



## Tissue Culture and Spawn Preparation of Edible Mushroom (*Agaricus Bisporus*)

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### ABSTRACT

Among the Higher Basidiomycota, the white button mushroom (*Agaricus bisporus*) is a crucial food and medicinal species that is utilized in the recycling of agricultural waste. includes debris from reed plants, wheat straw, and rubbish Paper, discarded tea leaves, oat straw, a few water plants, and others. There are several uses for *Agaricus bisporus* in human diets and due to its mix of key components, pharmaceutical carbs, fatty acids, amino acids, and low calorie vitamins, trace minerals, and unprocessed fibers. This paper demonstrates the process of tissue culture and spawns preparation for the cultivation of white button mushroom (*Agaricus bisporus*) Potato dextrose agar is used as the culturing media. The mushroom culture can be prepared in both slant and Petri plates. Various methods should be followed for the production of spawn and the standardized spawn will be prepared after the growth of mycelium in pure culture and subculture with the help of wheat mixed with calcium carbonate and calcium sulfate. Two types of spawning techniques have been used alternate spawning method and the top layer spawning method. As a result, the alternating spawning method gives good results in the harvesting fruit body of mushrooms.

### INTRODUCTION

*Agaricus bisporus*, is the most familiar mushroom for most of us. it is the commercial mushroom sold in stores and put on pizza. It comes in various forms: button versions, brown versions and large portobello versions all of which are varieties of the same species. Its popularity is not so much a consequence of flavor as it is a consequence of its suitability for commercial production. *Agaricus* does grow wild, typically in fields or lawns but all of the *Agaricus* mushrooms sold in stores are grown on a controlled medium and in a controlled Environment. (Barros et al., 2008). Mushrooms have a unique texture have good aroma, taste and flavor that differs mushroom from other food crops. Edible species of mushrooms found abundantly in indigenous forests. Mushrooms are highly nutritive, low-calorie food with good quality proteins, vitamins and minerals. Mushrooms are an important natural source of foods and medicines. By virtue of having high fiber, low fat and low starch, edible mushrooms have been considered to be ideal food for obese persons and for diabetics to prevent hyperglycaemia. They are also known to possess promising anti oxidative, cardiovascular, hypercholesterolemia, antimicrobial, hepato-protective and anticancer effects. Fekadu Alemu (2014)

### MATERIALS AND METHODS

#### Preparation of bacteriological media:

#### Potato Dextrose Agar

Readymade Potato Dextrose Agar base media (Hi Media) was used. Agar powder was added to basal media.

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## Collection of Mushroom samples

One species of mushrooms, *Agaricus bisporus* (were collected from local markets placed in sterile Polyethylene bags and kept at a temperature of 4 °C until use). The material was brought to the laboratory and preserved at room temperature.

## PREPARATION OF MOTHER CULTURE OF MUSHROOM

**Mother culture production:** Fruiting bodies of white fungi *Agaricus bisporus* strain were used for producing pure mother culture by the method of tissue culture in Petri dishes contained the Potato Dextrose Agar (PDA) media. Then, the dishes were transferred to an incubator with a degree of  $25\pm 1^{\circ}\text{C}$  for three weeks with continuous monitoring for mycelium growth and the exclusion of contaminated dishes. Optimal conditions determination to produce the mother culture: The fungi isolated, purified from the previous step, was developed on two types of media, which is the commercial media Potato Dextrose Agar, which was used to stimulate and preserve the isolates and for comparison.

Effect of the media type on the growth rate of fungi:

The media were mixed well, and its pH was set to 6.5, sterilized in the autoclave device at  $121^{\circ}\text{C}$  for 15 minutes, and it cooled to  $55^{\circ}\text{C}$ , then it was distributed in Petri dishes with a diameter of 8.5 cm. The prepared dishes are inoculated with a piece of stimulated fungi growth on the PDA media, by placing that piece in the center of the dish, and then the dishes were tightly closed with Parafilm. The dishes were incubated at a temperature of  $25^{\circ}\text{C}$ , and the growth of mycelium was monitored until it was fully grown in the dish

## Preparation of subculture

Mycelial agar cultures (8 mm diameter) were, taken from the margin of seven days old fungal colonies were inoculated individually in the centre of plates containing PDA medium. The plates were incubated at  $28 \pm 2^{\circ}\text{C}$  for seven days.

Preparation of subculture in slants

Mycelial agar cultures (8 mm diameter) were, taken from the margin of seven days old fungal colonies were inoculated individually in the centre of slants containing PDA medium. The slants were incubated at  $28 \pm 2^{\circ}\text{C}$  for seven days.

## PREPARATION OF SPAWN

### Substrate preparation

Good quality wheat grains which were free from pest and moulds were selected. Boiled grains were submerged in clean water for 20 – 30 minutes. When the grains become soft, remove and spread evenly on a cotton cloth to drain out the water and cool the grains. We added 20g of  $\text{CaCO}_3$  and 20 gm of  $\text{CaSO}_4$  to the grain mixed well. Fill 250 gms of grain in cleaned and dried polypropylene bags and plug the mouth of the bottle tightly with non-absorbent cotton. Sterilized the grain bags in autoclave exposed to  $121^{\circ}\text{C}$  for 20 minutes. After cooling transfer the bags to inoculation chamber.

