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EFFECT OF THERMALLY COMPOSTED CULTURE MEDIA ON THE GROWTH OF HYPHAE FROM VARIOUS PARTS OF THE BASIDIOCARP OF BUTTON MUSHROOM, *AGARICUS BITORQUIS* (QUELET) SACC

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Abstract

This research was carried out to study the hyphal growth initiation from various parts of *Agaricus bitorquis* on yeast extract agar media composted for different periods. Gills showed more radial growth 1.63 cm than that of all other tissues of basidiocarp followed by joint, cap and stalk with radial growth, 1.15 cm, 0.85 cm and 0.23 cm respectively after twelve days of growth initiation. Stalk showed poor radial growth of all. Thermal composting showed inconsistent effect on radial growth of hyphae taken from selected tissues of basidiocarp. However, thermal composting up to three days was found best for the colony development.

Introduction

Mushroom is one of the useful, delicious and mysterious member of the biosphere (Verma *et al*., 1987a, b). The recent number of edible mushrooms reported is 2166 and only 470 of them are considered to have useful properties (Boa, 2004). Yet, only 80 mushrooms of them are grown experimentally. Among them 40 are cultivated and only 5 or 6 have reached industrial scale (Change, 1987). Button mushrooms contribute almost 80% of the total mushroom produced worldwide. These are naturally found in meadows and called meadow mushrooms (Raven & Johnson 1992). Mushrooms are considered a good source of proteins, vitamins, fats, carbohydrates, amino acids and minerals. Average protein value of the mushrooms is twice as that of asparagus and potatoes, four times as that of tomatoes and carrots, six times as that of oranges. The protein contents of mushrooms vary between 4 to 44%. Mushrooms contain vitamins i.e., thiamin; riboflavin, niacin, biotin and ascorbic acid, all are essential for human health. The common fats available in different mushrooms are palmitic, steric, oleic and linoleic acids. The carbohydrate contents vary from 3 to 28 %, as xylose, ribose, rhamnose, glucose, sucrose and mannitol. Mushrooms also contain minerals such as calcium, phosphorus, potassium, iron, potassium, sodium, magnesium, manganese, chlorine, silicon, sulfur and aluminum (Rambelli & Menini, 1985; Konuk *et al,* 2006).

Mushrooms are also important for their medicinal uses. The mixture of mushrooms has been used for healing purposes for thousands of years. Mushrooms are recommended to diabetic and anemic persons, owing to their high folic acid content. Some have been used for antibiotic activity, anti-allergic, surgical dressing, anesthesia, swollen glands, and epilepsy. Mushroom extract also inhibits the growth of some viruses like influenza. Cardiotoxic proteins are present in different edible mushrooms, which lower the blood pressure, and are also active against tumor cells and are anti cancer (Cochran, 1978).

Mushrooms also play an important ecological role. Some mycorrhizal mushrooms are used for establishment of forests, to improve the soil fertility, for reclamation and for introduction of exotic plant species; others are predatory and used as bio control agent and pesticide (Jiskani, 2003). Mushrooms cultivation has not been given due importance in Pakistan, although it has tremendous prospect in Pakistan. Khyber Pakhtunkhwa has a rich forest area and share more than 70% of the total mushroom production of Pakistan. About 50 tons of dried morels are collected each year by about 150,000 forest dwellers, mainly children and women (Iqbal, 1991*)*, worth value Rs. 130 to 150 million in foreign exchange (Shah, 1991). According to 1997-98 survey about 532,280 kg cultivated mushrooms were produced in some areas of Punjab, Khyber Pakhtunkhwa and Azad Jammu & Kashmir (Anon., 2001).

Farmers hesitate to cultivate mushroom on commercial basis because of unavailability of cheap and good quality spawn and selection of species adaptable to the existing geographical zone and weathers. All the cultivated mushrooms require temperature range from 20 C to 25 C and difficult to cultivate in the summer. As we have long summer period; so *A. bitorquis* a thermo-tolerant mushroom species which could be considered for cultivation in Pakistan.

