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Effect of nutrients on mycelial growth and sclerotia formation in *Morchella esculenta*

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ABSTRACT: Basic biology and life cycle of morels has established the sclerotium as the central point in the morel fruiting. Late isolated sclerotia (LIS) have temperature requirements, cold resistance and biochemical properties similar to the sub-terranean mycelial structure connected with the ascocarp in nature. Six culture media viz; Malt Extract Agar (MEA), Potato-dextrose-agar (PDA), Complete Medium+Yeast Extract (CYM), Medium A, Medium B and Czapex's Dox Agar were tested for radial mycelial growth and sclerotia formation with four isolates *of M. esculenta.* Malt Extract Agar followed by complete Medium+Yeast Extract was found the best culture medium to support the maximum radial growth in all the test isolates. As regards sclerotia formation. Malt Extract Agar medium was the only medium on which all the test isolates *of M. esculenta* could form sclerotia in plenty. The rest of the culture media though supported radial mycelial growth but failed to initiate sclerotia formation uniformly in all the test isolates *of M. esculenta.* The present study indicates that nutrient poor conditions are not essential for sclerotia production as they could be produced on nutrient rich media also.

1 INTRODUCTION

The genus *Morchella* is well represented in India. Its various species grow abundantly in Jammu and Kashmir, Himachal Pradesh and hilly tracts of Uttar Pradesh (Jandaik & Sharma, 1995; Kaul, 1997). Mycologists have been making sustained efforts since early times for cultivating morels. Break through in this direction came with the description of development of morel ascocarp and subsequent patent of a cultivation process by Ower *et al.* (1986, US patent 4594809). This led to detailed study of basic biology and life cycle of morels and establishment of sclerotium as the central point in morel fruiting (Ower *et al,* 1986; Volk & Leonard 1989, 1990). Amir *et al* (1993) used split plate method for its culture upto sclerotia formation. Buscot, (1993) reported two types of sclerotia i.e., 'early encrusting sclerotia (EES) and 'late, isolated sclerotia (LIS). EES initiation was promoted by growth interruptions and are very small in size, whereas LIS initiation is related to ageing of cultue and are bigger in size. They reported that LIS have temperature requirements, cold resistance and biochemical properties (quality and type of mycosporin) similar to the subterranean mycelial structure connected with the ascocarp in nature. The latter were reported to function as storage and resting structures from which resources are redistributed to fructification in the spring (Buscot 1989; Buscot & Bernillon, 1991). Recently Singh et al. (1999) reported a modified jar technique for mass production of carpogenic selerotial spawn in *Morchella esculenta* under *in vivo* controlled conditions.

The nutritional requirements for sclerotia formation by *Morchella* spp. have received little attention. Volk & Lenoard (1989) found that although vegetative growth *of Morchella* sp. on most media was excellent, the production of sclerotia was relatively poor. The few small sclerotia (EES) that developed did not contain enough nutrient reserve to support the formation of fruiting bodies.

This paper presents effect of different culture media on mycelial growth and sclerotia formation in *Morchella esculenta*, the most common species of the genus.

2 MATERIALS AND METHODS

2.1 *Culture Medium Preparation*

Six culture media viz., Potato dextrose-agar (PDA), Malt Extract Agar (MEA), Complete Medium+Yeast Extract (CYM), Medium A, Medium B and Czapex Dox Agar were steam sterilized before use. Most of these culture media are commonly used in mycological laboratory except Medium A and Medium B compositions of which have been described by Buscot (1993). Medium A comparised (gH) agar,10, malt extract, 5, glucose, 10. Medium B comprised (gH) agar, 10, yeast extract, 4, strach, 15, glucose, 10 KH_2PO_4, 1, Mg $SO_4.7H_2O$, 0.5. The pH was adjusted to 6.5 before autoclaving (20min./121°C).

