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THE SPAWN DEVELOPMENT IN *Agaricus bitorquis* (Quél.) Sacc. MYCELIUM GERMINATED AT DIFFERENT TEMPERATURES*

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Abstract

Agaricus bitorquis (Quél.) Sacc. basidiocarps collected from nature were grouped as A,B,C,D. The seconder mycelium was obtained by the development of primer mycelium at 25°C, 28°C, 30°C, 32°C, 35°C and 38°C. The spawn was prepared from the seconder mycelium developed at different temperatures . In the development of spawn; as the mycelium began to develop, the shaking time, the mycelium covering the wheat grains completely and the period of incubation were taken as criteria.

Key Words *Agaricus bitorquis*, spawn, development of vegetative mycelium

1. Introduction

The seconder mycelia of mushrooms are generally referred to as spawn. Spawn is a pure culture of a mycelium growing on a solid substrate such as cereal grains (1-6). Grain spawn was invented by Sinden in 1931 (7). In 1932 Sinden patented a new spawn making process using cereal grain as the mycelial carrier. Since then rye has been the most common grain employed although millet, milo and wheat have also been used (8). When compared with manure spawn, the grains with the mycelium on the surface offer the advantage that the spawn can readily be mixed evenly throughout the compost. The most widely used grains are rye and millet, while success has also been reported with wheat and sorghum (2,6,9). Small grains such as millet, give a greater number of inoculation points per liter than large grains such as rye (2). Therefore those who use millet claim it makes better spawn (9). Spawn is usually prepared with wheat in Türkiye because of wheat was grown very common in the country (3,4). In preparing grain spawn, it is important to consider carefully both the origin and strain of the grain to be used. The mycelium chosen for spawn production must be of first class quality, that it must be healthy and show no signs of degeneration (2). This papers reports the development of spawn prepared from *Agaricus bitorquis* (Quél.) Sacc. mycelium germinated at different temperatures.

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2. Material and Methods

A- Used Organism

In this study, *Agaricus bitorquis* basidiocarps collected from Aşağı Hacıosmanoğlu village (Polath,Ankara) in May 1995 were used. The collected mushrooms were grouped as 20 groups previously. The basidiospores of these groups were germinated on the wheat agar in the petri dishes and they were incubated for 20 days. At the end of the periods, the best developed 10 groups were taken for development of mycelium. The mycelial agar discs were taken from these groups and they were inoculated on the wheat agar. At the end of the incubation periods of 20 days, the mycelium were developed as rhizomorphic and healty at the 4 groups and these were grouped as A,B,C,D. The fructifications were dried after taking spores in sterile inoculation cabin. They were kept in the refrigerator at +4°C in the paper bags.

B- The Preparation of Agarmedia

In the study, the wheat agar was used (3,10). For preparing wheat agar, 125 g wheat was boiled for 2 hours in 4 liters distilled water and it was kept for 24 hours in the water. After filtering the liquid part and 4 liters distilled water was added. It was heated until the boiling point after adding 2% agar. They were filled in the erlenmeyers of 500 cc before freezing. The erlenmeyers were closed by cotton and the aluminium paper. They were sterilized for 15 minutes at 120°C temperature in autoclave and poured into sterile petri dishes and then cooled (3).

C- The Preparation of the Main Culture

The basidiospores of A,B,C,D groups were germinated by multispore method (3,11-13) in the agar and primer mycelium obtained. The mycelial agar discs were taken from primer mycelium and transferred to wheat agar into the petri dishes. They were developed at 25°C, 28°C, 30°C, 32°C, 35°C and 38°C temperatures and seconder mycelium were obtained. For a healthy and productive study mycelium transfers were made in each 15 days. For the mycelial developments, optimal temperature was pointed out as 30°C (14-17) and these developments were thought as control group. In this study, the groups were shown for example as C30. This expression shown that the mycelium of group C were developed at 30°C.

D- The Preparation of Spawn

The spawn used in this study was obtained from the covering of the wheat grain. Ten kg wheat was boiled for 20 minutes and filtered for this aim. The wheat were left to dry on a place. For the pH media, 50 gr chalk and 200 gr gypsum were added in order not to stick to each other and they were mixed altogether. They were filled in the bottles of 1 lt until 2/3 volume of it. The bottles should be resistant to temperature. They were closed with the cotton and the thick paper and sterilised in the autoclave at 125 °C for one and a half hour. They were placed in the sterile room and allowed to cool (2-6,8,18,19). Two mycelial agar discs that were taken from main cultures were put into bottles separately and then closed in the sterile inoculation cabin. They were incubated in 90-100% humidity and 28-30°C temperatures.

