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Mycelial Growth Rate of Some Morels (*Morchella spp*.) In Four Different Microbiological Media

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Abstract: In this study, growth rates of mycelium of six wild *Morchella* species (*M. costata*, *M. esculenta*, *M. esculenta* var. *rigida*, *M. esculenta* var. *rotunda*, *M. intermedia* and *M. pseudoumbrina*) obtained from different regions of West Anatolia were investigated in different culture medium. Potato Dextrose Agar (PDA), Hagem medium (HM), Minimal medium (MO) and Malt Extract Agar (MEA) were used as microbiological media. Mycelial growth rate was measured in different periods. Mycelial growth rates of all isolates were decreased in MM and HM media. PDA and MEA are suitable media for mycelial growth of Morchella spp in this study.

Key words: Morels % Mycelial growth % *Morchella spp.*

INTRODUCTION

About 14.000 species of mushrooms are now known in the world. Recently, macrofungi have become attractive as functional foods and a source of physiologically beneficial medicine. Among the most desirable edible wild macrofungi, morels are well-known around the world [1, 2]. Many researches investigated into growing them in both the liquid media and on solid substrates [3, 4]. Despite many attempts, the commercially cultivation of morels has not been exactly possible up to now.

Nowadays morels are very important non-wood forest product for peasant who is living the nearby forest area. *Morchella spp*, which are appear from the late April until the end of May are widely distributed in the temperate zones of the world. In general, ascocarps of morel species occur in a variety of habitats, including riverbanks, mountain slopes, pastures, burned-out forests and near plants that have been injured. They emerge in sand, moist soil with abundant organic matter and in mud [5].

Cultures on agar and in agitated liquid media are entirely artificial systems which are excellent models and are indispensable for research and other purposes. Microbiological medium used for mycelial growth is often highly specific for a given macrofungi [6, 7]. It has been reported that several mechanisms in fungi protected fungal cells against several stress factors. Healthy and active mycelial growth in used medium play crucial role for protecting themselves from the stress [8, 9]. Many researches reported on cultural characteristics, physiological properties *in vitro* and complex genotyping factors of *Morchella spp*. [10, 11, 12].

According to previous experiments, mycelial growth is strongly influenced by *in vitro* conditions, such as incubation time, temperature, pH, inoculum size, etc. [13]. We strongly believe that it is critical to search for the key influencing factors from the mentioned above for further study. The present work focuses on determination of mycelial growth rate of six morels (*M. costata*, *M. esculenta*, *M. esculenta* var. *rigida*, *M. esculenta* var. *rotunda*, *M. intermedia* and *M. pseudoumbrina*) in Petri plate containing four different microbiological media, Potato Dextrose Agar, Hagem medium, minimal medium and Malt Extract Agar.

MATERIALS AND METHODS

Fungal Material: Fruit bodies were collected from different regions of West Anatolia, Turkey in spring 2006 and 2007. The morphological and ecological characteristics of these macrofungi were recorded and photographed in their natural habitats and then brought to the laboratory. Their spore prints were taken and spores were photographed. Dried specimens were numbered and placed in locked bags. All ascocarps of morels were deposited in the Fungarium of Mugla

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Table 1: Collection numbers and names of morels

Collection NO.	Name
MCC 3736	*M. esculenta* var. *rigida*
MCC 3739	*M. costata*
MCC 3740	*M. intermedia*
MCC 3742	*M. pseudoumbrina*
MCC 3744	*M. esculenta* var. *rotunda*
MCC 3747	*M. esculenta*

MCC: Macrofungi culture collection

University, Turkey. The specimens were identified with the help of macroscopic and microscopic features at species level whenever possible according to the related literatures [14, 15, 16]. Collection numbers of these morels are given in Table 1.

