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EXPRESSION AND PRODUCTION OF ENZYME LACCASE FOR POTENTIAL INDUSTRIAL APPLICATIONS

Syed Sajid Jan, Sikander Ali*, Muhammad Waqas and Noaman Yaseen

Institute of Industrial Biotechnology, GC University Lahore, Pakistan.

***Corresponding Author: Sikander Ali**
Institute of Industrial Biotechnology, GC University Lahore, Pakistan.

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ABSTRACT

Laccases are polyphenol oxidases containing copper atoms, catalysing the oxidation of a broad spectrum of complex polyphenols and aromatic diamines compounds. They are increasingly being used in biotechnological and industrial application. In food industries laccases have been progressively more used to improve and modify the appearance, colour, and texture of various beverages and food products. Laccases have many other application in paper and textile industries and is being used frequently in bleaching and dye synthesis. To overcome its current need, higher amount of laccase availability and an adequate production systems must be developed. This review article presents new advancement about the mechanism of action of laccase catalyzing the oxidation reaction, its overexpression in various heterologous hosts such as bacteria, fungi, and yeast, various factors affecting its production, different type of fermentation processes for its overproduction, and its purification on large scale. Some of the important industrial and biotechnological application have also been discussed.

KEYWORDS: Laccase, Expression, Fermentation, Purification, Application.

INTRODUCTION

Laccase was discovered for the first time in the latex of Japanese or Chinese lacquer trees by Yoshida in 1883. These enzymes appear to be involved in wound curing of herbivore as part of defense mechanism against pathogens, in iron metabolism, and in lignification.[1] Laccase was discovered in 19th century.[2] Almost all fungi have the potential to produce laccase, and have also been recorded in numerous bacteria and plants.[3] Laccases (oxidoreductases: 1,4-benzenediol EC 1.10.3.2) is a member of a wide group of enzymes named as polyphenol oxidases that contain copper atoms in center of their catalytic site, and therefore it is also known as multicopper oxidases. Typically, it contains copper atoms of three different variety, of which one give a distinctive blue color, and those that do not have blue copper atom are termed as white or yellow laccases. Laccases as a whole are globular monomeric proteins of about 60–90 kDa contain 15– 30% carbohydrate with isoelectric point (pI) 4.0.

There is a great diversity of Genes for laccase that are found in bacteria, fungi, plants and, insects and various other laccase isoenzymes have been documented in numerous species. They have been recorded in numerous vegetables and fruits such turnip, potatoes, cabbages, pears, apples and in various other plants. Existence of laccases is widespread in deuteromycetes, basidiomycetes, and ascomycetes, predominantly plentiful in fungi that can metabolize lignin.[4] Few compounds that shows "laccase like properties" such as phenoloxidases have been isolated from cuticles of various insects that possibly have role in the process of sclerotization.[5] The genes for laccase production have also been documented in both gram-positive and gram-negative bacteria, as well as bacterial species that are extremophiles. Researchers have reported the presence of laccase genes in some species of actinomycetes.[6] In fungi, laccases perform important role in carrying out various physiological and biological functions including lignin degradation, stress defense, fungal plant-pathogen interactions, plant host interaction, and morphogenesis.[3] Most of the laccases are excreted outside the cells, and around 10 to 25% are glycosylated, while in some cases glycosylation may reach up to 30%. In bacteria laccases play role in sporulation, homeostasis, or pigmentation of spores that provide resistance to harsh conditions such as hydrogen peroxide or UV radiation.[7]

Laccase oxidizes a broad range of substrates including hydroxyindols, aromatic diamines, methoxy-substituted phenols, polyphenols, and various other compound by a radical catalyzed reaction and use oxygen as an electron acceptor. Additionally, numerous other organic compounds that comprise amino, hydroxil, or acid

groups can act as a substrates for laccase.[8] Each molecule of laccase possess four copper ions in three sites according to their spectroscopic properties that pairs the four oxidations of electron of phenolic compound to reductive cleavage of four electron of the dioxygen bond and reduce oxygen to water.[3] When laccase oxidize a substrate, the substrate loses an electron and generally produce a free radical that may experience additional non-enzymatic reactions or oxidation together with polymerization, disproportionation, and hydration.[9] Typically laccases are polymeric and generally contains metal content of one type-1 copper, one type-2 and two type-3 copper ions subunit/center, whereas the type-2 and type-3 are jointly arranged creating a trinuclear cluster (TNC). The blue color is due to the type 1 site copper, whose strong interaction to cysteine create a solid SCys → Cu (II) transfer transition charge at about 600 nm, giving the characteristic blue color to laccase. A typical electron paramagnetic resonance (EPR) spectrum is exhibited by type 2, evidently different from that of the type 1 EPR, while type 3 coppers are EPR-silent ions and anti-ferromagnetically coupled. Several 3-dimensional structures of laccases have been resolved. All laccases have similar molecular structure arranged in three consecutively cupredoxin-like domains.[3] The type 1 site is positioned in domain 3, whereas the cluster TNC is entrenched amid domains 1 and 3, through both domains delivers residues for organization of coppers. Due to the advanced redox capacity of laccases from fungal origin (more than 800mV), as compared to bacterial or plants laccases, they are concerned in quite a lot of biotechnological applications particularly in the catabolism of lignin. Laccases, owns an outstanding potential for the processing of food in diet industry. Laccases has a vital role in various industries such as paper and pulp industries, food industries, cosmetics, textile industries, soil bioremediation, treatment of endocrine disruptors, and biodegradation of phenolic pollutant from environment. Laccases can also be used for degradation of insecticide or pesticide, pulp delignification, waste detoxification, organic synthesis food technological uses, textile dye transformation, and analytical and biosensor applications.[9] Researchers have also applied laccase successfully to Nano-biotechnology owing to its competence to carryout oxidation and the allocation of electron without need of additional cofactors.

