

Mycelial biomass, antioxidant, and myco-actives of mycelia of abalone mushroom *Pleurotus cystidiosus* in liquid culture

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ABSTRACT

The mycelial biomass production of four strains (WS218-1, WS218-2, CST01, and CST02) of *Pleurotus cystidiosus* in liquid culture using coconut water as a medium was evaluated. Mycelia were extracted and the 1,1-diphenyl-2-picrylhydrazyl radical scavenging activities and total phenolic contents were analyzed. The mycochemicals of the best strain were also screened using a thin-layer chromatography spot test. Results revealed that WS218-2 strain produced the heaviest mycelial biomass (6.57 g fresh wt. and 0.39 g dry wt.) while CSC02 and WS218-1 strains registered the lowest fresh and dry weight, respectively. Ethanolic extract of the WS218-2 strain showed the highest radical scavenging activity (79.01%) and contained the highest phenolic content (95.67 mg gallic acid equivalents/g of sample). Eleven mycochemicals including essential oil, triterpenes, anthraquinones, tannins, flavonoids, phenols, fatty acids, alkaloids, steroids, sugars, and coumarins were detected in WS218-2 mycelia. *Pleurotus cystidiosus* mycelia could be a valuable source of natural antioxidants and bioactive metabolites and it is strain-dependent.

1. INTRODUCTION

In Central Luzon, Philippines, *Pleurotus* species have been commercially cultivated using different formulations of agro-industrial substrates such as rice straw, sawdust, rice bran, among others. These commercially cultivated mushrooms include *Pleurotus florida*, *Pleurotus ostreatus*, *Pleurotus cystidiosus*, and *Pleurotus sajor-caju*. Cultivation of mushrooms provides nutritious food and livelihood for the Filipino farmers and promotes environmental protection through bioconversion of lignocellulosic wastes. Some exotic *Pleurotus* species such as *Pleurotus djamor*, *Pleurotus citrinopileatus*, *Pleurotus cornucopiae*, and *Pleurotus pulmonarius*, acquired from other countries, are currently under evaluation for their optimal growth requirements under the tropical conditions of the Philippines by the Center for Tropical Mushroom Research and Development.

With our intention to increase the number of mushrooms to be cultivated, we continuously search and collect for new species and/or better strains of Philippine wild mushrooms. One of the mushroom

species that was collected is *P. cystidiosus*. This basidiomycetous fungus has unique characteristic and that is the ability to produce cystidia—a black-headed coremia stalk-like cells whose tops are fitted with liquid droplets of black spores, which are produced abundantly in culture. In the previous work, we established the optimum mycelial growth conditions of *P. cystidiosus* in submerged culture and elucidated the lipid compositions, namely, cholesterol, triglycerides, free fatty acids, and polar lipids [1]. Moreover, this mushroom could also play a vital role in bioremediation as it accumulates lead from the lead-contaminated substrate [2].

Herein, we successfully rescued the cell lines of wild strains of *P. cystidiosus* from the forest area of Central Luzon State University and compared their biomass production performances in liquid culture, radical scavenging activity, and total phenolic content. The best strain in terms of production and the antioxidant property was subjected further in mycochemical analysis.

2. MATERIALS AND METHODS

2.1. Source of Strains and Tissue Culture

Wild fruiting bodies of *P. cystidiosus* were collected from the forest area of Central Luzon State University, Science City of Munoz,

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Nueva Ecija, Philippines and brought to the laboratory to rescue the mycelia. Internal tissues from the premature basidiocarp were obtained and inoculated onto potato dextrose agar plates. Culture plates were labeled properly as WS218-1, WS218-2, CST01, and CST02, and incubated at room temperature to allow mycelial ramification for 7 days. Mycelial disks in the pure culture were prepared using a flame sterile 10 mm-diameter cork borer and these were used as an inoculant in the evaluation of mycelial biomass production in liquid culture.

2.2. Mycelial Biomass Production in Submerged Culture

In the evaluation of mycelia biomass production of *P. cystidiosus*, coconut water from mature coconut was served as the medium in liquid culture. Fifty ml was dispensed into each glass bottle, plugged with cotton, and covered with paper. Ten replicates per strain were done. Culture media were sterilized in an autoclave at 121°C, 15 psi for 30 minutes. After cooling, 10 mm-diameter mycelial disk was inoculated into each medium. Cultures were incubated at 30°C for 15 days to allow mycelia growth. The mycelia were harvested, air-dried, and weighed.

2.3. Ethanol Extraction

Five grams of powdered air-dried mycelia of each strain were extracted in 500 ml of 80% ethanol for 48 hours. Extracts were filtered using Whatman filter No. 2 and concentrated to dryness using rotary evaporator. The extracts were harvested and subjected to assay.

