Survey of Prokaryotes

Kingdoms Archaebacteria and Bacteria

Learning Objectives

By the end of this exercise you should be able to:

1. Describe distinguishing features of members of kingdoms Archaebacteria and Bacteria.
2. Describe the major differences between bacteria and cyanobacteria.
3. Identify representative examples of archaebacteria, bacteria, and cyanobacteria.
4. Perform a Gram stain.

MULTI-MEDIA Please visit www.mhhe.com/vodopich10e to review multi-media resources tailored to this lab.

Cellular organisms have evolved along two lines. Species with cells lacking membrane-bound organelles are **prokaryotes** (table 24.1). Those with membrane-bound organelles are **eukaryotes** and include plants, animals, fungi, and protists. About 5000 species of prokaryotes have been described, and many more await identification and description.

Prokaryotes were long thought to be a unified group commonly called bacteria. However, genetic analysis as recently as 1996 of the DNA of prokaryotes revealed two groups with surprisingly different DNA sequences. Both were strikingly different from the DNA sequences of eukaryotes. This has led to recognition of three **domains** of organisms (fig. 24.1).

Domain Archaea includes **kingdom Archaebacteria,** which are all prokaryotes. Archaebacteria often inhabit but are not restricted to extreme and stressful environments on Earth. **Domain Bacteria** includes **kingdom Bacteria,** which are all prokaryotes and the most abundant organisms on Earth (fig. 24.2). **Domain Eukarya** includes **kingdoms Fungi, Plantae, Animalia,** and the polyphyletic (multiple origins) **protists** (see Exercise 25 for the current status of protistan classification). All of the kingdoms in domain Eukarya are eukaryotes and are described in Exercises 25–31 and 36–40. This classification of living organisms into three domains and five kingdoms is now widely accepted, but much phylogenetic information remains to be revealed. Classification is an exciting and ongoing process.

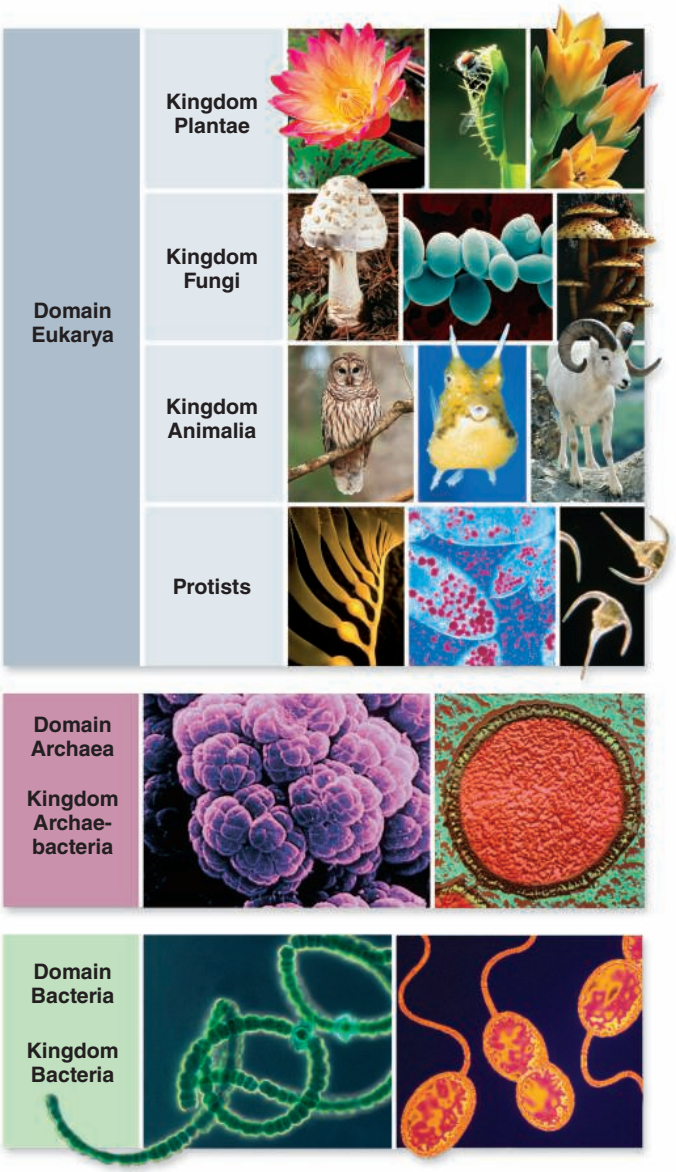
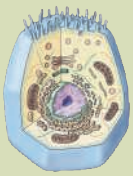
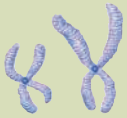
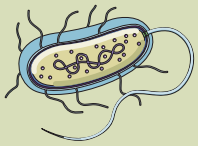
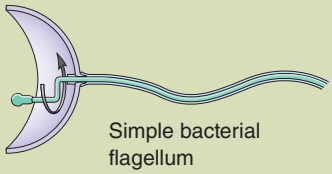
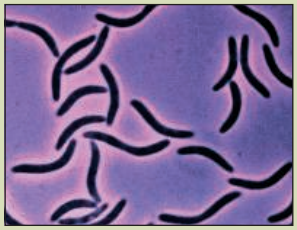


Figure 24.1
The diversity of life. Biologists categorize all living things into three overarching groups called domains: Bacteria, Archaea, and Eukarya. Domain Eukarya is composed of protists and three kingdoms: Plantae, Fungi, and Animalia.

Table 24.1

Prokaryotes Compared to Eukaryotes

Feature	Example
Unicellularity. All prokaryotes are basically single-celled. Even though some bacteria may adhere together or form filaments, their cytoplasm is not directly interconnected, and their activities are not integrated and coordinated as is the case in multicellular eukaryotes.	 Prokaryotic cell
Cell Size. Most bacterial cells are only about 1 micrometer in diameter, while most eukaryotic cells are over 10 times that size.	 Bacterial cell  Eukaryotic cell
Chromosomes. Prokaryotic DNA exists as a single circle in the cytoplasm, while in eukaryotes, proteins are complexed with the DNA into multiple chromosomes.	 Bacterial genome  Eukaryotic chromosomes
Cell Division. Prokaryotic cells divide by binary fission. The cells pinch in two. In eukaryotes, microtubules pull chromosomes to opposite poles during the cell division process, called mitosis.	 Binary fission in bacteria  Mitosis in eukaryotes
Internal Compartmentalization. Unlike eukaryotic cells, bacterial cells contain no internal compartments, no internal membrane system, and no cell nucleus.	 Bacterial cell
Flagella. Prokaryotic flagella are simple, composed of a single fiber of protein that spins like a propeller. Flagella in eukaryotes are complex structures that whip back and forth, rather than rotating.	 Simple bacterial flagellum
Metabolic Diversity. Prokaryotes possess many metabolic abilities that eukaryotes do not; some prokaryotes can perform several different kinds of anaerobic and aerobic photosynthesis, obtain their energy from oxidizing inorganic compounds, or fix atmospheric nitrogen.	 Chemoautotrophs

KINGDOM ARCHAEBACTERIA

Archaebacteria of domain Archaea may be the oldest forms of life on earth, and domains Bacteria and Eukarya probably diverged from Archaebacteria independently. Archaebacteria are diverse prokaryotes that share ribosomal RNA sequences as well as several important biochemical characteristics quite distinctive from those of all other types of organisms. Archaebacteria are significantly different from the prokaryotes of kingdom Bacteria. Archaebacteria have distinctive membranes, unusual cell walls, and unique metabolic cofactors.

