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Effects of culture media and physical factors on the mycelial growth of the three wild strains of *Volvariella volvacea* from Ecuador

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ABSTRACT

Volvariella volvacea is an edible and nutraceutical tropical mushroom species. This study reports the optimal culture conditions for efficient growth of mycelia of the three wild strains of *V. volvacea* (La Clementina, Vinces, and Montalvo) from Ecuador. The mycelial growths were evaluated on the three culture media, pH, aeration, illumination, and temperature. Mycelia of the three strains grew best on Sabouraud dextrose agar at pH 6.5–7.5 when incubated in either sealed and unsealed conditions at 26–28°C and 32°C. Vinces and Montalvo strains favored dark and lighted conditions, respectively, whereas La Clementina favored both. Therefore, the three exotic strains of *V. volvacea* could be commercially cultivated in the Philippines and utilized in various applications.

1. INTRODUCTION

Volvariella volvacea (Bull.: Fr.) Singer is the most popular edible mushroom because this is the first mushroom species that was introduced in the Philippines [1]. Among Filipinos, it is commonly known as *kabuteng saging* once found growing on decaying banana leaves or *kabuteng dayami* when growing on rice straw. In the past, this mushroom is being collected from the wild by farmers and mushroom hunters for food. However, because of the generated production technologies, this mushroom can now be year-round cultivated. The fruiting body production requires substrate (either rice straw or dried banana leaves) with 65% moisture content in a bed-type pile and incubation conditions with very minimal aeration and illumination at 30–35°C for 14 days from the time of spawning [2].

Fruiting body of *V. volvacea* has grayish-to-black egg-shaped volva at young and rupture to expand the pileus up to nearly flat. Nutritionally, the fruiting bodies contain carbohydrates, sugar, protein, crude fiber, ash, fats, vitamins, organic acids, and bioactive metabolites that contribute immensely to umami taste and aroma and notable biological properties such as anti-hypertensive, antimicrobial, antioxidants, anti-inflammatory, anti-cancer, and anti-coagulant [3-6]. Moreover, *V. volvacea* is an ideal source of vitamins and minerals [7].

Given the vast agricultural and industrial lignocellulosic waste materials and very fine climatic conditions in the Philippines, mushroom cultivation is of great interest. Thus, it is imperative to assess the growth and acclimatization of exotic strains under the Philippine condition to have a variety of species and strains of mushroom for a maximum production. Herein, we optimized the culture conditions for efficient mycelial growth of the three *V. volvacea* strains from Ecuador in our objective to establish basic information necessary for the generation of successful technology for fruiting body production and for submerged cultivation of mycelia of these wild strains.

2. MATERIALS AND METHODS

2.1. Mushroom Strains Source

The wild *V. volvacea* fruiting bodies [Figure 1] growing on the decaying banana leaves [Figure 1] were collected from La Clementina (1°44'18.6"S, 79°19'45.3"W), Vinces (1°34'44.0"S, 79°44'13.9"W), and Montalvo (1°48'29.6"S, 79°17'13.4"W) in Ecuador. The collected fruiting bodies were tissue cultured using potato dextrose agar (PDA) as culture medium [1]. Successful cultures were used as source of mycelial inoculant in the optimization study.

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2.2. Influence of Culture Media and pH

The procedures on the evaluation of culture media and pH, as described by Kalaw *et al*. [8], were followed with minor modification. Three dehydrated mycological culture media, namely, Sabouraud dextrose agar (SDA), potato dextrose agar (PDA), and malt extract agar (MEA) were evaluated in this study. Media were prepared following the product instructions, adjusted to pH 6, dispensed into 500 mL capacity flask plugged with cotton, covered with aluminum foil, and sterilized at 15 psi, 121°C for 30 min. The sterilized media were pour-plated, solidified, and inoculated with 10 mm diameter mycelia discs from the 7-day-old culture. Each treatment was replicated 3 times. Assay plates were incubated at 30°C. The diameter of mycelial growth was measured every 24 h until the full mycelial ramification. To evaluate the effect of pH, the identified best medium was adjusted to varying levels of pH using 1 M NaOH and HCl. Sterilization of pH media, inoculation, and incubation was the same as described in the preceding section.