The core objective of this review paper is to evaluate and abridge the data present in up-to-dated literature about laccases, its overexpression, fermentation, mechanism of action and its uses in different industries. Laccase has numerous uses such as stabilization of different types of beverages, bioremediation, baking, and various other industries. This review would contribute in overall features of laccase in an attempt to produce an up-to-date information that could help the practices of laccase in various industries.

Sources of Laccases

Usually laccase occurs in fungi and higher plants but it has also been isolated from bacteria including *Marinomonas mediterranea, S. lavendulae,* and *S. cyaneus*. However, laccase is documented more in fungi than the higher plants. Several species of Trichoderma has the potential to produce laccase that comprises *Trichoderma longibrachiatum, T. harzianum,* and *T. atroviride.*[10] Some species of basidiomycetes that includes *Lenzites, Betulina, Theiophora terrestris, Phanerochaete chrysosporium,* and white-rot fungus for example *Trametes versicolour, Pleurotusostreatus,* and *Phlebia radiate* produce laccase. Laccase from *Pycnoporus sanguineus* act as phenol oxidase whereas *Pycnoporus cinnabarinus* produces laccase as ligninolytic enzyme.[11] Some species of ascomycetes such as *Monocillium indicum, Podospora anserine, Melanocarpus albomyces, Ophiostoma novo-ulmi, Neurospora crassa, Magnaporthe grisea, Monocillium indicum, Gaeumannomyces graminis,* and *Mauginella,* produce laccase.[12] Laccase production has also been described for soil ascomycete species from the genera penicillium, curvularia, and aspergillus, and in few freshwater ascomycetes.[13] However, there are more than a few groups of fungi that seemingly do not synthesize laccase. Production of laccase has not been documented in lower fungi, such as chytridiomycetes and zygomycetes.[14] In fungi it take part in plant pathogenesis, pigment production, sporulation, fruiting body formation, and delignification while in plants, laccase has role in lignifications.[15]

Mechanism of Laccase Action

The enzyme laccase catalyzes oxidation by reducing one oxygen molecule to water coupled by loss of electron "oxidation" of a wide-ranging variety of complex compounds such as aromatic amines, methoxy-substituted monophenols, and polyphenols.[16] As a result of this oxidation, an oxygen-centered free radical is produced that can be changed to quinone. Laccases have four atoms of copper named as Type-1 Copper (where the reducing substrate binds) and Type-2/Type-3 copper trinuclear clusters (TNC). Oxidation by laccase is accomplished in three steps: (1) The reduction of type 1 Cu by oxidation of substrate; (2) The transfer of electron from type-1 Copper to type-2 Copper and then to type 3 Copper TNC; (3) The reduction of molecular oxygen to water at TNC.[17] The enzymatic activity of laccase has showed that Type 2 Cu and Type 3 Cu in TNC has catalytic activity. The molecule of oxygen typically binds to TNC for irregular activation, and then reduced to water.[9] Laccase functions like a battery, taking electrons from each oxidation reaction and loads it to an oxygen molecule. Therefore, it need four substrates to be oxidized in order to completely reduce molecular oxygen to water. Free radicals are produced when laccase oxidizes a substrate. The lignin catabolism is continued by radicals of phenoxy, which then breaks the bonds between α-carbon and β-carbon or just oxidize α-carbon. Due to oxidation, a free radical is produced, which is

then transformed into quinone by another enzyme-catalyzed reaction. The free radicals and the quinone can undertake polymerization.[18]

Moreover, laccase can also catalysis the oxidation of nonphenolic substrate in the presence of certain mediators. These mediators are low molecular weight organic compounds, which are oxidized through laccase forming a cation radical that is highly active and capable of oxidizing a nonphenolic compound. Some common mediators for laccase are 2,2-azino bis-3-ethyl thiazoline-6-sulfonat (ABTS), 1-hydroxy benzo triazole (HOBT) and N-hydroxy phthalimide (NHPI).[19]

Recombinant Expression of Laccase

The production of laccases from its native source is too less, most of microorganisms are incompatible with the normal conditions and processes of industrial fermentation, and its yield from natural host cannot meet the growing market demand. Through recombinant DNA technology, proteins are expressed in easily handling and cultivable hosts that allow it's over expression and higher productivity in relatively shorter time, as well as reducing the production costs.[20] The productivity and purification of enzyme is increased by manipulating its genes with a solid promoter and strong signaling sequences, correctly designed to transfer the enzyme outside the cell, hence make downstream processing simpler. Moreover, the specificity, activity and stability of enzyme can be enhanced by protein engineering, therefore modified enzymes can be created according to the users need.[21]

Now a day's researchers have a growing interest in the field of recombinant expression of laccase, where its genes are expressed in Heterologous hosts such as in filamentous fungi, yeasts, bacteria, and plants, as well as some homologous expression.[22] Bacteria such as Escherichia coli has been used for expression, to solve the problem in which the laccase cannot be easily produced and obtained from its natural hot. The recombinant expression and production of *Streptomyces coelicolor* laccase (SLAC) in *Streptomyces lividans* has produced substantial a great amount of laccase with high purity.[23] Fungal laccase from a ligninolytic fungus such as *Cyathus Bulleri* has also been expressed in *E. coli*.[24] The Table 1 shows a list of recombinant laccases produced in bacterial hots.

