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Full Length Research Paper

# Production of high temperature-resistant strains of *Agaricus bitorquis*

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In this study, the culture mushroom *Agaricus bitorquis* (Quel.) Sacc. was examined for growth of mycelia and fructifications under high temperature. The spores taken from the mushrooms that were collected from nature were grouped as A, B, C, D and E. These spores were inoculated into malt extract agar and incubated at 30°C and primer mycelium was produced. The mycelium discus taken from primer mycelium in 8 mm diameter were inoculated into the center of malt extract agar and incubated at 30°C, 32°C, 34°C, 36°C, and 38°C, separately. During the incubation period the growth of the mycelia were measured. The best mycelia growth for all groups was seen at 30°C. At 36°C, the E group mycelia and at 38°C other group's mycelia did not grow. These temperatures were determined as thermal lethal point for the groups. From all the mycelia produced spawn was prepared and inoculated into compost and incubated at 30°C and 32°C. The harvested mushrooms were inspected morphologically.

**Key words:** *Agaricus bitorquis*, mycelial growth, high temperature.

## INTRODUCTION

*Agaricus bitorquis* (Quel.) Saccardo like most cultivated edible fungi is Basidiomycetes and is widespread in nature (Raper, 1978). This species has specific characteristics and grows at higher temperatures than strains of *Agaricus bisporus* (Hasselbach and Mutsers, 1971). It is resistant to the virus that causes the dreaded dieback (Dieleman-van Zaayen 1972). Another advantage characteristics of this species is its resistance to pressure and bruising (Vedder, 1978).

One of the most important problems that many countries' mushroom producers have is to cool down the temperatures of the production rooms during summer months. However, the air conditioning units are very expensive; and the small and medium scale producers do not have these air conditioning units. As there are no heat resistant strains, these small

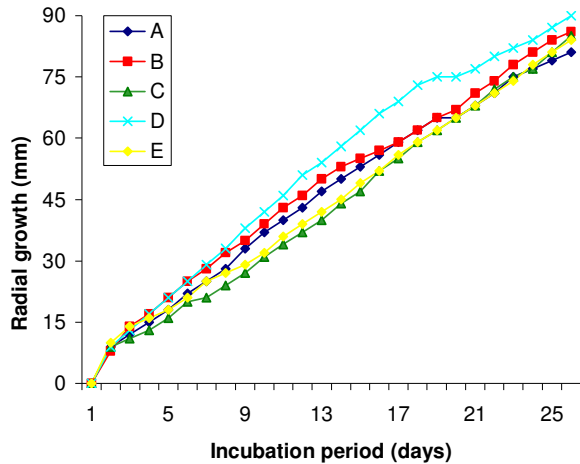
producers stop production during summer season leaving only the bigger producers which causes the cost to increase.

## MATERIAL AND METHODS

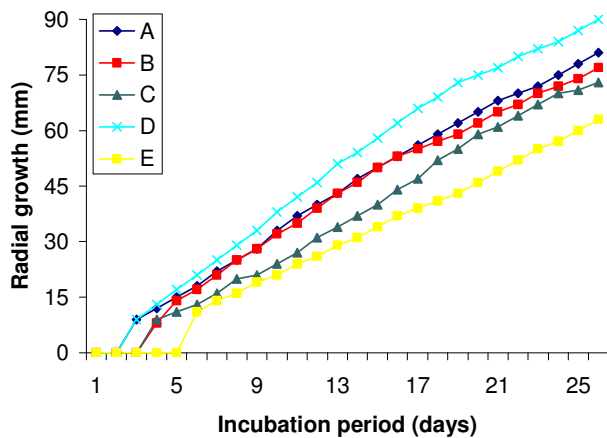
In this study, 2% malt extract agar (MEA) was used. The samples, which are collected from the field, were divided into ten groups. The spores, which are taken from each group, were inoculated at the agar center by multispore method (Fritsche, 1972). At the end of the 20 day incubation period, the homokaryon primer mycelium were obtained and five groups which have the best mycelium development were named as A, B, C, D and E. The homokaryon mycelium was transferred to the agar center as a single pellet and heterokaryon mycelium were obtained. The spore germination and mycelium development were carried in dark at 30°C, and main cultures were obtained.

