REVIEW

Influence of Different Inducers on Ligninolytic Enzyme Activities

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White rot fungi are important for their efficient, various and complex ligninolytic enzyme system, which is able to degrade wide variety of compounds including lignin. These enzymes are desirable for using in various industrial and bioremediation applications. However, ligninolytic enzymes are produced by fungi only in small quantities, so their use in biotechnological processes is limited due to low productivity and high economic costs. Thus, there is a great demand in induction, enhancement and stabilization of ligninolytic enzymes. The main scope of this review is to briefly summarize general and specific concepts about induction of ligninolytic enzymes produced by white rot basidiomycetes.

Keywords: white-rot fungi; enzyme activity; inducer;

1. Introduction

Enzymes have a wide range of technological applications in various fields of human activities such as food, textile, paper and pharmaceutical industries. Enzymes also play very important role in biological remediation [1], the process leading to the removal, detoxification or transformation of various organic pollutants in environment. They are also applied in nanobiotechnology, where they are used as biosensors, the analytical tools for the analysis of bio-material samples [2]. The use of enzymes for this purpose, however, entails certain limitations. These are mainly the high cost of commercial preparations and therefore are constantly looking for new, cheaper and natural sources.

One of the potencial enzyme producers are fungi that have broad enzymatic equipment. They are currently the focus of considerable attention due to their diverse application [3].

Fungi are able to decompose, or cause to deteriorate a huge variety of materials and compounds such a different type of wood, textile, stored paper, plastics, leather and diverse materials using for wrapping [4].

Species of basidiomycetes are considered to be a very interesting group of fungi including different ecological groups such as white rot, brown rot, and leaf litter fungi [5, 6]. Among them, only the white rot fungi are able to efficiently decompose lignin due to the production of ligninolytic enzymes. Lignin, a complex aromatic biopolymer, makes structural rigidity to wood and protects it from microbial attack [7] and it is extremely recalcitrant to degradation [8]. Ligninolytic enzymes are also capable of degrading various environmental pollutants, including polycyclic aromatic hydrocarbons, synthetic dyes, pesticides, polychlorinated biphenyls, herbicides and many other xenobiotics. However, ligninolytic enzymes from white rot fungi are only secreted in small amounts, so their using in industrial applications has been limited due to low productivity and high economic cost [9, 10]. A higher enzyme activity guarantees a higher and faster transformation of the target substrate and improves the applicability and effectiveness of enzyme-catalyzed processes [11].

The main scope of the review is to briefly encompass general and specific concepts about possibilities, how to increase enzyme activity of white rot fungi using natural or synthetic inducers.

2. Ligninolytic Enzymes of White Rot Fungi

Each of white rot basidiomycetes produced different enzymes, depending on cultivation conditions. Secretion of enzymes is influenced by different aspects, such as fungal species, culture type, aeration and cultivation time [12, 13, 14, 15]. These enzymes are usually produced extracellulary as secondary metabolites and they can degrade different plant materials and can colonize lot of environmental parts [16].

Fungi can secrete various isoforms of the same enzyme [14]. These isoenzymes are different in their stability, optimal pH and temperature and affinity for different substrates [16, 17].

The most important ligninolytic enzymes of white rot fungi are phenol oxidase laccase (Lac, E.C. 1.10.3.2) [18] and three heme peroxidases: lignin peroxidase (LiP, E.C.1.11.1.14), Mn dependant peroxidase (MnP, E.C. 1.11.1.13) [19] and versatile peroxidase (VP, E.C. 1.11.1.16) [20]. Other enzymes, secreted by white rot fungi, are associated with ligninolytic enzymes in lignin degradation but are unable to degrade lignin alone. For example, glyoxal oxidase (E.C. 1.2.3.5) and superoxide dismutase (E.C. 1.15.1.1) produce the H_2O_2 required by LiP and MnP [21].

Laccase is blue copper oxidase which contains four copper atoms per molecule in the catalytic center and catalyzes the four electron reduction of oxygen to water [22, 23]. Laccase is able to oxidize a huge variety of organic or inorganic compounds, including phenols (e.g. catechol, hydroquinone, 2,6-dimethoxyphenol and syringaldazine) [24, 25], aromatic amines and ascorbate [26]. Laccase is often produced in the form of numerous isoenzymes [27].

Lignin peroxidase is glycosylated protein containing heme. In the presence of endogenously produced peroxide, LiP catalyzes the oxidation of aromatic non-phenolic lignin structures to give aryl-cation radicals [28]. The oxidative lignin degradation requires the presence of veratryl alcohol, which is the substrate for LiP and also secreted fungal metabolite [29].

