

Diversity— Kingdoms Eubacteria, Archaeobacteria, and Protista

LABORATORY

22

OVERVIEW

Biologists currently recognize three *domains* of living things: **Bacteria**, **Archaea**, and **Eukarya**. The domain Bacteria is comprised of one kingdom, **Eubacteria** (true bacteria). The domain Archaea is also composed of one kingdom, **Archaeobacteria** (ancient bacteria). The domain Eukarya includes all other living organisms: kingdoms **Protista** (unicellular animals and plants, slime molds, and algae), **Fungi**, **Plantae** (multicellular plants), and **Animalia** (multicellular animals).*

Among the diverse organisms in these kingdoms you will find two major types. **Prokaryotes** are unicellular (single-celled) organisms characterized by the absence of a membrane-bound nucleus and membrane-bound organelles. In contrast, **eukaryotes** have both a well-formed nucleus and many other types of membrane-bound organelles.

Prokaryotes and eukaryotes differ also in the chemical composition of their cell walls (if present), the organization of their genetic material, and the structure of their flagella. The kingdoms **Eubacteria** and **Archaeobacteria** are composed of prokaryotic organisms. Eukaryotic organisms are found in the other four kingdoms.

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency.

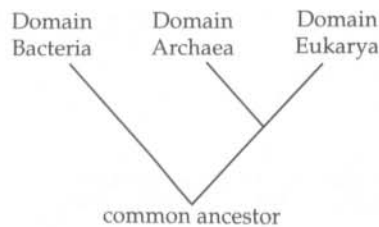
PART I DOMAINS BACTERIA AND ARCHAEA

Prokaryotes represent the oldest and simplest living things. Prokaryotes are clearly different from eukaryotes, but ordering them taxonomically into subgroups of organisms according to anatomical and physiological affinities is difficult. Based on comparison of DNA and RNA sequences, it appears that prokaryotes diverged early in evolutionary history into two distinct lineages now recognized as domains, Bacteria and Archaea (Figure 22I-1).

Belonging to the domain Archaea, the kingdom Archaeobacteria is composed of four phyla of organisms that live at environmental extremes: methanogens (bacteria that use H_2 to reduce CO_2 to

*Because scientists are continually learning more about the structure and origins of organisms, the assignment of organisms within a particular classification may change. Indeed, the number of phyla and even the number of kingdoms have changed during the past decade. It is possible that your instructor will adopt a different classification scheme than that used in the following exercises, but the principle of classification will remain the same—the grouping of organisms according to similarities that indicate evolutionary relationships.

Figure 22I-1 According to present thinking, the domains Eukarya and Archaea share a common ancestry and thus are more closely related to each other than to the domain Bacteria.



methane), thermoacidophiles or hyperthermophiles (bacteria that live where it is both hot and acidic), extreme halophiles (salt-loving bacteria), and thermoplasma. Belonging to the domain Bacteria, the kingdom Eubacteria is composed of 12 phyla, among which are gram-negative, gram-positive, and photosynthetic bacteria. These include myxobacteria, rickettsias, desulfovibrio, purple nonsulfur bacteria, rhodospseudomonas, purple sulfur bacteria, spirochetes, actinomycetes, clostridia, and mycoplasmas.

Bacteria are virtually ubiquitous. Not only do bacteria surround you, they thrive within you. Most of these bacteria are nonpathogenic and actually keep your body free from more harmful bacteria through competition. However, conditions sometimes make the difference between a pathogen and a nonpathogen. For example, *Staphylococcus aureus*, a common bacterium, can cause severe infections if introduced into an open cut. The same bacterium is also thought to be an agent in toxic shock syndrome.

Other bacteria play a large role in the environment and in the economy. Some bacteria are important as decomposers, recycling dead material into components required by living organisms. In sewage treatment plants, they promote the breakdown of solid wastes. Many foods, such as vinegar, sour cream, yogurt, and cheeses, are made with the help of bacteria. Certain antibiotics are produced by bacteria. Some bacteria can even be used to clean up oil spills. Bacteria are also a main focus of genetic and biochemical research.

✓ EXERCISE A Morphology of Bacteria

Most bacteria may be classified into one of three major morphological groups: rods (bacilli), spheres (cocci), or spirals (spirilla). You can observe the morphology of these groups both microscopically and macroscopically, by observing growth forms.

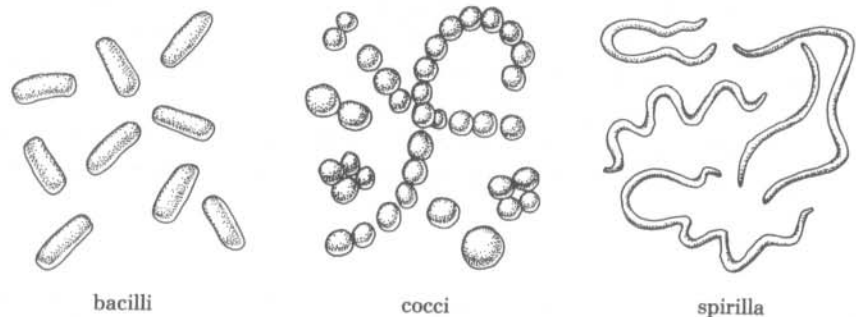
■■■■ Objectives ■■■■

- ☐ Recognize the different morphological forms of bacteria.

■■■■ Procedure ■■■■

1. Check the demonstration microscopes and observe the basic morphological forms of bacteria (Figure 22A-1). Draw the bacterial forms and identify each in the space that follows.

Figure 22A-1 Morphology of bacteria.



Demonstration A

Demonstration B

Demonstration C

Type _____ Type _____ Type _____

- a. *Strep throat* is caused by streptococcal bacteria. What shape would you expect these bacteria to have?

 - b. The prefix “strep” means strip or chain. How would you expect streptococcal bacteria to be arranged? _____
 - c. How are the bacterial cells arranged in the demonstration slides you have observed? As single cells? Clusters? Strands? A _____ B _____ C _____
2. Gram stain is a specific stain (purple) used to test for gram-positive bacteria. In these bacteria, the cell wall contains a small amount of lipid combined with a thick layer of a peptide/sugar material (peptidoglycan). The peptidoglycan molecules trap Gram stain and the cell walls retain a purple color. The cell walls of gram-negative bacteria contain a great deal of lipid but little peptidoglycan and the purple Gram stain washes out. A second stain, safranin, stains the gram-negative bacteria pinkish-red. What color were the bacteria on the demonstration slides? Were they gram-positive or gram-negative?

