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Original Research Paper

Optimization of liquid culture conditions of Philippine wild edible mushrooms as potential source of bioactive lipids ☆

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ABSTRACT

With remarkable bioactivities and delightful taste, mushrooms have been a commercial nutraceutical around the world. Mushrooms are cultivated on solid materials. Here we report the successful cultivation of four Philippine edible mushrooms in liquid medium. This work highlights the optimal liquid culture conditions with reference to the nutritional and physical growth factors, and preliminarily elucidated their wide variety of lipids by thin-layer chromatography. Sabouraud dextrose broth (SDB) was the most suitable culture medium for all studied mushrooms. Maximum mycelial biomasses of *Ganoderma lucidum*, *Pleurotus cystidiosus*, *Volvariella volvacea* and *Schizophyllum commune* favorably produced in SDB at pH 7, 7, 6 and 8, respectively, when incubated at 28 °C and 30 °C as optimum temperatures. The mycelia biomass yields obtained in the present study 0.8 g/30 ml are superior to the reported biomass yields of other basidiomycetes such as *Russula* sp. and *Pycnoporus cinnabarinus* at 0.3 g/50 ml (Shittu et al., 2005). Agitation did not improved mycelial growth of mushrooms except *G. lucidum*, which the biomass yield was peaked at 100 rpm (500 ml medium in 2.8 L) shake-flask culture. Cholesterol, triglycerides, free fatty acids, and polar lipids were detected in all the four mushrooms. The potential biological activities as well as molecular species of these lipid materials from the four basidiomycetes are currently under investigation.

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1. Introduction

Mushrooms are non-timber forest products which are often found naturally growing on cellulosic substrates in the temperate and tropical regions. Nutritionally, the fruiting bodies of mushrooms contain carbohydrate, protein, ash, and fats which make them suitable nutraceutical food resource. They also act as an effective source of anti-diabetic, antiviral, antioxidant, antitumor, antibacterial, immunomodulating, cardiovascular protective, radical scavenging, anti-Alzheimer, anti-hypercholesterolemic, anti-parasitic, anti-malarial, anti-HIV, and antifungal agents (Huang et al., 2011; Kim et al., 2011; Wasser, 2011; De Silva et al., 2013). Similarly, some Philippine wild edible mushrooms have also been studied for their nutritional and functional activities. Fruiting bodies of mushrooms like *Schizophyllum commune, Lentinus tigrinus, Lentinus sajor-caju, Ganoderma lucidum, Collybia reinakeana* and *Paneaolus antillarium* exhibited anti-diabetic, antibacterial, anti-inflammatory, antioxidant, antihypertensive, and anti-coagulative properties (Reyes et al., 2013; Dulay et al., 2014a; Eguchi et al., 2014; Dulay et al., 2015). With remarkable bioactivities and delightful taste, mushrooms have been gradually commercialized in the Philippines and being recognized by the government as one of the priority crops in the recent road-mapping for agricultural development. Cultivation of mushroom fruiting bodies by either small or large scale growers is based on the developed production technologies using various agro-industrial materials and cellulosic residues (Reyes et al., 1993; Reyes et al., 2004; Dulay et al., 2012a, 2012b; Magday et al., 2014; Dulay et al., 2014b). Apart from the substrate, other important factors such as moisture, temperature, pH, aeration, and illumination are also considered in the cultivation process.

Submerged cultivation is a promising method for efficient production of mushroom biomass and obtaining pharmaceutical

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compounds (De Silva et al., 2013). In the Philippines, biotechnological production of mushroom mycelia and their metabolites is less intensively practiced. Some developed innovative technology for instance was the production of schizophyllan, a bioactive therapeutic β-glucan, from *S. commune* in a submerged cultivation using coconut water as based medium (Reyes et al., 2009). Production of *S. commune* from distiller's dried grains was also reported (Sutivisedsak et al., 2013). Leathers et al. (2015) reported the use of enzymatic method for modification of schizophyllan.

