

## Biology 204 Laboratory 6. Fungal Diversity and Evolution

### Kingdom Fungi

There are approximately 80,000 species of true fungi. Until recently, the kingdom Fungi contained many additional organisms (such as egg water molds and slime molds) that are now classified as protists because of their closer phylogenetic relationship to other protist groups. The kingdom as treated in this lab is a monophyletic group. Although the kingdom Fungi includes one primitive phylum of aquatic organisms (Chytridiomycota) and some members that have “returned” to the aquatic environment, the kingdom is largely terrestrial. The Basidiomycota is generally regarded to be the phylum with the most advanced characteristics.

The fungi are defined by having eukaryotic **hyphae**, non-motile **spores** (except the phylum Chytridiomycota), and **absorptive, heterotrophic** nutrition (enzymatic digestion of their food is external to their "bodies"). The hyphae of the fungi form an extensive and diffuse **mycelium**, the "body" of an individual fungus. It is this mycelium that is ecologically active, playing one of three roles in specific habitats: **decomposer**, **parasite** (mostly on plants), or **mutualist**. Although mycelia can be active throughout the year, the spore-producing structures they make are usually formed during restricted times. The kingdom Fungi includes one aquatic phylum, Chytridiomycota, and three terrestrial phyla, Zygomycota, Ascomycota and Basidiomycota, distinguished by details of their sexual stages. Some fungi in each phylum are microfungi (mycelium only; little or no macroscopic structures) and some are macrofungi (mycelium produces macroscopic "**fruit bodies**" for spore production).

In their general characteristics, each of the three terrestrial phyla of fungi, the Zygomycota, the Ascomycota and the Basidiomycota, show, in turn, more adaptations to coping with the stresses of living on land. It is generally believed that the fungi evolved from aquatic protists, arriving on land about the same time as the plants in the middle Paleozoic era. Some mycologists even believe that the success of early plants was aided by their associations with fungi in structures much like the mycorrhizas that we see today (and for which there is abundant fossil evidence). Our survey will be taxonomic.

### Objectives

1. Understand the distinctive characteristics shared by organisms in the kingdom Fungi
2. Understand the advantages fungi have when relating ecologically with plants
3. Become familiar with the 3 phyla of terrestrial fungi and how each group deals with living on land.
4. Examine fungi exhibiting various fungal life styles such as yeasts, molds, lichens and mycorrhizas and realize that these life styles are carried out by fungi in more than one phylum.
5. Be able to identify an organism on display into its proper kingdom and phylum, and know why it is classified there.

### Key Themes

The fungal “body” (mycelium made of hyphae) is usually composed of a loose collection of tubes and is sometimes difficult to study and understand. However, it is this mycelium that relates to the environment and it is the structure of this hyphal “body” that is unique among organisms and explains, in part, why the fungi are successful in doing what they do.

Fungal hyphae grow at their tips and invade environments where they release enzymes that function external to the fungus.

At some point in the life of a fungus, the mycelium produces spores. Asexual spores repeat the genetic type of the individual that produces them. Sexual spores are produced after the exchange of genes between two partners. The precise nature of how the sexual spores are formed is the most important character to use in classifying a fungus to its correct phylum.

Two “life styles” and two types of symbioses have evolved several times independently among different groups of fungi that expand their ecological capabilities. These include growing as yeasts or molds, and forming lichens mycorrhizas.

### **Key Terms**

hyphae	decomposer (saprobe)	thallus
mycelium	mutualist	crustose
sporangium	zygosporangium	fruticose
spores	ascus (asci)	foliose
heterotrophic	basidium (basidia)	mold
parasite	dikaryotic	mycorrhiza

### **Samples (refer to the chalkboard for updates)**

prepared slides	
slides and coverslips for fresh preparations	
forceps	representative lichens
representative fungi from 3 phyla	representative mycorrhizas
yeast culture	

### **What to do**

You will start this lab by examining representatives from phyla of the true fungi. Then you will study examples of "alternate lifestyles." Finally, you will study two symbioses involving fungi. Fresh material (cultures and specimens) will be available for you to study. By examining the specimens and answering the questions accompanying each section of the lab, you should come to understand the general characteristics of the fungi and the specific characteristics that define each phylum, life style, or symbiosis. Whenever possible, examine fresh material to get the best microscopic view of the features to study. Use the illustrations in your text or in the Photo Atlas as a guide to what to find. Be sure to make accurate labeled drawings with explanations whenever necessary.

### **Phylum Chytridiomycota: "Chytrid Fungi"**

This phylum includes a small number of aquatic “water molds” and their allies. They are characterized by the production of flagellated spores, each with a single, posterior, whiplash flagellum; this is unlike the pattern seen in the Oomycota, “Egg Water Molds”, which are now classified as protists in a group related to the brown algae. The Chytrid fungi are common decomposers of aquatic materials and are often seen on dead or dying freshwater algae such as Spirogyra. Although the typical chytrid has a small and simple thallus with a very short mycelium-like root embedded in its host, there are some with more complex mycelia. Unless

you happen to see one on algae in pond water elsewhere in this course, you will not have a chance to study this interesting group. Please read about them in your text.

