FUNGAL LAB ANCILLARY MATERIAL

So far, you have been introduced to microbiology from the bacterial perspective. However, there are comparatively less known, but biologically important taxa that belong to different microbial kingdoms. Although we will not have time to explore the relevance and biology of all these kingdoms, we will attempt to examine several **microfungi**. Fungi can be parasitic, **saprophytic**, **commensalistic**, **mutualistic**, or even predatory (yiiikes!) . . . or any combination of all these lifestyles. For example, some microfungi called mycorrhiza can form mutualistic relationships with plants during the summer months when nitrogen is scarce and parasitic when nitrogen is abundant.

The genetics, ecology, physiology and chemistry of fungi are complex and remarkably interesting. For example, did you know that the source of the first mass-produced antimicrobic (penicillin) was a lowly mold called *Penicillium notatum*? Many microfungi, including many species of *Penicillium*, don't have sex (bummer). These microfungi may form "platonic" and mutualistic relationships with other microfungi or other taxa including humans (Have you tasted **tempeh**? Bit into blue cheese? Gorged on Gorgonzola? Slurped on soy sauce? . . . no? How about bread, yogurt, wine?).

Some terms you may not know

Commensalistic- An external, mutual relationship between two or more organisms where one organism may gain more than the other.
Microfungi- non-bacterial microbes belonging to the kingdom *Myceteae* (sometime referred to as the fungal kingdom)
Mutualistic- An external, mutual and equally beneficial relationships between two or more organisms.
Phylogeny- The evolutionary or genetic development or groups of organisms.
Saprophytic- a system of gathering energy that involves the organisms consuming dead or dying organic material.
Tempeh- Any of the family of bean products made into a patty or curd and infected with *Rhizopus oligosporus* and allowed to age. Commonly consumed in India and Southeast Asia

The study of fungi is called **mycology**. As with most biological disciplines, it has its own lingo that is used by **mycologists** to study fungi. Many of the terms you will need to understand this lab can be found on Exercise 10-1 of your laboratory manual. Make sure you read this exercise before coming to class as it will help you understand some of the terms and concepts discussed during the laboratory period.

We will superficially examine four of the most well known or economically important fungal groups (and any that you can come up with yourself). I have chosen not to present the more well-known Basidiomycetes which are macrofungi (you know, the stuff you eat on your pizza). Since this is a microbiological lab, sticking to the microfungi seemed reasonable.

1. Myxomycetes (Myxostelids). Slimy things growing on dead logs or leaf litter. Really cool! This group has historically been studied by mycologists, but are actually more genetically related to protists (kingdom: Protista). They are believed to represent the beginnings of the **phylogenetic** evolution that ended with true fungi. I have included diagram of the life-cycle for your convenience. Your laboratory table should contain one low nutrient agar plate with one myxostelid (*Physarum sp.*; the "sp." designation after the scientific names implies that although we know what genus this organism belongs to, we do not know the species, and therefore the species epithet is usually written as "sp.") and

some bits of oatmeal. This organism is considered one cell!!! able to move by a process called **protoplasmic streaming**. If it were many cells we could call it cellular streaming (e.g., your blood vessels show **cellular streaming**).

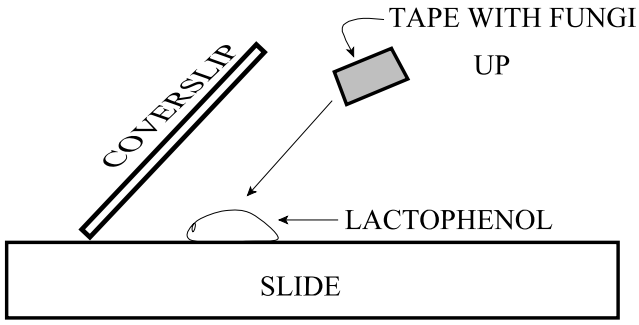
-Place the entire low nutrient agar plate under the LOWEST power of your objective scope. Examine the **protoplasmic streaming** that occurs. 1) Why do you think this is considered one cell and not a collection of cells? 2) How does it eat? 3) Does the protoplasmic streaming only go in one direction?

Some terms you may not know

Cellular streaming- movement of many cells from one location to another.
Conidiophore- Fungal structure that bears the asexual spores or conidia.
Protoplasmic streaming- Fluid movement of the protoplasm, or the contents of one cell (cf. cellular streaming - movement of cells).

