

## Effect of Culture Media, pH and Temperature on Mycelial Growth of *Agaricus Bisporus* Strains

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The several fungi are edible mushrooms and are saprophytic basidiomycetes, which have been successfully cultivated at commercial level worldwide using lignocellulose wastes as substrates for their cultivation. In the present investigation that were the studies of effect of different culture media, pH and temperature on mycelia growth of strains (S-79, A-15 and Delta) of *A. bisporus*. Five culture media were tested for the radial growth and measured the mycelia growth on PDA of three strains S-79, A-15 and Delta were obtained fast and full growth (9 cm) completed in 8, 10 and 12 days respectively. The effect of different pH were tested for the radial growth and measured the mycelial growth on 9 pH of all three strains S-79, A-15 and Delta were obtained fast and full growth (9 cm) completed in 12, 12 and 14 days respectively. The growth of mycelial in 8pH was good performance in all strains of *A. bisporus* followed by 7 pH. Mycelial growth was much slow on 6 pH and full growth completed in 18 days. The effect of different temperature was evaluated on mycelia growth of strains of *A. bisporus*. Mycelial growth of all three strains on 25°C was obtained fast and full growth (9 cm) completed in 8, 10 and 14 days respectively, followed by growth of mycelial at 20°C and 30°C were similar performance in all strains of *A. bisporus* and the mycelial growth was much slow on 15°C and full growth completed in 18 days.

**Key words:** *A. bisporus* strains, mycelial growth, culture media, pH and temperature.

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*Agaricus bisporus* (Lange) Sing is popularly known as the button mushroom. This mushroom is extensively cultivated throughout the world and contributes about 40% of the total world production of mushroom. Button mushroom is one of the largely growing mushrooms and has the good demand in the market and world trade too. By keeping this view in mind the choice of the farmers for growing of any crops variety depends upon its yielding ability. It means the cost-benefit (C: B) ratio should always be in favour of the farmer. Scientist are suggesting to farmers for growing the high yielding mushroom strains. To make the farmer's aware about the best strains for the cultivation of mushroom although most of the farmer's using especially for edible mushroom so

little effort has been made "to innovate the farmer's about the effect of best strains on the growth, number of sporophores and yield of button mushroom or obtained high output". Prakasam and Singh (2008), stated that investigate strains were studied on the basis of growth on MEA medium, the different characters of their fruiting body. S-130 showed maximum growth on MEA medium whereas NCB-13 produced higher fruit body weight and stipe width. Lesser stipe length and maximum pileus diameter were observed in strain Delta. Maximum pileus thickness was observed in CM-5.

Andrade, *et al.* (2010), the *in vitro* mycelium growth of *Agaricus bisporus* strains ABI-05/03, ABI-06/04, ABI-04/02, ABI-06/05 and ABI-01/01 was evaluated performed by means of measurements of four diameters of the colonies, every 48 hours, during 12 days of incubation in darkness under 20 and 25°C.

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In mushroom cultivation, mushroom mycelium absorbs food from the natural or semi-synthetic composts on which they grow. The mycelium branches and produces enzymes that digest complex carbohydrate, lipids and protein, which are then easily absorbed by the hyphae. The mycelium penetrate the compost during spawn run stage, mushroom growth take place, and energy stored until fruiting bodies are formed. The fruiting stage is the formation of the primordial, formed from an aggregation of mycelium after casing soil. Therefore, present investigation was based on the effect of different culture media, pH and temperature for the mycelia growth of three strains of button mushroom (*Agaricus bisporus*).

## MATERIALS AND METHODS

### Collection of mushroom culture

Three strains S-79, A-15 and Delta of *Agaricus bisporus* were obtained from G.B.P.U.A.T, Pantnagar (Uttarakhand). These cultures were sub-cultured and maintained on PDA medium in a B.O.D. incubator at  $25 \pm 2^\circ$  C temperature.

### Preparation of culture media

The pure culture of cultivated mushrooms can be obtained on the following media. These media generally used as substrate for isolation, sub-culture, maintenance and preservation of mushroom culture.

Contents of media	Medium				
	PDA	MEA	WEA	CEA	YPDA
Peeled potato	250g	-	-	-	250g
Malt extracts	-	25g	-	-	-
Wheat grain	-	-	100g	-	-
Ready synthetic compost	-	-	-	100g	-
Yeast extracts	-	-	-	-	1.0g
Dextrose	20g	-	-	-	20g
Agar agar	20g	20g	20g	20g	20g
Distilled water	1L	1L	1L	1L	1L

PDA- Potato dextrose agar, MEA- Malt extract agar, WEA- Wheat extracts agar, CEA- Compost extract agar, YPDA- Yeast extract potato dextrose agar, g-Gram, L-Litre.