Tissue culture and spawn production are preliminary steps toward mushroom production. Tissue culture development involve two main components i.e. culture media and a fungal part i.e. mycelia. Hence, interaction between culture media, fungal hyphae and amendments to these components would be assessed to produce high quality and viable culture, spawn and subsequently high mushroom production. This paper is to assess the growth potential and viability of hyphae from various parts of basidiocarp i.e. cap, gills, stipe, joint (where cap attaches stalk), and the subsequent effect of thermally composted culture media on colony development of *A. bitorquis.*

Materials and Methods

This study was carried out to find suitable culture media for thermo- tolerant button mushroom (*Agaricus bitorquis*). These investigations were conducted out in 3 dimensions simultaneously i.e., the effect of thermally composted yeast agar medium ranging from one day to 5 days consecutively at room temperature on various parts of basidiocarp of *A. bitorquis.*

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The medium (yeast extract agar) was used to assess the desired parameters. The medium was composed of 10 g yeast extract, twenty grams dextrose and fifteen grams agar each in one liter of distilled water. Yeast extract agar medium was sterilized at 121°C for 15 minutes in autoclave to completely dissolve the ingredients and avoid contamination.

To control bacterial contamination streptomycin sulphate @ 2 g/l was added to sterilized media. Media was poured into disposable plastic petri-plates for cooling down to solidification. The solidified media was stored at room temperature to get desirable thermal composting ranging from 1 day to 5 days consecutively. Mushroom fruiting bodies of *A. bitorquis* were obtained from Abid mushroom farms Muree road, Islamabad.

Hyphae were taken from different parts of basidiocarp (Gills, Joint, cap and stalk) with the help of scalpel, placed on thermally composted yeast agar medium plates and wrapped with squash tape to avoid contamination and maintain moisture of medium. Fresh yeast extract agar medium was used as control. These plates were placed in incubator at 25°C. Experiment was laid out by using 2 factors randomized completely blocked design having 6 replications to fulfill the desired studies.

Data recording and analysis: Radial growth was measured in cm after each 48 hour. The radial growth was recorded up to 12 days after the first growth initiation was seen. Data were analyzed statistically by using (ANOVA) test and means were separated with Fisher's protected least significant difference (LSD) test.

Results

Hyphae from various parts of basidiocarp are listed against and their relative radial colony development on yeast extract agar medium, thermally composted for different period of time indicated by corresponding figures (Table 1).

Table 1. The relative effect of thermally composted yeast extract agar medium on radial growth of various types hyphae of basidiocarp.

Composting	C*- 0	C- 1	C- 2	C- 3	C- 4	C- 5	Mean
Pileus	0.00 E	0.00 E	1.45BC	1.07 D	1.34BCD	1.28CD	0.86 B
Joint	0.00 E	1.61 B	1.45BC	1.29CD	1.34BCD	1.23CD	1.15 B
Gill	1.53BC	1.44BC	1.65 B	1.49BC	1.52BC	2.15A	1.63 A
Stipe	0.00 E	0.00 E	0.00 E	1.40BC	0.00 E	0.00 E	0.23 C
Mean	0.38C	0.76BC	1.14AB	1.31A	1.05AB	1.17A	

C*-0 = Fresh yeast extract agar medium, CV = 28.37%, Significance ($p<0.05$) Grand mean= 0.97

Growth potential of selected tissues of basidiocarp on thermally composted medium: Radial growth was measured for 12 days after the first growth initiation was seen. Gills showed maximum radial growth (1.63 cm) followed by joint (1.15 cm), Pileus (0.86 cm) and stipe showed the poor radial growth (0.23 cm) of all. The fresh medium prompted an average radial growth (0.38 cm), 1 day composted medium average radial growth (0.76 cm), 2 day composted medium (1.14 cm), 3 days composted medium (1.31 cm) and 4 days composted medium showed the mean radial growth (1.05 cm) and 5 days composted medium show (1.17 cm) growth. The effect of fresh and composted medium on each individual part is discussed in detail as under.

