Determination of Some Characteristics of Wild *Agaricus bisporus* Collected from Turkey

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Abstract: Nature, as a bank of genetic resources, available for biodiversity and genetic research, serves as the origin of various characteristics. The button mushroom, *Agaricus bisporus*, is one of the world's most economically important crops. Four wild sporocarps of *A. bisporus* were collected from different regions of West Anatolia during 1998 and 1999. In this study, morphological features, basidiospore germination time, mycelium growth rate and colony morphology were determined. Morphological and spore germination time analyses among the wild samples revealed slight variability. In the course of our research, we isolated 32 strains from early-germinated spores. Colony morphologies of the mycelia from the 32 isolates showed high variability. Different mycelial growth rates were also measured in some strains. It is important that new strains having different properties, are isolated from nature for the further investigation of *A. bisporus*.

Key words: *Agaricus bisporus*, wild-type isolates, Turkey, mushroom, genetic resources, rapidly growing strains

1 Introduction

A wild population of mushrooms serves as the souce of new native traits and is very important for the development of commercial species. The white button mushroom, *A. bisporus*, is one of the world's most popular edible mushrooms with major economic value.[1] Wild populations of this organism have been discovered from different countries and preserved by Dr. R.W. Kerrigan who is leader of the *Agaricus* Resource Program. Researchers have characterized extensively each of collected samples. As a result, all strains of *A. bisporus* now grown commercially are derived from only a few popular European strains.[2]

2 Materials and Methods

Basidiocarps collected during field research in Turkey were harvested, photographed and immediately transported to the laboratory. After macroscopic and microscopic examinations were completed, taxonomical analyses were performed according to Cappelli.[3] On the same day as collection, whole basidiocarps were placed in a vertical position in sterile Petri dishes in order to allow overnight collection of spores. After the spores were collected on the petri dishes, 5 ml sterile water was added under aseptic conditions. The Petri dishes containing 5 ml of water were gently shaken to collect most spores. The basidiospore suspensions were then transferred to sterile test tubes. For preparation of a spore suspension, 1 ml aliquots from the basidiospore suspension were transferred to sterile test tubes containing 9 ml sterile water (1:10). Serial dilutions were made until a 1:10000 dilution was reached. Spore numbers in the 1 ml dilutions were counted with a haemocytometer in three replicates (Table 1).
Two different agar media were used in this study; CYM medium (complete yeast medium: 2 g peptone, 2 g yeast extract, 20 g glucose, 1 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.46 g KH_2PO_4 and 15 g Difco Bactoagar per litre

of distilled water) as described by Horgen et al,[4] and Malt Extract Agar (MEA) (20 g malt extract and 15 g Difco Bactoagar in one liter distilled water) with 25 µg/ml streptomycin. One millilitre of each spore suspension was aseptically transferred on to the agar media. Three replicates were prepared and all plates were incubated at 25 +1°C for 30 days. Rapidly germinating basidiospores were detected with the naked eye with the aid of a fluorescent lamp and recorded as early germinating spores.

Table 1. Number of the spores in 1 ml dilutions

	Dilutions			
Code	1/10	1/100	1/1000	1/10000
D_1	700000	75000	8000	900
D_3	550000	57000	6000	700
H	930000	95000	10000	1200
Y	980000	100000	11000	1000

Germinated spores in two different culture media were transferred to fresh Petri dishes containing malt agar (2%) to measure mycelial growth rate and to determine mycelial morphology. All Petri dishes were incubated at 23 +1°C for 30 days. On the 10th, 16th, 24th and 30th day, the radius of each mycelial colony was measured in millimetres. Mycelia that had grown to cover half of the plate were photographed to reveal morphological features.

3 Results and Discussion

Three of the four wild samples had a brown-cream cap while the fourth had a white cap color. All the collected sporocarps had white flesh. They contained two-spored basidia and the spores were pale brown and broadly ellipsoidal (mean dimensions: 5.2 x 4.2 µm). Basidiospores from all basidiocarps used in this study were successfully collected and germinated on two different agar media. Basidiospore germination was not detected in some dilutions during the 30-day period. Table 2 shows basidiospore germination time of spores that were collected from four wild fruit bodies of *A. bisporus* from Turkey in the two different culture media.

Table 2. Basidiospore germination time (day) in two different media

a. CYM

Dilutions	H	D_1	D_3	Y
1/10	13	8	9	14
1/100	12	9	10	15
1/1000	26	11	14	19
1/10000	none	24	27	23

b. Malt Extract Agar

Dilutions	H	D1	D3	Y
1/10	16	10	10	15
1/100	16	10	10	16
1/1000	none	12	14	none
1/10000	none	none	none	none

Early germinated basidiospores among wild samples were observed with D_1 (1/10 dilution) on CYM medium at 8 days and D_3 (1/10 dilution) with D_1 (1/100 dilution) on CYM medium at 9 days. Late germinating basidiospores of wild samples were D_3 (1/10000 dilution) in CYM medium at 27 days. Spores obtained from wild

samples H and Y did not germinated in 1/1000, 1/10000 dilutions on MEA. Also, basidiospores in suspensions containing less than 900 spores for all wild samples did not germinate in the same medium. As can be seen from Table 3, different radial growth rates were observed with different germinated basidiospores. Spores collected from the same basidiocarp (they have also been collected from the same geographic location) showed different radial growth rates and mycelium morphology. This is important and the first stage in the selection of new strains that have different genotypes.[2, 5] Several strains, especially $D_{1\text{-}5}$, $D_{3\text{-}9}$ and Y_5, have been vigorously grown in two different media. They can be designated as rapidly growing strains for further research on the mycelium.