### Inoculation of substrate

The grain bags on platform were allowed to dry for 1 hr after that, placed the bags in laminar air flow for 20 to 30 min for UV exposure. Inoculation was done by using prepared mother culture. Mycelial agar cultures were placed in bags using inoculation loop. Bag was mixed well and lightly closed with cotton plugs.

### Production of spawn

The inoculated bags were shifted to spawn room having temperature range of  $25 - 30^{\circ}\text{C}$ . Check the bags regularly and discard contaminated one immediately. Within 15 – 20 days of inoculation mycelial growth covers entire substrate and the spawn is ready for use.

## RESULTS AND DISCUSSION



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## MOTHER CULTURE

Inoculated mushroom tissue were fully grown in PDA plates in 7days forming the mother culture.



Figure 1 A) the growth of mushroom in subculture plates B) the summary of spawn production C) Growth of mushroom subculture D) Prepared spawn

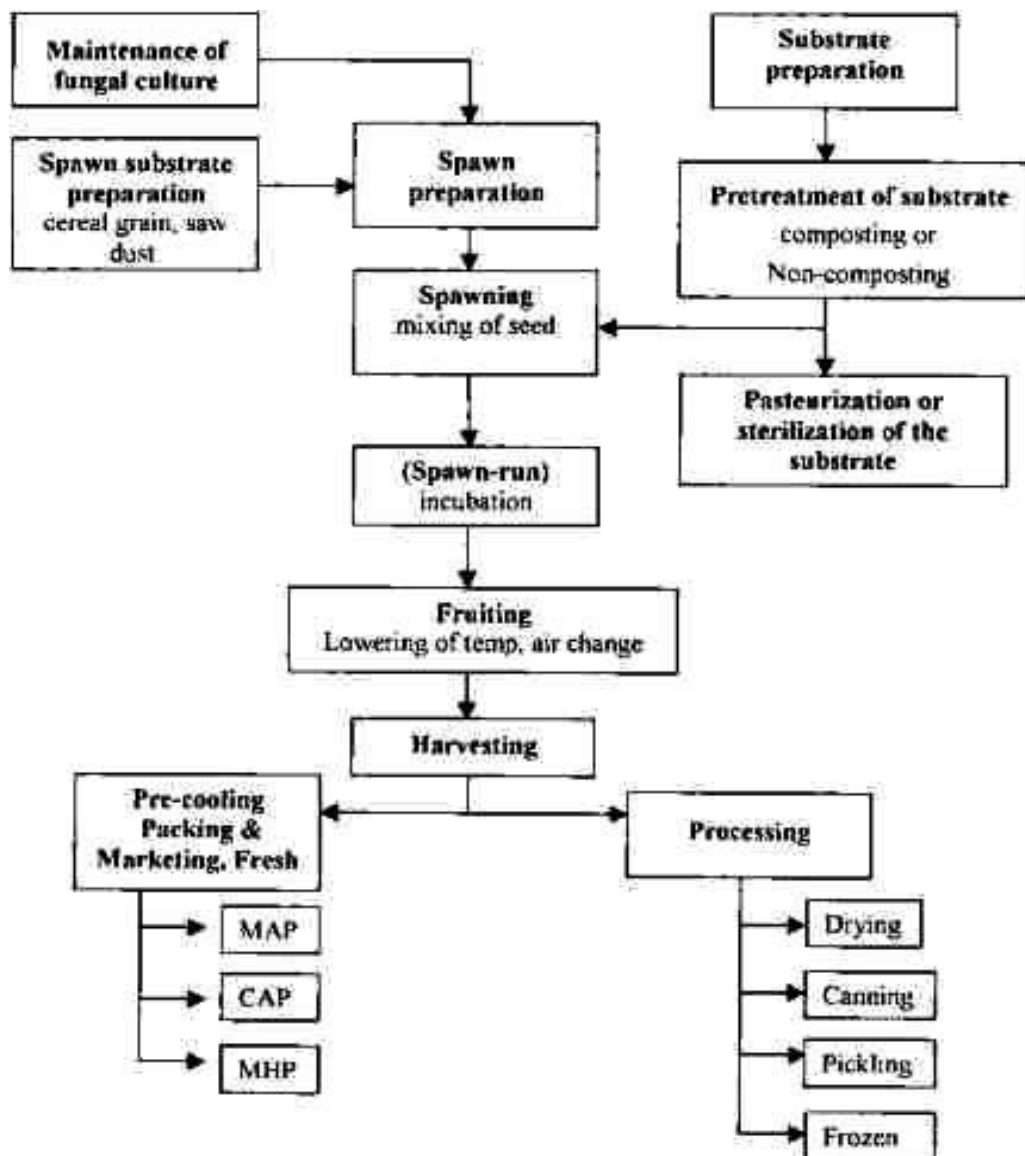
## Spawn preparation

After 2-3 weeks the mushroom mycelium fully colonizes (covers) the wheat grains. To begin with, spawn is prepared in bags using the culture grown in the test tube and this is called Mother Spawn. The first generation grain spawn prepared is Mother Spawn.

Using potato dextrose agar is for developing mushroom culture. The contaminated plates were discarded. And the spawn bags and the mushroom beds which are contaminated and damaged during the growing period and sterilization were discarded. Using the completely cover mycelium covered spawn the bags were prepared and the spawns and the beds were s during the incubation period. The contaminants were discarded to avoid the spoilage to the nearer bags. mushroom shed were maintained at correct temperature. The mycelium covered within 15 – 25 days in mushroom beds.

## CONCLUSION

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