Lab IX-1

Fungi Survey

Equipment and Materials

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.) If you are preparing your own live specimens, do so starting a week or so before you intend to do this lab session.

Materials from Kit

- □ Goggles
- □ Centrifuge tube, 50 mL
- □ Coverslips
- □ Inoculating loop
- □ Magnifier
- □ Pipettes
- □ Reaction plate, 24-well
- □ Scalpel
- □ Slides, flat
- □ Slides, deep well
- □ Stain: eosin Y
- □ Stain: Gram's iodine
- □ Stain: methylene blue
- □ Stain: safranin O
- □ Yeast

Materials You Provide

- □ Gloves
- □ Bags, plastic zip
- □ Bread (without preservatives)
- □ Butane lighter (or other flame source)
- □ Microscope
- □ Microtome (optional)
- □ Mushroom (fresh)
- □ Orange slice
- □ Paper towels
- □ Slides, prepared (optional; see text)
- □ Stereo microscope (optional)
- □ Sugar

Background

Fungi are a eukaryotic monophyletic group characterized by some unique features, as well as by other features that they share with plants or animals. Like plants, fungi possess a cell wall and vacuoles, and can reproduce sexually and asexually. Most fungi are *sessile* (fixed rather than motile) and often grow in soil. Like simple plants—mosses and ferns—fungi produce spores and, like mosses and algae, most fungi have haploid nuclei. Some fungi form fruiting bodies that

resemble those of plants, and some fungi form *rhizomorphs* that resemble plant roots and perform similar functions. Like animals and some protists, fungi are heterotrophs.

The primary feature unique to fungi is the composition of their cell walls, which contain both *glucans* (a class of polysaccharides) and *chitin*. Although both plants and animals use glucans—for example, the starch used in plant cells for energy storage and the glycogen used in animal cells for the same purpose are both glucans—and some animals use chitin for exoskeletons, beaks, and radulae, fungi are the only organisms that combine glucans and chitin in their cell walls. (Animal cells, of course, are contained by a cell membrane rather than a cell wall.)

In the obsolete two-, three-, and four-kingdom taxonomies, fungi were classified as *Plantae* because they obviously weren't *Animalia* (or, later, *Bacteria* or *Protista*). Over the next 200 years, as the differences between plants and fungi gradually became more difficult to ignore, most taxonomists became convinced that fungi belonged in a kingdom all their own. When Robert and Barbara were in high school in the 1960's, fungi were still officially classified as plants, but that changed in 1969 when Whittaker proposed his five-kingdom system, with *Fungi* as the fourth and final eukaryotic kingdom. (The prokaryotic *Bacteria* and *Archaea* were assigned to the fifth kingdom.) Whittaker's five-kingdom system was rapidly accepted by most biologists, and formed the basis for the later six-kingdom systems.

> The study of fungi is called *mycology*, and a biologist who specializes in fungi is called a *mycologist*. Oddly, many biology books, including recent ones, continue to treat mycology as a sub-discipline of botany, which is the study of plants, although DNA analysis shows indisputably that fungi are more closely related to animals than to plants.

Although it's been more than 40 years since fungi were assigned their own kingdom, a great deal of fundamental disagreement remains about how they should be classified within that kingdom. Even the number of phyla remains in dispute. What is not in dispute is that *Fungi* indeed form a *monophyletic group* (a single group of related organisms with a shared common ancestor). *Slime molds* (myxomcetes) and *water molds* (oomycetes), which are structurally similar to and were formerly classified as fungi are now classified in kingdom *Protista*.

The most recent proposal groups true fungi into seven phyla: *Ascomycota* and *Basidiomycota* (which are grouped into the subkingdom *Dikarya*), *Microsporidia*, *Chytridiomycota*, *Blastocladiomycota*, *Neocallimastigomycota*, and *Glomeromycota*.

> In 2001, the polyphyletic phylum *Zygomycota* was broken up, and the former order *Glomales* was promoted to the phylum *Glomeromycota*. About 230 members of *Zygomycota* were reassigned to this new phylum, with the remaining ~750 members assigned to other existing phyla. Despite the fact that it has been deprecated for a decade, many biology books (and biologists) continue to use phylum *Zygomycota* because it provides a convenient morphological grouping of *saprophytic* fungi with *aseptate hyphae*.