Today's Archaebacteria are probably survivors of ancient lines that have persisted in habitats similar to those present when bacteria first evolved. These habitats are often extremely acidic, hot, or salty. Thus, many Archaebacteria are called **extremophiles.** Many Archaebacteria can live in an anaerobic atmosphere rich in carbon dioxide and hydrogen as well as the more benign environments typical of bacteria and eukaryotes. See Exercise 16 for a procedure describing *Halobacterium salinarum,* a common archaebacterium that inhabits salty environments.

KINGDOM BACTERIA

Bacteria of kingdom Bacteria are distributed more widely than any other group of organisms. Individual bacterial cells are microscopic (1 μm or less in diameter, figs. 24.2 and 24.3); a single gram of soil may contain over a billion bacteria. Bacteria have cell walls, which give them three characteristic shapes (fig. 24.4).

- Bacillus (rod-shaped)
- Coccus (spherical)
- Spirillum (spiral)

Most bacteria are **heterotrophic,** meaning that they derive their energy from organic molecules made by other organisms. Heterotrophic bacteria are **decomposers** because they feed on dead organic matter and release nutrients locked in dead tissue.

Question 1

a. Why is it important that decomposers such as bacteria release nutrients?

b. What term best describes heterotrophic bacteria that feed on living tissue?

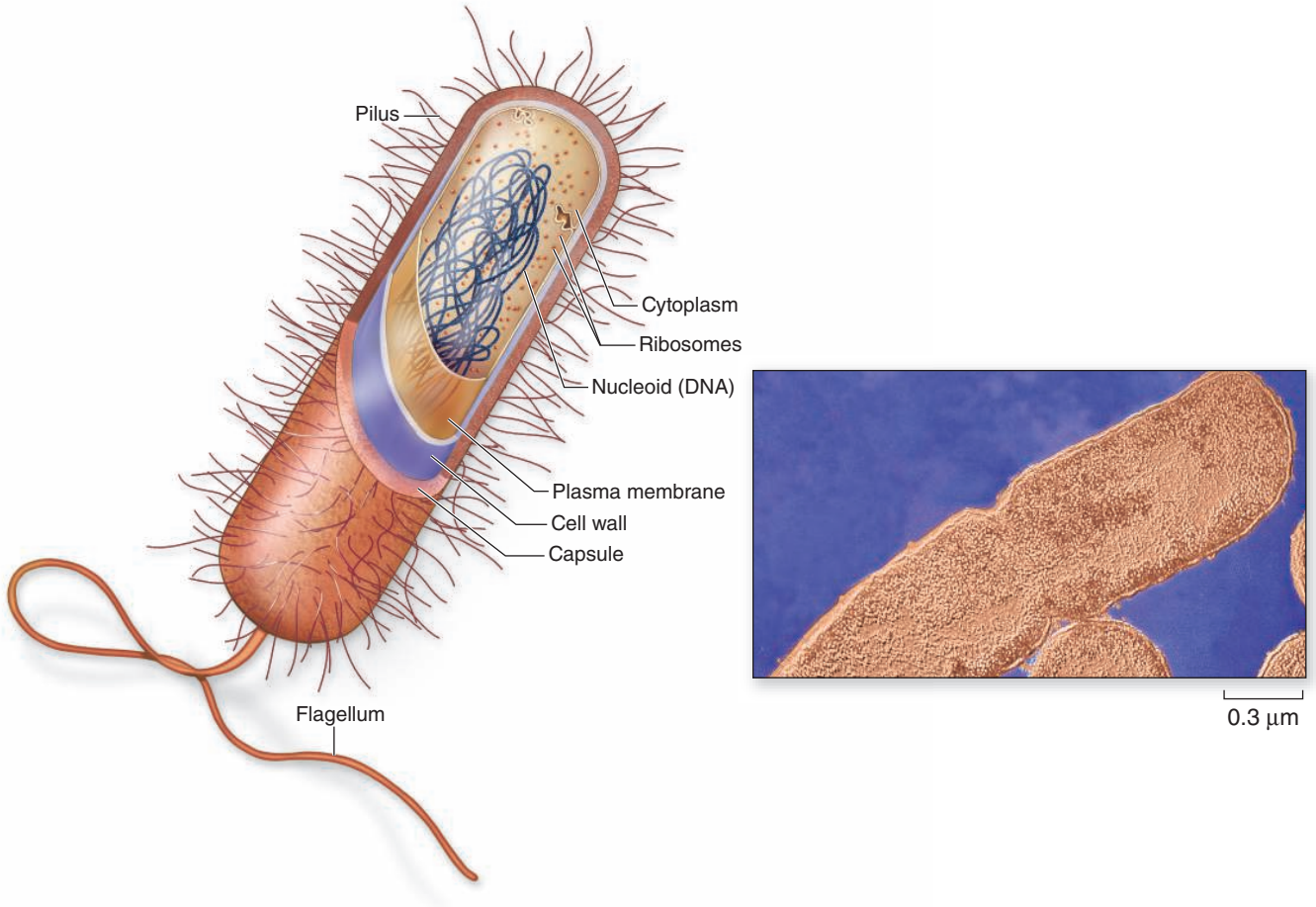


Figure 24.2
The structure of a bacterial cell.

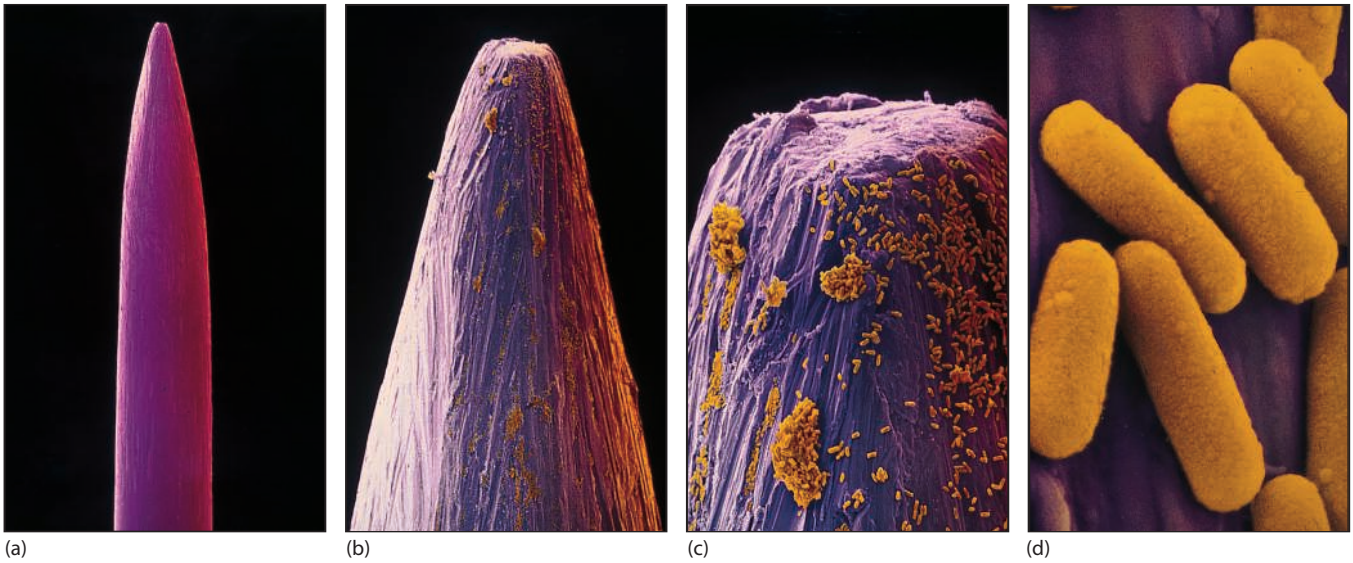


Figure 24.3
Four views of a contaminated pin, which would seem an unlikely site for bacteria to grow. (*a*) The tip of the pin, magnified 7×. When scanning electron micrographs are shown at increasing magnifications—(*b*) 35×, (*c*) 178×, and (*d*) 4375×—you see rod-shaped bacteria growing there.