### **Phylum Zygomycota: "Zygote Fungi"**

Members of the Zygomycota are characterized by producing coarse haploid hyphae that usually lack cross walls, by the production of asexual spores inside asexual **sporangia** (spore sacks), and by the production of **zygosporangia** as the distinctive sexual stage (fertilization and meiosis occur in the zygosporangium).

This phylum includes the black bread mold, *Rhizopus* and other common molds that decompose a variety of substrates in soil. *Rhizopus* grows well on any medium rich in starch. It reproduces rapidly by forming asexual spores. Later, if two compatible mating types (designated + & -) are present, *Rhizopus* produces the sexual zygosporangia.

Obtain a Petri plate with potato dextrose agar medium that has been inoculated with two mating types of *Rhizopus* separated by several centimeters. Examine the cultures under the dissecting microscope using light from the incident spotlight. Identify the hyphae, asexual sporangia, and asexual spores. **Prepared slide (# 76)** includes *Rhizopus* (along with two unrelated molds - see p. 5). Look at this slide first, before proceeding to the next paragraph.

Once two suitable mating types of hyphae near one another, they become mutually attracted and proceed through several changes, ultimately fusing to form zygosporangia. On the culture plate, see if you can distinguish the sexual stage (zygosporangia) that will occur among older hyphae where the two mating types have met. Remove a small amount of agar where zygosporangia have formed and make a wet mount of the material. Try and include both asexual sporangia and zygosporangia in the same preparation. When viewing, be sure you understand the relationships of the structures you see. Do not misinterpret the swollen, internal septum (cross wall) that divides the asexual sporangium from its supporting hypha. Draw and label structures.

### **Ascomycota: "Sack fungi"**

All members of this phylum produce sack-shaped sexual sporangia called **asci** (singular, ascus) at some point in their life cycles. They often also reproduce asexually early in their life by means of asexual spores (we'll discuss this further on in the section on molds). Ascomycota range from unicellular forms with no hyphae (yeasts) to complex forms with asci contained in fruiting bodies (both above and below ground - the latter, the true truffles). The dominant part of the life cycle is the haploid, mycelial phase, but (unlike Zygomycota) there is also hyphal growth in the dikaryotic phase (see discussion in text and below) but only associated with fruiting body formation.

Examine the living "cup fungus" material if available (perhaps the genus *Peziza*, *Aleuria*, or *Morchella*). The cups (apothecia) are produced on the surface of the substrate in which the mycelium is living (this mycelium may be saprobic, parasitic or mutualistic). Make a wet mount in water and examine a small section from the upper surface of the apothecium to see the arrangement of the ascospores, asci and the sterile hyphae among the asci. The individual cells can be more easily seen if the section is gently squeezed apart by tapping the coverslip with a pencil point until the tissue spreads out. How many ascospores are in an ascus? Once you have viewed the living material, compare what you saw with that on the prepared slide of a cross section of *Peziza* (**prepared slide # 73**) and look for the hyphae that make up the structure of the cup, the asci, and the ascospores within them. Because the sections are thin, you may not see

complete asci, but only parts of them. However it is easier to see the arrangement of the tissues in the fruiting body in this prepared slide.

### **Basidiomycota: "Club fungi"**

All members of this phylum produce club-shaped cells called **basidia** during sexual reproduction. Four spores are produced outside each basidium on small spines. Primitive members of the phylum, the rusts and smuts, are nasty plant parasites. In the more advanced members (e.g., mushrooms, puffballs and shelf fungi) the basidia are contained in a fruiting body. In contrast to the Ascomycota and Zygomycota, Basidiomycotes have a long-lived **dikaryotic** mycelial phase. The union of two haploid mycelia occurs at an early stage. In the resulting mycelium, the nuclei remain paired but unfused (the dikaryotic condition). Such mycelia periodically produce fruiting bodies in which sexual spores are made. For the first exercise below, use the commercial mushrooms provided, or any particularly good wild species as suggested by your lab instructor.

*Agaricus*. This genus includes the species of the common commercial mushroom (*Agaricus brunesceus*) and a number of wild relatives, some of which are edible (and some, not). Examine a button and note the cap, stipe ("stem"), and the veil (a thin membrane between the margin of the cap and the stipe). The underside of the cap is formed into numerous, flap-like "gills" (lamellae) to increase the spore-bearing surface. Examine a slightly older mushroom: the gills become darker as the dark spores mature. Spore color is one of the most important features used to identify mushrooms.