In these lab sessions, we'll examine some characteristics of representative fungi from three of these phyla, *Ascomycota*, *Basidiomycota*, and *Glomeromycota* (*Zygomycota*).

The phylum *Ascomycota*, also called *sac fungi*, contains roughly 30,000 species, including some *molds*, *morels*, *truffles*, and *yeasts*. The phylum is named for the sac-shaped reproductive structures called *asci* (singular, *ascus*) present in members of this phylum. Figure IX-1-1 shows a specimen representative of *Ascomycota*, *Morchella deliciosa*, whose common name is *white morel*. Figure IX-1-2 shows the most costly fungus in the world, *Tuber*

melanosporum, AKA the black truffle, which commonly sells for $5,000 or more per kilo.

Insert figure here. Use file White_Morel_IMG_0399.JPG

Figure IX-1-1. Morchella deliciosa (white morel)

Insert figure here. Use file black_truffle.jpg

Figure IX-1-2. Tuber melanosporum, the black truffle

Although most fungi are know to reproduce both sexually and asexually, the names assigned to classic fungal phyla are based on the structures used for sexual reproduction.

The phylum *Basidiomycota*, also called *club fungi*, contains roughly 25,000 species, including some *mushrooms*, *puffballs*, *shelf fungi*, and many important plant pathogens such as *smuts* and *rusts*. The phylum is named for the microscopic club-shaped reproductive structures called *basidia* (singular, *basidium*) present in members of this phylum. Figure IX-1-3 shows an unidentified species of *Amanita* mushroom growing in a suburban yard.

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Figure IX-1-3. Amanita sp.

The word *toadstool* is used in casual conversation for toxic (or at least inedible) mushrooms. Biology makes no distinction between mushrooms and toadstools.

The deprecated phylum *Zygomycota* contains roughly 750 species, many of them common bread molds. Members of this group possess sexual reproductive structures call *zygosporangia*, for which the original phylum was named. Figure IX-1-4 shows an unidentified mold growing on pita bread.

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Figure IX-1-4. Bread mold

Lichens are composite organisms, made up of a photosynthetic alga or cyanobacterium in symbiosis with a fungus. About two dozen species of algae and cyanobacteria are found in various lichens, but the species present in the vast majority of lichens is either the green alga *Trebouxia* or the cyanobacterium *Nostoc*. Similarly, although exceptions exist, the fungal

species present in the vast majority of lichens is one of the *Ascomycete* species.

> Although they are found in similar environments and may appear superficially similar, lichens and mosses are two distinct classes of organisms. Mosses are simple plants.

Lichens encompass about 25,000 “species”, many of which are quite colorful. Lichens are informally grouped according to their *growth forms*.

- *Crustose lichens* are flat (crust-like), with the *thallus* (body) growing close to the surface of a substrate such as bark or stone. Figure IX-1-5 shows the crustose lichen Rhizocarpon geographicum, vernacular name Map lichen.

Insert figure here. Use file Map_lichen.jpg

Figure IX-1-5. Rhizocarpon geographicum

- *Filamentous lichens* are hair-like, with thin tendrils or filaments.
- *Foliose lichens*, like those shown in Figure IX-0-6, peel away from the substrate, forming sheets that may superficially resemble leaves.
- *Fruticose lichens* are three-dimensional, with parts of the thallus forming stalks that protrude from the substrate surface.
- *Gelatinous lichens* are gel-like.
- *Leprose lichens* are powdery.
- *Squamulose lichens* are scale-like.

Insert figure here. Use file Yellow_lichen.jpg

Figure IX-1-6. A foliose lichen, probably Xanthoria parietina or X. polycarpa

Reproduction in lichens is complicated because technically lichen do not themselves reproduce. Instead, their component algae or bacteria and fungus reproduces—sexually, asexually, or both—according to the characteristics of their particular phyla. In practice, though, lichens can be considered to reproduce asexually because they release fragments or packages that contain both algal (or bacterial) and fungal cells, which cells go on to reproduce using their own methods. The new algae (or bacteria) and fungi may or may not associate symbiotically as new lichen individuals.

