Fungal Laccase Enzyme Applications in Bioremediation of Polluted Wastewater

Nil Kılıç, Farnoud Nasiri, and Demet Cansaran-Duman

Abstract Environmental pollution had emerged by the beginning of urban life and increased parallel to the industrial development. Chemicals are being produced and used largely in the branches of mainly textile industry with today's technology such as leather tanning, paper industry, food technologies, agricultural investigations, hair dyes, and many other branches, mainly the field of cosmetics. Various amounts of pollutants found in the wastewaters are the chemicals that cause color pollution in waters. In addition, they threaten the photosynthetic activity of the life in water and are also hardly decomposed. The classical methods used in the treatment (refinement) of wastewater (classical precipitation, ion exchange, ozone treatment, coagulation, flocculation, adsorption, etc.) are far from being practical and economical because of their investment and management costs and also reemergence of new pollutants after a certain period. The ability of laccase enzyme to oxidize many different forms of substrates made them to be used in different industrial and biotechnological applications as biocatalysts. Laccase activity and occurrence of laccases in fungus species were demonstrated in these studies. In addition, determination of the expression levels of the gene coding for laccase enzyme which is thought be very important in defense against oxidative stress will give information about the mechanism of the enzyme and will illuminate the development of the production of laccase-based methods. This result is going to form a major step for the studies that will provide the fungus species to be used as biosorption agents for the detoxification purposes of the wastes mainly of textile and petrochemical industries.

Keywords Wastewater • Laccase • Gene expression

1 Introduction

The increase in urbanization and industrial activity has led to harmful ecological impacts in recent years. All industry sectors compared with the textile industry which volume and composition of waste has the capacity to produce the most

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pollutant source. In particular, the accumulation of wastewater resulting from industrial activity has led to toxic and persistent pollutants produced in large quantities. Textile waste is significant amount of substances such as dyes, additives, salts, and detergents, and it is quite a threat to primarily human and all biological organisms' health. The provide of clean freshwater is serious to the future of man and biosphere [1, 2]. Nowadays, public awareness and unconscious situation to this subject have influenced all industry sectors and governments to take actions to control the situation [2, 3]. There has been various research in industrial wastewater treatments to propose more effective technologies and to reduce the release of toxic and polluting substances in water courses [2].

Traditional technologies that include different physical and chemical methods for wastewater treatments are not preferred to use due to expensive, inefficient, and often do not reduce the toxic effect. But in recent years, innovative physical, chemical, and biological methods by using treatment process are obtained to higher effective results. Especially, innovative biological methods have enabled low cost, less energy intensive, easy handling, environmentally safe, and rapid degradation thus they could be possible for wastewater treatment and provide enough information on the serious effects of pollutants on the wastewater. Biological species show different sensitivity to vary sources of pollution. Many different biological organisms that including bacteria and fungi have been standardized for ecotoxicity studies in recent years. In particular, fungi species are always proposed for toxicity monitoring of wastewaters [2, 4, 5].

Fungi, mainly white rot fungi, have long been recommended for their ability to degrade a synthetic dye, through the use of relatively nonspecific, extracellular oxidative enzymes [6, 7]. This enzymatic system is involved in lignin degradation, consists mainly of oxidative enzymes like laccases (Lac), lignin peroxidases (LiP), and manganese peroxidases (MnP) that have been known as effective against an industrial dyes [8]. White rot fungi has able to the low efficiency of dye removal by mixed bacterial communities and the high rates of dye decolorization. In this respect, many researchers suggest a combination of both processes as an option of treatment of textile wastewater containing dyes and high concentrations of organic compounds [2, 9].

2 Laccase Enzymes and Its Applications in Industrial Areas

Fungal ligninolytic enzymes have broad biotechnological applications. Especially, laccase enzyme has been developed up to pilot scale for degradation of pollutants in water in recent years. Laccase (E.C.1.10.3.2, p-benzenediol:oxygen oxidoreductase) is a copper-protein belonging to a small group of enzymes denominated blue oxidase [10]. Copper, which is located in the active center of the enzyme, plays an significant role during the catalyzed reaction [11, 12]. The catalytic core of the enzyme involve to the cluster of four copper atoms. It carries out four single electron oxidations of the substrate to a four electron reductive cleavage of the dioxygen

bond. The laccase molecule could be formed four copper atoms distributed to three sites and four type copper ions [13]. Laccase is a crucial role for oxidoreductase able to catalyze the oxidation of various aromatic compounds with the concomitant reduction of oxygen to water [14]. Additionally, in the presence of primary substrates [2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) or 1-hydroxybenzotriazole (1-HBT)] which act as electron transfer mediators, the substrate range can be extended to non-phenolic compounds [12].

