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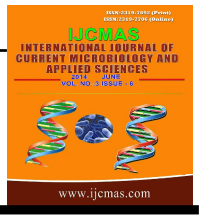

Original Research Article

Screening and Evaluation of *Agaricus bisporus* (Lange) Sing. strains for temperature variability

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Keywords

Agaricus bisporus, strains, temperature variability, growth, yield.

A B S T R A C T

Agaricus bisporus (Lange) Sing. strains were screened for their ability to grow at different temperatures and for their yield potential with the objective of selecting strains capable of mitigating the effects of climatic change. Twenty four strains of *Agaricus bisporus* (Lange) Sing. (SSI 01/12- SSI 15/12, AVT 01- AVT 06, P1, U3 and S11) were evaluated for linear growth and biomass production at five different temperatures (15° to 27°C) on potato dextrose medium. At 15 and 18°C, strains SSI 06/12 (3.53 mm/day) and SSI 15/12 (4.21 mm/day) showed maximum growth, respectively. At 21, 24 and 27°C, strain AVT 06 showed consistently maximum growth i.e. 4.65 mm/day, 4.79 mm/day and 4.62 mm/day, respectively. Biomass production was maximum (6.5 g/l) at 15°C for strain SSI 06/12 while at 18, 21 and 24°C, strain SSI 15/12 showed maximum biomass production i.e. 29.5 g/l, 15.0 g/l and 25.0 g/l, respectively. The strain SSI 01/12 showed maximum biomass production (21.5 g/l), at 27°C. All the mushroom strains were cultivated on short and long method compost to assess their yield potential. Strains SSI 04/12 (18.22 kg/100 kg compost), SSI 08/12 (17.94 kg/100 kg compost) and AVT 02 (17.48 kg/100 kg compost) gave maximum yield on wheat straw based short method compost. Strains SSI 10/12 (13.24 kg/100 kg compost) and SSI 09/12 (10.60 kg/100 kg compost) were maximum yielders on long method compost.

Introduction

Mushrooms have been esteemed as a food delicacy for centuries. Button mushroom, *Agaricus bisporus* (Lange) Sing. is the most extensively cultivated mushroom in the world. It belongs to phylum Basidiomycota, class Basidiomycetes, order Agaricales and family Agaricaceae. It accounts for nearly 40% of the total world production and about 90% of the total mushroom production in India (www.greencarcongress.com, 2007).

Consumption of mushrooms has increased substantially due to their delicacy, flavor and nutritional value. Conventional breeding in *A. bisporus* is a time consuming process due to secondary homothallic nature of the species, absence of clamp connections in mono and dikaryons, and lack of well-defined morphological difference in fertile and non-fertile cultures. One of the simplest technique to obtain improved strain is to isolate and

evaluate single spore isolates (SSIs). The frequency of identification of high yielding isolates is low. It is practically impossible to screen all the single spore isolates for their yield performance. It is thus important to look for selection criterion at early stages. Non-significant differences between mycelial growth on media and yield have been reported earlier implying that growth on agar media may not be an indicator of yield potential (Anonymous 1987, Mehta 1988).

Due to ozone layer depletion and global warming there are changes in climatic conditions. The temperature of earth's environment is increasing and the summer season is extending. So, there is a need to for screening and evaluation of high temperature tolerant strains of *A. bisporus* with respect to their yield and quality. Although *A. bisporus* is the most cultivated edible fungus worldwide, its potential ability to fruit at higher temperature (FHT) has received little attention (Wang *et al* 2003, 2004). Therefore present study planned to screen *A. bisporus* strains for wider adaptability to changing environmental conditions for mitigating the effects of climatic change and selection of high yielding strains.

Materials and Methods

Procurement of cultures of *Agaricus bisporus* (Lange) Sing.