Most lichens are extremely hardy because they combine the advantages of cyanobacteria or algae (photoautrophism) and fungi (durable chitinous cell walls). Accordingly, lichens can be found in nearly any environment from the hottest, driest deserts to frigid Antarctic zones. One exception to that hardiness is the extremely high sensitivity of most lichens to air pollutants, probably because lichens have evolved to absorb nutrients efficiently from the atmosphere. For that reason, ecologists consider lichens to be “trigger species”, much like a canary in a coalmine. Lichens often suffer mass die-offs at pollution levels low enough that other species are apparently entirely unaffected.

Although you can purchase live specimens of various fungi and molds from Carolina Biological Supply or another vendor, there's really no need to do so. Fungi and mold spores are ubiquitous, and growing them is less a problem than keeping them from growing.

As a representative of *Zygomycota*, we'll use ordinary bread mold, *Rhizopus* sp. To grow a thriving colony, obtain some bread that does not contain preservatives. Bread from a bakery is a good source, as are those pop-and-bake tubes of bread dough sold at supermarkets. (Make sure the label indicates that no preservatives are present.)

> Most bread from the supermarket is loaded with preservatives. When we attempted to grow bread mold on supermarket white bread, we found after two weeks under ideal conditions that no growth had occurred. Those preservatives really do work.

To grow bread mold, simply expose a slice of bread to the air in your kitchen overnight to ensure that it's covered with mold spores and then place it and a wet paper towel in a sealed plastic bag or similar container at room temperature. Keep it in a dark location, or at least out of direct sunlight, and observe the growth over a period of several days. If you have a suitable camera, shoot images every 12 hours or so—say, morning and evening—as the mold develops. Note the

variety of textures and colors, each of which represents a different species of mold. The black (or very dark) mold is *Rhizopus nigricans* or *R. stolonifer*. We'll use that in this session, but it's also worth observing and attempting to identify any other mold species growing on your bread.

As a representatives of *Ascomycota*, we'll use the ubiquitous mold *Penicillium* spp. and common baker's or brewer's yeast, *Saccharomyces cerevisiae*. There's a good chance you'll find at least one bluish mold colony on your bread, which is almost certainly a species of *Penicillium*. If not, you can obtain a *Penicillium* specimen from any bleu cheese, such as Camembert or Roquefort. You can culture *Penicillium* obtained from a cheese by inoculating a slice of orange with a small amount of the bluish material from the cheese and incubating it at room temperature in a plastic bag with a wet paper towel. Culturing *S. cerevisiae* is trivially easy. Simply dissolve a teaspoon of table sugar in some tap water in a 50 mL centrifuge tube, transfer a small amount of baker's or brewer's yeast to the tube, fill the tube to the brim with water, and allow the tube to incubate in the dark at room temperature for several days.

As a representative of *Basidiomycota*, we recommend using an edible mushroom from the supermarket, which has the advantage of being known-safe. Using a wild mushroom instead has the advantage of allow you to obtain the entire mushroom, including the volva and other structural components that may be partially or completely beneath the surface. If you do collect wild mushroom specimens, treat them as though they are deadly poisonous; they may well be. Do not allow them to contact bare skin, do not inhale spores, and obviously do not ingest them.

Fungi are structurally simple. As you examine your specimens, look for the following (usually) microscopic structural features. Note that not all of these features are present in all species.

Hyphae (singular *hypha*) are the fundamental structures that make up fungi other than yeasts, which are unicellular. Hyphae are (generally) transparent, branching, filament-like structures that comprise cytoplasm and nuclei contained by a chitinous cell wall. Some hyphae, called *septate hyphae*, are divided into cellular sections by walls called *septa* (singular *septum*), with each section containing one nucleus. Other hyphae, called *aseptate hyphae* or *coenocytic hyphae*, have either incomplete or absent septa, with multiple nuclei present within a single cell wall.

A *stolon* is a septate hypha that connects *sporangiophores* (*sporophores* that produce spores within an enclosure) and may also anchor *rhizoids* (also called *holdfasts*), root-like hyphae that are embedded in the soil or other substrate where the fungus is growing. Hyphae used by parasitic fungi to penetrate the cells of the host to obtain nutrition are called *haustoria* (singular *haustorium*).

