Sciencia Acta Xaveriana
ISSN. 0976-1152

Volume 1
No. 1
pp. 49–55

Laccase Mediated Degradation of Cypermethrin and Endosulfan by Using Ganoderma lucidum

A. Cholarajan[1*] **and Ahamed John. S**[2]

[1]P.G. Department of Microbiology, Meenaakshi Chandrasekaran College of Arts and Science, Pattukkottai–614 626, Thanjavur (DT), India

[2]PG and Research Department of Botany, Jamal Mohemad College, Trichirapalli–620020, India

Abstract. Laccase activities were found after GLC analysis of culture liquid of mycelium extract *Ganoderma lucidum* grown on malt extract medium with or without inducers. These laccase enzyme (E.C.1.10.3.2 paradiphenol: oxygen oxidoreductase) catalysis the oxidation and polymerization of aromatic compounds in the presence of molecular oxygen. Laccase activity in the malt extract medium had on pH 3–4 and was stable from pH 3–10 during 24 hr at room temperature. This enzyme had wide substrates specificity on hydroquinone, methoxy-substituted monophenols and aromatic amines. The laccase activity was found only with compounds having a redox potential lower than 0.5 mV. The highest activity was obtained with methoxy, methyl-substituted p-hydroquinone and aromatic diamines. Some activity also occurred with the aliphatic compounds 3, 5–cyclohexadiene-1, 2-diol.

(Received: 09 December 2009)

1 Introduction

Laccases (paradiphenol: oxygen oxidoreductases) are produced by *Ganoderma lucidum*. Laccases were able to catalyze the oxidation of a range of inorganic and aromatic substances by the removal of electrons with the concomitants reduction of oxygen (O_2) to water [1]. Fungal laccase are generally active at low pH values (pH 3). Laccases are glycoproteins with molecular weight of 50–130 kD. The carbohydrate moiety of themajority of laccases consists of mannose, *N*-acetylglucosamine, and galactose and constitutes about 45% of the protein mass in laccases of plant origin [2]. Fungal laccases have lower carbohydrate contents (10-20%). In laccases from the basidiomycete *P. eryngii*the carbohydrate moieties are 1 and 7%, whereas in laccases from *C. fulvocinerea*and *B. cinerea* carbohydrate contents are 32 and 49%, respectively, i.e. are high for fungal laccases [3]. Many researchers think the carbohydrate moiety of the molecule is responsible for the stability of the protein globule: deglycosylation of laccase from *Ganoderma lucidum*completely inhibited the activity [4]. Laccases belong to the class of blue oxidases and contains four copper atoms/molecules, distributed three different types [5]. The type 1 site is responsible for immerse blue colour of the enzyme due to maximum absorbance to 605 nm; type 2 and type 3 site in corporate, two copper centers and it is responsible for a band near 330 nm. The optimal temperature range of the fungal Laccase activity is 30°C to 60°C [2].

Cybermethrin. Molecular formula : $C_{12}H_{19}Cl_2NO_3$.

The IUPAC name is (RS)–α-cyano-3 phenoxy benzyl (IRS)-cis, trans-3-(2,2-chlorovinyl)-2,2-cyclopropane carboxylate, in C.A usage (RS) cyano (3-phenoxy phenyl) methyl (IRS)–cis, trans 3-(2,2-dichloro ethyl cyclopropane carboxylate. The acute oral LD 50 is: for rates 300–412 mg/kg. It is a slight skin irritant, a mild eye irritant and can cause skin sensitization [6]. It is relatively toxic to honey bees.

Endosulfan. Molecular formula: $C_9H_6O_3S$.