Color	Gram + or Gram -
A	
B	
C	

- d. Are all coccal bacteria gram-positive? _____ (Hint: Review the slides on demonstration.)
3. Streptococcal bacteria are gram-positive. Physicians use Gram stain to identify specific bacteria.
- e. What color would Gram-stained streptococcus be? _____
4. Penicillin is usually an effective antibiotic for treating infections caused by gram-positive bacteria. Penicillin affects the ability of bacterial cells to synthesize peptidoglycan.
- f. Would penicillin be an effective way to treat strep throat? _____
5. You were given an agar-filled Petri dish with instructions to expose it to a potential source of bacteria in your environment, for example, keys, money, shoes; air in various locations (inside the house, in a bathroom; outside, in moist or dry environments); your hands before washing, after washing with regular soap, after washing with an “antibacterial” hand soap; the foot of a

cat, dog, or bird. Examine your dish and those of your classmates. *Do not remove the lids.* Note the number of bacterial colonies in each plate. These will have the appearance of small, shiny masses. The “fuzzy” masses that may appear on some plates are fungi. Theoretically, each colony of bacteria or fungi arose from a single bacterium or fungal spore (though closely adjacent individual colonies may coalesce and appear as one). The appearance of a bacterial colony on agar is used in classification. Record your observations of the number of different colonies on each of four plates, and the shape, color, texture, and size of each type of colony.

Plate 1 _____

Plate 2 _____

Plate 3 _____

Plate 4 _____



EXERCISE B Characteristics of Bacteria: Sensitivity to Antibiotics

Since the number of basic bacterial morphologies is limited, identification of species is largely dependent on biochemical and physiological characteristics. Composition of the cell wall, DNA or RNA sequencing, sensitivity to antibiotics, mode of metabolism, and mode of reproduction can all be used to distinguish among bacterial types. These characteristics are the topics of this exercise and Exercises C through D.

The bacterial cell wall and ribosomes represent two major sites for the action of antibacterial agents. As you have already learned, many of the common antibiotics treat bacterial infections by inhibiting the synthesis of the essential structural polymer, peptidoglycan, in the bacterial cell walls. Recall that penicillin is an example of one of these antibiotics. But how effective are other antibiotics against gram-positive bacteria? And what about gram-negative bacteria?

Objectives

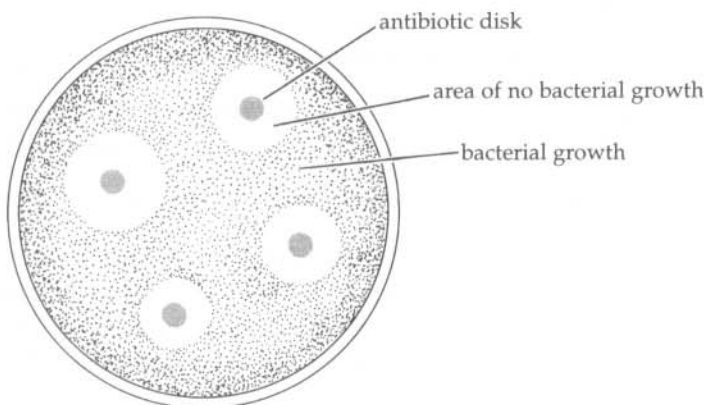
- ☐ Determine the sensitivity of selected bacteria to several antibiotics.
- ☐ Describe the action of antibiotics.

There are two general types of antibiotics. **Narrow-spectrum antibiotics** target a limited number of bacterial species, while **broad-spectrum antibiotics** are effective against a wider spectrum of bacterial types. This exercise will show the relationship between bacterial cell-wall structure, as indicated by Gram stain, and susceptibility to different types of antibiotics.

Procedure

1. Examine the Petri dishes on display. In preparing these, the agar surfaces were first covered with a suspension of a known type of bacteria—one plate with gram-positive and the other with gram-negative. Paper disks impregnated with an antibiotic or antiseptic were then placed on top of the bacteria (Figure 22B-1). After an incubation period, bacterial growth will be

Figure 22B-1 Bacteria growing on an agar plate demonstrate sensitivity to antibiotics. Clear areas indicate inability of bacteria to grow in the presence of an antibiotic. The diameter of the zone of inhibited growth depends on the relative diffusibility of the antibiotic and the sensitivity of the particular bacterium.



visible unless inhibited by the substance in the disk. (This procedure is often used to determine which drug would be most effective against a particular bacterium.)

2. Record the results in Table 22B-1. List the antibiotics in the left-hand column, then record the effects of each antibiotic on each of the two types of bacteria. Use the following symbols to record your observations of the dishes: $-$, growth not inhibited; $+$, growth slightly inhibited; $++$, growth greatly inhibited.

Table 22B-1 Effects of Antibiotics

Antibiotic	Effect on Growth	
	Plate 1: Gram $-$	Plate 2: Gram $+$
A.		
B.		
C.		
D.		
E.		

3. In Table 22B-2, list the antibiotics and the types of bacteria against which they are effective, and then decide whether each antibiotic has a broad or narrow range, or spectrum, of action.

Table 22B-2 Spectrum of Antibiotic Activity

Antibiotic	Bacteria Affected	Spectrum
A.		
B.		
C.		
D.		
E.		

- a. Under what circumstances might a doctor prescribe a narrow-spectrum antibiotic?

A broad-spectrum antibiotic? _____

EXTENDING YOUR INVESTIGATION: HOW EFFECTIVE IS YOUR SOAP?

Why do you use the brand of soap that you do? Is your soap effective in clearing bacteria from your hands or face? Would water be just as effective? Is medicated or deodorant soap better than "just plain soap"? Is alcohol an effective disinfectant? What about commercial disinfectants such as Lysol or Mr. Clean? When antibacterial substances are used on living organisms, they are called **antiseptics**. Those used to clean surfaces are called **disinfectants**.

Formulate a hypothesis concerning the effectiveness of an antiseptic or disinfectant of your choice.

HYPOTHESIS:

NULL HYPOTHESIS:

What do you **predict** will be the effect of the soap or the antiseptic or disinfectant solutions you have chosen?

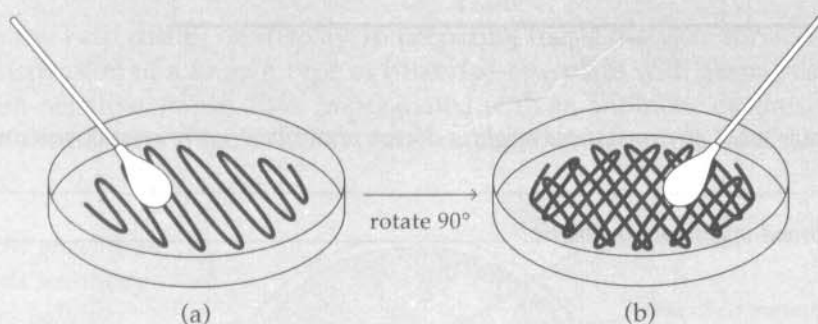
What is the **independent variable**?

What is the **dependent variable**?

The following materials will be available to you: an *S. aureus* (gram-positive) culture or plate, an *E. coli* (gram-negative) culture or plate, detergents, soaps, and disinfectants (or bring your own). Use these materials to design an experiment based on the general procedure that follows.

PROCEDURE: Your instructor may provide you with agar plates that have been inoculated with bacteria. If not, inoculate the plates as in steps 1–3. Then proceed to testing your selected detergents, soaps, or antiseptics (steps 4–10).

1. Wipe your laboratory area clean with a 10% Clorox solution.
2. Obtain an agar plate and a broth culture of *S. aureus* or *E. coli*.
3. Prepare a lawn of bacteria as follows:
 - a. Lift the lid of the agar plate and hold it above the plate.
 - b. Use a sterile cotton swab to remove some bacterial culture from one of the flasks. Make sure the cotton is saturated, but not dripping.
 - c. Apply the bacteria to the agar plate in a zig-zag motion over the entire surface, as shown below (a).
 - d. Rotate the plate 90° and repeat this motion (b).