Asexual reproduction in fungi occurs via different mechanisms and using various specialized structures. Yeasts reproduce by *budding*, shown occurring in Figure IX-1-8, during which mitosis occurs and the cell cytoplasm splits into two uneven portions. The smaller portion detaches from the larger, and eventually matures as a new individual organism. The similar process called *fragmentation*, resembles budding with a more even initial distribution of cytoplasm. Most fungi reproduce asexually by mitotic production of haploid vegetative cells called *spores*. Spores are produced by specialized hyphae called *conidiophores* and *sporangiophores*, which produce spores in enclosures (*sporangia*, singular *sporangium*) such as sac-shaped *asci* (singular *ascum*) and club- or stool-shaped *basidia* (singular *basidium*).

Mycelia (singular *mycelium*) are intertwined masses of hyphae that make up the vegetative “body” of a fungus. A *monokaryotic mycelium* (also called a *homokaryotic mycelium*) results when one spore germinates; such mycelia cannot reproduce sexually. If two monokaryotic mycelia grow together and merge, forming a *dikaryotic mycelium*, that mycelium can reproduce sexually, forming *fruiting bodies* (e.g., mushrooms), which produce and disperse spores.

Depending on the species, mycelia may range from microscopically small to gigantic, weighing thousands of tons.

> Use extreme care when handling fungi samples. Fungi release spores, which may be pathogenic or toxic. Many environmental molds are potent allergens. If you collect fungi, such as mushrooms or molds, wear gloves and goggles at all times. An N100 respirator mask protects you against inhaling spores. Unless you know they are safe (e.g., edible mushrooms or yeast from the supermarket), when you have handled fungi of any type, always wash your gloves with soap and water before removing them and then wash your hands thoroughly. Obviously, do not ingest any unknown fungi.

Procedure IX-1-1: Observing Zygomycota

To begin, use the magnifier (or a stereo microscope, if you have one) to examine closely the various molds growing on your bread specimen.

1. Note the color and texture of each species, and record your observations in your lab notebook.
2. Using the Internet or printed reference material, attempt to identify each of the species present, or at least their genus.
3. For each species present, examine and identify any structures visible at low magnification. Are hyphae present that are modified as sporangiophores and sporangia? Is pigmentation evenly distributed throughout each mycelium, or concentrated in specific structures? If you have a suitable camera, record an image, ideally at low magnification through the microscope.

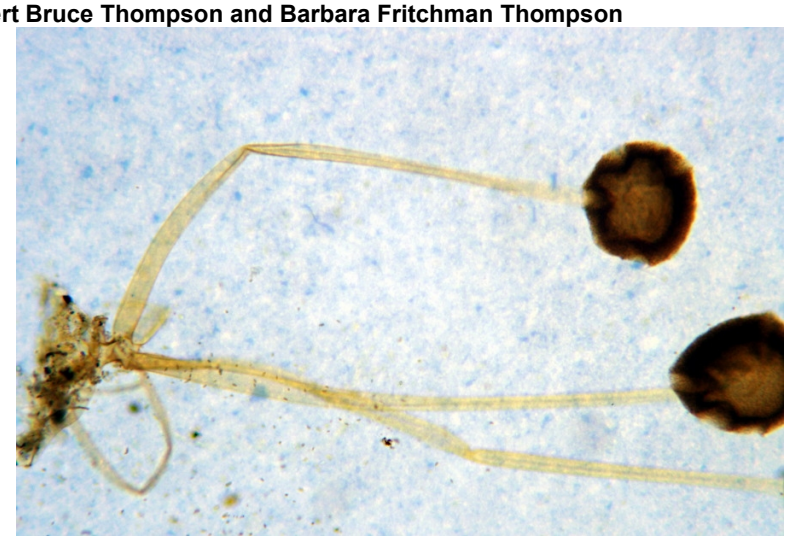
Unless you are very unfortunate, your bread should have at least one dark-colored colony of *Rhizopus* sp. growing on it. We'll use material from that colony in the following steps. If you have time, you can repeat these steps for some or all of the other species present on your bread specimen.

4. Flame sterilize the inoculating loop and use it to transfer a tiny amount of material from that colony to each of five flat slides. Make smear mounts of each slide by adding a drop of water, using the edge of a clean slide to smear the material, and then heat-fixing the specimen.
5. Retain one of the slides as an unstained specimen. Stain each of the other four slides using one of the following stains: eosin Y, Gram's iodine, methylene blue, and safranin O. For each slide, add a small drop of the stain to the specimen area, allow the stain to work a minute or so, and then rinse off the stain with a very gentle stream of tap water.
6. For each slide, position a coverslip, scan at low power to locate a populated area, and observe the specimen at medium and high magnifications. Determine which, if any, of the stains are helpful in revealing additional detail versus the unstained slide. Record your observations in your lab notebook.

