Biotechnol Lett (2010) 32:1523–1527
DOI 10.1007/s10529-010-0328-3

ORIGINAL RESEARCH PAPER

Biotechnology of morel mushrooms: successful fruiting body formation and development in a soilless system

Segula Masaphy

Received: 7 April 2010 / Accepted: 7 June 2010 / Published online: 19 June 2010
© Springer Science+Business Media B.V. 2010

Abstract *Morchella* spp. ascocarps (morels) are some of the world's most sought-after mushrooms. Successful cultivation of morels is still a rare and difficult task despite over 100 years of effort. Here we provide the first report of successful *Morchella rufobrunnea* fruiting body initiation and development in laboratory-scale experiments. Mushroom initials appeared 2 to 4 weeks after first watering of pre-grown sclerotia incubated at 16 to 22°C and 90% humidity. Mature fruiting bodies reached 7 to 15 cm in length and were obtained after the five morphological developmental stages of this *Morchella* species: sclerotium formation, scelerotium germination, asexual spore formation, formation of initial knots and development of the fruiting body.

Keywords Fruiting body · *Morchella rufobrunnea* · Morel · Morphological development · Mushroom cultivation · Soilless culture

Purpose of the work The purpose of this work was to study the option of cultivating the saprophytic species *Morchella rufobrunnea* in a soilless system and its different developmental stages during the process.

S. Masaphy (✉)
Applied Microbiology and Mycology Department, MIGAL, P.O. Box 831, 11016 Kiryat Shmona, Israel
e-mail: segula@migal.org.il

S. Masaphy
Tel Hai Academic College, 12210 Upper Galilee, Israel

Introduction

Morels (*Morchella* spp.) are naturally growing and commercially important edible mushrooms with a delicate taste and a unique appearance that make them one of the world's most sought-after mushrooms (Carluccio 1989). In the western world, morels are considered a gourmet food, resulting in their increased harvesting from the wild. In nature, morels have a short season, usually on the order of a few weeks, mainly in spring, and fresh mushrooms can only be found in the markets for a few weeks per season. This, and the increasing number of reports on accumulation of metal in ascocarps that are picked from natural habitats (Isildak et al. 2004; Shavit 2008), have resulted in the need to develop a biotechnological process to cultivate morels under controlled conditions.

Although such efforts began over 100 years ago (Roze 1882), it was only in 1982 that the first indoor cultivation of morels was reported (Ower 1982), with *Morchella esculenta* being the first *Morchella* species, followed by others, reported as culturable by the same author (Ower et al. 1986). However, growth of morel ascocarps under controlled conditions and repeating Ower's success have proven to be difficult tasks. The failure to cultivate morels has been suggested to be partly related to insufficient knowledge of the factors controlling fruiting body initiation and development, but also to insufficient knowledge of which species are suitable for cultivation, the latter being an important aim.

In the past, morels were divided phenotypically into two groups, the Black and Yellow morels (Boudier 1897), and were studied as such for their indoor cultivation options (Ower et al. 1986). Recently, a new group of morels has been characterized: the red-brown blushing morel represented by the species *Morchella rufobrunnea*. It differs morphologically from the known Yellow and Black morel species (Kuo 2008; Masaphy et al. 2009; Pilz et al. 2007) in having a conical head with pale tan pits and reddish brown ridges when mature. This species has been found in different sites throughout the world, and confirmed as genetically distinct from the Yellow and Black morels (Guzman and Tapia 1998; Kuo 2008; Masaphy et al. 2009; Pilz et al. 2007). The various habitats in which it has been discovered—disrupted soil in a healthy forest, the forest after a fire, and road beds—imply its saprophytic ecotype.

Here we provide a first report on the successful initiation and development of fruiting bodies of *M. rufobrunnea* in a soilless controlled process which has been conducted at MIGAL's mushroom experimental cultivation growth facility since 2002. The developmental changes in the fungus's morphology during the cultivation process are also reported.

Materials and methods

Fungal source

The *M. rufobrunnea* (MIGAL strain MS3-730) described in this report has been reported previously (Masaphy 2005).The isolate has been preserved in the author's collection at MIGAL Institute (Kiryat Shmona, Israel).

