**Fungi**

The fungi lack photosynthetic pigments. As a group, they are heterotrophic and must obtain their food either from dead organic matter (saprophytically) or from living organisms (parasitically). Approximately 100,000 species have been described, and these are very widely distributed, particularly on land, although there are a number of common water-growing species.

What were once fungi have been divided into six phyla: Myxomycophyta and Chytridomycota (now in kingdom Protista), Oomycota (now in kingdom Chromista), and Zygomycota, Ascomycota, and Basidiomycota (the true kingdom Fungi). Classification of the three phyla of true fungi is controversial and based primarily on their sexual reproduction. A seventh artificial phylum, Deuteromycota, includes all species for which no sexual stages are known at present. Mycologists consider it their duty to discover the sexual stages and classify these species into the natural phyla...easier said than done. Most are clearly members of Ascomycota and a few are clearly members of Basidiomycota.

The true fungi produce a vegetative body which is composed of masses of fine branched filaments often having a cottony appearance. A single filament is called a hypha, while a mass of hyphae makes up the mycelium. Mycelia may form reproductive structures, usually both asexual and sexual. While the mycelia of different species are very much alike in gross appearance, the reproductive structures are distinctive.

You will look at a few rather typical representatives of kingdom fungi. Make a record that will give some idea of the diversity of form that occurs among the fungi and will point out some of the differences among the kinds of fungi. You should practice making life cycle diagrams for these taxa as good practice in understanding their natural history.

**I. Bread Molds: (Zygomycota)**

A two-week old plate culture of Phycomyces is available for examination. This organism is a zygomycete fungus. **DO NOT OPEN THE CULTURE!** Examine through the lid of your culture with the dissecting microscope.

Your culture will likely show asexual reproduction; notice the obvious, sporangia borne at the ends of upright branches of the coenocytic mycelium. These contain or produce a great many, small, spores which are easily wind distributed (**DO NOT OPEN THE CULTURE**)! These spores may contaminate the building and cause allergic reactions in some persons). The upright sporangiophores may be connected to each other by stolons and to the medium by rhizoids. Most of these species are heterothallic, so two strains are required for successful sexual reproduction. This Potato Dextrose Agar (PDA) plate has the + and – mating types inoculated at opposite edges of the plate (note the markings on the bottom of the plate).

Examine the plate culture under the dissecting microscope. Check the edges where most of the mycelium is of one mating type. Then compare mid-way across the plate where the two mating types have converged. Where the two strains have grown together, you may be able to observe a line composed of structures called zygospores. In the zygomycetes, hyphae of the two strains contact each other and the hyphal tips each divide off to form a gamete cell. The two gamete cells, which are in contact, fuse to form the zygote. The zygote develops a thick, sometimes dark wall, and enters a resting period as a zygospore. Meiosis occurs inside this resting spore or inside a sporangium that grows out of it.
In the space below, illustrate what you have found of the life cycle of this zygomycete. If you cannot find both sexual and asexual reproductive structures, ask the instructor what to do about that.

Life Cycle of Phycomyces (8 points)

II. Ascomycota--the sac fungi

This is the largest group of fungi and includes many economically-important species: some are pathogens of higher plants, others produce valuable substances such as alcohol or penicillin. All are characterized by a sac-like structure, called an ascus, which contains ascospores. The ascus is produced after sexual fusion, normal within an ascocarp, a structure of characteristic size and shape formed by an aggregation of hyphae. The asci occur in large numbers, forming a continuous layer, the hymenium, in the ascocarp; the ascospores are produced by meiosis. Asexual reproduction is usually by means of conidiospores cut off mitotically at the ends of certain special hyphae.

