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Effect of physicochemical components on mycelial growth of *Agaricus bisporus*- a popular edible mushroom

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Abstract

In this investigation, the effect of physicochemical components on mycelial growth of 11 strains of *Agaricus bisporus* was studied to provide more credible references for successful artificial cultivation. The suitable temperature for mycelial growth (22.8 mm) was 25ºC after 10 days of incubation. In terms of total average growth (15.5 mm), the minimum mycelial growth was found 11.8 mm at 15ºC. Among the tested fungi, *A. bisporus* str. 1955 and str. 2178 showed the highest (19.5 mm) and lowest (8.9 mm) average mycelial growth, respectively. In case of pH, the most remarkable growth was found 21.5 mm at pH 6 whereas, the lowest growth was recorded 8.4 mm at pH 4 in comparison with total average growth (16.9 mm). Considering growth phenotype of mycelia, Lilly and YM media were the most suitable and Hennerberg and Hoppkins media were the most worst among the tested media for the mycelial growth of *A. bisporus* strains. Out of the tested carbon sources; sucrose and dextrin were the most suitable, whereas galactose and lactose were the most unfavorable for the fungal mycelial growth. Among the used nitrogen sources, glycine and arginine were the best supplement as nitrogen sources and histidine, alanine and urea showed the lowest performance on mycelial growth of the tested fungi. These results may be useful for commercial farming of *A. bisporus* mushroom in Bangladesh.

Keywords: *Agaricus bisporus*, artificial cultivation, phenotypic growth, physicochemical parameters.

INTRODUCTION

Agaricus bisporus is one of the very popular edible mushrooms in the world, usually known as white button mushroom. The mycelial mass and fruiting bodies of the mushroom have been used as foods and food-flavoring materials in many centuries due to their unique and delicate flavors. The morphological bunches have also become attractive as functional foods and as a source of bioactive components. Apart from this, it has been reported that *Agaricus* spp. have many therapeutic properties which are significant for human health (Kim *et al*., 2005; Kimura *et al*., 2004; Souza-Paccola *et al*., 2004; Rodrigues *et al*., 2003; Mizuno, 2002; Oshiman *et al*., 2002).

The success and feasibility of commercial cultivation of mushroom is mainly depending on the success of mycelial culturing. The substrates colonized with mushroom mycelia called spawns are used for mushroom farming to inoculate the suitable compost used. After colonization with mycelia, compost covered with the supplemented peat and $CaCO_3$ on top of the colonized compost in a 5 cm thick casing layer. This casing layer is invaded by mushroom mycelia and then developed fruit bodies by lowering the temperature and CO_2 concentration in the growing room. Duration of mycelial growth is not only of direct economic importance, but also non-fully colonized compost and casing compost are

sensitive to fungal and bacterial contamination. Moreover, a precise knowledge of different phases of growth is necessary to analyze growth anomalies in mycelial cultures that are presumably linked with degeneration of *A. bisporus* (Fritsche, 1966 & 1970; Elliott, 1985). However, a few studies have been focused on the characteristics of mycelial growth with respect to *A. bisporus*. To shorten the period of mycelial growth and gain thick mycelial mass with fast growth rate, many factors should be considered including culture species, carbon-nitrogen sources, carbon-nitrogen ratio, temperature, pH and growth regulators. Considering this obligation, this work was designed to investigate the influence of physiochemical parameters on mycelial growth of selected *A. bisporus* strains to provide useful information for commercial farming of studied mushroom.

MATERIALS AND METHODS

Fungal strains used:
The mycelial cultures of 11 strains of *Agaricus bisporus* were obtained from the Culture Collection and DNA Bank of Mushrooms (CCDBM) in the Department of Biology, University of Incheon, Korea. These strains of *A. bisporus* were transferred to potato dextrose agar (PDA) plates and incubated at 25°C in the dark condition until they showed a full growth and then kept at 4°C for further use.