Mangan dependent peroxidase is also enzyme containg heme. It catalyzes H_2O_2 dependent oxidation of Mn^{2+} to highly reactive Mn^{3+}. After the cation Mn^{3+} subsequently oxidizes phenolic parts of lignin to produce free radicals. The high reactivity of Mn^{3+} is stabilized by chelators (oxalate, malonate, maleate), which are secreted by fungus [30].

Versatile peroxidase has catalytic properties of LiP and MnP. VP secreted by P. eryngii showed high sequence and structural homology with LiP, but also comprises a binding site for Mn^{2+}. It is not yet clear how much VP are expanded, but their presence suggests a close relationship with LiP and MnP [30].

Some white rot basidiomycetes contain all of these lignin-modifying enzymes, while others contain only one or two of these enzymes [31].

3. Induction of Fungal Enzyme Activity

Enzyme activity can be affected by many factors. The most critical factors are concentration of carbon, nitrogen and inducer agent [32]. The inducer is a specific molecule that induces synthesis of the relevant inducible enzyme and is usually a substrate for a given enzyme. The inducers are natural (wood, wheat, straw, fruit, etc.) [33, 34, 35, 36] or synthetic compounds (2,5-xylidine, guaiacol, ferulic acid, 2,6- dimethoxyphenol etc.) [37, 28, 38, 39, 40], which depending on the enzyme.

3.1 Nitrogen, and Carbon Sources, and Natural Inducers

Amount of carbon, nitrogen and inducer is considered to be limiting for large scale production of fungal enzymes [10]. It is well known that the white rot fungus, Phanerochaete chrysosporium, cultivated in synthetic medium, produces LiP and MnP only under nitrogen-limited conditions [41]. However, it was found that in the presence of lignocellulosic substrate, a high nitrogen concentration stimulates these enzymes production [34, 42, 43]. Previous studies have proved that both the nature and

concentration of nitrogen sources are powerful nutrition factors regulating ligninolytic enzyme secretion [44, 45, 46].

Songulashvili et al. [34] tested several inorganic and organic nitrogen sources in submerged fermentation of wheat bran to improve enzyme production by Ganoderma lucidum. The maximal value of laccase activity was revealed in supplementation of culture medium with KNO_3. The same compound slightly stimulated MnP accumulation. Among organic compounds, peptone appeared to be the appropriate nitrogen source for laccase accumulation. Levin et al. [47] used different nitrogen sources (amino acids, yeast extract, peptone) and found that the highest laccase activity was with L-glutamic acid by Trametes trogii.

The concentrate of carbon in nutrient medium and lignocellulosic substrate play important role in enzyme expression [48, 49]. Galhaup et al. [48] studied that the expressive production of laccase by Trametes pubescens started when the glucose concentration in the growth medium was a certain low, critical concentration. Cellobiose and glucose that were efficiently and quickly utilized by T. pubescens resulted in high levels of laccase activity [12]. According to Bettin et al. [50] the laccase activity in Pleurotus sajor-caju cultivation in glucose media was higher than those obtained with lactose. The contrary, lactose appeared to be the best carbon source for the laccase production by Pseudotrametes gibbosa and good for the enzyme secretion by Cerrena unicolor and Fomes fomentarius. In addition, glycerol also had significant accumulation of laccase by these three fungi [12].

Many authors used wheat straw, sawdust or bran as a substrate for increase enzyme activity [34, 51, 52, 53, 54]. Stajic et al. [35] studied that the medium carbon sources mandarine peels and grapevine sawdust for Pleurotus eryngii and Pleurotus ostreatus were the best for highest laccase activity. Makela et al. [55] found the maximal LiP activities and noticeable levels of MnP, when they used wood as a carbon source with milled alder as inducer. Bazanella et al. [56] tested agricultural and food wastes as substrate for Pleurotus pulmonarius. The highest activities of laccase were found in wheat bran, pineapple peel and orange peel. The highest activities of Mn peroxidase were obtained in pineapple peel cultures.

3.2 Synthetic Inducers

Enzyme activity can be increased not only by addition of the natural inducers, but also using synthetic inducers. It has been reported [37, 49, 57] that the addition of aromatic compound 2,5-xylidine induces several times more the laccase activities of white rot basidiomycetes. Another aromatic compounds such as ferulic acid, guaiacol, veratryl alcohol and 1-hydroxybenzotriazole have been used to increase laccase secretion [58, 59, 60]. Kuhar et al. [39] and Wang et al. [61] used ferulic acid as inducer for the highest laccase activity.