3. Results

The development of seconder mycelium prepared from *Agaricus bitorquis* basidiocarps were examined at different temperatures. In the group A, the development of rhizomorphic mycelium was observed at 25°C, 28°C, 30°C and 32°C. In the same way, the development of mycelium that grew parallel to the agar surface was observed at 25°C, 28°C, 30°C, 32°C and 35°C in the B,C,D groups, but the abnormal mycelium development was determined at 35°C in the B,C,D groups. In these groups the mycelium formed miscellaneous and cottony aerial hyphae. In the A group, the development of mycelium was not observed at 35°C. Therefore, the spawn was prepared in all the groups except the group A35. The

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and on the 14th day of development. In this culture, the wheat grains were completely covered by the mycelium on the 24th day of incubation.

The fastest development in the group B was seen at the spawn that improved at 32°C. In this group, the mycelium development began on the 4th day of incubation and the first shaking was on the 9th day of incubation and on the 6th day of the development. The second shaking was on the 15th day of incubation and on the 12th day of development. The mycelium development was completed on the 21st day of incubation (Table 1).

c) The development of spawn prepared from the mycelium in group C

The slowest development in the study was determined in the spawn cultures improved in the mycelium of group C except the group C30. In the group C, the mycelium development began on the 6th day of development in all the temperatures except the control group as C30.In the spawn cultures improved at 25°C the first shaking was on the 14th day of incubation and on the 9th day of the development. The second shaking was on the 21st day of incubation and on the 16th day of development. The mycelium development was completed on the 27th day of incubation.

In the spawn cultures improved at 28°C the first shaking was on the 12th day of incubation and on the 7th day of the development. The second shaking was on the 19th day of incubation and on the 14th day of development. The mycelium development was completed on the 25th day of incubation.

In the spawn cultures improved at 32°C the first shaking was on the 13th day of incubation and on the 8th day of the development. The second shaking was on the 20th day of incubation and on the 15th day of development. The mycelium development was completed on the 26th day of incubation (Table 1).

d) The development of spawn prepared from the mycelium in group D

The fastest development in the study was observed in the spawn cultures improved in the mycelium of group D. In the group D, the mycelium development began on the 4th day of development in all the temperatures. In the spawn cultures improved at 25°C the first shaking was on the 8th day of incubation and on the 5th day of the development. The second shaking was on the 14th day of incubation and on the 11th day of development. The mycelium development was completed on the 19th day of incubation.

In the spawn cultures improved at 28°C the first shaking was on the 9th day of incubation and on the 6th day of the development. The second shaking was on the 16th day of incubation and on the 13th day of development. The mycelium development was completed on the 20th day of incubation.

The slowest development in the group D was obtained in the spawn cultures improved at 32°C. In this group, the first shaking was on the 10th day of incubation and on the 7th day of the development. The second shaking was on the 17th day of incubation and on the 14th day of development. The mycelium development was completed on the 22nd day of incubation (Table 1).

Table 1. The calendar of spawn development prepared from the mycelium groups A,B,C,D.

Groups	Temp.	The incubation period (day)																									
	°C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	25	-	-	-	+	+	+	-	1S	-	+	-	+	2S	-	+	+	+	BK									
A	28	-	-	-	+	+	+	-	+	-	1S	-	+	-	-	-	+	2S	+	+	+	-	BK					
	30	-	-	-	-	-	+	+	1S	-	-	+	-	-	+	2S	+	+	+	+	+	BK						
	32	-	-	-	+	-	-	+	+	-	+	-	+	1S	-	+	+	+	+	2S	-	+	+	+	BK			
	25	-	-	-	-	-	-	-	+	-	+	1S	-	-	+	+	+	2S	+	+	+	+	+	BK				
	28	-	-	-	-	+	-	+	-	-	+	1S	+	-	+	+	+	-	2S	+	+	+	+	+	BK			
B	30	-	-	-	-	+	+	-	1S	+	+	+	-	+	-	2S	+	+	+	+	+	BK						
	32	-	-	-	-	+	+	-	-	1S	+	-	+	+	-	2S	+	+	+	+	BK							
	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	-	-	-	-	-	-	+	1S	-	+	+	-	+	2S	+	+	+	-	+	BK	
	28	-	-	-	-	-	+	-	-	-	+	-	1S	+	+	-	+	+	+	2S	+	-	+	-	+	BK		
C	30	-	-	-	-	+	+	+	1S	-	-	+	+	-	-	2S	+	+	+	+	+	BK						
	32	-	-	-	-	-	+	-	+	-	-	-	+	1S	-	+	+	-	+	+	2S	+	+	+	+	-	BK	
	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	+	-	1S	+	+	+	-	-	2S	-	-	-	-	BK								
	28	-	-	-	-	-	-	+	-	1S	+	-	+	+	+	+	2S	+	-	BK								
D	30	-	-	-	+	+	+	1S	+	-	+	+	2S	+	-	+	-	+	+	BK								
	32	-	-	-	+	-	+	-	-	-	1S	-	+	-	-	-	+	2S	+	+	+	+	BK					
	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) = Positive development

