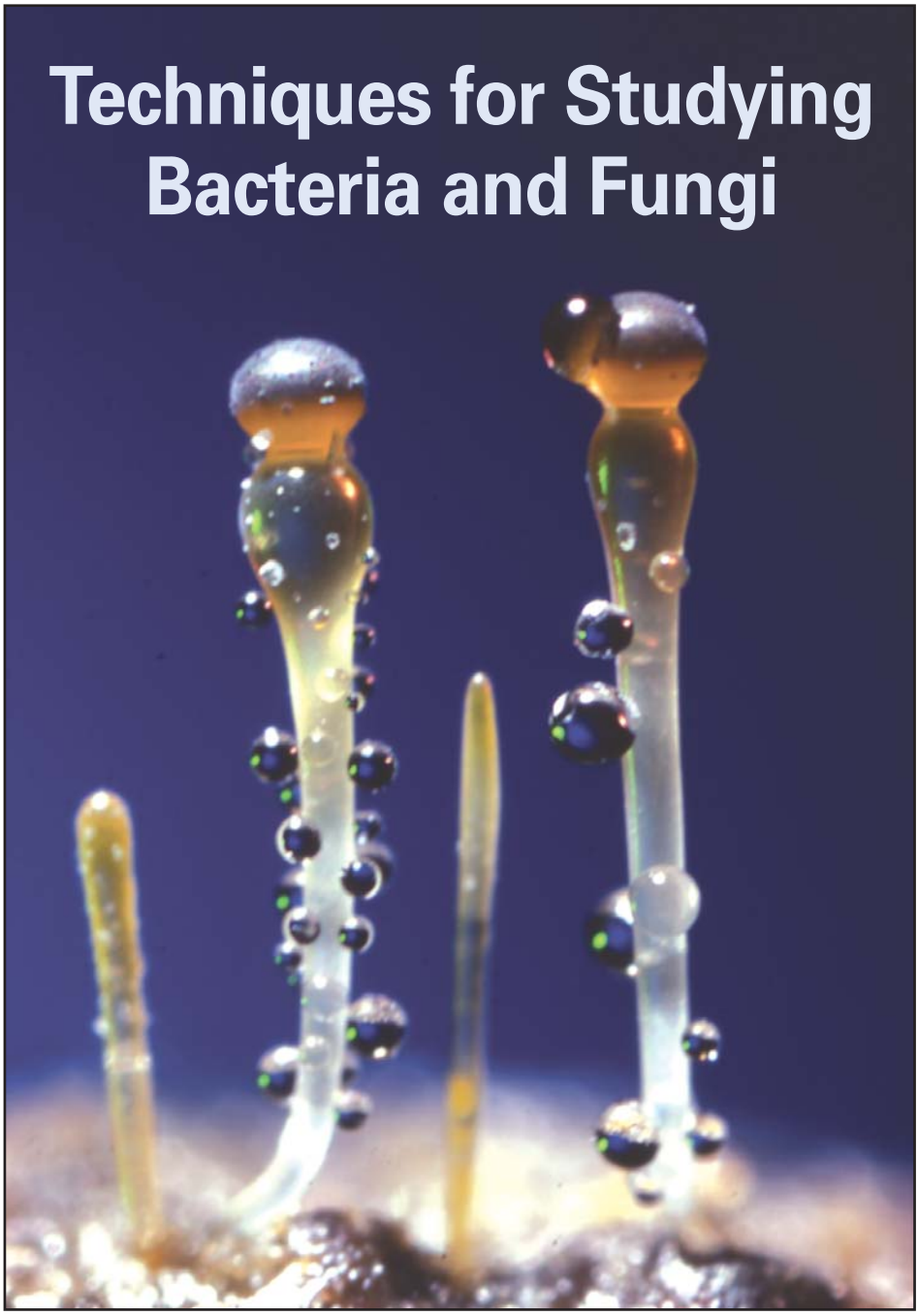
# Techniques for Studying Bacteria and Fungi



CAROLINA

*World-Class Support for Science & Math*

# Techniques for Studying Bacteria and Fungi



**Juliana T. Hauser**
**Microbiology Department**
**Carolina Biological Supply Company**

# 1. Introduction

If the human eye could resolve images as well as the light microscope, we would see bacteria and fungi virtually everywhere. They grow in air, water, foods, and soil, as well as in plant and animal tissue. Any environment that can support life has its bacterial or fungal population.

Bacteria and fungi affect man in various ways. Some cause human diseases such as typhoid fever, syphilis, athlete's foot, tuberculosis, and leprosy, while others cause plant diseases such as Dutch elm disease, corn smut, late blight of potatoes, soft rots of vegetables, and crown gall, which is characterized by tumor formation. Most microorganisms do man little or no harm, however, and many are vital to our well-being and continued existence on earth. Bacteria and fungi are involved in the recycling of matter, purification of sewage, and filtration of water in the soil. They are essential to the production of cheeses, sauerkraut, pickles, alcoholic beverages, and breads. Biotechnology firms use microorganisms to produce antibiotics, amino acids, interferons, enzymes, and human growth hormones.

Bacteria and fungi are convenient organisms for research in genetics, physiology, cytology, and biochemistry because they grow rapidly, are easy to manipulate, and require only minimal laboratory space compared to mice or guinea pigs. As prokaryotes, bacteria have the advantage of being relatively simple organisms. On the other hand, fungi, which are eukaryotes and thus much more complex genetically, grow so quickly that a number of generations can be obtained in only a short period of time.

# 2. General Techniques

## Aseptic Technique

In most microbiological procedures, it is necessary to protect instruments, containers, media, and stock cultures from contamination by microorganisms constantly present in the environment. Aseptic technique involves the sterilization of tools, glassware, and media before use as well as measures to prevent subsequent contamination by contact with nonsterile objects.

## Equipment and Work Area

To culture bacteria or fungi, you need the following materials:

1. Disinfectant solution such as 70% ethanol, 4% household bleach solution, or Lysol®.
2. Alcohol or gas (Bunsen) burner.
3. Inoculating loop for bacteria, yeasts, and fungi with abundant spores; scalpel or half-spearpoint needle for other fungi.

4. Stock culture (the original culture from which other cultures will be started).
5. Sterile medium in petri dishes or culture tubes.
6. Soap for washing hands.
7. Lab coat or old, clean shirt, especially while you are staining cultures.

Before working with bacterial or fungal cultures, always wash your hands with soap and water. Next, prepare a work area. Select an area that is as free from drafts as possible. Turn off the air-conditioner and fans, and close all windows and doors. Wipe the work area with 70% ethanol or a similar disinfectant solution. Arrange your materials conveniently on the clean work surface. Do not smoke, eat, or drink while working with cultures.

## Media Preparation

The first step in media preparation is to assemble the equipment and ingredients. You will need a balance, spatula, weighing paper, 1-L graduated cylinder, glass stirring rod, a large flask or beaker, and culture tubes or petri dishes. You can either use a recipe to prepare a particular medium from scratch or purchase any of the commercially available dehydrated media. The media most commonly used are nutrient agar (bacteria), potato dextrose agar (fungi), and Sabouraud dextrose agar (fungi). Recipes for a number of special media can be found in Chapter 5.

After assembling the equipment and ingredients, weigh the dry ingredients *accurately*. Place a sheet of weighing paper on the pan to protect the balance and to facilitate transferring the material into a flask. Using the weighing paper as a funnel, pour the dry ingredients into a large flask or beaker. Add the proper amount of distilled water and swirl the flask to dissolve the dry material. Agar-containing media must be heated slowly, just to boiling, to dissolve the agar. Gently agitate the medium during the heating process by either stirring or shaking the flask. Watch the flask carefully: agar burns easily and boils over quickly.

Pour the liquid agar or broth into bottles or culture tubes and cap them loosely. Autoclave the medium in the bottles or culture tubes to sterilize it. If the medium is to be used to pour dishes, autoclave it in the plugged flask in which it was mixed. When sterilization is complete, lay the tubes on a slant tray. Tighten the tops for storage only after the agar solidifies.

If plates are to be poured, disinfect the work area and stop all air drafts. Let the flask cool until it is easy to handle (20 to 40 minutes). Lay out sterile petri dishes and light a Bunsen burner. Remove the stopper from the flask and flame the mouth. Lift the cover of the dish at just enough of an angle to pour in the medium. Pour the agar slowly to avoid bubble formation; if bubbles do form, pass the burner flame quickly over the surface of the agar several

times, which should cause the bubbles to burst. Pour enough agar to fill the dish about one-half full, replace the cover, and allow the dish to stand undisturbed until the agar solidifies.

## Sterilization Procedures

Many microorganisms produce highly resistant spores that remain viable even after exposure to dry heat or boiling water for several hours. Steam under pressure is used to increase the temperature enough to kill any contaminating microorganisms. Steam penetrates wrappings and loosely capped articles, sterilizing the contents. The home pressure cooker works on this principle. An autoclave is, in essence, a large, self-contained pressure cooker that goes through the heating, sterilizing, and cooling cycles automatically.

If an autoclave is not available, you can use a large pressure cooker on a stove as long as you follow a few rules:

1. Read the directions for your brand of cooker and follow them carefully.
2. Make sure there is sufficient water in the cooker.
3. Don't start timing until 15 pounds per square inch (psi) have been reached.
4. At the end of 15 minutes, allow the pressure cooker to cool slowly.