- e. Dispose of the cotton swab in the container provided.
4. Pour small amounts of your selected liquid antiseptics or disinfectants into shallow dishes or other containers. If you are testing a solid material such as a bar of soap or a soap powder, make a liquid solution.

5. Using a pair of forceps, submerge a paper disk in a solution. Drain off excess liquid by touching one end of the disk to a paper towel.
6. Place the paper disk on your agar plate for 2 minutes.
7. While the disk is on the agar, mark the undersurface of the agar plate with a circle to show the placement of the disk and the name of the substance tested.
8. Repeat this procedure for three more disks impregnated with different solutions. Remember to mark and label the agar plate for each disk position. Remove the disks and dispose of them in the container provided.
9. Incubate the dishes, upside down, until the next laboratory period. Be sure that your name is on the culture plate.
10. After you complete your work, wash your work area with 10% Clorox. Also, wash your hands with soap and water!

RESULTS: A clear area around the position of a paper disk indicates that bacterial growth has been inhibited. Quantify your results by measuring the diameter of this region and noting it in the following table.

Test Substance	Zone of Inhibition (Diameter)

Do your results support your hypothesis?

Your null hypothesis?

What do you **conclude** about the effectiveness of the disinfectant, antiseptic, or soap solutions you tested?



EXERCISE C Nitrogen-Fixing Bacteria

Although all organisms require nitrogen for the production of proteins and nucleic acids, eukaryotic organisms cannot use atmospheric nitrogen (N_2) directly. It must first be converted to a compound such as ammonia (NH_3). Only bacteria and cyanobacteria are capable of **nitrogen fixation**, the conversion of N_2 into NH_3 . This capacity is the basis of a significant relationship between bacteria and eukaryotes.

Legumes, the family of plants to which peas and beans belong, form a symbiotic relationship with bacteria of the genus *Rhizobium*. *Rhizobium* live in the root nodules of the legumes and supply the plants with usable nitrogen by converting atmospheric N_2 into NH_3 . The plants supply *Rhizobium* with sugar, the product of photosynthesis.

Objectives

- ☐ Recognize *Rhizobium* bacteria on the root nodules of legumes.

Procedure

- Examine the two groups of soybean plants provided. One group was inoculated with *Rhizobium*.
 - Which group of plants is more robust? _____
 - Examine the roots. Which group of plants has root nodules? _____
 - Which group of plants has a symbiotic relationship with *Rhizobium*? _____
- Observe *Rhizobium* bacteria on a demonstration slide if available. Make a sketch in the space below.
 - Morphologically, how would you classify *Rhizobium* bacteria? _____

**EXERCISE D Bioluminescent Bacteria**

Some marine bacteria are capable of producing light in the presence of oxygen. Several marine fishes are equipped with bizarre light organs inhabited by these bacteria. The fish provide the bacteria with nutrients and oxygen and use the light produced by the bacteria primarily to attract prey.

Bioluminescence in bacteria is the result of a reaction between a *luciferin* (an aldehyde compound), reduced flavin mononucleotide, or FMNH₂ (FMN is a component of the electron transport chain), and O₂ in the presence of the enzyme *luciferase*.

Objectives

- ☐ Observe the bioluminescent properties of bacteria.

Procedure

- Your instructor has prepared a broth culture of the bioluminescent marine bacterium *Vibrio fischeri*. Obtain a tube of the culture and place a rubber stopper in the end of the tube.
- In a darkened room or closet, shake the tube vigorously.
 - What do you see? _____ What color does the light appear to be? _____
 - Why did you have to shake the tube? _____
- Stop shaking the tube.
 - What happens? _____
 - Which part of the tube darkens first? _____ Why? _____
 - How is the supply of oxygen normally maintained in the seas? _____

✓ EXERCISE E Diversity and Structure of Cyanobacteria

Traditionally, cyanobacteria have been called "blue-green algae." However, they are not algae, but prokaryotes: gram-negative photosynthetic bacteria. Cyanobacteria contain chlorophyll *a* (as do photosynthetic eukaryotic green plants and algae), but it is characteristically masked by blue, red, and purple pigments. These pigments (**phycobilins**) enhance light absorption by the cells and serve as nitrogen reservoirs. All cyanobacteria are unicellular, but individual cells are commonly attached to each other by a gelatinous sheath, thus producing filaments or colonies. Like some other bacteria, many cyanobacteria are able to fix atmospheric nitrogen.

Cyanobacteria share with other bacteria the ability to inhabit the most inhospitable locations on earth, such as hot springs and bare rocks. Cyanobacteria can be desiccated for many years yet resume growth when water is again present.

■■■■ Objectives ■■■■

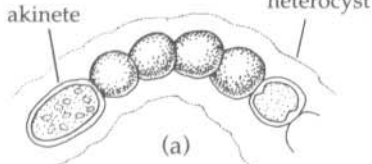
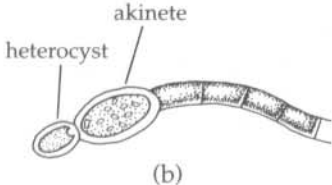
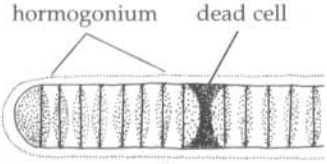
- ☐ Recognize several types of cyanobacteria and compare these to other types of bacteria.
- ☐ Recognize several cell types common to cyanobacteria.

■■■■ Procedure ■■■■

Work in pairs. Prepare and examine material on a wet-mount slide and then exchange slides with your partner. Depending on the material available, examine one or more examples of cyanobacteria. Refer to Table 22E-1, and add further information to the table where necessary. Make sketches if instructed to do so, adding labels where possible.

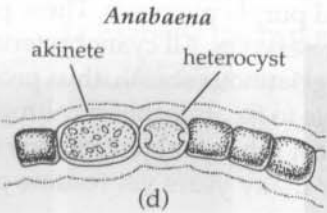
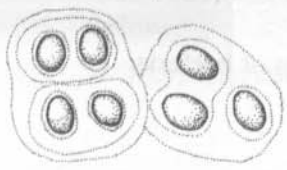
Most cells of cyanobacteria are structurally undistinguished, but a few specialized cell types can be recognized. **Heterocysts** are round or oval, *clear* cells that allow cyanobacteria to fix atmospheric nitrogen. Many cyanobacteria can fix nitrogen when they are in an anaerobic environment, but heterocysts are

Table 22E-1 Characteristics of Cyanobacteria

Type	Morphology	Distinctive Features
<p><i>Nostoc</i></p>  <p>(a)</p>	Filaments of round cells; gelatinous sheath surrounds filament.	Can combine in large gelatinous balls containing hundreds of filaments. Reproduce by fission or fragmentation.
<p><i>Cylindrospermum</i></p>  <p>(b)</p>	Filaments of rectangular cells; length greater than width.	Heterocysts at the ends of filaments function in nitrogen fixation. Reproduce by fission or fragmentation. Akinetes are special sporelike reproductive cells resistant to adverse environmental conditions.
<p><i>Oscillatoria</i></p>  <p>(c)</p>	Filaments of rectangular cells covered by a sheath; width greater than length.	Oscillate, seek specific conditions in water. Reproduce by fragmentation only. Hormogonia are short fragments between dead cells where fragmentation takes place.