Sliced potato was boiled in distilled water for 20-25 minutes till these become soft. Extract was filtered with a muslin cloth and add 20g dextrose and 20g agar powder to the filtrate over a hot plate by stirring. The final volume of the medium was adjusted to one liter by adding required amount of distilled water. The medium was taken in flask were plugged with non-absorbent cotton and sterilized in autoclave at  $121^\circ\text{C}$  (15lb/sq. inch pressure) for 30 minutes.

Wheat grains were boiled in 1 liter distilled water for one hour. Filter the extract after 24 hour. Agar was added to the supernatant by stirring with a glass rod over a hot plate. The final volume of medium was adjusted to 1 liter by adding required amount of distilled water. The medium was then autoclaved as above.

Malt extract medium was prepared by malt extracts 25g and Agar 20g was dissolved in 1liter warm distilled water. Compost extract agar medium

was prepared by same method of wheat grain extract agar medium. Yeast Extract Potato Dextrose Agar (YPDA) was also prepared by PDA medium.

### Maintaining temperature

In case of temperature effect was different strains (S-79, A-15 & Delta) of *Agaricus bisporus*. The inoculums were transferred in Petri-plates which poured by PDA in laminar flow. Then the inoculated Petri-plates took in incubator where different temperature were  $15^\circ\text{C}$ ,  $20^\circ\text{C}$ ,  $25^\circ\text{C}$  and  $30^\circ\text{C}$  and then observing the radial growth of fungi or mushroom.

### Maintaining pH in medium

The pH was maintaining in potato dextrose agar media with the help of base and acids. Firstly make the medium without agar and adjusted the pH (6, 7, 8 & 9) with the help of pH meter. After adjusting the pH mixed the agar-agar and then sterilized the medium in autoclave at  $121^\circ\text{C}$  (15lb/sq. inch pressure) for 25-30 minutes. After

autoclaved melt medium pour in sterilized Petri-plates in laminar flow and took for the solidification of media. After solidifying of medium in laminar flow then inoculate culture in Petri-plates and kept in incubator where temperature was 25°C.

## RESULTS AND DISCUSSION

### Effect of culture media

The results presented in Table 1 revealed that the studies of effect of different culture media on mycelia growth of strains (S-79, A-15 and Delta) of *A. bisporus*. Five culture media were tested for the radial growth and measured two days interval. The result showed the mycelial growth on PDA of all three strains S-79, A-15 and Delta were obtained fast and full growth (9 cm) completed in 8, 10 and 12 days respectively. The growth of mycelial in YPDA medium was good performance in all strains of *A. bisporus* followed by the WGEA and SCA culture media. The mycelial growth was very slow on MEA medium and full growth completed in 18 days.

The similar works of Furlan *et al.* (1997) had been observed mycelial growth on different growth media and under different culture media conditions was investigated in 7 strains of edible fungi. Mycelial growth rates were investigated higher on WDA (wheat/dextrose/agar) medium than on PDA (potato/dextrose/agar) or MPA (malt/Soya peptone/agar) media in all strains.

### Effect of temperature

The results presented in Table 2 revealed that the studies of effect of different temperature on mycelia growth of strains (S-79, A-15 and Delta) of *A. bisporus*. Four different temperatures were tested for the radial growth and measured two days interval. The result showed the mycelial growth of all three strains S-79, A-15 and Delta on 25°C was obtained fast and full growth (9cm) completed in 8, 10 and 14 days respectively, followed by the growth of mycelial at 20°C and 30°C were similar performance in all strains of *A. bisporus* and the mycelial growth was much slow on 15°C and full growth completed in 18 days. The data recorded was subjected to statistical analysis. The differences in data in the various experiments were tested for their significance by employing RBD. The temperature differs for mycelium growth on different strains of *A. bisporus*.