Temperature and pH effect on the mycelial growth:
To screen the optimum temperature for the mycelial growth of the mushroom, different temperatures such as 15, 20, 25, 30 and 35ºC were used. A 5 mm diameter agar plug removed from 10 day old culture grown on PDA and placed in the centre of each plate filled with 20 ml of PDA. The medium was adjusted to pH 6 and incubated for 10 days at 15, 20, 25, 30 and 35ºC separately. Radial growth of mycelia on each Petri dish was measured at three directions and average diameter was calculated. To calculate final mean value of mycelial growth of each strain, four replications were used. To determine optimum pH, the medium was adjusted to pH 4, 5, 6, 7, 8 and 9 with the addition of 1 N NaOH or HCl; inoculated with agar plug (5 mm) centrally and incubated for 10 days at 25ºC. The measurement of mycelial growth was performed following the technique mentioned before.

Effect of solid media on the vegetative growth:
Ten different culture media e. g. Czapek Dox, Hamada, Hennerberg, Hoppkins, Glucose peptone, Glucose tryptone, Lilly, Mushroom complete, PDA and YM were used to investigate the mycelial growth of the selected *A. bisporus* strains. All the media were adjusted to pH 6 before autoclaving. Inoculation of culture plate with each medium was done. After 10 days of incubation at 25ºC, measurement of mycelial growth was performed like the same manner as before.

Effect of carbon and nitrogen sources on the vegetative growth:
To screen carbon and nitrogen sources for optimal mycelial growth of the selected *A. bisporus* strains, the basal medium (Sung *et al.*, 1993) was supplemented with each of carbon sources i.e. dextrin, fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, sucrose and xylose and nitrogen sources alanine, ammonium acetate, ammonium phosphate, arginine, calcium nitrate, glycine, histidine, methionine, potassium nitrate and urea separately. The basal medium was prepared with $MgSO_4$ (0.05 g), KH_2PO_4 (0.46 g), K_2HPO_4 (1.0 g), thiamine-HCl (120 µg), agar (20 g) and distilled water (1000 ml). Each carbon source (0.1 M/L) with 5 g of peptone was added to the basal medium separately and was mixed thoroughly (Shim *et al.*, 1997). In case of nitrogen sources, each nitrogen source (0.02 M/L) with 20 g of glucose was supplemented to the basal medium. In both cases, the basal medium was adjusted to pH 6 before autoclaving. To measure the mycelial growth on the media, the same method was followed as described earlier.

RESULTS AND DISCUSSION

Effect of temperature on the mycelial growth:
The effect of temperature on the mycelial growth of the selected *A. bisporus* strains was studied. The maximum mycelial growth was found 22.8 mm at 25ºC and the minimum mycelial growth was found 11.8 mm at 15ºC while total average growth was recorded 15.5 mm after 10 days of incubation. Among the tested fungi, *A. bisporus* str. 1955 and str. 2178 showed the highest (19.5 mm) and lowest (8.9 mm) average mycelial growth, respectively (**Table 1**). Ma *et al*. (2014) studied the biological characteristics of *A. bisporus* and found temperature 22-24ºC was the best for mycelial growth of the studied fungus. This result completely correlates to the present findings where suitable temperature was recorded from 20-25ºC.

Table 1. Effect of temperature on the mycelial growth of the selected *A. bisporus* strains on PDA medium at pH 6 after 10 days of incubation

Strain number	Mycelial growth (mm)					Mean
	15°C	20°C	25°C	30°C	35°C	
A. bisporus str. 1946	10.3	20.3	26.7	21.7	0	15.8
A. bisporus str. 1947	9.3	12.7	16.7	15.3	0	10.8
A. bisporus str. 1948	10.3	21.7	25.3	22.3	0	15.9
A. bisporus str. 1955	16.7	24.7	30.3	25.7	0	19.5
A. bisporus str. 1956	10.0	17.3	21.3	16.7	0	13.1
A. bisporus str. 2029	13.3	19.0	29.7	22.3	0	16.9
A. bisporus str. 2097	9.0	14.3	21.3	14.7	0	11.9
A. bisporus str. 2102	19.7	23.7	16.0	8.0	0	13.5
A. bisporus str. 2107	21.7	20.0	23.7	8.0	0	14.7
A. bisporus str. 2177	13.0	17.0	24.7	21.7	0	15.3
A. bisporus str. 2178	8.0	10.7	14.7	11.3	0	8.9
Mean	12.8	18.3	22.8	17.1	0	