Culture	Source
AVT 01-AVT06	DMR, Solan
SSI 01/12- SSI15/12	DMR, Solan
S11, P1, U3	Department of Microbiology, PAU Ludhiana

Linear growth and biomass

Twenty four strains of *A. bisporus* were grown on potato dextrose medium (agar and broth) at five different temperatures (15, 18, 21, 24 and 27°C) for linear growth and biomass determination for fifteen days. For linear growth, diameter of the growing colony in the petri plate was measured on every third day. For biomass production, the fungal mycelia biomass was filtered on pre weighed Whatman No.1 filter paper after 15 days of incubation and dried in an oven at 60°C for 48 hours. The difference in weight as biomass was recorded.

Cultivation

Spawn preparation

For cultivation trial, wheat grain spawn was prepared using the standard methodology of Garcha (1994). Wheat grains were washed boiled for 35-40 minutes and then excess water was drained. The grains were then mixed with 2% $CaCO_3$ and 4% $CaSO_4$ powder, filled in bottles and steam sterilized at 20 psi for 90 minutes. After cooling overnight, the bottles were inoculated using 14-15 days old culture bit of size 3 x 1 cm, incubated at 25±1°C until the mycelial growth impregnated the grain (@ 20 days incubation).

Composting and Spawning

Wheat straw based compost was prepared using long as well as short method of composting (Khanna and Kapoor 2007). The spawning was done using polythene bags (20"x 24") filling 10 kg compost in each bag and spawned @ 70 g/ bag by thorough mixing. Four replicates of each strain with ten bags for AVT 01-AVT 06 strains and 6 bags for each SSI01/12- SSI15/12, P1, S11 and U3 strains were laid in random block design (RBD).

Casing

Casing soil was prepared by mixing well decomposed (2 yrs old) FYM and spent

compost (SC, 2 yrs old) in 2:1 (v/v) ratio adjusted to pH 7.5 with $CaCO_3$. Bags fully impregnated with mycelium were covered with the casing soil to make 4 cm thick uniform layer using 2 kg casing soil per bag. The mushrooms were harvested by gentle twisting of the fruit body. A record of total yield, number of opened mushrooms in each harvest and average fruit body weight was made to determine the quality of mushrooms produced.

Results and Discussion

Linear growth

The linear growth of mycelium at 15°C, ranged between 0.10 to 3.53 mm/day with maximum in strains SSI 01/12, SSI 06/12, SSI 09/12 and SSI 15/12. At 18°C, linear mycelium growth ranged between 0.28 to 4.21 mm/day with maximum in P1, SSI 09/12, SSI 15/12 and AVT 06. At 21°C, linear mycelium growth ranged between 0.23 to 4.65 mm/day with maximum in strains U3, SSI 02/12, SSI 09/12, SSI 15/12 and AVT 06. The linear growth rate for P1, SSI 01/12, SSI 02/12, SSI 04/12, SSI 09/12 and AVT 06 was consistently high at both 24° and 27°C. However, at 24°C, AVT 02 and AVT 05 showed maximum growth at par with other strains. At 27°C, SSI 05/12, SSI 06/12 and SSI 15/12 also showed maximum growth statistically at par with other mentioned strains (Fig.1a & 1b).

Biomass

The biomass harvested from *A. bisporus* strains grown on potatoes dextrose broth for 15 days at 15° to 27°C ranged between 0.04 to 6.5 g/l at 15°C, with maximum from strain SSI 06/12. The biomass at 18, 21 and 24°C ranged between 0.05 to 29.5 g/l, 0.06 to 15.0 g/l and 0.08 to 25.0 g/l, respectively with maximum biomass from strain SSI 15/12. At 27°C, the biomass ranged between 0.10 to 21.5 g/l with maximum from SSI 01/12. In general biomass production was maximum when the strains were grown at 24°C (Fig 2a & 2b).

Singh and Kamal (2011) reported linear mycelial growth of *Agaricus bisporus* on malt extract agar and observed maximum variation in linear growth among the strains as compared to other morphological traits. Prakasam and Singh (2008) also compared *A. bisporus* strains for their radial growth and colony characters to report maximum radial growth (43 mm) and growth rate (2.86 mm/day) in strain CM-10. Mata and Estrada (2005) compared wild and commercially cultivated strains of *A. bisporus* for their biomass and laccase production on different media with more biomass and enzyme production in commercial strains.