2.2 *Pure Culture and growth studing*

Four isolates of *M. esculenta* namely, OE 1; ME-1; ME-2 and ME-3 were collected from hilly region of Northern India (Himachal Pradesh). Multispore isolations were obtained from the inner walls of fresh ascocarp on Malt Extract Agar Culture Medium (MEA) at 25 ± 2 C. Five petriplates/isolate/culture medium were used for testing different culture media and fungal isolates. A 5mm circular mycelial disc was carefully transferred under asceptic conditions in the centre of each petriplate. All the inoculated petriplates were then incubated at 25 ± 2 C and observed daily for radial mycelial growth and sclerotia formation. Time taken for completing radial mycelial growth (90 mm) and initiation of sclerotia formations was recorded in days.

3 RESULTS

The results of the effect of different culture media on radial mycelial growth in *M. esculenta* isolates are presented in Table 1. It is evident from the data that the radial mycelial growth of different *Morchella* isolates viz., OE-1, ME-1, ME-2 and ME-3 were greatly influenced by the nutrients of different culture media. Out of the six culture media tested, malt extract agar followed by complete medium + yeast extract was found to be the best culture medium to obtain the maximum radial growth in all the test isolates. Potato dextrose agar medium was also found suitable for the growth of the test isolates except ME-3 in which radial growth could not be completed upto an extended period of 16 days. The cultrue media viz., Czapek's Dox Agar, Medium A and Medium B were not found suitable as all the test isolates took comparatively more times to complete the radial growth. The isolate ME-3 could not complete the radial growth on Medium B and Czapex's Dox Agar Medium.

As regards initiation of sclerotia (LIS) formation in *M. esculenta*, six culture media were tested and results are shown in Table-2. Despite mycelial growth in Medium A, Medium B and Czapek's Dox Agar formation of sclerotia was not initiated. Malt Extract Agar culture medium was found to be the best for sclerotia formation by all the test isolates. This was followed by Potato-dextrose-agar in which all the test isolates except ME-3, exhibited formation of sclerotial bodies. Whereas, Complete Medium + Yeast Extract Culture Medium, despite faster mycelial run did not support sclerotia formation in OE-1 isolate but was recorded best for LIS production in isolates ME-1 and ME-2.

4. DISCUSSION

Present investigation exhibits that the mycelial run was comparatively faster on MEA and complete medium + yeast extract being rich in nutrition. Paries et al (1996) also reported denser mycelial growth on MEA- side than on plates with growth originating from the CLA-

Table-1. Effect of different culture media on rate of mycelial growth in different isolates of *Morchella* species.

Culture Medium	No. of days to complete radial mycelial growth (90mm)			
	O I M M E - 1		ME-2	ME-3
Potato-Dextrose- Agar	6.6 (13.63)	5.0 (18.0)	5.8 (15.51)	(-)
Malt-Extract-Agar	5.6 (16.07)	5.0 (18.0)	5.1 (17.64)	6.0 (15.0)
Complete Medium + Yeast Extract (CYM)	5.8 (15.51)	5.0 (18.0)	5.0 (18.0)	7.0 (12.58)
Mediium A	7.4 (12.16)	5.8 (15.51)	7.5 (12.0)	9.7 (9.27)
Medium B	7.8 (11.53)	12.0 (7.5)	10.3 (8.73)	(-)
Czapek's Dox Agar	10.7 (8.41)	9.7 (9.28)	11.0 (8.18)	(-)

() = Values in parenthesis represent mean growth rate/day.
(-) = Radial mycelial growth could not be completed in 3 weeks.

Table-2. Effect of different culture media on initiation of scelerotia formation in *M.esculenta* isolates.

Culture Medium	Days for initiation of late isolated sclerotia formation			
	OE-1	ME-1	ME-2	ME-3
Potato-Dextrose- Agar	9.0	8.0	10.4	-
Malt-Extract-Agar	9.0	11.8	9.4	6.3
Complete Medium + Yeast Extract (CYM)	(-)	8	8	7.4
Mediium A	(-)	(-)	(-)	(-)
Medium B	(-)	(-)	(-)	(-)
Czapek's Dox Agar	(-)	(-)	(-)	(-)

(-) = Sclerotia formation did not initiate within three weeks.

side in split plate tests due to the availability of more nutrients.