Table 1: Laccase expressed in bacteria from different microbial sources.

Source	Laccase	Bacteria
Cyathus bulleri	Cbu-laccase	
Streptomyces ipomea	SilA	
Bacillus licheniformis	CotA	
Streptomyces coelicolor	SLAC	*Streptomyces*
Bacillus halodurans	Lbh1	
Thermus thermophilus HB27	Tth-laccase	
Streptomyces coelicolor	SLAC	
Streptomyces lavendulae REN-7	STSL	*Escherichia coli*
Aquifex aeolicus	McoA	
Streptomyces griseus	EpoA	
Marinomonas mediterranea	PpoA	
Bacillus subtilis	CotA	

Yeast such as *Pichia pastoris* and *Saccharomyces Cerevisiae* have often been used as an excellent species for production of recombinant laccase. Plant laccases has also been produced in these two yeast species.[25] However *P. pastoris* is better in term of production than *S. cerevisiae*.[26] Expression in yeast has several advantages over bacteria, it provide ease of gene manipulation and has the ability to carry out post-translational modification, such as glycosylation, disulfide bridge formation, and proteolytic processing. The use of yeast for laccase production is economical, require less time and effort, and generally give high yields. Moreover non-conventional yeasts *Yarrowia lipolytica*, *Kluyveromyces lactis* and *Pichia methanolica* have been used and has similar yield as those obtained from other yeasts.[27] Filamentous fungi can be used as host for the recombinant expression of laccases, and the choice of fungi is usually based on its ability to produce large amounts of proteins into the media.[28] Saprotrophic fungi and basidiomycetes are the utmost common species that are capable of yielding considerable quantity of laccase in variable amount. However only a small figure of fungal host has been studied so for. Examples of fungi that has been used as hosts for production of laccase are the asexually reproducing *Trichoderma reesei*, *Aspergillus sojae*, *Aspergillus nidulans*, *Aspergillus oryzae* and *Aspergillus niger*. Additionally the yield of laccase in much higher in filamentous fungi than those obtained from yeast, mostly in the range of 100 mg^{-1}. Researchers have also used plants as a hot for recombinant expression of plant and fungal laccases. Potato laccase (PPO) has been overexpressed in tomatoes to provide a greater and improved resistance against pathogenic bacteria. Insect Sf9 cells has also been used for expression of laccase from tobacco hornworm, *Manduca sexta*, through a *baculovirus* expression system.[29]

Production of Laccase

Laccases are produced by fermentation process usually using filamentous fungi that are extracellularly secreted into media. They are usually synthesized through the host secondary metabolism.[30] Different factors can influence the production of laccase such as concentration of microelements, carbon limitation, nature of cultivation (either solid state or submerged), and source of nitrogen.[31]

Effect of nitrogen and carbon

Fungi for laccase production are cultured in a specific media that is composed of 1% w/v sources of carbon and nitrogen and 0.1% w/v yeast extract. Fructose, mannose, lactose glucose, and maltose are the generally used as carbon sources while urea, peptone, $NaNO_3$, and $(NH_4)_2SO_4$ are common source of nitrogen. Researchers have investigated that in several fungal species, high concentration of glucose and sucrose have inhibitory action on the production of laccase.[32] Polymeric substrates such as cellulose can be used as a carbon source instead of glucose or sucrose, and hence the inhibition of laccase production do not occur.[32] The activation of laccase production is generally achieved by depleting the concentration of nitrogen. However some studies suggest that concentration of nitrogen has no effect on the activity of enzyme. It has been documented that high laccase activity can be induced by low nitrogen to carbon ratio. Nevertheless some investigators have reported that high nitrogen to carbon ratio can significantly increase the production of laccase.[33]

Application of inducers

Laccase production can be significantly increased by providing different inducer to the media. Use of xenobiotics such as veratryl alcohol, lignin and 2,5 xylidine induce and enhance the activity of laccase.[34] Furthermore, laccase activity can be enhance up to nine folds through supplementation of 2,5 xylidine to growth media after 24 hours of cultivation. The aromatic compound, veratryl alcohol addition to culture media can considerably increase the production of laccase.[35] Moreover Lee *et al.* (1999) has documented that alcohol induced laccase activity more and is much effective than xylidine. In another study, it has been reported that laccase activity can be considerably encouraged by cellobiose in certain Trametes species. Additionally, less concentration of copper (Cu^{+2}) in media can encourage high level of laccase synthesis, 50 times more than a basal medium.[36]

Influence of temperature

The temperature has limited effect on the production of laccase. The Optimum temperature vary from strain to strain. It is reported that in the dark, the best temperature is 25°C while in the light it is about 30°C.[37] Thus the optimum temperature for production of laccase varies among 25°C to 30°C. Farnet *et al.* (2000) investigate that activity of laccase was greatly increased by pre-incubating the enzymes at 40°C. Furthermore, laccase produced by *P. ostreatus* is nearly completely active in a wide range of temperature between 40°C to 60°C, with best activity at 50°C.[38] Moreover Nyanhongo *et al.* (2002) documented that the production of laccase by *T. modesta* is almost very stable at 40°C and completely active at 50°C, however the half-life reduced to two hours at higher temperature then 60°C.

