

# Biophysiology of Himematsutake (*Agaricus blazei* strain 2)

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*Abstract* — The mycelia growth performance of *Agaricus blazei* strain 2 was evaluated in this study. Evaluation of influence of physical factors (pH, aeration and light); nutritional factors (types of indigenous culture media), mother spawn materials (types of grain spawn materials) and formulation of sugarcane bagasse and mud press ratios as substrate material. Corn grit decoction sucrose gulaman having a pH of 6.5 favors the growth of this mushroom. Plate cultures in terms sealed and unsealed, light and dark condition, unsealed and dark incubated plates has significant effect on growth compared to sealed and lighted plates. Sorghum seed was highly significant to other treatment when used as mother spawning materials for *Agaricus blazei* strain 2. And 20% sugarcane bagasse and 80% mud press was the best formulation using sugarcane based substrate as spawning material of *Agaricus blazei* strain 2.

## I. Introduction

A mushroom is the fleshy, [spore](#)-bearing [fruiting body](#) of a [fungus](#), typically produced above ground on soil or on its food source. (Wikipedia .com)

Mushrooms have been esteemed a delicacy for several thousands of years, but only within the last three hundred years did the French discover how to cultivate them. Towards the seventeenth century someone whose name has not been recorded evolved a method of treating horse-manure and planting it with spawn of wild mushroom (Atkins, 1966)

The cultivation of mushroom for food has developed into an industry of considerable proportions in the United States, and is continually growing. Although only one species of mushroom (*Agaricus campestris*) is cultivated extensively for food, many of the wild species are equally good or better in flavor, and are highly prized by connoisseurs (Alexopoulos 1979).

In Central Luzon, species of *Agaricus* have been grown or cultivated by mushroom growers to supply their needs for daily consumption, not only for food but also for nutraceuticals or for functional uses as well. However, as the demand goes up, growers decide to produce more yields to have a good profit as well. Therefore, many researches for new strains of mushroom are being examined and developed; one of these introduced strains is *Agaricus blazei* strain 2.

New fungi are being brought into cultivation, notably truffles (*Tuber spp.*), boletes (*Boletus spp.*) and oyster mushroom (*Pleurotus spp.*) for direct consumption (Burnett, 1975).

A number of technologies for the production of other locally growing wild edible mushroom such as *Schizophyllum commune*, and *Collybia reinakeana* have been developed (Reyes et al 2003, 2004, Reyes et al 2005).

### **Statement of the Problem**

This study aimed to determine the biophysiology of *Agaricus blazei* strain 2 on the different nutritional and physical factors for growth.

Specifically, it intends to determine:

1. The mycelial growth performance of *Agaricus blazei* strain 2 using different culture media such as rice bran decoction sucrose gulaman, and corn grit decoction gulaman
2. The mycelial growth performance of *Agaricus blazei* strain 2 as influenced by physical factors such as different pH levels of the media, illumination, condition and aeration requirement and;
3. The mycelial growth performance of *Agaricus blazei* strain 2 on palay, corn grits, milled rice and sorghum based mother spawning materials and;
4. The mycelial growth and fruiting body performance of *Agaricus blazei* strain 2 as influenced by the difference rice straw-based substrate formulations

### **Significance of the Study**

Considering our current situation, the necessity for portable products is rising in demand. Mushroom production can be great help to solve this crisis many research studies are being conducted to develop technology for wild edible species of mushroom in the Philippines. one of these mushroom is *Agaricus blazei* strain 2 which was studied under Central Luzon University condition. The study of biophysiology of *Agaricus blazei* strain 2 will serve as our guide and information for the development of production technology.

### **Scope and Limitation of the Study**

This study was guided by the following limitations

1. Different indigenous culture media such as rice bran decoction sucrose gulaman, coconut water gulaman, potato sucrose gulaman and corn grit decoction sucrose gulaman was used for the mycelial growth performance of *Agaricus blazei* strain 2

2. Physical factors such as pH levels of the media, illumination condition and aeration requirement in the mycelial growth performance of agari sajour caju
3. The difference mother spawning materials was used such as palay seed, milled rice, corn grits, and sorghum seed in the mycelial growth performance of *Agaricus blazei* strain 2
4. The different rice straw based substrate formulation was used for the production of fruiting bodies of *Agaricus blazei* strain 2

### Definition of Terms

**Biophysiology** biological and processes that govern the growth of mushroom

**Strain** genetic variant of the mushroom

**Substrate** material which mushrooms grow and from which they draw nutrients

**Mycelia** form the main body of mushroom

## II. Methodology

This chapter presents the research design, sampling design, data-gathering procedure, and data analysis.

### Source of Strain

Culture of *Agaricus blazei* strain 2 was obtained from the culture collection of the Center for Tropical Mushroom Research and Development (CTMRD), Department of Biological Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija.