Buscot & Bernillon (1991) reported that late isolated sclerotia and subterranean masses exhibited similar contents of biochemical compounds which indicates that both represent a similar early stage in the fructification process. This biochemical analogy is consistent with their common ecophysiological features (Buscot, 1989), and confirms that late isolated sclerotia are a cultural form of the subterranean mycelial masses found in nature, which are storage and frost resistant organs, at the expense of which acocarps develop. Paris *et al.* (1996) also reported CYM as the optimum agar medium for sclerotia production. They also reported that more sclerotia formed in split plate culture when mycelia grew from carnation leaf agar (CLA) culture medium to malt extract agar (MEA) than when they grew from MEA to CLA. Out of 42 isolates tested, 12 of them formed sclerotia within 6 weeks and 30 of them after 6 weeks. They reported that there were no signifcant difference (P=0.05) in the number or size of sclerotia produced by the three isolates. In contrast with the findings of Amir *et al.* (1992) and Philippoussis and Balis (1995) who both reported sclerotia production on both the nutrient-rich and nutrient-poor media sides. Present study indicates that nutrient poor condition is not essential for sclerotia production as they could be produced on nutrient rich media viz., MEA, CYM and PDA also.

REFERENCES

Amir, R., Lavanon, D., Hadar, Y., & Chet, I. 1992. Formation of sclerotia by *Morchella esculenta :* relationship between media composition and turgor potential in the mycelium. *Mycol Res.* 96 : 943-948.

Amir, R., Lavanon, D., Hadar, Y. & Chet, I. 1993. Morphology and physiology of *Morchella esculenta* during sclerotia formation. *Mycol. Res.* 97 : 683-689.

Buscot, F. 1989. Field observations of growth and development of *Morchella rotunda* and *Mitrophora semilibera* in relation to forest soil temperature. Canadian Journal of Botany 67: 589-593.

Buscot, F. 1993. Mycelial differentation of *Morchella esculenta* in pure culture. *Mycol. Res.* (2): 136-140.

Buscot, F. & Bernillon, J. 1991. Mycosporins and related compounds in field and cultured mycelial structures *of Morchella esculenta. Mycological Res.* 95:752-754.

Paris, H., Broderick, A. & Nair, N.G. 1996. Occurrence and intitial observations *of Morchella* in Australia in *Mushroom Biology* and *Mushroom Products.* Royse (ed.)Penn State Univ.

Jandaik, J.L. & Sharma, S.R. 1995. Present status of *Morchella* in India. *In* advances in Horticulture Vol. 13. (Ed) K.L. Chadha and S.R. Sharma. Malhotra Publishing House, New Delhi- 110 064.

Kaul, T.N. 1997. Recent developments in morel biology *in* adnvaces in Mushroom biology and production (Rai, Dhar & Verma, eds.), Mushroom Society of India, NRCM, Solan.

Ower, R., Mills, G. & Malachowshi, J. 1986. Cultivation of *Morchella* US patent No. 4,594, 809.

Phillippoussis, A. & Balis, C. 1995. Studies on the morphogenesis of sclerotia and subterranean mycelial network of ascocarp in *Morchella* species. *Mushroom Science* 14: 847-855.

Singh, S. K., Dhar, B. L. & Verma, R. N. 1999. Mass production of carpogenic sclerotial spawn in *Morchella esculenta* - An attempt at its domestication, *in* proceedings of 3rd International Conference on mushroom biology and mushroom products (Ed. Andrew Broderick & Tan Nair) 12-16 Oct., 1999, Sydney (Australia).

Volk, T.J. & Leonard, T.J. 1989. Experimental studies on the morel. I. Heterokaryon formation between monoascosprous strains *of Morchella Mycologia* 81 : 523-531.

Volk, T.J. & Leonard, T.J. 1990. Cytology of the life-cycle of *Morchella Mycol. Res.* 94(3): 399-406.