Fungi

This lab is designed to acquaint you with the basic characteristics and techniques used to identify fungi. Fungi are increasingly important not only as human pathogens but as serious environmental pests to both cultivated and native species. One fungus, *Batachochytrium*, appears to be responsible for the extinction of two species of frog (1). On the positive side, fungi are important in a variety of industrial processes and bioremediation. They are also essential for breaking down dead plant material freeing up the nutrients. Plant productivity is tied to the presence of fungi associated with the plant roots.

Fungi are identified on the basis of the reproductive structures present in their natural environment and their morphology when grown in isolation on media. Your text has an excellent discussion of the fungi life cycles and spore production. Be sure to read it **BEFORE** you come to lab. There are also excellent web sources including Tom Volk's mycology web site, www.TomVolkfungi.net. There are many excellent keys and photographs of macro fungus (mushrooms and toadstools) available. Be sure to also visit www.mykoweb.com.

Because fungi are so successful in propagating themselves through airborne spores we will be observing mostly preserved material. And because they are so successful at dispersing, most of the preserved fungi we will be looking at are of non-pathogenic organisms. We need to follow extra precautions when examining our "live" samples to avoid contaminating the lab with fungal spores. We will be using BSL 2 procedures.

Safety Protocol

Some cultures should not be opened and will be sealed with shrink-wrap.

- Yeast cultures may be handled the same as bacterial cultures.
- Any fluffy cultures bearing fruiting bodies should be opened slowly and only enough to extract the sample. Unnecessary movement near the culture should be avoided while it is open.
- Use a plastic loop to avoid aerosols generated by flaming. An incinerator may also be used.
- Dissecting microscope observations of the plate should be done with the lid on if there is not too much humidity.
- Do not chew gum while you are working with the fungi.
- NEVER sniff a fungal culture to determine its odor.

Lab Protocol

There are three parts to this lab. All your observations should be recorded in your lab book. Don't forget to include the magnification. You will be looking at micro fungus (mold members of the Zygomycota), macro fungus (fleshy fungus representatives of Basiodomycota and Ascomycota), and yeast (single cell representatives of the Ascomycota).

1. Observations of your samples and selected strains. You will note the morphology of your unknown fungi and try to determine at least the phlya of fungus using the Xeroxed handout and/or the posters provided in the lab. Pick at least one fungi to identify. You also need to observe the plates on the front desk. There will be representatives of all three phyla - either in plates or as individual macro fungus.

a. Is it a filamentous bacterium, yeast-like, thermally dimorphic, or a thermally monomorphic mold (2)? You will need to examine the plate macroscopically through a dissecting scope. Then prepare a tease mount, a cellophane tape mount, or a Mycomount of your sample. Your instructor will tell you which one to do.

   i. Tease mount. Place a drop of lactophenol cotton blue (LPCB) on a glass slide. Using the loop end of a disposal inoculating needle, remove a small portion of the colony and place it in the drop of LPCB. Use two needles to tease apart the mycelium. Cover with a coverslip and observe at 100 and 400 X.

   ii. Cellophane tape-mount. Loop a 4 cm strip of cellophane tape, sticky side out. Hold the loop with forceps or your gloved fingers and press the loop very firmly onto the surface of the edge of the colony. Place the tape on a small drop of LCPB on a slide and press the whole piece down. The tape serves as a coverslip. Observe under 100X and 400X. Use the Xeroxed guide to find a close match.

   iii. MycoMount. "A plastic strip with adhesive for the collection of fungal fruiting structures for observation under the microscope". These are pretty nifty and unfortunately a little pricey. Instructions will be provided in lab.

b. Record the color of the surface and reverse underside of the colony. Use the color plates in the reference manuals in lab to find a similar colony.

2. Prepare a wet mount. You will be given an actively growing culture of yeast, *Saccharomyces cerevisiae*. Place a drop on a slide, cover with a coverslip and look at the general morphology and for the presence of budding. Also look for a sort of mushy looking cell, called schmoos. These are the mating forms of yeast. You may need to dilute your culture for easier observations. There may also be plates of *Candida albicans* for you to observe.

3. Prepared slides. You have a tray full of representative fungal species. You are responsible for identifying the asexual and sexual structures characteristic of those phlya. You will find the BioCam posters in the lab exceedingly useful in identifying your slides. There are also pictures available on the website under Bug Info > Survey Organisms. Click on the links below to see representative pictures of each fungus. Most of these slides have been stained to enhance their features. Do not memorize them by their color. Know what group of fungi each specimen belongs to, basidomycotes, zygomycotes or ascomycotes and which specific structure you are looking at ascospores, zygospores or basdiospores, conidia or sporangia. Check the board during class for additional, specific information.

Fungi Survey Slides

- Aspergillus
- *Candida albicans*
- Coprinus
- Penicillium
- Rhizopus
- Saccharomyces

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1. Salyers, A.A. & Whitt, D.D. (2001) Microbiology: diversity, disease, and the environment. Fitzgerald Science Press, Inc. Bethesda, Maryland.
2. Monomorphic species have the same appearance regardless of the temperature they are cultured. Thermally dimorphic species are filamentous at 25-30°C and yeast-like at 35-37°C. Since we did not culture our samples at a higher temperature, you will only be able to compare their morphology at 25°C.