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Germplasm collection and culture media optimization for cell lines of edible macrofungi from Bicol Natural Park, Region V, Philippines

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Abstract In the part of Bicol National Park (BNP), twelve edible macrofungi were collected and identified from Sipocot, and Lupi, Camarines Sur. Two species namely, *Auricularia auricula-judae* and *Schizophyllum commune* are familiar and being consumed by the locals. Its mycelial cell lines were successfully rescued and subjected to media optimization. The longest mycelial increment and shortest day for full mycelial ramification of *A. auricula-judae* was significantly noted in Coconut Water Gulaman, with 30.47 mm having very thick mycelial growth within five days of incubation. On the other hand, fastest mycelial colonization of *S. commune* was observed in Corn Grit Sucrose Gulaman with a total increment of 23.36 mm which significantly colonized within seven days among evaluated medium. Additionally, it was noted that all the evaluated media were completely ramified by *A. auricula-judae* and *S. commune*. The ideal fruiting substrate based on the locally available and abundant materials in the region were being conducted at this moment.

Keywords: Bicol natural park, Edible mushrooms, Culture media, Macrofungi

Introduction

The Philippines is endowed with different biological resources; being included in the 17 mega bio-diverse countries globally it is considered as one of the biodiversity hotspots in the world (USAID, 2016). It is also known for its forests which serve as a home to various flora and fauna species (Guia, 2013). One of the forested areas in the country that conserve nature's biodiversity is the Bicol Natural Park.

The Natural Park is a protected area with rough hills and mountains (Nepal, 1995). According to Proclamation No. 665 signed on December 23, 1940, by Manuel L. Quezon, it covers a total area of 5,201 hectares (12,850

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acres), which lies between the two provinces of the Bicol Region, namely: Camarines Norte, and Camarines Sur. Being rich with various wildlife, the Natural Park has different types of trees such as white lauan (*Shorea contorta*) and red lauan (*Shorea negrosensis*), apitong (*Dipterocarpus grandiflorus*), yakal (*Shorea astylosa*), etc., and other premium species of trees (DENR, 1992). Being an area with vast forest products, Bicol Natural Park indeed has high plant debris that can be considered as organic wastes. Mushrooms are found in decaying organic materials in the park. Being a decomposer, these macrofungi degrade large varieties of lignocellulosic wastes and use it as their source of nutrition. Generally, some macrofungi mushrooms are known to be consumed because of their palatability and high nutritional attributes (Chang and Miles, 2004; Ergonul *et al.,* 2013; and Dutta, 2013). Additionally, Sahoo (2014), claimed that edible mushrooms and macrofungi convert these organic wastes into food. It contains proteins, minerals, polysaccharide, crude fat, crude fiber, triterpenoids, phenols, nucleotides, glycoproteins, sterols, and significant content of vitamins such as B_1, B_2, B_{12}, C, D, and E (Chang, 1996; Heleno *et al.*, 2010; and Matilla *et al.,* 2001). Also, mushrooms possess more than 100 medicinal functions, and the key uses are antidiabetic, antioxidant, anti-allergic, anticancer, cardiovascular protector, immunomodulatory, anticholesterolemic, antibacterial, antiviral, antifungal, antiparasitic, and hepatoprotective effects, and they also give protection against the development of tumor and inflammation (Chang and Wasser, 2012; Finimundy *et al*., 2013; Yu *et al*., 2009; Zhang *et al*., 2011). With these advantages, healthy cell lines of wild edible mushrooms are being collected and rescued to optimized their biological potential.

In the Philippines, mushroom cultivation provides employment opportunities and offers a source of nutrition. There are technologies, materials, and media which can be used for mushroom cultivation; however, most of these synthetic materials are expensive and does not guarantee availability at all year round. Meanwhile, there are indigenous materials that can be used as an alternative media for mushroom cultivation which are readily available and abundant in the locality. Thus, this study aimed to rescue the healthy cell lines of edible macrofungi from the Bicol Natural Park and evaluate the mycelial growth performance of the collected edible species in locally available indigenous media.