Observe a slide culture of Penicillium, the Fungi Imperfecti stage of an ascomycete, which is a saprophyte commonly occurring on rotting fruit, etc. Observe under the microscope. These imperfect stages are haploid in most cases. (6 points)

Sketch:

Conidia

Is the mycelium septate or coenocytic? [ ] septate [ ] coenocytic

Conidiophore

Do you find any hyphae producing conidia? [ ] yes [ ] no

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Yeast (Saccharomyces) is also an ascomycete, although it does not produce a typical mycelium. Obtain a drop from the yeast culture and observe under the microscope. If the culture is actively growing, the cells will be budding, a form of asexual reproduction by cutting off small cells at the surface. Yeast breaks down sugars to alcohol and carbon dioxide (fermentation) and is used commercially to produce both products; for example, in wine and bread making. A plate culture contains cells that have fused to form a zygote that becomes an ascus and ultimately contains ascospores. Make a wet mount of a tiny bit of the plate culture from a confluent growth area to see if you can find asci containing ascospores. (5 points)

Sketch: ascus ascospores

How many ascospores are in each ascus here? 1 2 4 8

A 10-day old plate of Sordaria should be available. Use a dissection needle or two to pluck one of the tiny black structures from the agar. This structure is a perithecium. This ascocarp is flask-shaped and has an apical opening pointing up toward the cover of the dish. Put two or three perithecia in a drop of water on a microslide. Add a cover slip and observe under low power. If water tension was sufficient, the perithecium may have ejected a bundle of asci. If not, you should remove the slide from your microscope and provide slight pressure on the cover slip with your thumb. Not too much pressure...just enough to crack open the ascocarp. Observe (10 points)

Sketch:

The vegetative hyphae are septate coenocytic

How many ascospores are in each ascus? 1 2 4 8

Sordaria is homothallic and produces a perithecium (ascocarp) even when only one strain is present. When two genetically different strains are present, they hybridize where the mycelia come into contact. Please notice that syngamy is a two-step process (plasmogamy and karyogamy). In most other organisms the two steps occur more-or-less simultaneously. In higher fungi (Ascomycetes and Basidiomycetes) these steps are separated in time and a protracted dikaryotic (heterokaryotic) phase results. The fusion cells (the results of plasmogamy) produce the dikaryotic ascogenous hyphae which produce the hymenium consisting of many asci. In each ascus, the two nuclei united (karyogamy occurred) and the diploid cell divided meiotically (four products) and then mitotically to produce eight haploid ascospores. The ascus contains these spores and, by virtue of small diameter, holds them in order of their production by meiosis. The combinations of black and tan spores held in a hybrid ascus of Neurospora (a related ascomycete) prompted the discovery of meiosis!

After observing the wild-type asci from the ascocarp demonstration plate, record some of the combinations you see in the asci inside perithecia taken from the Sordaria mating plate. The hybrid perithecia should be taken from the borders between the zones of wild-type (black) and mutant (tan) mycelia.

15-
Shade the ascospores appropriately in each ascus and provide a tally the number of asci you find having each combination of ascospores: (16 points)

<table>
<thead>
<tr>
<th>Unmated Combinations</th>
<th>Parental Combinations</th>
<th>Recombinant Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

These are only the beginning of the complexity of forms in ascomycetes. Before you finish your observations of this group, look at prepared slides of some larger ascocarps:

*Morchella*, the morel mushroom. This one is considered a delicacy to eat. The hymenium is highly convoluted and in the form of a cone on the end of a stalk. Examine the hymenium closely for asci. Count the ascospores in several asci and depict the correct number in your sketch. Remember you are seeing a thin slice so all asci might not be “typical,” and remember that all asci may not be at the same stage of development!

*Tuber*, the truffle. This is also a large edible fungus. The ascocarp is hypogeous and is a cleistothecium. Dogs and pigs are used to locate them by scent. Huge prices (higher than gold) are paid per ounce for gourmet varieties of truffles. Make a sketch with the correct number of spores in the ascus.

**Sketches:**

*Morchella* (9 points)

- haploid cells
- dikaryotic cells
- ascus
- ascospores
- There are ____ spores per ascus

*Tuber* (7 points)

- haploid cells
- ascus
- ascospores
- There are ____ spores per ascus
III. Basidiomycota--The club fungi

While this division is not as large as Ascomycota, it is probably better known to you. It includes the common mushrooms, toadstools, bracket fungi, puffballs, and the rusts and smuts. The latter two groups are parasitic on many of our important crop plants. We are rarely aware of the vegetative mycelium; it grows in the organic matter of soils, in rotting logs, etc. The mushroom, etc., with which we are familiar, is the reproductive structure or basidiocarp formed by an intricate mass of interwoven dikaryotic hyphae. Basidiocarps vary greatly in appearance in different genera, but all bear basidia which are usually arranged in a layer of hymenium. The basidium, like the ascus, is the site of karyogamy and meiosis. The basidium is a club-like cell at the end of which are four short stalks (sterigmata), each of which extrudes one of the four basidiospores.