Influence of pH

Many reports showed initial pH value is usually established between pH 4.5 to pH 6.0 before the inoculation, however the value of pH is usually not fixed during cultivation and fermentation process.[33] It has been documented that an initial pH of 7.0 was the finest for maximum production of laccase. Additionally the pH has a partial influence on the production of laccase. Its value varies from substrate to substrate because different substrate has different reaction for laccase. The effect of pH on laccase activity was reported by Cord *et al.* (2007) using syringaldazine as a substrate, and determined that the enzyme activity ranges from 3.0 to 8.0. The L1 isoenzyme of laccase has an optimum pH 4.0 while L2 has an optimum pH 5.0. On the other hand Han *et al.* (2005) has isolated laccase from *Trametes versicolour,* and found that the boosted laccase activity could occurs at a wide range, however concluded the optimum pH of 3.0 at 50°C. Filamentous fungi can produce laccase in excess when grown at pH 5.0, though most researchers have reported that optimum pH should be at 4.5 to 6.0 for laccase production.

Influence of agitator

Laccase production can be affected by agitation. Hess *et al.* (2002) studied the production of laccase by fungus *Trametes multicolour* and reported that fungal mycelia are damaged by agitation, when it is cultivated in a stirred tank and the yield of laccase was noticeably reduced. More over the laccase production by fungus *Bjerkandera adusta* has dramatically decreased when grown on stirred tank. However some researcher reports that using the fungus *T. versicolour*, agitation has no effect on the production of laccase.[44]

Cultivation Type

Laccases are produced on industrial scale by Solid State and Submerged modes of fermentation. Numerous cultivation techniques are applied to produce laccase on a large-scale, using filamentous fungi. Chief features of solid state and submerged fermentation has been discussed in forthcoming section.

Submerged Fermentation

Submerged fermentation encompasses the encouragement of fungi in aerobic condition in a liquid medium having suitable amount of nutrients. Viscosity of broth is a most common issue in submerged fermentation. Additionally, the growth of fungal mycelium can hinder impeller action that slowdown of mass transfer and oxygen absorbance. This problem has been resolved by a pulsed system designed by Hess *et al.*

(2002) that uses white rot fungus *Trametes versicolor*, and decolorizes the synthetic dye, letting the bioreactor to work in higher efficiency for an extended period of time in a continuous mode.[45] Furthermore, another technique "Cell Imoobiliztion" is implemented to increase oxygen absorbance, reduce the viscosity and ease the mass transfer. Luke and Burton. (2001) documented that continuous production of laccase can be produced without enzyme deactivation for up to four months by immobilizing the fungus *Neurospora crassa* on membrane supports. Sedarati *et al.* (2003) examined that, in stainless steel and fixed bed bioreactors, there was higher laccase activity by using a diverse synthetic carriers for immobilizing the fungus *Trametes hirsute*. Other factors such as fed-batch culture can also significantly increase the production of laccase. Using the fungus *T. pubescens*, researchers have investigated that fed batch method can enhance the production laccase as well as enzyme activity up to two folds.[47]

Solid State Fermentation

Solid state fermentation (SSF) is the type of fermentation process that contain very less amount of liquid, and composed of natural organic substrate or synthetic inert substrates as a solid support that provide the condition for filamentous fungi under which it naturally grows.[48] Different kind of lignocellulosic residues has been studied as a solid substrates for numerous enzymes including laccases. These natural residues have cellulose/hemicellulose and lignin that are rich in sugar enhancing fungal mycelial growth as well as acts as an inducer for different enzymes production. Nevertheless on of the major limitation of SSF is absence of any conventional bioreactor designs. A lot of literature have discussed several bioreactor designs for SSF, however they having many major drawbacks of mass and heat transfer. However different configuration of bioreactor for laccase production has been studied. For instant, Couto *et al.* (2003) has investigated try, immersion and expended bed configurations of bioreactor having barley bran (non-inert) and nylon (inert) support for the production of laccase from fungus *T. versicolor*. They concluded that tray configuration has the finest result for laccase production. Moreover, Rosales *et al.*, (2007) has also documented similar result of tray configuration of solid media for laccase production from *T. hirsute* raised on orange peels.

