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An Investigation about the Effect of UV-Ray on Activity of Laccase and Manganese Peroxidase in Pleurotus Florida Shell Fungus

Sara Saadatmand[1], Fatemeh Sohrabi[2]

[1]Ph.D of Plant Physiology, Assistant Professor in Department of Biology,
Islamic Azad University, Science and Research Branch, Tehran, Iran

[2]M.Sc in Plant Physiology, Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran

Abstract: ***Lignin is the second most abundant compound on the earth and the most resistant one against decomposition. Fungi are able to produce Ligninases and to secrete them to their surroundings. Among them, white rot Basidiomycetes are very important. Pleurotus species are one of the Basidiomycetes. In the current research, the effect of UV-B ray on activity of Laccase and Manganese Peroxidase in Pleurotus Florida shell fungus is studied. In this regard, the Mycelium of Pleurotus Florida fungus was cultured in a liquid environment containing mineral elements and Guaiacol, during a ten days incubation period and after fifth days from the start of culturing UV-ray was applied to Pleurotus Florida fungus in four periods of time (0, 1, 2, 3, and 4 hours). The obtained results show that applying UV-ray in 1, 2, and 3 hours periods have not considerably changed wet weight and dry weight of Mycelium but during 4 hours of UV-ray applying, these weights are significantly reduced. At the other hand, applying UV-ray to Mycelium of fungus causes to significant increase in activities of Laccase and Manganese Peroxidase.***

Keywords: Pleurotus Florida, Laccase, Manganese Peroxidase, UV-Ray, Shell Fungus, Plant Physiology, Biology

1. Introduction

Lignocellulose is one of the renewable organic materials that have an important structural role in plants. Lignocelluloses are remained from various industries such as industries related to jungle, preparation and production of pulp and paper, agriculture and food industries. It consists of three important compounds including Cellulose, Hemicelluloses and Lignin. In addition, there are small amounts of Protein and Pectin in Lignocellulose, based on its origin of production [1-16]. Among these compounds, Lignin is the second most abundant biopolymer in the earth; a heterogenic polymer with aromatic structure. Lignin consists of three aromatic radicles including Coniferyl, Sinapyl and P-Coumaryl [17]. These prefabrics consists subunits of Lignin so that the oxidations of these aromatic alcohols make complex structure of Lignin which is very resistant against decomposition [18]. Connection of Lignin with Hemicellulose and Cellulose makes Lignin as a resisting barrier against decomposition. Fungi have decomposing enzymes of Lignin. Some species of Ascomycetes such as Trichoderma Reesei and Basidiomycetes causing white rot (Phaneromycetes Chrysosporium) and brown rot (Fomitopsis Palustris) are able to decompose Lignin. The ability of decomposing Lignin compared to other compounds of wood depends on environmental conditions and specie of fungus. Basically, Lignin can be decomposed by Lignin Peroxidase (Lip,E.C.1.11.1.14), Versatile Peroxidase (Vp,E.C.1.11.1.16), Manganese Peroxidase (MnP,E.C.1.11.1.13), Phenol Oxidase (Pox) and Laccase (E.C.1.10.3.2) [19, 20]. Laccase is an enzyme belongs to a family from Polyphenol Oxidase (PPO) containing Copper which is usually named as Multicopper Oxidases. Proteins with Copper atom as cofactor have an important role in many cell metabolism reactions such as photosynthesis, phosphorylation oxidative, metal ions Homeostasis and various food Catabolism and toxic chemical compounds. Laccase is one of the Proteins which have Copper. It is from the family of Oxidoreductases [21, 23, 25, and 26]. Laccase catalyzes the oxidation of various Phenol compounds by molecular Oxygen as electron recipient [21-39]. When a compound is oxidized by Laccase, renewable substrate loses a single electron and converts to, usually, a free radical. This unstable radical usually experiences a catalyzed oxidation by Laccase or subjects to non-enzyme reactions such as hydration and polymerization. In industry, Laccase is used for separating Lignin from pulp, decomposing multi-cycle aromatic compounds, decomposing pesticides or insecticides and synthesizing organic compounds. Laccase enzyme is a Protein containing large blue Copper which can be found in a wide range of life including super plants, fungi and bacteria. Laccases are separated from Ascomycetes, Deuteromycetes and Basidiomycetes fungi. Previous studies related to role of Laccase in decomposition of Lignin are mainly focused on Basidiomycetes causing white rot. This group of Basidiomycetes perform maximum amount of Lignin decomposition which involved enzymes in Lignin decomposition are Lignin Peroxidase, which catalyzes oxidation of Phenol and non-Phenol units, Manganese Peroxidase and Laccase, which oxidize Phenol compounds and cause to producing Phenoxy radicals and Quinines. A huge amount of Laccase is produced by white rotting fungi and at the present time, a number of genes related to Laccase are separated and recognized [40-53]. Some species of Pleurotus make Laccase [54-67]. Four Isoenzymes of Laccase including LCC1, LCC2, LCC3 and LCC4 are recognized which synthesize by Pleurotus Ostreatus. In the current research, the effect of UV-ray on synthesizing and activity of Laccase and Manganese Peroxidase enzymes on Pleurotus Florida shell fungus is investigated.