Growth conditions

Growth conditions were as described in an earlier work (Masaphy 2005). For the sclerotial production phase of the process, the fungal mycelium was inoculated into a sterile (1 h at 120°C) of potting soil buffered with limestone. The inoculated medium was placed over a layer of nutritionally rich medium based on wheat grains, and incubated for 2–3 weeks at 18–25°C. The sclerotium-containing layer was kept at 4°C until its use for the second phase of the process, in which sclerotia were subjected to continuous watering for 5–24 h. The induced sclerotia were then incubated at 16–22°C for 2–4 weeks for carpogenic initiation and fruiting body development. The different morphological developmental stages were followed during the growing process and photographed.

Scanning electron microscopy

Conidial production was studied by scanning electron microscopy (SEM) according to Masaphy (2005). A sample of the conidial layer was transferred to a solution of 4% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h. The sample was dehydrated in a graded series of ethanol washes (10, 25, 50, 70 and 100% ethanol for 60, 15, 15, 60, 60, 15 min, respectively). After dehydration, the samples were critical-point-dried through liquid CO_2 adhered to stubs, coated with a gold–palladium layer, and observed under a scanning electron microscope (SEM) (JEOL JSM 35C, Japan).

Results and discussion

In nature, morel fruiting body formation is associated with a broad range of environmental stress conditions, some of which have yet to be defined. This conclusion is based on the high diversity of habitats in which morels are found (Pilz et al. 2007), and the high number of *Morchella* species reported (Gessner 1995).

The *M. rufobrunnea* isolate used in this study produced nutritionally rich sclerotia on the growth medium (Fig. 1).Generally, fungal sclerotia are multicellular storage structures that are also found in the soil in nature (Miller et al. 1994). *Morchella* sclerotia have been suggested to protect the fungal cells from the harsh environmental conditions in winter (Miller et al. 1994; Volk and Leonard 1989). In the present study, *M. rufobrunnea* production of sclerotial biomass was obtained before triggering the fungal biomass to produce fruiting bodies. Sclerotia developed on the surface and within the potting soil layer after exploitation of the nutrients from the cereal-rich medium by translocating them to the sclerotia, as reported by Amir et al. (1995) and Volk and Leonard (1989). The produced sclerotia varied in

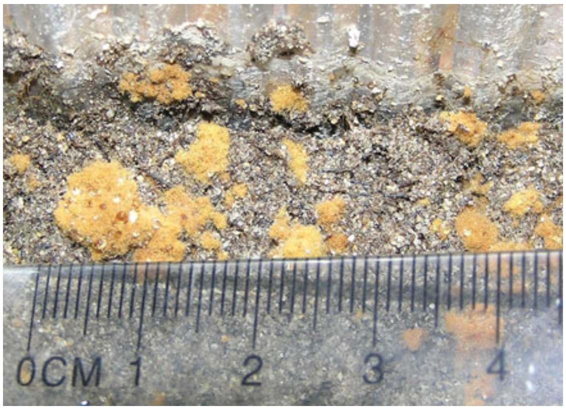

Fig. 1 Mature sclerotia produced by *M. rufobrunnea* on the surface of potting soil, 1 month after inoculation. Ruler is added to provide scale for sclerotial size

size and shape, but they became brown upon maturation, reaching over 1 cm in size (Fig. 1).

In the second phase of the growth process, after a period of incubation at 4°C, the sclerotium-containing medium was subjected to induction of fruiting body initiation and development. This was achieved by continuous watering of the mature sclerotia as suggested by Ower et al. (1986). Watering is known to influence carpogenic germination of sclerotia in other sclerotium-producing fungi, such as *Sclerotinia sclerotiorum*, where irrigation levels affected direct apothecial production from the sclerotia (Twengström et al. 1998). Volk and Leonard (1990), in describing the full life cycle of morels, suggested that after watering, morel sclerotia may germinate into vegetative mycelium, which may then produce the carpophores; alternatively, the sclerotia may undergo direct carpogenic germination as in other ascomycetes (Twengström et al. 1998). In our study, within 3–6 days after watering, the *M. rufobrunnea* sclerotia germinated into vegetative mycelium, but no direct carpophore production from the sclerotia was observed in any of the experiments. Once the vegetative mycelium covered the medium surface, it produced a dense white layer of asexual conidia. SEM observation (Fig. 2) showed that the conidia are borne on phialides which are produced in a whorl around each conidiophore, a single spore per phialide. This is the first report of asexual spore production by *M. rufobrunnea*; however, the condiophore and conidial arrangement was similar to that described in Weber (1995) and Pilz et al. (2007) for other *Morchella* species. The conidial stage has never been reported for natural morel-bearing sites although this stage is similar to the conidial arrangement of *Constantinella cristata* Matr., described by Constantin (1936), which was suggested to be the *Morchella* anamorph stage.