The mycelium of A, B, C, D and E groups were incubated at 30°C, 32°C, 34°C, 36°C and 38°C. Radial growth of mycelium development was measured. Heterokaryon mycelium was developed at the wheat grain and spawn of each group were obtained separately.

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**Figure 1.** The mycelium development curve of all groups of *Agaricus bitorquis* at 30°C.



**Figure 2.** The mycelium development curve of all groups of *Agaricus bitorquis* at 32°C.

### The productivity trials

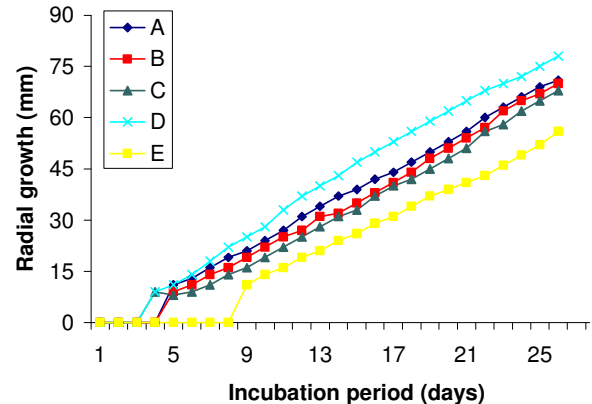
#### A-1<sup>st</sup> Mycelium growth period

The prepared spawn culture was inoculated to the compost bags (1 kg ea), (Günay, 1995; Erkel, 1993; Boztok, 1987; Elliott, 1985; Fritsche, 1981). The top of the compost bags were covered and incubated at 30°C, 32°C, 34°C and 36°C for different periods. During the first mycelium period, air humidity was kept at 90%.

#### B- 2<sup>nd</sup> Mycelium growth period

After the completion of the first mycelial growth, the surface of compost bags were covered by disinfected soil, which has approximately 3.0-3.5 cm thickness.

During the second mycelial growth period, the irrigation with 2% formaldehyde was done for 3 days with sprinkling. During this period, the humidity of incubation room and its temperature were



**Figure 3.** The mycelium development curve of all groups of *Agaricus bitorquis* at 34°C.

preserved at 90-95% and 28°C, respectively. After the mycelium was seen over the cover soil, the room temperature was decreased to 23°C each day. The 14<sup>th</sup> day of the application period of covered soil, fresh air was passed through the incubation room.

### C-Harvest period

The mushrooms, which have 3-5 cm cap width, were collected. The cap width, the thickness of cap tissue, stalks lengths and stalk thickness were measured.

## RESULTS

### Development of mycelia

In all of the groups, the mycelia started to develop from the center and grew parallel to agar surface. They started to develop after 12 h of inoculation. The development at 30°C, which is the best temperature for mycelium interactions, served as control (Zadrazil et al., 1973; Vedder, 1975, 1978; Raper, 1978) (Figure 1). At 32°C, the mycelia development of A, B, C, D and E groups started at different days after incubation. For instance, 3<sup>rd</sup>, 4<sup>th</sup>, 4<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup> days respectively. The mycelia of D group had covered all of the Petri dishes at 26<sup>th</sup> day of incubation, while for the others groups, the mycelium development was very slow (Figure 2). The mycelia development of A, B, C, D, E groups had also started at the different days at 34°C. Here the mycelia development was cottony (Figure 3). At 36°C, there was no mycelial growth in group E. The experiment was repeated again three times for the E group but the results were same. Therefore, 36°C was taken as the lethal temperature for the E group. For the A, B, C and D groups, the mycelia developed as mixed, cottony and aerial hyphae from agar medium surface. The mycelial development also started at different days (Figure 4). There was no development of mycelium in A, B, C and D groups at 38°C.

