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The growth and laccase activity of edible mushrooms involved in plastics degradation

Ong Ghim Hock*, Hing Wai Lum, Ding De Qin, Wong Kok Kee and Wong Ling Shing

Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.

ABSTRACT

The accumulation of plastic materials leads to serious environmental issues as these materials degrade at a very slow rate. Many approaches have been proposed to reduce the plastic materials, including ways to increase the rate of the degradation process. In this study, mycoremediation of plastic material using commonly available edible mushrooms (*Pleurotus ostreatus*, *Pleurotus abalones* and *Agaricus bisporus*) is investigated. The mushrooms were grown in optimized salinity and temperature with two plastics materials -polystyrene (PS) and polyethylene (PE); both served as their sole carbon sources. The changes in laccase activity and biomass were determined after 7 days of incubation. The results showed the presence of laccase activity and growth in biomass, indicating that degradation of plastic materials had taken place. The results showed that *P. ostreatus*, *P. abalones* and *A. bisporus* were able to utilize PS and PE as carbon sources. Among all three mushrooms, *A. bisporus* was found to be the best candidate to be used in the degradation of PS and PE.

KEYWORDS: edible mushroom, plastic degradation, laccase enzyme.

INTRODUCTION

Plastics have been produced in large scale since the 1950s to cater for a wide range of societal benefits, especially in the fields of industry, construction, medicine and food preservation [1]. However, mass production of plastics resulted in growing environmental concerns due to inadequate methods of waste disposal, ubiquitous distribution of plastic waste and slow degradation rates [2]. Two of the most prevalent plastic materials commonly found in the environment are PS and PE.

PS is a tough material, is water resistant, has low electrical conductivity and can be manufactured cheaply [3], and is thus useful for production of disposable utensils, storage containers, laboratory equipments [4], and protectants [5]. PE is safe to be used as packaging material for food, cosmetics, medicines and flammable liquids due to its toughness, low electrical conductivity, chemical stability and water-resistant characteristics [5]. With the increasing cases of illegal dumping, PS-based and PE-based products can now be found everywhere including oceans and rivers [6].

The conventional ways to reduce plastic wastes include recycling, burning or landfill disposal [1]. But according to the statistics, only 9% of the plastics is being recycled while the rest is either burnt or accumulated in landfill, causing ill effects to the environment like air and water pollution [7]. Other more environment-friendly methods include replacing the current plastics with a more degradable plastic material like cellulose acetate [8] or limiting the usage of plastics [9] to reduce plastic pollution in the environment. However, plastic pollution still remains as an issue due to lack of sustainable and environment-friendly approaches to reduce it.

*Corresponding author: ghimhock.ong@newinti.edu.my

Therefore, plastic biodegradation using the mycoremediation approach could be the solution.

Mycoremediation is an effective approach for environment waste management [10, 11]. It can be used for plastic degradation as well [12]. Most of the potential fungi species belong to the phylum of Ascomycetes, Basidiomycetes and Deuteromycetes [13]. Fungi are good decomposers because they can produce various types of extracellular enzymes including laccase [14] that are responsible for degrading different types of xenobiotics including plastics [15]. Laccase is a blue multi-copper oxidase that is able to oxidize different aromatic or non-aromatic compounds [16]. However, the complete profile of fungi usage in biodegrading has not been reported. The capability of fungi to survive and tolerate stress conditions in the presence of plastics is still unknown.

A study conducted by Viswanath *et al*. [17] stated that production of laccase is affected by different abiotic factors, including temperature and salinity. Thus, the objective of this study was to determine the growth and laccase enzyme activity of three commonly found edible mushrooms (*Pleurotus ostreatus*, *Pleurotus abalones* and *Agaricus bisporus*) during plastic degradation under different temperature and salinity levels.

METHODS

Preparation of mushroom culture

Oyster mushrooms (*Pleurotus abalones* and *Pleurotus ostreatus*) and white button mushroom (*Agaricus bisporus*) were purchased from a fresh market at Nilai, Malaysia. The inner part of the fresh mushrooms' caps was picked by using sterilized forceps. Then, mushroom samples were transferred onto freshly prepared potato dextrose agar (PDA) and incubated at 25 °C for 1 week, as recommended by Nasim *et al*., The process was repeated to isolate the young and pure cultures [18]. All the culturing work was conducted in aseptic conditions.