(continued)

Table 22E-1 (continued)

Type	Morphology	Distinctive Features
<p><i>Anabaena</i></p>  <p>(d)</p>	Barrel-shaped vegetative cells held in a gelatinous matrix.	<p>Heterocysts are integral or terminal and function in nitrogen fixation.</p> <p>Reproduce by fission or fragmentation. Akinetes are dispersed among vegetative cells.</p>
<p><i>Gleocapsa</i></p>  <p>(e)</p>	Spherical cells; single or groups of 2 to 8; each cell surrounded by its own sheath; colony surrounded by sheath.	<p>Can fix nitrogen despite absence of heterocysts.</p> <p>Reproduce by fission.</p>

Sketches

necessary for aerobic nitrogen fixation. **Akinetes** are generally larger, usually oval, densely packed, sporelike reproductive cells that are resistant to adverse conditions.

- What are the basic cyanobacterial cell shapes? _____ How are these individual cells combined to form colonies and filaments? _____
- Which of the specialized cell types can you recognize in each of the types of cyanobacteria? _____ What does the presence of these cells (heterocysts, akinetes) indicate about the environment of these organisms? _____
- Cyanobacteria grow prolifically in streams and lakes with low oxygen levels and high nutrient concentrations. How might the presence or absence of cyanobacteria be used as an index of pollution in lakes? _____
- How can you determine, from your microscope observations, whether cyanobacteria are prokaryotes or eukaryotes? _____ Name three differences between prokaryotes and eukaryotes. _____
- Cyanobacteria have a peptidoglycan cell wall. Would you expect them to be sensitive to antibiotics? Explain. _____
- Prokaryotes have membrane-bound organelles. Did the cyanobacteria that you examined contain chloroplasts? _____ Chlorophyll? _____

PART II KINGDOM PROTISTA

With the kingdom Protista,* we begin our study of eukaryotes. The cells of eukaryotic organisms contain both a nucleus and membrane-bound organelles. All other eukaryotic organisms (including fungi, plants, and animals) probably originated from the primitive protists.

For our purposes, protists can be divided into three broad groups, usually based on modes of nutrition.

Protozoa Unicellular heterotrophs, typically animal-like.

Fungus-like protists (slime molds) Sometimes referred to as the “lower fungi” because they may be multinucleate, as are fungi, during some part of their life cycle. They are classified with protists because of their similarities to protozoans.

Algae Unicellular and multicellular plant-like organisms.

PROTOZOA

Protozoans are unicellular organisms. Most are motile. Protozoans can be found in free-living and parasitic forms and in freshwater or marine environments.

✓ EXERCISE F Identifying Protozoans

There are many phyla of protozoans. Some of the most common forms are represented below. They can be distinguished by body form and mode of locomotion (Table 22F-1).

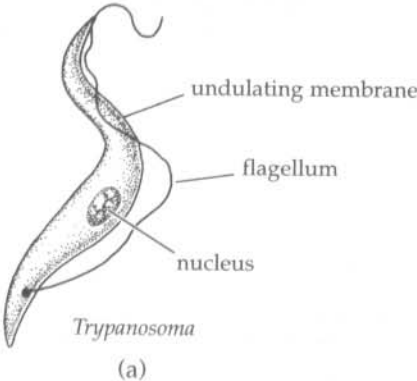
■■■■ Objectives ■■■■

- ☐ Identify and classify representative protozoans.

■■■■ Procedure ■■■■

Observe material using prepared slides or, if fresh material is available, make temporary wet-mount slides according to directions in Table 22F-1. Label structures and make notes or sketches of any identifying characteristics or behaviors.

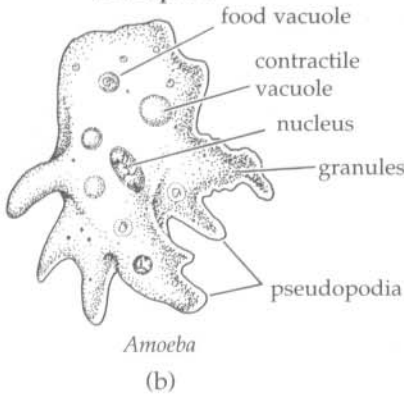
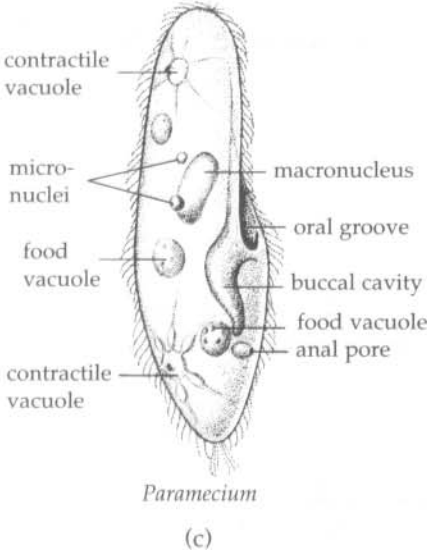
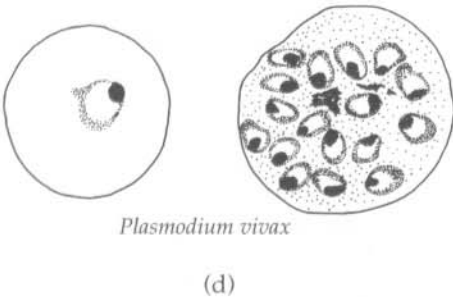
Table 22F-1 Characteristics of Protozoans

Phylum/Representative	Method of Observation	Mode of Locomotion
Zoomastigophora  <p><i>Trypanosoma</i> (a)</p>	Study a prepared slide.	Flagellar movement. A single flagellum is united basally with the body of cell by an undulating membrane. Amoeboid extensions (pseudopodia) are also found in many flagellates. <i>Trypanosoma gambiense</i> is the causative agent of African sleeping sickness.

(continued)

*Originally, only unicellular organisms were assigned to the kingdom Protista, but in recent years it has been suggested that the kingdom be expanded to include some multicellular organisms—the multicellular algae and fungus-like organisms that lack some of the important characteristics of true fungi. The name *Protoctista* has been proposed for this “expanded kingdom.”

