International Journal of Current Trends in Pharmaceutical Research

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Research Article **Open Access**

Assessment of Xenobiotic Toxicity by Laccase Enzyme from *Pleurotus Fungi*

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A B S T R A C T

Most of the known laccases are of fungal origin, in particular from the white rot fungi (*Pleurotus Fungi)*. These fungi secrete lignolytic enzymes, which generate radical species that cause the complete biodegradation of lignin polymers. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumour growth and inflammation, have hypoglycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities. The objectives of this study was to assess the ability of the obtained crude laccase in the enzymatic degradation of xenophytic compound 2,4-D. Laccase production from this fungal strain is reduced.

Keywords: *Pleurotus Fungi, Laccase, xenobiotics and 2,4-D.*

A R T I C L E I N F O

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Article History: Received 29 March 2016, **Accepted** 25 April 2017, **Available Online** 15 May 2017

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Manuscript ID: IJCTPR3239

PAPER-QR CODE

Citation: Suja. D, *et al.* Assessment of Xenobiotic Toxicity by Laccase Enzyme from *Pleurotus Fungi*. *Int. J. Curnt. Tren. Pharm, Res.*, 2017, 5(3): 94-97.

1. Introduction

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. 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 [1]. 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 [2]. This oxidation results in an oxygen-centred free radical, which can then be converted in a second enzyme-catalysed reaction to quinone. The quinone and the free radicals can then undergo polymerisation. 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 [3].

The white-rot basidiomycetes are the most efficient degraders of lignin and also the most widely studied. The enzymes implicated in lignin degradation are: (1) lignin peroxidase, which catalyses the oxidation of both phenolic and non-phenolic units, (2) manganese-dependent peroxidase, (3) laccase, which oxidises phenolic compounds to give phenoxy radicals and quinones; (4) glucose oxidase and glyoxal oxidase for H_2O_2 production, and (5) cellobiose-quinone oxidoreductase for quinone reduction. The veratryl alcohol oxidase and some esterases may also play roles in the complex process of natural wood decay. In plants, laccase plays a role in lignification, whereas 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].

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[5]. In presence of ABTS oxygen uptake by laccase is faster than the HOBT. In case of *Pycnoporus cinnabarinus*, laccase was the only ligninolytic enzyme which degrades lignin. But the laccase producing capability of brown-rot fungi is not known, and no laccase has been purified. Recently it was found that brown-rot fungus *Coniophora puteana* oxidizes the syringaldazine and supports the oxidation of ABTS in *Laetiporus sulphureus*[6]. Several factors influence laccase production such as type of cultivation (submerged or solid state), carbon limitation, and nitrogen source[7] .Degradation of 2,4-D 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) [8]. The enzymes participating in the 2,4-D degradation pathway have been purified and characterized, and genes that code for these have been sequenced [9] .

Laccase is widely distributed in higher plants and fungi and has been found also in insects and bacteria. Laccase is important enzyme to oxidizes both the toxic and nontoxic substrates. It is utilized in textile industry, food processing industry, wood processing industry, pharmaceutical industry, and chemical industry. This enzyme is very specific, ecologically sustainable and a proficient catalyst. Fungal laccases have widespread applications, ranging from effluent decolouration and 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 [10]. The biotechnological use of laccase has been expanded by the introduction of laccase-mediator systems, which are able to oxidise non-phenolic compounds that are otherwise hardly or not oxidised by the enzyme alone.

Objective of the Work

The objectives of this study were to evaluate the ability of the laccase in the enzymatic degradation of xenophytic compound 2,4-D.

2. Materials and Methods

All glassware (Borosil and Pyrex, Kimmax, USA.) were immersed in 10% potassium dichromate solution in 25% Sulphuric acid cleaning solution for an hour and washed thoroughly with tap water. Then they were washed with diluted commercial detergent (Liqui-nox USA), rinsed with tap water and finally rinsed in distilled water. Dried glassware and media were sterilized in an Amsco-3021 (Gravity steam sterilizer, USA) for 25 min at15 lb/sq inch pressure.

Culture Maintenance

The studies of white rot fungi Pleurotus species was obtained from university of Madras, Chennai and it was cultured in Potato Dextrose Agar Medium (PDA). It was maintained at 4^{o} C in slants as a mother culture.

Media preperation

Boil 200g of peeled and sliced potatoes for one hour in one liter of water. Filter and make up the volume to one liter and add 3g of glucose (dextrose), agar and heated until agar gets dissolved and then the media should be sterilized using an autoclave. This media composition is used for the culture *Pleurotus* species. The BL medium and 10 ml vitamin solution (biotin, folic acid, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, cyanocobalamine, nicotinic acid, DL-calcium pantothenate, p -aminobenzoic acid, thioctic acid) as described [11] for cultivation of Trametes sp.

Screening of extra cellular laccase activity Solid plate assay:

100 ml of Nutrient media was prepared with 200 micro litre of guaiacol as substrate. Actively grown mycelial disc were inoculated on the middle of the plate. Those plates were incubated at room temperature for 48 hrs. Formation of dark brown colour was observed presence of dark brown

colour indicates the production of extra cellular laccase activity by the white rot fungi.

