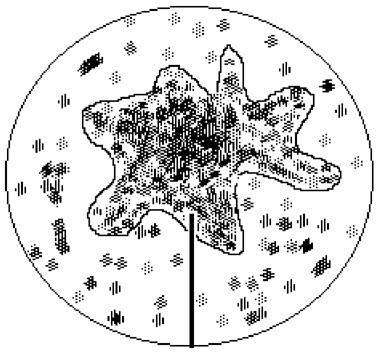

Fungi and Microbial Culturing

In this lab, you will study the microscopic world. All life on Earth depends on the actions of organisms that are too tiny to see without a microscope. These organisms are responsible for generating oxygen, releasing nutrients and minerals from dead plants and animals, and serving as the food source at the base of many food chains.

Objectives:

1. You should be able to correctly operate a compound microscope, know its basic parts, and be able to put it away properly. If your lab section has not already done so, you may be asked to complete a short lab on microscopy before proceeding with this lab.

2. You should be able to differentiate between microscopic organisms at the Kingdom level (Monera[1], Protista, Fungi, Animalia, Plantae) on sight.

3. You should be familiar with the taxonomic classification system used for the Monera, the Fungi, and the Protista.

4. You will learn about sterile techniques.

5. You should be able to recognize the major groups of Fungi on sight.

Synopsis of the Lab Exercise:

You should read through the introductory material below *before* lab. You should also read Sections 20.1-20.3, 20.5, Chapter 21, and Chapter 23 in your textbook (Lewis et al. 2002). There is a **pre-lab assignment** on pages 9 & 10 of this handout to complete **before** coming to lab.

Week 1:

1. Your group will inoculate plates with microbiological samples from the environment.

Week 2:

2. You will examine several different types of fungi and again make detailed observations and drawings.
3. Your group will analyze the results from the plates that you streaked the previous week.

Please bring your textbook to lab.

[1] Your textbook uses a 3 domain system (see page 908). For compatibility with other texts, including those used in advanced courses, we are grouping the organisms in the domains Bacteria and Archaea into the Kingdom Monera (actually, in lab we will really only discuss the bacteria anyway).

Microorganisms in the Environment

Microorganisms such as Monera, Protista and Fungi are found in almost every conceivable place in the environment. Every surface (including the skin of animals, and the outer surface of plants) is covered with a thin film of organic molecules, bacteria, and, in some cases, other microscopic organisms including fungi, algae, cyanobacteria and protozoa. In some cases, there are even microscopic multicellular animals such as mites (Phylum Arthropoda: Class Arachnida) obtaining their food from these 'biofilms'. Also mixed in with this film are a variety of living organisms in "resting stages", that is spores of molds, protozoa, rotifers, etc. that may be activated by the proper environmental conditions - often an increase in moisture.

These films form complex ecosystems in their own right. Among the organisms found in the films are autotrophs and heterotrophs. **Autotrophs** obtain energy either from simple inorganic chemicals in the environment or by photosynthesis. **Heterotrophs** get their energy from complex chemicals produced by other organisms (**organic chemicals**). Humans are heterotrophs; we obtain energy from plants (which are autotrophs who get their energy from the sun via photosynthesis), or from other animals (which are heterotrophs and obtain their energy from either plants or other animals). Heterotrophs that feed on plants are called **herbivores**, and heterotrophs that feed on animals are called **carnivores**. There is another major source of energy for heterotrophs; it is in the organic chemicals that are no longer associated with living organisms. Such chemicals are usually found in the feces of other animals (it is rare that an animal extracts all the energy from the food it eats), or in the decaying bodies of plants and animals. The heterotrophs that exploit these food sources are known as **detritivores** or **decomposers**.