### Preparation of Culture Media

Fresh matured coconut water was gathered from the market and was filtered using cheesecloth to remove dirt. One liter of the filtered coconut water was boiled. Twenty grams of white gulaman were also added and stirred constantly until homogenized. Forty milliliters of the prepared medium were dispensed into newly blanched dried flat bottles. And allowed to stand up open for a few minutes in order to evaporate the moist and then it was covered with cotton plug. It was wrapped with clean recycled paper and sealed with rubber band. The media were then sterilized for twenty minutes at 121 °C or 15 pounds per square inch. After sterilization, the bottled substrates were allowed to cool and solidify in a slanted position preventing the cotton plug to get wet.

## Revival of Pure Culture

Approximately one square millimeter (mm<sup>2</sup>) of a pure culture of *Agaricus blazei* strain 2 was aseptically transferred into previously prepared coconut water gulaman in plates. This was kept at room temperature to allow the proliferation of mycelia.

### **Sub-study 1: Nutritional Requirements for Mycelial Growth on Indigenous Culture Media**

This sub-study has seven treatments with three corresponding replicates each. The treatments are as follows:

$T_1$  = Rice (*Oryza sativa*) bran decoction sucrose gulaman

$T_2$  = Corn (*Zea mays*) grits decoction sucrose gulaman

$T_3$  = young coconut (*Cocos nucifera*) water gulaman

$T_4$  = matured coconut (*Cocos nucifera*) water gulaman

$T_5$  = Potato (*Solanum tuberosum*) sucrose gulaman

$T_6$  = Sorghum (*Sorghum bicolor*) decoction sucrose gulaman

$T_7$  = Mudpress decoction gulaman

One liter of coconut water were boiled and then added with twenty grams of gulaman. Two hundred fifty grams of fresh potato were boiled in 1000 mL of water until tender. After boiling, potatoes were separated by straining with the use of cheesecloth. Twenty grams of gulaman and 10 grams of sucrose were dissolved into the strained decoction with constant stirring. Approximately 10 mL of previously prepared potato sucrose gulaman were dispensed in properly labeled petri plates.

Fifty grams of rice bran, corn grits sorghum and mud press were boiled separately in 1000 mL of water. After boiling, each decoction was strained and added with twenty grams of gulaman and 10 grams sucrose (except for the mud press). It was dissolved through constant stirring. Approximately 10 ml. of each previously prepared medium were dispensed in properly labeled Petri plates. The pH of the media were adjusted to pH 7.0 using 0.1 M NaOH and 0.1 M HCL. All the prepared media were put in a flask and sterilized under autoclave at 15 psi at 121 °C for 20 minutes. After sterilization, it was partially cooled before transferring into sterile Petri plates. Approximately 10 ml. of media were dispensed on properly labeled Petri plates. Ten millimeter of fungal disc of *Agaricus blazei* strain 2 was inoculated into previously prepared media in plates. It was stored at room temperature to allow the proliferation of the mycelia. The data to be gathered are the total days of total mycelial colonization, daily mycelial growth being measured using digital vernier caliper and mycelial density were noted and rated using the legend:

(+) thin, (++) thick, and (+++) thick and cottony.

### **Sub-study 2: Physical Requirements for the Mycelial Growth of *Agaricus blazei* Strain 2**

**pH.** The most appropriate medium obtained in sub-study 1 will be subjected to different pH levels: pH 5.0, 5.5, 6.0, 6.5, and pH 7.0, using 0.1 M NaOH or 0.1 M HCl. All treatments have three replicates and each was inoculated with approximately 10 mm fungal disc to allow the ramification of mycelia.

**Aeration.** From the most appropriate medium and pH level, the lid of the previously inoculated plates of *Agaricus blazei* strain 2 were sealed with parafilm twice and the unsealed plate will serve as control. The plates will then be incubated at room temperature to allow the full ramification of mycelia.

**Light Requirements.** The most appropriate medium and pH level obtained from the previous study were subjected to light and dark conditions. In light condition, the plates were placed in a chamber with artificial light (fluorescent light) under room temperature. In dark condition, the plate will be placed in a chamber covered with carbon paper at room temperature.

All the three study under this sub study were gathered with data namely daily mycelial growth, total day of total mycelial colonization and mycelial density.

### **Research Design**

The study utilized experimental design will lead out in completely randomized design with three replications. Diameter of mycelial growth of *Agaricus blazei* strain 2 on the different culture media. The number of days of total mycelial ramification from the different physical requirements of the media such as pH, illumination condition and aeration. Number of days of total mycelial colonization of *Agaricus blazei* strain 2 in different spawning materials. Number of days of total mycelial ramification of *Agaricus blazei* strain 2 in different rice straw-based substrates formulation. Weight of *Agaricus blazei* strain 2. Computation of biological efficiency of *Agaricus blazei* strain 2 in different substrate formulation.