After decline of the conidial layer, small regular initial knots gradually appeared on the medium surface, followed by production of the fruiting body primordia. The early developmental stages of primordium development, monitored by SEM, were described in an earlier report (Masaphy 2005). Following primordium initiation, the fruiting body gradually developed, resulting in mature fruiting bodies within 2–3 weeks of initiation (Fig. 3a–d). During the course of development, the head of the

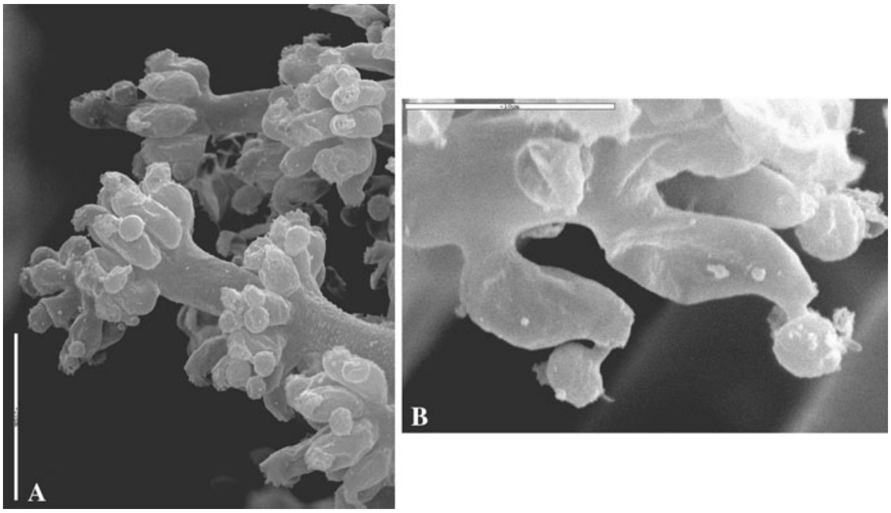

Fig. 2 SEM image of asexual spores produced *M. rufobrunnea* after watering the sclerotial layer. **a** Conidiophore with conidia. **b** A single conidium on a phialide. *Bars* represent 20 and 10 µm for images **a** and **b**, respectively

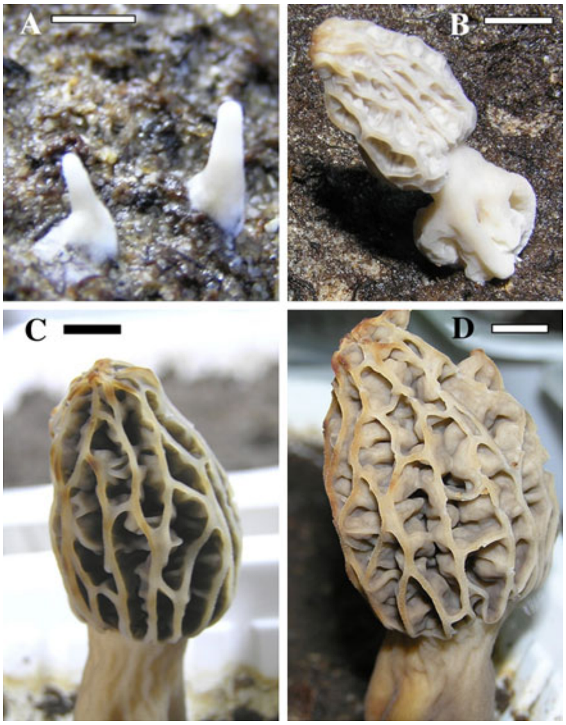

Fig. 3 *M. rufobrunnea* fruiting body development in controlled conditions growth system. **a** 2-day-old white initials, **b** 7-day-old white fruiting body, **c** 15-day-old reddish brown fruiting body, and **d** 18-day-old mature fruiting body. *Bars* represent 1 cm

fruiting body changed in size and color. Under the indoor conditions, at the early stages of fruiting body growth, the whole head was white, including ridges and pits, and in some cases this coloring persisted until the fruiting body reached 5 cm (Fig. 3a, b). Upon fruiting body maturation, the ridge and pits turned light brown to beige (Fig. 3c, d), while its shape was either conical or rounded. The later stages of fruiting body development under the controlled conditions were similar in head morphology (conical to round shape) and head color to the natural mushrooms (Fig. 3). The mature fruiting body reached 7–15 cm in length. In nature, as under controlled conditions, these phenotypic features—fruiting body size, shape and color, are affected by, and vary with developmental dynamics and environmental conditions.