Media should be sterilized for 15 minutes at a temperature of 121°C and a pressure of 15 psi. Glassware and contaminated articles like old stock cultures should be autoclaved for 30 minutes at 121°C and 15 psi.

## Transferring Tube Cultures

### *Slants*

After wiping the work area with a disinfectant and washing your hands with soap and water, light the alcohol or gas burner. Hold the stock culture tube and a sterile agar slant tube in the palm of one hand (Fig. 1a). Pick up the inoculating loop with the other hand, grasping it a little farther back than you would a pencil. Hold the wire in the flame until it glows red (Fig. 1b). Pass the lower end of the handle through the flame several times. Any part of the wire or holder that will be inserted into the tube *must be flamed*.

Remove the caps or cotton plugs from the stock tube and the sterile tube with the "loop" hand (Fig. 1c). *Do not lay the loop or caps down or allow them to touch anything.* Sterilize the mouths of the tubes by passing them through the flame several times (Fig. 1d). Insert the inoculating loop into the stock culture tube. Touch the loop to the top of the slant to cool it. Pick up a small quantity of bacteria, yeast, or fungal spores from the stock culture tube with the loop (Fig. 1e). Remove the loop from the culture tube, being careful not to touch the sides, and insert it into the sterile tube. Streak the loop back and forth from the bottom to the top of the slant (Fig. 1f).

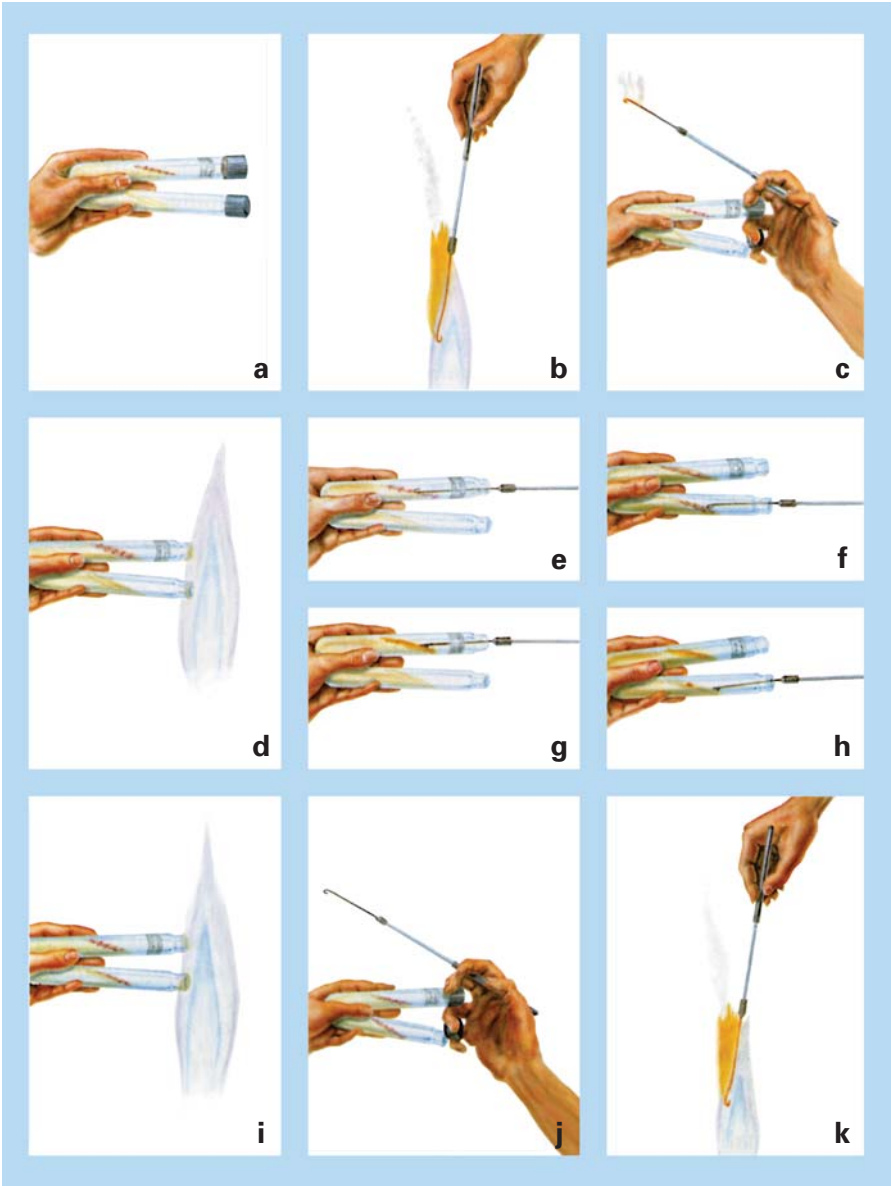



**Figure 1.** Transferring slant tube cultures of bacteria and fungi. (a) Hold the stock culture tube and the sterile agar slant tube in the palm of one hand. (b) Sterilize the inoculating loop by flaming it. (c) Remove the cap from each tube and (d) flame the mouths of the tubes. (e) Pick up a small quantity of bacteria, yeast, or fungal spores from the stock culture tube. (f) Insert the loop of bacteria into the sterile tube and streak back and forth from the bottom to the top of the slant. (g) For other fungi, use a half-spearpoint needle to remove the block of agar containing mycelium or fruiting cultures. (h) Place the block of agar into the sterile tube face down near the bottom of the slant. (i) Withdraw the loop or needle and flame the mouths of the tubes. (j) Replace the caps and (k) flame the loop or needle.

Withdraw the loop and flame the mouths of the tubes (Fig. 1i). Replace the caps or cotton plugs (Fig. 1j) and flame the loop until it glows red (Fig. 1k). Place the loop in a holder or lay it on the workbench. Label the new tube with your name, the name of the organism, the medium used, the incubation temperature, and the date.

When transferring other fungi, use the half-spearpoint needle or bent inoculating needle to cut a small block of agar containing mycelium or fruiting structures (Fig. 1g). Remove the needle with its block of agar, being careful not to touch the sides of the tube with the agar block. Insert the agar block of fungi into the sterile tube, placing the agar piece face down near the bottom of the slant (Fig. 1h). Flame the mouths of the tubes. Replace the caps or plugs. Flame the loop and label the inoculated tube as described above.

### *Broth Cultures*

*Never pipet microorganisms by mouth.* Use a pipet with a rubber bulb or a pipetting device such as a Pi-Pump®.

Light the burner. Hold the stock tube securely between the thumb and forefinger of one hand and agitate it by gently tapping or stroking the end of the tube with the other hand. Hold the tube of sterile medium in the same hand as the stock tube. Remove the caps but *do not lay them down.* Flame the mouths of the tubes. Draw about 0.1 mL of the microorganism-containing suspension into the pipet. Insert the end of the pipet into the sterile tube and release the contents into the broth. Remove the pipet from the tube, flame the mouths of both tubes, and replace the caps. Gently agitate the inoculated tube and label it. Place plastic pipets in autoclavable bags; sterilize reusable glass pipets in a disinfectant solution.

A broth culture may also be transferred with a loop. In order to inoculate with enough of the stock culture, several loopfuls of stock suspension should be transferred to the tube of sterile medium.

## Transferring Plate Cultures

### *Concentrated Growth*

Clean all work surfaces with a disinfectant solution. It is *essential* to reduce airflow as much as possible during the transfer of plates to avoid contamination.

To transfer bacteria, yeasts, or fungi with abundant spores, place the stock tube in the palm of one hand. With the other hand, flame an inoculating loop. Remove the cap or cotton plug with the "loop" hand and flame the mouth of the tube. Insert the cooled loop into the stock tube and pick up a small quantity of the culture (more than for transfer to a tube). Replace the cap or plug. Gently raise the cover of the petri dish. Touch the loop to the top of the dish and streak from side to side all the way to the bottom edge. The finished plate will have a zigzag pattern from edge to edge (Fig. 2). Lower the cover

and flame the loop. Label the dish as you would a tube. All petri dishes incubated at temperatures above 25°C should be placed upside down to prevent condensed water from dripping onto the agar and causing colonies to run together.

### *Isolation Streaking*

The isolation streaking technique (Fig. 3) produces individual colonies for observing morphology or separating mixed suspensions of bacteria as described in Chapter 3, "Separation of Unknowns" (page 14).

Hold the stock tube in one hand. Flame the loop with the other hand and remove the cap or plug from the tube. Flame the mouth of the tube. Insert the cooled loop into the tube and remove a small quantity of the culture (about the same as for a tube). Replace the cap or plug. Raise the cover of the petri dish at just enough of an angle to insert the loop. Streak only the top one-fourth of the plate (Area 1) in a zigzag pattern and replace the cover. Remove the loop, flame it, and allow it to cool. Turn the dish 90° (Area 2). Lift the cover and touch the loop to the center of the agar to make sure that it is cool. Make one streak from Area 1 into Area 2. Then streak Area 2 in a zigzag pattern until one-fourth of the plate is covered. Remove the loop and flame it. Then repeat the above steps twice more, streaking from Area 2 to Area 3 and from Area 3 to Area 4.