Much of the energy in the biofilms comes from organic chemicals no longer associated with living organisms; therefore, most of the organisms in the films are detritivores. The organisms in the film compete for the nutrients available. They try to grow over each other and produce chemicals that are toxic to their competitors (we call these compounds antibiotics because they are "against life"). For a long time, biologists have found the incredible complexity of these films simply too much to deal with as a unit, and our study of the films has been based on isolating individual species and studying them one by one.

Isolating the organisms involves moving them from the films to an environment suitable for growth, and physically separating the organisms. This is typically done in a simple procedure called plating. A plate of nutrient medium, containing agar (a gel that comes from red algae), along with other nutrients, is prepared using sterile or aseptic techniques. Aseptic (*a* - Gr. without; *septi* - Gr. rotten) techniques include heating the medium to kill any organisms already in it. To inoculate (add organisms) to the plate, a sterile cotton swab (*swab* - Eng. Q-tip), moistened with sterile water from a test tube, is rubbed over the environmental biofilm. The swab picks up an assortment of organisms from the film, and it is then rubbed lightly over the surface of the agar. The diluted organisms will thus be spread evenly over the surface of the plate.

The plate is then incubated (held at some controlled temperature) until the individual organisms scattered over the surface of the medium have time to reproduce. Each organism divides repeatedly to form a mass composed of only one species of organism. Such a pure mass of organisms is called a colony. Each colony contains millions of cells, and these can later be removed from the agar to start new, pure colonies on another plate. The organisms from the biofilm are now isolated.

The initial stage of isolation on rich nutrient media seeks to provide an environment ideal for the growth of the majority of organisms in the film. Later stages often consist of restreaking on more specialized media in order to limit growth and thus learn something about the organisms involved. For instance, agar may be prepared that is lacking a certain nutrient, and any organisms that grow on this agar can be presumed to be able to produce this nutrient themselves. Other agar may be laced with antibiotics to test the resistance of the organisms to those antibiotics. Since many of the organisms in the biofilm are so small, and since they are difficult if not impossible to separate on the basis of their physical appearance, such chemical differentiation is often used to classify them. Of course, there are variations on the simple scheme presented here. For instance, a nutrient-deficient or antibiotic-laced agar may be used in the initial isolation to "screen" for organisms with certain characteristics.

You should be aware that the simple techniques available to us in a general biology laboratory have many drawbacks. For instance, many times it is difficult to get good physical separation of cells in the streaking technique (especially for beginners). Colonies that are not separated enough can merge, reducing the apparent number of colonies. In many cases, colonies composed of different species of bacteria will look alike. And, not all of the bacteria in the environment grow well in the presence of oxygen and on an agar plate. To avoid these problems, modern attempts to look at biofilms are often more holistic, seeking to learn about the characteristics of the organisms as they exist together in the film - such characteristics are often vastly different when compared to the characteristics of the organisms in isolation. Techniques used to study the group characteristics of biofilms include radioisotopes, high-performance chromatography, and scanning electron microscopy.

Kingdom Fungi

Ecological Roles

Fungi are among the most important decomposers because they are one of the few types of organisms that can digest two of the tougher materials used in living organisms. Both **chitin** and **cellulose** are **polysaccharides**; that is they are composed of sugar molecules in long chains. Unlike the polysaccharide starch, however, chitin (used in the exoskeleton of insects) and cellulose (the principle component of wood) are very difficult to digest. It has been found that many animals that feed on decaying leaves or wood actually are feeding on the fungi. It is the fungi that do most of the work of decompostion.

Likewise, fungi play an important role in the nutrition of many plants. By forming symbiotic, mutualistic relationships with the plant roots, the fungi can bring the plant nutrients such as phosphorous, as well as water. In return, the plant roots furnish the fungi with sugars. This is called a **symbiotic** (literally living together) relationship because of the close association; it is **mutualistic** because both of the organisms benefit. The fungi that associate with plant roots are called **mycorrhizae**; many of our prized edible mushrooms, truffles and morels come from such associations. It has also been found that after strip mining, plants whose roots have been "infected" with the mycorrhizal fungi are better able to survive the harsh soil conditions in the reclaimed strip mine. Modern foresters raising seedlings to reforest strip mines have had to become mycologists too!