Effect of thermally composted medium on growth of hyphae of pileus: Pileus hyphae turned brown on fresh and one day composted medium and developed (1.45 cm) radial growth on 2 days composted medium, (1.07 cm) on 3 days composted medium,(1.34 cm) on 4 days composted medium and (1.28 cm) 5 days composted medium

Effect of thermally composted medium on growth of hyphae of joint: Joint could not grow on fresh medium (000 cm), developed a colony of (1.61 cm) on 1 day composted medium, (1.45 cm) on 2 days composted medium, (1.29 cm) on 3 days composted medium, (1.34 cm) on 4 days composted medium and (1.23 cm) on 5 days composted medium.

Effect of thermally composted medium on growth of hyphae of gill: Gills developed colony of (1.53 cm) on fresh medium, (1.44 cm) on 1 day composted medium, (1.65 cm) on 2 days composted medium, (1.29 cm) on 3 days composted medium, (1.34 cm) on 4 days composted medium and (2.15 cm) on 5 days composted medium.

Effect of thermally composted medium on growth of hyphae of stipe: Stipe showed zero growth on all treatments except 3 days composted medium (1.40 cm).

Accumulative effect of composted yeast and various types of hyphae: The accumulative effect of yeast extract agar and hyphae of various parts of basidiocarp on the radial growth is not significant as its grand mean is 0.97 cm only.

Discussion

Thermo tolerant button mushroom (*Agaricus bitorquis*) is a saprophytic mushroom but zymogenous by its nutritional mode and need readily available food source. Therefore medium was amended by exposing to thermal composting to assess whether thermal composting inflict any effect of on the availability of nutrients. Mushroom fruiting body includes different kinds of hyphae with respect to morphology and fertility (Smith, 1966). So hyphae from different parts of basidiocarp (cap, joint, gill and stalk) were considered in this study to find the fertile and viable tissues for obtaining best quality tissue cultures.

Gill tissue showed the best growth as they contain spores which are reproductive structure. They are more viable and withstand harsh conditions as they are in lag phase and possess more ATP than that of hyphae. These spores have specialized morphology and physiology which, make these spores more enduring than mycelia. Spore are important feature of the lifecycle of fungi as they posses the inherited capability and responsibly to develop into a new generation. Spore is not affected by the environmental condition as they remain dormant in the unviable condition for their growth. There is self-inhibitory mechanism of dormancy which prevents the spore germination in detrimental condition and so keeps the spore viable through the fatal period and assures its germination in favorable environment.

Contrasting to the above mentioned specialized features of gill, other parts of mushroom contain mycelia only. The viability of the mycelium depends on its morphlogical and physiological features. Mycelia remain in log phase for most of the time and require more energy to maintain its growth. The viability of mycelial fragments is also determined by their length, the type of mycelium and degree of septations (Marfenina *et al*., 1994.)

The hyphae taken from stipe failed to grow in most of the treatments as indicated by the results. Hyphae of the stipe are thick walled and lactiferous (Smith, 1966). So the hyphae of stipe are sterile and do not develop colony on culture media because septation in stipe dissolves soon after the fruiting body development. But some time the fertile hymenial hyphae approach the stipe and shows growth on culture media as indicated. Joint and pileus showed significant results as they contain septated and fertile hyphae so have the capability to develop into a colony on culture media. The septated mycelium has the capability to develop branch as the viability is affected by critical compartment size.

Thermal composting is considered positive as it boosts the growth of the fungus. Thermal composting up to 3 days showed positive effect on radial growth but later on its effect is inconsistent on different parts of the mushroom fruiting body as they are different from one another. It has an indirect positive effect on colony development by restricting bacterial contamination as it appears to enhance the efficacy of antibiotic (streptomycin sulphate) consecutively up to 3 days and onward and that's why hyphae from different parts of basidiocarp had maximum radial growth on media thermally composted for three days. Fungi can only grow in that environment that is not favourable for bacterial growth.

The overall effect of yeast extract agar medium is not appreciable on colony development as yeast extract agar medium restricts the vegetative growth of hyphae and induces sporulation in fungi. Yeast extract agar trigger the spore germination that's why gills which already contain spore, were prompted to develop a good colony. Plastic made Petri plates also appear to affect the growth of mushroom tissues as they release some chemical (Di-2 hydroxyethyl methyl dodycyammonium), (Holt, 2008)

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