Figure IX-1-7 shows *Rhizopus nigricans* (black bread mold) at 100X. Rhizoids are visible at the far left of the image, with sporangiophores supporting the sporangia visible on the far right.

Insert figure here. Use file Rhizopus-nigricans-wm-100X.jpg

Figure IX-1-7. Rhizopus nigricans, wm, 100X



Procedure IX-1-2: Observing Ascomycota

In this procedure, we'll observe *Ascomycota* (sac fungi), many species of which closely resemble various species of *Zygomycota* with the exception of their spore-forming structures. Many *Ascomycota* species are economically valuable, critical to the production of antibiotics, beer and other alcoholic beverages, cheeses, livestock feed supplements, and many other important products.

The first of the *Ascomycota* we'll observe, ordinary yeast, does not form spores at all, instead reproducing asexually by budding. Other *Ascomycetes* reproduce asexually by forming spores called *conidia* on the surface of *conidiophores*—hyphae adapted for reproduction—in contrast to the enclosed sporangia spore sacs formed by *Rhizopus* and other *Zygomycota*. *Penicillium* spp. and *Aspergillus* spp. are two ubiquitous examples of *Ascomycota*.

1. Carefully open the centrifuge tube that contains your *S. cerevisiae* culture. Note the appearance of the culture and any odor present, and record your observations in your lab notebook.
2. Transfer about 2 mL of the yeast suspension to a well in the reaction plate, add one drop of methylene blue stain, and stir to mix the solutions.
3. Transfer one drop of the unstained live yeast suspension to a flat slide, position a coverslip, scan the specimen at medium magnification to locate budding cells, and then switch to high-dry magnification to observe any visible detail. If you have an oil-immersion objective, also observe the specimen at high magnification. Record your observations in your lab notebook.
4. Repeat step 3 with one drop of the live yeast culture that you stained with methylene blue.

Figure IX-1-8 is a 1000X differential interference contrast (DIC) microscopy image of *S. cerevisiae* budding.

Insert figure here. Use file S_cerevisiae_under_DIC_microscopy.jpg

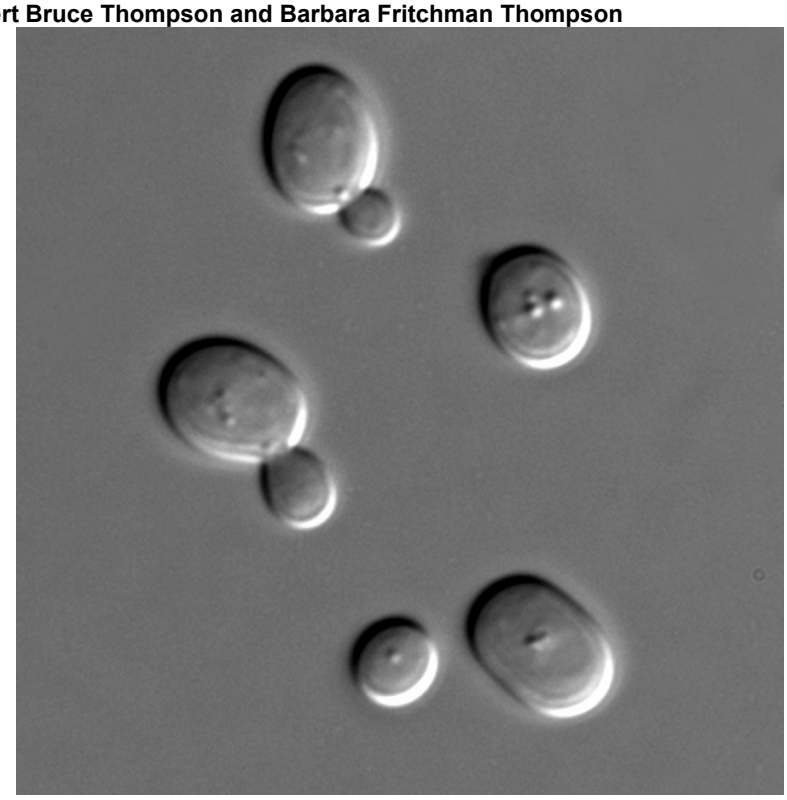


Figure IX-1-8. Saccharomyces cerevisiae budding, 1000X (DIC microscopy)