This has upgraded the status of mushroom production and has changed the mindset of Filipinos towards mushroom, from gourmet to nutraceutical food source. As promising nutraceutical agents, mushrooms are becoming more indispensable in the diet, and many researchers have investigated the biochemical components present in mushrooms responsible for several biological activities. Major compositions of mushrooms such as polysaccharide (β-glucan), proteins and polysaccharide-protein complexes have been widely and comprehensively studied for their effective nutritional and pharmacological significance (Silva et al., 2012; Li et al., 2012; Synytsya and Novak, 2013). Nevertheless, the amino acid profile and functional activities of some native and exotic species of Philippine edible mushrooms namely, *S. commune, L. tigrinus, Lentinus sajor caju, G. lucidum, Pleurotus florida,* and *C. reinakeana* have been established in our laboratory in Philippines (Reyes et al., 2010; Reyes et al., 2013). Lipids or fats are another valuable constituent of mushrooms. Generally, dried fruiting body contained 5.7% of fats (Beluhan and Ranogajec, 2011), which attributed to oils, sterols, triglycerides, terpenoids, phospholipids, and fatty acids. Several studies have elucidated the fatty acids composition of wide variety of wild edible mushrooms around the world (e.g. Ribeiro et al., 2009; Ergönül et al., 2013) in order to establish their functions not only in the human diet but most importantly for pharmacological purposes.

To our knowledge, there are no comprehensive reports about the optimization of liquid culture conditions for production of mycelial biomass and lipid composition of the Philippine local strains of wild edible mushrooms. Herein, we optimize liquid culture conditions of *G. lucidum, Pleurotus cystidiosus*, *Volvariella volvacea*, and *S. commune* as influenced by culture broth media, pH, temperature and shaking condition for the production of mushrooms. Lipid materials were identified from the solvent extractable fractions of the four Philippines mushrooms. The potential biological activities as well as molecular species of these lipid materials are currently under investigation.

2. Materials and methods

2.1. Source of strains

Pure cultures of the Philippine wild edible mushrooms were obtained from the culture collections of the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. Wild fruiting bodies (Fig. 1) were originally collected from the forest of different localities in Central Luzon.

2.2. Culture inoculant

An agar block from the pure culture of each mushroom was aseptically transferred into sterilized potato dextrose agar (PDA) plates. Cultures plates were incubated at 28 °C to allow mycelia growth. After 7 days of incubation, mycelial discs were prepared using a flame sterile 7 mm-diameter cork borer. Mycelial discs served as culture inoculant in the evaluation of the optimum growth conditions.

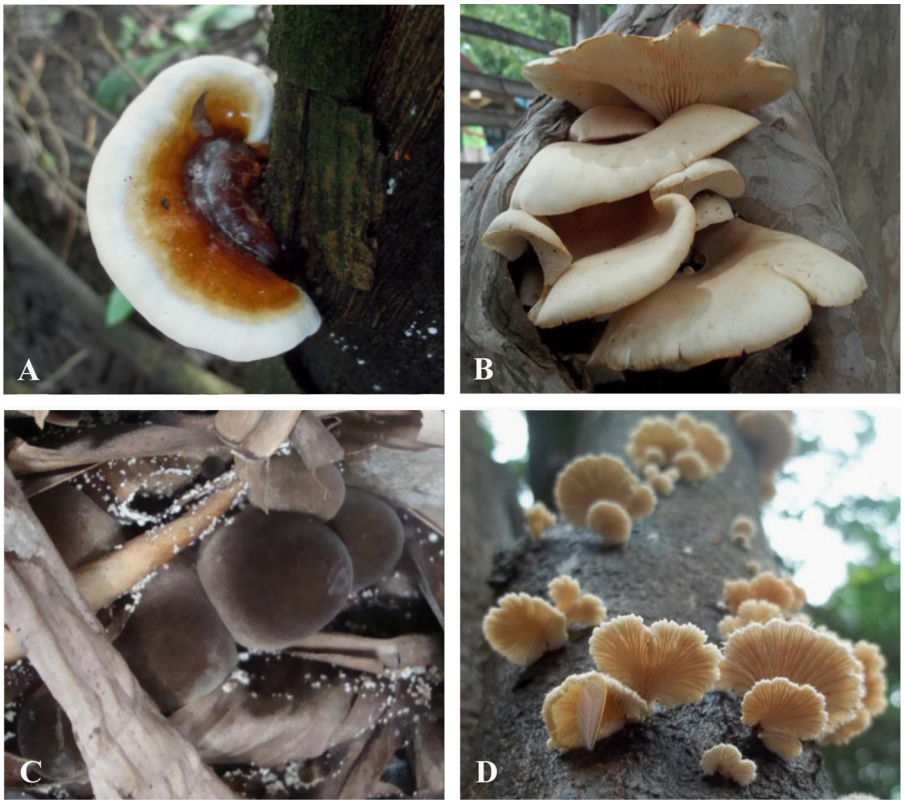

Fig. 1. Wild fruiting bodies of Philippine edible mushrooms namely, (A) *G. lucidum*, (B) *P. cystidiosus*, (C) *V. volvacea*, and (D) *S. commune*.