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TISSUE CULTURE AND SPAWN PREPRATION 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, andothers. 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 fibre. 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)

Agaricus, and nearly all of the fungi that would be described as mushrooms, i.e. that produce stalked structures with a cap, are club fungi = basidiomycetes fungi (Phylum Basidiomycota). Most mushrooms have 'gills' on the underside of the cap where spores are produced, and *Agaricus* show this feature. Historically all mushrooms with gills were put in a taxonomic entity (usually an order, the Agaricales) but recent molecular analysis has demonstrated that gills are not a sound feature on which to base a phylogenetic classification. Although there still is an order Agaricales, named for *Agaricus,* it does not contain all gilled mushrooms and it does include a number of fungi that do not possess gills. Like nearly

all fungi and nearly all club fungi, the bulk of a mushroom's structure is a network of branching filaments, one cell thick (hypae) that permeate a substrate (Hrudayanath T et al., 2012). In the case of wild *Agaricus,* the substrate is the soil; for cultured *Agaricus* the substrate is a prepared compost, typically manure with other material added to it. In club fungi the filaments are cellular, meaning that there are individual cells delineated by the presence of cross-walls (septa). The septa are not complete but usually have a pore in the middle that allows cytoplasm to move from one cell to another. The fruiting body of *Agaricus* is a consequence of drastic change in the behavior of hyphae. Instead of growing in a diffuse manner and spreading throughout their environment, they grow close to each other and intertwine, forming a solid structure that emerges from the substrate it is growing in and produces the familiar mushroom structure. Manjunathan et al*.,* (2014)

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.

Fig 1 Represents PDA media

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 ºC 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±1^{0}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°C for 15 minutes, and it cooled to 55°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°C, and the growth of mycelium was monitored until it was fully grown in the dish as shown in fig 2.

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 ± 2°C for seven days. Growth of subculture can be shown below.

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 slantswere incubated at 28 ± 2°C for seven days. Growth of subculture can be shown below. fig 2