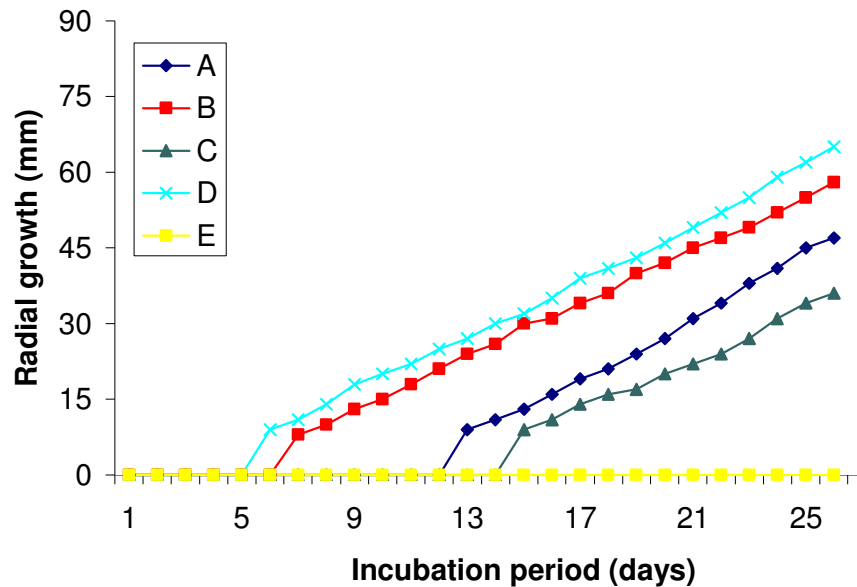


Figure 4. The mycelium development curve of all groups of *Agaricus bitorquis* at 36°C

The experiment was also repeated three more times for these groups but the results were same. Therefore, 38°C was lethal temperature for all the groups.

### Spawn development

The spawn was developed at different temperatures (30°C, 32°C, 34°C and 36°C) for the A, B, C and D groups. At the 38°C, spawn was not prepared because the mycelia were not developed. Also, for the E group the spawn was not prepared at 36°C as the mycelia did not develop.

### Mycelia development at compost

After 3 days of inoculation, mycelial development at the compost started at B, C and E groups. For groups A and D, mycelial development started after 1 day of inoculation. At the 2<sup>nd</sup> week of inoculation, the mycelia covered the compost completely and compost turned into white.

One week after covering the soil, the mycelium advanced into the soil up to 2-3 cm. After covering the soil, the primordia was seen in A, B, C, D and E groups at 15<sup>th</sup>, 17<sup>th</sup>, 18<sup>th</sup>, 15<sup>th</sup> and 17<sup>th</sup> days, respectively. At this period, the temperature of the incubation room was 28°C, compost temperature was 30°C and air humidity was 85-90%.

### Harvest period

The temperature of culture room was regulated to 30°C, 32°C and 34°C at the different periods and the fructifications were investigated. When the temperature of culture room was at 34°C, the mycelium development was observed at compost and covered soil but no primordium was observed. At this period, the mushrooms, which have cap width of 3.0 - 3.5 cm were harvested and cap width, cap tissue thickness, stalk thickness and stalk lengths, were examined. At the control temperature (30°C), the D group the mushrooms have smaller cap, thicker cap tissue, thicker and shorter stalk (Table 1). The mushrooms of A group have big cap and the thickest cap tissue at 32°C. At this temperature, the mushrooms of C group have the thinnest cap tissue and shortest stalk (Table 2)

### DISCUSSION

The rule of *A. bisporus* cultivation which was proposed by Zadrazil et al. (1973), Vedder (1978), Fritsche (1981), Stamets and Chilton (1983), Boztok (1987), Erkel (1993), Stamets, (1993), Günay (1995), Quimio (2002) was modified for mycelial development and yield of *A. bitorquis*. 30°C was optimum temperature for mycelium development of *A. bitorquis* (Zadrazil et al., 1973; Vedder, 1975, 1978; Raper, 1978), and in this study it was accepted as control temperature. The earliest mycelial development was at 30°C and started second day in all of the groups. However, the latest mycelium

