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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 30ºC 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 30ºC, 32ºC, 34ºC, 36ºC, and 38ºC, separately. During the incubation period the growth of the mycelia were measured. The best mycelia growth for all groups was seen at 30ºC. At 36ºC, the E group mycelia and at 38ºC 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 30ºC and 32ºC. 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 seasonleaving only the bigger producers which causes the cost to increase.

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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 30ºC, and main cultures were obtained.

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

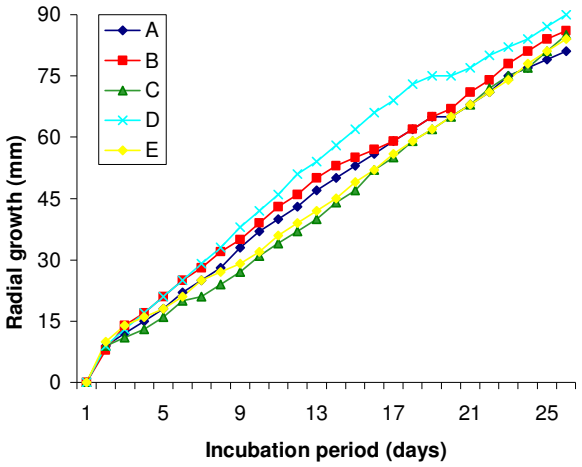

Figure 1. The mycelium development curve of all groups of *Agaricus bitorquis* at 30°C.

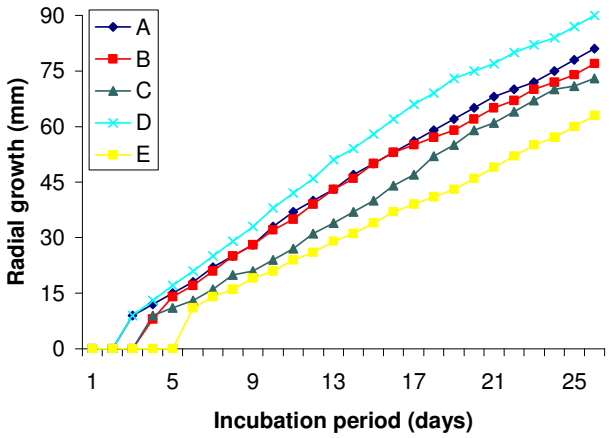

Figure 2. The mycelium development curve of all groups of *Agaricus bitorquis* at 32°C.

The productivity trials

A-1st 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 30ºC, 32ºC, 34ºC and 36ºC for different periods. During the first mycelium period, air humidity was kept at 90%.

B- 2nd 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

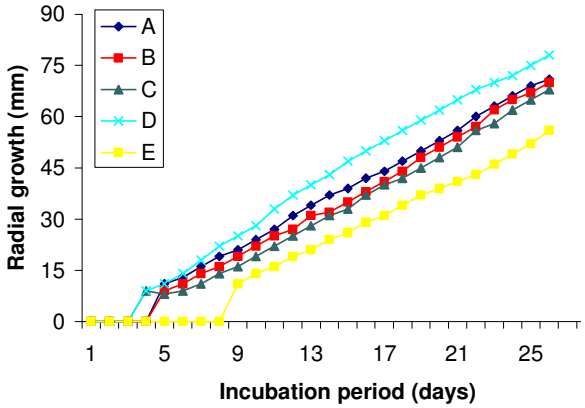

Figure 3. The mycelium development curve of all groups of *Agaricus bitorquis* at 34°C.

preserved at 90-95% and 28ºC, respectively. After the mycelium was seen over the cover soil, the room temperature was decreased to 23ºC each day. The 14th 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 30ºC, which is the best temperature for mycelium interactions, served as control (Zadrazil et al., 1973; Vedder, 1975, 1978; Raper, 1978) (Figure 1). At 32ºC, the mycelia development of A, B, C, D and E groups started at different days after incubation. For instance, 3rd, 4th, 4th, 3rd, 6th days respectively.The mycelia of D group had covered all of the Petri dishes at 26th 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 34ºC. Here the mycelia development was cottony (Figure 3). At 36ºC, 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, 36ºC 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 38ºC.

