

PRACTICAL 7: MICROSCOPIC EXAMINATION AND GENERAL MORPHOLOGY OF FUNGI

Introduction

A considerable amount of information can be obtained by careful microscopic examination of microorganisms. Two techniques are employed to study microbial cells: (i) examination of living cells and, (ii) the study of stained cells. Living cells can be studied to determine the natural size and shape of cells and cellular arrangement. However, observation of cells in their natural or unstained state is sometimes difficult because microbial cells are semitransparent.

Objectives

- i. To gain skill in mounting specimens of fungi for microscopic examination
- ii. To observe the general external and internal morphology of fungi

Requirements

14-21 day-old cultures of two fungi you isolated and purified in practical three (3), clean glass slides, Cover slips, Mounting needles, Distilled water, Cotton blue in lactophenol in a dropper bottle, Spirit lamp or Bunsen burner, 70% alcohol, Nail polish, Microscopes, Mounting needles.

iii. Temporary wet mount technique for microscopic observation of fungi

Procedure

- i. Place a small drop of distilled water on the centre of a clean glass slide
- ii. Using an inoculating needle, transfer a small amount of fungal growth onto the drop of water on glass slide
- iii. Hold a cover slip by its edges and place it on the drop
- iv. Press the cover slip gently with the end of a pencil.
- v. Observe under objective x20 and 40 of microscope

Observations

Note the size, shape and characteristics of the fungus (spores, hyphae)

2. Cotton blue in lactophenol mounting of fungi

Introduction

Cotton blue in lactophenol stain is commonly used for making semi-permanent microscopic preparations of fungi. It stains the fungal cytoplasm and provides a light blue background, against which the walls of hyphae can readily be seen. The stain contains four constituents: (i) phenol, which serves as a fungicide; (ii) lactic acid, which acts as a clearing agent, and (iii) cotton blue, which stains the cytoplasm of the fungus; and (iv) glycerine, which gives a semi-permanent preparation. A permanent preparation may be made by incorporating polyvinyl alcohol in place of glycerine into the mounting medium. Lactophenol alone (without cotton blue) may be used in case of dark coloured fungi.

Procedure

1. Place a drop of cotton blue in lactophenol on a clean slide
2. Transfer a small tuft of a well-sporulated fungus into the drop, using a flamed, cooled mounting needle.
3. Gently tease the material using the two mounting needles.
4. Mix gently the stain with the fungal material
5. Place a cover slip over the preparation, taking care to avoid trapping air bubbles in the stain. A gentle pressure can be applied to the cover slip by tapping with back of mounting needle or pencil in order to spread the fungal structures and expel air bubbles.

6. Examine the preparation under low –power (X10 and X20) and high-power (x40) objectives.
7. Sealing lactophenol mounts to keep the slides for many years – the cover slip is sealed with nail clear varnish as follows:
 - a. Remove all air bubbles from the preparation by pressure, gentle heating or addition of more cotton blue in lactophenol.
 - b. Remove the excess stain from around the cover slip with 70% alcohol on cotton swab or with blotting paper.
 - c. Apply a thin layer of nail varnish around the edge of the cover slip.
 - d. Allow the preparation to dry overnight.
 - e. Apply a second coat of nail varnish over the first coat.

The fungal cytoplasm is seen as a lightly stained blue region forming a layer inside the unstained cell wall of hyphae, conidiophores, and conidia that is surrounded by light blue background on the slide.

Questions

- i. From the observations of the fungal cultures and under the microscope, list at least four (4) ways the fungus you isolated differs from the bacteria you observed in the earlier class.
- ii. How do the specimens mounted with water differ from those stained with cotton blue in lactophenol when observed under microscope?
- iii. List four (4) ways in which the staining of the fungi specimens differed from that you did for bacteria.
- iv. How many times was the fungal specimen magnified when you observed under microscope – explain how you arrived at the magnification you have indicated.
- v. Draw a large, clear diagram of what you have observed and label to indicate hypha, sporangiophores, sporangiospores, conidia, conidiophores and septa