Using rice bran as substrate, the production of laccase is usually higher in both submerged and soli-state fermentation. The laccase production is induced by the presence of phenolic compounds such as vanillic acid and ferulic acid in the rice bran. Various other organic agricultural wastes has been used as substrate for laccase production such as wheat bran, molasses waste water, cotton stalk, barley bran, grape stalks, and grape seeds.[51]

Purification of Laccase

Fractional precipitation by ammonium sulphate has been frequently used for laccase purification. This is accompanied with other purification techniques such desalt/buffer exchange of protein, gel filtration chromatography, and anion exchange chromatography. However single step technique for purification of laccase from *Neurospora crassa* has been successfully applied, using celite chromatography and up to 54 fold laccase purification with enzyme activity of 333 U mg^{-1} was obtained.[52] Laccase from the strains LLP13 purified using column chromatograpy followed by gel filtration for polishing.[53] Laccase produced by *T. versicolour* is purified with specific activity of 91,443 U mg^{-1} using frictional precipitation by ethanol, followed by Sephadex G-100, DEAE- Sepharose, and Phenyl Sepharose chromatography. [43] Furthermore, laccase from *Tinea Versicolor* was also purified by Ion exchange chromatography for initial capturing and intermediate purification and then followed by Gel filtration chromatography for polishing purpose and an enzyme activity of 101 UmL^{-1} and purification of 34.8 fold was obtained.[54] Moreover, the laccase from fungus *Sterum ostrea* was also purified upto 70 fold, by ammonium sulphate precipitation and final polishing by Sephadex G-100 colum chromatography. In the same way, laccase has also been purified from the fruiting bodies by frictional precipitation with ammonium sulphate salt, up to 40 to 70 percent saturation that was then followed by DEAE cellulose chromatography.[55]

Applications of Laccase

Laccase is a vital enzyme that can oxidize both toxic and nontoxic substrates. Its mains application are in the field of chemical industry, pharmaceutical industry, wood processing industry, food processing industry, textile industry and in numerous other areas. In this review, some of the important application are described.

Food industry

Laccases are being used for improving or modifying the colour, appearance and texture of various drinks and food products. The areas of food industry where laccase are used for food processing includes wine stabilization, juice processing and in baking. Its main application includes the removal of unwanted phenolic compounds that can create haze, browning and turbidity in clear wine, bear, and fruit juices. Additionally, laccases have the ability to cross-link biopolymers and are being used in baking industry. Laccases from whit-rot fungus *Trametes hirsuta* decreased the dough extensibility and enhanced the resistance of dough in both fluten and flour dough.[56] Researchers have also documented numerous application of laccase in various processes of food industry for example, in baking, sugar beet pectin gelation, beverage processing, bioremediation, ascorbic acid determination and as a biosensor.[57] However, there is need of more research on lowering the cost by improving laccase production techniques for industrial application.

Paper and pulp industry

Degradation and separation of lignin in wood pulp is essential for industrial production of paper but they are usually based on of polluting and conventional procedures that uses chemical oxidants that are oxygen based, and chlorine for bleaching and delignification. However, handling of wood pulp with ligninolytic enzyme such as laccase would deliver a cleaner and milder approach to delignification that will be more environmental friendly.[58] Laccase are used in specific alteration of wood fibers by producing radicals that are reactive in lignin. For instance, laccases can also be applied to produce complex materials that are lignocellulose based such as fiberboards. Laccases can activate lignin that are fiber bound during industrial production of composites, hence, can be used in boards with better mechanical and physical features without any harmful adhesives.[59]

Textile industry

Dyes used in textile industry are resistant to fading and decolourization on exposure to different chemicals, water, and light due to their synthetic origin. Numerous dyes are made of carcinogenic chemicals such as aromatic compounds and benzidine.[60] The dye wastewater from textile industries is difficult to treat and is usually not economical. Therefore, the ability of laccase to degrade various dyes of complex chemical structure including synthetic dyes has got more coverage. Laccase-based products have been established and are used in numerous industries for degrading different type of dyes. In addition to its ability to decolourise textile effluent and to bleach, they are also used in synthesise of various dyes.[61]

Nano biotechnology

Laccases are used in biosensor detection as they are capable of catalysing reactions that involves the transfer of electron without the need of supplementary cofactors. They are being practised for the detection of numerous phenolic compound. Furthermore, biosensor for the detection of codeine and morphine, plant flavonoids, electro-immunoassay and catecholamines have also been developed.[62] Laccases can also have applications in immunochemical reporting such as Nucleic acid-detection, Histochemical, Cytochemical and Western blotting by covalently bonded to a bio-binding molecule. Nanotechnology has developed more effective and well organized biosensors that have highly controlled specific adsorption and deposition of biomolecules on various type of surfaces attaining micro and nanometre order.

Other applications of laccase

Soil bioremediation

Xenobiotics and polycyclic aromatic hydrocarbons are the main contaminations in the soil, that have numerous adverse effect on environment and therefore, their degradation is of very important. These complex compounds can be degraded by using the catalytic activity of laccases. Hence, laccase causes the detoxification of munition residues by facilitate the conversion and joining of reduced 2,4,6-trinitrotoluene (TNT) metabolites to organic soil matrix.[63]

Synthetic chemistry

Sooner or later laccase might also become of a great consideration in the field of synthetic chemistry, where it can be used for synthesis of complex polymers, oxidative deprotection and as a medical agents.[64] Lately, Mustafa *et al*. (2005) manufactured a phenolic colourants with the help of an industrial laccase Suberase® (Novo Nordisk A/S, Denmark).

