THE MYXOMYCETES

Morphology and Taxonomy

Materials:

Alcohol 90%
Lactophenol
Glycerine or glycerine jelly (Gelatin 1.0 g; Glycerine 7.0 g; water 6.0 g; Phenol 0.14g)
Hydrochloric acid 1%

Procedure:

1. Classify the given materials according to sporangial type: Aethalium, pseudoaethalium, plasmodiocarp, sporangium, stipitate or sessile.

   Classification of higher taxa is based on: 1. spore colour; 2. presence of lime; 3. distribution of lime; 4. presence or absence, and structure of capillitium.

2. Examine carefully under the dissection microscope and note the colour, shape and other gross characters.

3. Mount a small portion (single sporangium if sporangiate) in a drop of water on a slide. Dissect carefully under the dissection microscope and observe (1) peridium: single or double; (2) capillitium: colour and structure; (3) presence or absence of a columella.

4. Separate the peridium, capillitium and stipe, and add a small drop of 1% HC1 to each, separately. Watch for effervescens, indicating the presence of lime. Now you can assign the specimens to the different orders.

5. Mount a portion, or a single sporangium, after wetting with a drop of alcohol, in (a) lactophenol if non-calcareous; (b) glycerine if calcareous. Examine under the microscope, noting the following characters:
   Total height; length and thickness of stipe; diameter of sporangium; nature of lime if present, i.e., crystalline or amorphous; capillitium: colour, degree of branching and anastomosis, relation to columella and peridium, nature and distribution of lime, ornamentation on the threads if any; spores: colour, shape, size and ornamentation.
   Identify to the genus, using the monographs of (1) Lister and (2) Martin & Alexopoulos.
   Seal your preparation, label and store them.

Spore Germination

Materials:

Watch glass, placed in Petri dish lined with blotting paper and sterilized – 2 Nos.
Cavity slides/van Tieghem rings – 3 Nos.
Petri dishes with Z rods – 3 Nos.
Tween 80 solution, 0.1%, sterile

Carrot decoction, 2%, sterile
Sterile water
Sterile pipettes: 1 ml – 2 Nos.
Sterile pipettes: 5 ml – 2 Nos.

Procedure:

Place spores of …….. in a watch glass containing 0.5 ml of sterile Tween 80 solution (1:1000). Leave for 10 minutes. Separate the spores using dissection needles, and add 2 ml of 2% carrot decoction. Take loopfuls of this suspensions and make hanging drops. Incubate in moist chambers* at 25°C and examine at intervals of 24 hours. Describe the method of germination and the swarm cell.

*The moist chamber is a Petri dish containing some sterile water or 20% glycerin, bearing a z-shaped glass rod to support the slide, or lined with moist blotting paper supporting the watch glass.

Culture

Media

1) Carrot Agar (CA) 2) Oat Agar (OA) 3) Water Agar (WA) 4) Oats on agar

1. Carrot agar

Fresh carrots	-	50 g
Agar	-	20 g
Tap water	-	1000 ml
pH 6.5		

The carrots should be scraped, cut into small bits and boiled with 300 ml of tap water for half an hour in a 500 ml Erlenmeyer flask. The agar must be melted in the remaining water and added to the strained carrot decoction and the volume made up to one litre. The pH adjustment to 6.5 can be done using 1N HC1.

2. Oat Agar

Rolled (porridge) oats	-	30 g
Agar	-	20 g
Tap water	-	1000 ml
pH 6.5		

Cook the oats in 300 ml water over a slow fire with constant stirring. The agar can be melted with the remaining water. The two must be mixed together and the volume made up to one litre. The pH adjustment to 6.5 to be made as in the case of carrot agar.

3. Water Agar

Agar	-	20 g
Tap water	-	1000 ml

Twenty grams of the agar is added to 1 litre of tap water and boiled to form molten agar.

4. Currently we are subculturing plasmodia on “Oat flakes on Agar” (to avoid excessive bacterial growth). This is done by sprinkling about half a teaspoonful of sterilized dry rolled oats on 50 ml of 2% water agar prersterilized in 250 ml Erlenmeyer flasks. The oats should be first ground to powder. A slight moistening of the powdered oats prior to sterilization reduces fungal contamination from oat flakes. If too moist, they should be dried in a drying over after sterilization.

All media are to be sterilized by autoclaving at 121°C for 15 min.