### *Fungal Plates*

Yeasts and fungi with abundant spores (e.g., *Penicillium*, *Aspergillus*, and *Rhizopus*) can be transferred in the same way as bacteria.

For nonsporulating fungi or those whose spores are enclosed within a fruiting structure (e.g., *Sordaria fimicola*), cut a block of agar with a flame-sterilized

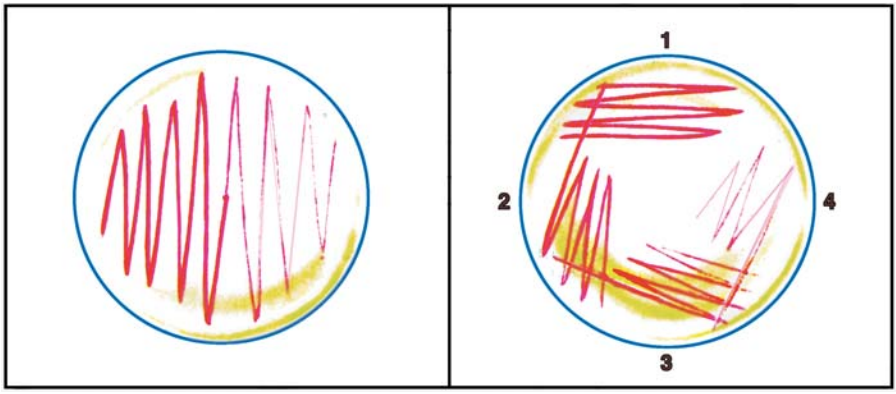



**Figure 2.** (Left.) Typical zigzag streaking pattern for inoculating plates.
**Figure 3.** (Right.) Isolation streaking technique. Streak the top one-fourth of the dish (Area 1). Flame the loop and make one streak from Area 1 into Area 2. Then continue streaking in a zigzag pattern until the second one-fourth of the dish (Area 2) is covered. Repeat above steps twice more by streaking Area 3 from Area 2 and Area 4 from Area 3.

half-spearpoint needle, bent inoculating needle, or scalpel. Lift the cover of a sterile agar dish only enough to insert the block of agar face down in the center of the dish.

## Care of Cultures

### *Temperature*

Most fungi grow well at room temperature (about 25°C). Most nonpathogenic (as well as some pathogenic) bacteria also grow well at room temperature. *Chromobacterium violaceum*, *Neisseria subflava*, *Spirillum volutans*, *Thiobacillus thioparus*, and most *Bacillus* and *Enterobacter* species grow best at a slightly higher temperature (30°C). Most bacterial pathogens, the enteric bacteria (e.g., *Escherichia coli*), and *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus* species grow well at body temperature (37°C). *Bacillus stearothermophilus* grows best at 55 to 65°C, temperatures that would be lethal to most other bacteria.

### *Storage and Maintenance of Stock Cultures*

For short-term storage of a few weeks, inoculate stock cultures into screw-cap tubes. With strict aerobes and fungi, leave the cap loose until growth is luxuriant and then tighten it. Store most cultures at room temperature. *Spirillum volutans* and *Thiobacillus thioparus* should be stored in an incubator at 30°C at all times. For anaerobic bacteria (*Clostridium* species) and microaerophiles (*Lactobacillus* species and *Spirillum voluntans*), screw the top down tightly after subculturing.

If cultures must be stored for several months and subculturing is not practical, screw-cap tubes containing luxuriant growth may be stored in the refrigerator. This is the cheapest and most practical method. Several stock cultures of each species should be stored, however, because some cultures do not survive refrigeration or may undergo genetic mutation.

Freeze-drying is now popular for extended storage of several years but requires specialized techniques and equipment. Carolina™ freeze-dried cultures contain lyophilized pure strains of viable bacteria and fungi. They can be stored for at least three years at 4°C and normally require only 24 to 48 hours to produce luxuriant growth after rehydration. To activate, dissolve the freeze-dried culture in rehydration medium and incubate it at the appropriate temperature for 24 hours. After incubation, inoculate to the appropriate growth medium, either broth or agar, and again incubate. Freeze-dried cultures should be subcultured twice before staining.

Most bacteria and fungi will remain viable for prolonged periods of time in culture if they are transferred to fresh medium every two to three weeks. *Spirillum volutans* and *Vibrio fischeri* must be transferred twice each week and *Thiobacillus thioparus* once a week. *Physarum polycephalum* needs to be transferred as soon as the organism has covered the agar surface.

## Cleanup and Disposal

After transfer work is completed, the area should again be cleaned with a disinfectant solution. Wash your hands thoroughly. If it is not possible to autoclave old stock cultures and glassware, cover them with 70% ethanol or a similar disinfectant overnight. The cultures should then be incinerated if possible.

Accidents do and will happen when working with bacteria and fungi. If a tube or petri dish breaks, report the accident to the instructor or assistant immediately. The spill should be covered with 70% ethanol for a few minutes. Then sweep up the spilled material very carefully and put it with other contaminated wastes to be autoclaved or incinerated. *Do not* pick up glass fragments with your fingers or stick your fingers into the culture itself. *Don't panic: bacteria and fungi are not vengeful and do not crawl across the floor to attack the one who dropped them!*

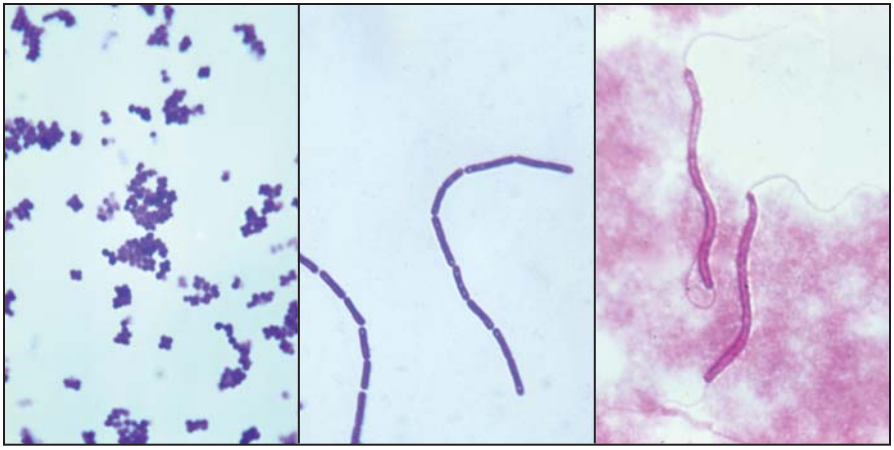


**Figure 4.** The three basic shapes of bacteria: coccus (left), bacillus (center), and spirillum (right).

# 3. Specific Techniques: Bacteria

## Morphology

Bacteria vary greatly in size, but their cell shapes are of three basic types (Fig. 4): coccus (sphere-shaped), bacillus (rod-shaped), and spirillum (spiral- or comma-shaped). Some bacteria exist singly, while others are attached in chains or packets.

## Staining

Bacterial cells can be colored with a stain to provide contrast with the background or to make cellular organelles visible.

### *Simple Stains*

A simple stain consists of an aqueous or alcoholic solution of a single dye. Some of the more commonly used stains are methylene blue, basic fuchsin, and crystal violet. The procedure for simple staining is as follows.

1. Place a drop of distilled water on a clean slide.
2. Flame the inoculating loop and the mouth of the culture tube.
3. Remove a small quantity of bacteria from the slant.
4. Flame the mouth of the tube and replace the cap.
5. Mix the bacteria with the water on the slide and spread thinly.
6. Allow the smear on the slide to air-dry.

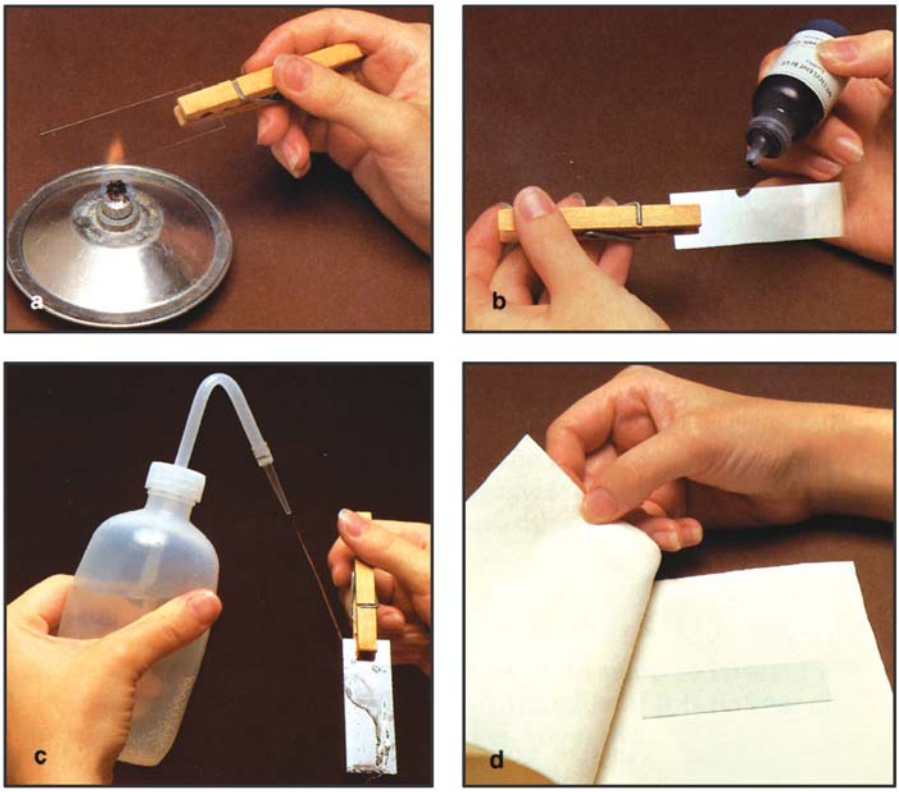



**Figure 5.** Staining a bacterial smear. (a) Pass the slide, smear side up, through a flame three times. (b) Flood the smear with a stain and let stand one minute. (c) Rinse the slide gently with water, making sure the stream of water does not strike the smear directly. (d) Carefully blot the slide dry.