The IUPAC name is 1,4,5,6,7,7-hexa chloro- 8,9,10 trinorbon-5-en, 2,3-xylene dimethyl sulphite, endosulfan is a mixture of 2 sterio isomers; alpha endosulfan, endosulfan(I),

the acute LD 50 is for rates 80–110 mg tech (in oil)/kg. The acute dermal LD 50 for rabbit is 359 mg/kg. It is highly toxic fish but, in practical use, should be harmless to wild life and honey bees

2 Materials and methods

Ganoderma lucidum was collected from the Department of Microbiology. Anbil Dharmalingam College and Research Institute, TNAU, Trichirapalli, Tamilnadu, India.

Cultivation

Inoculate the matured *Ganoderma lucidum* culture to 500 ml of sterilized malt extract medium in 1000 ml of conical flask. It was incubated at 25°C for 5–7 days. This culture was used for enzyme assay.

2.1 Enzyme assay

The collected cultures were filtered through muslin cloth and centrifuged at 500 rpm for 25 min. The supernatant was used for enzyme assay. The assay of mixture consisting of 5 ml of 0.1M Guaiacol in 0.1M Sodium phosphate buffer at pH 6.0., 0.1 of enzyme source was added to the mixture and incubated for 5 min. The absorbance was determined at 412 nm; one unit is equivalent to change in absorbance of 0.10 per min [4].

Determination of chemical pesticides detoxification

The degradation of chemical pesticides were identified by has liquid 0.4% of Cypermethrin and Endosulfan were prepared by mixing 0.04 ml of Endosulfan and Cypermethrin in 10 ml of double distilled water. The pure culture *Ganoderma lucidum* was inoculated into pesticide containing container. In 15 days of time intervals the sample was harvested and for performing Gas Liquid Chromatography (GLC).

GLC analysis

The concentration of the reactant and the cross-coupling products were determined using GLC (Gas Liquid Chromatography), (Chemito Model 3865, India, Integrator; Shimadzu Model CR3A). The column was a 3% OV 1718–100mesh (1 m $\times$ 1.5 cm) for Endosulfan and 3% Dexi/300. 100–120 mesh (0.75 m $\times$ 1.5 cm) for Cypermethrin was used. The GLC parameters and mobile phase are listed below.

Mobile phase

Nitrogen Gas	40 ml/min
Zero air	30 ml/min
Hydrogen Gas	15 ml/min

Calculation

$$\frac{\text{Weight of Technical} \times \text{Area of Sample}}{\text{Weight of Sample} \times \text{Area of Technical}} \times \text{Purity}$$

Operational procedure for GLC

The required column was fitted in the port 3% OV 17 m, 80–100 mesh (1 m $\times$ 0.05) for Endouslfan and 3% Dexi/300; 100–120 mesh (0.75 mm $\times$ 1.5 mm) for Cypermethrin. Then the required temperature in the injection port, detection port and oven was setted. Open the carrier gas (Mobile phase) Nitrogen at 40 ml/min. Time was allowed for the instrument to show the ready lamp. Then open the zero air at 30 ml/min. Hydrogen cylinder was opened and the flow rate was maintained at 15 ml/min. Ignition switch was pressed. Then integrator was switched on. After the ready lamp was on 2 μl of sample was injected into the injector port. The peak gets printed in the integrator. The printed chromatogram was collected and calculated by using area printed.

3 Results and discussion

The growth on malt extract broth showed oyster like mycelium growth after 14th day of incubation. *Ganoderma lucidum* producing laccase enzyme was assayed. The assay revealed that the extra cellular laccase accounted nearly 90%of the total medium. The laccase was found to gradually accumulate in the mycelium from 8th day onwards. (Table 1)

Table 1: Laccase enzyme assay.

Days of sampling	OD value at 412 nm
8	1.09
10	1.10
12	1.11
14	1.12
16	1.13
18	1.14
20	1.15
Control	1.00

The toxic concentration of pesticides varied according to the substitute groups. The amount of Cypermethrin and Endosulfan were found to be decreased when the *Ganoderma lucidum* was added to the malt extract medium containing the pesticides. The analysis of sample at regular intervals (0th, 15th, 30th, 45th days) in GLC showed that the pesticide concentration has increased to a non-toxic level due to the degradation activity of white- rot fungi. The laccase secreted by the organism thus played an important role in degrading the pesticides. *Ganoderma lucidum* producing laccase enzyme had shown the degradation activity on Cypermethrin (Table 2).