Cut through the pileus and determine the arrangement of the gills. Make a thin cross section of several gills. Look for basidia in a layer that covers the surface of the gill. Basidia are recognizable by the terminal "spines" on each cell since the spores often break off. Sketch the parts of the mushroom and a basidium with basidiospores (note that this species *Agaricus* has atypical basidia with two rather than four spores per basidium). In many mushrooms, including *Agaricus*, the veil forms a ring on the stipe, another of features identifying mushroom genera. Examine the **prepared slide (# 77)** of a cross section of *Coprinus* (inky cap mushroom) gills, looking for the basidia and spores. Fertilization of the two paired dikaryotic nuclei occurs in the basidium, followed by immediate meiosis to give four haploid nuclei. These migrate into the developing spores.

Because the long-lived mycelium of the Basidiomycota is dikaryotic, this group of terrestrial organisms has "reinvented" a condition (diploidy) that both the animals and higher, vascular plants have found useful for terrestrial life. What is the advantage of having two sets of genes?

Examine the other examples of Basidiomycota, making note of the great diversity in fruiting body structure. Especially, look closely at the "polypores," in which the basidia line the interior of hard, cylindrical "pores." Many polypores have perennial fruiting bodies, producing a new layer of spore-producing tissue each year on an old fruiting body. In the puffballs, the basidia disintegrate, leaving only spores and some sterile hairs within. An external force, such as raindrops or animal consumption must disperse the spores.

### **Fungal life styles**

The fungi in the above phyla can live their lives along specialized "life styles" adapted to specific conditions. We give these fungi common names, that aren't necessarily taxonomic. Truffles and false truffles (below-ground fruit bodies) are two we won't have time to study. In this part of the lab you will investigate four others: yeasts, molds, lichens and mycorrhizas. Keep in mind that each life style is shared by fungi from several phyla.

## Yeasts

Some members of all three of the fungal phyla live as unicells. The unicellular life style is an adaptation to living in plant sap. *Saccharomyces cerevisiae* is the common brewer's or baker's yeast that is able to ferment sugar solutions into ethanol and carbon dioxide. Examine a drop of yeast culture and note the size and shape of the cells. Look for and observe cells undergoing budding, the asexual reproduction of this yeast. Although asci are rarely produced by this yeast, it is known to be an Ascomycote. There are also yeast-forming fungi that form basidia and zygosporangia. Where would you expect yeasts to be found in nature? Why is *Saccharomyces* valuable in brewing and baking?

## Molds

“Mold” is a general term for any rapidly-growing, asexually reproducing (occasionally sterile) fungi. Such fungi are extremely common, functioning as saprobes and parasites in many habitats. Molds often do not reproduce sexually, or do so only after an extended period of time.

How are molds classified? If the mold eventually reproduces sexually, it can be classified in one of three fungal phyla by reference to its sexual structures. If this is not possible, the mold is placed in the “holding tank” phylum **Deuteromycota** (= "Fungi Imperfecti") and classified by features of its asexual spores. This is really for the convenience of those who work with these fungi, since their true sexual names can't be easily (or perhaps at all) determined. Actually, if the mold produces asexual spores inside a sporangium, then it is automatically known to be a Zygomycote since only this phylum produces asexual sporangia.

**Instructions for mold culture the week before this lab:** Prepare mold cultures to look at during the next week as follows. Each pair of students obtain one Petri plate prepoured with Potato Dextrose Agar. Inoculate the plate with spores by doing any of the following: 1) open the lid and expose the plate for 5 minutes in the room of your choosing (lab, hallway, classroom, etc.), 2) scrape or blow dust from a table, clothing rug, chair or other surface onto the plate, 3) crumble a small amount of decaying leaf litter over the plate, or push it into the center of the plate, or 4) be creative. Incubate for a week at room temperature. **Note:** in general, not much grows from surfaces in the bathrooms. These rooms are disinfected almost daily and are probably the cleanest in the building.

Observe your cultures of molds under the dissecting scope (remove the lids only to quickly wipe the water droplets off the inside with a paper towel). Determine the extent of each mold's mycelium and observe the hyphal tips extending out from the center at the edge of each culture. A variety of means of asexual spore production will usually cause each “colony” to change color or texture a short distance from the margin: see if you can see individual spores under the dissecting ‘scope in these areas. How many asexual spores do you think are being produced on each colony? Is there a way you could estimate this? Make two vertical slices across the margin of a colony and remove a very thin "slab" of agar that includes a portion of the mold culture. Mount this, first dry, on a slide and carefully examine using the 10x objective of your microscope. Note the relationship of any spore-producing structures to the hyphae of the mold mycelium. Add a drop of water and a coverslip and squash the sample before moving to higher power.

