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Cultural and Antibacterial Studies of *Hypsizygus tessulatus* (Buna-shimeji)

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Abstract

Hypsizygus tessulatus also known as buna-shimeji is an endemic Japanese edible and medicinal mushroom. Attempt to grow this species under Philippine condition was undertaken from November 2013-March 2014 and grown under laboratory condition using different cultural parameters and its antibacterial activity was also determined. Different culture media, temperature and pH, spawning and growing substrates are utilized. The highest rate of radial mycelia growth among ten different culture media was observed in compost agar measuring (78.67 mm) in diameter, optimum level of pH (6 and 7) and temperature (20-25° C) were attained. Profuse mycelia colonization was attained on 18 days after inoculation using black beans as spawning substrate however, sorghum spawn inoculated in paddy straw and sawdust- rice bran was found to be the most suitable fruiting substrate for initiating mycelia growth (135 mm). Methanol extract of oven-dried mycelia mat of *H. tessulatus* (HMME) was used against two Gram positive (+) and Gram negative (-) bacteria for antibacterial study. An average inhibition zone exhibited by *H. tessulatus* mycelia extract in *Staphylococcus aureus* (15.67 mm). *H. tessulatus* exemplifies moderate and fair antibacterial activities.

Vegetative studies show that *H. tessulatus* grows at optimum temperature of 25-27° C under laboratory condition. Furthermore, buna-shimeji mushroom cultivation requires for its fructification stage in lower temperature and higher relative humidity.

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Introduction

Mushrooms are especially known for their attractive flavour, texture and represent one of the world's greatest untapped resources of nutritious and palatable food. Mushrooms have been used as food and medicine in many parts of the world since time immemorial. Although mushrooms are often grouped with vegetables and fruits, they are actually fungi. They are macro-fungi which belong either to Basidiomycetes or Ascomycetes and they are very distinct from plants, animals and bacteria (Mushigeni and Chang, 2001). Mushroom served as the important biosources of food and medicinal industry for many years.

Mushroom cultivation technology is friendly to the environment. The production of edible and medicinal mushrooms utilizing, for example, paddy, straw, cotton wastes, coffee waste, water hyacinth, tree sawdust, sugar cane, bagasse, wild grasses and various categories of refuse and lignocellulosic wastes, could readily be adopted in Asia and Pacific communities in sophisticated, but low technology approaches. Various environmental factors affect morphogenesis in agaricomycetous mushrooms. Physical and chemical factors such as temperature, light, moisture, and chemicals serve as activators for spore germination and as stimulator or inducers for fructification.

As an Asian country, Philippines is already adopting different approaches in cultivating edible and medicinal mushrooms such as oyster, shiitake, ganoderma, rat ear, and paddy straw. Considering Philippines is a tropical country, there are unfavourable conditions that might other mushrooms cannot adapt such as temperature or climate. It is evidently clear that the growing interest in the cultivation of mushrooms can help in solving many problems of global importance such as protein shortage as well as improving the health and well-being of people, considering that mushrooms are valuable health foods which are low in calories and provide essential minerals.

Materials and Methods

Composition of Culture Media

The following agar based media were used to study the mycelia growth rate (diameter in mm) of *H. tessulatus* together with cultural and physiological characteristics. Optimum pH is administered and adjusted to the media. The agar-based media were sterilized in a pressure cooker for 20 min at 15 psi, 121°C then cooled at 45°C and poured into sterilized petri dishes. The plates were inoculated 1.5 cm agar plug of *H. tessulatus* under sterile condition and incubated in the dark at laboratory temperature range from 20-25°C. Radial mycelia growths were observed and measured after 3 days of inoculation. Measurements of the mycelia growth were recorded every 3 days until full ramification attained.

Effect of Different Spawn Substrates

Different grains (ground corn, sorghum, green peas) were used to observe the mycelia growth characteristics of *H. tessulatus* for spawn production. The spawn bottles were sterilized in an autoclave for 1 hour at 15 psi, at 115°C. All spawn bottles were inoculated with 1.5 cm agar plug of *H. tessulatus* and incubated at laboratory temperature of about 25°C. Measurements of the mycelia growth were recorded every 7 days until full spawn run was attained.

Effect of pH

The effect of pH on mycelia growth of *H. tessulatus* was determined using potato dextrose agar medium the pH was adjusted to 4, 5, 6 and 7 by adding 0.1 N KOH or HCL and incubated for 15 days at 25°C. The measurement of mycelia growth was performed to determine the optimum initial pH of culture media for vegetative growth.

Effect of Temperature

To evaluate the optimum temperature of mycelia growth of *H.tessulatus*, four different temperature (10, 15, 20, 25, °C) were evaluated using PDA medium and at pH 6. And incubated for 10 days at (10, 15, 20, 25, °C). The measurement of mycelia growth was performed using Kadiri method.