The present study had shown that at the initial stage, the concentration of endosulfan was 4.86%. After 15 days of incubation, the concentration of Endosulfan was reduced to 3.65%, Endosulfan concentration was analyzed on 30th and 45th days was 1.76% and 1.20% respectively. The result revealed that the concentration of endosulfan was reduced gradually by activity of laccase enzyme (Table 3).

Table 2: Degradation of Cypermethrin by laccase enzyme.

Days	Concentration of Cypermethrin percentage in *Ganoderma lucidum*
0	5.49
15	2.76
30	1.26
45	1.12

Table 3: Degradation of Endosulfan by laccase enzyme.

Days	Concentration of Endosulfan percentage in *Ganoderma lucidum*
0	4.86
15	3.65
30	1.76
45	1.20

In recent years the pesticide pollution is a major problem so that the biodegradation of pesticide has gained importance, environmental pollution were neutralized by this biodegradation methodology. The work has been focused on the degradation of pesticides like Endosulfan and Cypermethrin by laccase from *Ganoderma lucidum* [4]. All the findings made were correlated with the majority of pesticide detoxification findings. This work was carried out only at laboratory scale and can be extended to the field level to effectively and economically control the agricultural pollutant like chemical pesticides. *Ganoderma lucidum* produces a type of copper containing extracellular enzyme called laccases. Which are capable of oxidizing chemical pesticide containing phenolic compound, the production of laccase enzyme in broth was increased during the mycelial development.*Pleurotus spp.* is a white-rot basidiomycetes fungus, commonly called oyster mushroom. It is able to degrade recalcitrant aromatic compound like polychlorinated biphenyl polycyclic aromatic hydrocarbon or pentachlorophenol in liquid culture [1]. In nature it grows well on different type of lignocellulosic mate-

rials converting them into more digestible and protein enriched substances suitable for use as animal feed. Artificially it can be cultivated in malt extract medium for its characters. The result reveal that the fungus has capability of degradation pesticides that by secreting the enzymes laccase and this study is helpful to exploit the extracellular laccase enzyme from *Ganoderma lucidum* [2] for the bioremediation of pesticide contaminated agricultural lands. Further studies are required to standardize the technology for effective utilization.

Acknowledgement

The authors are greateful to the Principal, Head, Department of Botany, J.M. College, Trichy and the Principal, Meenaakshi Chandrasekaran College of Arts and Science, Pattukkottai–614 626, Thanjavur (DT), S.India.

References

[1] D.C. Eaton, *Mineralization of polychlorinated biphenyl's by Phancrocharte chrysosporium a lignolytic fungus*, Enzyme Microb. Technol., **7** (1984) 194–196.

[2] R. Bourbonnais, and M.G. Paice, *Enzymatic delignification of Kraft pulp using Laccase and mediator*, TAPPI, **79** (1996) 199–204.

[3] P.J. Collins, M.J. Kotterman, A. Field, and A.D. Dobson, *Oxidation of antrhacene and benzo (a) pyrene by laccase from Trametes versicolor*, Appl. Environ. Microbiol., **62** (1996) 4563–4567.

[4] I. Dill, and G. Kraeplin, *Palo Padrido: model for Extensive Deliginification of wood by Ganoderma aplantun*, Appl. Environ. Microbiol. **52** (1986) 1305–1312.

[5] F.R. Guenther, R.M. Parris, and L.K. Hipert, *Determination of phenolic compound in alternate fuel matrices*, J. Chromatogr., **207** (1981) 256-261.

[6] B. Reinhammar, in *Copper Proteins and Copper Enzymes*, Vol. 3 (R. Lontie, ed.) CRC Press, Boca Raton, (1984) pp. 1–35.