BCCM/MUCL - (Agro)Industrial Fungi & Yeasts Collection
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*Protocol of cryopreservation of ectomycorrhizal fungi.*

In

I. Lalaymia, S. Cranenbrouck and S. Declerck.
Maintenance and preservation of ectomycorrhizal and arbuscular mycorrhizal fungi
Mycorrhiza (2013) – DOI 10.1007/s00572-013-0541-8

Adapted from
C. Crahay, S. Declerck, J.V. Colpaert, M. Pigeon, F. Munaut
Viability of ectomycorrhizal fungi following cryopreservation
Fungal Biology (2013) 11: 103-111.

Protocol:

(1) Isolates are grown on agar medium in Petri plates and incubated in the dark at 22–23 )C for 2 to 4 weeks.

(2) A mycelium plug of ~4 mm diameter is sampled from the margin of the growing colony and inoculated into a 2-ml sterile polypropylene cryovial containing 750 µl of sterilized (121 °C for 15 min) MFM agar medium poured in a slope.

(3) Cryovials are incubated at 22– 23 °C in the dark for 7 to 9 weeks and

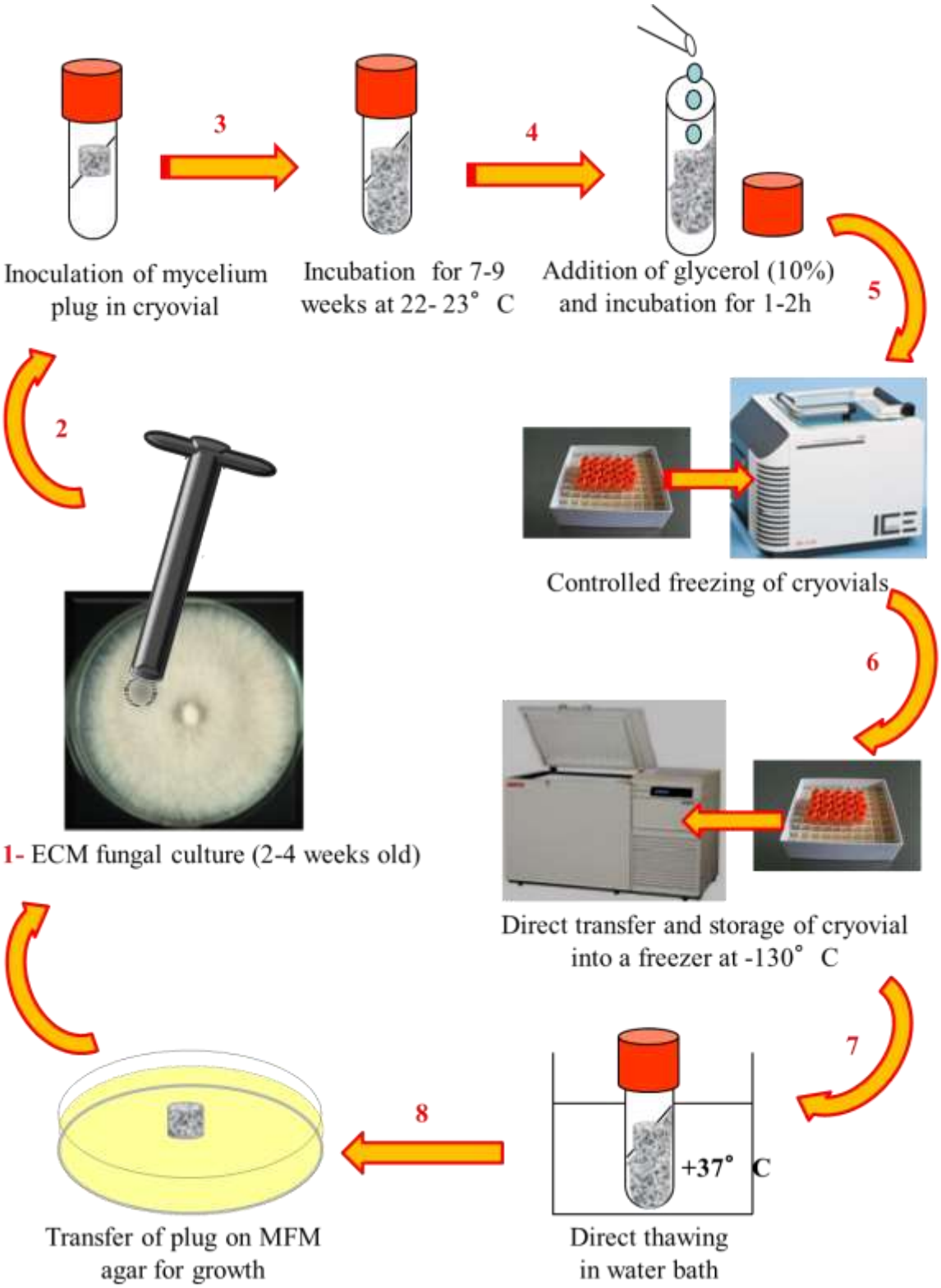
(4) 500 µl sterilized (121 °C for 15 min) glycerol cryoprotectant solution (10%v/v) is added into the cryovial for 1 to 2 h before cryopreservation.

(5) Cultures are cryopreserved by controlled decreases in temperature (8 °C min−1 from + 20 to +4 °C; 1 °C min−1 from +4 to −50 °C; 10 °C min−1 from −50 to −100 °C).

(6) The cultures are directly transferred into a freezer at −130 °C.

(7) For revival, the isolates are directly thawed in a water bath at +38 °C for 2 min.

(8) Viability is checked by transferring plugs of cultures on 30 ml MFM in a Petri dish and incubated at 22–23 °C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+37° C
Inoculation of mycelium plug in cryovial
Incubation for 7-9 weeks at 22- 23° C
Addition of glycerol (10%) and incubation for 1-2h
Controlled freezing of cryovials
Direct transfer and storage of cryovial into a freezer at -130° C
Direct thawing in water bath
Transfer of plug on MFM agar for growth
1- ECM fungal culture (2-4 weeks old)