**Table 1.** The morphological characteristics of of *Agaricus bitorquis* mushroom at 30°C.

Groups	Cap width	Cap tissue thickness	Stalk thickness	Stalk length
A	3.61*±0.05x3.88**±0.06	1.07±0.02	2.10±0.09	1.13±0.04
B	3.59±0.02x3.82x0.07	1.06±0.01	1.54±0.04	1.11±0.02
C	3.57±0.02x3.81±0.06	1.04±0.02	1.98±0.05	1.07±0.02
D	3.44±0.04x3.74±0.07	1.09±0.02	2.31±0.06	1.03±0.03
E	3.55±0.04x3.74±0.06	1.06±0.02	1.56±0.03	1.07±0.02
	*LSD %5= 0.11 **LSD %5= 0.17	LSD %5=0.06	LSD %5=0.14	LSD %5=0.08

**Table 2.** The morphological characteristics of of *Agaricus bitorquis* mushroom at 32°C.

Groups	Cap width	Cap tissue thickness	Stalk thickness	Stalk length
A	3.61*±0.05x3.88**±0.06	1.09±0.02	2.31±0.07	1.09±0.04
B	3.59±0.02x3.82x0.07	1.06±0.01	1.54±0.04	1.11±0.02
C	3.57±0.02x3.81±0.06	1.04±0.02	1.98±0.05	1.03±0.02
D	3.55±0.04x3.74±0.07	1.07±0.02	2.02±0.06	1.13±0.03
E	3.54±0.04x3.74±0.06	1.06±0.02	1.56±0.03	1.07±0.02
	*LSD %5= 0.11 **LSD %5= 0.17	LSD %5=0.06	LSD %5=0.14	LSD %5=0.08

development started at 36°C in the C group and at 15<sup>th</sup> day. At the other temperature groups, the beginning of mycelial development was in 3<sup>rd</sup> - 5<sup>th</sup> days. At 36°C, this situation was between 6<sup>th</sup> and 15<sup>th</sup> days for groups A, B, C and D, and no mycelial development was seen at group E. At 38°C, no mycelial development was seen at A, B, C and D groups. Therefore, the thermal lethal point for the A, B, C and D groups is 38°C and for the E group it is 36°C. Other studies have indicated that 28°C (Lemke and Claron, 1978; Fritsche, 1981) and 28-30°C (Isik, 1996) are suitable temperatures for mycelium development. Fritsche (1977) improved the mycelia of *A. bitorquis* at 26°C and 30°C and she showed that the mycelium development was very abundant at 30°C than 26°C. Raper (1976) expressed that the vegetative mycelium was faster at 30°C than 24°C. Song (1975) reported that 39°C was the optimum temperature for *A. bitorquis* and showed that the vegetative mycelia declined under 15°C and over 40°C. The thermal lethal point was indicated for *A. bitorquis* mycelium as 33-34°C by Vedder (1975) and as 48°C by Van Zayen and Rutjens (1981).

For spawn development in the compost, the mycelia started to develop after the 1<sup>st</sup> day of inoculation at 30°C and for other temperatures after 2 days. The first mycelial development was completed in 15<sup>th</sup>-17<sup>th</sup> day. The latest mycelial development was at 34°C. The mycelium development in the compost was excellent at D group bags. Mycelial growth in compost at 30°C was reported

by Vedder (1975, 1978) as 12<sup>th</sup>-14<sup>th</sup> days and as 14 days by Zadrazil et al. (1973). Mycelial growth period in compost at 28°C was also observed by Arkan and Guler (1996). Isik et al. (1997) showed that the most development was at 28°C-30°C.

Interest in the cultivation of *A. bitorquis*: is due to several of its valuable quality including resistance against virus diseases (Vedder, 1975, 1978; Van-Zaayen, 1976; Steane, 1980) and its higher CO<sub>2</sub> concentrations (Vedder (1975, 1978, Steane, 1980). *A. bitorquis* fructifications are strong and have tight tissue (Steane, 1980; Gibson, 2003) and it can be cultivated at higher temperatures than *A. bisporus*.

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