Effect of Different Cultivation Substrates

To determine the optimum cultural characteristic of *H. tessulatus* two different fruiting substrates were used ,sawdust and rice Straw. Composition for the substrate were as follows. Sawdust with 78% of the total composition, supplemented with 20% rice bran, 1% CaCO3 and 1% sugar. On the other hand, Paddy straw 78% of the total mixture and supplemented with 20% rice bran, 1% CaCO3 and 1% sugar.

Each fruiting substrate were filled on polypropylene bags (500 g each) and sterilized at 121oC for 1 hour. After sterilization the bags, medium was cooled down and were inoculated using different spawn substrates.

Antibacterial Screening

The oven-dried and pulverized mycelia mats of *H. tessulatus* weighing 10 g were soaked separating in 100 mL of 80% methanol and distilled water in a 500 mL sterile flask for 48 hours at ambient temperature range 25-30°C and occasional agitation. The extracts were filtered using cheese cloth and filtered again using a Whatman filter no 1. The filtrate was evaporated in water bath for 30 min to 1 hr enough to evaporate the alcohol and the extracts acquired were stored in airtight flask.

The screening for antibacterial activity of the extract was performed using two Gram (+) bacteria *B. subtilis* and *S. aureus* and two Gram (-) bacteria *E. coli* and *P. aeruginosa*. Agar disc diffusion assay was carried out for the determination of antibacterial activity of the extracts. The inoculum was added to a plate agar, agar dilution method was used. Afterwards, the inoculum was incubated into plates with a moisten surface. Then, Whatman filter paper no.

1discs were soaked in mycelia extracts (methanol) of *H. tessulatus* to completely saturate the discs and placed in bacterial culture seeded plates. Each sample was performed in triplicates to determine the consistency of the possible antibacterial activity. The inoculated petri dishes were placed at room temperature and incubated at 25-30°C for 24 hours. The antibiotic Streptomycin sulphate (10mg/L) was used as a control. The efficacy of extract in assay was evaluated by measuring the diameter of zone of inhibition in millimeters.

Results & Discussion

Ten different culture media were used to determine and screen the mycelia growth of *H. tessulatus*. The different growth rates exhibited by *H. tessulatus* on different culture media indicated that the fungus utilizes the given supplements at different growth rates. Results revealed that radial mycelia growth was high in Compost Agar (CoA), Rice Bran Agar (RBA) Potato Dextrose agar (PDA), Sorghum Decoction Agar (SDA), Wheat Decoction Agar (WDA), Potato Yeast dextrose Agar (PYDA) compared to Standard Method Agar (SMA), Carrot Agar (CA).

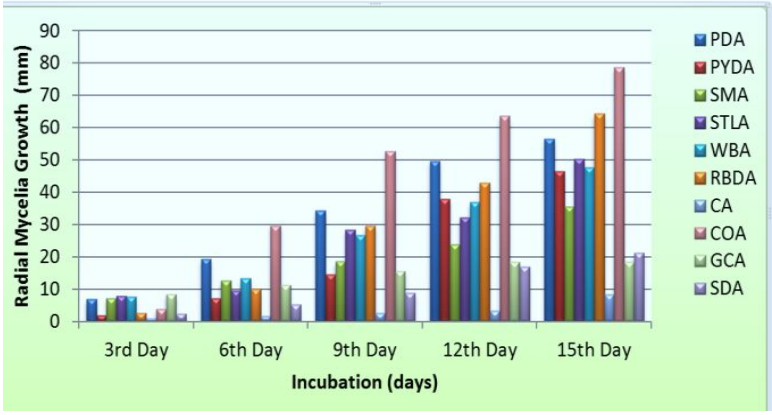

Figure 1. Comparative growth of *H. tessulatus* on different culture media after 15 days of incubation.

Characteristics of mycelia on different substrates showed variations among the seven substrates sorghum grains, black beans, and soy bean was found to have abundant mycelia growth. Sweet sorghum grains has very thick mycelia, followed by green monggo bean with moderate growth. Feed wheat had thin growth on the two weeks of incubation, but showed thickness on the terminal week. Whole corn recorded the thinnest vegetative growth.

Temperature is found to be an important environmental factor that controls the growth and cultivation of mushroom. *H. tessulatus* was able to tolerate temperature range of 15 °C to 25°C.Poor growth was recorded at 10 °C .According to the result the optimum range of temperature for the growth of *H. tessulatus* was at 25 °C. (Fasidiet. al1996).

