The Pharma Innovation Journal 2021; 10(10): 2404-2406

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2404-2406

www.thepharmajournal.com
Received: 13-08-2021
Accepted: 19-09-2021

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Estimation of Laccase enzyme in different *Pleurotus* spp.

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Abstract
Lignin is the major component followed by cellulose and hemicellulose that are abundantly present in plant biomass. Laccase is the key enzyme which can be produced naturally by various organisms. Among them *Pleurotus* spp. dominated in the secretion of laccase and plays a key role in the lignin degradation. This study aims to explore the quantitative estimation of laccase enzyme between two different *Pleurotus* spp. (*Pleurotus djamor* var MDU 1 and *Pleurotus djamor* isolate woody 1) from the agricultural waste. The results obtained from the present investigation depicted that *Pleurotus djamor* var MDU 1 has recorded the maximum production of laccase enzyme by utilizing the paddy straw as substrate.

Keywords: *Pleurotus* spp., laccase, lignin degradation, agricultural waste

1. Introduction
Agricultural wastes are produced from the remnants of the raw and processed agricultural products. They are mainly obtained from the plants under field conditions and from industries during processing. They are mainly composed of 35 – 50% cellulose, 25 – 35% hemicellulose, 10 – 25% lignin and rest with ash and others (Kumla *et al.*, 2020). Though, cellulose and hemicellulose are predominant that can be easily degraded and extracted from the resources because of simple sugars. Besides cellulose and hemicellulose, lignin is one of the vital components and offers structural integrity to the plants and defend against abiotic stress. It is a highly branched and complex polymer consisting of various functional groups that resist biodegradation (Kim *et al.*, 2021) [4]. Laccase is the key enzyme belongs to the group of oxidative enzymes that involved behind the lignin degradation. Even though, laccase can be produced by plants, arthropods, microbes such as bacteria and fungi, the basidiomycetous fungi plays the key role in lignin degradation (Batal *et al.*, 2014). Interestingly, mushroom cultivation is going trendly at present and expected to be at future. Globally, the mushroom fungus belongs to the genus such as *Agrocybe, Agaricus, Pleurotus, Tremella, Lentinus, Volvariella* and among them *Agaricus, Pleurotus, Lentinus, Volvariella and Flammulina* were the most cultivated mushrooms (Ma *et al.*, 2018) [5]. Due to its versatility *Pleurotus* spp. are diversified under different agroclimatic conditions. It is highly saprophytic in nature and serve as a potential secretor of lignin degrading enzymes (Naraian *et al.*, 2014) [7]. In this study, naturally isolated *Pleurotus djamor* isolate woody 1 and commercially cultivating *Pleurotus djamor* var MDU 1 were examined for lignolytic enzymes as they differ by morphological characters.

2. Materials and Methods
2.1 Collection of mushroom strains
Different mushroom strains were collected from different localities of Tamil Nadu *viz Pleurotus djamor* isolate woody 1from Agricultural college and Research Institute, Killikulum and *Pleurotus djamor* var MDU 1 Agricultural college and Research Institute, Madurai from respectively.

2.2 Isolation of mushroom culture
Fresh, fully matured and disease free basidiocarp of oyster mushroom was collected and surface sterilized with 70% ethanol. The sporocarp was divided into two halves by splitting longitudinally with sharp sterilized blade. At the junction of stipe and pileus portion of split opened basidiocarp, a few small pieces of plectenchymatous tissues were taken and these

tissue bits were further sterilized with 70% ethanol for 10 seconds and subsequently were washed three times with sterile water and later the redundant moisture had been removed by placing the tissue bits in sterile filter paper. Meanwhile the streptomycin sulfate of 100 ppm was added to the sterilized PDA medium and was poured in petri dishes and allowed to cool and solidify at room temperature. The tissue bits were inoculated on the PDA media at equilateral triangular position and incubated for 28℃ till the mycelia covered the entire surface of media. The actively growing tip of the fungal mycelia was introduced into freshly prepared PDA media and slants for the observation of morphological differences and growth pattern and for the qualitative and quantitative estimation of enzyme studies.

2.3 Production of Lignolytic enzymes

Mushroom fungus can degrade lignolytic compounds present in the ecosystem. Moreover, laccase is the predominant enzyme produce by mushroom fungus. For production of enzymes, paddy straw was used as substrate. Paddy straw was taken and dried before processing. Paddy straw was ground and sieved to less than five mm size. Ten gram of paddy straw was taken into 250 ml of conical flask containing 75 ml of sterile water. Then the substrate was autoclaved at 121℃ for 20 min. Simultaneously, 25 ml of potato dextrose broth was prepared and autoclaved at 121℃ for 20 min. The fungal discs of parent and hybrid strains were inoculated into the liquid broth and incubated at 15,000 rpm and temperature of 28 ℃ for 10 days. Then the liquid culture was transferred to the paddy straw used as substrate and incubated at 150 rpm and temperature of 28 ℃. The extracellular enzymes were separated by centrifuging the liquid culture at 12,000 rpm for 15 min at 4℃ and the supernatant was collected. The enzyme activity was performed with the collected supernatant.