-If you follow along with your life cycle you will notice that these organisms also produce structures that resemble true fungal spores. These structures are called sporangia (plural, sporangium–singular) and are usually produced as a means to disperse or survive in their environment. When times get rough (lack of food, water or cable TV, etc.), most slime molds will produce resistant spores to wait for the next "time of plenty." There may be a demo on the stereoscope that will show sporangia of our *Physarum sp*.

Live cultures of molds (a word often used to denote microfungi that produce asexual spores). I will have a variety of agar plates with different types of molds at each bench. In most cases the sporulating structures are important in classifying fungi into an ARTIFICIAL taxonomic scheme (i.e. fungal taxonomy does not necessarily describe true phylogeny). You are to look for the sporulating structures with your compound scope (low to medium power usually works well). Since the sporulating structures are often brittle and easily destroyed we will attempt to look at the sporulating structures intact and in place by using a small piece of cellophane tape. Without digging into the agar, firmly dab a small (3 mm X 3 mm) piece of tape near the edge of the fungal colony. By doing so you may get intact sporulating structures to adhere to the tape. The piece of tape with some sporulating structures stuck to it should be inverted and placed, sticky/moldy side up, on a drop of stain (Meltzer's or acid fuchsin works pretty good) placed previously on a slide. This allows you to look through the sporulating structures first, rather than through the tape. After the small piece of tape is placed on the slide, place a cover-slip on the tape and look through the scope using low power first. I have included several diagrams of some of the most common types of sporulating structures to help you identify the samples used this semester. Species of *Penicillium* and *Cladosporium* are usually the most difficult to find, but some patience and perseverance usually pays off with a decent **conidiophore**.



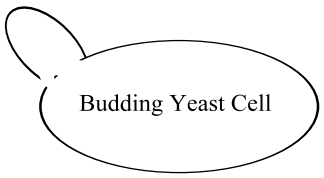
2. Zygomycetes - Fluffy stuff on the agar plate that occasionally gets on my loaf of bread. I will provide some Zygomycetes (*Radiomyces sp.*, *Absidia sp.,* or *Rhizopus sp*.). Some of these forms are **homothallic** (*Radiomyces sp*.). That is, **gametangia** of the same strain are required to form a **Zygospore**. And others are **heterothallic** (*Absidia sp.* and *Rhizopus sp*., i.e., "bread mold").

3. Ascomycetes - Most yeasts and stuff that produces sexual spores in sacs. Necessary in <u>really important</u> stuff, like beermaking. I will place one yeast species used to produce beer on display. Make a wet mount of a loopful/needleful of any of the colonies. Yeasts are a polyphyletic group of organisms that can (and do) change from a yeast growth form to a mycelial one, depending on their physiology or ecology. Wine can be made into wine vinegar simply by placing a mycelial growth form ("mother") of certain types of yeasts and bacteria into some wine and letting the wine breathe (letting the alcohol be turned into acetic acid). Once you have looked at your yeast under the scope you should observe, "budding" : a form of asexual reproduction that involves the production of a new individual by blowing out a portion of the cell wall of the parental cell (see figure below) . You should see budding of the yeast cells and if we are lucky we may even see both mycelial and yeast growth forms.

Some terms you may not know

Gametangia- In fungi, a specialized hyphae that functions as a gamete. Usually fuses with other gametangia to produce a zygote.
Heterothallic- In fungi, a reproductive system that requires the fusion of two gametangia from the different and genetically distinct organisms.
Homothallic- In fungi, a reproductive system that requires the fusion of two gametangia from the same organism.
Sporangia- Fungal structure that bears asexual spores of Zygomycetes
Zygospore- A sexual zygomycete spore produced by the fusion of two gametangia.

4. Deuteromycetes ("Imperfects")- All the stuff that produces tons of spores and usually adds color to my fridge but don't have sex . . . strange! There should be 3 very common imperfects (*Aspergillus sp*., *Penicillium sp*., & *Cladosporium*.) and any others that you can bring in. Although these genera are classified as imperfects (not having a sexual stage), from time to time, a sexual stage is discovered. The sexual stage of the individual species is given and additional (not new) name. Confusing? . . . it is. Nevertheless, I will have an *Aspergillus* species that has both the sexual (bright yellow, globular cleistothecia) and the asexual (dull green, conidia, or asexual spores) form on the same colony on the computer monitors. Compare the sporulating structures with the figures in exercise 10-1 and your handouts. Can you determine the genus? Make sure you understand and can recognize the different genera of fungi because you will be expected to identify those genera in an identification practical the following week. Feel free to ask about the defining features of a mold or, better yet, look them up yourself! Or even better yet, thanks to some Teaching Assistants (aren't they cool!!) the computers in the laboratory should have images of most of the sporulating structures of the genera you are asked to identify. Use these powerpoint presentations to determine what you should look for in your microscope. Good luck, & please let me know what can be improved.