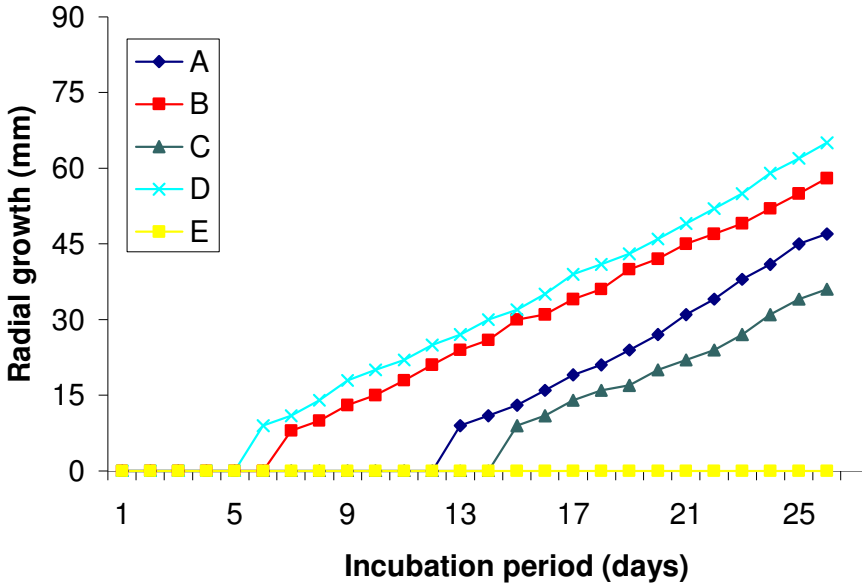

Figure 4. The mycelium development curve of all groups of *Agaricus bitorquis* at 36°C

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

Spawn development

The spawn was developed at different temperatures (30ºC, 32ºC, 34ºC and 36ºC) for the A, B, C and D groups. At the 38ºC, spawn was not prepared because the mycelia were not developed. Also, for the E group the spawn was not prepared at 36ºC 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 2nd 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 15th, 17th, 18th, 15th and 17th days, respectively. At this period, the temperature of the incubation room was 28ºC, compost temperature was 30ºC and air humidity was 85-90%.

Harvest period

The temperature of culture room was regulated to 30ºC, 32ºC and 34ºC at the different periods and the fructifications were investigated. When the temperature of culture room was at 34ºC, 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 (30ºC), 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 32ºC. 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*. 30ºC 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 30ºC 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 36ºC in the C group and at 15th day. At the other temperature groups, the beginning of mycelial development was in 3rd - 5th days. At 36ºC, this situation was between 6th and 15th days for groups A, B, C and D, and no mycelial development was seen at group E. At 38ºC, 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 38ºC and for the E group it is 36ºC. Other studies have indicated that 28ºC (Lemke and Claron, 1978; Fritsche, 1981) and 28-30ºC (Isık, 1996) are suitable temperatures for mycelium development. Fritsche (1977) improved the mycelia of *A. bitorquis* at 26ºC and 30ºC and she showed that the mycelium development was very abundant at 30ºC than 26ºC. Raper (1976) expressed that the vegetative mycelium was faster at 30ºC than 24ºC. Song (1975) reported that 39ºC was the optimum temperature for *A. bitorquis* and showed that the vegetative mycelia declined under 15ºC and over 40ºC. The thermal lethal point was indicated for *A. bitorquis* mycelium as 33-34ºC by Vedder (1975) and as 48ºC by Van Zayen and Rutjens (1981).

For spawn development in the compost, the mycelia started to develop after the 1st day of inoculation at 30ºC and for other temperatures after 2 days. The first mycelial development was completed in 15th-17th day. The latest mycelial development was at 34ºC. The mycelium development in the compost was excellent at D group bags. Mycelial growth in compost at 30ºC was reported by Vedder (1975, 1978) as 12th-14th days and as 14 days by Zadrazil et al. (1973). Mycelial growth period in compost at 28ºC was also observed by Arkan and Guler (1996). Isık et al. (1997) showed that the most development was at 28ºC-30ºC.

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_2 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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