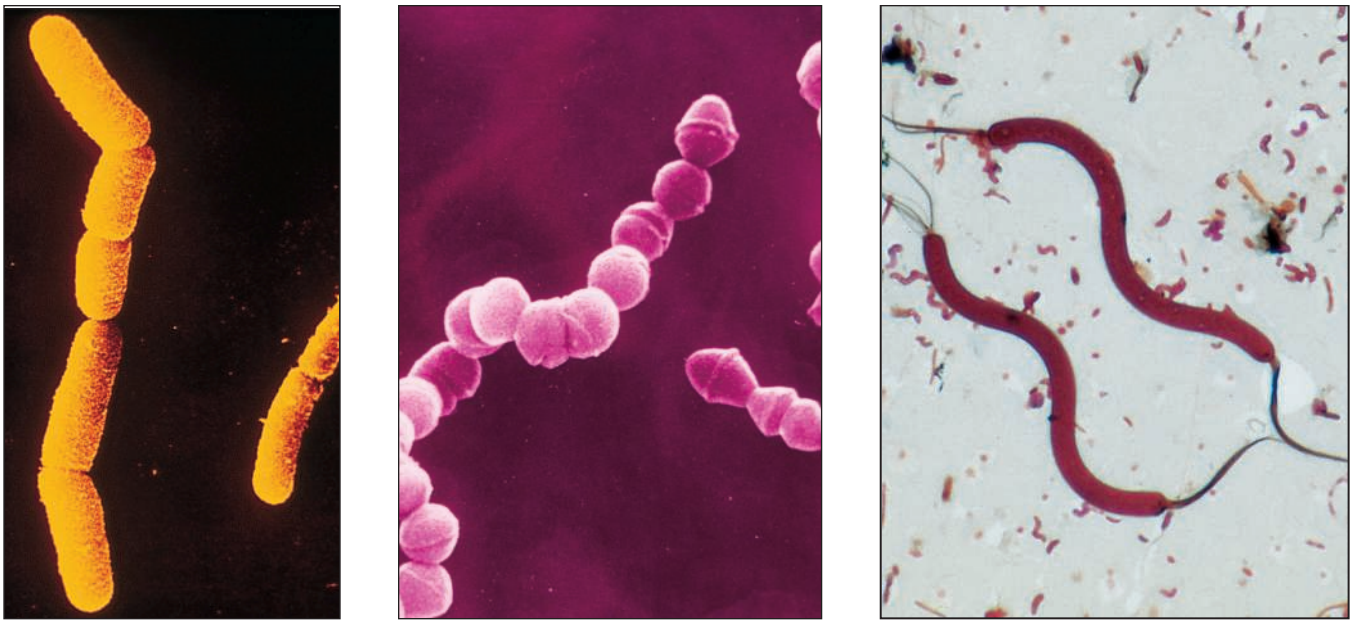


Figure 24.4
The three basic shapes of bacteria: (*a*) bacillus (*Pseudomonas*); (*b*) coccus (*Streptococcus*); and (*c*) spirillum (*Spirilla*), 400×.

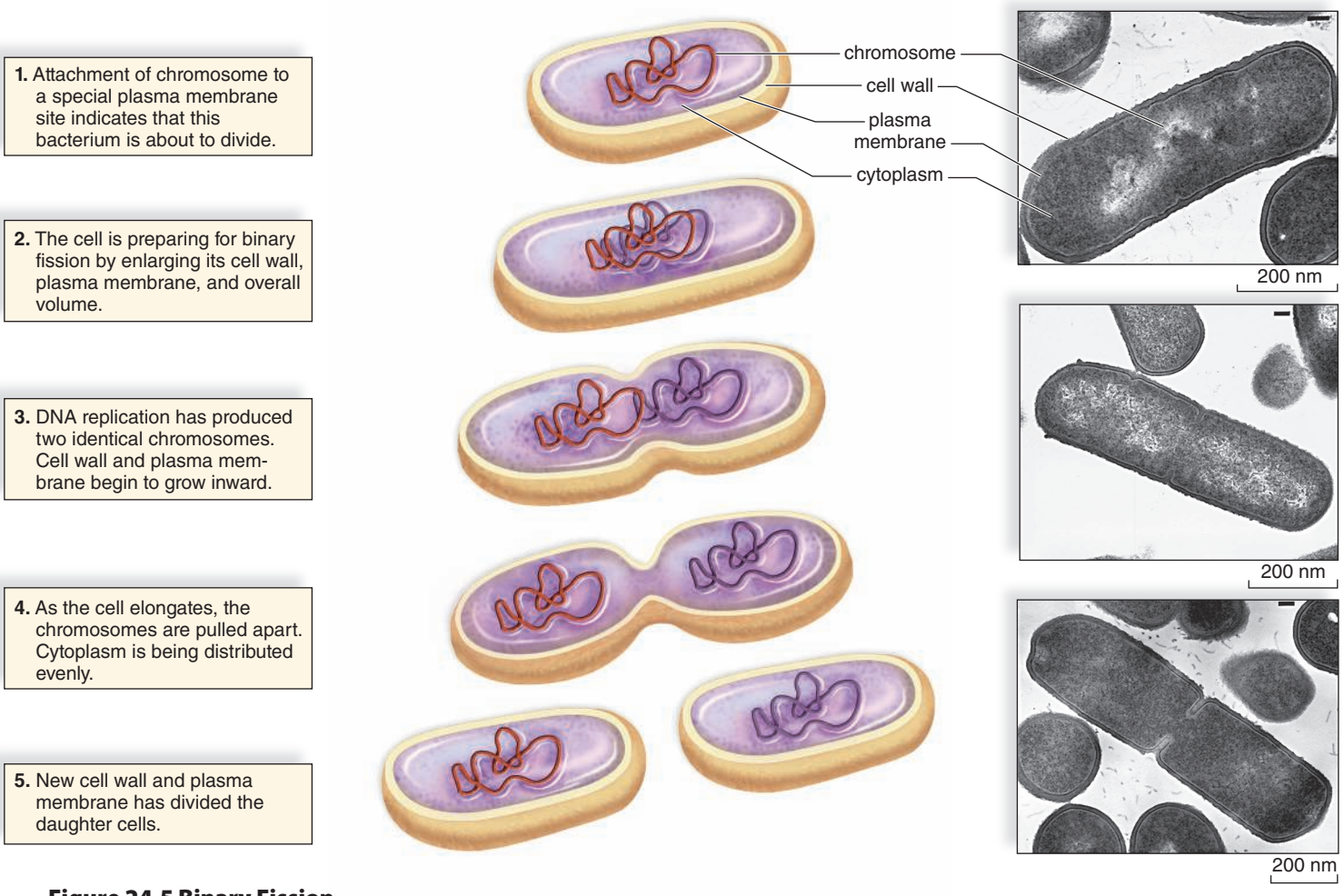


Figure 24.5 Binary Fission
First, DNA replicates, and as the cell lengthens, the two chromosomes separate, and the cells become divided. The two resulting bacteria are identical.