(-) = Negative development

1S= First shaking

2S = Second shaking

BK = Put into refrigerator

4. Discussion

In this study, the development of spawn which was prepared from *A. bitorquis* mycelium germinated at different temperatures was examined.

The mycelium of *A.bitorquis* were grouped as A,B,C,D and they were incubated at 25°C, 28°C, 30°C, 32°C, 35°C and 38°C. As a result the main cultures were obtained. The mycelium of group A was developed at 25°C, 28°C, 30°C and 32°C, but they were not developed at the 35°C and 38°C. In the same way, in the groups B,C,D, the mycelium development was observed at 25°C, 28°C, 30°C, 32°C and 35°C, but in these groups, the mycelium development was not obtained at 38°C. That is way, the thermal lethal point for the mycelium of group A was determined as 35°C. Likewise, the thermal lethal point for the mycelium of groups B,C,D was determined as 38°C. Later, the spawn was prepared from these main cultures. As shown in the Table 1, in this study, the fastest mycelium development was observed in the mycelium of group D and the slowest mycelium development was observed in the mycelium of group C. In the spawn prepared from the groups of B,C,D germinated at 35°C; the mycelium development was not obtained. Therefore, the thermal lethal point for spawn in the groups of B,C, D was determined as 35°C.

prepared culture bottles were put into the incubation room, that had the conditions of temperature between 27°C and 29°C and 80% humidity. The incubation period showed the differences according to the development in the groups. During the incubation period, the first and second shaking was made at the spesific days for the homogeneus distrubition of mycelium. Günay (1995) (3) reported that the first shaking is 10-15 days and second shaking is 20-25 days after the inoculation. In this study, the development of the spawn and the days of shaking were shown at the Table 1 which formed the development calendar of mycelium.

1. The development of mycelium in the control group

As we mentioned before for the mycelial development, optimal temperature was pointed out as 30°C (14-17) and these developments were thought as control group. In this temperature, the mycelium development began on the 4th day of the incubation in all spawn bottles. For a homogeneus development, the first shaking was on the 8th day and the second shaking was on the 15th day of the incubation. After the 21 daily incubation, the mycelium covered the wheat grains and the development was completed (Table 1).

2. The development of spawn prepared from the mycelium improved in the different temperatures

a) The development of spawn prepared from the mycelium in group A

In the group A, the development began in all the temperatures on the 4th day of incubation. The fastest spawn development was seen at the mycelium that improved at 25°C. In these cultures, the first shaking was on the 8th day of incubation and on the 5th day of the development. The second shaking was on the 13th day of incubation and on the 10th day of development. The wheat grains were completely covered by the mycelium on the 18th day of incubation. In the spawn cultures developed at 28°C, the first shaking was on the 10th day of incubation and on the 7th day of the development. The second shaking was on the 17th day of incubation and on the 14th day of development. The mycelium development was completed on the 22nd day of incubation. The slowest development in the group A was seen at the spawn that improved at 32°C. In these cultures, the development was completed on the 24th day of incubation and the first shaking was on the 13th day of incubation and on the 10th day of the development. The second shaking was on the 19th day of incubation and on the 16th day of development (Table 1).

b) The development of spawn prepared from the mycelium in group B

In the mycelium of group B; in the spawn cultures improved at 25°C and 28°C, the development began on the 5th day of incubation. In the each two groups, the first shaking was on the 11th day of incubation and on the 7th day of the development.

In the spawn cultures improved at 25°C, the second shaking was on the 17th day of incubation and on the 13th day of development. In this culture, the mycelium development was completed on the 23rd day of incubation. In the spawn cultures improved at 28°C, the second shaking was on the 18th day of incubation