Materials and methods

Source of macrofungi

The fruiting bodies of edible mushrooms were obtained from Bicol Natural Park specifically, from the part of Tible, Sipocot, Camarines Sur (13.8893 ºN, 122.9719 ºE) and Bahi, Lupi, Camarines Sur (13.922 ºN, 122.942 ºE) with a range of two kilometers sampling site per area. The collected edible mushrooms were labelled and placed in a sealed container.

Identification of Collected Mushrooms on Bicol Natural Park

All obtained mushroom specimens were subjected to morphometric characterization to determine their species classification. The mushroom samples were identified with the use of the book entitled "A Field Guide to Mushroom" by Kent H. McKnight and Vera B. Mcknight and were also verified through mushroom specie data-based online.

Selection of mushroom species for evaluation

The mushroom species subjected for media optimization were selected based on the availability of culture media, growing substrates, market demand, and its suitability in the climate conditions in the locality (Hanko, 2001). Additionally, the selected edible mushrooms were known to be consumed by the locals in Bicol Natural Park.

Media preparation and sterilization process

One liter of coconut water was filtered using cheesecloth and transferred to an Erlenmeyer flask. Subsequently, it was added with 20 grams of yellow gulaman bar and boiled with continuous stirring until a homogenous mixture was attained. On the other hand, 50 grams each of corn grit, sorghum, rice bran, and 250 grams of potato cubes were separately decocted in one liter of tap water until they became tender. After boiling, each decoction was strained using cheesecloth and dispensed in an Erlenmeyer flask. Each decoction was then added with 20 grams of yellow gulaman bar and 10 grams of white table sugar plugged with cotton and covered with aluminum foil.

For the sterilization process, different prepared culture media were autoclaved for 20 minutes at 121℃/15 psi. Then, sterile test media were then separately dispensed into sterile Petri plates and they were allowed to cool and solidify.

Inoculation and incubation

A seven-day-old, 10-mm mycelial block was inoculated at the center of the test media with three replications each using a sterile cork borer (Aldave *et al.,* 2021). Subsequently, petri plates with inoculated media were then sealed with cling wrap, and incubated at room temperature.

Mycelial growth evaluation of the collected mushroom

Daily mycelial growth were recorded in terms of mycelial increment (every 8:00 AM, 12:00 PM, and 5:00 PM), mycelial density, and days of incubation (Cañal *et al.,* 2020). Mycelial increment and shortest number of days for mycelial ramification were observed to identify the ideal culture media. Analysis of Variance (ANOVA) with 0.05 level of significance was employed to evaluate the collected data.

Results

Identified macrofungi species at Bicol Natural Park (BNP), Region V, Philippines

Twelve edible species of macrofungi were identified from two collection sites of Bicol Natural Park which belongs to the genus *Auricularia, Neolentinus, Lentinus, Polyporus, Aleuria, Cookeina, Sarcoscypha, Schizophyllum*, and *Lyophyllum*. The study subjected *A. auricula-judae* and *S. commune* in media optimization, since these mushroom species are the most widely known to be consumed by the locals in the area, have abundant substrates in the locality, and high market demand.

Mycelial density of the collected edible macrofungi in Bicol Natural Park

Results revealed that Coconut Water Gulaman and Rice Bran Sucrose Gulaman test media elucidated the thickest mycelial density of *A. auricularia-judae*. Meanwhile, it has been observed that both Coconut Water Gulaman and Potato Sucrose Gulaman obtained the thickest mycelial density of *S. commune* after five days of incubation. Whereas the thinnest mycelial ramification of *A. auricularia-judae* and *S. commune* was recorded in Corn Grit Sucrose Gulaman and Sorghum Sucrose Gulaman respectively.