Fungi play other roles in the ecosystem as well. In human terms, however, we are most likely to notice fungi in a few situations. We eat some of them of course; others are important in processing our food. For instance, we use yeasts for baking (where they produce carbon dioxide gas that causes dough to rise) and brewing (where they ferment sugars into alcohol); other fungi help us make cheese. Some fungi cause diseases (athlete's foot, thrush); others produce antibiotics that are used to treat diseases. In nature, of course, the fungi produce these antibiotics to kill potential competitors. Fungi are important in natural ecosystems because they break down detritus; but when the fungi are rotting the wood in your house or the bread for your sandwich, you tend to forget their natural usefulness. Finally, some fungi have changed the course of human history. The potato blight which starved millions of Irish and caused millions more to emigrate to the United States is one example of this phenomenon.

Structure of Fungi

As already mentioned, for most multicellular fungi the basic unit of the "body" is the **hypha** (plural hyphae). Hyphae are small, threadlike filaments that grow to, around and through food materials. Here they secrete digestive enzymes to break down the food into small molecules that can be absorbed. When the hyphae are densely packed they form a whitish mass called the **mycelium**; when it is time to reproduce, the mycelium form a **fruiting body**. Mushrooms are one type of fruiting body.

Fungal Reproduction

In most fungi, the hyphae are **haploid**, with a single set of chromosomes. Often, the cell walls between adjacent cells in the hyphae are incomplete or missing, therefore there may be many nuclei in a single "cell". However, since each nucleus has but one set of chromosomes, we call the hyphae haploid. When a haploid hypha of one type grows next to a haploid hypha of a different type, the two may fuse to form **diploid** hyphae, and a fruiting body will be formed. In the fruiting body, **meiosis** takes place to produce haploid spores. Spores are produced in great numbers and blow on the wind, hopefully to new food sources. When they germinate, spores produce new haploid hyphae by regular cell division or **mitosis**. Some fungi apparently have no sexual life cycle at all. These latter fungi (which include *Penicillium,* the source of the antibiotic penicillin) reproduce **asexually**.

Taxonomic Groupings of Fungi

Your book divides the Fungi into 4 phyla. The **Chytrids** (Phylum Chytridiomycota) are found in water and in moist habitats on land. Many plant diseases are caused by water molds. The organism which causes the potato blight was formerly placed in this phylum, but is now classified with the protists.

Common Molds (Phylum Zygomycota) include a number of fungi including the familiar bread mold. Other species are also saprophytic (feeding on decaying material) or even parasitic. These fungi reproduce sexually with a simple structure being formed when two hyphae of different types meet.

The **Sac Fungi** are placed in the Phylum Ascomycota. This is a very diverse group. As mentioned, it includes various yeasts (which, under the right conditions, reproduce sexually using asci), however this group also includes truffles, morels and pathogens like the ones that cause Dutch Elm disease and Chestnut Blight. A host of species in this group produce important antibiotics. Sac fungi form their spores in a sac or ascus.

Club Fungi, including **mushrooms,** are placed in the Phylum Basidiomycota. Other fungi in this group are the bracket or shelf fungi found growing on trees, coral fungi, bird's nest fungi, rusts, smuts, and some puffballs. Basidiomycota form spores by budding them off structures called basidia.

Many books have a 5^{th} phylum, Phylum Deuteromycota or **Imperfect Fungi**. Fungi in this group have never been observed to reproduce sexually, and since it is the nature of the structures formed during sexual reproduction that is used to divide fungi into phyla, there is no way to know where to place these fungi. Some of them, like *Penicillium*, are quite well studied, and it is likely that this fungus, at least, simply has no sexual reproduction. Recent molecular data has placed *Penicillium* into the sac fungi, and your book follows this classification. Other imperfect fungi are not as well studied, however, and closer examination will sometimes reveal sexual stages that allow mycologists to classify the organism into one of the other 4 phyla.