Table 24.2

Important Bacterial Diseases That Affect Humans

Disease	Pathogen	Vector/Reservoir	Epidemiology
Anthrax	*Bacillus anthracis*	Animals, including processed skins	Bacterial infection that can be transmitted through contact or ingested. Rare except in sporadic outbreaks. May be fatal.
Botulism	*Clostridium botulinum*	Improperly prepared food	Contracted through ingestion or contact with wound. Produces acute toxic poison; can be fatal.
Chlamydia	*Chlamydia trachomatis*	Humans, STD	Urogenital infections with possible spread to eyes and respiratory tract. Occurs worldwide; increasingly common over past 30 years.
Cholera	*Vibrio cholerae*	Human feces, plankton	Causes severe diarrhea that can lead to death by dehydration; 50% peak mortality if the disease goes untreated. A major killer in times of crowding and poor sanitation; over 100,000 died in Rwanda in 1994 during a cholera outbreak.
Dental cavities	*Streptococcus*	Humans	A dense collection of this bacteria on the surface of teeth leads to secretion of acids that destroy minerals in tooth enamel—sugar alone will not cause cavities.
Gonorrhea	*Neisseria gonorrhoeae*	Humans only	STD on the increase worldwide. Usually not fatal.
Hansen's disease (leprosy)	*Mycobacterium leprae*	Humans, feral armadillos	Chronic infection of the skin; worldwide incidence about 10–12 million, especially in Southeast Asia. Spread through contact with infected individuals.
Lyme disease	*Borrelia bergdorferi*	Ticks, deer, small rodents	Spread through bite of infected tick. Lesion followed by malaise, fever, fatigue, pain, stiff neck, and headache.
Peptic ulcers	*Helicobacter pylori*	Humans	Infects the stomach, where it causes ulcers. About 40% of the world's population harbors *H. pylori*. Barry Marshall, who isolated the bacterium in 1982, drank a culture of *H. pylori*; he got an ulcer.
Plague	*Yersinia pestis*	Fleas of wild rodents: rats and squirrels	Killed ¼ of the population of Europe in the 14th century; endemic in wild rodent populations of the western United States today.
Pneumonia	*Streptococcus, Mycoplasma, Chlamydia*	Humans	Acute infection of the lungs, often fatal without treatment.
Tuberculosis	*Mycobacterium tuberculosis*	Humans	An acute bacterial infection of the lungs, lymph, and meninges. Its incidence is on the rise, complicated by the development of new strains of the bacteria that are resistant to antibiotics.
Typhus	*Rickettsia typhi*	Lice, rat fleas, humans	Historically a major killer in times of crowding and poor sanitation; transmitted from human to human through the bite of infected lice and fleas. Typhus has a peak untreated mortality rate of 70%.

Bacteria that derive their energy from photosynthesis or the oxidation of inorganic molecules are **autotrophic.** However, photosynthesis in bacteria is often different from that in eukaryotes, because molecular sulfur rather than oxygen is sometimes produced as a by-product.

A laboratory culture of bacteria usually consists of a tube of liquid nutrients (broth) containing growing bacteria or a tube or plate of solidified agar with bacteria growing on the surface.[1] The jellylike agar is melted, mixed with nutrients, and poured into tubes or plates to solidify. Many species of bacteria can be cultured in nutrient broth or on a layer of nutrient-rich agar. It may surprise you to know that most species of bacteria are *not* culturable in vitro. We just don't know enough about the nutrient and environmental requirements of these bacteria to grow them in the lab.

Bacteria reproduce asexually via **binary fission,** in which a cell's DNA replicates and the cell pinches in half without the nuclear and chromosomal events associated with mitosis (see Exercise 14) (fig. 24.5). Some bacteria have genetic recombination via **conjugation,** in which all or part of the genetic material of one bacterium is transferred to another bacterium and a new set of genes is assembled.

Some bacteria are pathogenic (table 24.2); that is, they cause diseases such as pneumonia and tuberculosis. However, most bacteria are harmless to humans. Indeed, many beneficial bacteria live in and on your body. Nevertheless, you should handle all bacterial cultures with

1 *Agar is a gelatinous polysaccharide used in culture media for microbiology labs. You'll learn more about agar and the red algae it comes from in Exercise 25.*

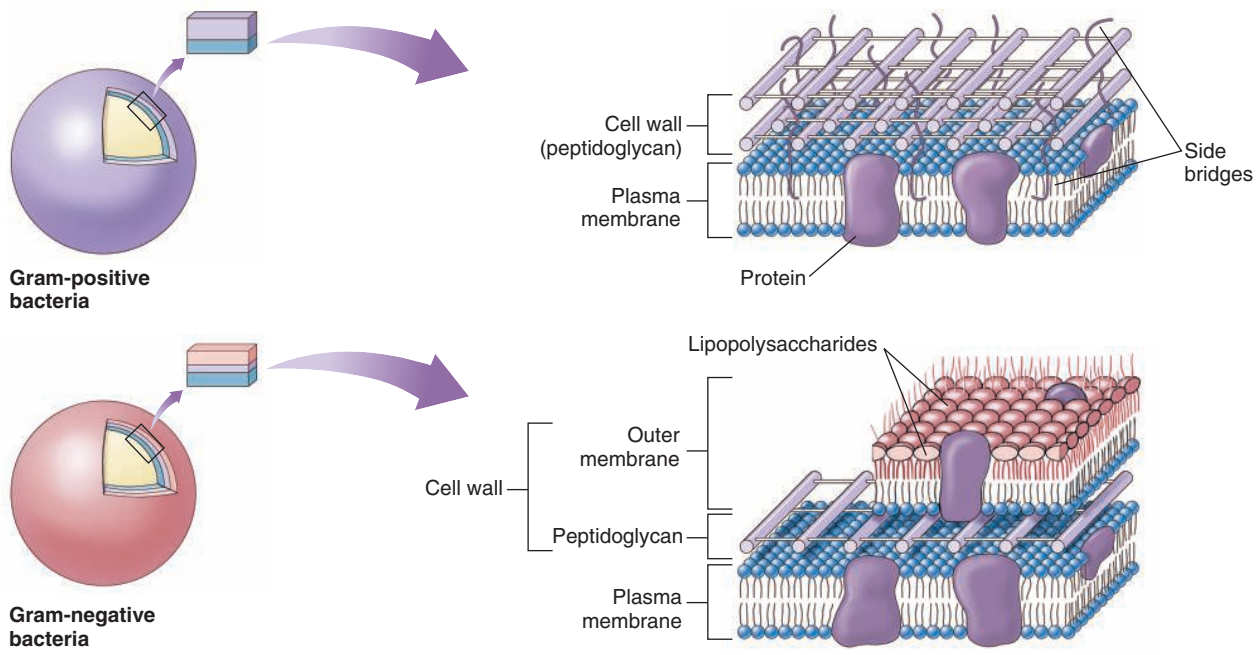


Figure 24.6

Gram stain technique. The surface of Gram-positive bacteria traps crystal violet dye in its peptidoglycan layer, so the bacteria stain purple in a Gram-stained smear (named after Hans Christian Gram, who developed the technique). Gram-negative bacteria have much less peptidoglycan, and the layer is beneath an outer membrane rich with lipopolysaccharides. The crystal violet doesn't stick to these bacteria, and therefore Gram-negative bacteria appear red from the red safranin background stain.

care. The preparation of wet mounts of bacterial cultures requires proper use of a transfer loop and sterilizing flame. Your instructor will demonstrate this aseptic technique.