5. Flame-sterilize the inoculating loop and use it to transfer a tiny amount of material from your *Penicillium* culture to a flat slide. Add a drop of water, spread the liquid, position a coverslip, and observe the slide at medium and high-dry magnification.
6. Add a drop of methylene blue at one edge of the coverslip, and use the corner of a paper towel to wick the stain under the coverslip. Allow the stain to work for a minute or so and then add a drop of water at the edge of the coverslip and wick out the excess stain. Observe the slide at high-dry and with the oil-immersion objective, if you have one. Note the appearance of the conidiophores and conidia, and record your observations in your lab notebook.
7. Using the same slide, repeat step 6 with a drop of eosin Y stain.
8. If you have an *Aspergillus* culture, purchased or home-grown, repeat steps 5 through 7 to observe *Aspergillus*. (Like *Penicillium*, *Aspergillus* sp. is ubiquitous, and is likely to be represented among the colonies growing on your bread specimen.)

Figure IX-1-9 is a 100X overview of a *Penicillium* prepared slide. Figure IX-1-10 shows the conidiophores and conidia at 1000X. Figure IX-1-11 is a 100X overview of an *Aspergillus* prepared slide. Note the similarities between Figures IX-1-9 and IX-1-11.

Insert figure here. Use file Penicillium-sp-wm-showing-conidia-100X.jpg

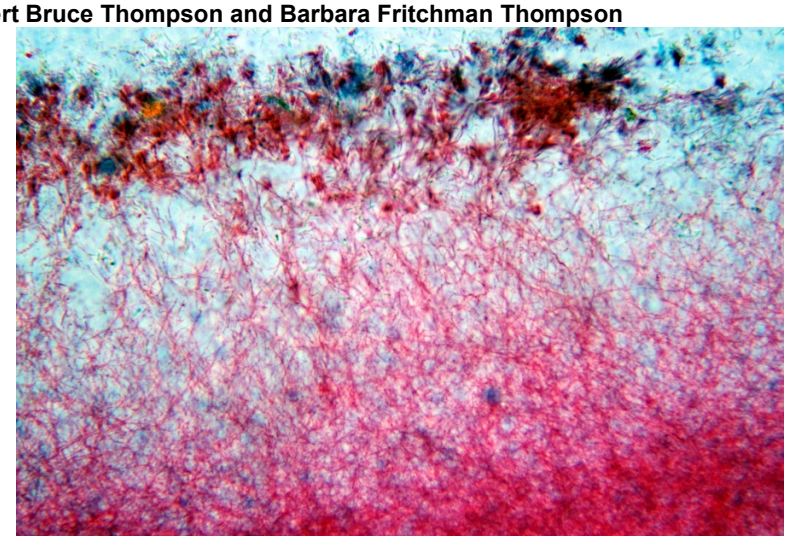


Figure IX-1-9. Penicillium sp. wm showing conidiophores, 100X

Insert figure here. Use file conidiophores-with-conidia-of-Penicillium-marneffei.jpg