Effect of pH on the mycelial growth:

Among the selected strains of *A. bisporus*, the best mycelial growth was found 21.5 mm at pH 6 whereas, the lowest vegetative growth was recorded 8.4 mm at pH 4 compared to total average growth of 16.9 mm. Among the strains, *A. bisporus* str. 1955 and str. 2107 showed the best and worst total average mycelial growth at pH 6 (**Table 2**). Ma *et al*. (2014) revealed that the biological characteristic for mycelial growth of *A. bisporus* was good at pH 7.0-7.5. This result does not support the present findings. Hur (2008) studied the cultural characteristics and log-mediated cultivation of *Phellinus linteus* and found that the pH value 6 was the best for the mycelial growth. Chi *et al*. (1996) also reported that mycelial growth of *P. linteus* was the best at pH 6 which corroborates the present findings.

Table 2. Effect of pH on the mycelial growth of the selected *A. bisporus* strains on PDA medium at 25ºC after 10 days of incubation

Strain number	Mycelial growth (mm)					Mean
	pH 4	pH 5	pH 6	pH 7	pH 8	
A. bisporus str. 1946	9.3	13.7	14.7	13.3	11.7	12.5
A. bisporus str. 1947	9.3	22.0	18.3	14.0	12.3	15.2
A. bisporus str. 1948	8.7	13.7	14.7	12.7	11.3	12.2
A. bisporus str. 1955	11.3	22.0	21.0	18.7	14.0	17.4
A. bisporus str. 1956	11.0	22.0	19.7	17.0	14.0	16.7
A. bisporus str. 2029	9.0	22.7	17.0	14.7	14.0	15.5
A. bisporus str. 2097	8.7	22.7	17.0	14.7	13.3	15.3
A. bisporus str. 2102	8.7	21.0	16.0	12.0	10.0	13.5
A. bisporus str. 2107	0	15.7	13.7	11.7	10.3	10.3
A. bisporus str. 2177	16.3	33.3	64.0	61.7	54.0	45.9
A. bisporus str. 2178	0	10.3	20.7	16.0	12.3	11.9
Mean	8.4	19.9	21.5	18.8	16.1	

Effect of various media on the mycelial growth:

The role of solid media on the mycelial growth of the selected strains of *A. bisporus* was also studied. Considering growth phenotype of mycelia of 11 strains, Lilly and YM media were the most suitable media and Hennerberg and Hoppkins media were not favorable among the tested media for the mycelial growth of *A. bisporus* mushroom. *A. bisporus* str. 2177 showed the exceptionally highest growth of mycelium (59.3 mm) whereas, rest of the strains showed the moderate mycelial growth ranging 15.5-25 mm (**Table 3**). Hur (2008) reported that the excellent mycelial growth of *P. linteus* was not similar to the present findings. In case of *Hericium erinaceus*, the highest mycelial growth was observed on PDA, YM, Hamada and glucose peptone media whereas, the lowest mycelial growth was obseved on Czapek Dox, Hoppkins, Lilly and Hennerberg media (Imtiaj *et al*., 2008).

Effect of carbon sources on the mycelial growth:

The mycelial growth of the tested strains of *A. bisporus* varied with different carbon sources. Among the tested carbon sources, sucrose and dextrin were the most suitable while, galactose and lactose were favorable for the mycelial growth of *A. bisporus* strains. The *A. bisporus* str. 2177 showed the highest total average growth (31.6 mm) among the tested strains (**Table 4**). Ma *et al*. (2014) studied the biological characteristics of *A. bisporus* and found glucose as the best carbon source for the mycelial growth of the tested fungus. This result is not in conformity to the present findings that could be because of different strains or isolates as well as different sources of carbon.