Conclusions

Here we provide the first report of successful *M. rufobrunnea* fruiting body production under controlled conditions, and *M. rufobrunnea*'s entire life cycle in the experimental controlled cultivation system is described. Some of these developmental stages have been previously described for other reported cultured *Morchella* species (Ower 1982; Ower et al. 1986). However, *M. rufobrunnea* was not mentioned in the list of cultivable species in those reports. The results may indicate identical life-cycle stages in different species of *Morchella*, within the groups of Black, Yellow or Red-Brown morels. Successful fruiting body initiation of *M. rufobrunnea* in soilless culture confirms that it is a saprophyte, and the search for other saprophytic species that can produce fruiting bodies under controlled conditions is therefore warranted. However, this goal will be easier to achieve once the species confusion surrounding the genus *Morchella* is resolved.

Acknowledgements The authors thank Prof. Dan Levanon, Dr. Doron Goldberg and Dr. Ofer Danai for their help. This work was supported by the Office of the Chief Scientist, Ministry of Agriculture, Israel.

References

Amir R, Levanon D, Hadar Y, Chet I (1995) Factors affecting translocation and sclerotial formation in *Morchella esculenta*. Exp Mycol 19:61–70

Boudier E (1897) Révision analytique des morilles de France (Taxonomic revision of the morels of France). Bulletin de la Société Mycologique de France (Bull French Mycol Soc) 13:129–153

Carluccio A (1989) A passion for mushrooms. Salem House Publishers, Topsfield

Constantin J (1936) La culture de la morille et sa forme conidienne (Morel culture and its conidial form). Ann Sci Nat Bot Ser 10:111–129 (in French)

Gessner RV (1995) Genetics and systematics of North American populations of *Morchella*. Can J Bot 73(Suppl 1):S967–S971

Guzman G, Tapia F (1998) The known morels in Mexico, a description of a new blushing species, *Morchella rufobrunnea*, and new data on *M. guatemalensis*. Mycology 90:705–714

Isildak O, Turkekul I, Elmastas M, Tuzen M (2004) Analysis of heavy metals in some wild-grown edible mushrooms from the middle Black Sea region, Turkey. Food Chem 86:547–552

Kuo M (2008) *Morchella tomentosa*, a new species from western North America, and notes on *M. rufobrunnea*. Mycotaxon 105:441–446

Masaphy S (2005) External ultrastructure of fruit body initiation of *Morchella* spp. Mycol Res 109:508–512

Masaphy S, Zabari L, Goldberg D (2009) New long season ecotype of *Morchella rufobrunnea* from Northern Israel. Mycol Apl 21:45–55

Miller SL, Pilar T, McClean TM (1994) Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia 86:89–95

Ower R (1982) Notes on the development of the morel ascocarp. Mycologia 74:142–144

Ower R, Mills G, Malachowski J (1986) Cultivation of *Morchella*. U.S. Patent No 4,594,809

Pilz D, McLain R, Alexander S, Villareal-Ruiz L, Berch S, Wurtz T, Parks C, Mark E, McFarlen E, Baker B, Molina R, Smith JE (2007) Ecology and management of morels harvested from the forests of western North America. USDA General Technical Report, Portland, OR. http://www.fs.fed.us/pnw/publications/gtr710/

Roze ME (1882) Adherence de la base d'appareils ascospores de *Morchella* sur *Helianthus tuberosus*. Bull Soc Bot Fr 19:166–167

Shavit E (2008) Arsenic in morels: morels collected in New Jersey apple orchards blamed for arsenic poisoning. Fungi 1:8–16

Twengström E, Köpmans E, Sigvald R, Svensson C (1998) Influence of different irrigation regimes on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. J Phytopathol 146:487–493

Volk TJ, Leonard TJ (1989) Physiological and environmental studies of sclerotium formation and maturation in *Morchella*. Appl Environ Microbiol 55:3095–3100

Volk TJ, Leonard TJ (1990) Cytology of the life cycle of *Morchella*. Mycol Res 94:399–406

Weber NS (1995) A morel hunter's companion: a guide to the true and false morels. Thunder Bay Press, Holt