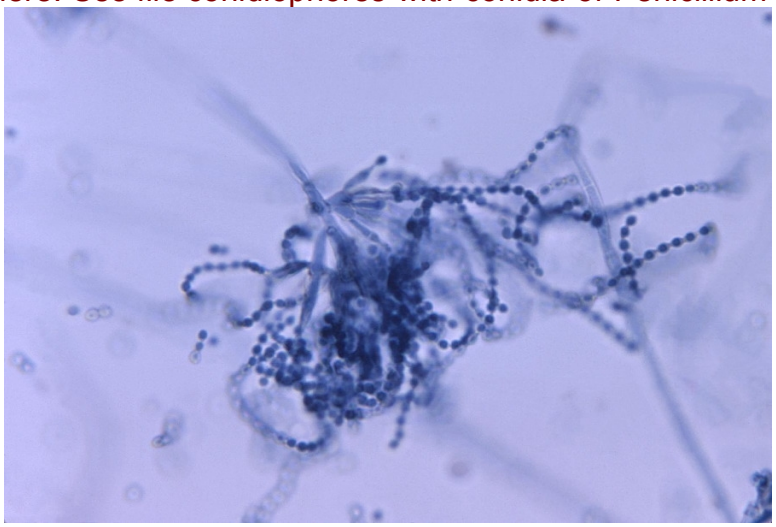


Figure IX-1-10. Penicillium conidiophores with conidia, 1000X

Insert figure here. Use file Aspergillus-showing-conidia.jpg

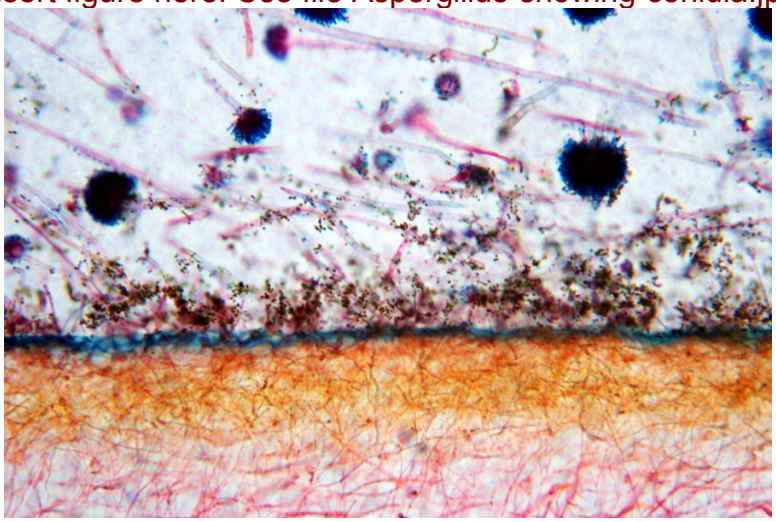


Figure IX-1-11. Aspergillus sp. wm showing conidiophores and conidia, 100X

Procedure IX-1-3: Observing Basidiomycota

Many *Basidiomycota* (club fungi) species are among the most familiar fungi. *Basidiomycota* include mushrooms, puffballs, and shelf fungi—some of which are edible, but most of which are inedible or even poisonous—as well as various microscopic plant pathogens, including rusts and smuts. Figure IX-1-12 shows a fine example of *Agaricus augustus*, a typical mushroom.

Insert figure here. Use file Agaricus_augustus_2011_G1.jpg

Figure IX-1-12. Agaricus augustus, a typical Basidiomycete

Begin by examining your mushroom specimen by naked eye and with the magnifier or a stereo microscope. Identify, name, and describe as many features as possible, including (if present) the *stipe* (stem) and any substructures present including the *annulus* or *volva*, the *pileus* (cap), and the *lamellae* (gills) present on the underside of the pileus. Record your observations in your lab notebook.

The most significant structures of a *Basidiomycete*, and the ones for which the group is named, are the basidia, microscopic spore-producing structures located on the gill. To observe basidia, take the following steps:

1. Cut off the stipe of your mushroom specimen, if present, and place the pileus flat on a hard surface, with the lamellae side (bottom of the cap) facing up.
2. Halve the pileus by using the scalpel or a single-edge razor blade to make a vertical cut through the pileus.
3. Place one of the halves aside, and use the scalpel to cut as thin a vertical section as possible through the remaining half near the edge of the pileus. Ideally, you want this vertical section to be so thin that it is almost transparent.
4. Transfer the section to a flat slide. Position a coverslip on the slide, place the slide on the stage, and observe it at low magnification to locate the basidiophores and basidia. Center that

area under the objective and observe it at medium and high-dry magnification. Record your observations in your lab notebook.

Figure IX-1-13 shows a prepared slide of *Agaricus* sp. at 100X. Figure IX-1-14 is a scanning electron microscope (SEM) image showing the stromal layer, basidophores, and basidia of *Agaricus bisporus*.