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 24.1

Culture common bacteria

1. Obtain a sterile cotton swab and a closed petri dish containing sterile nutrient agar.
2. Open the packaged swab and drag the tip over a surface such as your teeth, face, or tabletop.
3. Open the petri dish and drag the exposed swab over the surface of the agar in the manner demonstrated by your instructor.
4. Close the lid and tape it shut. Label the dish with a wax pencil.
5. Turn the dish upside down and place it in the incubator or in a warm area.
6. After 24–48 h examine the agar for bacterial growth. Record your observations.

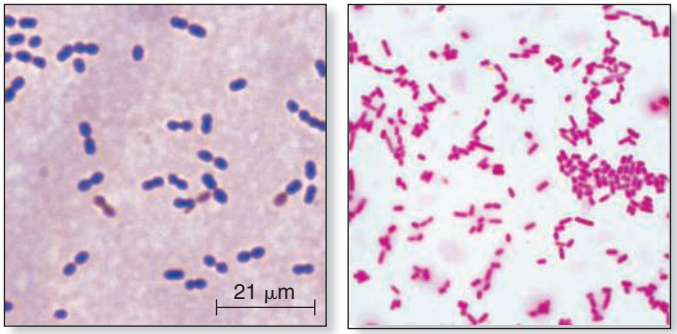
Question 2

What is the shape and size of each bacterial colony?

Gram Stain

One of the most important techniques to classify bacteria is the **Gram stain,** based on the different structural and chemical compositions of bacterial cell walls (fig. 24.6). Gram staining is important because it often correlates with the sensitivity of a bacterium to antibiotics. **Gram-positive** bacteria (e.g., *Streptococcus, Micrococcus*) have a thick cell wall that retains a purple dye, whereas **Gram-negative** (e.g., *Escherichia coli, Serratia*) bacteria have a much thinner cell wall that does not retain the dye.

During the Gram stain technique, crystal violet and iodine are applied to stain all of the bacteria purple. Then alcohol is used to remove the stain from the surface of the Gram-negative cell walls that do not bind the stain. Finally, safranin is used to counterstain the Gram-negative cells with a red color contrasting to purple Gram-positive cells (fig. 24.7). In the following procedure, you will Gram-stain



(a) Gram-positive bacteria (b) Gram-negative bacteria

Figure 24.7

Gram-positive and Gram-negative bacteria. (*a*) *Streptococcus pneumoniae* stains positive (purple) with the Gram stain. (*b*) *Escherichia coli* stains negative (pink) when the Gram-stain procedure is applied.

some of your bacteria to see the difference between Gram-negative and Gram-positive organisms.

Procedure 24.2

Observe stained bacteria with oil-immersion magnification

1. Obtain a microscope and a small bottle of immersion oil. Recall from Exercise 3 that the resolving power of a lens depends, among other things, on the amount of light that it gathers. More light improves resolution, and light is scattered when it passes through air. If a drop of immersion oil, a fluid with the same refractive index (ability to bend light) as glass, is placed between the objective lens and the specimen, then the lens can gather more light.
2. Examine the microscope, and verify with your instructor that the microscope is equipped with an oil-immersion objective. This objective can resolve micrometer-sized particles such as bacteria.
3. Rotate the low-power objective into observation position.
4. Obtain some prepared slides of stained bacteria from your instructor. These may be commercially prepared slides or slides with bacteria that your instructor has stained to help you practice with your microscope.
5. Place a slide on the stage with the specimen centered over the light path through the hole in the stage.
6. While watching from the side, slowly rotate the low-power objective as close as possible to the slide without the objective touching the slide. Adjust the diaphragm for medium light-intensity.
7. Look through the oculars and slowly adjust the coarse adjustment to increase the working distance. Stop when you see the color of the stained bacteria and are roughly focused on the smear of bacteria.
8. Improve the illumination and sharpen the image as much as possible with the fine-adjustment knob. At this low magnification you will only see small dots, at best.
9. Rotate a higher-power objective into position and refocus.
10. Rotate the nosepiece so that the alignment is halfway between the oil-immersion objective and the next lowest-power objective. There should not be an objective in correct position for observation. This position will allow you to place a drop of oil on the slide.
11. Put one drop of immersion oil on the coverslip directly over the spot of the light path. Do not touch the dropper to the slide or it will contaminate the oil when the dropper is returned to the bottle.
12. Rotate the oil-immersion lens directly into observation position and directly into the drop of oil.
13. While looking from the side, use the fine-adjustment knob to lower the objective until it *gently* touches the coverslip.
14. Look through the oculars and *slowly* rotate the fine-adjustment knob to increase the working distance. This rotation should be counterclockwise. Stop when the stained bacterial color appears. Slowly rotate the fine-adjustment knob back and forth until the bacteria are in focus.
15. Improve your resolution by adjusting the diaphragm.
16. Examine the sizes, shapes, and stains of the bacteria on the slide.
17. Repeat this entire procedure for each of the slides offered by your instructor.
18. When you finish your work, clean the oil from the slides and objectives with the lens paper provided.

Procedure 24.3

Use known bacterial cultures to prepare and observe a Gram stain

1. Obtain a slide, coverslip, transfer loop, alcohol burner, and a culture of living bacteria.
2. Available cultures should include the following bacteria, among others:

   Bacillus megaterium—a large bacterium resistant to radiation, desiccation, and heat.

   Rhodospirillum rubrum—a photosynthetic purple bacterium.

   Escherichia coli—found in the human intestine; the most intensively studied of all bacteria.

   Staphylococcus epidermidis—a cocci found among the normal flora of skin.