Media and Culture Conditions: Four different microbiological media, Potato Dextrose Agar (PDA-Merck, 1.10130.0500), Hagem medium (HM-Malt extract 4 g, yeast extract 1 g, D-glucose 5 g, NH_4Cl 0,5 g, KH_2PO_4 0,5 g, $MgSO_4$ $7H_2O$ 0,5 g, %1 $FeCl_3$-0,5 ml, 100 ppm Thiamin-0.125 ml, distilled water 1000 ml. pH 5.40-5.50) [17] minimal medium (MM-urea 0.036 g, KH_2PO_4 2 g, $MgSO_4$ $7H_2O$ 0,5 g, $CaCl_2$ 0.099 g, D-Glucose 1 g, distilled water 1000 ml. pH 5.20-5.40) [18] and Malt Extract Agar (MEA-Merck, 1.05398.0500) were used to select a simple and suitable medium for determination of the mycelial growth rate. Cultivation on solid media was carried out at 25°C in Petri dishes (90mm diameter) containing above mentioned microbiological media. The dishes (five parallels) were inoculated with mycelial plugs (6 mm diameter) cut from actively growing mycelia. Mycelial growth was followed by measuring radial extension of the mycelium measured, as described in Weitz *et al*. [19] with a calliper gauge along two diameters at right angles to one another and the average diameter for each plate calculated.

RESULTS AND DISCUSSION

Variation in colony diameter of different isolates of *Morchella spp*. was presented in Table 2 and Fig. 1. Among the microbiological media used in this study, it was indicated that PDA and MEA are more suitable media for mycelial growth of *Morchella spp*. than others. Mycelia of all isolates, except for *M. esculenta* var. *rigida*, reached the edges of Petri dishes (90mm Ø) containing MEA for 5 days.

While *M. esculenta*, *M. intermediate* and *M. esculenta* var. *rotunda* were showed similar rapid mycelial growth in MEA, same result was not obtained for *M. pseudoumbrina*, *M. esculenta* var. *rigida* and *M. costata*. *M. costata* has been showed slow mycelial growth at 7th of incubation in MEA. It has been reported that maltose has a good carbon source for mycelium of morels [11, 12].

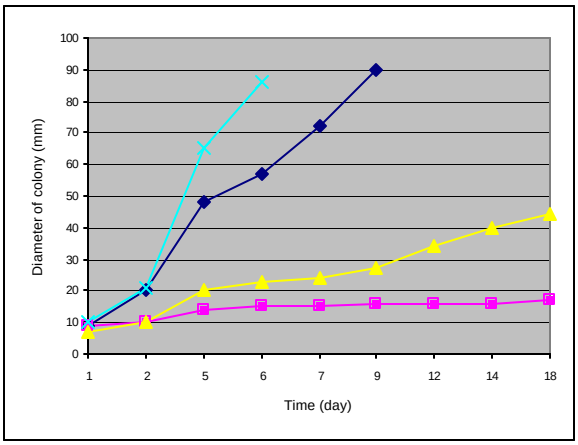
All isolates retarded mycelial growth rates in the media, MM and HM. Especially, mycelium of *M. intermedia* growing in MM and HM has been showed slow growth as compared the other isolates in the same media. Best growth rates obtained in this study for MM medium was obtained from *M. esculenta*. Moreover mycelium of *M. esculenta* var. *rigida* growing in HM medium also showed the slowest growth. Increases of mycelial growth rate of all strains, growing in HM medium is very interesting. Although HM medium was contained several carbon sources such as glucose, yeast extract and malt extract, the best growth was not determined in this medium. It has been previously noted variable growth in some morel isolates [20]. In one recent study, Winder pointed out in variations between slower and faster growing *M. elata* isolates in different media. Moreover, in the same study, some isolates of *M. elata*

Table 2: Mycelial growth rate of six wild morels in four different media

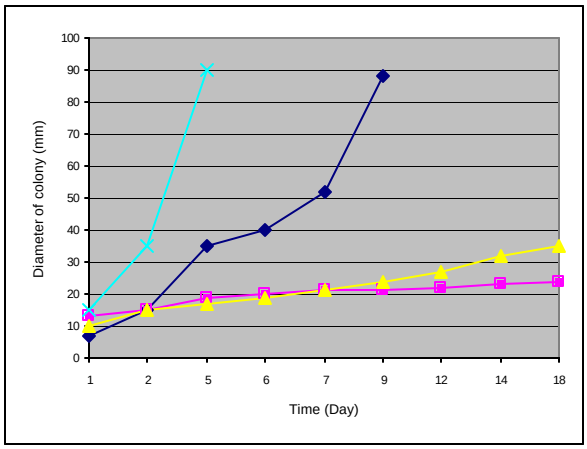
	Culture media																							
	3736				3739				3740				3742				3744				3747			
Days	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	9	9	7	10	7	13	10	15	10	13	10	11	8	8	8	8	10	12	10	16	10	11	10	10
2	20	10	10	21	15	15	15	35	24	16	11	29	15	9	9	16	19	14	12	30	20	12	15	29
5	48	14	20	65	35	19	17	90	60	18	18	90	52	19	12	85	55	16	17	90	61	15	34	90
6	57	15	23	86	40	20	19		68	19	18		61	20	14	90	64	17	18		70	16	38	
7	72	15	24	90	52	21	21		84	20	20		72	21	15		84	18	19		90	16	43	
9	90	16	27		90	21	24		90	20	20		90	22	16		90	18	20			18	44	
12		16	34			22	27			21	21			24	19			19	24			20	47	
14		16	40			23	32			21	24			25	20			20	26			22	49	
18		17	44			24	35			23	25			29	22			24	27			23	50	