Cultivation

On Short method compost

The cultivation trial was carried out on short method compost for all the *Agaricus bisporus* strains (AVT 01-06, P1, U3, S11 and SSI 01/12-15/12). Four replicates each with ten bags for each strain were laid in random block design (RBD) to accommodate 240 bags. Beds were cased after 22-25d of spawning. Pinheads appeared in 17-20d after casing. The first crop was harvested between 19-22d after casing and lasted for 4 weeks. AVT 01- 06 were compared on short method compost because these are advanced variety trials The yield data (Table 1) indicated maximum yield for the strain AVT-02 (17.48 kg/100 kg compost compost) and the number of fruit bodies (1314 no.) followed by AVT-06. Strain AVT-01 gave minimum yield (5.16 kg/100 kg compost). The average weight of a mushroom ranged between 11.8 to 14.8g (Fig 3).

Table.1 Cultivation of *Agaricus bisporus* strains on short method compost

Strain No.	Spawn run (d)	Case run (d)	Pinning after casing (d)	First harvest (d)	Last harvest (d)	Yield (kg/100kg compost)	NFB (no/ 100kg)	Av.wt. of a FB (g)
AVT-01	24	19	20	22	28	5.16	355	14.5
AVT-02	23	17	19	21	28	17.48	1314	13.3
AVT-03	25	19	20	21	28	9.10	728	12.5
AVT-04	25	19	20	22	28	11.90	1008	11.8
AVT-05	22	16	18	19	28	8.74	590	14.8
AVT-06	24	15	17	20	28	14.02	1023	13.7
P1	23	16	18	19	28	5.94	606	9.8
U3	23	14	17	20	28	15.18	1632	9.3
S11	23	15	17	19	28	9.24	1087	8.5
CD5%						1.46	148	

Bag size: 20”x 24” (Polythene, 150 gauge); No. of replicates: 4 each for one strain with 10 bags (10kg compost/bag); Experimental design: RBD; Date of spawning: 6.1.13-8.1.13; Rate of spawn: 0.7% wet compost (70g/bag of 10kg compost); Days of spawn run: 22-25d; Casing: FYM +SC (2:1 v/v); Date of casing: 28.1.13 – 31.1.13; Days of case run: 15-19 d; Days for pinning: 17-20 d after casing; Days for first harvest : 18-22 d after casing; Days for last harvest: 4 weeks crop data ; NFB: number of fruit bodies.

Table.2 Cultivation of *Agaricus bisporus* strains on short method compost

Strain	Spawn run(d)	Case run(d)	Pinningafter casing(d)	First harvest (d)	Last harvest (d)	Yield (kg/ 100 kg)	NFB (no/ 100kg)	Av. wt. of a FB (g)
SSI01/12	15	16	18	20	21	13.46	1246	10.8
SSI02/12	15	16	18	20	21	14.80	1203	12.3
SSI03/12	18	17	19	22	21	5.64	440	12.8
SSI04/12	15	19	20	23	21	18.22	1612	11.3
SSI05/12	17	16	18	20	21	5.44	375	14.5
SSI06/12	18	17	18	22	21	13.20	892	14.8
SSI07/12	18	19	20	22	21	10.14	700	14.5
SSI08/12	17	18	20	21	21	17.94	1401	12.8
SSI09/12	21	19	20	21	21	5.40	357	15.1
SSI10/12	18	19	20	22	21	7.20	507	14.2
SSI11/12	18	19	20	22	21	6.54	467	14.0
SSI12/12	18	19	20	22	21	7.70	616	12.5
SSI13/12	17	19	20	23	21	7.62	544	14.0
SSI14/12	16	18	20	22	21	10.64	744	14.3
SSI15/12	18	19	20	22	21	5.04	403	12.5
CD (5%)						2.68	205	

Bag size: 20”x 24” (Polythene, 150 gauge); No. of replicates: 3 each for one strain with 4 bags (10kg compost/bag); Experimental design: RBD; Date of spawning: 15.1.13-18.1.13; Rate of spawn: 0.7% wet compost (70g/bag of 10kg compost); Days of spawn run: 15-18d; Casing: FYM +SC (2:1 v/v); Date of casing: 30.1.13 – 2.2.13; Days of case run: 16-19 d; Days for pinning: 18-20 d after casing; Days for first harvest: 20-23 d after casing; Days for last harvest: 4 weeks crop data; NFB: number of fruit bodies.