Cosmetics

Laccases substitutes hydrogen peroxide as oxidising agent in dye formulation. Therefore, it has numerous uses in cosmetics industries. Laccases can be used for hair dying that are less harsh, have reduced nuisance and can be handled easily as compared to current non-laccase based dyes.[66] Furthermore, some dermatological and cosmetic preparation have used laccase in various products for lightening of skin.[67]

CONCLUSION

Laccases are synthesized by a broad spectrum of bacteria, fungi and plants. It has the capability of oxidizing both phenolic and nonphenolic lignin-related complex compounds that makes it of a great interest in biotechnological and industrial applications. To meet the industrial demand, laccases are overexpressed in different heterologous host to improve their catalytic activity as well as its yield. Optimization of fermentation media and application of suitable inducers can significantly increase its production and expending less resource to make it economical. Both solid state and submerged fermentation techniques have been used for its optimal yield. Laccases can act on highly recalcitrant environmental pollutants, capable of detoxifying and decolorizing the industrial effluents, can be used effectively in bioremediation, xenobiotic degradation, textile industries, and in paper and pulp industries, along with its use in Nano-biotechnology and as a biosensors.

REFERENCES

1. Hoopes JT, Dean JF. Ferroxidase activity in a laccase-like multicopper oxidase from *Liriodendron tulipifera*. Plant Physiology and Biochemistry, 2004; 42(1): 27-33.
2. Laborde J. Sur la casse des vins. *CR Hebd Seances Acad Sci*, 1896; 123: 1074-5.
3. Giardina, P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G. Laccases: a never-ending story. *Cellular and Molecular Life Sciences*, 2010; 67(3): 369-385.
4. Leontievsky A, Myasoedova N, Pozdnyakova N, Golovleva L. Yellow'laccase of *Panus tigrinus* oxidizes non-phenolic substrates without electron-transfer mediators. *FEBS letters*, 1997; 413(3): 446-448.

5. Dittmer NT, Suderman RJ, Jiang H, Zhu YC, Gorman MJ, Kramer KJ, Kanost MR. Characterization of cDNAs encoding putative laccase-like multicopper oxidases and developmental expression in the tobacco hornworm, *Manduca sexta*, and the malaria mosquito, *Anopheles gambiae*. *Insect Biochemistry and Molecular Biology*, 2004; *34*(1): 29-41.
6. Molina-Guijarro JM, Pérez Torres J, Muñoz-Dorado J, Guillén Carretero F, Moya Lobo R, Hernández Cutuli. M, Arias Fernández ME. Detoxification of azo dyes by a novel pH-versatile, salt-resistant laccase from *Streptomyces ipomoea*, 2009.
7. Martins LO, Soares CM, Pereira MM, Teixeira M, Costa T, Jones GH, Henriques AO. Molecular and Biochemical Characterization of a Highly Stable Bacterial Laccase That Occurs as a Structural Component of the *Bacillus subtilis* Endospore Coat. *Journal of Biological Chemistry*, 2002; *277*(21): 18849-18859.
8. Baldrian P. Fungal laccases–occurrence and properties. *FEMS microbiology reviews*, 2006; *30*(2): 215-242.
9. Cruz JFO. Banana skin: a novel material for a low-cost production of laccase. *arXiv preprint arXiv*, 2008*; 0801.3337*.
10. Velázquez-Cedeño MA, Farnet AM, Ferré E, Savoie JM, Variations of lignocellulosic activities in dual cultures of *Pleurotus ostreatus* and *Trichoderma longibrachiatum* on unsterilized wheat straw. *Mycologia*, 2004; *96*(4): 712-719.
11. Viswanath B, Chandra MS, Pallavi H, Reddy BR. Screening and assessment of laccase producing fungi isolated from different environmental samples. *African Journal of Biotechnology*, 2008; *7*(8).
12. Palonen H, Saloheimo M, Viikari L, Kruus K. Purification, characterization and sequence analysis of a laccase from the ascomycete *Mauginiella sp*. *Enzyme and Microbial Technology*, 2003; *33*(6): 854-862.
13. Junghanns C, Moeder M, Krauss G, Martin C, Schlosser D. Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. *Microbiology*, 2005; *151*(1): 45-57.
14. Morozova, OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI. "Blue" laccases. *Biochemistry (Moscow)*, 2007; *72*(10): 1136-1150.
15. Yaver DS, Berka RM, Brown SH, Xu F. The presymposium on recent advances in lignin biodegradation and biosynthesis. *Vikki Biocentre, University of Helsinki, Finland*, 2001; 40.
16. Bourbonnais R, Paice MG. Oxidation of non-phenolic substrates: an expanded role for laccase in lignin biodegradation, 1990; *FEBS letters*, *267*(1): 99-102.
17. Gianfreda L, Xu F, Bollag JM. Laccases: a useful group of oxidoreductive enzymes. *Bioremediation Journal*, 1999; *3*(1): 1-26.
18. Thurston CF. The structure and function of fungal laccases. *Microbiology*, 1994; *140*(1): 19-26.
19. Gochev VK, Krastanov AI, Fungal laccases. *Bulgarian Journal of Agricultural Science*, 2007; *13*: 75-83.
20. Ferrer-Miralles N, Domingo-Espín J, Corchero JL, Vázquez E, Villaverde A. Microbial factories for recombinant pharmaceuticals. *Microbial cell factories*, 2009; *8*(1): 17.
21. Demain AL, Vaishnav P. Production of recombinant proteins by microbes and higher organisms. *Biotechnology advances*, 2009; *27*(3): 297-306.
22. Waterman SR, Hacham M, Panepinto J, Hu G, Shin S, Williamson PR. Cell wall targeting of laccase of *Cryptococcus neoformans* during infection of mice. *Infection and immunity*, 2007; *75*(2): 714-722.
23. Dubé E, Shareck F, Hurtubise Y, Daneault C, Beauregard M. Homologous cloning, expression, and characterisation of a laccase from *Streptomyces coelicolor* and enzymatic decolourisation of an indigo dye. *Applied Microbiology and Biotechnology*, 2008; *79*(4): 597-603.
24. Garg N, Baranwal R, Chhabra M, Mishra S, Chaudhuri TK, Bisaria VS. Laccase of *Cyathus bulleri*: structural, catalytic characterization and expression in Escherichia coli. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 2008; *1784*(2): 259-268.
25. Wang GD, Li QJ, Luo B, Chen XY. Ex planta phytoremediation of trichlorophenol and phenolic allelochemicals via an engineered secretory laccase. *Nature Biotechnology*, 2004; *22*(7): 893-897
26. Colao MC, Lupino S, Garzillo AM, Buonocore V, Ruzzi M. Heterologous expression of lcc1 gene from *Trametes trogii* in *Pichia pastoris* and characterization of the recombinant enzyme. *Microbial Cell Factories*, 2006; *5*(1): 31.
27. Piscitelli A, Giardina P, Mazzoni C, Sannia G. Recombinant expression of *Pleurotus ostreatus* laccases in *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, 2005; *69*(4): 428-439.
28. Nevalainen KH, Te'o VS, Bergquist PL. Heterologous protein expression in filamentous fungi. *Trends in Biotechnology*, 2005; *23*(9): 468-474.
29. Dittmer NT, Gorman MJ, Kanost MR. Characterization of endogenous and recombinant forms of laccase-2, a multicopper oxidase from the tobacco hornworm, Manduca sexta. *Insect Biochemistry and Molecular Biology*, 2009; *39*(9): 596-606.
30. Vasconcelos AFD, Barbosa AM, Dekker RF, Scarminio IS, Rezende MI. Optimization of laccase production by *Botryosphaeria sp.* in the presence of veratryl alcohol by the response-surface method. *Process Biochemistry*, 2000; *35*(10): 1131-1138.