* See Table 1 for abbreviations of morels

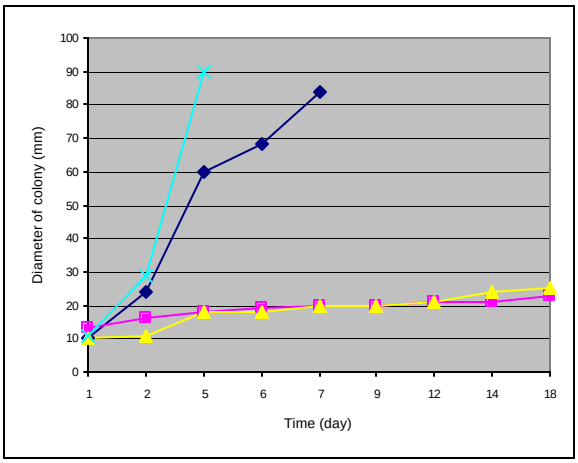
M. esculenta var. *rigida*



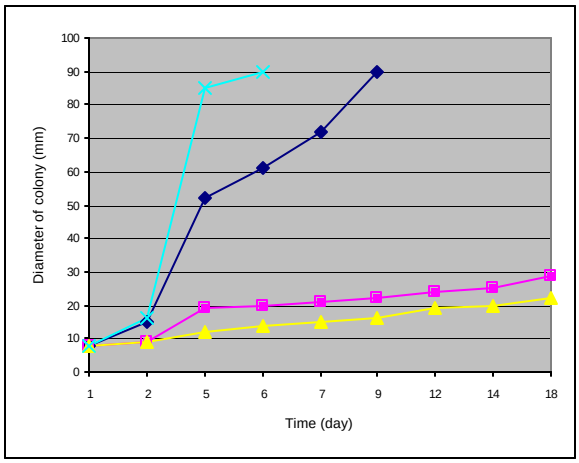
M. costata



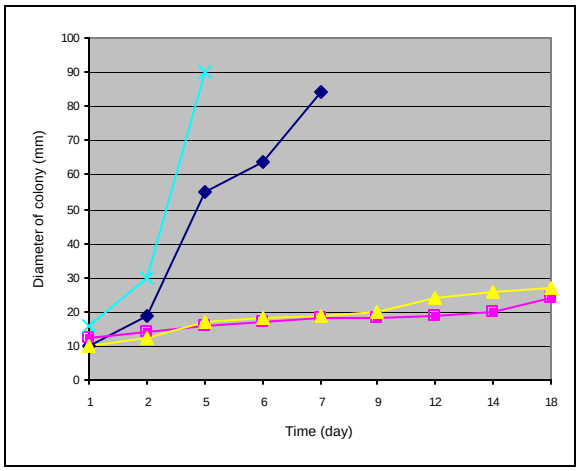
M. intermedia



M. pseudoumbrina



M. esculenta var. *rotunda*



M. esculenta

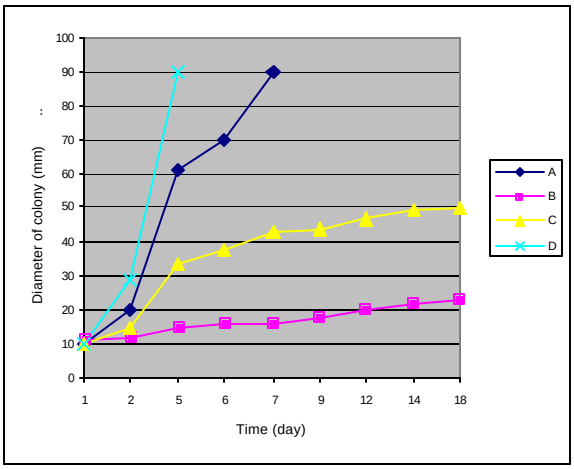


Fig. 1: Mycelial growth rate of six wild morels in four different media

grew optimally in maltose, whereas others did not show same performance in the same medium [21].