Observe the gross aspects of a common edible mushroom (Agaricus). In the arboretum you learned that the basidiocarp of some mushrooms include a volva, a stipe, an annulus, and a pileus (cap). Look at the under-surface of the cap of the mushroom; note the gills with lamellae radiating from the center to the periphery. The surface of the gills is a hymenium made up of closely packed basidia bearing basidiospores. Sketch:

<table>
<thead>
<tr>
<th>Agaricus (10 points)</th>
<th>Coprinus (10 points) use highest magnification!</th>
</tr>
</thead>
<tbody>
<tr>
<td>pileus</td>
<td>basidiospores</td>
</tr>
<tr>
<td>lamellae</td>
<td>sterigmata</td>
</tr>
<tr>
<td>annulus</td>
<td>basidium</td>
</tr>
<tr>
<td>stipe</td>
<td>dikaryotic cells</td>
</tr>
</tbody>
</table>

Examine a prepared slide of Coprinus, a mushroom, which shows sections of gills lined by basidia with basidiospores. Most of the latter may have become detached from the basidia during the preparation of the slide. Note the intertwined hyphae which make up the tissue of the gills.

Are these hyphae septate or coenocytic? [septate coenocytic]

Closely examine basidia along the surface of the lamellae (gills) to find one showing all four basidiospores on the tips of four sterigmata (tips). If you find a good one, call the instructor over so the rest of the class can see this…and include that basidium in your sketches above.

Schizophyllum is a bracket fungus usually found on tree bark. The white basidiocarp is fan-shaped and usually grows out over the surface of the bark with gills facing the bark. This species has been studied widely in fungal physiology, genetics, and morphogenesis.

Some mushrooms you picked up on our hike in the arboretum did not have gills under their caps. They may have a sponge-like hymenium composed of many small pores. These are the so-called polypore mushrooms. Examine a prepared slide of some of this hymenium in Polyporus. Locate basidia and basidiospores. Sketch (4 points):

| basidia | basidiospores |

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Fungal Physiology

Observations: Nearly all organisms require a source of chemicals to provide energy for maintenance and growth. Of these organisms, nearly all rely upon some kind of carbohydrate. Textbooks indicate that glucose and fructose are found along the glycolytic pathway. In the absence of oxygen, yeasts utilize the alcoholic fermentation pathway to produce energy (in ATP), ethyl alcohol, and carbon dioxide. Of these products, carbon dioxide is a gas and can be measured volumetrically as you did in Durham tubes.

Question: What type of carbohydrate might serve as the best energy fuel for an organism?

Hypothesis: Yeast make the enzymes required for fermentation of all of these carbohydrates.

Prediction: If the hypothesis above is true, then carbon dioxide gas should accumulate equally among yeast samples when given each of the carbohydrates.

Experiment: A mixture of 2.5 ml yeast suspension (Saccharomyces cerevisiae) with 2.5 ml of 10% carbohydrate solution can be held in an inverted 2-ml graduated pipette if the tip end of the pipette is plugged with modeling clay. At your station you will find some 13x100 mm test tubes, a test tube rack, some 2-ml pipettes, a pipette filler, some 3 mL transfer dropper pipettes, and a small lump of clay. NEVER PIPETTE BY MOUTH!!

Results: Record the meniscus location:

<table>
<thead>
<tr>
<th>Carbohydrate Treatments</th>
<th>10% Sucrose</th>
<th>10% Glucose</th>
<th>10% Lactose</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time = 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Analysis: Calculate a rate for each treatment = \( \frac{\text{ml of gas}}{\text{elapsed time}} = \frac{\text{start mark} - \text{end mark}}{\text{hr}} \)

Units

Rate ( )

Decision:

The hypothesis above is rejected

Conclusions:

The yeast cells had used up their initial substrate.

The yeast cells did produce invertase.

The yeast cells did not produce lactase.