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2. Materials and Methods

(a) Mycelium preparation:
The pure Mycelium of Pleurotus Florida was prepared and reproduced by culturing the tissue from registered laboratory sample [68-76].

(b) Preparing a liquid culturing environment to produce Laccase and Manganese Peroxidase enzymes:
To produce Laccase, the Mycelium of Pleurotus Florida was cultured in a liquid environment consisting of KH_2PO_4 $1gL^{-1}$, $MgSO_4.7H_2O$ 0.5 gL^{-1}, NH_4NO_3 $0.2gL^{-1}$, $CaCl_2$ $0.01gL^{-1}$, $CuSO_4.5H_2O$ $0.0006gL^{-1}$, $FeSO_4.7H_2O$ $0.001gL^{-1}$, $MnSO_4$ $0.001gL^{-1}$, Guaiacol 0.02ml, Glucose $10gL^{-1}$ and zymogenic extract $0.1gL^{-1}$.

Then, in order to better producing the Laccase, pH of culturing environment was reached to 6.5 using Sodium Succinate. The culturing environment was distributed to 100 ml flasks after autoclaving. To prepare the liquid synthetic culturing environment for producing Manganese Peroxidase enzymes, 175 gr of rice bran was autoclaved in 1000 ml of water for 15 minutes at 121 degree of Celsius. Then, pH of the environment was set to 5.8 using 0.1% salts, 0.05% $MgSO_4.7H_2O$ (KH_2PO_4).

(c) Culturing the Mycelium and pre-treatment:
A disc containing pure Mycelium was transferred to each flask. All culturing incubated in dark condition for ten days at 28 degree of Celsius.

(d) Measuring wet and dry weights:
Ten days culturing was filtered. The filtered solution was used for measuring the activity of Laccase enzyme and the remained sediment was used for measuring wet and dry weights of Mycelium. Firstly, wet weight of Mycelium was measured and then, samples were dried in freezer drier for 24 hours and their dry weight were calculated.

(e) Measuring the activity of Laccase by spectrophotometry:
To measure the activity of Laccase enzyme, 3 ml of Sodium Acetate buffer 10mM, 1 ml of Guaiacol 10mM and 1 ml of filtered liquid culturing environment was used. Then, for preparation of Spectrophotometer, blank sample with wavelength of 470 nm was set in apparatus. Thereafter, wavelength of 530 nm was considered for measuring the activity of enzymes in samples.

(f) Extracting for measuring Manganese Peroxidase enzyme:
The process was performed using a solution containing Guaiacol 0.4 mM, Sodium Lactate buffer 50 mM (pH 4.5), $MnSO_4$ 0.2 mM and Hydrogen Peroxide 0.1 mM. The total volume of the solution, in addition to filtered culturing environment solution, was 1 ml.