7. Using a clothespin or similar holding device, pass the slide, smear side up, through a flame three times (Fig. 5a) to fix the bacterial cells. Fixing kills the bacteria and causes them to stick to the slide.
8. Allow the slide to cool.
9. Flood the slide with basic fuchsin, methylene blue, or crystal violet (Fig. 5b) and allow to stand one minute.
10. Rinse the slide gently with tap water (Fig. 5c). Do not let the stream of water strike the smear directly, or you will wash off the stained cells.
11. Carefully blot the slide dry with bibulous paper (Fig. 5d).
12. Slides can be made permanent with mountant and a coverslip.
13. Observe under an oil immersion lens.

### *Gram Stain*

Differential stains, which are more complex than simple ones, are used to divide bacteria into groups. Bacteria stain differentially because they differ in cell wall composition. The Gram stain separates almost all bacteria into two large groups: the Gram-positive bacteria, which stain blue (Fig. 6), and the Gram-negative bacteria, which stain pink (Fig. 7). This classification is basic to bacteriological identification.

1. Prepare the smear, air-dry, and heat-fix by following Steps 1 through 8 in the "Simple Stains" staining instructions above.
2. Flood with Hucker ammonium oxalate crystal violet for 60 seconds.
3. Rinse with tap water.
4. Flood with Gram's iodine solution for 60 seconds.
5. Rinse with tap water.
6. Decolorize with 95% ethanol. Allow the ethanol to drip across the slide until the runoff is almost clear.
7. Rinse with tap water.
8. Flood with safranin for 60 seconds.
9. Rinse with tap water.
10. Blot carefully.
11. Observe with an oil immersion lens.

Morphological observations and the Gram stain are the first steps in identifying an unknown bacterium. Differential media are then used for definite identification.

**Figure 6.** Gram-positive bacteria.

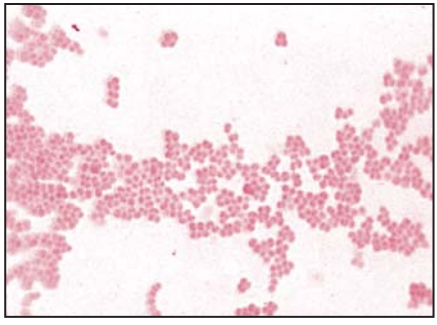


**Figure 7.** Gram-negative bacteria.

## Biochemical Properties

Because of their microscopic size, bacteria are difficult to identify by direct observation. A more precise method is to determine whether or not the bacteria utilize a particular biochemical pathway. Many bacteria use carbohydrates as energy sources. The Bacterial Fermentation Kit (15-4710) allows students to differentiate among several bacterial species by observing whether the bacteria can ferment various carbohydrates (Fig. 8). Students can further classify bacteria by determining whether they can hydrolyze starches (Fig. 9), lipids, and proteins with the Bacterial Biochemical Identification Kit (15-4715).

## Separation of Unknowns

For a student exercise in separating unknown bacteria, we offer two broth cultures of mixed bacteria (15-4760 Mixed Suspension of Introductory Bacteria and 15-4765 Mixed Suspension of Pigmented Bacteria). To separate the bacteria, first perform a Gram stain. Then, streak a loopful of broth on a nutrient agar dish, as described in Chapter 2, "Isolation Streaking." Incubate for five to seven days at room temperature. Observe daily. The colors of the colonies will depend upon the bacteria in your culture (Fig. 10):

Red colony: *Serratia marcescens*.

Yellow colony: *Sarcina lutea*.

White colony: *Bacillus subtilis*.

Pinkish-gray colony: *Rhodospirillum rubrum* (grows very slowly).

Perform Gram stains of each colony to confirm the results.

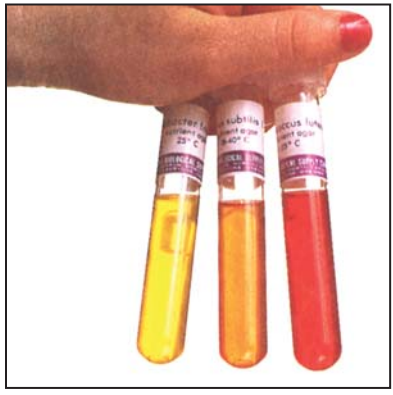


**Figure 8.** Ability of different bacteria to ferment the carbohydrate dextrose. Some bacteria produce acids as end products of dextrose fermentation (center). Others produce both acid and gaseous end products (left). Some cannot ferment dextrose at all (right).

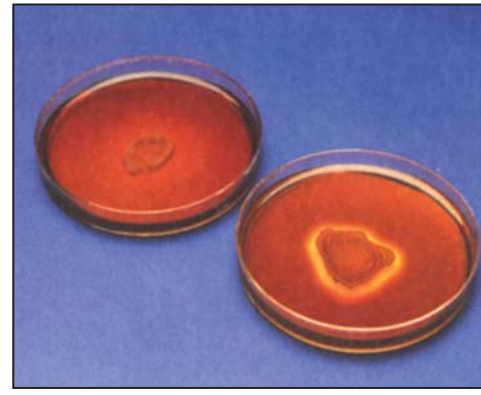


**Figure 9.** Starch hydrolysis. Some bacteria do not hydrolyze starch (left), while others do (right), leaving a clear ring in the agar around the bacterial culture.

**Figure 10.** A plate streaked for isolation.

## Laboratory Activities

### *Effects of Environment on Growth*

Bacteria grow when environmental conditions are favorable. If conditions are not suitable, growth occurs slowly or not at all, and death may even occur. Some factors that affect growth are water, food, oxygen, pH, and temperature. The Bacterial Anaerobe Culture Kit (15-4676), pH Tolerance of Microbes Kit (15-4716), and the Carolina™ Germicidal Effects of UV Light Kit (15-4640 and 15-4641) allow students to investigate specific environmental factors. With the Bacterial Investigative BioKit® (15-4727) students test for the presence of bacteria in different environments and observe the effects of different temperatures and media on bacterial growth. Students can also explore the effects of osmotic pressure (15-4714 Tastefully Shrinking Microbes Kit), boiling (15-4717C Carolina™ Spore Wars Kit), natural inhibitors (15-4723 The Spicy Inhibitors Kit), and chemical preservatives (15-4662 Foiling Spoilage with Chemical Preservatives Kit).

### *Effects of Antibiotics and Disinfectants*

Many ways have been devised to kill bacteria in order to prevent contamination or spread of disease. These include physical methods (heat, ultraviolet light) and chemical means (disinfectants, antibiotics). Disinfectants are chemical substances that kill or retard the growth of microorganisms. The Disinfectant Sensitivity BioKit® (15-4735; Demonstration Kit 15-4734) allows students to test the effects of common household disinfectants on the growth of bacteria. Antibiotics are substances produced by living organisms that inhibit the growth of microorganisms. The Antibiotic Sensitivity BioKit® (15-4740) allows students to test the effects of eight antibiotics on bacterial growth (Fig. 11). The Antibiotic Production Kit (15-4739) demonstrates the production of penicillin and streptomycin by living microorganisms and the effects of these two antibiotics on bacterial growth.

**Figure 11.** Antibiotic sensitivity test from 15-4740 Antibiotic Sensitivity BioKit®.

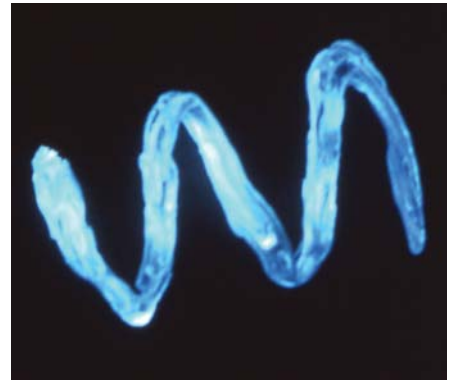


**Figure 12.** *Vibrio fischeri* photographed in total darkness using only light emitted from the bacteria.

### *Bioluminescing Bacteria*

*Vibrio fischeri* (15-5722) (Fig. 12) is a bioluminescing marine bacterium that commonly inhabits fish. It requires some salt in the medium in order to grow, and it is usually cultured on saltwater agar or, preferably, photobacterium agar. *V. fischeri* should be inoculated more heavily than other bacteria. The cultures should be placed *in the dark* (e.g., a cleaned, disinfected cabinet or a covered box) at room temperature with caps loosened. A subculture should be made 18 to 24 hours before bioluminescence is to be observed. Allow at least five minutes for the eyes to adjust to the dark in a room with no light leakage. (**Note:** A classroom with the lights out and the shades drawn will not provide enough darkness).

