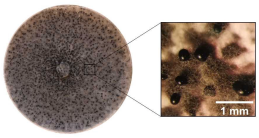
Aug 01, 2024

Protocol for the production and storage of Diplodia sapinea pycnidiospores



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.

DOI
dx.doi.org/10.17504/protocols.io.kqdg3271pv25/v1

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DOI: dx.doi.org/10.17504/protocols.io.kqdg3271pv25/v1

Protocol Citation: Anne Geertje Oostlander, Laura Brodde, Miriam von Bargen, Rasmus Enderle, Marco Leiterholt, Dagmar Trautmann, Malin Elfstrand, Jan Stenlid, André Fleißner 2024. Protocol for the production and storage of Diplodia sapinea pycnidiospores. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg3271pv25/v1**

Manuscript citation:
Oostlander AG, Brodde L, Bargen M von, Leiterholt M, Trautmann D, Enderle R et al. A reliable and simple method for the production of viable pycnidiospores of the Pine pathogen *Diplodia sapinea* and a spore-based infection assay on Scots Pine. Plant Dis 2023; 107(11):3370–7. doi.org/10.1094/PDIS-01-23-0107-RE

Protocol status: Working
We use this protocol and it's working

Created: July 25, 2024

Last Modified: August 01, 2024

Protocol Integer ID: 104068

Keywords: Diplodia sapinea, Diplodia tip blight, in vitro sporulation, pycnidiospores, Sphaeropsis sapinea

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 $1x10^6$ and $2x10^6$ 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 µl of the spore suspension and 500 µl 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.