Table 3. Mycelial growth rates (mm) and morphological characteristics of the new strains

Strains/Days	10	16	24	30	Morphology
H_1	20/24	34/39	50/16	65/76	Threadlike/Linear
H_2	17/18	27/30	50/58	66/74	Threadlike/Linear
H_3	19/17	35/29	55/46	75/56	Threadlike/Powdery
H_4	19/19	30/27	50/48	64/62	Threadlike/Threadlike
H_5	17/15	30/28	49/46	66/64	Threadlike/Fluffy
$D_{1\text{-}1}$	19/22	32/37	50/54	67/70	Threadlike/Powdery
$D_{1\text{-}2}$	22/25	36/40	59/60	74/75	Linear/Linear
$D_{1\text{-}3}$	20/24	34/39	50/61	65/76	Circular;Threadlike/ Threadlike;Fluffy
$D_{1\text{-}4}$	23/29	37/45	55/61	74/76	Threadlike/Linear
$D_{1\text{-}5}$	19/31#	36/49#	54/74#	65/90#	Threadlike/Linear
$D_{1\text{-}6}$	24/18	38/30	62/47	73/65	Linear/Linear
$D_{1\text{-}7}$	35/13	54/21	76/38	90/51	Fragmented;Fluffy/Linear;Powdery
$D_{1\text{-}8}$	23/22	35/36	52/50	66/61	Circular/ Linear
$D_{1\text{-}9}$	23/17	34/29	54/45	67/57	Linear;Fluffy/Powdery
$D_{1\text{-}10}$	22/21	35/31	57/51	73/70	Linear/Linear
$D_{3\text{-}1}$	13/20	25/29	41/38	56/50	Linear/Threadlike
$D_{3\text{-}2}$	15/21	25/30	40/39	48/54	Linear;Fluffy/Linear
$D_{3\text{-}3}$	12/18	24/25	45/33	63/49	Linear/Fluffy
$D_{3\text{-}4}$	10/14	22/23	39/36	50/73	Threadlike/Linear
$D_{3\text{-}5}$	11/12	20/21	41/39	57/75	Threadlike/Linear
$D_{3\text{-}6}$	10/14	21/19	38/30	55/70	Linear;Fluffy/Linear
$D_{3\text{-}7}$	14/20	26/21	45/32	60/57	Linear/Fluffy
$D_{3\text{-}8}$	11/22	29/22	43/32	55/63	Linear;Fluffy/Linear
$D_{3\text{-}9}$	10/21#	27/29#	42/43#	55/75#	Threadlike/Linear
$D_{3\text{-}10}$	17/24	28/29	49/39	69/60	Linear/Linear
$D_{3\text{-}11}$	11/20	20/22	38/32	58/59	Threadlike/Threadlike*
$D_{3\text{-}12}$	15/21	21/22	33/32	60/64	Linear/Linear
Y_1	17/15	23/20	38/26	46/35	Powdery/Powdery*
Y_2	15/16	20/24	39/32	38/40	Fragmented/Powdery
Y_3	15/14	24/19	34/27	42/33	Circular/Fluffy
Y_4	11/10	16/13	21/19	26/25	Powdery/Powdery*
Y_5	19/20#	36/35#	57/61#	73/77#	Linear/Linear

Malt agar medium/Complete yeast medium
#Rapidly growing strains in columns of days. *Aberrant growth patterns

Genetic diversity was revealed in established culture collections using RAPD analyses. Callac et al[2] have investigated the Mediterranean populations of *A. bisporus* and have extensively characterized the properties of collected wild samples from Greece, Israel and Italy. These experimental analyses on mushrooms are very

important to elucidate genetic variability in the wild samples collected from cross-border countries. Moreover, data from investigations on biodiversity can be used for studying population structure, relationships and movement of species from country to country.

References

[1] Sonnenberg ASM. Genetics and breeding of *Agaricus bisporus*. In L.J.L.D. Van Griensven (ed.), Mushroom Science XV: Science and cultivation of edible fungi, Vol. 2. Rotterdam: Balkema, 2000.
[2] Callac P, Imbernon M, Guinberteau, et al. Discovery of a wild Mediterranean population of *Agaricus bisporus*, and its usefulness for breeding work. In L.J.L.D. Van Griensven (ed.), Mushroom Science XV: Science and cultivation of edible fungi, Vol. 2. Rotterdam: Balkema, 2000.
[3] Cappelli *A. Agaricus* L. Fr. ss. Karsten, Traduzioni inglese di E. Grili, Tavole a colori di L. V. Musumeci, Milano. 1984.
[4] Horgen PA, Kokurewicz KF, Anderson JB. The germination of basidiospores from commercial and wild collected isolates *of Agaricus bisporus* (=*A. brunnescens*). Can. J. Microbiol. 1989, 35:492-498.
[5] Jennings DH, Lysek G. Fungal biology: Understanding the fungal lifestyle, 2nd Edition, New York:Bios Scientific Publishers Ltd, 1999, pp119-133.