2.4 Laccase

Laccase is one of the major lignolytic enzyme produced by the Basidiomycota fungus which can be determined by using Guaiacol was used as substrate. Oxidation of guaiacol by laccase produces red color which is an indicator for production of laccase enzyme. (Monssef *et al*., 2016) [8]

One ml of guaiacol and three ml of sodium acetate buffer were added to the test tubes and one ml of the enzyme extract was added and mixed to the test tube. For blank solution, one ml of water was added instead of enzyme solution. The test solution and the blank solution were incubated at 30℃ for 15 min and the absorbance was read at 450 nm.

The enzyme activity was expressed as International Units, where 1 IU is the amount of enzyme required to oxidize 1µmol of guaiacol per min. the laccase activity in U/ml was calculated by the following formula,

$$E.A = A \times V/t \times e \times \nu$$

Where,

E.A is the enzyme activity

A is the absorbance

V is the total mixture volume (ml)

ν is the enzyme volume (ml)

t is the incubation time

e is the extinction coefficient for guaiacol (0.6740µM/ cm)

2.5 Statistical analysis

Analysis of variance was analysed to find the differences between each treatment and Duncan Mean Range Test were analysed for comparison of mean by using SPSS 16.0 software.

3. Results and Discussion

3.1 Morphological characterization of mycelial growth on PDA

The results obtained from this study showed that the different morphological characters exhibited by two *Pleurotus* spp. *Pleurotus djamor* var MDU 1 exhibited thick, cottony white, compact and rhizomorphic mycelial growth (Fig 1 & Table 1) which is similar to Praveen *et al.*, (2018) [5] and *Pleurotus djamor* isolate woody 1 had thin, sparse, light white and non-rhizomorphic mycelial growth on Potato dextrose agar (Samundeeswari *et al.*, 2020) [10].

3.2 Production of Laccase enzyme

The colour change of enzyme substrate mixture from brown to red depicted the confirmation of laccase enzyme production by the two *Pleurotus* spp. (Fig 2). *Pleurotus djamor* var MDU 1 recorded the highest laccase enzyme production approximately 3- fold when compare to *Pleurotus djamor* isolate woody 1 which was showed (Table 2).

Kalmis *et al.*, (2008) showed that *Pleurotus ostreatus* was recorded for the maximum laccase activity of 62.9 U/L whereas *Pleurotus citrinopileatus* was recorded for the minimum laccase activities of 1.68 U/L. Godinaz *et al.*, (2016) worked with the enzyme activity of three wild mushrooms namely *Lentinula boryana*, *Pleurotus djamor* var. *roseus* and *Pycnoporus* sp. From the experiment, *Lentinla* showed the maximum production of extracellular laccase activity followed by *Pleurotus* whereas *Pycnocarpous* showed the least laccase activity.

Table 1: Morphological characters of mycelium on PDA

S. No	Mushroom varieties	Mycelial Phenotype
1.	*Pleurotus djamor* var MDU 1	Thick, cottony white, compact and rhizomorphic mycelium
2.	*Pleurotus djamor* isolate woody 1	Thin, sparse, light white and non-rhizomorphic mycelium

Table 2: Estimation of Laccase enzyme

S. No	Mushroom varieties	Laccase activity Uml^{-1}
1.	*Pleurotus djamor* var MDU 1	0.1421 ± 0.01482^{a}
2.	*Pleurotus djamor* isolate woody 1	0.0358 ± 0.00104^{b}
3.	Control (Distilled water)	0.0002 ± 0.00014^{c}

± denotes the standard deviation

a, b, c denotes that data are significantly different at P=0.05 from their respective unsupplemented set in the same column

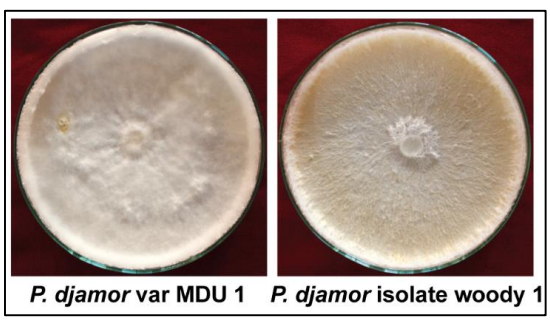

Fig 1: Morphological characters of different *Pleurotus* spp.

Fig 2: Production of laccase enzyme

4. Conclusion

From this study, *Pleurotus* spp. exhibit a wide range of habitat and have the ability to degrade the biodegradable resources to various extents. So that *Pleurotus* spp. can be effectively utilized for bioremediation in future.

5. Acknowledgement

The authors are thankful and showing gratitude to Tamil Nadu Agricultural University for giving facility.

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