The effects of some environmental parameters on mycelial growth of two ectomycorrhizal fungi, *Tricholoma caligatum* and *Morchella angusticeps*

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Abstract. A comparative evaluation was conducted to assess the effects of some environmental parameters such as pH, type of carbon source and temperature on the mycelial growth of two species of ectomycorrhizal fungi, *Tricholoma caligatum* and *Morchella angusticeps.* All carbon sources were found to be equally beneficial for mycelial growth. However fructose and sucrose were better sources of nitrogen. Maximum mycelial growth in Petri dishes was achieved at 25 °C after 8 and 20 days for *T. caligatum* and *M. angusticeps* respectively. Growth was reduced significantly below 15 °C and above 35 °C. Different pH levels (4.5 to 8.0) markedly affected the mycelial growth of the fungi.

Keywords: ectomycorrhiza, *Morchella angusticeps,* mycelial growth, *Tricholoma caligatum*

Introduction

During the last two decades, commercially important wild mushrooms, including chanterelles, morels, *Tricholoma, Lactarius,* and truffles have been harvested from forests around the world. *Tricholoma caligatum,* harvested from *Cedrus* forest in south-west Anatolia in Turkey and *Morchella angusticeps,* also called the black morel fetch high prices and are very important non-wood forest products for the economy of local communities in western Turkey. These mushrooms often form symbiotic relationships with trees in forest ecosystems. Despite many attempts, the commercial cultivation of *T. caligatum* or *M. angusticeps* has not been achieved to date (Ohta 1998; Yamada *et al.* 2001). Almost all families living in villages in Mugla City, Turkey, are actively involved in gathering wild mushrooms; during November and December for *T. caligatum* and April and May for *M. angusticeps.* It is possible for one person to collect almost 4-5 kg of mushroom daily (personal communication with a local farmer).

Ectomycorrhizal fungi are physiologically capable of absorbing amino acids and small peptides from the soil, due to the presence of specific transporter proteins in the plasma membrane (Chalot & Brun 1998; Treseder 2006). Many studies have determined the optimal growth conditions and nutritional requirements of different fungi (Buscot 1992; Ohta 1994; Sanchez *et al.* 2001). The mycelial growth of some ectomycorrhizal fungi has been shown to decline when potassium was limiting but increase when phosphorus was limiting (Wallander & Nylund 1992; Ekblad *et al.* 1995). It has been also reported that *Cantharellus cibarius* (golden Chanterelle) grew best in well-drained forest soils with low nitrogen content and a pH range of 4.0-5.5 (Danell 1994).

The aim of this study is firstly to investigate the effects of various environmental conditions such as pH, carbon sources and temperature on mycelial growth of *T. caligatum* and *M. angusticeps* and, secondly to determine optimum growth conditions in vitro for these organisms.

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Materials and Methods

Culture conditions and storage

Fruiting bodies of *Tricholoma caligatum* and *Morchella angusticeps* used in this study were originally collected from south-west Anatolia in Turkey. After the completion of the macroscopic examinations both on site and in the laboratory, all fruiting bodies were cleaned in wet paper with sterile water, and several pieces of tissue, obtained from the hymenium of *T. caligatum* and the ascocarp of *M. angusticeps,* were transferred to sterile Petri dishes containing approximately 10 ml of liquid modified Hagem's medium; 4 g malt extract, 1 g yeast extract, 5 g D-Glucose, 0.5 g NH_4Cl, 0.5 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 ml $FeCl_3$ (1 % aqueous solution), 100 ml biotin (50 mg/ml aqueous solution), 100 ml thiamine (1 mg/ml aqueous solution) in 1 l dd H_2O (Gregory & Matthew 2002). All cultures were incubated in the dark at 25 °C. Mycelia obtained from tissue were transferred on Potato Dextrose Agar (PDA) and maintained in this medium +4 °C.

Carbon Sources

Fructose, glucose, galactose, sucrose, maltose, lactose, xylose, and arabinose were selected as carbon sources and added at a concentration of 1 % to Kirk's Basal Medium (KBM) (Sanchez *et al.* 2001). Carbon sources were filter sterilized separately and concentrated to prevent possible heat damage.

Temperature and pH

The effect of five temperatures, 15, 20, 25, 30, and 35 °C and eight pH settings, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 on fungal growth was assessed. Cultures of *T. caligatum* were grown on KBM containing 1 % fructose, and *M. angusticeps* on KBM containing 1 % sucrose.

Agar plugs 6 mm in diam., were taken from actively growing colonies on PDA and transferred to the center of Petri dishes. Five replicates were prepared and incubated at 25±1 °C for 30 days. The radial extension of the mycelium was measured, as described by Weitz *et al.* (2001), with a caliper gauge along two diams at right angles to one another and the average for each plate calculated.

Table 1. Effect of carbon source, temperature, and pH on mycelial growth for *Tricholoma caligatum*[a]

Days	Carbon source effect		Temperature effect		pH effect	
	Carbon source	Mycelial growth (mm)	°C	Mycelial growth (mm)	pH	Mycelial growth (mm)
8	**Fructose**	**85 ± 1.254**	15	40 ± 1.421	4.5	62 ± 1.739
	Glucose	78 ± 1.625	20	74 ± 1.365	5.0	76 ± 0.869
	Galactose	80 ± 1.457	**25**	**85 ± 0.856**	**5.5**	**85 ± 1.520**
	Sucrose	70 ± 1.865	30	82 ± 1.012	6.0	80 ± 0.627
	Maltose	62 ± 1.568	35	39 ± 1.652	6.5	78 ± 1.847
	Lactose	68 ± 1.874			7.0	64 ± 2.550
	Xylose	80 ± 1.054			7.5	60 ± 3.217
	Arabinose	52 ± 1.965			8.0	58 ± 2.203
	N. C.	39 ± 1.385				

[a]Best growth for each tested parameter has been indicated with the bold characters in table.