2.3. Influence of Physical Factors

The best medium with optimum pH was used as basal medium in the evaluation of the influence of aeration, illumination, and temperature. To evaluate the aeration requirements, inoculated plates were sealed with parafilm while other plates remained unsealed. In lighted condition, the inoculated plates were exposed to artificial white light (322.92 lumens/m^2), while the other plates were maintained in total dark by covering with black paper, both at 30°C. For temperature, culture plates were incubated in required aeration and illumination conditions, and at different temperature conditions: 9°C, 26–28°C, and 32°C. Mycelial growth diameters were measured. All evaluations were done in triplicate.

2.4. Statistical Analysis

Analysis of variance was employed to analyze the data. Comparison among treatment means was done using Tukey's HSD at 0.05 significance level. T-test was used for the comparison of means with only two treatments.

3. RESULTS AND DISCUSSION

3.1. Effect of Culture Media and pH

In nature, mushrooms are commonly growing on lignocellulosic substrates such as fallen logs, dead trunks and branches of trees, pile of leaves, stumps, on soil, and other agro-industrial waste. Hence, mushrooms require suitable nutrients for their efficient growth. In mushroom production, the success of fruiting body development is dependent on the good quality mycelial culture. Thus, the present work evaluated the growth of mycelia of the three *V. volvacea* strains on the three culture media as source of nutrients. Interestingly, the three strains exceedingly favored SDA followed by PDA [Figure 2]. In contrast, MEA recorded the lowest mycelial growth diameter. Tukey's HSD analysis revealed the significant difference of the three culture media. Looking at the nutritional components of the three culture media evaluated. SDA has higher amounts of mycological peptone (10 g/L) than malt extract agar (3 g/L) as source of nitrogenous compounds. Both SDA and PDA contain dextrose as a major carbon source. The higher amount of peptone and the presence of dextrose as ingredients of SDA make this medium superior than the other media used. The present study suggests that the three *V. volvacea* strains have greater preference for the combination of peptone and dextrose in the medium. This finding conforms with the study of Dulay *et al*. [9] who reported that Sabouraud dextrose broth produced the highest biomass yield of *Schizophyllum commune*, *Ganoderma lucidum*, *V. volvacea,* and *Pleurotus cystidiosus* in submerged culture. However, in the evaluation of mycelial growth using indigenous culture media, the two strains of *V. volvacea* (Rang-ayan and CLSU) showed the maximum mycelial growth on potato sucrose gulaman (PSG) and corn grit gulaman (CGG) [8]. In addition, Ukoima *et al*. [10] demonstrated that the highest growth of *V. volvacea* mycelia was observed on palm fiber culture medium. Accordingly, aside from the commercially available dehydrated culture media, indigenous culture media derived from natural sources could also be used as suitable media for the excellent mycelial growth of *V. volvacea*, which are dependent on their components, their nutrient concentration, and preparation of the medium.

The growth performance of mycelia at varying pH levels was also determined. Figure 3 shows the growth of mycelia of the three *V. volvacea* strains on SDA at varying pH levels. It can be seen that the three strains could grow from pH of 5.5–8.0, but with optimum pH at 6.5–7.5 for La Clementina and Vinces and at 6.5–7.0 for Montalvo. Similarly, Rang-ayan and CLSU strains of *V. volvacea* showed favorable mycelial growth at a wide range of pH with very thick mycelia at pH 7.5–8.0 [8]. In liquid culture, the optimum pH of *V. volvacea* was at pH 6.0 [9]. However, other

Figure 1: Wild fruiting body of *V. volvacea* taken from decaying banana leaves collected in Ecuador

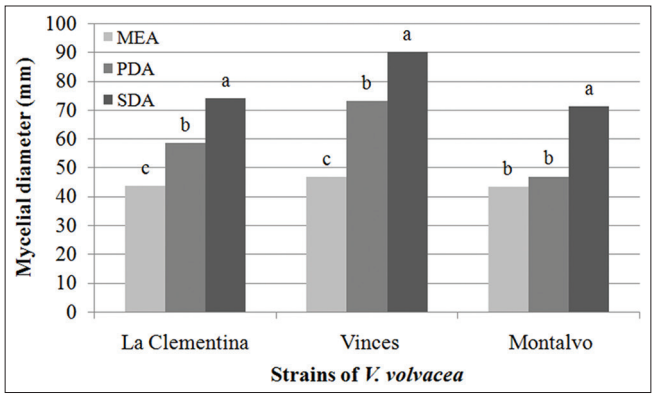

Figure 2: Mycelial growth diameter of the three strains of *V. volvacea* on the different culture media at pH 6 in 4 days of incubation. MEA: Malt extract agar, PDA: Potato dextrose agar; SDA, Sabouraud dextrose agar. Means with the same letters of superscript are not significantly different according to Tukey's HSD ($P < 0.05$)

species of *Volvariella* such as *V. esculenta* could produce good quality mycelia at pH 6 [11], and *V. speciosa* mycelia could grow at pH 3–9 [12]. Therefore, optimum pH of the genus *Volvariella* is species and strain dependent.

