fungi. Curr Genomics. 12, pp.104–112.

58. Ribeiro, D.S., Henrique, S.M.B., Oliveira, L.S., Macedo, G.A., and Fleuri L. F. (2010). Enzymes in juice processing: a review. International Journal of Food Science and Technology. 45, 4, pp. 635–641.

59. Riva, S. (2006). Laccases: blue enzymes for green chemistry. Trends in Biotechnology. 24, 5, pp. 219–226.

60. Robene-Soustrade, I., and Lung-Escarmant, B., (1997). Laccase isoenzyme patterns of European Armillaria species from culture filtrates and infected woody plant tissues. European Journal of Forest Pathology. 27, 2, pp.105–114.

61. Romero, S., Blanquez, P., and Caminal, G. (2006). Different approaches to improving the textile dye degradation capacity of Trametes versicolor. Biochemical Engineering Journal. 31, 1, pp. 42–47.

62. Rosales, E., Couto, R.S., and Sanroman, M.A. (2002). New uses of food waste: application to laccase production by Trametes hirsuta. Biotechnol Lett. 24,pp.701–704.

63. Schlosser, D., and Hofer, C. (2002). Laccase-catalyzed oxidation of Mn2+ in the presence of natural Mn3+ chelators as a novel source of extracellular H_2O_2 production and its impact on manganese peroxidase. Applied and Environmental Microbiology. 68, 7, pp. 3514–3521.

64. Schueler, T. (1995). Urban pesticides: from the lawn to the stream. Watershed Protect Tech; 2,1,pp.247–253.

65. Shleev, S.V., Morozova, O.V., Nikitina, O.V., Gorshina, E.S., Rusinova, T.V., Serezhenkov, V.A., Burbaev, D.S., Gazaryan, I.G., and Yaropolov, A.I. (2004). Comparison of physico-chemical characteristics of four laccases from different basidiomycetes. Biochimie. 86, pp. 693-698.

66. Solano, F, Garcia, E, Perez de Egea, E., and Sanchez-Amat, A. (1997). Isolation and characterization of strain MMB-1 a novel melanogenic marine bacterium. Appl Environ Microbiol. 63, pp. 3499–3506.

67. Souza, C., Zilly, A., and Peralta, R.M. (2002). Production of laccase as the sole phenoloxidase by a Brazilian strain of Pleurotus pulmonarius in solid state fermentation. Journal of Basic Microbiology. 42, 2, pp. 83–90.

68. Stajic, M., Persky, L., Friesem, D., Hadar, Y., Wasser, S.P., Nevo, E., Vukojevic, J. (2006). Effect of different carbon and nitrogen sources on laccase and

peroxidases production by selected Pleurotus species. Enzyme Microb Technol. 38, pp.65–73

69. Suzuki, T., Endo, K., Ito, M., Tsujibo, H., Miyamoto, K., and Inamori, Y. (2003). Thermostable laccase from Streptomyces lavendulae REN-7: purification, characterization, nucleotide sequence and expression. Biosci Biotechnol Biochem. 67, pp. 2167-2175.

70. Thakker, G.D., Evans, C.S., and Koteswara Rao, K. (1992). Purification and characterization of laccase from Monocillium indicum Saxena. Applied Microbiology and Biotechnology. 37, 3, pp. 321–323.

71. Tong, P., Hong, Y., Xiao, Y., Zhang, M., Tu, X., and Cui, T., (2007). High production of laccase by a new basidiomycete. Biotechnology Letters. 29, 2, pp. 295–301.

72. Udayasoorian, C., and Prabu, P.C. (2005). Biodegradation of phenols by lignolytic fungus Trametes versicolour. Journal of Biological Sciences. 5, pp. 824–827.

73. Ullah, M.A., Bedford, C.T., and Evans, C.S. (2000). Reactions of pentachlorophenol with laccase from Coriolus versicolor. Appl Microbiol Biotechnol. 53,pp.230–234.

74. Upadhyay, P., Shrivastava, R., Agrawal, P.K. (2016). Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech. 6, pp.189–199.

75. Valeriano, V.S., Silva, A.M.F., Santiago, M.F., Bara, M.T.F., and Garcia, T.A. (2009). Production of laccase by Pycnoporus sanguineus using 2,5-xylidine and ethanol. Brazilian Journal of Microbiology. 40, 4, pp. 790–794.

76. Vantamuri, A.B., and Kaliwal, B.B. (2016). Production of laccase by newly isolated Marasmıus sp. BBKAV79 in solid state fermentation and its antiproliferative activity. International Journal of Pharmaceutical Science and Research. 7,12, pp. 4978–4987

77. Viswanath, B., Subhosh Chandra, M., Pallavi, H., and Rajasekhar Reddy, B. (2008). Screening and assessment of laccase producing fungi isolated fromdifferent environmental samples. African Journal of Biotechnology. 7, 8, pp.1129–1133.

78. Xiao, Y.Z., XM, Tu., Wang, J., Zhang, M., Cheng, Q., Zeng, W.Y., and Shi, Y.Y. (2003). Purification, molecular characterization and reactivity with aromatic compounds of a laccase from basidiomycete Trametes sp. strain AH28-2. Appl Microbiol Biotechnol. 60, pp.700–707.

79. Xin, F., and Geng, A. (2011). Utilization of Horticultural Waste for Laccase Production by Trametes versicolor Under Solid-State Fermentation. Applied Biochem Biotechnol. 163, 2, pp. 235-246.

80. Yaver, D.S., Berka, R.M., Brown, S.H., and Xu, F. (2001). The presymposium on recent advances in lignin biodegradation and biosynthesis, Vikki Biocentre, University of Helsinki, Finland. June 3-4 June, pp.40.

81. Zilly, A., Dos Santos Bazanella, G.S., Helm, C.V., Araujo, C.A.V., De Souza, C.G.M., Bracht, A., and Peralta, .R.M. (2012). Solid-state bioconversion of passion fruit waste by white-rot fungi for production of oxidative and hydrolytic enzymes. Food Bioprocess Technol. 5, pp.1573–1580.

82. Zoppellaro, G., Huang, H.W., and Sakurai, T. (2000). Kinetic studies on the reaction of the fully reduced laccase with dioxygen. Inorganic Reaction Mechanisms. 2, pp. 79–84.