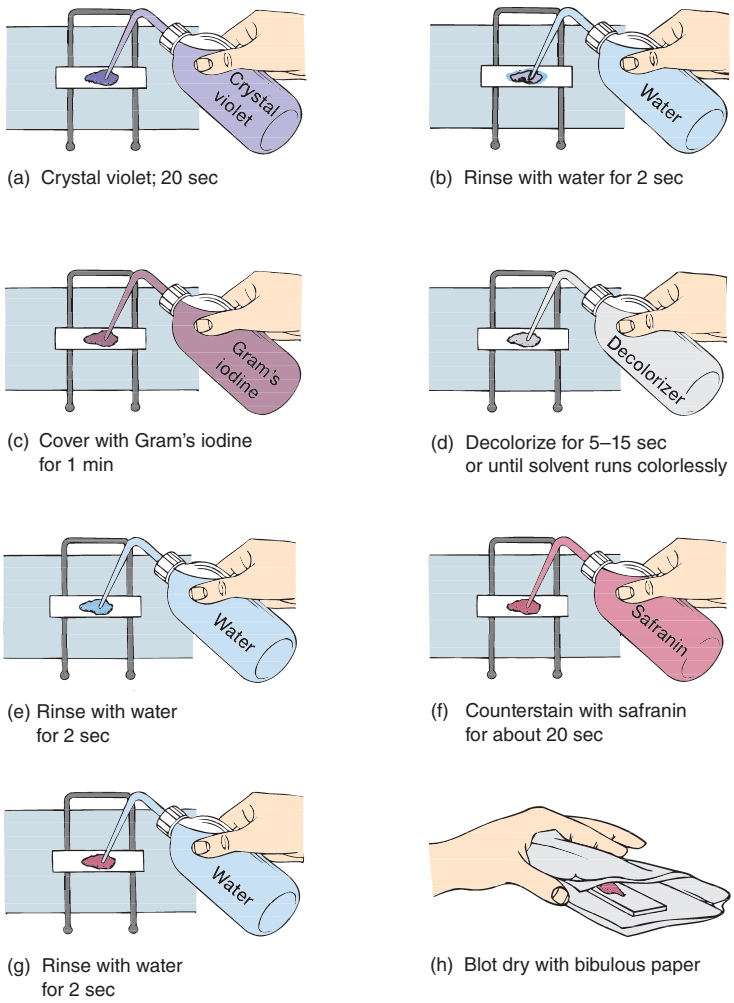


Figure 24.8
Gram staining procedure.

3. Apply a loop of bacteria to a drop of water on a slide. If your cultures are in liquid broth, then add one drop of culture medium to your slide. Your instructor will demonstrate how to use sterile technique to open, sample, and close the culture of bacteria. Do not add a coverslip to the slide.
4. Heat the slide gently by holding it with a clothespin and passing it over the top of a flame three to four times. Drying time is critical to success. Check with your instructor to avoid heating the slide too little or too much. Be careful not to break the slide. If hot plates are available, hold the slide to the hot surface for 10 sec. This heat will adhere the bacteria to the slide.

Be careful not to inhale or spill on your skin crystal violet or any other biological stain used in the following steps.

5. Examine figure 24.8 and become familiar with the staining procedure. When the slide has cooled, gently drench the bacterial smear with drops of crystal violet for 20 sec.
6. Rinse the slide for 2 sec with a gentle but steady stream of water from a squirt bottle.
7. Gently drench the bacterial smear with drops of iodine for 1 min.
8. Drop 95% alcohol (decolorizer) on the smear with an eyedropper until no purple shows in the alcohol coming off the slide. Quickly rinse the slide with water to remove the alcohol.
9. Gently drench the bacterial smear with drops of safranin for 20 sec.
10. Gently rinse the slide with water. Air dry the slide, or blot gently if necessary. Add a coverslip.
11. Observe the smear with your microscope using low power and then high power and/or the oil-

Deadly Food! Beware!

The bacterium *Clostridium botulinum* can grow in food products and produces a toxin called botulinum, the most toxic substance known. Microbiologists estimate that 1 gram of this toxin can kill 14 million adults! The good news is that *C. botulinum* requires anaerobic conditions for growth, which limits its prevalence. The bad news is that *C. botulinum* is extremely tolerant to stress; it can withstand boiling water (100°C) for short periods, but is killed at 120°C in 5 min. This tolerance makes *C. botulinum* a serious concern when people can vegetables. If home canning is not done properly, this bacterium will grow in the anaerobic conditions of the sealed container and be extremely poisonous. Several adults and infants die every year from botulism in the United States.

Tolerance to stress is enhanced in *C. botulinum* and many other bacteria by the formation of thick-walled **endospores** that surround their chromosome and a small portion of the surrounding cytoplasm. These highly resistant endospores (fig. 24.A) may later germinate and grow after decades or even centuries of inactivity. The endospores of *Clostridium botulinum* can germinate in poorly prepared canned goods, so never eat food from a swollen (gas-filled) can of food; you risk contracting botulism leading to nerve paralysis, severe vomiting, and death.

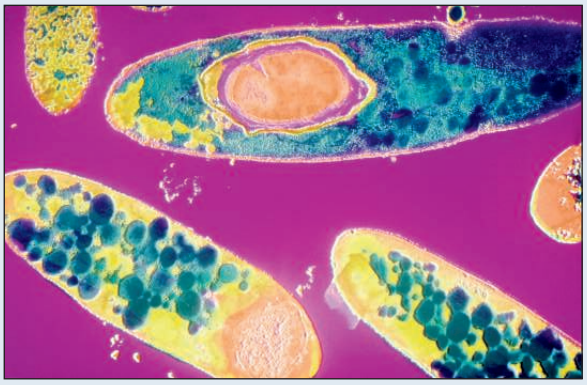


Figure 24.A

Endospores. The round orange circle in the upper cell is an endospore forming within a cell of *Clostridium botulinum*, the bacterium that causes the disease botulism. These resistant endospores enable the bacterium to survive in improperly sterilized canned and bottled foods.

Table 24.3

The Relative Size and Shape of Some Common Bacteria

Bacterial Species	Gram Stain (+/−)	Relative Size	Shape

immersion objective. You will not need a coverslip for heat-fixed slides with oil immersion.

12. Determine if the bacteria are Gram positive or Gram negative.

13. Repeat the Gram-stain procedure using other known bacteria that you obtain from culture tubes.

14. Record your observations in table 24.3.

Procedure 24.4

Use a Gram stain to observe living bacteria from your teeth

1. Obtain a slide, coverslip, transfer loop, alcohol burner, and a culture of living bacteria.

2. Use the wide end of a toothpick to scrape your teeth near the gum line.

3. Thoroughly mix what's on the tip of the toothpick in a small drop of water on a microscope slide.

4. Allow this bacterial smear to dry.

5. Repeat steps 4–13 of procedure 24.3.

Question 3

a. Which type of bacteria is most prevalent in the sample from your teeth?

Table 24.4

An Evaluation of Bacterial Colony Morphology

Plate ID	Species	Colony Diameter (mm)	Color	Form	Elevation	Margin
A						
B						
C						
D						
E						
F						

