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Proizvodnja i primena enzim lakaze u tekstilnoj industriji

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Sažetak

Lakaze je bakar-polifenol oksidaza koja deluje na širokom spektru podloga. Postoje različite izvore organizama za proizvodnju lakaze poput bakterija, fungi i biljaka.
Fungi iz *deuteromycetes, ascomycetes, basidomycetes* su poznati proizvogjači lakaze, posebno bele truležne gljive. *Trametes versicolor, Chaetomium thermophilium and Pleurotus eryngii* su dobro poznati proizvogjači lakaze. Tekstilne industrije otpuštaju ogromnu količinu otpada na životnu sredinu, a odlaganje ovog otpada pretstavlja veliki problem. Enzimi mogu da detoksifikuju ovaj otpad a nisu stetni po okolinu. Lakaze koriste kiseonik a proizvode vodu kao nuz proizvoda. Njihova osobina da deluju na nizu podloga napravili su ih da budu upotrebljivi za više namena u mnogim industrijama, uključujući tekstilne industrije. Cilj ovog pregleda je da okupi naucne informacije za glavnu biotehnolosku proizvodnju fungalne lakaze i njihovu primenu u tekstilnoj industriji.

Ključne reči

enzim Lakaze, cvrsta površinska fermentacija (SSF), oksidacija, fungi.

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Production and application of laccase enzyme in textile industry

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Abstract

Laccase is a copper-containing polyphenol oxidase that acts on a wide range of substrates. There are diverse sources of laccase producing organisms like bacteria, fungi and plants. Fungi from the *deuteromycetes, ascomycetes, basidomycetes* are the known producers of laccase, especially white rot fungi. *Trametes versicolor, Chaetomium thermophilium and Pleurotus eryngii* are well known producers of laccase. Textile industries discharge a huge quantity of waste in the environment, and the disposal of this waste is a big problem. Enzymes can detoxify these wastes and are not harmful to the environment. Laccases use oxygen and produce water as by product. Their property to act on a range of substrates have made them to be usable for several purposes in many industries including textile industries. The aim of present review is to gather the scientific information for mainly biotechnological producing of fungal laccases and their application in textile industry.

Keywords

Laccase enzyme, solid state fermentation, oxidation, fungi.

1. Introduction

White Biotechnology, is the modern use and application for the sustainable processing and production of chemicals, materials and fuels. Biotechnological processing uses enzymes, micro-organisms and plants to make products in a wide range of industrial sectors including chemicals, pharmaceuticals, food and feed, paper and pulp, textiles, energy, materials and polymers. Some of these enzymes are used in detergents, allowing lower washing temperatures and reducing the consumption of water and energy, and others are creating new opportunities for the production of fine chemicals via biotechnological processes. Biotechnology offers new ways to improve the environmental impact of industrial processes in various sectors. In parallel, the economy will benefit as biotechnology enables the introduction of more efficient and less energy-intensive processes.

Although oxidation reactions are essential in several industries, most of the conventional oxidation technologies have the following drawbacks: non-specific or undesirable side-reactions and use of environmentally hazardous chemicals. This has impelled the search for new oxidation technologies based on biological systems such as enzymatic oxidation. These systems show the following advantages over chemical oxidation: enzymes are specific and biodegradable catalysts and enzyme reactions are carried out in mild conditions.

Laccase (benzenediol: oxygen oxidoreductases; EC 1.10.3.2, p-diphenol oxidase) is one of a few enzymes that have been subject of intensive research in the last decades. Laccases are oxidoreductases which oxidize a variety of aromatic compounds using oxygen as the electron acceptor and producing water as by-product. The interest for these old enzymes has progressively increased due to their outstanding biotechnological applicability [1]. Laccase is most widely distributed in a wide range of higher plants, bacteria, fungi, and insects [2].

The laccase molecule is a dimeric or tetrameric glycoprotein, which usually contains four copper atoms per monomer distributed in three redox sites. This enzyme catalyses the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins and aryl diamines as well as some inorganic ions coupled to the reduction of molecular dioxygen to water [3].