### *Photosynthesizing Bacteria*

*Rhodospirillum rubrum* (15-5300) is a photosynthetic bacterium. It grows anaerobically (a tightened screw cap) in sunlight and aerobically in the dark. *R. rubrum* multiplies slowly, requiring five to seven days for growth to be visible along inoculated areas. It should be inoculated more heavily than most other bacteria. As its name implies, *R. rubrum* is spiral-shaped.

### *Nitrogen-Fixing Bacteria*

Members of the genus *Rhizobium* (15-5270) have the ability to utilize atmospheric nitrogen when living in a symbiotic relationship with the roots of a host leguminous plant like clover, alfalfa, or soybean. Most other bacteria as well as higher plants must have nitrogen compounds present in the medium or in the soil. The *Rhizobium* Inoculum with Clover Seeds (15-4720) may be used to demonstrate the nitrogen-fixing nodules that form on the roots of the host clover plant.

## Halobacterium *sp. NRC-1*

*Halobacterium* belongs to the most recently identified domain of life, the Archaea. As such, it is phylogenetically distinct both from the Bacteria and the Eukaryota. Like bacteria, it is a prokaryote without a nuclear envelope. *Halobacterium* cells are rod-shaped and, like bacteria, its cells are much smaller than most eukaryotic cells. However, some of its characteristics are distinctly different from those of bacteria and more similar to those of eukaryotes.

Most known Archaea are extremophiles; that is, they are organisms that thrive in and even require extreme environments. This includes extremes of pH, pressure, and temperature. Most methanogens require an anaerobic environment. It is difficult to safely provide these extreme conditions in most teaching labs or classrooms, which has until now severely restricted the use of Archaea in education. However, *Halobacterium* thrives in an extreme salt environment, which can be easily and safely provided. In nature, *Halobacterium* occurs in such hostile environments as the Great Salt Lake, the Dead Sea, and solar salt pools.

**Figure 13**. *Halobacterium* plate culture.

In the laboratory, *Halobacterium* can be handled using the same techniques, streaking, etc., as described for bacteria. However, *Halobacterium* grows on a hypersaline medium on which almost no other microorganisms can even survive (Fig. 13). In fact, *Halobacterium* can survive *only* in hypersaline environments. This allows beginning students to practice and master basic skills of sterile technique with little chance of contaminating their cultures or work area. Additionally, *Halobacterium* is not known to cause disease in humans. However, we recommend that standard microbiological safety procedures be followed whenever using *Halobacterium* or any other microbe. Cultures can be incubated at 20°C to 45°C, with the optimal growth at 42°C. *Halobacterium* is a model organism both for basic courses and for advanced research.

# 4. Specific Techniques: Fungi

The members of the Fungi Kingdom (Myceteae) are parasitic or saprophytic organisms that exist in either a unicellular or filamentous form (hyphae) surrounded by a cell wall. Fungi either absorb or engulf their food. The Myceteae are subdivided into three divisions.

## Division I. Gymnomycota

The Gymnomycota, commonly called the slime molds, exhibit phagotrophic nutrition, i.e., they engulf their food.

Found in nature under cool, humid, dark conditions, the slime mold *Physarum polycephalum* (15-6190, 15-6192, and 15-6193) (Fig. 14) offers students a unique opportunity to work with living protoplasm. *Physarum* is easy to culture and handle and exists in two forms: as a motile, multinucleate mass of protoplasm called a plasmodium and as a dry, resistant structure called a sclerotium. With the Introduction to *Physarum* Kit (15-5829) students observe cytoplasmic streaming and plasmodial fusion and investigate factors influencing plasmodial growth and sclerotia formation. The Chemotaxis in *Physarum polycephalum* Kit (15-5825B) presents methods and procedures that enable students to design and conduct active investigations of chemotaxis in slime molds.

**Figure 14.** *Physarum polycephalum* plasmodium.

The plasmodium can be cultured on 2% water agar, fed oat flakes, and kept in the dark. Reactivate a piece of dried sclerotium by placing it on damp filter paper or 2% water agar. When the active plasmodium begins to appear, feed it by placing oat flakes at its margins. After 24 to 48 hours, a portion of the plasmodium may be transferred to another plate of 2% water agar; continue feeding with oak flakes. The streaming of protoplasm in the veins of the plasmodium may be observed on an agar dish at the edges of the growth using a low-power objective.

*Dictyostelium discoideum* (15-5995 and 15-5996) is a cellular slime mold. Unlike *Physarum*, the cells of *Dictyostelium* always retain their individuality even though they aggregate in a mass called a pseudoplasmodium. *Dictyostelium* is cultured in lactose agar dishes or tubes inoculated with bacteria as a food source. A nonmucoid strain of *Escherichia coli* (15-5066) is inoculated on lactose agar. Several sporocarps from a mature culture of *Dictyostelium* are then streaked across the agar in the area inoculated with bacteria. Individual amoebae will be present in 12 to 24 hours, and the first

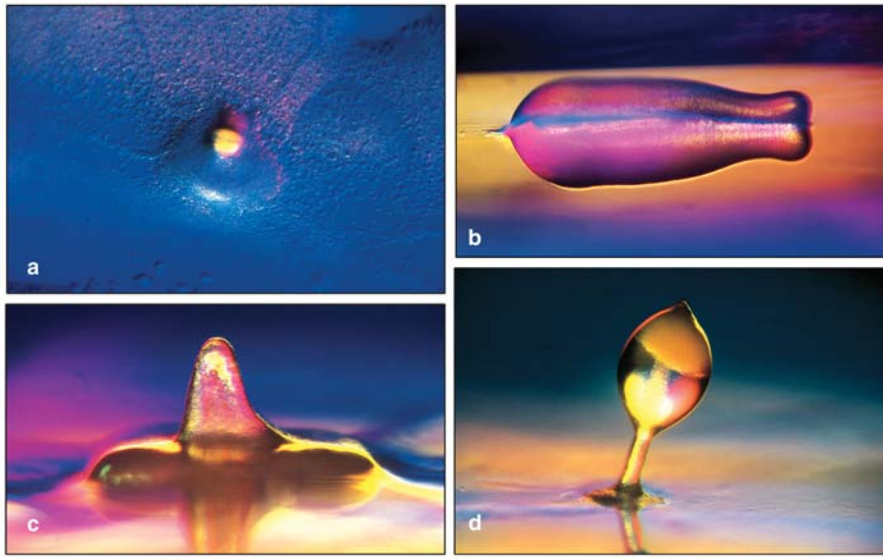



**Figure 15**. *Dictyostelium discoideum.* (a) Aggregation of amoebae into pseudoplasmodia (slugs). (b) Migrating pseudoplasmodium. (c) Formation of sporocarp. (d) Mature fruiting sporocarp.

aggregations of amoebae into pseudoplasmodia (slugs) (Fig. 15a) can be observed within this period of time. Pseudoplasmodia will be present in 36 to 48 hours, and many of them will have begun to migrate (Fig. 15b). After 48 to 72 hours, the pseudoplasmodium stops migrating and formation of the sporocarp begins (Fig. 15c). By 72 hours mature sporocarps should be present (Fig. 15d).

## Division II. Mastigomycota

The Mastigomycota exhibit absorptive nutrition, i.e., food in the environment is digested and then absorbed. They produce motile cells during their life cycles.

### *Class Chytridiomycetes*

The Chytridiomycetes (chytrids) differ from all other fungi in that their motile cells (zoospores) have a single, posterior flagellum. The chytrids require the presence of a thin liquid film for zoospore maturation and dispersal (Fig. 16). Add about 0.3 mL of sterile water to a slanted agar screw-cap tube. Inoculate the water with a chytrid species like *Hyphochytrium* (15-6055), *Phlyctochytrium* (15-6170), or *Rhizophydium* (15-6220) and place the fresh culture on a slant tray with the cap slightly loosened. After six to ten days in the dark, the culture will have established itself along the whole slant, and the excess water will have evaporated. Tilt the tray daily to allow water to wash over the entire agar surface.