It is important to elucidate parameters effect on mycelial growth biotechnological researches which are dealing with large-scale production of mycelium (biomass) to obtain anti-cancer and antimicrobial substances, volatile compounds, biomass for food industry and enzymes production etc.

REFERENCES

1. Tsai, S.Y., C.C. Weng and S.J. Huang, 2006. Nonvolatile taste components of *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Food Sci. Technol., 39: 1066-1071.
2. Wasser, S.P., 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiol. Biotechnol., 60: 258-274.
3. Kosaric, N. and N. Miyata, 1981. Growth of morel mushroom mycelium in cheese whey. J. Dairy Res., 48: 149-162.
4. Volk, T. and T. Leonard, 1989. Physiological and environmental studies of sclerotium formation and maturation in isolates of *Morchella crassipes*. Applied Environ. Microbiol., 55: 3095-3100.
5. Friedman, S.A., 1986. Morels, may apples and the meaning of life mushroom. J. Wild Mushrooming, 4: 5-9.
6. Jennings, D.H. and G. Lysek, 1999. Fungal Biology: Understanding the fungal lifestyle. Chapter 3, Bios Scientific Publishers Ltd.
7. Steele, G.C. and A.P.J. Trinci, 1975. The extension rate of mycelial hyphae. New Pathologist, 75: 583-587.
8. Herdeiro, R.S., M.D. Pereira, A.D. Panek and E.C. Eleutherio, 2006. Trehalose protects *Saccharomyces cerevisiae* from lipid peroxidation during oxidative stress. Biochimica et Biophysica Acta, 17(60): 340-346.
9. Ruijter, G.J.G., M. Bax, H. Patel, S.J. Flitter, P.A. Vondervoort, R.P. Vries, P.A. Kuyk and J. Visser, 2003. Mannitol is required for stress tolerance in *Aspergillus niger* conidiospores. Eukaryotic Cell, 2: 690-698.
10. Buscot, F., 1993. Mycelial differentiation of *Morchella esculenta* in pure culture. Mycol. Res., 97: 136-140.
11. Amir, R., D. Levanon, Y. Hadar, I. Chet, 1994. The role of source-sink relationships in translocation during sclerotial formation by *Morchella esculenta*. Mycol. Res., 98: 1409-1414.
12. Amir, R., 1993. Morphology and physiology of *Morchella esculenta* during sclerotial formation. Mycol. Res., 97: 683-689.
13. Kalmis, E. and F. Kalyoncu, 2008. The effects of some environmental parameters on mycelial growth of two ectomycorrhizal fungi, *Tricholoma caligatum* and *Morchella angusticeps*. Mycologia Balcanica, 5 (3).
14. Marchand, A., 1973. Champignons du nord et du midi. Tome II: Les meilleurs comestibles. Societe Mycologique des Pyrenees Mediterraneennes, Perpignan.
15. Breitenbach, J. and F. Kranzlin, 1984. Fungi of Switzerland. Vol. 1. Ascomycetes, Verlag Mycologia, Luzern.
16. Solak, M.H., F.Y. Ersel, E. Kalmı̂0 and F. Kalyoncu, 2005. Morphological and anatomical characterization of *Morchella eximia* f. *schizocostata* Jct. recorded for the first time in Turkey. Acta Edulis Fungi, 12: 92-94.
17. Dighton, J., 1983. Phosphatase production by mycorrhizal fungi. Plant and Soil, 71: 455-462.
18. Kirk, T.K. and H.H. Yang, 1979. Partial delignification of unbleached kraft pulp with ligninolytic fungi. Biotechnol. Lett., 1: 347-352.
19. Weitz, J.H., A.L. Ballard, C.D. Campbell and K. Killham, 2001. The effect of culture conditions on the mycelial growth and luminescence of naturally bioluminescent fungi. FEMS Microbiology Letters, 202(2): 165-170.
20. Hervey, A., G. Bistis and I. Leong, 1978. Cultural studies of single ascospore isolates of *Morchella esculenta*. Mycologia, 70: 1269-1274.
21. Winder, R.S., 2006. Cultural studies of *Morchella elata*. Mycol. Res., 110: 612-623.