All substrates cannot be directly oxidized by laccases, either because of their large size which restricts their penetration into the enzyme active site or because of their particular high redox potential. To overcome this hindrance, suitable chemical mediators are used which are oxidized by the laccase and their oxidized forms are then able to interact with high redox potential substrate targets [4].

Laccases have many biotechnological applications because of their oxidation ability towards a broad range of phenolic and non-phenolic compounds. The laccase-mediated catalysis can be extended to non-phenolic substrates by the insertion of mediators. Mediators are low-molecular-weight organic compunds that are oxidized by laccase. The highly active cation radicals oxidize the non-phenolic compounds that laccase alone cannot oxidize. 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. Textile industry utilizes large volume of water and chemicals for wet processing. These chemicals range from inorganic compounds to organic compounds.

Laccase is used in commercial textile applications to improve the whiteness in conventional bleaching of cotton and the biostoning process. Laccase also can be used for denim processing, to convert dye precursors for better, more efficient fabric dyeing, for cleansing, such as cloth- and dishwashing, in a detergent to eliminate the odor generated during cloth washing, for anti-shrink treatment of wool and etc.

2. Laccase production

Laccases are the enzymes which are secreted out in the medium extracellularey by several fungi during the secondary metabolism but not all fungal species produce laccase [4].

The plants in which the laccase enzyme has been detected include lacquer, mango, peach, prune, mung bean, pine, sycamore, cabbages, potatoes, pears, apples, beets, asparagus, and various other vegetables [5]. Laccase was first discovered in the sap of the Japanese lacquer tree (*Rhus vernicifera*) and was first described laccase in 1883 [6]. Laccase was characterized as a metal-containing oxidase [7].

Laccase production occurs in various fungi as *deuteromycetes, ascomycetes and basidomycetes* [8]. *Trametes versicolor, Chaetomium thermophilium and Pleurotus eryngii* are well known producers of laccase. The white-rot fungus *Trametes pubescens* MB 89 is a source of the laccase production at industrial level [9]. When using an optimized medium containing glucose (40 g/L), peptone from meat (10 g/L), and $MgSO_4 \cdot 7H_2O$ and stimulating enzyme formation by the addition of 2.0 mM Cu, maximal laccase activities obtained in a batch cultivation were approximately 330 U mL^{-1} [10].

Laccase has been discovered in a number of bacteria including *Azospirillum lipoferum, Bacillus subtilis, Bordetella compestris, Escherichia coli, Pseudomonas syringae, Yersinia pestis and etc.*[11]. Laccase in bacteria is present intracellularly and as periplasmic protoplast [12].

Several factors influence laccase production such as type of cultivation (submerged or solid state), carbon limitation, and nitrogen source [13]. By the filamentous bacteria *Streptomyces psammoticus* MTCC 7334 is production of laccase in submerged fermentation [14]. Incubation temperature, incubation period, agitationrate, concentrations of yeast extract, $MgSO_4 \cdot 7H_2O$, and trace elements were found to influence laccase production significantly.

Submerged and solid-state modes of fermentation are used intensely for the production of laccase. Submerged fermentation involves microorganisms in high oxygen concentrated liquid nutrient medium. When fungal cell grows, mycelium is formed which hinders impeller action, due to this limitation occurring in oxygen and mass transfer. Bioreactor operates in continuous manner for obtaining high efficiency. Broth viscosity, oxygen, and mass transfer problems are solved by cell immobilization. The immobilization of the *Neurospora crassa* on membrane takes place continuous laccase production without enzyme deactivation for a period of 4 months [15].

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 [16,17]. The lignin, cellulose and hemicelluloses are rich in sugar and promote fungal growth in fermentor and make the process more economical [18].