The chytrid *Allomyces* can be used to demonstrate alternation of diploid (2n) and haploid (n) generations. The sporophytic plant (2n) and the gametophytic plant (n) are easily identified on a plated culture. The sporophyte produces colorless asexual zoosporangia (the structures producing zoospores) and numerous brown, resting sporangia. The gametophyte produces colorless oogonia (the structure producing female gametes) and orange antheridia (the

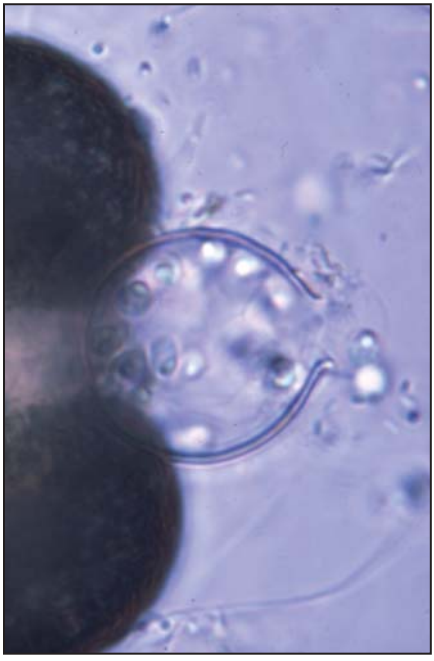


**Figure 16.** The chytrid *Rhizophydium* exhibiting escaping zoospores.

**Figure 17.** The chytrid *Allomyces javanicus*. Antheridium (top); oogonium (center).

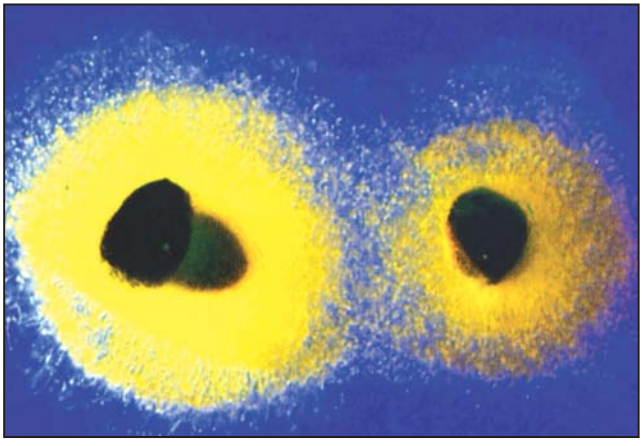


**Figure 18.** The water mold *Saprolegnia* growing on split hemp seed.

structures producing male gametes) in close proximity (Fig. 17). *Allomyces arbuscula* (15-5914) and *Allomyces javanicus* (15-5916 and 15-5917) are both cultured on Emerson Yp Ss agar.

### *Class Oomycetes*

The Oomycetes, commonly called water molds, are either unicellular or filamentous. The hyphae of the filamentous Oomycetes are coenocytic (without cross-walls). The Oomycetes produce motile asexual spores, the zoospores, which are biflagellate, as well as nonmotile sexual spores, the oospores.

*Achlya* (15-5901) and *Saprolegnia* (15-6271) may be cultured in cornmeal agar dishes. For ease of manipulation in the classroom, it is often desirable to culture the water molds in water on split hemp seed (Fig. 18) or cucumber seed. Using a sterile razor blade or scalpel, cut a small section of water mold and agar from the edge of the water mold stock culture and place it on an appropriate agar dish. Inoculate the culture at 20 to 25°C. After the mycelium has covered part of the dish, place sterile split hemp seeds or cucumber seeds (autoclaved for 30 minutes at 121°C and 15 psi) on their cut sides along the outer edge of the new growth.

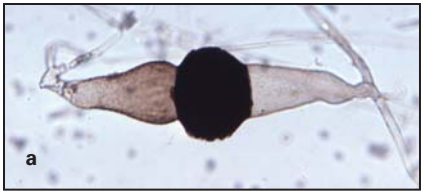
*Saprolegnia* takes about three days for good new growth and *Achlya* takes about four days. After a few days, notice the mycelium attaching to the inner portions of the seed. With sterile tweezers, lift hemp or cucumber seeds with mycelia attached from the agar plate and place four of them in sterile petri dishes containing about 20 mL of sterile lake water. Change the water every day for three to seven days to observe sporangia, which contain the zoospores, and oogonia, which contain the oospores.

## Division III. Amastigomycota

The Amastigomycota exhibit absorptive nutrition like the Mastigomycota, but they differ in that they do not produce motile cells during their life cycles. The Amastigomycota are divided into classes by the type of sexual reproduction exhibited.

**Figure 19**. Sporangia of the shotgun fungus *Pilobolus crystallinus.*

**Figure 20**. *Rhizopus* sporangia.





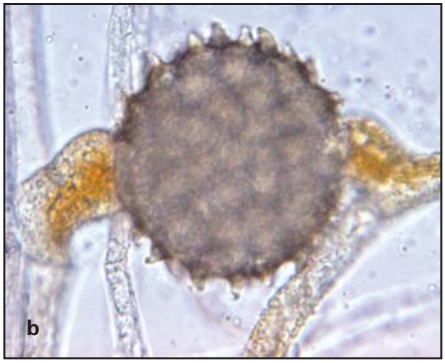



**Figure 21.** Zygospores.
(a) *Rhizopus stolonifer.*
(b) *Mucor hiemalis.*
(c) *Phycomyces blakesleeanus.*

### *Class Zygomycetes*

The Zygomycetes or conjugation fungi reproduce sexually by the fusion of gametes (conjugation) from two opposite mating types to form a thick-walled zygospore.

#### *Phototropism*

*Pilobolus crystallinus* (15-6207) (Fig. 19), called the shotgun fungus, is found in nature growing on dung. The common name comes from the forceful discharge of the sporangia, which can be shot upward to a height of 1.8 m (6 ft). The *Pilobolus* sporangiophore (i.e., the hyphae bearing the sporangium) is phototropic and often discharges the sporangium with enough accuracy to strike within 3 to 5 mm of a point light source. The *Pilobolus* Kit (15-5800) is designed to demonstrate this phototropic ability.

*Pilobolus* is cultured on rabbit dung agar plates. Mature sporangiophores are present in five to eight days following subculture. To subculture, cut a block of agar with sporangia and place it upside down on a fresh plate of rabbit dung agar.

#### *Conjugation*

*Rhizopus stolonifer* (15-6222 and 15-6224) is an excellent organism for demonstrating conjugation and formation of zygospores. Using aseptic technique, place a few sporangia of two opposite mating strains of *Rhizopus* on opposite sides of a cornmeal-dextrose-peptone agar dish. Incubate at room temperature. The asexual sporangia begin forming around the edge of the plate two days after inoculation (Fig. 20). The zygospores form a day or two later on the line where the hyphae of the two strains meet. When looking for zygospores (Fig. 21a) with a microscope, it may be necessary to part the mycelium with forceps or a teasing needle. Zygospores that have formed on the agar surface may be overgrown by and hidden under the mycelium.

*R. stolonifer* spores are so light and easily disseminated that other cultures in the laboratory often become cross-contaminated. *Mucor hiemalis* (15-6112 and 15-6113) (Fig. 21b) and *Phycomyces blakesleeanus* (15-6180 and 15-6181) (Fig. 21c) are easier to work with and their zygospores readily observed (Fig. 22).

### *Class Ascomycetes*

The Ascomycetes or sac fungi produce sexual spores called ascospores borne in a bag-shaped structure, the ascus. The presence or absence of an ascocarp (a fruiting body containing the asci) and ascocarp shape are used in classifying the Ascomycetes.

*Ascomycete Fruiting Types*

*Schizosaccharomyces octosporus* (15-6281) produces naked asci, i.e., asci not enclosed in an ascocarp (Fig. 23). All stages of *Schizosaccharomyces*, from ascus formation to mature ascospores, can be observed as described in the following section on yeasts.

*Eurotium chevalieri* (15-6032) develops cleistothecia, which are completely closed ascocarps. Greenish-gray conidia (asexual spores) of *E. chevalieri* form first, three to four days after inoculation. The yellow cleistothecia form in five to seven days. Cleistothecia can be scraped off the agar and placed in a drop of water on a slide. Gently crush the cleistothecia under the coverslip to observe ascospores inside the globular asci scattered among the hyphae.

*Anthracobia muelleri* (15-5926A) is an ascomycete that demonstrates the development of apothecia, which are open, cuplike ascocarps (Fig. 26). It fruits on rabbit food agar. After inoculating, place the culture near a light source and maintain at room temperature. In about 14 days, tiny fruiting structures begin to

**Figure 22**. Plate showing line of zygospores formed at the juncture of plus and minus mycelia of *Phycomyces blakesleeanus.*

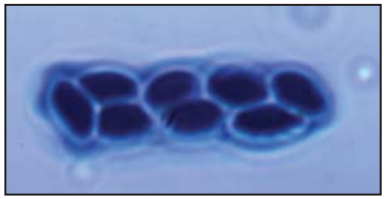


**Figure 23.** Naked ascus of *Schizosaccharomyces octosporus*.

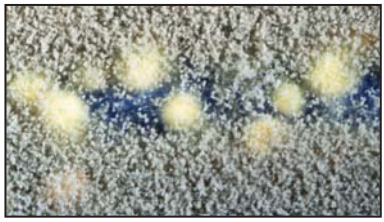


**Figure 24.** *Talaromyces flavus*, an ascomycete similar to *Eurotium chevalieri*, exhibiting gray conidia and green cleistothecia.

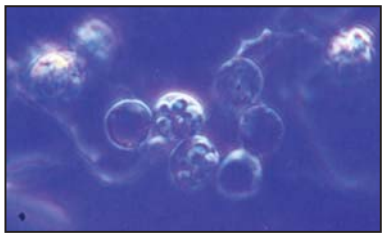


**Figure 25.** Squashed cleistothecium of *Talaromyces flavus*, an ascomycete similar to *Eurotium chevalieri*, exposing globular asci. Note the various stages of ascospore formation.