(g) Measuring the activity of Manganese Peroxidase enzyme by spectrophotometry:
The activity of enzyme was measured in wavelength of 465 nm.

3. Results and Discussions

Utilizing UV treatment for one hour significantly reduced wet weight of Mycelium compared to instance sample ($P \leq 0.05$). However, two and three hours UV treatments have not considerable effect on wet weight of Mycelium. Applying UV-ray for four hours severely reduced wet weight of Mycelium of fungus during incubation which is significant compared to instance sample ($P \leq 0.05$).

Dry weight of Mycelium in various times of radiating. Applying UV-ray for four hours lead to a considerable decrease in dry weight of Mycelium of fungus but in one and two hours UV treatments, this reduction of dry weight is less considerable, although it is meaningful compared to instance sample. In three hours UV treatment, decrease in dry weight of Mycelium is approximately same as instance sample.

In one, two and three hours UV-ray treatments, the activity of Laccase enzyme is considerably increased so that increasing the applying time of UV-ray leads to increasing the activity of enzyme but in four hours UV treatment, the activity of Laccase is same as to instance sample and has not a meaningful difference with instance sample. Figure 1 confirms this issue as applying UV-ray for one; two and three hours lead to discoloring of culturing environment of Mycelium of fungus to brown.

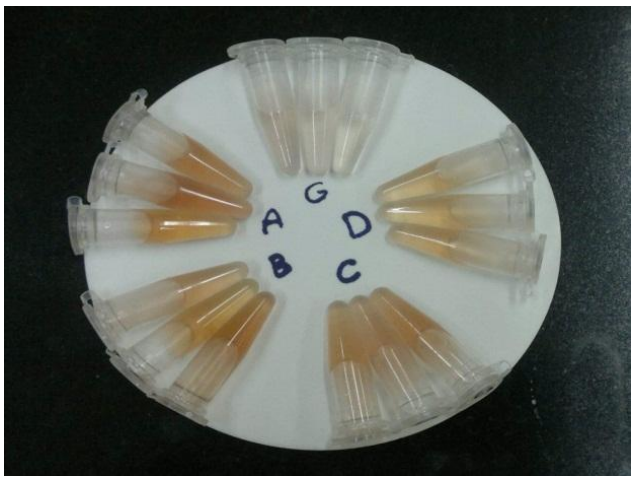


Figure 1: Comparing the discoloring of culturing environment of fungus in instance (G1), one hour UV treatment (A), two hours UV treatment (B), three hours UV treatment (C), and four hours UV treatment (D) samples to measure the activity of Laccase enzyme.

Applying UV-ray on liquid culturing environment of Pleurotus Florida fungus lead to significant increase in activity of Manganese Peroxidase enzyme and by increasing the radiation time, activity of enzyme also increased. However, the increase in activity of enzyme in four hours UV treatment is lower than other treatments. Figure 2 also shows that culturing environment of fungus is discolored toward brown due to applying UV-ray.

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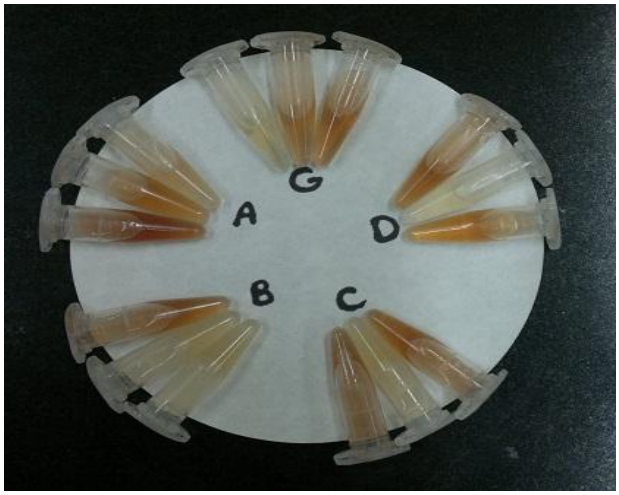


Figure 2: Comparing the discoloring of culturing environment of fungus in instance (G1), one hour UV treatment (A), two hours UV treatment (B), three hours UV treatment (C), and four hours UV treatment (D) samples to measure the activity of Manganese Peroxidase enzyme.