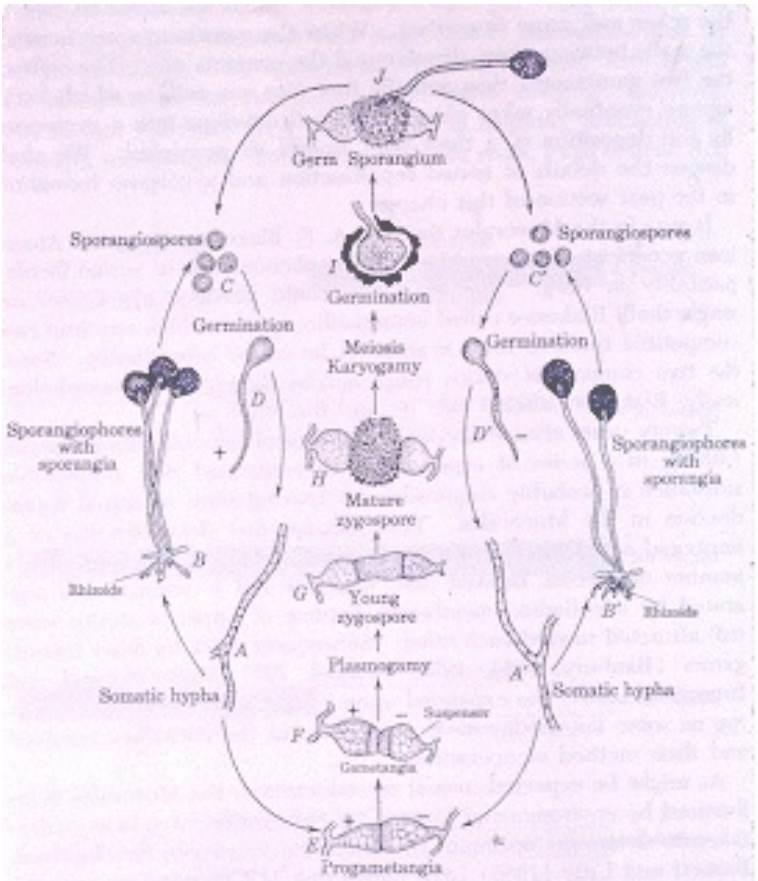
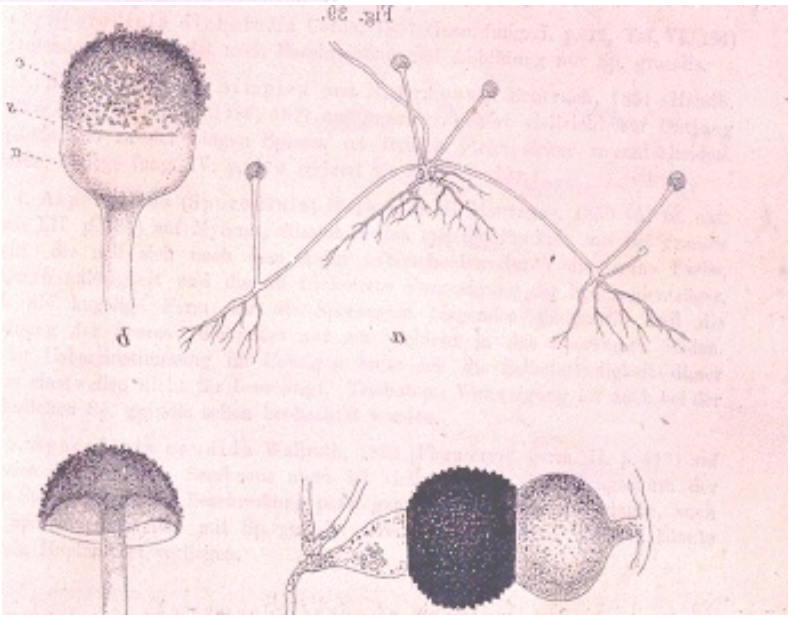


Figure 70. Life cycle of *Rhizopus stolonifer* (*R. nigricans*). *I*, redrawn from Cutter, 1942, *Bull. Torrey Bot. Club*, 69:592–616.



NOTES AND SKETCHES TO HELP YOU REMEMBER