31. Keyser P, Kirk TK, Zeikus JG. Ligninolytic enzyme system of *Phanaerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. *Journal of bacteriology*, 1978; *135*(3): 790-797.
32. Lee KH, Wi SG, Singh AP, Kim YS. Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus *Coniophora puteana*. *Journal of Wood Science*, 2004; *50*(3): 281-284.
33. Monteiro MC, De Carvalho MEA. Pulp bleaching using laccase from Trametes versicolor under high temperature and alkaline conditions. In *Biotechnology for Fuels and Chemicals* (983-993). Humana Press, 1998.
34. Lee IY, Jung KH, Lee CH, Park YH. Enhanced production of laccase in *Trametes vesicolor* by the addition of ethanol. *Biotechnology Letters*, 1999; *21*(11): 965-968.
35. Barbosa AM, Dekker RFH, St Hardy GE. Veratryl alcohol as an inducer of laccase by an ascomycete, *Botryosphaeria sp.*, when screened on the polymeric dye Poly R-478. *Letters in Applied Microbiology*, 1996; *23*(2): 93-96.
36. Assavanig A, Amornikitticharoen B, Ekpaisal N, Meevootisom V, Flegel TW. Isolation, characterization and function of laccase from Trichoderma. *Applied Microbiology and Biotechnology*, 1992; *38*(2): 198-202.
37. Thurston CF. The structure and function of fungal laccases. *Microbiology*, 1994; *140*(1): 19-26.
38. Farnet, AM, Criquet S, Tagger S, Gil G, Petit JL, Purification, partial characterization, and reactivity with aromatic compounds of two laccases from *Marasmius quercophilus* strain 17. *Canadian Journal of Microbiology*, 2000; *46*(3): 189-194.
39. Nyanhongo GS, Gomes J, Gübitz G, Zvauya R, Read JS, Steiner W. Production of laccase by a newly isolated strain of *Trametes modesta*. *Bioresource Technology*, 2002; *84*(3): 259-263.
40. Cord L, Minussi RC, Freire RS, Durán N. Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. *African Journal of Biotechnology*, 2007; *6*(10).
41. Han M, Choi H, Song H. Purification and characterization of laccase from the white rot fungus *Trametes versicolor*. *JOURNAL OF MICROBIOLOGY-SEOUL-*, 2005; *43*(6): 555.
42. Valeriano VS, Silva AMF, Santiago MF, Bara MT, Garcia TA. Production of laccase by *Pynoporus sanguineus* using 2, 5-Xylidine and ethanol. *Brazilian Journal of Microbiology*, 2009; *40*(4): 790-794.
43. Hess J, Leitner C, Galhaup C, Kulbe KD, Hinterstoisser B, Steinwender M, Haltrich D. Enhanced formation of extracellular laccase activity by the white-rot fungus *Trametes multicolor*. In *Biotechnology for Fuels and Chemicals* (229-241). Humana Press, 2002.
44. Tavares AP, Coelho MA, Agapito MS, Coutinho JA, Xavier AM. Optimization and modeling of laccase production by *Trametes versicolor* in a bioreactor using statistical experimental design. *Applied Biochemistry and Biotechnology*, 2006; *134*(3): 233-248.
45. Blánquez P, Caminal G, Sarra M. Vicent T. The effect of HRT on the decolourisation of the Grey Lanaset G textile dye by *Trametes versicolor*. *Chemical Engineering Journal*, 2007; *126*(2): 163-169.
46. Hess J, Leitner C, Galhaup C, Kulbe KD, Hinterstoisser B, Steinwender M, Haltrich D. Enhanced formation of extracellular laccase activity by the white-rot fungus *Trametes multicolor*. In *Biotechnology for Fuels and Chemicals* (229-241). Humana Press, 2002.
47. Galhaup C, Wagner H, Hinterstoisser B, Haltrich D. Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*. *Enzyme and Microbial Technology*, 2002; *30*(4): 529-536.
48. Pandey A, Selvakumar P, Soccol CR, Nigam P. Solid state fermentation for the production of industrial enzymes. *Current science*, 1999; *77*(1): 149-162.
49. Couto SR, Moldes D, Liébanas A, Sanromán A. Investigation of several bioreactor configurations for laccase production by *Trametes versicolor* operating in solid-state conditions. *Biochemical Engineering Journal*, 2003; *15*(1): 21-26.
50. Rosales E, Couto SR, Sanromán MA. Increased laccase production by *Trametes hirsuta* grown on ground orange peelings. *Enzyme and Microbial Technology*, 2007; *40*(5): 1286-1290.
51. Marques De Souza CG, Zilly A, Peralta RM. Production of laccase as the sole phenoloxidase by a Brazilian strain of *Pleurotus pulmonarius* in solid state fermentation. *Journal of Basic Microbiology*, 2002; *42*(2): 83-90.
52. Grotewold E, Taccioli GE, Aisemberg GO, Judewicz ND. A single-step purification of an extracellular fungal laccase. *World Journal of Microbiology and Biotechnology*, 1988; *4*(3): 357-363.
53. Kiiskinen LL, Rättö M, Kruus K. Screening for novel laccase-producing microbes. *Journal of Applied Microbiology*, 2004; *97*(3): 640-646.
54. Cordi L, Minussi RC, Freire RS, Durán N. Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. *African Journal of Biotechnology*, 2007; *6*(10).
55. Khammuang S, Sarnthima R. Lacease Activity from Fresh Fruiting Bodies oìGanoder/na sp. MK05: Purification and Rcmazol Brilliant Blue R Decolorization. *J. Biol. Sci*, 2009; *9*(1): 83-87.

