Conidial production from M. fijiensis mycelium

Mar 28, 2019

Working

PLOS One

Leticia Peraza[1], Cecilia Rodríguez[1], Dalia Zapata[1]

[1]Centro de Investigación Científica de Yucatán

dx.doi.org/10.17504/protocols.io.sgmebu6

Leticia Peraza Echeverría
Centro de Investigación Científica de Yucatán

ABSTRACT

Continuous *in vitro* conidial production from *Mycosphaerella fijiensis* mycelium. A method for *in vitro* production of conidia is described. *M fijiensis* was grown on culture medium containing V8 juice. Mycelium was macerated until a fine suspension was produced; 2 mL was carefully spread on V8 sporulation medium. The Petri dishes were incubated for 6 days at 20 °C, under continuous cool-white fluorescent and black light. After the incubation period, 2.4 mL of 1% gelatin was added to each Petri dish, the conidia carefully dislodged with a camel hair brush. Conidial production was notably stimulated by brush harvesting mycelial cultures. After 6 days of culture the first harvest was made and the same batch of petri dishes were put in the same culture conditions to stimulate new conidial production. Significant cumulative conidial production was observed after the second harvest.

EXTERNAL LINK

https://link.springer.com/article/10.1071/AP08042

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Peraza-Echeverría, L., Rodríguez-García, C. M., & Zapata-Salazar, D. M. (2008). A rapid, effective method for profuse in vitro conidial production of Mycosphaerella fijiensis. *Australasian Plant Pathology*, *37*(5), 460-463.

PROTOCOL STATUS

Working

GUIDELINES

-V8 juice from Herdez company
- Unflavored gelatin
- Camel hair brush

MATERIALS

NAME	CATALOG #	VENDOR
Calcium carbonate	C-5929	Sigma – Aldrich
Agar-agar	1.01614.1000	

SAFETY WARNINGS

All Petri dishes with *M fijiensis* should be sterilized after its last use.

BEFORE STARTING

1.- The mycelium colonies used for the obtention of the conidia should have before its use between 3 to 4 weeks on V8 growth medium.
2.- V8 sporulation medium ready

V8 sporulation (V8S) medium

1 Calcium carbonate
0.2 g per litre

2 V8 juice

100 ml per litre

3 Mix step 1 and 2 in 1 L of distiller water for 15 min

00:15:00

4 Measure pH and adjut to pH 6

5 Add Agar-agar

20 g per litre

6 Sterilize at 1 pressure atmosphere and 120° C for 15 min

7 Pour 25 mL of V8 sporulation medium into each Petri dish,

wait 3 days to see if any contamination was present then it can be used

Inoculation of ground mycelium in V8 sporulation medium for conidia production

8 Ground mycelium using a mortar and pistile in a laminal flow cabinet

- cut 1 cm^2 of mycelium and put it into a mortar
- add 2.5 mL of destilled sterile water
- grind to a non-pasty liquid consistency
- pour 2.5 mL of the grind to each Petri dish containing V8 sporulation medium
- the ground mycelium is distributed making circles, until the entire Petri dish is full of suspension
- let the Petri dish for 20 min to dry, then seal with kleen pack

9 The Petri dishes were put in the folowing culture condition for sporulation

- Room temperature 20 °C
- Continuous light with cool-white fluorescent and black light (General Electric 15W/18) emitting radiant energy in the near-ultraviolet or black light spectral range
- Time in this conditions: for the firs harvest 5 days for the second harvest 3 days (same batch of petri dishes).

Harvest of conidia

10 Harvest of conidia in a laminal flow cabinet

1. Take 2400 µL of grenetine (1%) and add it to the mycelium grown in the V8S medium
2. Sweep gently with the camel bruch
3. Tilt the petri dish and with the help of the micropipette wash the mycelium with the same suspension
4. After harvesting put all the suspension in a Falcon 50 mL tube, the resulting suspension must be cloudy

Conidia count

11 After the harvest take a sample for counting, if necessary you can dilute the suspension 1:10

1. Use the Sedgwick-Rafter chamber to count the conidia (it could be the Neubauer chamber) using a
2. The following formula is used to calculate the concentration of conidia

$$\text{cells or spores/mL} = \frac{\Sigma N \times 1000\ mm^3}{A \times D \times F} \times \text{dilution factor}$$

Were:

N=Conidia counted
A= field area 1 (mm^2)
D= depth of field (1 mm)
F= numbers of counted fields (25)

3. Once the concentration of conidia is calculated, then the concentration of the original suspension is adjusted to 200 conidia per uL

4. If the conidia are not going to be used immediately, they can be stored at 4 ° C