### **Sampling Design**

The experiment will be laid out in completely randomized design with three replications

### **Research Instrument**

In this study, an experimental approach is employed. *Agaricus blazei* strain 2 will subject on the different indigenous culture media in the mycelial growth performance of *Agaricus blazei* strain 2

### Data Gathering Procedure

1. The influence of the different indigenous culture media in the mycelial growth performance of *Agaricus blazei* strain 2
  - Incubation period (no. of days of total mycelial colonization)
  - Daily mycelial growth in diameters every 24 hours (mm.)
  - Mycelial density
2. Number of days of total mycelial ramification from the different physical requirements (pH, aeration and light requirements).
  - Incubation period (no. of days of total mycelial colonization)
  - Daily mycelial growth in diameters every 24 hours (mm.)
  - Mycelial density
3. Number of days of total mycelial colonization and mycelial density of *Agaricus blazei* strain 2 in different mother spawning materials.
4. Number of day of total ramification of *Agaricus blazei* strain 2 on sugarcane bagasse and mud press based substrates for spawning material.
  - Incubation period (no. of days of total mycelial colonization)
  - Mycelial density

### Data Analysis

All treatments in each sub study will be laid out in a Complete Randomized Design with 3 replicates per treatments.

Analysis will be done using the analysis of variance technique in a one-way classification analysis.

The general statistical model will be followed:

$$Y_{ij} = \bar{x} + \tau_i + \varepsilon_{ij}$$

Where:

$Y_{ij}$  = individual observation of the  $j$ th replicate of the  $i$ th treatment.

$\bar{x}$  = over all mean

$\tau_i$ = treatment effect

$\epsilon_{ij}$ = random error

Comparison among means will be the Least Significant Difference (LSD) test for the Sub study 1, sub study 2, sub and study 3 while Duncan's Multiple Range Test (DMRT) will be employed in sub study 4 to determine the specific significant treatment comparisons. All tests of significance will be done at 5 % level of significance.

### III. Results and Discussion

#### Sub-study I: Nutritional Requirements for Growth using Indigenous Culture Media

Mycelia are a vegetative structure produce by mushrooms. Sometimes it was considered as the root of the mushroom which attached to the substrates. These are strands that are interwoven to each other. In Mushroom production mycelial culture is very important as stock culture during production. Supplementation of nutrients helps improved the growth of the mycelia such as organic matter from the substrate that may be taken through absorption.

In this study, seven indigenous culture media are evaluated for the efficiency of growth of *Agaricus blazei* strain 2, such as rice bran decoction sucrose gulaman, corn grits decoction sucrose gulaman, young and matured coconut water gulaman, potato decoction sucrose gulaman, sorghum decoction sucrose gulaman, and mud press decoction gulaman. As shown in table 1, it was observed that corn grits decoction sucrose gulaman which obtained the highest mean of 69.17mm which is highly significant than all other treatments.

### IV. Conclusion

The growth performance of *Agaricus blazei* strain 2 as influenced by nutritional requirements, physical factors such as pH, aeration and illumination were evaluated. Mother spawning materials (palay, sorghum, milled corn and mud press. Were also evaluated to determine the best substrate for the mycelial production of *A. blazei*. Formulation and evaluation of sugar cane bagasse and mud press based substrate for spawning or mass production of the mycelia was also evaluated.

The growth performance of the mycelia of this mushroom on indigenous culture media such as rice bran decoction sucrose gulaman, corn grit decoction sucrose gulaman, young and matured coconut water gulaman, potato sucrose gulaman, sorghum decoction sucrose gulaman and mud press decoction gulaman having a pH of 7.0 was evaluated. On the other hand, the other physical factors such as pH (5.0, 5.5, 6.0, 6.5 and 7.0) aeration (sealed and unsealed) and

illumination (light and dark condition) were also evaluated. Growth performances of this mushroom in grain spawn material (palay, sorghum, milled corn and mud press) was also noted.

*Agaricus blazei* strain 2 favorably grew on corn grit medium having a pH of 6.5. It grew rapidly and produced cottony in this medium incubated at room temperature. A *blazei* when grew in an unsealed plate were faster in terms of fully ramifying the plates than the sealed and dark condition favored the efficient mycelial growth sorghum yields the best mycelial growth in terms of mother spawn compare to palay, milled corn and mud press. Treatment 9 having 20% sugar cane bagasse and 80% mud press was the best ratio in production of mycelia. So this ratio can be used in the production of the fruiting bodies of this mushroom using sugarcane based substrates.

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