Table 22F-1 (continued)

Phylum/Representative	Method of Observation	Mode of Locomotion
Rhizopoda 	<p>Examine living amoebas. You can see the organism on the bottom or side of the culture dish. Remove an amoeba with a pipette and place it on a glass slide. Observe without a coverslip. Adjust light. If motion does not occur, add coverslip.</p>	<p>Amoeboid movement—pseudopodia. Cytoplasmic extensions change in size.</p>
Ciliophora 	<p>Examine living paramecia. Place a small drop of culture medium on a clean glass slide. Mix in a drop of Protoslo (methylcellulose). Cover with a coverslip and observe. Is ciliary movement coordinated? _____ Can you see food vacuoles? _____ Do you see any contractile vacuoles? _____ How often do they fill and empty? _____</p>	<p>Ciliary movement. Cilia have the same internal structure as flagella, but are shorter.</p>
Apicomplexa 	<p>Study a prepared slide. Locate sporozoites. See life cycle, Figure 22F-1.</p>	<p>Nonmotile phases predominate. Blood parasites.</p>

(continued)

Table 22F-1 (continued)

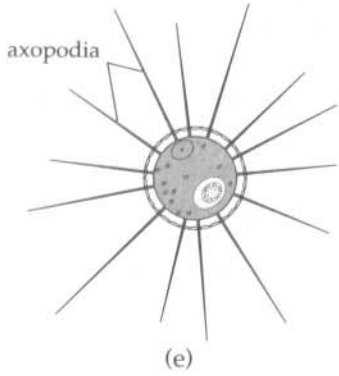
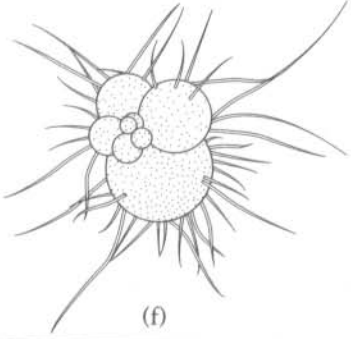
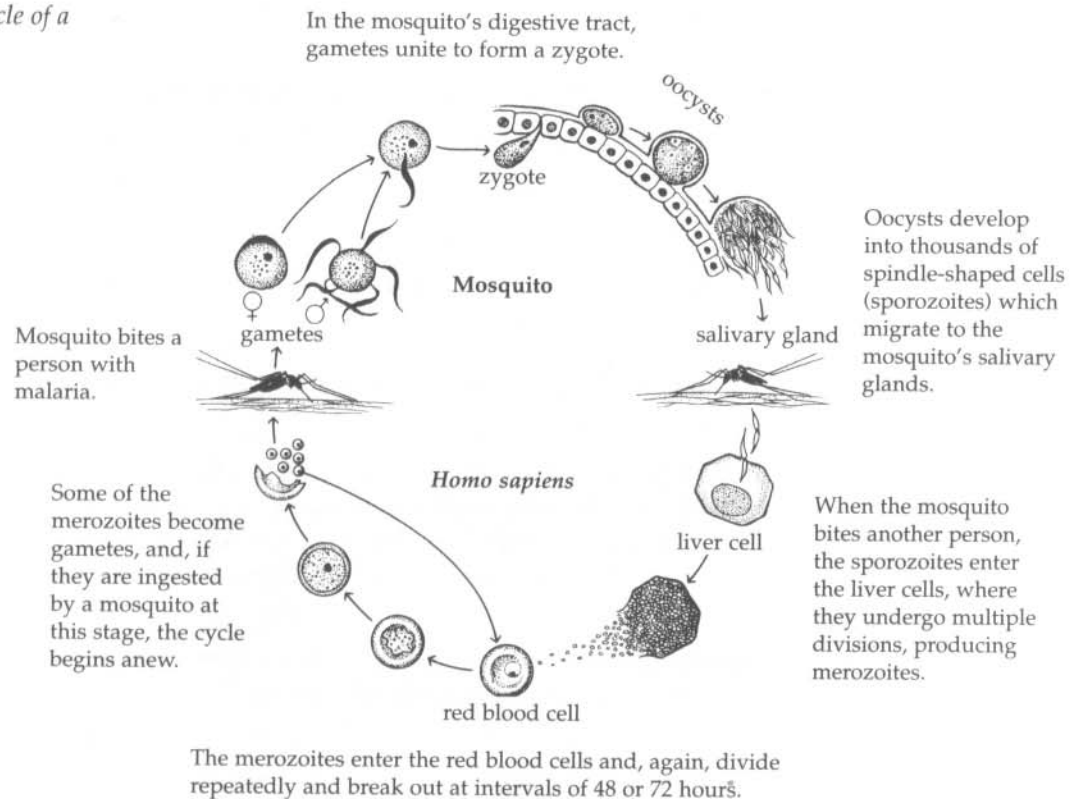
Phylum/Representative	Method of Observation	Mode of Locomotion
Actinopoda  (e)	Not studied in this laboratory.	Slender pseudopodia called axopodia (reinforced by microtubules) help the organisms to float and feed. Phylum includes heliozoans and radiolarians.
Foraminifera  (f)	Not studied in this laboratory.	Foraminiferans (forams) are named for their porous shells. Strands of cytoplasm extend from holes in the shell for swimming and feeding.

Figure 22F-1 Life cycle of a *Plasmodium*.

How does *Plasmodium*'s lack of motility affect the mechanism required for infection in humans? _____



EXERCISE G Symbiosis in the Termite: A Study of Flagellates

A very good source of protozoans is the gut of a termite. Termites are well known for their ability to eat their way through wooden buildings. However, these organisms cannot, on their own, digest the cellulose of wood, even though it is the major constituent of their diet. The guts of termites are well populated by flagellated protozoans, and at least some of these have the enzyme required to digest cellulose. The termite–flagellate relationship is an example of **symbiosis**.

Symbiosis is the close living relationship of two organisms. A symbiotic relationship that benefits both organisms, as is seen in the termites and flagellates, is called **mutualism**. For example, one organism may obtain nourishment from another and in turn provide a nutrient that is necessary to the host. In some mutualistic relationships, each organism becomes so dependent on substances or services provided by the other that neither can survive alone; this is *obligatory mutualism*.

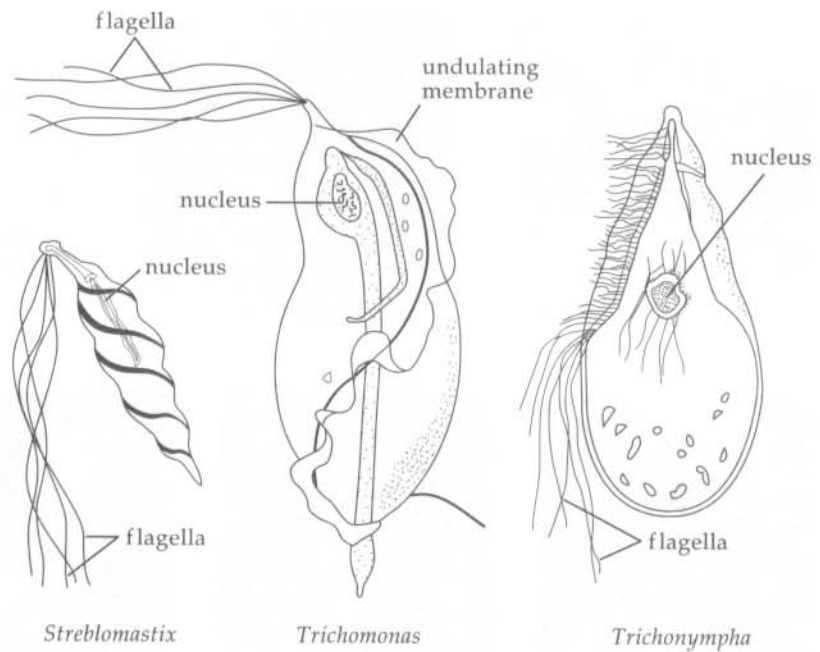
In another type of symbiosis called **commensalism**, one organism provides something of value to the second, but is neither harmed nor helped by the relationship.