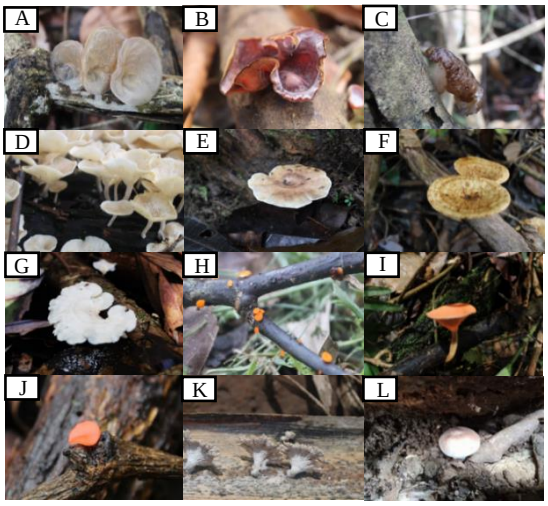

Figure 1. A) *Auricularia auricula-judae*; B) *Auricularia polytricha;* C) *Neolentinus ponderosus;* D) Lentinus tigrinus; E) Lentinus sajor-caju; F) *Lentinus spp*.; G)*Polyporus grammocephalus;* H)*Aleuria aurantia;* I)*Cookeina speciosa;* J) *Sarcoscypha coccinea;* K) *Schizophyllum commune;* L) *Lyophyllum connatum*

Figure 2. Mycelial Density of (A) *A. auricula-judae* and (B) *S. commune* after 5 days of incubation

Daily mycelial increment and evaluation of edible macrofungi collected at Bicol Natural Park in Different Indigenous Media

Mycelial growth of *A. auricula-judae* was noted to have the longest and fastest increment in Coconut Water Gulaman with a total growth of 30.37 mm, while it was revealed that shortest length of mycelial increment among the test media was observed in Rice Bran Sucrose Gulaman with 25.67 mm after five days of incubation (Table 1). Furthermore, there was no significant (p =0.05) difference among culture media from the first to the fifth day of *A. auricula-judae* mycelial incubation.

Table 1. Mycelial growth increment (mm) and evaluation of *A. auricula- judae* in various media

Treatment	Number of Days					Total Mycelial Increment
	1	2	3	4	5	
T1-Coconut Water Gulaman	4.46 a	6.96 a	6.17 a	6.63 a	6.25 a	30.47
T2-Potato Sucrose Gulaman	3.63 a	6.00 a	6.38 a	5.75 a	6.25 a	28.01
T3-Rice Bran Sucrose Gulaman	3.25 a	6.17 a	5.29 a	4.67 a	5.29 a	24.67
T4-Corn Grit Sucrose Gulaman	3.83 a	5.67 a	5.75 a	5.58 a	6.33 a	27.16
T5-Sorghum Sucrose Gulaman	3.33 a	5.92 a	5.63 a	5.75 a	6.46 a	27.09

[1]/Data presented are means of three replicates.
[2]/Means with the same letter are not significantly different at 5% level of significance using Analysis of Variance (ANOVA).

It was revealed that the longest incubation period of *A. auricula-judae* mycelia to completely ramify the test media was recorded in Potato Sucrose Gulaman, which took 7.33 days of observation. While, Coconut Water Gulaman only took an average of 5.67 days to be fully colonized by the mycelia, this treatment noted the shortest ramification period of the *A.* auricula-*judae* mycelia as compared to the rest of the test media. Additionally, it was revealed that there was no significant (0.05) difference among the different tested media in terms of complete mycelial ramification as shown in table 2.

Table 2. Full mycelial ramification period of *A. auricula-judae* in different test media

Treatment	Number of Days
T1- Coconut Water Gulaman	5.67 a
T2-Potato Sucrose Gulaman	7.33 a
T3-Rice Bran Sucrose Gulaman	7.00 a
T4-Corn Grit Sucrose Gulaman	7.00 a
T5-Sorghum Sucrose Gulaman	6.67 a

[1]/Data presented are means of three replicates.
[2]/Means with the same letter are not significantly different at 5% level of significance using Analysis of Variance (ANOVA).

The highest total mycelial growth increment of *S. commune* was noted in Corn Grit Sucrose Gulaman with 34.22 mm, whereas the lowest total growth increment of 18.57 mm was observed in Rice Bran Sucrose Gulaman (Table 3). On the other hand, results revealed that on the first, fourth, and fifth day of incubation there was no significant (0.05) difference among the test media. However, on the second and third day of mycelial ramification, data showed that there is a significant (0.05) difference in the mycelial growth increment of *S. commune* cultured in Rice Bran Sucrose Gulaman and Corn Grit Sucrose Gulaman.