Table 2. Effect of carbon source, temperature, and pH on mycelial growth for *Morchella angusticeps*[a]

Days	Carbon source effect		Temperature effect		pH effect	
	Carbon source	Mycelial growth (mm)	°C	Mycelial growth (mm)	pH	Mycelial growth (mm)
21	Fructose	82 ± 1.029	15	42 ± 1.877	4.5	55 ± 2.961
	Glucose	80 ± 1.454	20	71 ± 1.407	5.0	69 ± 2.122
	Galactose	79 ± 1.312	**25**	**85 ± 1.302**	5.5	77 ± 1.286
	Sucrose	**85 ± 1.692**	30	80 ± 1.362	**6.0**	**85 ± 1.469**
	Maltose	66 ± 1.390	35	40 ± 2.137	6.5	80 ± 1.087
	Lactose	70 ± 1.880			7.0	72 ± 2.139
	Xylose	76 ± 1.708			7.5	65 ± 2.732
	Arabinose	50 ± 1.848			8.0	57 ± 2.778
	N. C.	35 ± 1.270				

[a]Best growth for each tested parameter has been indicated with the bold characters in table.

Statistical Analysis

All statistical analyses were performed using Systat 11 (SPSS Inc., Chicago, IL, USA) on a PC running Windows XP.

Results

Tricholoma caligatum showed a greater comparative mycelial growth rate than *M. angusticeps* under all test conditions. Furthermore, none of the media inhibited mycelial growth of the two fungi.

Effects of carbon sources on mycelial growth

As shown in Table 1, all carbon sources tested in this study were found to similarly effective while fructose, galactose and xylose were the best nitrogen sources for *T. caligatum.* Sucrose, fructose and glucose were the best nitrogen source for *M. angusticeps* (Table 2).

Temperature effects

25 and 30 °C were shown to be the most effective temperature for mycelial growth of *T.* caligatum and *M. angusticeps.* There was little mycelial growth at 15 and 35 °C, approximately half of that measured at 25 °C. The mycelial growth rate of *T. caligatum* at 25 °C (as well as 30 °C) was greatest between the days 4 and 8 (20 mm diam. per two days). *M. angusticeps* exhibited more regular mycelial growth at 25 and 30 °C (Tables 1-2).

pH effects

The effects of pH on mycelial growth was determined at eight different pH levels. Both *T. caligatum* and *M. angusticeps* grew over the pH range of 4.5-8.0 with the greatest growth rate at pH 5.5 for *T. caligatum* and pH 6.0 for *M. angusticeps* (Tables 1-2). The mycelial growth of *T. caligatum* reached 85 mm at pH 5.5-6 on day 8 and *M. angusticeps* reached 85 mm at pH 6-6.5 on day 21.

Discussion

Ectomycorrhizal fungi exploit carbon and other essential organic substances from their host tree and in return assist the host tree absorb mineral salts, water and metabolites. In this comparative study, two different ectomycorrhizal macrofungi, *T. caligatum* and *M. angusticeps,* were examined for their mycelial growth in various culture conditions, pH, temperature, carbon sources. Arabinose was found to be ineffective on mycelial growth. Several studies have reported a considerable difference in the utilization of mannose and cellobiose among *Suillus luteus* and *S. grevillei* (Chu-Chou & Grace, 1988; Bending *et al.* 2002). It was also reported that five different species of ectomycorrhizal mushrooms were unable to utilize both mannose and cellobiose (Hatakeyama & Ohmasa 2004).

In culture conditions and in nature, many basidiomycetes that are ectomycorrhizal with forest trees, form a sheath of mycelium covering the root and penetrating the cortex, and are able to utilize peptides and proteins, as sources of nitrogen and carbon. The fastest and most extensive degradation of complex carbon and nitrogen sources is caused by fungi. Basidiomycetes are most effective, but some ascomycetes and anamorphic fungi are also effective.

It has been reported that mycelium of *Tricholoma robustum,* an ectomycorrhizal macrofungus related to *T. caligatum* and *T. matsutake,* was not able to grow in a medium containing sucrose as a sole carbon source (Iwase 1992). The secretion of brown pigments into the culture medium, which may inhibit growth, has been reported for different ectomycorrhizal fungi, including *T. robustum* and *T. bakamatsutake* (Ogawa 1981). No mycelial pigmentation was observed in this study.

As noted by Ogawa (1978), mycelium of *T. caligatum* obtained from northern African and southern European fruiting bodies grew well below 25 °C. Mycelial growth was inhibited above at 30 °C. It is interesting to note that mycelium of *T. caligatum* used in this study showed weak growth at 35 °C.

To our knowledge the present study is the first report on *in vitro* utilization of various carbon sources by two economically important ectomycorrhizal macrofungi. Different levels of carbon utilization and responses to pH and temperature change may be indicate an underlying genetic diversity of strains that may have potential for future exploitation.

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