A **prepared slide (# 76)** compares asexual structures of three common molds: *Aspergillus*, *Penicillium* (which are known to be Ascomycota) and *Rhizopus* (which you have already seen is a Zygomycote). How would you know that *Rhizopus* is a zygomycote just by looking at this slide?

## Lichens

Lichens are mutualistic symbioses in which a fungus is associated with a green alga and/or a cyanobacterium. They are classified by the fungal partner (usually Ascomycotes, but some Basidiomycotes also form lichens). The fungi found in lichens rarely live apart from their photosynthetic partners. Each species forms a distinctive **thallus** combining the two or three partners. Thalli are categorized among three growth forms: **foliose** (leaf-like, flattened, usually with a distinctly different upper surface), **fruticose** (shrub-like, often round in cross section), or **crustose** (paint-smear like, firmly attached, without a lower surface).

Asexual reproduction is common among lichens and usually takes the form of specialized structures (soredia – powdery granules or isidia – tiny wart-like growths) that include all partners in the mutualism. If available, examine the examples on display that illustrate these structures. What is the adaptive significance of this type of reproduction?

Examine the examples on display and try to categorize the growth form of each. Can you find any evidence that the lichens on display are Ascomycota? Describe the structures you used to make your decision. Asexual reproduction is common among lichens and usually takes the form of specialized structures (soredia or isidia) that include all partners in the mutualism. What is the adaptive significance of this type of reproduction?

Fresh material of a large, foliose species will be available for further examination. Make a thin cross section of the thallus and examine it in a wet mount with the compound microscope. Using the photo atlas or your textbook, find the following structures: upper cortex, algal layer, medulla, lower cortex (if the lichen is *Peltigera* it will not have a lower cortex). Where in the lichen are the photosynthetic cells? How can you tell?

Examine the **prepared slide (# 75)** of the cross section of a lichen, and look for the following structures: upper surface, algal layer, medulla (inner hyphal tissue), lower surface. Where in the lichen are the photosynthetic cells? How can you tell?

## Mycorrhizas

Many Zygomycota, Ascomycota and Basidiomycota form mutualistic symbioses with the roots of higher plants. The structure that combines the tips of specialized roots with the ends of specialized hyphae from the mycelia of these fungi are known as **mycorrhizas** (literally, "fungus roots"). These structures are quite diverse in form and specific function, but the fungal partner usually provides the host with increased efficiency in nutrient and water uptake and the host provides the fungus with fixed carbon.

Examine and sketch the macroscopic habit of ectomycorrhizal roots on display. The mycorrhizas are usually short, thicker or fluffier, side-roots. Make thin cross sections of the mycorrhizas. Mount the sections in water, and refer to your text or lecture notes for help in finding structures: hyphal mantle, root epidermal cells, fungus net, and root cortex cells. Do the fungal hyphae penetrate into the root cells or just lie between the cells?

Examine a **prepared slide (Slide # 80)** of ectomycorrhizas and look for the same structures as above.

Examine a **prepared slide (Slide # 78)** of vesicular-arbuscular endomycorrhizae. Look for root cortex cells, and fungal structures. How are these mycorrhizas different from ectomycorrhizas?

## Organism Observation Check-List

## Lab 6 Fungi

1. Cultures of + & - strains of *Rhizopus* on PDA plates
2. Prepared slide # 76 – mold types *Aspergillus*, *Penicillium*, *Rhizopus* w.m.
3. Representative Ascomycota, including 1 fresh or preserved *Peziza*, *Morchella* or *Aleuria*
4. Prepared slide # 73 – *Peziza* apothecium with asci x.s.
5. Store-bought mushrooms; young and old white button mushrooms (*Agaricus brunescens*) some with gills browning due to spores [~2 per lab should be more than enough]
6. Prepared slide # 77 – *Coprinus* mushroom w/ basidia x.s.
7. Representative Basidiomycota of the season: mushrooms, shelf fungi, puffballs
8. Beaker of baker's yeast in water (+ ¼ tsp sugar) made fresh night before
9. Mold cultures on PDA plates prepared the week before (1 per 2 students)
10. Prepared slide # 76 – molds (same as #2)
11. Representative dry lichen thalli from three growth forms: foliose, fruticose, crustose
12. At least one of these should be a large foliose species that is maintained moist (in hydrated crisper) such as *Peltigera*, *Lobaria* or *Platismatia*
13. Prepared slide # 75 –lichen thallus (foliose) x.s.
14. Demonstration ectomycorrhizas – these are best collected in conifer forests on conifer roots from the organic – mineral soil interface, most under the drip zone. Kept moist in hydrated crisper for sectioning. [see photos below of typical local ectomycorrhiza types]
15. Prepared slide # 80 – ectotrophic (*Pinus*) mycorrhizal root x.s.
16. Prepared slide # 78 – V-A (endotrophic) mycorrhizal root x.s.

### Representative mycorrhizas