The most extreme form of symbiosis is **parasitism**. The parasite is detrimental to its host, sometimes to the point of causing the host's death.

Procedure

1. Use a dropper to place a drop of insect Ringers (a saline solution isotonic to the tissues of the termite) onto a glass slide.
2. Place a termite into the Ringers and observe using the dissecting microscope.
3. Use two dissecting needles or fine forceps to open the termite. Place one point at the posterior end and one at the anterior end and pull in opposite directions.
4. The intestine is long and tubular. Locate it and move the remaining material to one side.
5. Use the dissecting needles to tear open the intestine.
6. Cover your preparation with a coverslip and observe using the compound microscope at 4× and 10×.
 - a. What do you observe? Is more than one type of organism present? _____ Do you see *Trichonympha* (Figure 22G-1)? _____ How many flagella are present on each organism? _____ In which phylum would you place these organisms? _____
 - b. Describe their movements. _____
 - c. Suggest the major function of *Trichonympha* in termites. (Hint: Consider the diet of the termite.) _____
7. On a separate sheet of paper, sketch several flagellates. Include flagella and indicate the direction of movement.
8. Allow your slide to air dry into a thin film, then place it on a staining trough or other suitable container and flood the slide with a few drops of giemsa stain. After 15 minutes, rinse off the excess with distilled water and air dry. Add a coverslip, using glycerol, and examine under the microscope again. Estimate the number of different flagellates present. Compare these to Figure 22G-1 and make tentative identifications.
9. In the space below, record your speculations on how symbiotic relationships such as that between termites and protists might have evolved.

Figure 22G-1 Some flagellated protists found in the termite gut.



d. What significant ecological role do termites and their symbionts perform (aside from destroying human-built wooden structures)? _____

e. Is their feeding niche shared by other organisms? _____ Name some examples.

FUNGUS-LIKE PROTISTS

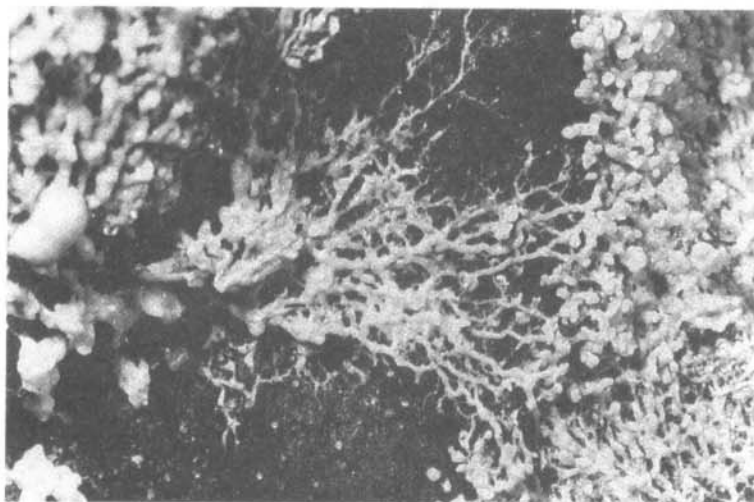


EXERCISE H Plasmodial Slime Molds

The slime molds are sometimes referred to as the “lower fungi” because they are multinucleate during part of their life cycle. Also, there are some ways in which slime molds resemble amoebas.

Some slime molds, known as **plasmodial slime molds** (phylum Myxomycota), are multinucleate masses of streaming protoplasm (Figure 22H-1). Others, the **cellular slime molds** (phylum Dictyostelida

Figure 22H-1 The plasmodial slime mold, *Physarum*.



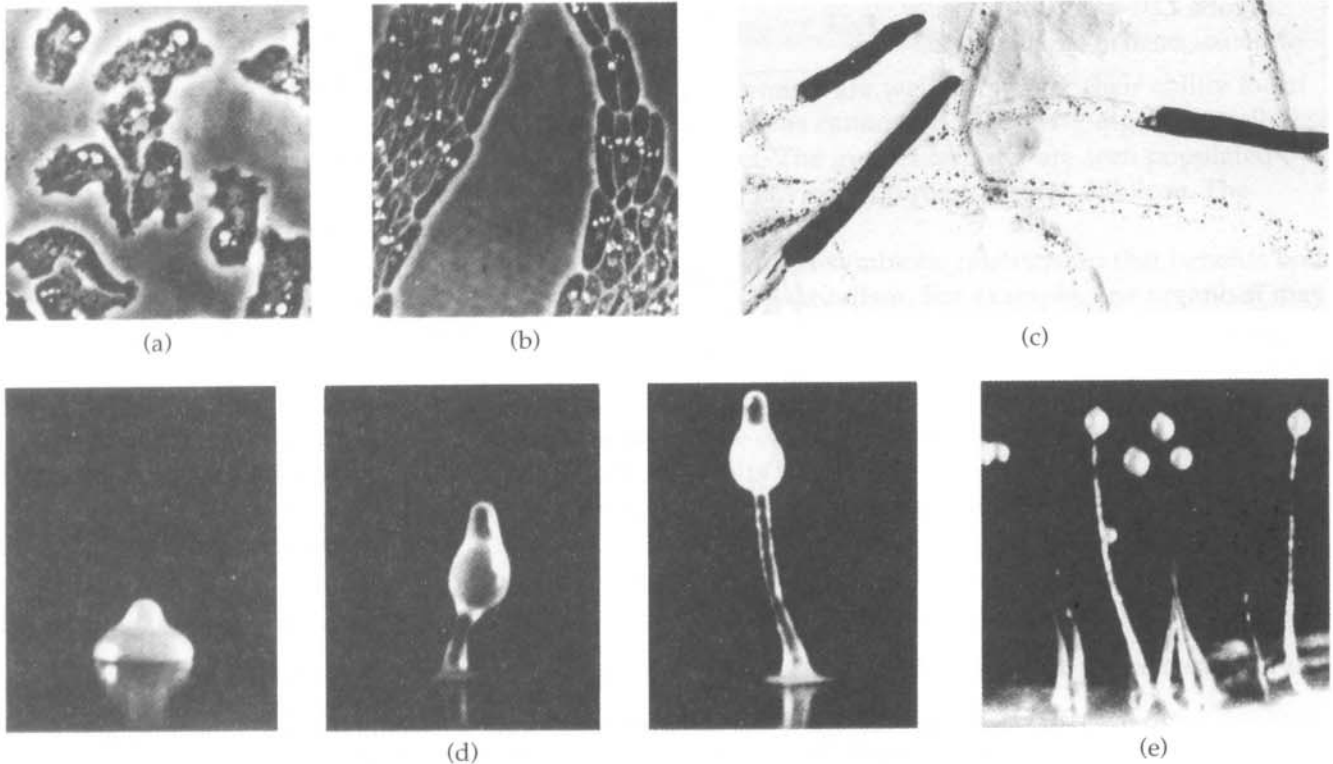


Figure 22H-2 Life cycle of a cellular slime mold, *Dictyostelium discoideum*. (a) Haploid (n) amoebas in the feeding stage. (b) Amoebas aggregating. (c) Migrating slugs (pseudoplasmodia). (d) At the end of migration, each pseudoplasmodium ($2n$) gathers together and begins to rise vertically, differentiating into a stalk and fruiting body (e). Meiosis restores the haploid condition.

and Acrasida), have bodies, or plasmodia, composed of aggregates of small cells called amoebas. These amoebas retain their identity as individual cells (Figure 22H-2).