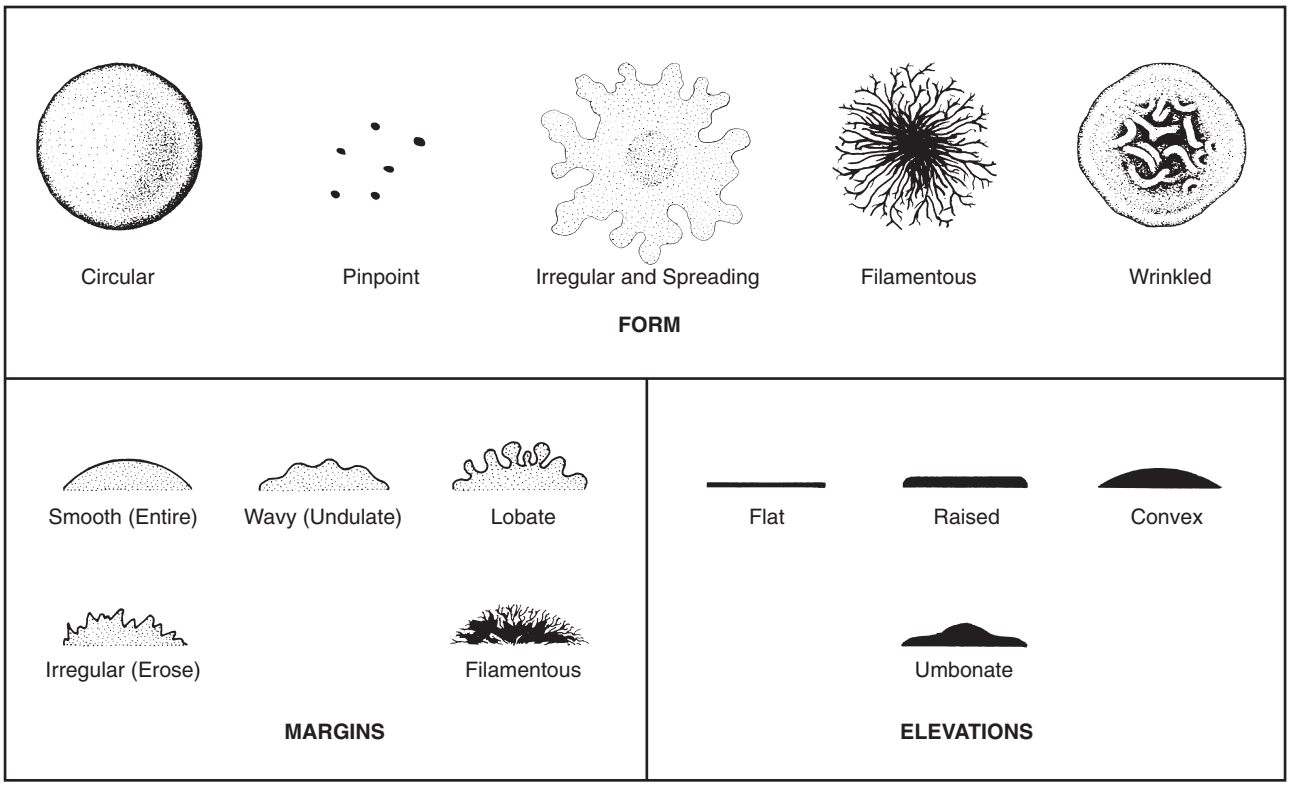


Figure 24.9
Colony characteristics. Colonies are classified by form, margin, and elevation as well as color and diameter.

Figure 24.10
Root nodules containing nitrogen-fixing bacteria.

b. Is *Bacillus megaterium* Gram positive or Gram negative? How do you know?

Bacterial Colony Morphology

A bacterial **colony** is a visible speck or patch of millions of bacterial cells that are typically the progeny of a single cell that reproduced on the agar's surface. A bacterial colony growing on the surface of nutrient agar often has distinctive characteristics depending on the species. Careful observation of the shape, color, size, texture, and margins of a colony is important for any bacterial identification. Figure 24.9 illustrates the most common features of bacterial colony morphology.

Procedure 24.5

Evaluate the colony morphology of bacterial species

1. Obtain 48-h growth plates of 4–6 bacterial species provided by your instructor for your evaluation.

2. Familiarize yourself with the colony morphologies shown in figure 24.9.

3. Use a stereomicroscope or hand lens to evaluate a representative colony on each of the plates. Record your observations in table 24.4.

4. When you have completed your analysis, your instructor will provide the species names associated with each of the plates and the accepted colony descriptions for that species. Use this information to evaluate the accuracy of your observations.

Nitrogen Fixation by Bacteria

Certain bacteria and cyanobacteria transform atmospheric nitrogen (N_2) into other nitrogenous compounds that can be used as nutrients by plants. This process is called **nitrogen fixation.** All organisms need nitrogen as a component of their nucleic acids, proteins, and amino acids. However, chemical reactions capable of breaking the strong triple bond between atoms of atmospheric nitrogen are limited to certain bacteria and cyanobacteria. This process uses an enzyme called nitrogenase along with ATP, energized electrons, and water to convert N_2 to ammonia (NH_3). Ammonia can be absorbed by plants and used to make proteins and other macromolecules.

Rhizobium is a bacterium that can fix nitrogen and can grow intimately with roots of some plants called legumes (e.g., clover, alfalfa, and soybeans). Such associations between *Rhizobium* and host roots form **nodules** on the roots (fig. 24.10). These resident nitrogen fixers provide ammonia to the plant while the plant provides sugars and other nutrients to the bacteria.

Procedure 24.6

Observe root nodules

1. Observe the root systems on display and note the nodules.

2. Examine a prepared slide of a cross section of a nodule.

Question 4

a. Where are the bacteria? Are they between cells or inside cells?

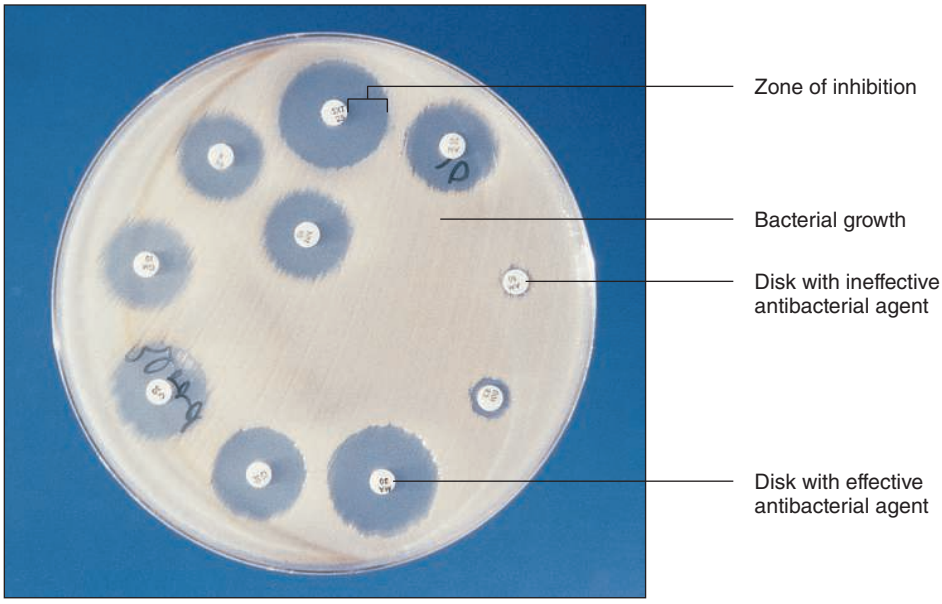


Figure 24.11

A sensitivity plate is used to determine the effectiveness of one or more antibiotics, each of which is on the surface of a paper disk. Any disk containing an effective antibacterial agent inhibits nearby bacterial growth on the agar. If the agent is ineffective, then bacteria will grow up to the disk.

Table 24.5

Inhibition of Four Bacterial Species by Various Growth Inhibitors

Antibiotic/Antiseptic	Plate 1 _____	Plate 2 _____	Plate 3 _____	Plate 4 _____

b. Why is this relationship between a plant and bacterium called a mutualism?

c. How does *Rhizobium* benefit from this association?

d. How does the host plant benefit from the association?

