

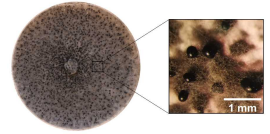


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Version 1

Protocol for the production and storage of *Diplodia sapinea* pycnidiospores V.1

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Macroscopic and microscopic appearance of a VMM cultivation plate after incubation under constant light (5000–6000 lx) for 21 d on VMM. Pycnidia release spore-containing fluid droplets.

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We use this protocol and it's working

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Abstract

An efficient, standardized protocol for the production and storage of highly viable pycnidiospores of *Diplodia sapinea*.

Image Attribution

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Materials

Material, media and solutions:

- Petri dishes (5.5 cm)
- *D. sapinea* (e.g. ex-type strain CBS 138184)
- Light shelf with cold white daylight (Osram Lumilux 18W/865), intensity from 5000 to 6500 lux
- Cleanbench
- Centrifuge

Minimal medium (VMM):

as described in Vogel, 1964

20 ml Vogel's solution

20 g sucrose

15 g agar

add 1 l water autoclave

Trace element solution:

50 g citric acid

50 g zinc sulfate (J.T. Baker)

10 g ammonium iron(II) sulfate

2,5 g copper sulfate

0,5 g manganese sulfate

0,5 g boric acid

0,5 g sodium molybdate

add 1 l water

Chloroform (1 ml) is added as a preservative, store at room temperature.

Vogel's solution:

125 g sodium citrate

250 g potassium dihydrogen phosphate

100 g ammonium nitrate

10 g magnesium sulfate

5 g calcium chloride

5 ml trace element solution

2,5 ml biotin solution

add 1 l water

Chloroform (2 ml) is added as a preservative, store at room temperature.

Biotin solution:

0,1 g biotin

add 1 l water

Store at -20°C



0,01 % (v/v) Tween:

0,1 ml Tween

add 1 l water

aliquod and autoclave

40% (v/v) glycerol

200 ml glycerol

add 1 l water

aliquod and autoclave



- 1 Inoculate a 5.5 cm Petri dish containing 10 to 15 ml of solid VMM with *D. sapinea* with an agar plug with mycelia from a preculture on VMM.
- 2 Incubate the plate for 21 d at 26 to 29°C with constant light (cold daylight at an intensity of 5000 to 650 lux). Pycnidia will form on the surface of the culture and open to release a droplet of liquid containing spores.
- 3 Harvest the spores from the plate by adding approximately 2 ml of 0.01% (v/v) Tween to the surface and rinse the surface of the colony several times by pipetting. Repeat the procedure for a higher yield. Typically, between 1×10^6 and 2×10^6 spores can be harvested per plate. Spores can be separated from the liquid by centrifugation for 10 s at 5000 rpm and then resuspended in your desired volume.
- 4 For storage, mix 500 μ l of the spore suspension and 500 μ l 40% glycerol in a 2 ml screw top tube or cryovial. Freeze the glycerol stock tube at -80°C.

Protocol references

Vogel HJ. A convenient growth medium for *Neurospora crassa* (Medium N). Microbial genetics bulletin 1956; (13):42–3.