Effect of nitrogen sources on the mycelial growth:

Among the used nitrogen sources, glycine and arginine were the best as supplemented nitrogen sources and histidine, alanine and urea were worst for the growth of mycelia. The *A. bisporus* str. 2177 and str. 2178 showed the highest and lowest mycelial growth, respectively in terms of total average growth (**Table 5**). According to the report by Ma *et al*. (2014) on biological characteristics, they focused that yeast extract was the best nitrogen source for the mycelial growth of *A. bisporus*. Shim *et al*. (2005) clarified that glycine was the most favorable and histidine, arginine and ammonium oxalate were the most unfavorable nitrogen sources for the mycelial growth of *M. procera*.

In conclusion, it may be concluded that moderate temperature and acidic medium was favorable for the mycelial growth of *A. bisporus*. Considering the growth phenotype, it was also noticed that disaccharide like sucrose and dextrin were favorable C-sources; and glycine and arginine were the best N-sources that can be supplemented with the medium for better growth of mycelia of the fungus. So, these findings may be helpful for researchers and mushroom farmers to mass scale production of mushroom that contributes to enhance food security.

Table 3. Effect of culture media on the mycelial growth of the selected strains of *A. bisporus*

Strain number	Mycelial growth (mm) on culture media										Mean
	Cza	Ham	Hen	Hop	GP	GT	Lil	MC	PDA	YM	
A. bisporus str. 1946	11.7	14.0	9.0	12.3	12.0	19.3	27.7	21.7	26.7	23.3	17.8
A. bisporus str. 1947	8.3	12.0	9.3	11.0	14.3	18.7	17.0	14.7	16.7	16.7	13.9
A. bisporus str. 1948	19.3	18.7	8.0	11.3	11.3	15.3	23.3	19.3	25.3	23.3	17.5
A. bisporus str. 1955	30.3	28.7	10.0	19.3	15.3	17.3	47.3	27.3	30.3	24.0	25.0
A. bisporus str. 1956	20.7	19.7	9.0	12.3	11.7	14.3	21.0	20.3	21.3	20.3	17.1
A. bisporus str. 2029	20.7	20.0	10.3	11.7	13.3	16.7	23.0	20.7	29.7	24.3	19.0
A. bisporus str. 2097	18.3	18.3	9.7	12.3	12.3	15.7	22.0	20.7	21.3	20.7	17.1
A. bisporus str. 2102	29.3	34.3	12.3	19.7	20.3	12.3	21.3	17.0	16.0	20.3	20.3
A. bisporus str. 2107	18.3	23.3	9.3	14.7	15.7	14.0	21.0	15.7	23.7	17.0	17.3
A. bisporus str. 2177	82.3	42.7	55.0	51.3	62.3	28.3	87.0	77.3	24.7	82.0	59.3
A. bisporus str. 2178	13.3	18.3	13.7	15.0	15.0	13.7	17.3	14.7	14.7	19.0	15.5
Mean	24.8	22.7	14.2	17.4	18.5	16.9	29.8	24.5	22.8	26.5	

Cza: Czapek Dox, Ham: Hamada, Hen: Hennerberg, Hop: Hoppkins, GP: Glucose peptone, GT: Glucose tryptone, Lil: Lilly, MC: Mushroom complete, PDA: Potato dextrose agar and YM: Yeast-malt extract agar.

Mycelial growth (mm) was calculated (n=3) after 10 days of incubation on potato dextrose agar (PDA) medium at 25ºC and pH 6.

Table 4. Effect of carbon sources on the mycelial growth of the selected strains of *A. bisporus*