Optimizing salinity and temperature

A total of 10 g of the mushroom culture was transferred into universal bottles containing 40 mL of potato dextrose broth (PDB) and incubated with different concentrations of NaCl (0, 0.01, 0.05, 0.10 M) at different temperatures (4 °C, 25 °C, 37 °C and 60 °C). The cultures were grown for 7 days. The optimum temperature and salinity for the growth of each species of mushroom was determined by identifying the highest biomass and laccase activity.

Laccase activity

The medium which contained laccase enzymes was transferred into centrifuge tubes. The tubes were centrifuged at 10000 g for 15 minutes. A volume of 1 mL of the collected supernatant was then transferred into test tubes and mixed with 1 mL of guaiacol and 3 mL of 10 mM sodium acetate buffer (pH 6.7). This mixture was incubated at 30 °C for 15 minutes, followed by spectrophotometer measurement at wavelength = 450 nm using a glass cuvette. Distilled water was used as blank. The enzyme activity was then calculated using Equation (1).

$$\text{Enzyme Activity (IU)} = \frac{A_{450} \times \text{Totalvolume (mL)}}{1 \text{ mL enzyme} \times 15 \text{ minutes} \times e(0.6740\ \mu\text{M/cm})} \quad \text{(Eq. 1)}$$

Plastic tolerance study

The plastic tolerance study was conducted by adding plastic materials into Bushnell Haas Broth (BHB). BHB does not contain carbon, and hence it was used to determine the ability of mushroom species to utilize plastic material as a carbon source.

An amount of 0.2 g of plastic materials -polystyrene (PS) and polyethylene (PE)- were ground into smaller pieces (around 0.5 mm mesh size) and mixed with BHB. The mushrooms' tissues were transferred to BHB that contains PS and PE, and this was followed by 7-day incubation. Then, the BHB medium was filtered through a filter paper (No. 1, Whatsman) to obtain the biomass. The medium with laccase was further used to determine the activity of the enzyme. Mushrooms were grown in BHB without plastic materials as negative control.

Statistical analysis

The analysis of biomass and enzyme activity was carried out using Statistical Package for the Social Sciences (SPSS). One-way analysis of variance (ANOVA), Dunnett T3 and Fisher's Least Significant Difference(LSD) tests were performed at 95%

confidence level to find out the significant differences among laccase activity and biomass weight for each species [19].

RESULTS AND DISCUSSION

After 7 days of incubation, *P. abalones*, *P. ostreatus*, and *A. bisporus* tissues formed spider web-like cottony mycelium on the PDA media, with an average diameter of 8 cm, 6 cm, and 1.5 cm from the top, respectively. The results showed that all three species of mushrooms grew at different rates on PDA.

This experimental study indicates that laccase activity in *P. abalones* was not significantly affected by salinity and temperature (Figure 1). However, *P. abalones* obtained the highest ($P < 0.05$) biomass of 0.213 g and 0.120 g under conditions of 25 °C temperature with 0.05 M salinity and 37 °C with 0.10 M salinity, respectively. *P. ostreatus* showed the highest ($P < 0.05$) laccase enzyme activity (0.437 IU) at 37 °C with 0.05 M salinity, and highest ($P < 0.05$) biomass obtained was 0.270 g at 37 °C with 0.01 M salinity (Figure 2). The highest ($P < 0.05$) laccase activity (0.307 IU) for *A. bisporus* was recorded at 37 °C with 0.05 M salinity (Figure 3), while the highest ($P < 0.05$) biomass obtained was 0.340 g at 37 °C with 0.05 M salinity. *P. abalones* had the highest laccase activity at the temperature of 37 °C and salinity level of 0.05 M.

According to Vivekanandan *et al.*, laccase activity was greatly reduced when the temperature rose above 50 °C and the optimum temperature for laccase activity is about 40 °C for *Aspergillus nidulans* [20]. The results of their report are in agreement with the results of this study, indicating that the optimum condition for laccase activity was achieved at 25 °C and 37 °C. When the temperature went higher, the enzymes started to break down and denature. The high temperature could damage the cell components as well by changing the structure of the mycelium membranes [21], which would further lead to the inhibition of the growth of the mushrooms.

Blatkiewicz *et al.* reported that the optimum salinity for laccase activity in *Cerrena unicolor* was below 0.4 M [22]. According to Nitheranont *et al.*, laccase activity is inhibited at higher concentrations of NaCl [23], which was reflected in this study, with lower laccase activity being observed at 0.1 M of NaCl. The presence of excessive ions in the cells affects the Cu activity in laccase, and leads to the disruption of the internal electron transfer process [24]. The presence of NaCl at the same time increases the osmotic pressure and leads to morphology changes in mycelium.

