IJBPAS, September, 2017, 6(9): 1829-1834

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MYCO-ACCUMULATION OF MERCURY BY MUSHROOM *Lentinus tigrinus* MYCELIA IN LIQUID CULTURE

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Received 7th Nov. 2016; Revised 19th Jan. 2017; Accepted 2nd April 2017; Available online 1st Sept. 2017

ABSTRACT

This paper reported the accumulation potential of *Lentinus tigrinus* on mercury in liquid culture. The effect of mercury on the mycelial biomass production of the mushroom was also determined. The mercury content of mycelia was analyzed using acid digestion/cold vapor atomic absorption spectrophotometer. Potato dextrose broth (PDB) with 10 ppm mercury recorded the highest yield of mycelial biomass (5.50 g), followed by PDB with 1 ppm mercury (5.18 g). These values were found higher than the yield of mercury-free PDB (3.42 g), indicating growth stimulatory effect of mercury on the biomass production of *L. tigrinus*. Results of analysis revealed that mycelia grown at PDB with 1000 ppm of mercury contained the highest content of mercury (4.50 ppm) while those at PBD with 10 and 100 ppm had 0.36 and 1.10 ppm of mercury, respectively. The mercury accumulation ability of *L. tigrinus* depends on the concentration of the mercury in the PDB.

Keywords: *Lentinus tigrinus*, mercury, liquid culture, mycelia

INTRODUCTION

Mushrooms are the foremost degraders of a wide variety of forest litters and residues and areimportant bioaccumulators of heavy metals, toxic pollutants and contaminants. To date, a number of mushrooms have been reported to mycoaccumulate heavy metals. For instance, *Lycoperdon perlatum, Macrolepiota rhacodes*, and *Lepista nuda* are the highly accumulating species whereas *Xerocomus badius, Suillus variegatus* and *Lactarius volemus* are the low accumulators of lead **[1]**. Moreover, *Laccaria laccata, Leucopaxillus giganteus, Russula rosea, Cantharellus cibarus, Tricholoma saponaceum, Agaricus arvensis, Boletus edulis, Clavulina rugosa, Hydnum repandum, Cantharellus tubaeformis,* and *Lepista nuda*collected from the wild were reported to hold considerable amounts of arsenic, cadmium and lead **[2]**. Some Philippine strains of mushroomare also reported to exhibit mycoaccumulation potential. The fruiting body of *Schizophyllum commune* accumulated lead and chromium but not cadmium from the contaminated substrate **[3]** while *Coprinus comatus* have accumulated the four heavy metals including lead, copper, chromium, and cadmium**[4]**.

Lentinus tigrinus, commonly known as tiger sawgill mushroom, is a white-rot fungus that naturally growing on fallen logs. Its production technology was developed using rice straw-sawdust based substrate formulations. This mushroom is also reported to demonstrate heavy metal tolerance and to accumulate lead, cadmium, and chromium from the treated substrate **[5, 6]**. In our intention to evaluate further the mycoaccumulation potentials of *L. tigrinus*, the present work investigated the effect of mercury on the mycelial biomass production and the mycoaccumulation potential of *L. tigrinus* in liquid culture using potato dextrose broth as basal medium.

MATERIALS AND METHODS

Source of Strain

Pure culture of CLSU strain of *L. tigrinus* was obtained from the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Munoz, Nueva Ecija. Philippines. This was revived in potato dextrose agar plate and incubated for 7 days in biological incubator.

Preparation of Liquid Media with Mercury

Potato dextrose broth (PDB) with varying concentrations of mercury was used in the evaluation of biomass production and mycoaccumulation capability of *L. tigrinus*. PDB (2.5 L) was prepared and 500 ml was allotted for the preparation of each concentration (0 ppm, 1 ppm, 10 ppm, 100 ppm, and 1000 ppm) of mercury. The 0 ppm served as the control for the study. Fifty ml was dispensed in a culture bottle, plugged with cotton and properly labeled. Ten replicates were prepared per concentration. These were sterilized in an autoclave at 121°C, 15 psi for 30 min. After cooling,bottled media were aseptically inoculated with mycelia discs from the 7-day old pure culture of *L. tigrinus*. Cultures were incubated at 30 °C to allow mycelial growth for 15 days. The volume loss and biomass yield was determined and the percentage inhibition of mycelial growth was calculated as follows: Growth stimulation (%) = [(growth diameter of treated – growth diameter of control) / growth diameter of treated] x 100.

Mercury Content Analysis

The mercury content of mycelia was analyzed using Acid Digestion/Cold Vapor Atomic Absorption Spectrophotometer.

Statistical analysis

Experiment was laid out in a Completely Randomized Design (CRD). Data were analyzed using analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to determine the significant difference of the treatments at 5% level of significance.