Strain number	Mycelial growth (mm) on carbon sources										Mean
	Dex	Fr	Ga	Gl	Lac	Mal	Man	Sor	Suc	Xy	
A. bisporus str. 1946	9.7	11.7	9.3	12.0	14.3	15.0	24.7	13.7	19.0	16.7	14.6
A. bisporus str. 1947	11.7	14.0	9.0	12.3	12.0	19.3	27.7	21.7	26.7	23.3	17.8
A. bisporus str. 1948	8.3	12.0	9.3	11.0	14.3	18.7	17.0	14.7	16.7	16.7	13.9
A. bisporus str. 1955	19.3	18.7	8.0	11.3	11.3	15.3	23.3	19.3	25.3	23.3	17.5
A. bisporus str. 1956	30.3	28.7	10.0	19.3	15.3	17.3	47.3	27.3	30.3	24.0	25.0
A. bisporus str. 2029	20.7	19.7	9.0	12.3	11.7	14.3	21.0	20.3	21.3	20.3	17.1
A. bisporus str. 2097	20.7	20.0	10.3	11.7	13.3	16.7	23.0	20.7	29.7	24.3	19.0
A. bisporus str. 2102	33.7	24.7	11.7	25.0	20.7	24.0	24.3	23.3	32.0	15.3	23.5
A. bisporus str. 2107	29.3	17.7	14.3	19.3	21.0	21.3	18.3	23.7	30.0	16.3	21.1
A. bisporus str. 2177	52.7	29.3	29.3	39.7	13.3	47.7	12.0	16.7	49.7	26.0	31.6
A. bisporus str. 2178	18.0	14.7	13.0	17.3	10.0	21.7	8.7	10.7	28.0	22.3	16.4
Mean	23.1	19.2	12.1	17.4	14.3	21.0	22.5	19.3	28.1	20.8	

Dex: Dextrin, Fr: Fructose, Ga: Galactose, Gl: Glucose, Lac: Lactose, Mal: Maltose, Man: Mannose, Sor: Sorbitol, Suc: Sucrose and Xy: Xylose. Each carbon source was added to the basal medium at the concentration of 0.1 M.

Mycelial growth (mm) was calculated (n=3) after 10 days of incubation on potato dextrose agar (PDA) medium at 25ºC and pH 6.

Table 5. Effect of nitrogen sources on the mycelial growth of the selected strains of *A. bisporus*

Strain number	Mycelial growth (mm) on nitrogen sources										Mean
	Ala	AA	AP	Arg	CN	Gly	His	Met	PN	Ur	
A. bisporus str. 1946	10.3	19.0	19.3	20.3	17.7	26.3	15.3	20.0	16.0	10.7	17.5
A. bisporus str. 1947	11.0	18.7	20.0	21.0	18.7	27.3	16.3	21.0	17.0	10.3	18.1
A. bisporus str. 1948	8.7	15.3	20.0	21.7	19.7	24.0	14.7	18.3	17.7	10.7	17.1
A. bisporus str. 1955	12.3	19.3	20.3	20.7	18.0	27.3	16.3	20.3	17.3	11.7	18.4
A. bisporus str. 1956	10.3	16.7	20.0	22.0	19.0	25.3	15.3	19.7	19.0	10.3	17.8
A. bisporus str. 2029	10.7	17.3	19.7	20.3	19.0	25.7	15.3	19.7	16.7	11.0	17.5
A. bisporus str. 2097	10.7	17.7	19.3	20.3	19.0	25.7	15.3	19.7	16.7	10.7	17.5
A. bisporus str. 2102	33.0	10.7	14.0	20.7	19.3	19.0	0	16.7	12.3	19.0	16.5
A. bisporus str. 2107	8.0	8.3	11.7	15.0	14.7	18.7	0	13.7	13.0	19.0	12.2
A. bisporus str. 2177	11.3	65.7	31.7	48.7	47.0	51.7	0	19.7	40.3	23.7	34.0
A. bisporus str. 2178	8.3	11.0	8.0	12.7	12.3	10.0	0	10.7	8.7	8.0	9.0
Mean	12.2	20.0	18.5	22.1	20.4	25.5	9.9	18.1	17.7	13.2	

Ala: Alanine, AA: Ammonium acetate, AP: Ammonium phosphate, Arg: Arginine, CN: Calcium nitrate, Gly: Glycine, His: Histidine, Met: Methionine, PN: Potassium nitrate and Ur: Urea. Each nitrogen source was added to the basal medium at the concentration of 0.02 M.

Mycelial growth (mm) was calculated (n=3) after 10 days of incubation on potato dextrose agar (PDA) medium at 25ºC and pH 6.

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