Table.3 Cultivation of *Agaricus bisporus* strains on long method compost

Strain No.	Spawn run (d)	Case run (d)	Pinning after casing (d)	First harvest (d)	Last harvest (d)	Yield (Kg/100 kg compost)	NFB (no./100 kg compost)	Av.Wt. of FB (g)
SSI01/12	26	DID NOT FRUIT						
SSI02/12	26	DID NOT FRUIT						
SSI03/12	26	19	21	24	28	1.66	170	9.76
SSI04/12	26	15	17	19	28	9.66	878	11.0
SSI05/12	26	DID NOT FRUIT						
SSI06/12	26	DID NOT FRUIT						
SSI07/12	26	DID NOT FRUIT						
SSI08/12	26	DID NOT FRUIT						
SSI09/12	26	18	21	23	28	10.60	883	12.0
SSI10/12	26	15	17	19	28	13.24	946	14.0
SSI11/12	26	DID NOT FRUIT						
SSI12/12	26	17	19	22	28	3.32	277	12.0
SSI13/12	26	15	17	20	28	9.96	743	13.0
SSI14/12	26	DID NOT FRUIT						
SSI15/12	26	14	17	20	28	8.30	664	12.5
CD (5%)						0.32	5.2	

Bag size: 20”x 24” (Polythene, 150 gauge); No. of replicates: 3 each for one strain with 2 bags (10kg compost/bag); Experimental design: RBD; date of spawning 16.1.2013; ; Rate of spawn: 0.7% wet compost (70g/bag of 10kg compost); Days of spawn run: 26d; Casing: FYM +SC (2:1 v/v); Date of casing: 11.2.2013; Days of case run: 14-19 d; Days for pinning: 17-21 d after casing; Days for first harvest: 19-24 d after casing; Days for last harvest: 4 weeks crop data; NFB: number of fruit bodies

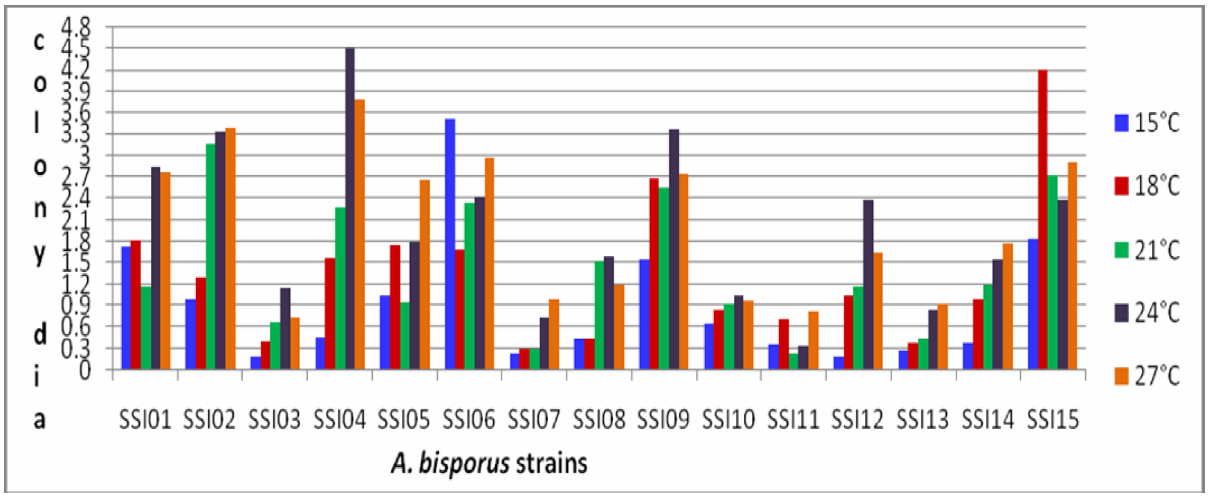

Figure.1a linear growth of *Agaricus bisporus* (Lange) sing strains at five different temperatures