A number of different types of fungi will be on display. Because the fungi can be difficult to place in groups without detailed examination, we will not expect you to be able to classify some of the "trickier" specimens. On the other hand, we would expect you to be able to identify the more obvious specimens, as well as the specimens on display in the lab.

MICROSCOPIC FUNGI. Fungi are often found when examining material under a microscope. While most people have little difficulty in distinguishing protozoa from algae, fungi sometimes present a difficulty. *Fungi are non-photosynthetic eukaryotes, and therefore are never green.* However, unlike protozoa, fungi are never motile, and are much less varied in cell shape. There are two principle groups of *microscopic* fungi, **YEASTS** and **MOLDS** (water molds and common molds). Yeasts are unicellular and roughly spherical in shape, most of them are in the sac fungi or club fungi. They reproduce by a process called "budding", whereby new cells pinch off an existing cell. *The filaments of molds can be distinguished from those of algae and cyanobacteria because molds are NEVER photosynthetic, and thus are not green.* A specimen of a mold typically will contain a mixture of hyphae and spores.

REVIEW OF CHARACTERISTICS OF MICROORGANISMS

(use this and the keys provided in lab to help identify microorganisms)

Organism	Cell structure	Kingdom	Photosynthetic (green?)
Cyanobacteria	Prokaryotic	Monera	Yes (blue-green)
Algae	Eukaryotic	Protista	Yes
Protozoa	Eukaryotic	Protista	No
Fungi	Eukaryotic	Fungi	No
Microscopic animals	Eukaryotic	Animalia	No

Lab Exercises

Week 1:

I. Macroscopic Fungi

A variety of Fungi will be on display during the first week of this lab. You should examine these specimens and be ready to identify them if you see them on a test.

You should also use the Pachyderm program to review fungi and any other organisms you have studied to date.

II. Microbiological Techniques - making an isolation from the "environment"

The purpose of this part of the exercise is to learn sterile technique and to reinforce your ability to plan and carry out a scientific experiment. To begin, you need to work with the others at your table. Discuss among yourselves what conditions would be ideal for microoganism growth and what conditions would inhibit such growth. Once you have decided on the conditions, try to identify places in the environment (inside or outside) where you would expect to see lots of microorganisms, few microorganisms, or no microorganisms based on these conditions.

With these thoughts in mind, develop a hypothesis and plan your sampling. You will have 9 plates, so you can make 3 replicate plates at each of 3 sites.

Each table will have a test tube of sterile water, 9 agar plates, and a container of sterilized cotton swabs. On the **bottom of the plates** use a marker or grease pencil to write your name, section number, and the place from which you took the sample. Record where you took the sample on page 13. While you are at the site, make sure it fits your assumptions as to the presence or absence of the conditions for growth that you discussed earlier.

Take the container of swabs and the test tube of sterile water to the site where you want to take the sample. Being careful to minimize contamination of the swabs in the container, remove one swab, moisten it with sterile water from the tube, and wipe it over the spot you have selected. Lightly rub the swab over the entire surface of the agar on the plate, being careful to use the sterile technique demonstrated during the lab lecture (hold the cover over the bottom plate while swabbing the surface). Tape your plate shut and place it with the others for incubation. Plates are typically incubated upside down (to prevent water condensing on the lid from dripping onto the medium thus swamping and mixing the colonies). ***Put your plate on the tray upside down.***

Clean up the lab by returning all tubes to racks for sterilization, and all swabs to the designated container for sterilization and disposal. Wash all surfaces of your lab table with disinfectant solution. Wash your hands thoroughly.

It will take about 2 days for the colonies to grow. At that point, they will be refrigerated and you can examine them during the second week of lab.

Week 2:

During the second week, examine any specimens that you did not have time to examine the first week.