■■■■ Objectives ■■■■

- ☐ Describe the structure of a typical plasmodial slime mold, *Physarum*.
- ☐ Determine behavioral responses of *Physarum* to heat, light, and overcrowding.

■■■■ Procedure ■■■■

1. Examine the well-developed body (**plasmodium**) of *Physarum* on demonstration. This multi-nucleate mass of protoplasm ingests food particles as it moves across the agar surface of the Petri dish. In the natural environment, plasmodia can be found on rotting stumps or fallen logs, usually underneath the bark.
2. Study the reproductive structures (**sporangia**) of those species on demonstration. The onset of unfavorable conditions—for example, the lack of water or food—triggers the formation of sporangia.
 - a. Are these reproductive structures plant-like or animal-like? _____
3. Obtain a piece of filter paper containing dried plasmodial material. Wet the paper and place it on the agar in the middle of a Petri dish.

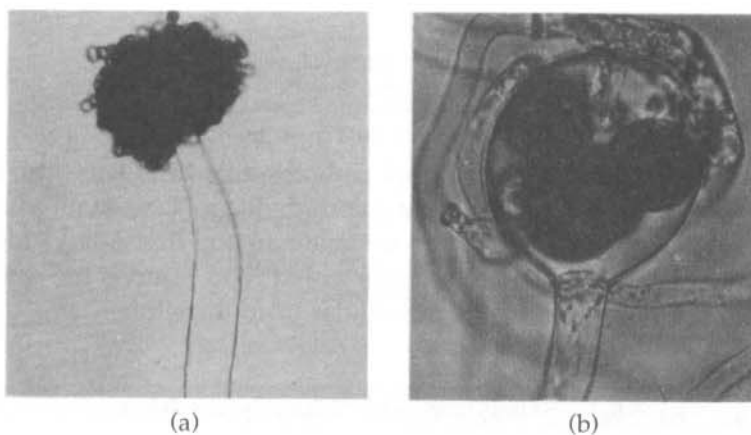
4. Place two or three oat flakes around the material.
5. Take this preparation home with you and observe what happens during the next week.
 - b. When the slime mold runs out of space, what happens? _____
 - c. Keep the slime mold in one place and observe the direction in which it moves. To what stimulus does it seem to be responding? _____
 - d. Punch a hole in the plasmodium. What happens to it? _____
 - e. Slightly warm one-half of the dish. What happens to the slime mold? _____
 - f. Illuminate one-half of the dish with your desk lamp and cover the other half with aluminum foil. Does *Physarum* react to light? Why or why not? _____
6. Summarize your experiments and their results on a separate sheet of paper. Be sure to include the hypotheses that determined the experimental procedures you used. Hand in your report, if assigned.



EXERCISE I Water Molds

Oomycetes, the “egg fungi” (phylum Oomycota), get their name from their sexual reproduction cycle in which large nonmotile eggs are produced inside a special structure called an **oogonium** (Figure 22I-1). Egg fungi are also called by several common names, including water molds, algae-like fungi, and downy mildews.

Figure 22I-1 (a) Asexual reproduction in the water mold *Achlya ambisexualis* is characterized by production of motile flagellated zoospores from sporangia located on the tips of the hyphae. (b) Sexual reproduction is characterized by formation of large nonmotile eggs within an oogonium (a type of female gametangium) to which tubal outgrowths on the antheridia (male gametangia) fuse, allowing sperm to enter the oogonium and fertilize the eggs. Zygotes develop thick coats and are called oospores.



Unlike other fungi, with cell walls composed of chitin, the cell walls of oomycetes are made up of cellulose. Another distinction is that the spores formed by oomycetes during asexual reproduction are flagellated, a distinctly protistan characteristic.

You are probably more familiar with the oomycetes than you realize. Since these molds can attack diseased or dying fish, you may have experienced a problem with *ick*—a disease caused by water molds—

in your aquarium. One oomycete, *Phytophthora*, was responsible for the potato famine in Ireland in the 1850s. Another, *Plasmopara*, almost destroyed the French wine industry.

■■■■ Objectives ■■■■

- ☐ Identify the structural characteristics typical of Oomycota, and distinguish between the structures of asexual and sexual reproductive phases.
- ☐ Explain the basis for the name "Oomycota."

■■■■ Procedure ■■■■

1. In the demonstration area, you will find water molds growing on dead seed. Note the cottony mass of filaments, or hyphae, which constitute the mycelium.
 - a. Are these oomycetes parasitic or saprophytic? _____
2. Examine (at 10× and 40×) a wet-mount slide of a portion of the water mold mycelium on demonstration. Look for denser areas at the tips of the hyphae. These individual cells, called **zoosporangia**, produce motile zoospores asexually.
3. Examine a prepared slide of the water mold *Saprolegnia*. Identify oogonia, antheridia, and zoosporangia. Use the space below to draw and label your observations.

ALGAE

A number of distinct lines of simple, photosynthetic eukaryotes, the **algae**, evolved more than 450 million years ago. All modern algae have chlorophyll *a* as their main photosynthetic pigment, and they also have accessory pigments. Distinctions are made among divisions of algae based on the type of accessory photosynthetic pigments; the nature of the stored food reserve; the composition of the cell wall, if present; and whether the body (**thallus**) is unicellular or multicellular.



EXERCISE J Studying and Classifying Algae

You will study six phyla of algae. Representatives range in size from microscopic to extremely large. Both freshwater and marine species exist.

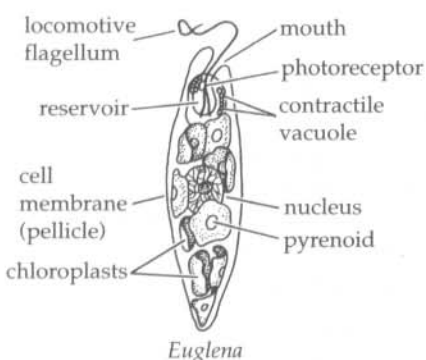
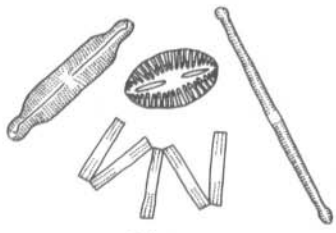
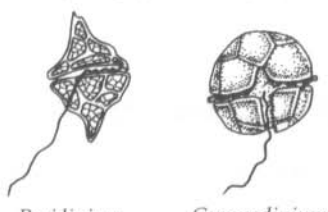
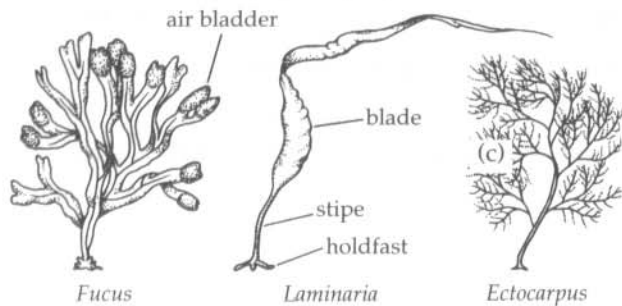
■■■■ Objectives ■■■■

- ☐ Identify types of algae based on morphological characteristics.