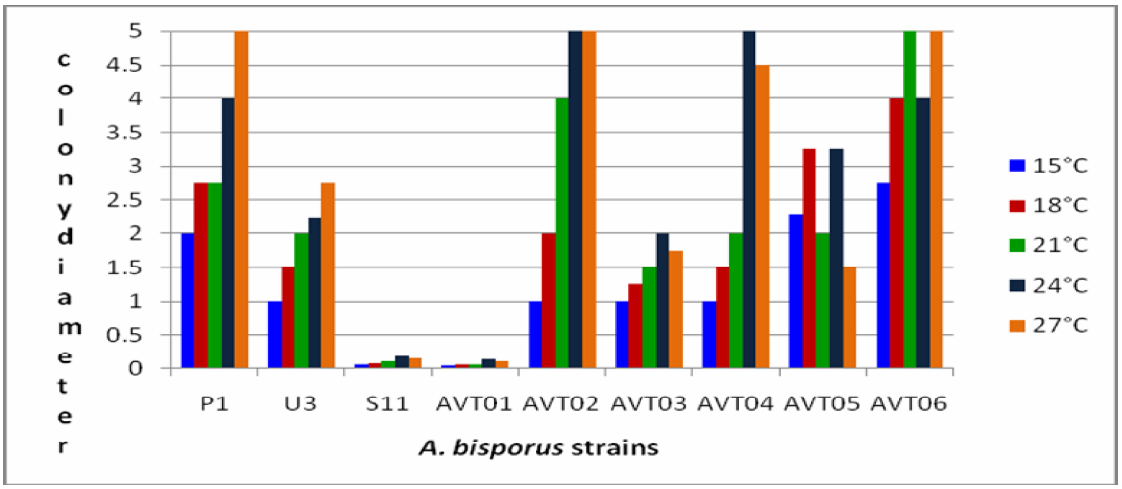

Figure.2b linear growth of *Agaricus bisporus* (Lange) sing strains at five different temperatures

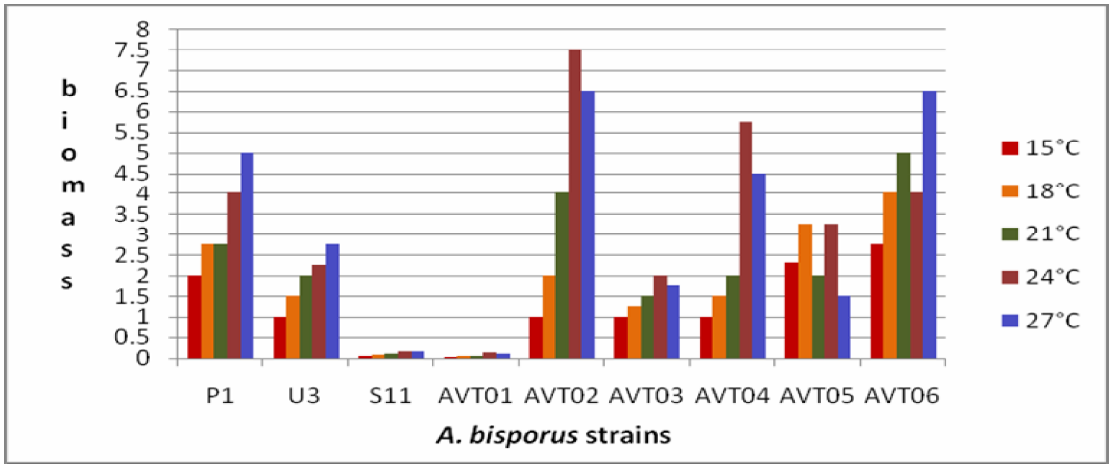

Figure.2a Biomass of *Agaricus bisporus* (Lange) sing strains at five different temperatures