The first laccase was identified in the latex of the lacquer tree *Rhus vernicifera* 130 years ago [15]. To date, laccases have been identified in plants [15], insects [16], some prokaryotes [17], and a few bacteria [18, 21]. Moreover, most known laccases are from fungi, especially from the white rot fungi. Among fungi species, the basidiomycetes, especially *Agaricus bisporus, Pleurotus ostreatus, Trametes versicolor, Phanerochaete chrysosporium*, and *Coprinus cinereus* produce various laccase isoforms [19, 20]. Finally, mention that although most laccases have been characterized from white rot basidiomycetes, other groups of fungi-producing laccases but they have been studied to a much lesser extent [13, 20]. Lichens are fungi often belonging to division Ascomycota or rarely Basidiomycota that together with live in green algae and cyanabacteria [21].

Lichen species can tolerate the environmental extremities, hence they are well known for tremendous abilities to adapt and survive under extreme conditions and for a rapid restoration of their metabolic activity [22]. A few reports exist for laccases in an important group of fungi, the lichenized ascomycetes [23, 24] demonstrated the presence of strong extracellular redox activity in some species of lichens. According to the examined study demonstrate that in lichenized Ascomycota, was occur high laccase activity especially species of in Peltigerineae family [25]. Recent studies support the view that the laccase activity was recently discovered in lichens of varies taxonomic and substrate groups [25, 26]. Lisov et al. purified the two main laccases from two different lichens species, which were *Solorina crocea* and *Peltigera aphthosa* after four sequential purification steps. Comparison of the molecular weight of these two laccases using SDS-PAGE and gel filtration chromatography demonstrated two lichen species were dimeric laccases [27].

Potentially important new application of lichen laccases are increasingly used in a growing number of industrial areas. Lichen laccases are shown to be promising alternative to their fungal counter partners for commercial applications in especially biotechnological areas. When compared to fungi and plant, bacteria, lichen enzymes, the high redox potential of copper Type 1 makes fungal laccases preferred for commercial application [28]. On the other hand, fungi are slow growers and therefore they make low production rate and contain low enzymes.

Due to the presence of copper, laccases are also named “blue enzymes” and defined as blue multi-copper oxidases (MCOs) [29]. By the reason of the efficient and low cost degradation of the pollutants properties, laccases have obtained great attention and largely used in various industry area [30–33]. Firstly, in the food industry, laccases are used for the selective removal of phenol derivatives to stabilize beverages like mainly beer, wine, and juices. Secondly, in the pulp and paper industries, laccases

are extensively usage for bleaching process, olive oil, dye and printing area of delignification of woody fibers [30]. Thirdly, common use of laccases is described biosensor, hair dyes for cosmetic industry and skin lightening [30]. Another interesting usage of laccases is also performed for decolorization of dyes, such as bleaching coupled with stone washing with cellulase of indigo dyed jeans [34].

3 Molecular Mechanisms of Wastewater Treatment

Molecular mechanism of oxidation by laccase enzymes was shown by Forootanfar and Faramarzi [35]. It was explained that the reaction catalyzed by laccase is based on the transfer of four electrons from a suitable substrate to the final acceptor molecular oxygen to form the corresponding reactive radical and water as a by-product [35–37]. The free radical may undergo additional enzymatic or spontaneous reactions to produce the final products [38]. A cluster of three copper sites containing T1 copper (blue), T2 copper (normal), and T3 copper (coupled binuclear coppers) in the catalytic core of the enzyme assists in the electron transition [35, 37, 39]. However, not all laccases have four copper ions in their active site.