Laccase production by both solid-state and submerged fermentation is higher in case of rice bran than other substrates. The rice bran inductive capability is based on the phenolic compounds which induce the laccase production. Many agricultural wastes such as grape seeds, grape stalks, barley bran, cotton stalk, molasses waste water and wheat bran [19,20] are also used as substrate for laccase production. Other alternatives options to increase laccase production have been the use to diverse food wastes as inducers, such as apple, orange and potato, which were screened for laccase production, under solid-state fermentation conditions, by the white-rot fungus *Trametes hirsute* [21].

3. Applications of laccase in textile industry

Laccases are diverse group of enzymes having great biotechnological potential and high market expectative due to their broad substrate specificity in the diverse fields of industrial applications such as in pulp delignification [22], textile dye bleaching [23], wastewater detoxification [24], xenobiotic detoxification [25], detergent manufacturing and transformation of antibiotics and steroids [26].

A few laccases are at present in market for textile, food and other industries, and more candidates are being actively developed for future commercialization. Being specific, energy-saving, and biodegradable, laccase-based biocatalysts fit well with the development of highly efficient, sustainable, and ecofriendly industries.

The textile industry accounts for two-thirds of the total dyestuff market and consumes large volumes of water and chemicals for wet processing of textiles. The chemical reagents used are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products. Synthetic dyes are widely used in industries as textile, leather, cosmetics, food and paper printing [27]. These dyes result in the production of large amounts of high-colored wastewater. Synthetic dyes are resistant to biodegradation [28]. Nowadays, environmental regulations in most countries require that wastewater must be decolorized before its discharge. Oxidation of toxic substrates by laccase with production of insoluble products and the subsequent separation of the precipitate is a promising approach for purification of industrial sewage.

Wastewater from textile dying process are usually treated by physical or chemical processes, which include electrokinetic coagulation, irradiation, precipitation, ozonation, or the use of active carbon and the mixture of certain gases [29]. The use of laccase in the textile industry is growing very fast. As part of the washing solution, laccase could quickly bleach released dyestuff, thus resulting in the reduction of processing time, energy, and water needed to achieve satisfactory quality of the textile.

Laccase is used in commercial textile applications to improve the whiteness in conventional bleaching of cotton and recently biostoning. Potential benefits of the application include chemicals, energy, and water saving. Laccases find potential applications for cleansing, such as cloth- and dishwashing [30]. Lantto et al. [31] found that wool fibers can be activated with laccase-mediator systems (LMS).

Replacing conventional chemical oxidants (hypochlorite), a laccase-based system has been shown capable of bleaching indigo dye in denim. By themselves, laccases cannot decolorize indigo on denim, but when combined with an appropriate mediator compound, rapid decolorization takes place. In this case, electrons are transferred from indigo to oxygen, via the enzyme and mediator compound. In denim processing applications, the combined laccase mediator system specifically decolorizes indigo. The benefits by laccase processing to hypochlorite and bleaching technologies are minimal strength loss, reduced time, reduced water consumption, reproducible performance, and a safe alternative [32]. The newest commercial product contains a solid laccase enzyme, mediator, buffer, and granulate components has been commercially available for the denim market since 1999 [32].

4. Conclusion

Laccases are produced by various sources like fungi, bacteria and insects. Laccases are a group of multi-copper enzymes that catalyze the one electron oxidation of phenols and are produced mainly by fungi. The broad commercial applications of laccases concern a great scientific interest on this group of enzymes. Many factors such as producer strain, cultivation conditions, composition of nutritive media, pH and temperature affect fungal laccases production.

They decolorize and detoxify the industrial effluents and help in wastewater treatment. They act on both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant environmental pollutants which help researchers to put them in various biotechnological applications. Recently several techniques have been developed for the immobilization of biomolecule which immobilize laccase and preserve their enzymatic activity.

For this reason the nowadays researches concerned on laccase are focused on the isolation of new fungal laccase producers, isolation and expression of laccase genes, genetic construction of super producers strains and development of low cost and effective biotechnological processes for laccase production. The use of inexpensive sources for laccase production is being explored in recent times. Besides solid wastes, wastewater from the food processing industry is particularly promising for that.

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