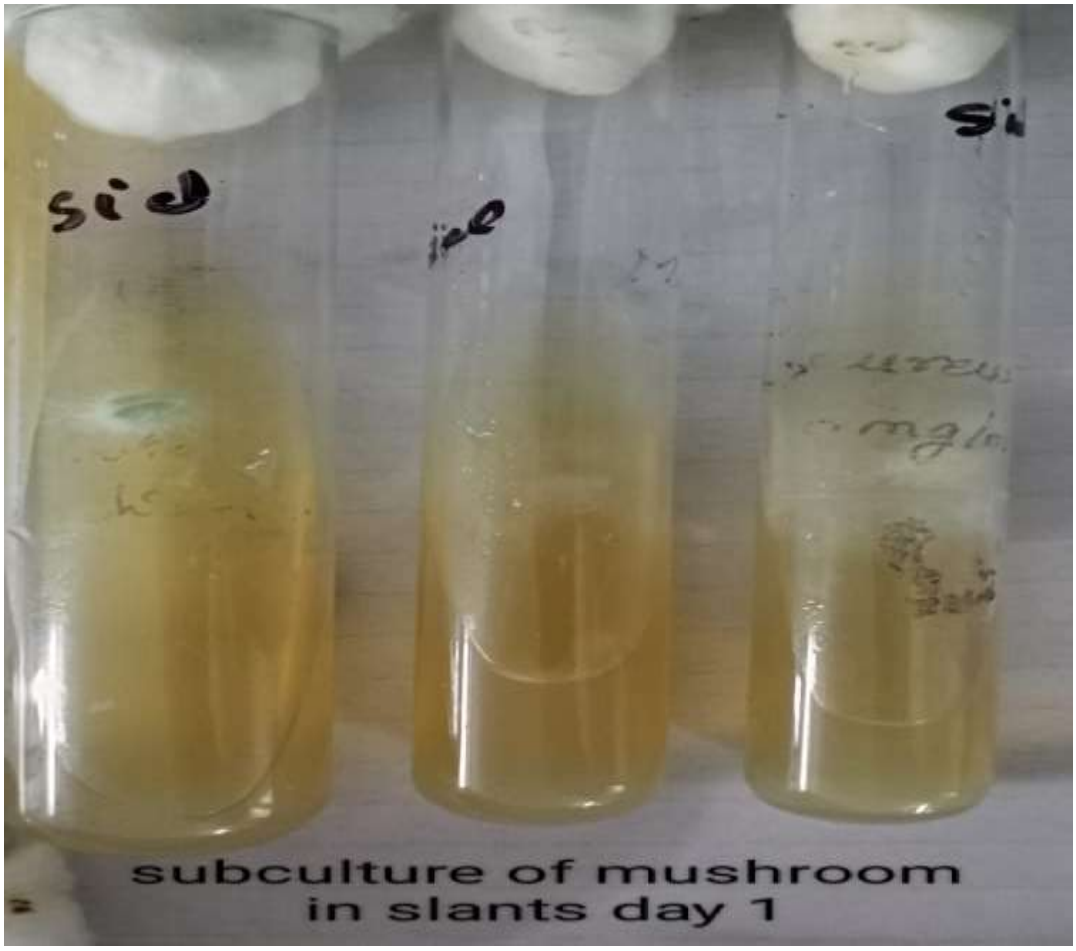


Fig 2 Represents growth of subculture in PDA slants

PREPARATION OF SPAWN .

Substrate preparation

Select good quality wheat grains free from pest and moulds. 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 $CaCO_3$ and 20 gm of $CaSO_4$ 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 121^0c for 20 minutes. After cooling transfer the bags to inoculation chamber.

Inoculation of substrate

We left the grain bags on platform 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

.Shift the inoculated Bags to spawn room having temperature range of 25 – 30 ^{0}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.

Cares to be taken

- Always keep the inoculation chamber and its surroundings very clean.
- Switch on UV tube in the inoculation chamber for 30 minutes before inoculation by keeping sterilized substrate, forceps, and cultures inside the chamber.
- Inoculation is always done near the spirit the spirit lamp flame to avoid contamination.
- The working person should swab his hands and inoculation chamber using alcohol.
- Spawn should grow fast in the bottles, should be silky white in colour and should never show fluffy growth.
- All grains should be covered by the mycelial growth and fresh spawn should have mushroom odour.
- Mother spawn should not be used beyond 3-4 generations as it starts degeneration. Fresh spawn gives higher yield; therefore spawn should never be stored for more than a month.

Fig 3 Represents the summary of spawn production

RESULTS AND DISCUSSION

MOTHER CULTURE

Innoculated mushroom tissue was fully grown in PDA plates in 7days forming the mother culture .

Fig 4 Represents the mother culture of mushroom

Subculture

Inoculated Mycelial agar cultures were fully grown in PDA plates and PDA slants Growth of subculture can be shown below. fig 5 and 6 respectively

