

DATE:
NAME:
TOPIC:

Culture of Wood Destroying Fungi

Methods of *in vitro* culture could also be used for multiplication, storage and distribution of the algae and the fungi taxons. Cultures of isolated mycelia of wood destroying fungi *Pleurotus ostreatus* a *Lentinus tigrinus*, which we have as a rest of former extensive experiments at the Department of Plant Physiology of Masaryk University, represent application of such methods. These cultures we use as an example of germplasm maintenance in minimal growth conditions as well as an example of the suspension culture.

Wood destroying fungi have fewer demands for culture medium in comparison with the explants of higher plants. The fungi as a heterotrophic organisms demand organic compounds of carbon. Universal, simple and testified medium represents malt extract solidified with agar.

MATERIAL: culture of the mycelium of wood destroying fungi (*Pleurotus ostreatus* and *Lentinus tigrinus*)

MEDIA: 3% malt extract solidified with 3% agar (test tubes and Petri dishes)
3% malt extract in Erlenmeyer flasks with glass beads
3% malt extract in flasks

PROCEDURE:

A. Maintenance of the Collection of Wood Destroying Fungi - Subculture of the Mycelium (Germplasm Maintenance in Minimal Growth Conditions)

1. Prepare the test tubes containing fresh medium (malt agar) and the tools (forceps, spatula, scalpel, etc.)
2. Transfer the test tubes containing the cultures of the fungi mycelium from the refrigerator into a laminar flow hood.
3. Pass the opening of the test tube through the flame.
4. Cut segment of the mycelium and transfer it on the middle part of the surface of the malt-agar medium in the test tube.
5. Pass the opening of the vessel through the flame carefully. Cap the vessels and label.
6. Culture in the dark, cold place (an refrigerator), the interval of subculture in such conditions could be quite long (to 1 year), which save both money and work.

B. Preparation of Inoculating Suspension of Wood Destroying Fungi from the Collection (An Example of the Suspension Culture)

1. Prepare the Petri dishes containing fresh medium (malt agar) and the tools (forceps, spatula, scalpel, etc.).

2. Transfer the test tubes containing the cultures of the wood destroying fungi mycelium from the refrigerator into a laminar flow hood.
3. Pass the opening of the test tube through the flame.
4. Cut segment of the mycelium and transfer it on the middle part of the surface of the malt-agar medium in the Petri dish.
5. Cap the Petri dish, save with a Parafilm or another similar foil and label.
6. Culture in the thermostat (at dark and 25°C) for 1 week.
7. Transfer new mycelium on the surface of glass beads with 3% malt extract in the Erlenmeyer's flasks.
8. Incubate in the thermostat (at dark and 25°C) for next week till the mycelium forms appropriate layer. In case of very thin mycelium layer, the growth is very slow. On the other hand, problems with disintegration of the mycelium layer to smaller segments could be observed, when the layer is too thick.
9. Divide the mycelium by glass beads shaking with the flask to small segments.
10. Pour of the suspension of malt extract with the segments of the mycelium and transfer carefully to the flask with fresh malt extract solution. Cap the vessel and mark.
11. Cultivate the suspension continuously shaking for about one week.
12. Suspension with the globular masses of the mycelium could be used for nature substrates inoculation for edible fungi production. Cold pretreatment of the mycelium could be used as an induction of the fruiting bodies formation.

EVALUATION

Control the contamination of the culture in the following cultivation period.

Compare the velocity of the mycelium growth at two different culture temperatures.

Test the procedure of the inoculum preparation

LITERATURE:

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2. Jennison MW, Richberg CG, Krikszens AE.: Physiology of Wood-rotting Basidiomycetes: II. Nutritive Composition of Mycelium Grown in Submerged Culture. - Appl Microbiol. 1957 Mar, 5(2): 87-95.
3. Scheffer T.C.: Relation of temperature and time to carbon dioxide production and growth in continuously aerated malt-agar cultures of *Polystictus versicolor*. - Plant Physiol. 1936 Jul; 11(3): 535-564.