The presence of PS and PE affected the laccase activity of certain species of mushrooms as shown in Table 1. Previous work done by Zeng *et al.* showed that laccase activity in fungi can be induced by phenolic or non-phenolic compounds in plastic materials [25]. *P. ostreatus* does not

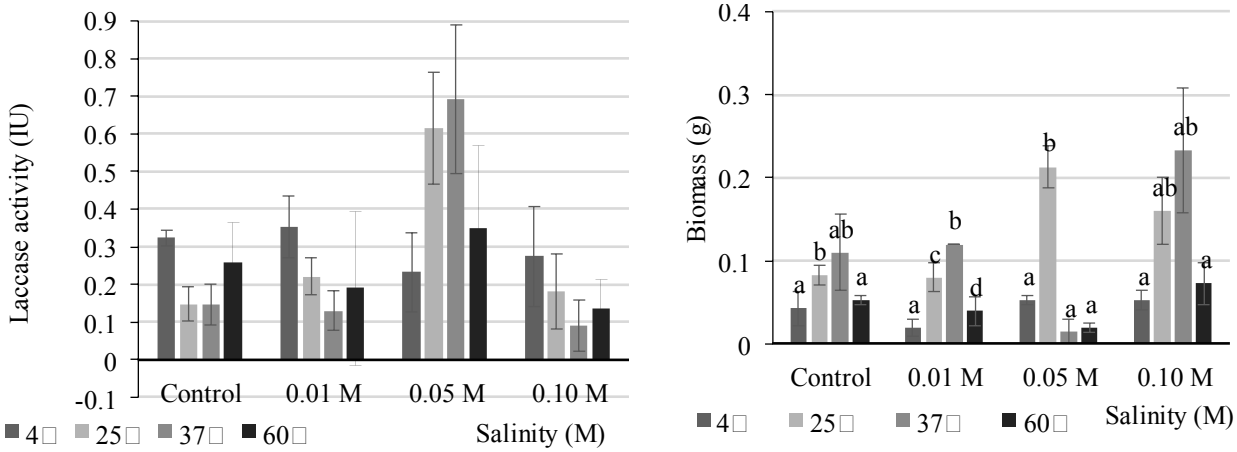

Figure 1. Laccase activity (IU) and biomass (g) of *P. abalones* at different temperatures (°C) and salinity levels (M) after 7 days in PDB.

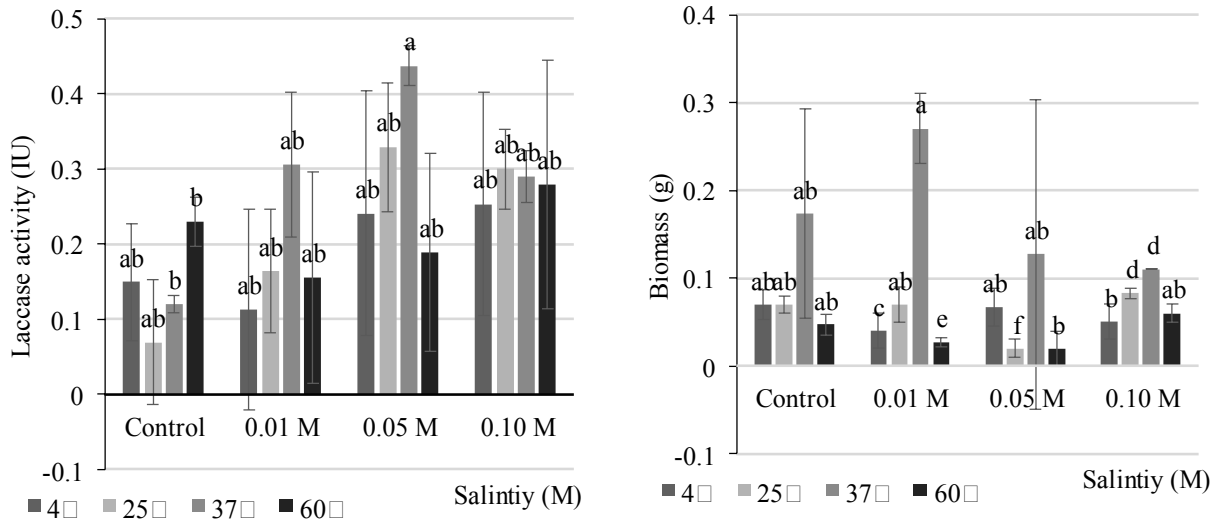

Figure 2. Laccase activity (IU) and biomass (g) of *P. ostreatus* at different temperatures (°C) and salinity levels (M) after 7 days in PDB.