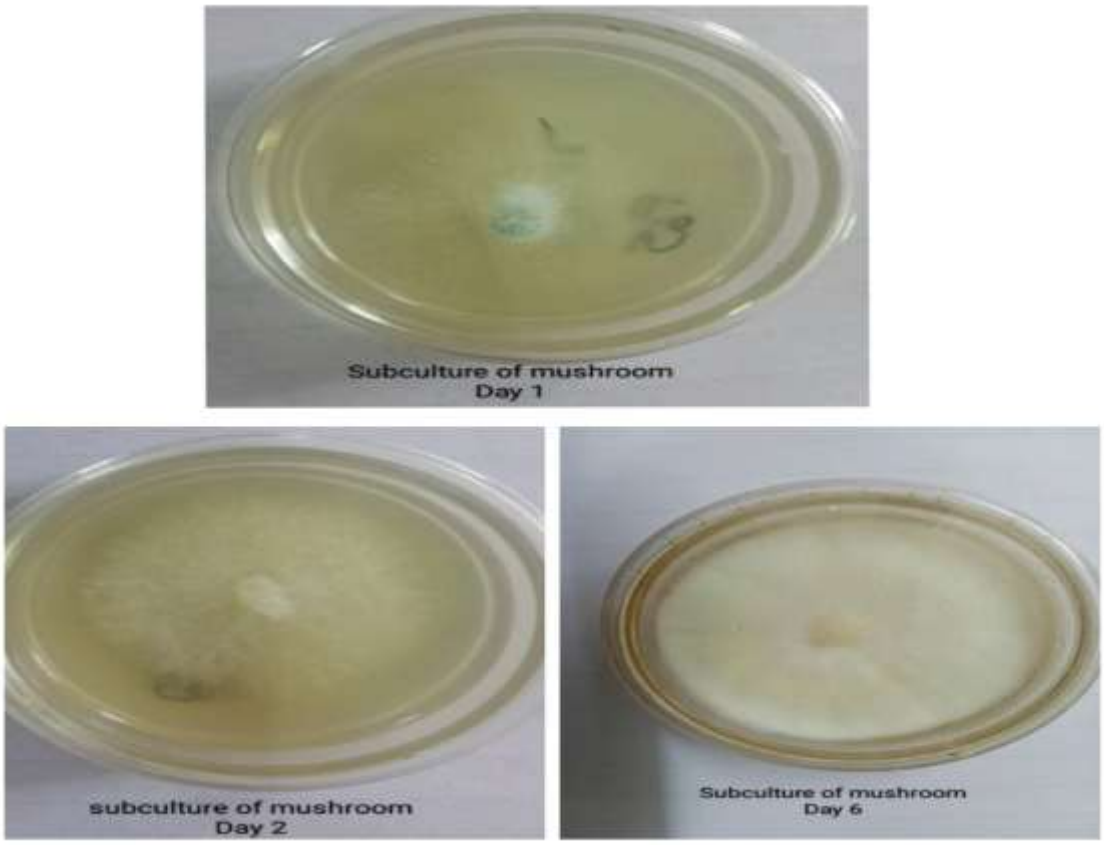


Fig 5 Represents the growth of mushroom in subculture plates

Fig 6 Represents growth of mushroom subculture

Spawn preparation

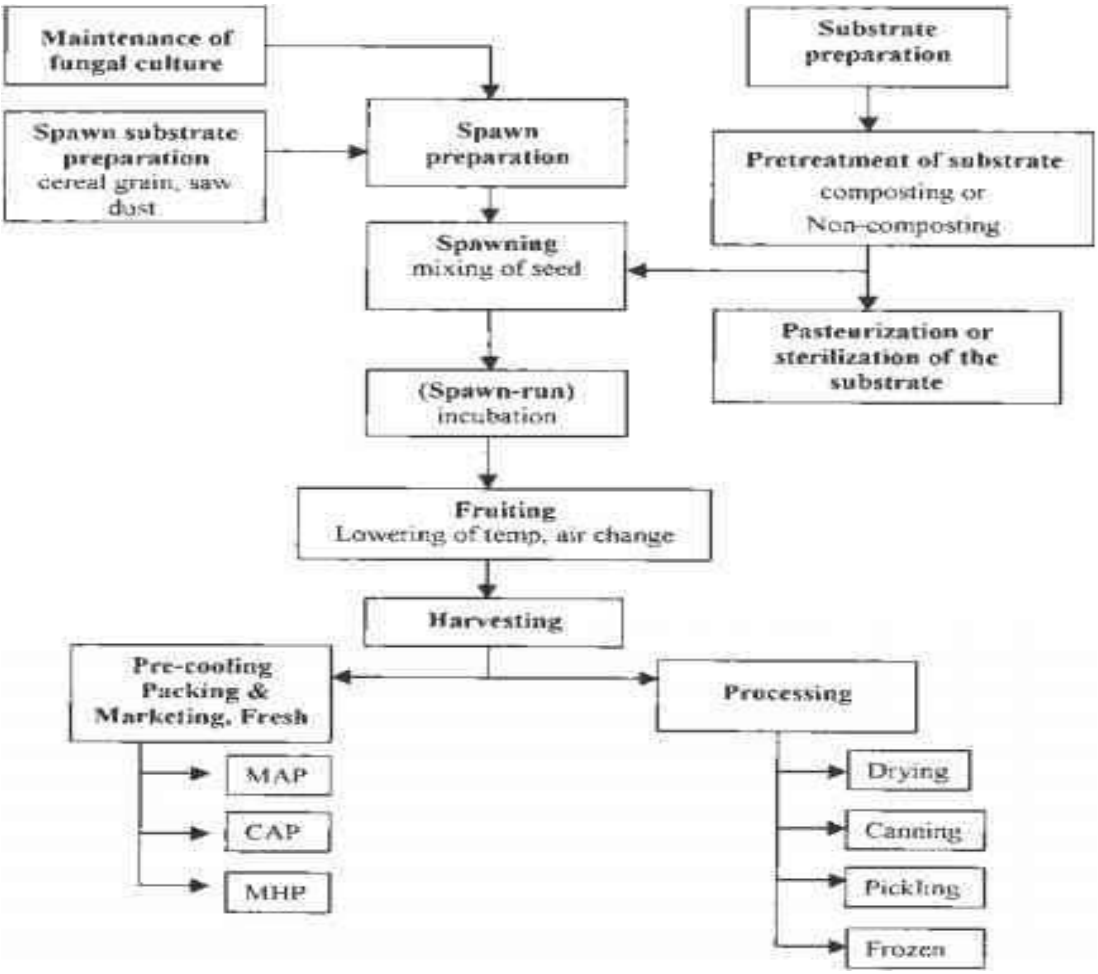
After 2-3 weeks the mushroom mycelium fully colonizes (covers) the wheat grains . To begin with, spawn is prepared in bags using the culture grown in the test tube and this is called Mother Spawn. The first generation grain spawn prepared is Mother Spawn.

Fig 7 Represents the prepared 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

Seed or planting material of mushroom i.e. spawn, consists of mycelia of the fungus multiplied on suitable substrates like cereal grains. The mycelia of mushroom fungus cannot be propagated as such; hence the mycelia are multiplied on a carrier like cereal grains (Bahukandi et al., 1989). Like in all other crop production systems, seed or spawn is the key input in mushroom cultivation. Non-availability of quality spawn is the major constraint in mushroom production. Good quality spawn conform to – i) high yield potential, ii) absence of contaminants, iii) better economic benefit. Spawn production primarily depend on the easy availability of spawn substrates.



