Protocol for Cryopreservation of ECM fungi

Adapted from:

Danell, E. & Flygh, G. *Cyropreservation on the enctmycorrhizal mushroom Cantharellus cibarius.* Mycol. Res 106 (11): 1340-1342 (November 2002).

Materials:

ECM fungal culture
2 Petri plates of Modified Melin-Norkrans (MMN) medium per isolate

Small ice bucket w/ ice
Lab tape
Blade
Cryopen
Sterile needle and syringe
Timer
Nalgene Cyro 1°C Freezing Container (Nalgene Cat. No. 5100-0001)
Isopropyl alcohol
1.8-ml sterile cryovials with lids (Nunc 377267)
200-µl filtered pipette tips
5-ml filtered pipette tip
1 sterile Petri plate per isolate
Metal spatula
Sterile DMSO

(Autoclave:)
Tip-up glass pipettes*
Tip-down glass pipettes*
Cryorack
250-ml flask w/ stainless lid (1 per isolate)
25-ml flask
50-ml graduated cylinder
Sterile wide-mouth screw-top test tube
4M sorbitol per isolate (2.22 ml per isolate)
Liquid MMN (20 ml per isolate)

*Burdsall, H.H., Jr., and E.B. Dorworth, Mycologia 86: 275-280, 1994

Prep:

Prepare axenic ECM cultures on MMN medium in 9-cm Petri dishes. Cultures should be less than two months old with enough mycelial growth to supply aprox. 6 sq. cm of surface biomass, preferably around the leading edge where new growth is occurring.

Autoclave all glassware (50-ml graduated cylinder for measuring media into flasks, 25-ml flask for measuring sorbitol, 1 250-ml flask per isolate, up-tips and down-tips).

Procedure:

Day1

In hood, flame tip-up glass pipets, cool and punch 24-36 plugs into mycelium around leading growth edge. Using tip-down pipets, lift each plug and place into 250-ml flask. Label flask with species name and ID number. Add 20ml of liquid MMN (no agar) and 105µl of 4M sorbitol every 3 min for 30 min. Replace lid and continuously swirl flask between additions. After 10 additions, replace lid, wrap flask in tin foil and place on rotary shaker at slow speed (ca. 90 rpm). Incubate on shaker at room temperature for 24 hours.

Day2

In hood, remove foil from flask and add 117µl of 4M sorbitol every 3 minutes for 30 minutes. Replace lid and continuously swirl flask between additions. After 10 additions, replace lid, wrap flask in tin foil and replace on rotary shaker on slow speed. Incubate on shaker at room temperature for 24 hours.

Day3

Allow at least 2 hours for this step.

In hood, using a sterile needle and syringe invert bottle of DMSO and push sterile needle through rubber stopper and extract 1.2ml per isolate. Place in sterile screw top test tube.

In hood, remove foil and place flask on ice. Add 117µl DMSO every 3 minutes for 30 minutes. Replace lid and continuously swirl flask between additions. After 10 additions, allow the flask to sit on ice, undisturbed, for 30 min. Dump contents of flask into sterile Petri plate and, using tip-down pipettes, place 3-4 mycelial plugs into each of 6 cryovials. If plugs have melted into agar slurry, you pipet fungus into cryotubes. Using pipette with 5ml plastic tip with cut edge (cut in the hood with flamed blade), add aprox. 1ml of agar slurry up to fill-line on cryovial. Place cryovial in cryorack and close cap tightly. Label tubes with species, ID #, and date. Place cryovials into Nalgene Freezing Container and place in freezer for 24 hours.

Day 4

See main Cryostorage Protocol step #3.