3.2. Effect of Physical Factors

Aside from the nutritional requirements, environmental factors such as aeration, illumination, and temperature are also essential for efficient mushroom growth. Hence, the effects of environmental factors on the growth of mycelia of the three *V. volvacea* strains were studied. In aeration, the mycelial growths of the three strains in the two aeration conditions were not significantly varied [Figure 4]. The results suggest that the mycelial growths of the three strains are not influenced by aeration. They could grow either in sealed and unsealed conditions. This response is in contrast with the observation of Reyes *et al*. [13] that *V. volvacea* favorably respond in sealed conditions. Likewise, the mycelia of *S. commune*, *Lentinus sajor-caju,* and *Lentinus tigrinus* showed luxuriant growth when incubated in either sealed and unsealed conditions [14-16].

The influence of illumination on the growth of mycelia of *V. volvacea* strains is shown in Figure 5. Mycelia of La Clementina and Montalvo strains favorably grew in lighted condition, whereas Vinces strain exceedingly favored dark condition. However, T-test revealed that the two conditions were significantly different in Vinces and Montalvo strains only. These results clearly indicate that the three strains have varying responses to illumination. Kalaw *et al*. [8] reported the excellent mycelial performance of two strains of *V. volvacea* in both lighted and dark conditions. However, Reyes *et al*. [13] confirmed that light has positive effect on the growth of *V. volvacea* mycelia.

Temperature is another important physical factor that influenced the mycelial growth. In general, fungi can be classified as temperate, semi-temperate, or tropical depending on their optimum temperature for mycelial growth [17]. The mycelial growth of the three mushroom strains as affected by temperature is presented in Figure 6. Noticeably, the maximum mycelial growth of the three strains was achieved when incubated at 26–28°C and 32°C. The mycelial growths of the three strains of *V. volvacea* on SDA at pH 6.5, incubated in the optimal physical conditions, are shown in Figure 7. In contrast, no growth of mycelia was observed when incubated at 9°C. The results suggest that the three strains of *V. volvacea* are tropical mushrooms. Since the Philippines is a tropical country, cultivation of these exotic strains would not require sophisticated temperature-controlled facility. The fruiting body production technology established for the Philippine strains of *V. volvacea* could be utilized for the production of these new strains from Ecuador.

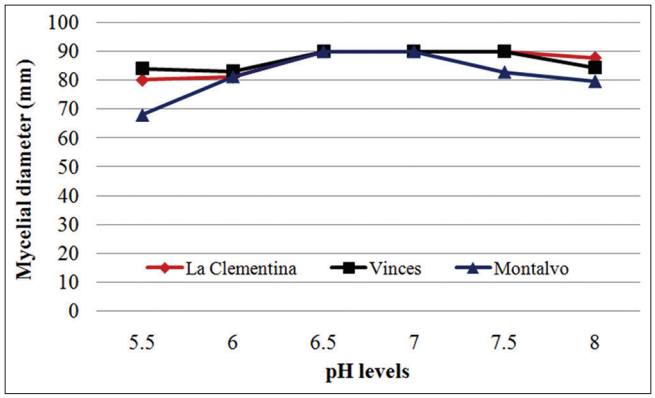

Figure 3: Mycelial growth diameter of the three strains of *V. volvacea* on Sabouraud dextrose agar at varying pH levels in 4 days of incubation

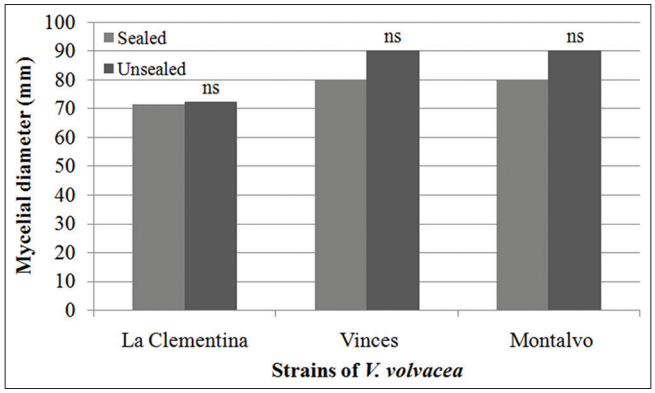

Figure 4: Mycelial growth diameter of the three strains of *V. volvacea* on Sabouraud dextrose agar at pH 6.5 incubated in sealed and unsealed conditions