Similar result given by Andrade *et al.* (2010), had been observed mycelium growth on different temperature, that investigated *in vitro* mycelium growth of *Agaricus bisporus* strains ABI-05/03, ABI-06/04, ABI-04/02, ABI-06/05 and ABI-01/01 was evaluated performed by means of measurements of four diameters of the colonies, every 48 hours, during 12 days of incubation in darkness under 20°C and 25°C, it was verified that:

**Table 1.** Effect of different culture media on mycelial growth of strains of *Agaricus bisporus*

Culture media	Radial full growth (9cm) of strains in days		
	S-79	A-15	Delta
PDA	8	10	12
YPDA	10	12	14
WGEA	12	14	16
MEA	14	14	18
SCA	14	14	16
SE	1.79	1.49	1.46
CD (0.05%)	3.69	3.07	3.01

**Table 2.** Effect of different Temperature on mycelial growth of strains of *Agaricus bisporus*

Temperature (°C)	Radial full growth (9cm) of strains in days		
	S-79	A-15	Delta
15	12	14	18
20	10	12	16
25	8	10	14
30	10	12	16
SE	1.48	1.45	1.05
CD (0.05%)	3.10	3.04	2.20

**Table 3.** Effect of different Temperature on mycelial growth of strains of *Agaricus bisporus*

pH	Radial full growth (9cm) of strains in days		
	S-79	A-15	Delta
6	14	16	18
7	14	14	18
8	12	12	16
9	12	12	14
SE	1.25	1.56	1.16
CD (0.05%)	2.62	3.27	2.43

mycelium growth of *A. bisporus* is influenced by the temperature of incubation; temperature of 25°C was more favourable to the mycelium growth of all *A. bisporus* strains; under temperature of 20°C, the best growth was obtained with strains ABI-06/05 and ABI-01/01 and, under temperature of 25°C, strain ABI-01/01 showed significantly higher growth than all other strains.

#### Effect of pH

The results presented in Table 3 revealed that the studies of effect of different pH on mycelia growth of strains (S-79, A-15 and Delta) of *A. bisporus*. pH were tested for the radial growth and measured two days interval. The result showed the mycelial growth on 9 pH of all three strains S-79, A-15 and Delta were obtained fast and full growth (9 cm) completed in 12, 12 and 14 days respectively. The growth of mycelial in 8pH was good performance in all strains of *A. bisporus* followed by the 7 pH. The mycelial growth was much slow on 6 pH and full growth completed in 18 days.

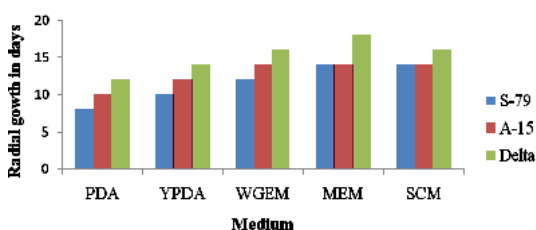


Fig. 1. Effect of culture media on different strains of *A. bisporus*

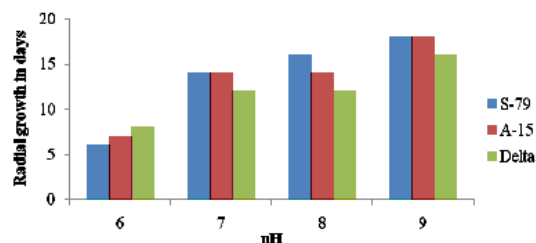


Fig. 2. Effect of pH on different strains of *A. bisporus*

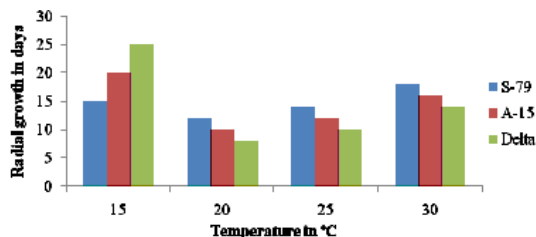


Fig. 3. Effect of temp on different strains of *A. bisporus*

Similar works giving by Shukla *et al.* (2003) had been observed mycelium growth on different pH, which investigated the suitable pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5), for the growth of button mushroom. For studies on the suitable pH, the fungi were incubated at 24±1°C. The best pH level for fungal growth was 6.0. The maximum mycelial dry weight (100 mg) was obtained at pH 6.0, followed by pH 5.5 with 86.66 mg mycelial dry weight.

Experimental findings of comparative evaluation of culture media, pH and temperatures for mycelial growth of tree strains of *Agaricus bisporus* and concluded that PDA medium temperature 25°C and pH 9 gave the fast growth of mycelium and appeared to better in maintenance of culture and sub-culture. This investigation will help for selection of culture media, pH and temperature for the maintenance of genetic materials and cultivation at commercial level.

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