Table 3. Mycelial growth increment (mm) and evaluation of *S. commune* in various media

Treatment	Number of Days					Total Mycelial Increment
	1	2	3	4	5	
T1-Coconut Water Gulaman	2.83	4.46 ab	7.84 ab	8.09 a	9.39 a	32.61
T2-Potato Sucrose Gulaman	2.92	3.75 bc	5.42 bc	5.34 a	7.83 a	25.26
T3-Rice Bran Sucrose Gulaman	1.46	2.30 c	3.59 c	4.75 a	6.47 a	18.57
T4-Corn Grit Sucrose Gulaman	2.13	6.5 a	8.34 a	7.33 a	9.92 a	34.22
T5-Sorghum Sucrose Gulaman	0	3.6 bc	5.8 bc	7.0 a	6.96 a	23.36

[1]/Data presented are means of three replicates.
[2]/Means with the same letter are not significantly different at 5% level of significance using Analysis of Variance (ANOVA).

Data showed that the shortest period of *S. commune* mycelial colonization was observed in Corn Grit Sucrose Gulaman which was significantly (0.05) colonized after five days of ramification period, while the longest ramification period took an average of nine days for Rice Bran Sucrose Gulaman to be completely ramified by the *S. commune* mycelia (Table 4).

Table 4. Full mycelial ramification period of *S. commune* in different test media

Treatment	Number of Days
T1- Coconut Water Gulaman (CWG)	5.33 c
T2-Potato Sucrose Gulaman (PSG)	7.00 b
T3-Rice Bran Sucrose Gulaman (RBSG)	9.00 a
T4-Corn Grit Sucrose Gulaman (CGSG)	5.00 c
T5-Sorghum Sucrose Gulaman (SSG)	8.00 ab

[1]/Data presented are means of three replicates.
[2]/Means with the same letter are not significantly different at 5% level of significance using Analysis of Variance (ANOVA).

Figure 4. Mycelial storage of the edible macrofungi A) *A. auricula-judae* and B) *S. commune*

Discussion

Mycelia is a white to pale white web-like structure of continuously growing filaments of hypha that permeates the lignocellulosic materials. In mushroom and macrofungi cultivation, one of the requirements that provides necessary nutrition which allows the growth of its mycelia is the culture media (Hoa & Wang, 2015). In terms of the total daily mycelial increment of *A. auricula-judae*, Coconut Water Gulaman recorded the longest mycelial growth, and shortest period for full mycelial ramification of *A. auricula-judae*. These results could be attributed in coconut water's high content of carbohydrates, protein, fat, ash, enzymes, and vitamins, likewise, it is also rich in macro and micro minerals such potassium, calcium, magnesium, sodium, phosphorous, manganese, copper, iron and zinc (Yong *et al.*, 2009; and Khan *et al*., 2003); which can hasten mycelial growth. Moreover, the findings of this study are congruent to the study of Aldave *et al*. (2021), and Cañal *et al*. (2020) that coconut water culture media facilitates the optimal growth of tropical mushrooms. On the other hand, findings revealed that Corn Grit Sucrose Gulaman attained the highest mycelial growth increment and shortest mycelial colonization period of *S. commune*. The longer mycelial increment and short mycelial ramification period of S. *commune* in Corn Grit Sucrose Gulaman could be associated to its high starch content and crude fiber (Ullah *et al*., 2010). The results of this study are similar to the findings of Kalaw *et al*. (2022), that corn grit gulaman is one of the favorable culture media for the mycelial growth of mushroom.

Furthermore, it was revealed that the *A. auricula-judae* have the thickest mycelial density in Coconut Water Gulaman and Rice Bran Sucrose Gulaman,

while *S. commune* thickest mycelia were recorded in Coconut Water Gulaman and Potato Sucrose Gulaman. These is congruent to the findings of Kalaw *et al*. (2016), that the nutrient composition of different culture media affects the mycelial growth rate of the mushroom strains. Furthermore, Chang and Miles (2004), stated that variations in the growth of mycelia could be different according to mushroom strain, physical growth condition, and chemical composition of the culture media.

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