



Workshop Activity 4

Slide Making & Fungal Observation

BACKGROUND

An important skill to master in plant pathology is slide making. Techniques vary according to the type of fungus being looked at. Most mycelium (ie. the cottony strands which make up the body of the fungus) is transparent if examined under the microscope. It is therefore common practice to stain mycelium before observation.

There are broadly two types of stain. Light stains, such as clear lactophenol, are used when observing fungi that have dark coloured colonies. Dark or coloured stains such as lactophenol cotton blue are used when observing fungi that form light coloured colonies.

RESOURCES

- plate culture of a fungus
- dissecting needles/scalpels/needles pushed into corks
- slides and coverslips
- bunsen burner or spirit lamp
- dropper bottles of appropriate stains
- microscope

PROCEDURE

1. Place a drop of the selected stain onto a clean microscope slide.
2. Take a needle and wave it through the flame to sterilise it.
3. Raise the cover from the plate culture of fungus. If the culture is to be re-used it is important to use sterile technique.
4. Use the needle to remove a very small piece of mycelium from the culture. If you are looking for spores the piece of mycelium should be taken from an older section of the colony. i.e. the centre of the colony.
5. Place the small piece of mycelium into the drop of stain.
6. Using two needles, tease the mycelium apart.
7. Move the slide quickly through a flame to gently warm the stain. Do not overheat the preparation. This warming helps the fungal cells to take up the stain and therefore makes them more easily visible under the microscope.
8. Lower a coverslip gently onto the drop of stain (touch one side down first and then carefully lower using a needle), and gently wipe away any excess with a tissue. This should prevent air bubbles from becoming trapped under the coverslip.
9. Observe the slide using a microscope.

EXPECTED RESULTS: *This technique should produce slides which can be placed under the microscope to find spores, mycelium and other fungal structures. Sometimes fungal spores are produced within the agar itself. It is then necessary to dig out a small piece of media from near the centre of the colony and place this on a slide, then treat as above. Some fungi require specific incubation conditions to form spores, hence you may not see any reproductive structures.*

KEEPING SLIDES: *If you wish to keep your slides for longer than a day, ring around the edge of the coverslip with some clear nail polish and allow to dry. A second coat will ensure a good seal.*