Enzymatic Degradation of 2.4-D by manganese peroxidase: The degradation of 2.4-D was performed in 20% acetone in 50 mM malonate buffer (pH 4.5) containing 15 µg ml−1 of each compound, 1 mM MnSO4, 30 mM glucose, 0.5 mM glutathione, 120 mUml−1 glucose oxidase and 850 mUml−1 of the enzyme. The reaction proceeded at 25 ◦C in the dark using agitation (90 rpm); five replicate samples were used for each compound. For metabolite analysis, the reaction mixture was acidified with HCl to pH ≈ 3 and the samples were extracted with three portions of ethylacetate. The extracts were concentrated using a rotary evaporator and injected into a gas chromatograph (GC). The intermediates were identified using gas chromatography coupled with mass spectrometry (GC-MS) with an ion trap detector (GCQ, Finnigan, USA). The GC instrument was equipped with split/splitless injector and an HP-5 column was used for separation (30 m, 0.25 mm inner diameter, 0.25 µm film thickness). The temperature program started at 60 ◦C and was held for 1 min in splitless mode. Then the splitter was opened and the oven was heated to 150^0C at a rate of 25 ◦Cmin−1. The second temperature ramp was up to 260 ◦C at a rate of 10^0C min−1, with this temperature maintained for 20 min. The solvent delay time was set to 5 min. The transfer line temperature was set to 280 ◦C. Mass spectra were recorded at 1 scan s−1 under electron impact at 70 eV, mass range 50-350 amu. The excitation potential for the MS/MS product ion mode applied was 0.5 V, and 0.9 V in the case of more stable ions. Methane was used as a medium for chemical ionization (CI).

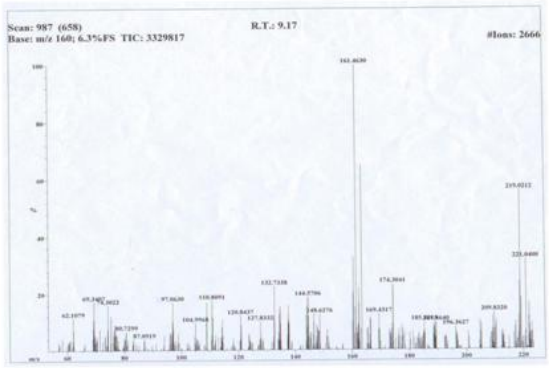
3. Results and Discussion

Enzymatic degradation of xenobiotic compound (2, 4-D degradation): To study degradation of selected xenobiotic 2, 4-D degradation tested their solubility in a mixture of acetone-water with various ratios and also measured laccase activity with respect to inhibition by acetone. Following these results selected for further experiments a mixture with 20% acetone that was sufficient to dissolve 15 µgml^{-1} of each xenobiotic compound and caused less than 20% enzyme inhibition. The activity of the enzyme during experiments in the water–solvent mixture was stable. The degradation results of individual compounds are shown in Fig-1. Xenobiotic tested only 2, 4-D degradation exhibited a concentration decrease after 168 h to 10.1 µgml^{-1} in the control treatment without the enzyme. The enzyme was able to decompose all the studied 2, 4-D degradation at different rates. The major degradation products of the studied 2, 4-D degradation, but current status were able to detect major distinct peaks only for 2, 4-D degradation. Their mass spectral characteristics are given in Fig-2 and they were identified 2, 4-D degradation respectively.

Discussion

Laccase-producing fungi have also been reported to be useful tools for xenobiotic removal in liquid effluents as well as in soil bioremediation [12]. The degradation of xenobiotic by Peroxidation enzyme was first described in *Phanerochaete chrysosporium* as a lipid peroxidation-dependent process [13]. It was later demonstrated that xenobiotic degradation by some MnPs also occurs directly: MnP from *Nematoloma frowardii* degrades anthracene, phenanthrene, pyrene, fluoranthene and benzo[a]pyrene, leading to partial mineralization [14]. The major degradation products of the studied 2, 4-D degradation, but current status were able to detect major distinct peaks only for 2, 4-D degradation. Their mass spectral characteristics are given in Fig-1 &2 and they were identified 2, 4-D degradation respectively. Benzo[a]pyrene and anthracene are also mineralized by MnP from the litter-decomposing fungus *Stropharia coronilla*. The oxidation of benzo[a]pyrene leads to benzo[a] pyrene-1,6-quinone as a temporal intermediate[15] . PAH degradation experiments showed that MnP isolated from *I. lacteus* was able to efficiently degrade three and four ring PAHs including PHE and FLT compounds with IP higher than 7.8 eV which are not degraded by the enzyme from *S. coronilla*. The MnP from *I. lacteus* had a tolerance to acetone comparable to that of MnP from *Bjerkandera adusta* and *Phanerochaete chrysosporium* [16].

Control



Treated

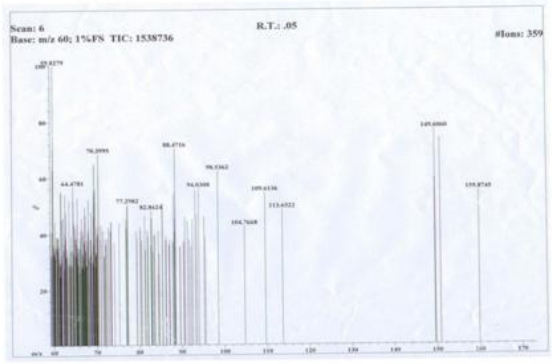


Figure 1 & 2: Enzymatic degradation of xenobiotic compound (2, 4-D degradation)

4. Conclusion

The ability of *Pleurotus* species to decolorize different dyes is evident, but the advantage of laccase treatment is a shorter treatment period. The *Pleurotus* species promising fungal strain since it produces a high laccase levels in the studied conditions. Currently, the optimization of laccase production from this fungal strain is being studied

xenobiotics compound 2-4 D being treated with the enzyme to check its potential use in detoxification of the effluents.

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