**Figure 26.** Mature apothecia of *Anthracobia muelleri*.

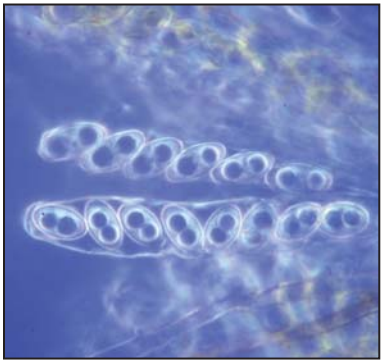


**Figure 27.** Squashed apothecium of *Anthracobia muelleri* revealing the ascospores inside the tubelike asci.

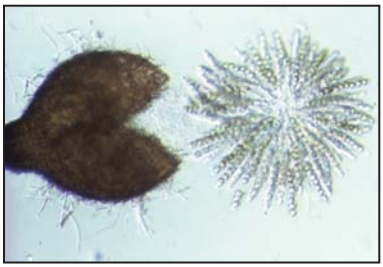


**Figure 28.** Squashed perithecium of *Sordaria fimicola* exposing asci.

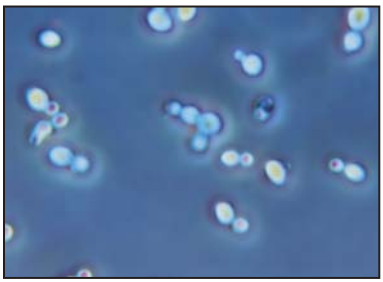


**Figure 29.** Budding *Saccharomyces cerevisiae* cells.

form at the periphery of the plate. The apothecia may be scraped off the agar, added to a drop of water on a glass slide, and gently crushed to reveal the tubelike asci (Fig. 27).

*Sordaria fimicola* (15-6291) produces a flask-shaped ascocarp called a perithecium that is closed except for a small opening at the apex. *Sordaria* forms several hundred perithecia within 5 to 10 days after inoculation on *Sordaria* culture agar. Within each mature perithecium are many asci clustered together (Fig. 28). The ascospores of *Sordaria* are forcibly discharged from the ascus and stick to the sides of the culture container.

The Ascomycete Fruiting Set (15-5822) of four plate cultures is available for demonstrating these fruiting types.

*Yeasts*

*Saccharomyces cerevisiae* (15-6252) and *Schizosaccharomyces octosporus* (15-6281) are two yeasts widely used in the laboratory. Both can be cultured on Sabouraud dextrose agar or any other good mycological agar.

*Saccharomyces cerevisiae* reproduces asexually by budding (Fig. 29). To observe budding, subculture onto fresh Sabouraud dextrose agar and incubate at 30°C for one to two days. Prepare a wet-mount slide of the yeast and stain with methylene blue if desired.

*Schizosaccharomyces octosporus* reproduces asexually by fission (transverse division). To observe fission, subculture onto yeast malt agar. Prepare slides daily over a period of four days to observe the fission into daughter cells (Fig. 30). Continue to observe a culture over a period of a week for the formation of ascospores inside the bag-shaped ascus (Fig. 23).

*Genetic Recombination*

*Sordaria fimicola*, a dung ascomycete, is used extensively in studies of mutation and biochemical genetics. Different mating types are found in *S. fimicola*. The *Sordaria* Genetics BioKit® (15-5847 classroom kit, 15-5859 demo kit) and the S*ordaria* Genetics Kit (15-5848) provide interesting exercises in fungal genetics for the laboratory.

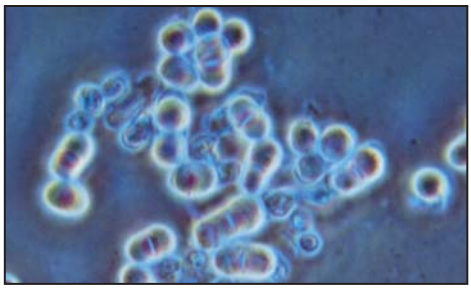


**Figure 30**. *Schizosaccharomyces octosporus* demonstrating fission.

If *S. fimicola* is crossed with either tan or gray mutants on *Sordaria* crossing agar (Fig. 31), hybrid asci are produced that contain four dark (wild-type) and four light (mutant) ascospores. The order of ascospores in the ascus reflects the order in which the chromosomes are segregated during meiosis. If crossing over has occurred, the sequence of mutant to wild-type spores will be 2:2:2:2 or 2:4:2. If crossing over has not occurred, the sequence will be 4:4.

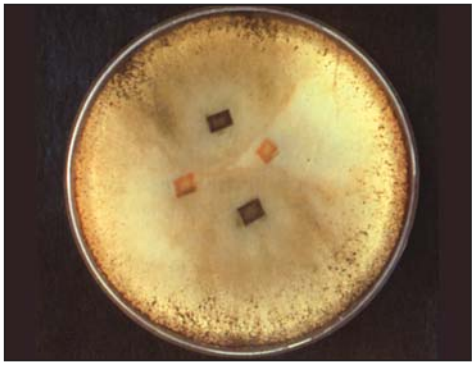


**Figure 31.** A crossing plate inoculated with wild-type (dark blocks) and mutant strains (tan blocks) of *Sordaria fimicola*.

To view the asci, use a toothpick to remove a few dark perithecia from the crossing dish. Place them in a drop of water on a microscope slide and add a coverslip. Gently press on the coverslip to crush the perithecia and reveal the asci with ascospores. A careful search of the dishes will reveal the location of perithecia containing hybrid asci.

### *Class Basidiomycetes*

The Basidiomycetes, often called club fungi, produce sexual spores on a club-shaped structure, the basidium. This class includes mushrooms, puffballs, and the rusts and smuts, which are plant pathogens. *Coprinus cinereus* (15-5979 and 15-5979B) is cultured on potato dextrose agar to which sterile rabbit dung has been added. *C. cinereus* is commonly called the "inky cap" mushroom because the cap dissolves into an inky black mass at maturity. Basidia can be observed by cutting a thin, wedge-shaped piece from a fresh cap. Basidiospores (the sexual spores) can be observed by preparing a wet-mount slide from the gills. The Mushroom Review Set (15-5792) consists of a large box culture of *C. cinereus* ready to fruit and 30 Gilled Fungi Bioreview® Sheets.

**Figure 32.** The basidiomycete *Schizophyllum commune*.

**Figure 33.** The nematode *Rhabditis* caught in the hyphal traps produced by *Arthrobotrys conoides*.

*Schizophyllum commune* (15-6275B) has a gray, fan-shaped fruiting body (Fig. 32). It has been used widely in physiology, genetics, and morphogenesis studies. It is easily cultivated on yeast malt agar and fruits one to two weeks after transfer.

### *Form-Class Deuteromycetes*

The Deuteromycetes or imperfect fungi have either a poorly understood or no known sexual stage. Since the sexual spores and fruiting bodies are characteristics used in classification, these fungi are placed in a class by themselves called a form-class.

*Arthrobotrys conoides* (15-5930) is a soil-inhabiting Deuteromycete that is carnivorous on nematodes (roundworms). When inoculated with the nematode *Rhabditis* (13-3258), *A. conoides* forms loops of hyphae that trap the nematode (Fig. 33). Once it is trapped, the fungus produces additional hyphae that penetrate the body of the nematode and then digest and absorb its contents. The Carolina™ Carnivorous Fungus Kit (15-5802) allows a classroom to observe this phenomenon.

# 5. General Media and Special Media

The following general media and special media are available through Carolina Biological Supply Company.

| General Media | Description |
|---|---|
| Nutrient Agar | General media for most bacteria. |
| Tryptic Soy Agar | General media for most fastidious bacteria. |
| Brain Heart Infusion Agar | General media for most fastidious bacteria. |
| Potato Dextrose Agar | General media for growing most molds. |
| Sabouraud Dextrose Agar | General media for growing yeasts, molds, and other aciduric fungi. |
| Yeast Malt Agar | General media for growing yeasts, molds, and other aciduric fungi. |

## Special Media

In addition to premixed dehydrated media, we use the following media in our laboratories. All are sterilized at a temperature of 121°C and a pressure of 15 pounds per square inch (psi) for 15 minutes. When preparing media, use only distilled water and always use bacteriological grade agar. Note that these are also available as prepared media. For ordering information including item numbers and pricing information, please refer to the most recent *Carolina™ Science* catalog, call toll free 800-334-5551, or visit the Carolina Biological Supply Company Web site at www.carolina.com.