2.4. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay following the standard method of Kolak *et al.* [3] with minor modifications was used to determine the antioxidant activities of the extracts. One ml of extract at 1,000 µg/ml (dilution in ethanol) concentration was mixed with 4 ml of 0.1 mM DPPH solution in the separate plastic cuvette. A 1,000 µg/ml concentration of catechin was also prepared and this served as the positive control. Triplicate test was done. The prepared mixtures were incubated in the dark at 37°C for 30 minutes. The absorbance readings were monitored at 517 nm using a UV-VIS spectrophotometer (Spectrumlab 752S, Hinotek Instrument Co., LTD, China). The ability to scavenge the DPPH radical was calculated using the formula:

$$\% \text{ Radical Scavenging Effect} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100.$$

2.5. Estimation of Phenolic Content

The total phenolic contents of the extracts were estimated using the Folin-Ciocalteu method of Sunita and Dhananjay [4] with modifications. Gallic acid was used as a standard and the total phenolics were expressed as mg gallic acid equivalents (GAE)/g of the sample. The different concentrations of gallic acid and the extract at 1 mg/ml (dilution in methanol) concentration were prepared. Each sample (0.5 ml) was introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. All tests were performed

in triplicate. The tubes were covered with parafilm and allowed to stand at 30°C for 30 minutes prior to absorbance reading at 760 nm using a UV-VIS spectrophotometer (Spectrumlab 752S, Hinotek Instrument Co., LTD, China).

2.6. Mycochemical Analysis

The mycochemical compositions of the fruiting bodies were detected following the procedures described by Guevara *et al.* [5].

2.7. Statistical Analysis

Data were analyzed using analysis of variance and the least significant difference at 5% level of significance was used to compare treatment means.

3. RESULTS AND DISCUSSION

3.1. Mycelial Biomass Production

Mycelial growth and biomass production of mushrooms are species- and strain-dependent. In our intention to determine and to select the best strain, we evaluated the mycelial biomass production of four strains of *P. cystidiosus* in liquid culture using coconut water as a medium. Table 1 presents the mean yield of mycelial biomass of the four strains of *P. cystidiosus* after 15 days of incubation. Apparently, the different strains respond differently in the coconut water medium. WS218-2 strain recorded the highest mycelial biomass (6.57 g fresh wt. and 0.39 g dry wt.), whereas CSC02 strain had the lowest fresh weight and WS218-01 had the lowest dry weight. These results strongly indicate that the mycelial biomass production of *P. cystidiosus* could vary and it is dependent on the type of strain. This finding substantiates the observation of Kalaw *et al.* [6] who reported that the two strains of each mushroom, namely, *Ganoderma lucidum*, *Lentinus tigrinus*, *Volvariella volvacea*, *Coprinopsis cinerea*, and *Schizophyllum commune* performed differently on the nutritional and physical factors for mycelial growth on solid medium. Moreover, the biomass production performances of the different strains of *Agaricus bisporus* significantly varied as affected by temperature and method of composting of the substrate [7]. Black (AMRL 63) *Morchella* strains had a higher growth rate on wheat grains, potato peels, and a mixture than yellow (AMRL 52) strain in solid-state fermentation [8].

3.2. Radical Scavenging Activity and Total Phenolic Content

The DPPH free radical scavenging activity assay is one of the most common methods in determining the antioxidant activity of the extracts or compounds being tested. The antioxidant activity is definitely attributed to the antioxidative agents including total phenolics. In this study, the radical scavenging activities and total phenolic contents of ethanolic extracts of mycelia of four strains of *P. cystidiosus* were analyzed (Table 2). Apparently, the four extracts exhibited different radical scavenging activities and total phenolic contents. WS218-2 mycelia extract significantly recorded the highest radical scavenging activity of 79.01% and phenolic content 95.67 mg GAE/g of the sample. Accordingly, the results strongly suggest that phenolics were the major antioxidant components in the mushroom extract, which contribute to the

Table 1: Yield of mycelial biomass of the four strains of *P. cystidiosus* grown in liquid culture using coconut water medium after 15 days of incubation.

<i>P. cystidiosus</i> strain	Mycelial biomass (g)	
	Fresh weight	Dry weight
WS218-1	5.20 ^b	0.25 ^c
WS218-2	6.57 ^a	0.39 ^a
CST01	6.30 ^{ab}	0.32 ^b
CSC02	4.80 ^c	0.30 ^b

Values are mean of 10 replicates. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

Table 2: Radical scavenging activity and total phenolic content of the four strains *P. cystidiosus* mycelia.