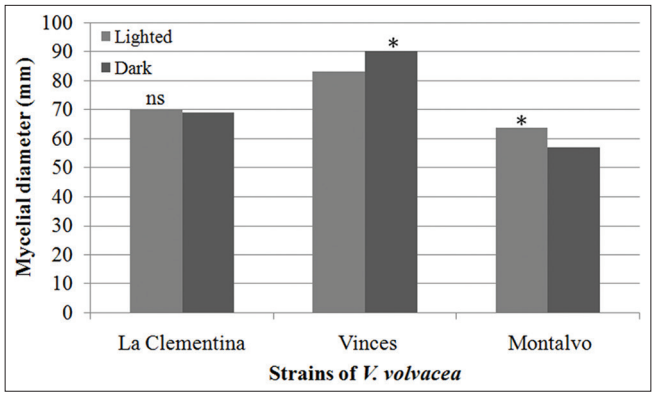

Figure 5: Mycelial growth diameter of the three strains of *V. volvacea* on Sabouraud dextrose agar at pH 6.5 incubated in lighted and dark conditions. Asterisks indicate significant difference of the two conditions using *t*-test

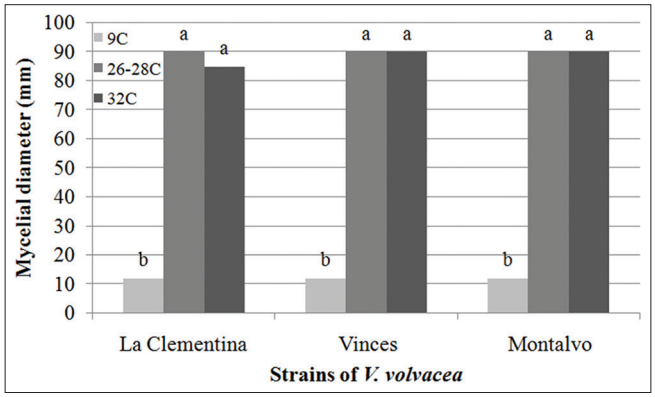

Figure 6: Mycelial growth diameter of the three strains of *V. volvacea* on Sabouraud dextrose agar at pH 6.5 incubated in three temperature conditions. Means with the same letters of superscript are not significantly different according to Tukey's HSD ($P < 0.05$).

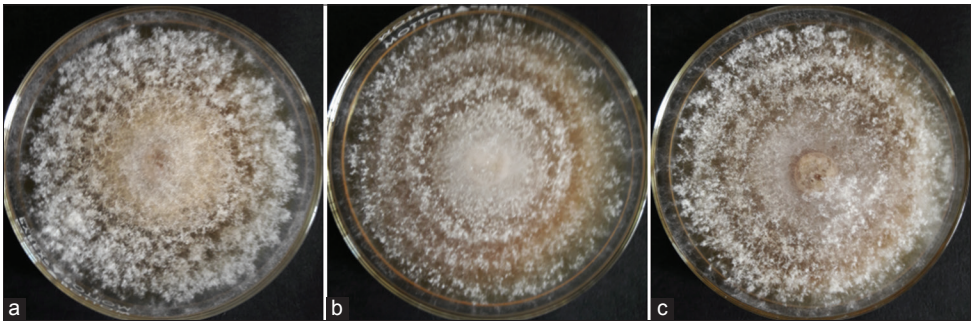

Figure 7: Mycelial growth of the three strains of *V. volvacea*; (a) La Clementina, (b) Vinces, and (c) Montalvo on Sabouraud dextrose agar at pH 6.5 incubated in the optimal physical conditions for growth

4. CONCLUSION

The present work has shown the cultural requirements for the growth of mycelia of the three wild strains of *V. volvacea* from Ecuador. Sabouraud dextrose agar at pH 6.5–7.5 incubated in either sealed and unsealed conditions at 26–28°C and 32°C were the best culture conditions for the three strains. However, the three strains showed different responses in illumination conditions. This information is very vital in the generation of successful mycelia and fruiting body production technologies of these exotic mushrooms. Production of fruiting body using banana leaves and rice straw substrates must be evaluated to compare and identify the best strains that can be utilized for large-scale production by our local mushroom growers and farmers.