REFERENCES

Bahukhandi d,and Munjal, R.L.Cultivation of Pleurotus Species on different Agricultural Residues. India Phytopath. 42(4), 1989.492-495.

Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira IC (2008) Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem. Toxicol 46(8): 2742-2747.

Fekadu Alemu (2014) Cultivation of lentinus edodes on teff straw (agricultural residue) at Dilla University, Ethiopia, Applied Microbiology 1(3): 49-59.=

Fekadu Alemu (2014) Cultivation of pleurotus ostreatus on grevillea robusta leaves at Dilla University, Ethiopia, Journal of yeast and Fungal Research 5(6): 74-83.

Fekadu Alemu (2015) Cultivation of Shiitake Mushroom (Lentinus edodes) on Coffee Husk at Dilla University, Ethiopia, Journal of Food and Nutrition Sciences 3(2): 63-70.

Manjunathan J, Kaviyarasan V (2010) Nutrient composition in wild and cultivated edible mushroom, Lentinus tuberregium (Fr.) Tamil Nadu, India. International Food Resource. Journal 18(2): 59-61.

Hrudayanath T, Sameer KS (2014) Diversity, nutritional composition and medicinal potential of Indian mushrooms, African journal of Biotechnology, Biju Patnaik University of Technology, Bhubaneswar, Odisha, India.