<i>P. cystidiosus</i> strain	Radical scavenging activity (%)	Total phenolic content (mg GAE/g of sample)
WS218-1	73.77 ^b	90.04 ^{ab}
WS218-2	79.01 ^a	95.67 ^a
CST01	58.95 ^c	81.50 ^c
CSC02	57.41 ^c	89.63 ^b

Values are mean of three replicates. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance.

antioxidative action via radical scavenging ability. This conforms with the report of Phan et al. [9] that the total phenolics present in the extracts were positively correlated to the free radical scavenging activities of two strains of *Pleurotus giganteus*. Among strains of *P. cystidiosus* evaluated, the WS218-2 strain could be a promising source of protective agents to help us reduce oxidative damage and the risk of cardiovascular diseases.

In contrast, CST02 had the lowest radical scavenging activity (57.41%), whereas CST01 contained the lowest phenolic content (81.50 mg GAE/g of sample). Although these values were found lower in the present work, these values are still higher when compared to the scavenging activities of methanolic extracts of specialty mushrooms (63.3%–67.8%) and commercial mushrooms (42.9%–69.9%) and the total phenolic content of methanolic extracts of specialty mushrooms (7.61–16.28 mg/g) and commercial mushrooms (6.27–15.65 mg/g) [10]. Moreover, DPPH free radical scavenging activities of *Pleurotus djamour* var. *djamour* (5.5 mg/ml), *Pleurotus djamour* var. *roseus* (7.5 mg/ml), *P. ostreatus* (12.0 mg/ml), *P. pulmonarius* (6.0 mg/ml), and *S. commune* (3.0 mg/ml) are far lower compared to the obtained values in the present study [11]. The maximum radical scavenging activity (18.94%) and total phenolics (26.59 mg AAE/g sample) of mycelial extract of *L. tigrinus* and the highest scavenging activity (16.94%) and total phenolics (25.60 mg AAE/g sample) of mycelial extract of *Lentinus sajor-caju* in liquid culture using rice bran decoction are also lower [12]. Based on the present results and findings of the previous works, it is noteworthy to say that the different strains of *P. cystidiosus* could exhibit superior antioxidant activity than among other mushrooms.

3.3. Mycochemical Composition

Mycochemicals are fungal-derived chemicals, which play major roles in several biological activities. The chemical components of some of the Philippine wild mushrooms have

Table 3: Mycochemical composition of mycelia of WS218-2 strain of *P. cystidiosus*.

Mycochemicals	WS 218-2
Essential oil	+
Triterpenes	+
Anthraquinones	+
Tannins	+
Flavonoids	+
Phenols	+
Anthrones	–
Fatty acid	+
Alkaloids	+
Steroids	+
Sugars	+
Coumarins	+
Amino acids	–

+ = positive, – = not detected.

been elucidated including *Trichaleurina celebica*, *Trametes elegans*, *Polyporus gramocephalus*, *Lentinus strigosus*, *L. tigrinus*, *C. cinerea*, and *Panaeolus antillarum* [13–19]. In our intention to elucidate the bioactive compositions of the best wild strain, WS218-2, mycochemical detection in thin-layer chromatography was also carried out. Table 3 presents the results of the mycochemical screening. Eleven mycochemicals, namely, essential oil, triterpenes, anthraquinones, tannins, flavonoids, phenols, fatty acids, alkaloids, steroids, sugars, and coumarins were detected in *P. cystidiosus* WS218-2. In the elucidation of bioactive compounds of *P. ostreatus* conducted by Mohamed and Farghaly [20], nine chemical groups including 2 metabolites related to acids, 5 alcohols, 27 alkane, 3 amides, 27 esters, 8 fatty acids, 4 terpenoids, 29 heterocyclic, and 2 phenols were detected and these were related to anti-cholesterol and anti-cancer activity of the mentioned mushroom.

Several research studies have focused on the elucidation of bioactive compounds from *Pleurotus* species (i.e., [20–23]) that can be isolated for nutraceutical and pharmacological purposes. Bioactive compounds from the mushrooms could possess antioxidant, antifungal, antibacterial, and antiviral activities [24]. Terpenoids, phenols, steroids, polysaccharides, fatty acids, and amino acid were reported to act as anticancer agents [25,26]. Phenolic compounds, especially flavonoid and organic acids, were known antioxidant metabolites [20]. The chemical constituents of *P. cystidiosus* obtained in the present work are very useful mycochemicals that revealed significant antioxidant properties and could provide interesting biological activities that need to be investigated in future studies.

4. CONCLUSION

The mycelial biomass production and antioxidant activity of *P. cystidiosus* are strain-dependent. WS218-02 strain recorded the most efficient mycelial biomass production and could be a promising source of antioxidants and other myco-metabolites with important biological activities. The other functional activities of the mentioned strain must be investigated in the next study.

CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

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