## Recipes for Special Media

### Acetobacter Agar
(For *Acetobacter aceti*)

| | |
|---|---|
| Yeast extract | 5.0 g |
| $CaCO_3$ | 10.0 g |
| Agar | 18.0 g |
| Dextrose | 5.0 g |
| D-Mannitol | 2.0 g |
| Distilled water | 1.0 L |

**Note:** Put in screw-cap tubes only. Tubes must be gently rotated just before slanting to resuspend precipitate.

### Azotobacter Supplemental Agar
(For *Azotobacter chroococcum*)

| | |
|---|---|
| Soil extract | 100 mL |
| *(see **Soil Extract** recipe below)* | |
| Dibasic potassium phosphate | 1.0 g |
| Magnesium sulfate heptahydrate | 0.2 g |
| Sodium chloride | 0.2 g |
| Ferrous sulfate heptahydrate | 0.005 g |
| Mannitol | 20.0 g |
| Distilled water | 900 mL |
| Agar | 20.0 g |

**Note:** Adjust pH to 6.0 using 1 Normal HCl (to lower pH) or 1 Normal KOH (to raise pH) before adding agar.

## Bread Crumb Agar
(For *Armillariella mellea*)

| | |
|---|---|
| Bread crumbs, plain/unflavored | 50 g |
| Agar | 9 g |
| Distilled water | 500 mL |

## Caulobacter Agar
(For *Caulobacter crescentus*)

| | |
|---|---|
| Peptone | 2.0 g |
| Yeast extract | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | 0.2 g |
| Agar | 10.0 g |
| Distilled water | 1.0 L |

## Cornmeal-Dextrose-Peptone
(For *Rhizopus* zygospore formation)

| | |
|---|---|
| Cornmeal agar | 17.1 g |
| Dextrose | 8.0 g |
| Peptone | 1.8 g |
| Distilled water | 1.0 L |

## Enriched Nutrient Agar
(For *Vibrio anguillarum*)

| | |
|---|---|
| Heart infusion broth | 12.5 g |
| Nutrient broth | 5.4 g |
| Yeast extract | 2.5 g |
| Agar | 20.0 g |
| Distilled water | 1.0 L |

## Gluconobacter Agar
(For *Gluconobacter oxydans*)

| | |
|---|---|
| Yeast extract | 5 g |
| Peptone | 3 g |
| Mannitol | 25 g |
| Agar | 20 g |
| Distilled water | 1 L |

## Halobacterium Agar
(For *Halobacterium* sp. NRC-1)

| | |
|---|---|
| Sodium chloride | 250 g |
| Magnesium sulfate, heptahydrate | 20 g |
| Trisodium citrate, dihydrate | 3 g |
| Potassium chloride | 2 g |
| Casamino acids | 5 g |
| Yeast extract | 5 g |
| Deionized water | 1.0 L |

**Note:** Adjust pH to 7.2 using 5 M NaOH or concentrated HCL. After adjusting pH, add:

| | |
|---|---|
| bacteriological grade agar | 20.0 g |

## Honey Peptone Agar
(For *Eurotium chevalieri*)

| | |
|---|---|
| Pure honey (made from clover) | 60.0 g |
| Peptone | 10.0 g |
| Agar | 20.0 g |
| Distilled water | 1.0 L |

## Lactose Agar
(For *Dictyostelium discoideum*)

| | |
|---|---|
| Lactose | 1.0 g |
| Peptone | 1.0 g |
| Agar | 20.0 g |
| Distilled water | 1.0 L |

## Mannitol Special Agar
(For *Azotobacter vinelandii*)

| | |
|---|---|
| Yeast extract | 5.0 g |
| Peptone | 3.0 g |
| Distilled water | 1.0 L |

**Note:** Adjust pH to 7.4 using 1 Normal HCl (to lower pH) or 1 Normal KOH (to raise pH) before adding agar. It takes approximately 3 mL of 1 Normal KOH to adjust 1 L of media. After adjusting pH, add:

| | |
|---|---|
| Mannitol | 25.0 g |
| Agar | 20.0 g |

## Oat Flake Agar
(For *Physarum polycephalum*)

Agar, 1.5% in distilled water
Old-Fashioned Quaker® Oats, 4 cm per tube

**Note:** Cover flakes with 1.5% melted agar. Steam until oat flakes are swollen. With an old inoculating loop handle, form flakes into a slant. Plug with nonabsorbent cotton and autoclave in a slanted position.

## Peptone-Succinate Agar
(For *Spirillum volutans*)

| | |
|---|---|
| $(NH_4)_2SO_4$ | 1.00 g |
| $MgSO_4 \cdot 7H_2O$ | 1.00 g |
| $MnSO_4 \cdot H_2O$ | 2.00 mg |
| $FeCl_3 \cdot 6H_2O$ | 2.00 mg |
| Succinic acid | 1.68 g |
| Peptone | 5.00 g |
| Agar | 1.50 g |
| Distilled water | 1.00 L |

**Note:** Adjust pH to 7.0 with KOH.

## Photobacterium Agar
(For *Vibrio fischeri*)

| | |
|---|---|
| Difco™ Bacto™ tryptone | 5.00 g |
| Yeast extract | 2.50 g |
| Ammonium chloride | 0.30 g |
| Magnesium sulfate | 0.30 g |
| Ferric chloride | 0.01 g |
| Calcium carbonate | 1.00 g |
| Monobasic potassium phosphate | 3.00 g |
| Sodium glycerol phosphate | 23.50 g |
| Sodium chloride | 30.00 g |
| Agar | 20.00 g |
| Distilled water | 1.00 L |

**Note:** Put in screw-cap tubes only. Tubes must be gently rotated just before slanting to resuspend precipitate.

## Rabbit Dung Agar
(For *Pilobolus crystallinus* and *Coprinus cinereus*)

| | |
|---|---|
| Fresh rabbit dung | 500 mL |

**Note:** Place in a dry flask. Autoclave for 30 minutes. Pour 4 to 5 pellets into a 16- × 150-mm test tube and 8 to 10 pellets into a dish, jar, or box. Cover with a thick layer of sterilized 2% agar.

## Rabbit Food Agar
(For *Anthracobia muelleri*)

| | |
|---|---|
| Rabbit food pellets | 25.0 g |
| Agar | 15.0 g |
| Distilled water | 1.0 L |

**Note:** Boil the rabbit food in the distilled water. Let steep 30 minutes. Filter through cheesecloth. Add agar to filtrate.

## Rhizobium-X Agar
(For *Rhizobium leguminosarum*)

| | |
|---|---|
| Soil extract | 200 mL |
| *(see **Soil Extract** recipe below)* | |
| Yeast extract | 1 g |
| Mannitol | 10 g |
| Distilled water | 800 mL |
| Agar | 20 g |

**Note:** Adjust pH to 7.2 using 1 Normal HCl (to lower pH) or 1 Normal KOH (to raise pH) before adding agar.

## S-6 Broth
(For *Thiobacillus thioparus*)

| | |
|---|---|
| $Na_2HPO_4$ | 1.20 g |
| $KH_2PO_4$ | 1.80 g |
| $MgSO_4 \cdot 7H_2O$ | 0.10 g |
| $(NH_4)_2SO_4$ | 0.10 g |
| $CaCl_2$ | 0.03 g |
| $FeCl_3$ | 0.02 g |
| $MnSO_4$ | 0.02 g |
| $Na_2S_2O_3$ | 10.00 g |
| Distilled water | 1.00 L |

## Soil Extract
(For Azobacter Supplemental Agar and Rhizobium-X Agar)

| | |
|---|---|
| Hyponex® African violet soil | 77.0 g |
| Sodium carbonate | 0.2 g |
| Distilled water | 200.0 mL |

**Note:** Autoclave for 60 minutes. Filter through 6 layers of cheesecloth.

## Sordaria Crossing Agar
(For *Sordaria fimicola*)

| | |
|---|---|
| Cornmeal agar | 17.0 g |
| Sucrose | 10.0 g |
| Dextrose | 7.0 g |
| $KH_2PO_4$ | 0.1 g |
| Yeast extract | 1.0 g |
| Distilled water | 1.0 L |

## Sordaria Culture Agar
(For *Sordaria fimicola*)

| | |
|---|---|
| Cornmeal agar | 17.0 g |
| Dextrose (glucose) | 2.0 g |
| Yeast extract | 1.0 g |
| Distilled water | 1.0 L |

## Tomato Juice-Yeast Extract-Milk Medium
(For *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactococcus lactis*)

**Note:** Filter the juice off canned whole tomatoes. Adjust the pH to 7.0 using 1 Normal HCl (to lower pH) or 1 Normal KOH (to raise pH). Tomato juice is typically pH 4.5.

| | |
|---|---|
| Low-fat skim milk | 100 g |
| Tomato juice | 1 L |
| Yeast extract | 5 g |
| Distilled water | 900 mL |

**Note:** Autoclave at 113°C for 20 min. This will keep the milk sugars from carmelizing.

## V8® Vegetable Juice Agar
(For *Chaetomium globosum*)

| | |
|---|---|
| V8® vegetable juice | 200.0 mL |
| $CaCO_3$ | 3.0 g |
| Agar | 20.0 g |
| Distilled water | 1.0 L |

**Note:** Add a strip of sterile filter paper to agar after inoculation.

# Further Reading

Alexopoulos, C.J., and C.W. Mims. 1979. *Introductory Mycology*, 3rd edition. John Wiley and Sons, New York.

Buchanan, R.E., and N.E. Gibbons, eds. 1974. *Bergey's Manual of Determinative Bacteriology*, 8th edition. The Williams and Wilkins Company, Baltimore.

Mycology Guidebook Committee, Mycological Society of America, Stevens, R.B., ed. 1981. *Mycology Guidebook*. University of Washington Press, Seattle.

Pelczar, M.J., Jr., and E.C.S. Chan. 1981. *Elements of Microbiology*. McGraw-Hill Book Company, New York.

Singleton, P., and D. Sainsbury. 1978. *Dictionary of Microbiology*. John Wiley and Sons, Chicester.

**Carolina Biological Supply Company**
2700 York Road, Burlington, North Carolina 27215
Phone: 800.334.5551 • Fax: 800.222.7112
Technical Support: 800.227.1150 • www.carolina.com
CB160680611