■■■■ Procedure ■■■■

Obtain fresh material or prepared slides to study representatives of the six divisions of algae. Refer to Table 22J-1 for characteristics and methods of observation.

Table 22J-1 Characteristics of Algae

Phylum/Representative	Characteristics/Method of Observation
<p>Euglenophyta*</p>  <p><i>Euglena</i></p> <p>(a)</p>	<p>Unicellular. True eye-socket algae.</p> <p>Flagellum attached within reservoir, distinct orange-red eyespot adjacent to the flagellum.</p> <p>A flexible protein layer (pellicle) rather than a rigid cell wall allows the organism to change its shape.</p> <p>Many bright green chloroplasts.</p> <p>Locomotion: swimming, creeping, or floating.</p> <p><u>Method of Observation</u> Make a wet mount of <i>Euglena</i>. Mix a drop of Protoslo with culture.</p>
<p>Chrysophyta</p>  <p><i>Diatoms</i></p> <p>(b)</p>	<p>Diatoms only: Unicellular or chains of rod (pennate) or circular (centric) shapes.</p> <p>Cell walls of silica with numerous holes.</p> <p>Walls make two overlapping halves (thecas) that fit together like the halves of a Petri dish.</p> <p>Cells are brownish yellow.</p> <p>Locomotion: attached, gliding, or floating.</p> <p><u>Method of Observation</u> Make a wet mount of diatomaceous earth, if available, or use material collected from stream rocks.</p>
<p>Pyrrophyta (Dinophyta)</p>  <p><i>Peridinium</i> <i>Gymnodinium</i></p> <p>(c)</p>	<p>Unicellular. Spinning flagellates.</p> <p>All members are biflagellate and motile. One flagellum wraps around the middle of the cell and allows it to spin; another flagellum trails and pushes the cell along.</p> <p>Cell wall composed of many interlocking plates, giving an armored appearance.</p> <p>Brownish color.</p> <p>Locomotion: floating or swimming.</p> <p><u>Method of Observation</u> Study prepared slide of <i>Peridinium</i>.</p>
<p>Phaeophyta (brown algae)</p>  <p><i>Fucus</i> <i>Laminaria</i> <i>Ectocarpus</i></p> <p>(d)</p>	<p>Multicellular, small to massive.</p> <p>Organisms called rockweeds, kelps, and brown seaweeds.</p> <p>All marine.</p> <p>Body differentiated into blade, stipe, holdfast, and, in some, air bladder floats.</p> <p>Cell walls contain mucilage, algin, used commercially as additive to food and cosmetics and as a thickener for some ice cream.</p> <p>Almost all are attached to the bottom (benthic) in coastal marine environments.</p> <p><u>Method of Observation</u> Study preserved specimens.</p>

(continued on next page)

Procedure

Use prepared slides or, if fresh material is available, make temporary mounts of the following organisms. Observe the progression in size and complexity illustrated in the green algae. Sketch the organisms in the spaces provided.

Chlamydomonas (class Chlorophyceae)
Unicellular thallus.

Gonium (class Chlorophyceae)
Spherical colony made up of 4 to 32 cells, depending on the species.

Volvox (class Chlorophyceae)
Spherical colony made up of 500 to 50,000 cells, depending on the species.

Zygnema (class Chlorophyceae)
Simple, unbranched filament (cell division occurs in a single plane).

Stigeoclonium (class Chlorophyceae)
Branched filament (cell division occurs in two planes).

Oedogonium (class Chlorophyceae)
Simple unbranched filament with netlike chloroplast. Oogamous.

Ulva (class Ulvophyceae)
"Sheets" that are two cells thick (cell division occurs in three planes).

Ulothrix (class Ulvophyceae)
Simple unbranched filament with only the basal cell differentiated into a holdfast. Isogamous.

Spirogyra (class Charophyceae)
Unbranched green alga with one or more ribbonlike chloroplasts helically arranged. Isogamous.

Desmids (class Charophyceae)
Unicellular (some multicellular). Cell wall is in two sections with a narrow construction, the isthmus, between them.

✓ EXERCISE L Recognizing Protists Among the Plankton

Plankton is a general term for small (mostly microscopic) aquatic organisms found in the upper levels of water where light is abundant. Plankton includes both plant-like photosynthetic forms (*phytoplankton*) and animal-like heterotrophic forms (*zooplankton*). A sample from enriched natural water, such as a fish pond, is an excellent source of algae and protozoans, as well as microscopic animals.

■■■■ Objectives ■■■■

- ☐ Identify diverse types of flagellates, ciliates, sarcodines, and algae.

■■■■ Procedure ■■■■

1. Place a small drop of the plankton sample on a slide and add a coverslip. Your instructor will provide some illustrations of types of organisms you are likely to see.
2. Identify as many organisms as possible. Various types of algae (diatoms, desmids, and, possibly, filamentous green algae) may be visible. Some of the flagellates you find may belong to the algal division Euglenophyta rather than Zoomastigophora, but they, too, illustrate the way in which flagellates, in general, move. Study this movement carefully.

a. Can you find any algae? _____ To which divisions might these algae belong?

How do you distinguish between algae of different divisions?

b. Are there any sarcodines? _____ c. Are there any ciliates? _____

d. Can you find any multicellular rotifers? (These are members of the phylum Rotifera, one of the animal phyla.) _____

3. Sketch representatives in the space provided below.

e. What might be the role of the plankton in the food chain of an ocean or lake?

f. Both phytoplankton and zooplankton can migrate to various depths in a lake or ocean. What might cause these organisms to surface or to move to greater depths within a lake or ocean environment?

Laboratory Review Questions and Problems

1. List three characteristics that distinguish prokaryotes from protists.
2. Why are the cyanobacteria included with the bacteria?

State the differences that distinguish cyanobacteria from green plants.

3. Fill in the table below, giving the distinguishing characteristics of each protozoan phylum and an example of a representative organism studied in the laboratory.

Protozoan Phylum	Distinguishing Characteristics	Example
Zoomastigophora		
Rhizopoda		
Ciliophora		
Sporozoa		

4. Slime molds and water molds are included among the Protista rather than in the kingdom Fungi. Why?
5. Fill in the table below, giving the distinguishing characteristics of each algal phylum and an example of a representative organism studied in the laboratory.

Algal Phylum	Distinguishing Characteristics	Example
Euglenophyta		
Chrysophyta		
Pyrrophyta		
Phaeophyta		
Rhodophyta		
Chlorophyta		

6. *Euglena* can be classified among the algae or among the flagellated protozoans. Explain why this organism is so difficult to classify.
7. A friend comments that you have more in common with an archaean than with a bacterium. Is this true? Why?