Procedure

Place a few sporangia in a sterile watch glass containing a few drops of sterile 0.01% Tween 80 (Polyoxyethylene sorbitan monoleate) for 7-10 min. When the sporangia become thoroughly wet, crush and tease them out using sterile dissection needles, to liberate the spores. Add about 5 ml of sterile water to prepare a spore suspension. A drop of the spore suspension should be viewed under the microscope to ensure adequate number of spores. The spore suspension is then inoculated into plates of the required media in 1 ml aliquots, using a sterile pipette. About 2 ml of sterile water is added to each plate and swirled to disperse the spores evenly. Three plates of CA and two plates of WA must be used for each sample.

All cultures must be incubated at 25 ± 2°C in a humid chamber under light intensity of 5 lux and examined daily from the fifth day onwards, for the appearance of plasmodia.

When the plasmodia have made sufficient growth on the agar surface, a small disc of the plasmodium along with the underlying agar must be cut from an advancing fan, using a sterilized cork borer of 1.0 cm diameter, and transferred to a plate of water agar. Transfer of inoculum can be made using an inoculation needle of nichrome wire, but a long-stemmed spatula is preferable.

The process of transferring on water agar should be repeated until there is no fungal growth. If there is excessive bacterial growth, plasmodial growth slows down. the unwanted bacteria can be eliminated by repeated transfers on plates of water agar. Three successive transfers on WA should be sufficient. Each plasmodium would be carrying its own bacterial associates.

Maintenance of Stock Cultures

Stock cultures are best maintained in 250 ml Erlenmeyer flasks of medium (2) or (4). Parallel cultures in duplicate test-tube slants of CA may be placed in the refrigerator (up to one month). Once the plasmodial cultures in the CA or OA flasks covers the surface of the agar medium and thick veins are prominently seen, subculturing should be done. This usually takes about a fortnight.

Fructifications might appear in some cultures if subculturing is delayed. These should be transferred to pieces of white card along with the underlying agar. When dry, they may be stored in small cardboard boxes, at a temperature below 25°C.

Note: Temperatures of 30°C and above, would cause drying of plasmodia and rapid loss of spore viability.

In case of loss of plasmodial culture, revival can be done from fructifications, if available.

Storage and Revival

As a back-up against unexpected contingencies, cultures can be dried (Sclerotized) and stored on filter paper. In case of loss of any culture, it can be revived either from the stored dry specimen or from fructifications.

Materials

Thick filter paper, cut into strips 1 x 8 cm
Culture tubes, 2 x 10 cm, 6 Nos.
Sterile water
Sterile Petri dishes

Procedure

Prepare two cultures on medium (2) or (4) in 250 ml conical flasks. When sufficiently grown, introduce vertically into each culture, 4-5 strips of sterile filter paper moistened by dipping in sterile water.

Leave for a few days to allow plasmodium to move up the paper.

Remove the strips bearing plasmodium, place in sterile Petri dish and allow to dry slowly over a few days. When dry, store in sterile culture tubes at <20°C.

For revival, place a strip on a plate of CA and add 2 ml of water over it. Observe daily for plasmodial growth. When plasmodia appear, transfer to flasks of fresh media.

The procedure for revival from cultured spores is the same as for making fresh cultures.

Sporulation

As in the fungi, the environmental requirements for sporulation in Myxomycetes differ from those for growth, humidity and temperature being the major factors. Generally, the plasmodium seeks a warmer and drier environment, as well as a drier and firmer substratum, when it is ready for sporulation. This readiness or commitment to sporulation, however, is determined by internal factors.

In culture, one can often recognize a plasmodium that is ‘committed to sporulation”. A phaneroplasmodium when ready to sporulate, shows a general condensation with highly thickened veins, an intensification of pigmentation if pigmented; a rapid, restless migration over

the agar surface, often covering the sides of the flask. In the aphanoplasmodium of *Stemonitis*, the condensation results in a coralloid formation, which also exhibits the same restless migration.

The culture vessel bearing the "committed" plasmodium should be transferred to a well-lit room or incubator at a temperature 2-3°C higher than the growth chamber. Petri dish cultures may be left slightly open to facilitate slow drying. A simple method to bring about slow drying and also to offer a firm foothold, is to insert a cone made from thick filter paper into the culture dish. Initially the cone should be moistened to induce the plasmodium to climb on to it. Once the plasmodium climbs on to it, the cone should be transferred to a dish containing water to a depth of 1-2 mm and left covered. The plasmodium would eventually sporulate on the filter paper. This enables easy storage of the fructifications, which otherwise would develop on the glass surface of the flask or Petri dish.

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