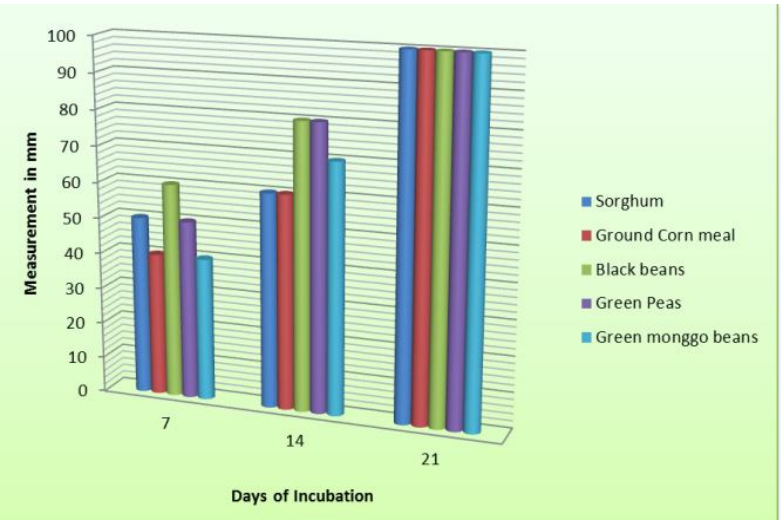

Figure 2. Comparative Mycelia Growth of *H. tessulatus* on Different Spawn Substrates.

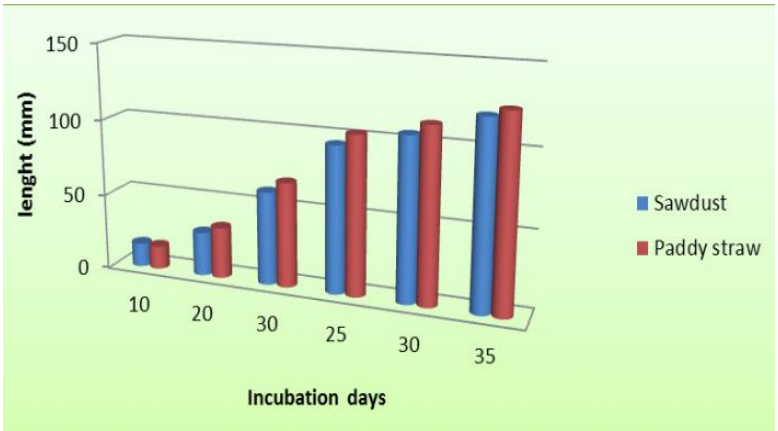

Figure 3. Mean comparison between the mycelia growth of sawdust and paddy straw.

After 10 days of observation, mycelia run on paddy straw measured 15.8 mm compared to 14.9 mm growth in sawdust. As observed, mycelia run on paddy straw was 34.64, 71.4, 108.4 mm on 20th, 30th ,40th days, respectively. While the growth was 24.27, 63.7, 104.6 mm with respect to the sawdust substrate with the same days of observation. Paddy straw promoted the fastest mycelia run on fruiting bags having all bags fully ramified on the 45th day of incubation. However the commonly and widely used substrate sawdust supported lesser mycelia growth after 40 days.

The results showed that *S. aureus* and E. coli had the wider zone of inhibition using the *H. tessulatus* extract which are (15.67 mm) and (9.67 mm) followed by *P. aeruginosa* (8.67 mm) and the least is *B. subtilis* (4.67 mm) in diameter. It also gave the same output with the control whereas *S. aureus* is appeared to have the wider inhibition zone then *P. aeruginosa, E.coli* and *B. subtilis.*

And almost the same study conducted by Moniraet.al,. that the H. tessulatus extract using chloroform fraction exhibited moderate activity towards most of the pathogen and the highest zone of inhibition was observed against *B. subtilis* and *S. aureus* (11 mm) at a concentration of 500)lg disc.

Conclusion

Cultivation of mushroom such as *Hypsizygus tessulatus*, a proficient for different valuable uses supplemented the most suited and finest culture media, spawn substrates and fruiting substrates. Experiment revealed that Compost agar, Potato dextrose agar, spent tea leaves agar, and Rice bran decoction agar and Wheat agar were the most suitable culture media in initiating growth of *H.tessulatus*. While, Potato yeast dextrose agar, Standard methods agar and Ground corn agar exhibited an average growth. On the other hand, Carrot agar and Sorghum decoction agar were the unfavourable media in growing *H.tessulatus*. For optimal requirements of culture media, the most suitable temperature for *H. tessulatus* ranges from 20-25° C while the range 10-15° C was determined as low efficient requirement. For the optimal pH the most favorable growth was observed at pH 6. Mycelia growth were also observed at pH of 5 and 7 signifying that *H. tessulatus* is prefer slightly basal conditions. For spawn substrate, *H. tessulatus* inoculated in black beans, green monggo beans and green peas promoted the most abundant mycelia run. Ground corn and sorghum grains gave the fastest but thinner growth. Moreover, paddy straw exemplified the fastest mycelia run on fruiting bags than sawdust which is used commonly.

Anti-bacterial assay revealed that both Gram (+) bacteria exhibited high susceptibility (B. subtilis and S. aureus) while the Gram (-) bacteria showed a minimal zone of inhibition againstH.tessulatus.

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