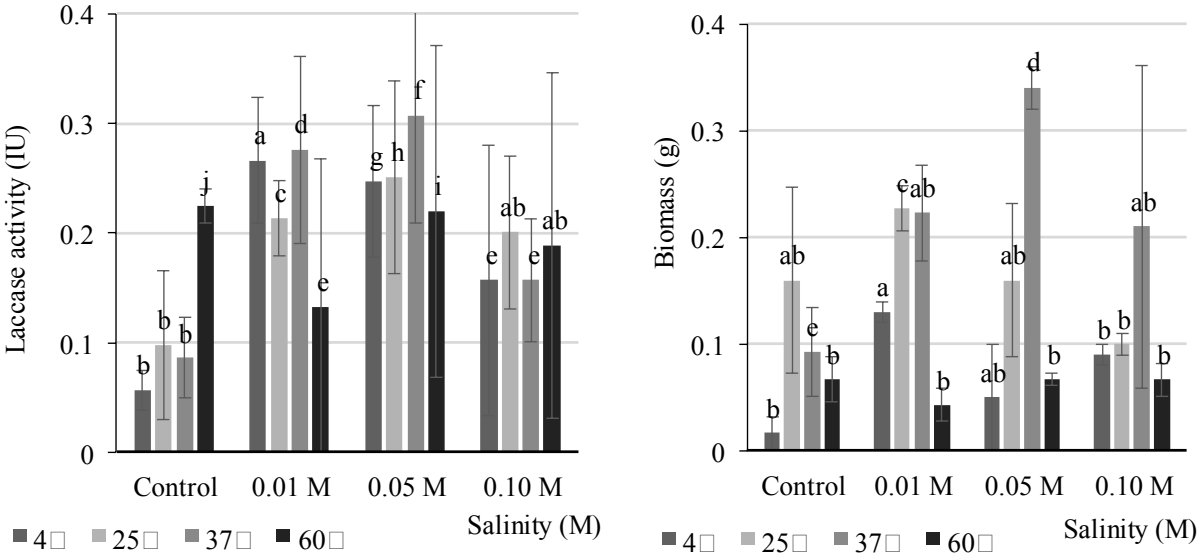

Figure 3. Laccase activity (IU) and biomass (g) of *A. bisporus* in different temperatures (°C) and salinity levels (M) after 7 days in PDB.

Table 1. Laccase activity and increase in biomass of the mushrooms in the presence of PS and PE after 7-day incubation.

Species	Laccase activity (IU)			Increase in biomass (g)		
	Control	PS	PE	Control	PS	PE
P. abalones	0.000	0.021	0.000	0.000	0.023	0.003
P. ostreatus	0.000	0.000	0.000	0.000	0.013	0.017
A. bisporus	0.000	0.027	0.019	0.000	0.030	0.030

show any laccase activity on PS and PE after 7 days of incubation. As suggested by da Luz *et al*., *P*. *ostreatus* required a longer time to colonize and degrade plastic [14]. Additionally the activity of laccase might be too low for spectrophotometry detection. Laccase activity was present in *A. bisporus* culture.

By comparison with the control (BHB only), increase in biomass was indicated for all three mushrooms, *P*. *abalones*, *P*. *ostreatus*, and *A*. *bisporus*, for both BHB + PS and BHB + PE (Table 1). As BHB is a type of media without carbon source, the increase in biomass indicates the ability of all three species to utilize both PE and PS as carbon sources [26]. From the results, *A. bisporus* had the highest biomass increment and highest laccase activity among all three species of mushrooms studied. Shah *et al*. suggested that laccase produced by mushrooms could be used to oxidize the PE chain into carboxylic acids, and then into acetyl-SCoA. Ho *et al*. reported that laccase could cleave the PS chain into styrene before the oxidation process [25]. These metabolites could be used in the tricarbocylic acid cycle to generate energy, carbon dioxide and water [27, 28].

CONCLUSION

In this study, the optimum conditions for increasing the laccase activity and biomass of *P*. *abalones*, *P*. *ostreatus*, and *A. bisporus* were determined as 37 °C and 0.05 M salinity. This study also confirmed that *P. ostreatus*, *P*. *abalones* and *A. bisporus* were able to utilize PS and PE as carbon sources to grow, even though the laccase activity in a few samples were too low to be determined by spectrophotometric approach. Among all three species of mushrooms, *A. bisporus* had the highest laccase activity, as well as the highest increase in biomass.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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