4. Conclusions

The obtained results show that UV-ray radiating leads to increase in activity of Laccase enzyme. Applying UV-ray causes to increase in activity of Laccase which is due to oxidation reaction defining Guaiacol which in turn, leads to brown to red color. The effect of UV-ray on production of Laccase is probably due to resisting Mycelium of fungus against UV-ray.

Laccases have various duties in the cell including participating in biosynthesizing Lignin, decomposing the walls of plant cell, creating pathogenic fungus characteristic for plants, creating Melanin in Bacteria, hardening the membrane of insects and creating human illness related to Melanin.

All of these duties of Laccases are because of oxidation of a wide range of aromatic compounds. Laccases have various, and sometimes anomalous, role based on this oxidation property. For example, plant Laccases oxide Monolignols to produce polymeric Lignin while Laccases belong to white rot fungi de-polymerized Lignin and hence, lead to decomposing Lignin. Under UV radiation, Melanin synthesizing increases in fungus cells. Melanin is a pigment that protects fungi from environmental stresses such as UV radiation, high temperature, anti-microbial factors and decomposer enzymes.

Four types of Melanin are produced in fungi which all of them are Phenol compounds that convert to Quinones due to enzyme oxidation. However, if this enzyme activity is not performed, those compounds polymerize and convert to Melanin pigment.
Melanin is very similar to Lignin from chemical point of view. So, white rot fungi are able to decompose Melanin by their oxidative enzymes. Hence, considering that Laccases catalyze single electron oxidations of Phenols or aromatic Amines to produce various products, it is possible that Laccases are involved in Melanin biosynthesizing and cause to producing Quinones instead of Melanin. It should be noted that the extracted Laccase from Lentinoula edodes fungus leads to oxidation of L-DOPA Melanin (β3 and β4 Dihydroxy Phenyl Alanin) to L-DOPA Quinones. Our results about the production of Melanin by Conodium of Aspergillus Nidulans fungus and describing Laccase gene are agreed.

There are numerous studies about the intensifying the activity of Laccase in fungi relating to composition of culturing environment were investigated the effect of Xenobiotics on the catalytic activity of organizational Laccases. In addition, they were used from metallic ions and organic compounds to consist induced Laccases.

UV-ray with lower than three hours radiation has not a considerable effect on growing the Mycelium of Pleurotus Florida but more than 4 hours applying UV-ray reduced wet and dry weights of Mycelium. Applying UV on Mycelium is a stress for fungus but in short times, the stress effects due to immunity system of plant are not sensible.

The physiological role of Laccase in Bacteria is protecting from toxic compounds of environment in response to respiratory stress. White rot fungi and their oxidative enzymes are able to decompose natural Xenobiotics. The oxidation of non-Phenol compounds, which are similar to Lignin, catalyzes by Lignin peroxidase to produce cationic Radicles of Aryl. Manganese peroxidase encourages peroxidation of unsaturated fats and in this condition, oxides non-Phenol compounds, which are similar to Lignin, but the activity of Laccase is limited to compounds with low ionization potential such as Phenols.

The effect of duration of UV-ray radiation on production of Manganese Peroxidase enzyme which increases as duration of UV-ray radiation increased. This increase is ascendant in 1, 2 and 3 hours and although this is reduced in 4 hours, it is still more than instance sample. Applying UV stress leads to peroxidation of fats which is same as to effect of Manganese Peroxidase. Generally, due to increasing the free radicals during stress applying, the activity of Anti Oxidant enzymes such as Peroxidases increases. Increasing the activity of Manganese Peroxidase also is probably due to resisting against stress effects.

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