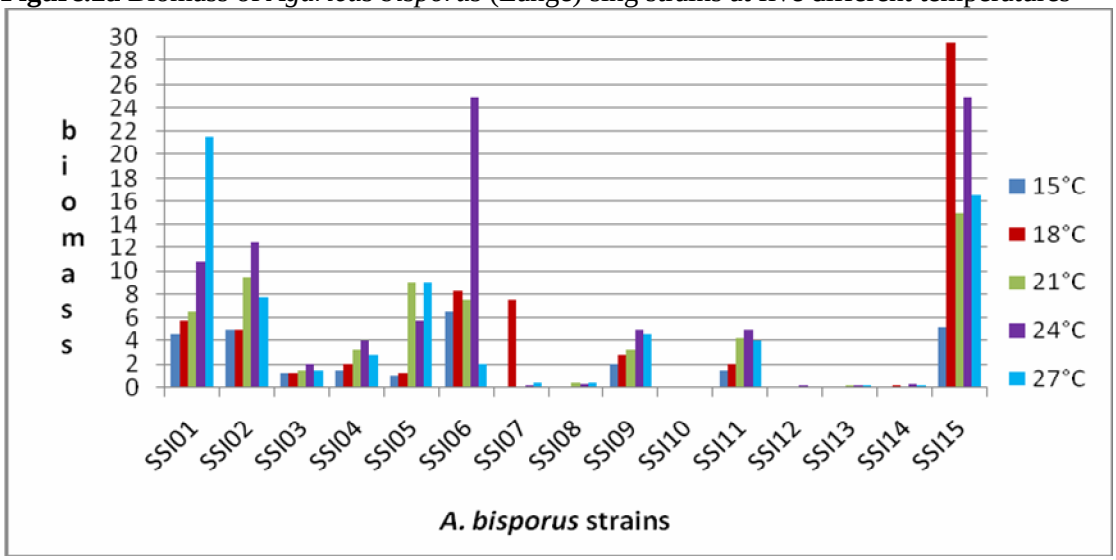

Figure.2b Biomass of *Agaricus bisporus* (Lange) sing strains at five different temperatures

Fig. 3 High yielding strains of *Agaricus bisporus*

Three commercial strains of *A. bisporus* P1, U3 and S11 were compared separately. Although spawn run was complete within 23 days but case run took 14-16 days to give first harvest between 19-20 days of casing. The yield potential from strain U3 was maximum (15.18 kg/100 kg compost and 1632 fruit bodies/100 kg compost each weighing 9.3 g).

On long method compost

As AVTs are advanced variety trials and these have already been accessed on long method compost. SSIs are single spore isolates and are yet to be accessed on long method and then further on short method compost. So, *A. bisporus* strains SSI 01/12 to SSI 15/12 were grown on wheat straw based compost prepared using long method of composting. The compost was spawned @ 0.7% wet compost. The spawned bags were cased after 26 days of spawn run. The pin head started appearing between 17-21 days after casing and mushrooms were first harvested in 19-24 days which lasted upto 28 days. The yield data indicated maximum harvest from strain SSI 10/12 (13.24kg/ 100 kg compost and 946 NFB/100 kg compost) followed by SSI 09/12 with minimum from SSI 03/12 (Table 3). Eight strains could not fruit on long method compost. The average weight of a fruit body ranged between 9.76 to 14.0 g.

Kumar and Singh (2013) reported maximum yield (16.1 kg/100 kg compost) in case of wheat straw based compost. Diamatopoulou and Philippoussis (2001) observed maxium yield (30.84 kg of edible mushroom biomass) in white strain 207 among five *Agaricus bisporus* strains. Baysal *et al* (2007) reported that the highest mushroom yield (1707.2 g) was recorded by wheat straw mixed with pigeon manure with the peat of Caykara and perlite mixture as casing material.

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