RESULTS AND DISCUSSION

Effect of Mercury on Mycelial Biomass

Mycelia are vegetative structure of mushrooms that directly involved in the absorption of nutrients from their substrate. The growth response of mycelia is dependent on the nature of the substrate and the availability of the required nutrients. In the present study, we determined the effects of varying concentration of mercury in the liquid medium on the biomass production of *L. tigrinus*. Table 1 presents the volume loss of the medium and biomass yield of *L. tigrinus* grown on potato dextrose broth with mercury after 15 days of incubation. Apparently, both volume loss and biomass yield were significantly affected by the varying concentrations of mercury. The highest volume loss was recorded in PDB with 1 ppm (18.33 ml), which was statistically comparable to mercury free PDB (17.33 ml). PDB with 1000 ppm registered the lowest volume loss of 3.33 ml. However, the highest yield of mycelial biomass was noted in PDB with 10 ppm (5.50 g), followed by PDB with 1 ppm (5.18 g). The yields of PDB with 1, 10, and 100 ppm mercury were superior when compared to the yield of mercury free PDB (3.42 g). These results strongly suggest that these concentrations of mercury in the medium stimulated the mycelial growth of *L. tigrinus*. PDB with 1000 ppm, on the other hand, inhibited the growth of mycelia with 25.73% inhibition. The quality of produced mycelia was also varied when grown on the different mercury concentrations as shown in Figure 1. Mycelia harvested from PDB with 1 and 10 ppm of mercury showed massive primordia or fruiting initial. This only means that the presence of mercury at low concentrations could also stimulate primordial development of *L. tigrinus*. Browning was distinctly observed in mycelia at mercury free PDB. Taken the results of mycelial evaluation, mercury at lower concentration has growth stimulatory effect while at higher concentration has inhibitory effect to *L. tigrinus*. Therefore, *L. tigrinus* has a strong tolerance to mercury, indicating its promising potential in accumulating mercury from the substrate.

Mercury Accumulation of *L. tigrinus* Mycelia

In our intention to determine the myco-accumulating potential of *L. tigrinus*, the mercury content of harvested mycelia grown on PDB with varying concentrations of mercury was analyzed. The results of analysis are presented in Table 2. It can be seen that the mercury content of mycelia had increased in increasing concentration of mercury in the PDB, indicating concentration dependent. The highest concentration (1000 ppm), where the growth of *L. tigrinus* was inhibited, significantly recorded the maximum accumulated mercury content of 4.50 ppm. Among the mercury treated media, the lowest mercury content was noted in mycelia at PDB with 1 ppm (0.10 ppm). Those mycelia at PBD with 10 and 100 ppm of mercury respectively accumulated 0.36 and 1.10 ppm of mercury. With this considerable ability of *L. tigrinus*, this white rot mushroom is promising candidate as agent in mycoremediation.

Table 1: Volume loss, biomass yield, and percent growth stimulation of *L. tigrinus* grown on potato dextrose broth (PDB) with varying concentrations of mercury after 15 days of incubation.

PDB with Mercury in ppm	Volume loss (ml)	Mycelial Biomass (g)	Growth Stimulation (%)
0	17.33^{a}	3.42^{cd}	0.00
1	18.33^{a}	5.18^{ab}	33.97
10	15.33^{b}	5.50^{a}	37.82
100	10.67^{c}	4.18^{bc}	18.18
1000	03.33^{d}	2.54^{d}	-34.64

Values are mean of 10 replicates. Means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT. Mycelial growth at PDB with 1000 ppm of mercury was 25.73% inhibited

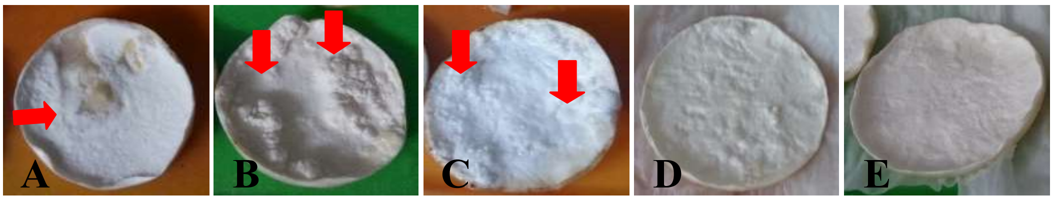

Figure 1: Mycelia of *L. tigrinus* harvested from the potato dextrose broth with varying concentrations of mercury: (A) 0 ppm; (B) 1 ppm; (C) 10 ppm; (D) 100 ppm; (E) 1000 ppm. Red arrow showing massive primordia or fruiting initials.

Table 2. Mercury content of mycelia grown on potato dextrose broth (PDB) with varying concentrations of mercury	
Mycelia grown on PDB with mercury in ppm	**Mercury content (ppm)**
0	0.00^{d}
1	0.10^{d}
10	0.36^{c}
100	1.10^{b}
1000	4.50^{a}
Means with the same letter of superscript are not significantly different from each other at 5% level of significance using DMRT.	

Falandysz et al. **[7]** reported the mercury accumulation abilities of 13 species of wild mushrooms from Zaborski Landscape Park, Poland. They found out that mercury concentrations in mushrooms varied ranges from 50 ± 20 to 3700 ± 1700 ng/g, dry matter, depending on the site and mushroom species. Fruiting bodies of *Lycoperdon perlatum* (3700 ± 1700 ng/g) and *Boletus edulis* (2600 ± 1200 ng/g) contained the highest concentrations of mercury. In addition, Alonso et al. **[8]** investigated the mercury content in some cultivated and wild species mushrooms in Province of Lugo NW Spain in relation to some factors. The mercury concentration of the studied mushrooms ranges from 0.35 to 33.1 ppm DW and 0.18 to 20.3 ppm DW for the hymenophore and the rest of the fruit body, respectively. These works support the results obtained in the present study, which provide evidence that mercury can be accumulated by various species of mushrooms. Fungi are known to have high heavy metal tolerance and myco-accumulating abilities due to their diverse mechanisms. These include transformation of metals, efflux, intracellular compartmentalization, biosorption, metabolism dependent accumulation and extracellular precipitation, complexation and crystallization, impermeability and sequestration **[9, 10]**.

CONCLUSION

Based on the results of the study, it can be concluded that the presence of mercury at varying concentrations in liquid medium affects the mycelial biomass production of *L. tigrinus*, which stimulated the biomass production when grown at 100 ppm and lower concentrations. The mercury accumulation ability of *L. tigrinus* depends on the concentration of the mercury in the liquid medium.

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