56. Selinheimo E, Kruus K, Buchert J, Hopia A, Autio K. Effects of laccase, xylanase and their combination on the rheological properties of wheat doughs. *Journal of Cereal Science*, 2006; *43*(2): 152-159.
57. Minussi, RC, Pastore GM, Durán N. Potential applications of laccase in the food industry. *Trends in Food Science & Technology*, 2002; *13*(6): 205-216.
58. Kuhad RC, Singh A, Eriksson KEL. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. In *Biotechnology in the Pulp and Paper Industry* (45-125). Springer Berlin Heidelberg, 1997.
59. Hüttermann A, Mai C, Kharazipour A. Modification of lignin for the production of new compounded materials. *Applied Microbiology and Biotechnology*, 2001; *55*(4): 387-394.
60. Baughman GL, Perenich TA. Fate of dyes in aquatic systems: I. Solubility and partitioning of some hydrophobic dyes and related compounds. *Environmental Toxicology and Chemistry*, 1988; *7*(3): 183-199.
61. Setti L, Giuliani S, Spinozzi G, Pifferi PG. Laccase catalyzed-oxidative coupling of 3-methyl 2-benzothiazolinone hydrazone and methoxyphenols. *Enzyme and Microbial Technology*, 1999; *25*(3): 285-289.
62. Ferry Y, Leech D. Amperometric detection of catecholamine neurotransmitters using electrocatalytic substrate recycling at a laccase electrode. *Electroanalysis*, 2005; *17*(2): 113-119.
63. Duran N, Esposito E. Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. *Applied Catalysis B: Environmental*, 2000; *28*(2): 83-99.
64. Nicotra S, Cramarossa MR, Mucci A, Pagnoni UM, Riva S, Forti L. Biotransformation of resveratrol: synthesis of trans-dehydrodimers catalyzed by laccases from *Myceliophtora thermophyla* and from *Trametes pubescens. Tetrahedron*, 2004; *60*(3): 595-600.
65. Mustafa R, Muniglia L, Rovel B, Girardin M. Phenolic colorants obtained by enzymatic synthesis using a fungal laccase in a hydro-organic biphasic system. *Food Research International*, 2005; *38*(8): 995-1000.
66. Lang G, Cotteret J, Hair dye composition containing a laccase. *L'Oreal, Fr.). Int Pat Appl WO9936036*, 1999.
67. Golz-Berner K, Walzel B, Zastrow L, Doucet O. Cosmetic and dermatological preparation containing copper-binding proteins for skin lightening. *Int Pat Appl WO2004017931*, 2004; *4*(04).