Novotný et al. [40] used Tween 80 and MnP activity was increased. Gassara et al. [62] observed that addition of Tween 80 caused the highest values of MnP activity produced by P. chrysosporium. Also in the work of Usha et al. [63] Tween 80 stimulated the production of ligninolytic enzymes secreted by Stereum ostrea. Munoz et al. [64] studied laccase of P. eryngii in the glucose medium, which contained ammonium-tartrate, and they obtained two isoenzymes with laccase activity.

Some solvents can be used to dissolve water-insoluble molecules for studies of their activity as enzyme inducers. For example dimethyl sulfoxide or ethanol are very often used as solvents to dissolve water-insoluble compounds for determination of enzyme activities. However, ethanol is also used as an active solvent to induce enzyme activity [65]. Lee et al. [66] added ethanol as a solvent to a medium containing glucose as the carbon source, laccase activity by T. versicolor increased. They also observed a mediators effect of ethanol on laccase production by Grifola frondosa and Coriolus hirsutus.

3.3 Metal Inducers

Some heavy metals are necessary for fungi, some aren't, but they can be toxic, when present in excess [33]. Usually the metals start to be toxic in concentration only a few times greater than those required [67]. The essential

fungal metals include copper, iron, zinc, nickel, manganese and molybdenum. Nonessential metals are chromium, cadmium, mercury lead and silver [68].

For white rot fungi the most important metal is manganese and copper. It has been reported that manganese plays a regulatory role in the expression of LiP, MnP, Lac and in the degradation of lignin [68, 69]. Copper is a cofactor in the catalytic center of laccase. Tinoco et al. [70] induce laccase secretion in Pleurotus ostreatus through culture medium optimization containing lignin and Cu^{2+} as inducers.

The positive synergistic effect was showed between Cu^{2+} and lignin, where the activity increased 60-fold. Metal responsive elements have also been identified in several laccase genes [33], what can explain the positive effect of copper ion on laccase level. Palmieri et al. [71] also observed supplementation of P. ostreatus cultures with Cu^{2+} and higher secretion of all laccase isoenzymes by the fungus. This result is similar to work of Khammuang et al. [72], where high levels of laccase activity after addition of Cu^{2+} were observed. The presence of copper in the catalytic center of the enzyme has been known for a lot time, but the importance of regulation role of copper in laccase production has just recently been studied [33]. The positive effect of the addition of metal ions on the production of enzyme was studied by lot of authors [42, 55, 62, 71-77, 83-85] (Table 1)

The induction of laccase by other metals, that are able to cause oxidative stress, was studied with T. pubescens by Galhaup et al. [78]. They also observed that laccase activity secreted by P. ostreatus was increased by the addition of 1-5 mM cadmium.

Extracellular enzymes must face high concentrations of metals, because they are not protected by the cell-associated metal-detoxication mechanism. When metals enter into the cell, they are able to influence the production of extracellular enzymes on the levels of transcriptional and translational regulation [33]. It seems, that low concentrations of essential heavy metals are necessary for the development of the ligninolytic enzyme system [79]. Addition of low concentrations of zinc and cooper into the metal-free synthetic cultivation medium increased the activity of LiP and MnP of P. chrysosporium [79].

4. Conclusion

White rot basidiomycetes have efficient, various and complex ligninolytic enzyme system. They have been successfully applied for treatment and decomposition of different phenolic compounds, dyes and other xenobiotics. The use of enzymes for the treatment or the removal of environmental and industrial pollutants has attracted increasing attention because of their high efficiency and high selectivity. The main issue of their implementation at industrial scale is the low yield of ligninolytic enzymes in most white rot fungi. For a more efficient secretion of these enzymes are necessary inducers of enzyme activity. Inducers have varies forms, they can be as natural substrates (straw, wood, lignin) or they can be added into medium as synthetic compounds (2,5-xylidine, ferulic acid, guaiacol, etc.). Induction of enzyme activity also can be cause by addition of some nitrogen source such as KNO_3, peptone, ammonium tartrate, etc. Many recombinant microorganisms overproduce many industrial enzymes, high expression of laccase and peroxidases in heterologous systems has not been achieved yet, they still have to be obtained from natural sources, also these enzymes are generally secreted in low quantities. It is evident that the potential applications of these enzymes in industrial and environmental technologies demand lot of amounts of these enzymes at low cost, that in future will increase the demand for potential inducers of enzyme activities.

Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform reguirements“ for biomedical papers.

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