4 Alternative Laccase Production Procedures

Laccase is produced by various organisms which mainly fungus species. The great potential and value in industrial and biotechnological applications have demonstrated strong interest in obtaining a large amount of laccase for practical use. However, these fungi produce laccase enzyme in small amounts and cannot meet the demand of practical applications in industry and biotechnology areas [40]. However, these fungi produce this enzyme in small amounts under normal conditions. Cheap and abundant production of laccase enzyme is very important for related areas. Thus, the main problem is to obtain sufficient laccase enzyme. Its production is dependent on various factors such as species and inducers, cultivation method [41–43]. For this purpose, many studies have been concentrated on expansion of the laccase production by inducing the laccase gene expression in fungi species. Study on the regulation of laccase gene expression may greatly contribute to the improvement of native laccase productivity in white rot fungi [19, 44].

Previous research has demonstrated that expression of laccase gene can be stimulated by some different external factors, for example, metal ions [42, 45–47], aromatic compounds structurally related to lignin or lignin derivatives [48–52], nutrient nitrogen [45, 53], and carbon [46, 54]. It was explained that the regulation of laccase gene expression by these factors previously occurs at the level of transcription [19, 44]. The effect of the same factor on the transcription of different laccase genes encoding various isoenzymes is also very different, with some being constitutively expressed and others being inducible [46]. Yang et al. show that the putative cis-acting-

responsive elements present in the promoter of laccase gene, like metal-responsive elements, xenobiotic-responsive elements could be involved in the transcriptional regulation of laccase gene [53–56].

Use of laccase need to induce both its expression and productivity through up-regulation of the enzyme-encoding genes. Contrary to an effective but complex and expensive tools of bioengineering, increasing the enzyme yield by adding inducers is perceived as simple and cost-effective [57, 58]. There are many different inducers for laccase production [59], but the most common of the effect of copper [60, 61]. Although research on the production, isolation, and expression optimization of laccases has brought many promising results in laboratories scale in the last 15 years, much more work to find the best and general conditions for the high level of heterologous expression of any laccase in yeast hosts is still needed [61].

Another promising approach; further research could be practice in nonsterile wastewater and scale-up in a bioreactor and to determine the metabolites produced during the dye decolorization process [62]. A more effective wastewater treatment of industrial scale was demonstrated by fungus species [62], and it can maintain the metabolic activity of the organism in very difficult conditions. The use of several bioreactors has been demonstrated for dye decolorization by white rot fungi [9]. However, for the establishment of a practical treatment process of textile effluents, several problems have to be overcome. Maintaining fungal growth under nonsterile operation of bioreactors represents important limitations of long-term biodegradative processes in immobilized fungal cultures that have to be overcome [63–65]. Moreover, despite the fact that the fungal process of decolorization of synthetic dyes has been too much studied, little attention has been paid to the possibility of its cooperation with the traditional biological wastewater treatment technology [66]. Another important and often underestimated aspect in related areas, laccase immobilization method were used to reduce the production cost of laccases in order to make their application more economical. Among such approaches laccase immobilization allows its reuse and improves its stability [67].

5 Conclusion

Laccases have a great importance for a wide range of industrial and biotechnological areas. Fungi and surprisingly lichen species nowadays seem to be operations such as easy handling, cheap cultivation media, and the possibilities of well-described genetic manipulations for improving the quantity and/or properties of the secreted enzyme for the industrial production of laccases [68]. Therefore, future studies will very likely focus more on in silico approaches for laccase engineering and subsequent construction of mutated and chimeric versions of laccase enzymes to improve their yields and properties [68]. A successful design of the specific heterologous production system and optimization of cultivation/fermentation conditions are fundamental for all kinds of industrial and biotechnological applications since it

is necessary to provide large-scale production and commercialization for related areas [21].

The design of improved this is omit laccase more appropriate to temperature and pH value, less dependent on metal ions, and less susceptible to inhibitory agents and aggressive hard environmental conditions [21, 67, 69]. As conventional bioremediation methods are costly with low efficiency, laccase enzymes could be good candidates to detoxify these compounds. Novel and engineered laccases are being developed to "green" biotechnological applications [31, 70] suggesting that this improved laccase is an environment-friendly candidate for use in the treatment of wastewaters from industrial area [31].

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