Each person should examine the inoculated plates and the group should complete the information and drawings starting on page 13 of this handout (ask your instructor for photocopies). When the last person in the group has examined the plate, place it in the proper area for disposal.

Once all the plates have been examined, the group should meet to share data and complete the table on page 14. Your instructor will inform you as to which of the additional assignments on page 14 you should complete.

Pre - Lab Assignment

---------> Name ________________________________

Complete the following *before* lab:

QUESTIONS

1. Complete this thought:

   Algae and cyanobacteria differ from fungi, because fungi ...

2. Describe three ways in which bacteria can obtain energy from the environment.

3. What are the 4 phyla of Fungi mentioned in your book? Which group are mushrooms placed in?

4. Give one example each of commensal, parasitic, and mutualistic symbioses of a bacterium or fungus *with another organism*. Example: *Neisseria gonorrhoeae* bacteria and humans – parasitic.

5. What do microorganisms need to grow? What kind of conditions favor the development of a biofilm? What conditions will kill most microogranisms? In a paragraph or two, answer these questions and describe some of the places on campus where you would expect to find a wealth of microorganisms, and some of the places that would be devoid of microorganisms.

In - Lab Assignment

---------> Name ________________________________

Complete the following *during* lab:

Fungi:

Organisms to examine: *Rhizopus* (a mold), *Saccharomyces* (a yeast), mushroom hyphae and spores, various preserved specimens and live cultures.

Make wet mounts of *Rhizopus* and *Saccharomyces*. Prepare large, detailed drawings of each:

Rhizopus	*Saccharomyces*

Take a piece of mushroom and dice it finely with a razor blade (be careful). Take a small amount of the diced material and spread it on a microscope slide with a drop of water. Add a cover slip. Likewise, place a drop of the mushroom spore suspension on a microscope slide and add a cover slip. Obtain a slide showing a cross-section of a mushroom cap. Examine these slides and make large, detailed drawings below:

Mushroom Mycelium	Spore Suspension
Cross-Section of Mushroom Cap	*Peziza* (ascomycete)

A variety of preserved Fungi and living Fungal cultures will also be on display in the lab. Be sure to examine each of these.

Data: Isolation of microorganisms from the environment:

Complete the following when you look at the plates after incubation:

Source of sample: ____

Use an empty petri dish to trace a circle below and draw the surface of one plate as it appears after incubation. Your group should examine all of the plates it inoculated and prepare one of these sheets for each.

How many colonies are there? ________ Do they all appear to be bacteria? _______

Based on differences in the appearances of the colonies, how many species would you estimate are present?

____

Compare your data with others at your table:

	Source	Number of Colonies				Number of Species			
		1	2	3	Avg.	1	2	3	Avg.
1									
2									
3									
4									
5									
6									
7									
8									
9									

Your instructor may assign one or more of these tasks for you to complete based on the microbiological sampling:

_____ 1. Prepare 2 graphs in Microsoft Excel comparing (1) the average number of colonies at each of the sites you sampled and (2) the average number of species at those sites.

Due Date: ______________________

_____ 2. Restate your hypothesis. Was the hypothesis supported by your results? Write a 1-2 page conclusion and explanation of results following the guidelines for a lab report; use citations to back up your arguments.

Due Date: ______________________

_____ 3. Prepare a procedures section of a lab report following the guidelines for lab reports outlining how you carried out the sampling and incubation for the microbiological techniques portion of the lab.

Due Date: ______________________

_____ 4. Using your results as a pilot study, prepare a 1-2 page proposal to expand the study and provide sufficient results to scientifically test your hypothesis. Your instructor will inform you on where to find the information on preparing a proposal.

Due Date: ______________________

_____ 5. With your team, prepare a ______ minute presentation of your results and conclusion for presentation to your classmates. Every member of your team should have an equal speaking part, and you should have the proper visual aids prepared in advance.

Presentation Date: ______________________