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6. CONFLICTS OF INTEREST

Authors declare that they do not have any conflicts of interest.

REFERENCES

1. Reyes RG, Abella EA, Eguchi F, Iijima T, Higaki M, Quimio TH. Growing paddy straw mushrooms. In: Mushroom Growers Handbook: Oyster Mushroom Cultivation. South Korea: Mushworld-HEINEART Inc.; 2004. p. 262-9.
2. Reyes RG. Indoor cultivation of paddy straw mushroom, *Volvariella volvacea*, in crates. Mycologist 2000;14:174-6.
3. Eguchi F, Kalaw SP, Dulay RM, Miyasawa N, Yoshimoto H, Seyama T, *et al*. Nutrient composition and functional activity of different stages in the fruiting body development of Philippine paddy straw mushroom, *Volvariella volvacea* (Bull.:Fr.) Sing. Adv Environ Biol 2015;9:54-65.
4. Mathew J, Sudheesh NP, Rony KA, Smina TP, Janardhanan KK. Antioxidant and antitumor activities of cultured mycelium of culinary-medicinal paddy straw mushroom *Volvariella volvacea* (Bull.: Fr.) singer (*Agaricomycetidae*). Int J Med Mushrooms 2008;10:139-48.
5. Da Silva RF, De Almaida Barros AC, Pletsch M, Argolo AC. Study on the scavenging and anti-*Staphylococcus* activities of the extracts, fractions and subfractions of two *Volvariella volvacea* strains. World J Microbiol Biotechnol 2010;26:1761-67.
6. Wu JY, Chen CH, Chang WH, Chung KT, Liu YW, Lu FJ. Anticancer effects of protein extracts from *Calvatia lilacina*, *Pleurotus ostreatus* and *Volvariella volvacea*. Evid Based Complement Alternat Med 2011;2011:982368.
7. Mshandete AM, Cuff J. Proximate and nutrient composition of three types of indigenous edible wild mushrooms grown in Tanzania and their utilization prospects. Afr J Food Agric Nutr Dev 2007;7:1-16.
8. Kalaw SP, Alfonso DO, Dulay RM, De Leon AM, Undan JQ, Undan JR, *et al*. Optimization of culture conditions for secondary mycelial growth of wild edible mushrooms from selected areas in Central Luzon, Philippines. Curr Res Environ Appl Mycol 2016;6:277-87.
9. Dulay RM, Ray K, Hou CT. Optimization of liquid culture conditions of Philippine wild edible mushrooms as potential source of bioactive lipids. Biocatal Agric Biotechnol 2015;4:409-15.
10. Ukoima HN, Ogbonnaya LO, Arikpo GE, Ikpe FN. Cultural studies of mycelia of *Volvariella volvacea*, *Pleurotus tuber-regium* and *Pleurotus sajor-caju* on different culture media. Pak J Nutr 2009;8:1052-54.
11. Jonathan SG, Fasidi IO, Ajayi EJ. Physico-chemical studies on *Volvariella esculenta* mass (singer), a Nigerian edible fungus. Food Chem 2004;85:339-42.
12. Fasola TR, Gbolagade JS, Fasidi IO. Nutritional requirement of *Volvariella speciosa* (Ff. Ex. Fr.) singer, a Nigerian edible mushrooms. Food Chem 2007;100:904-8.
13. Reyes R, Eguchi F, Iijima T, Higaki M. Physiological considerations for the efficient colonization of fukurotake, *Volvariella volvacea*. J Wood Sci 1998;44:408-13.
14. Bulseco MG, Abella E, Reyes R. Morphogenesis of *Schizophyllum commune*, a wild edible mushroom of Mt. Nagpale, Abucay, Bataan, Philippines. J Nat Stud 2005;4:20-8.
15. Cuevas MJ, Reyes R, Kalaw S. Biophysiology of *Lentinus sajor-caju*. J Trop Biol 2009;7:48.
16. Dulay RM, Kalaw SP, Reyes RG, Cabrera SP, Alfonso NF. Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull.) Fr., a new record of domesticated wild edible mushroom in the Philippines. Philippine Agric Sci 2012;95:278-85.
17. Lin Z. Grass (juncao). In: Mushroom Grower's Handbook 1: Oyster Mushroom Cultivation. Seoul, Korea: Mush World-Heineart Inc.; 2004. p. 107-13.

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