Insert figure here. Use file Agaricus-sp-cs-of-gills-showing-basidiospores-100X.jpg

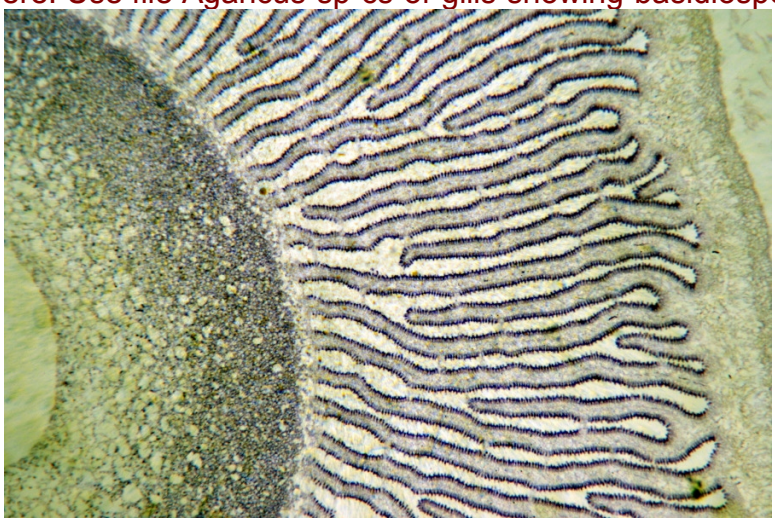


Figure IX-1-13. Agaricus sp. cs of gills showing basidiospores, 40X

Insert figure here. Use file Agaricus_bisporus_spores_SEM_1.jpg

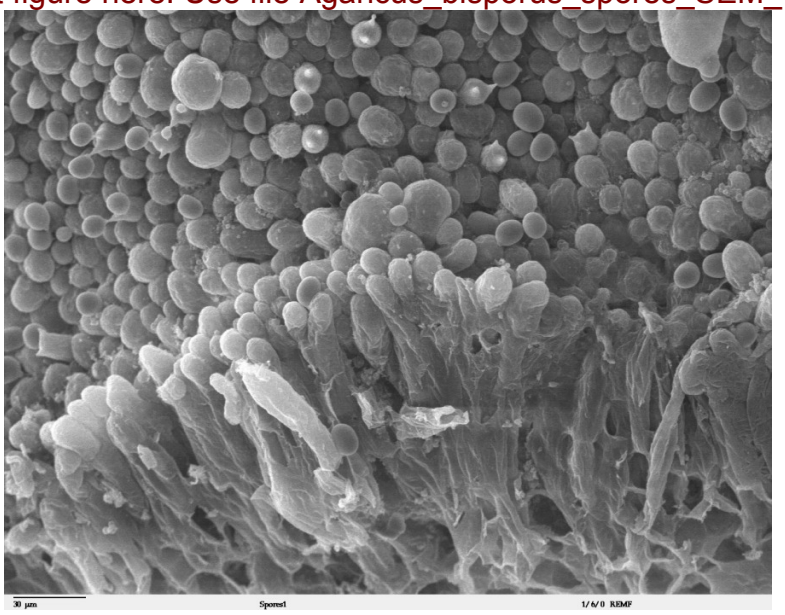


Figure IX-1-14. SEM image of Agaricus bisporus, showing stromal layer and basidiospores

Review Questions

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1. Why are fungi not classified as algae? As plants?

Fungi are not classified as algae because fungi do not contain chlorophyll and are therefore heterotrophs. All algae are autotrophs. Fungi are not classified as plants because fungi do not have real tissues, but only specialized hyphae that perform some of the functions performed by differentiated tissues in plants.

2. In session IX-1-1, which fungal structures were you able to identify? What structure(s) of *Rhizopus* sp. are responsible for its common name, black bread mold?

   Answers may vary, but with sufficient magnification hyphae (including rhizoids and stolons), sporangiophores, and sporangia should be readily visible. The coloration in *Rhizopus* sp. is caused by the dark color of the sporangia and the spores they contain.

3. In session IX-1-2, what odor did you notice when you opened the yeast culture tube? To what is that odor attributable?

   There should be a noticeable odor of alcohol present, caused by fermentation as the yeast consume the sugar by anaerobic respiration, producing ethanol as a waste product.

4. In session IX-1-2, which of the stains you tested was most helpful in revealing detail?

   Answers will vary depending on the particular species used, experimental conditions, and personal preferences. Most or all of the stains will provide at least some benefit, but many students will probably choose methylene blue.

5. In session IX-1-2 while observing the unstained *Penicillium* culture, what did you observe?

   Answers will vary, but should include a mass of white or translucent hyphae and blue to blue-green conidia.