Bacterial Sensitivity to Inhibitors

Growth of some bacterial species is more sensitive to inhibitors such as antibiotics than is growth of other species. For example, an antibiotic may be more effective against *Staphylococcus* than against *Streptococcus.* This is important information to a physician who must select one of many available antibiotics to treat a bacterial infection.

To determine the most effective antibiotic, a medical laboratory may set up a **sensitivity plate.** A sensitivity plate is a petri dish of solid medium that has been uniformly inoculated on its entire surface with a known bacterium or an unknown sample from an infected patient. After inoculation, four to eight small paper disks—each soaked in a different antibiotic—are placed equidistant from each other on the culture surface. After 24 h, an effective antibiotic will produce a visible halo of clear surface around the disks where it inhibited growth of the bacteria (fig. 24.11). If the antibiotic was ineffective, the bacteria will grow to the edge of the paper disk.

You've probably seen television commercials for products such as mouthwash or disinfectant that "kills germs on contact." Many of these products are developed and tested using sensitivity plates. The mouthwash is effective if no bacteria grows around a paper disk soaked in the mouthwash.

Procedure 24.7

Examine sensitivity plates

1. Obtain from your instructor one of each type of prepared sensitivity plate.

2. Examine each plate, note the bacteria used to inoculate the plate, and note the types of disks distributed on the plate.

3. Determine which disks inhibited bacterial growth strongly, weakly, or not at all.

4. Record the bacterial species and your observations in table 24.5.

Question 5
Based on their appearance, which drugs or chemicals inhibit the growth of bacteria?

Cyanobacteria (Blue-Green Algae)

Cyanobacteria are a major group of photosynthetic bacteria that grow in many environments. Most cyanobacteria are free-living, whereas others live symbiotically with plants and other organisms. Cyanobacteria are photosynthetic, and their pigments include **chlorophyll *a*** and the accessory pigments **phycocyanin** (blue) and **phycoerythrin** (red). Because of various proportions of these pigments, only about half of the cyanobacteria are blue-green in color; other cyanobacteria range in color from brown to olive green.

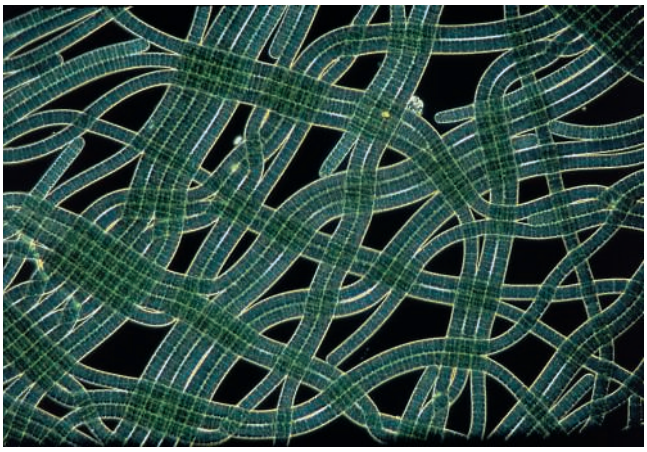
Cyanobacteria reproduce by fission and are often surrounded by a jellylike **sheath.** Because cyanobacteria are prokaryotes, they are not related to other algae, all eukaryotic. Cyanobacteria such as *Oscillatoria* often cause many of the disagreeable tastes, colors, and odors in water.

Procedure 24.8

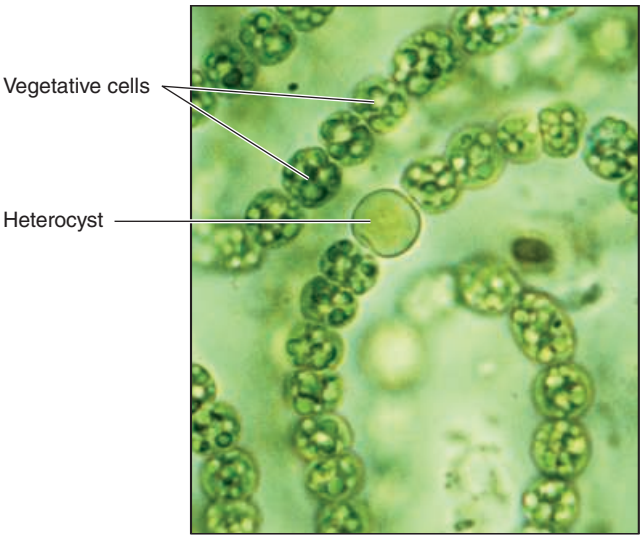
Examine cyanobacteria

1. With your microscope, examine living material and prepared slides of *Oscillatoria* (fig. 24.12*a*). *Oscillatoria* grows as long chains of cells called **trichomes.**

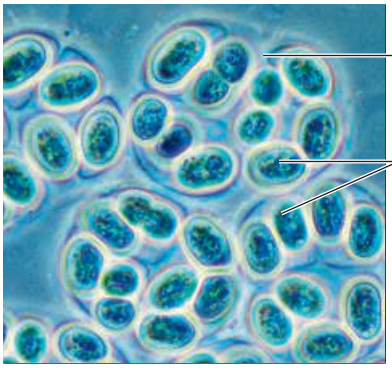
Question 6
Do all cells of a trichome of *Oscillatoria* appear similar?



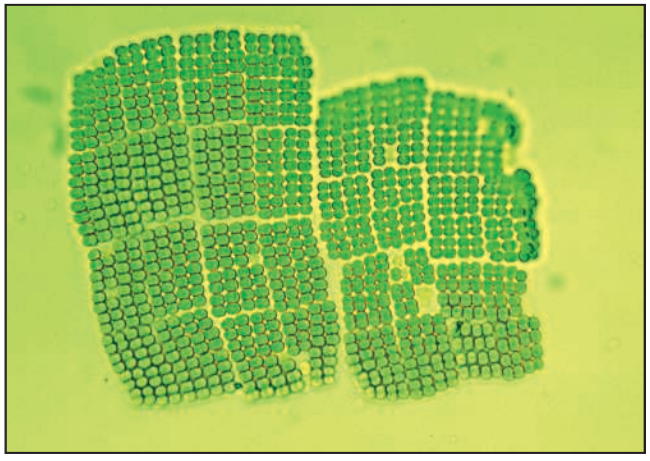
(a)



(b)



(c)



(d)

Figure 24.12
Cyanobacteria. (*a*) *Oscillatoria* (200×); (*b*) *Nostoc* (300×); (*c*) *Gloeocapsa* (400×); and (*d*) *Merismopedia* (400×).

2. Examine living material and a prepared slide of *Nostoc* (commonly called witch's butter or starjelly) (fig. 24.12*b*). *Nostoc* forms large, grapelike colonies. Trichomes of *Nostoc* consist of small vegetative cells and larger, thick-walled **heterocysts,** in which nitrogen fixation occurs.

3. Examine a wet mount of living *Gloeocapsa,* characterized by a thick, gelatinous sheath (fig. 24.12*c*).

4. Add a drop of dilute India ink to the slide of *Gloeocapsa* so that the sheath will stand out against the dark background. Often *Gloeocapsa* forms clusters of cells and therefore has a colonial body form. Locate one of these colonies.

5. Add a drop of methylene blue to a fresh slide of *Gloeocapsa* and determine if it enhances your observation more than does India ink.

Question 7

a. Do adjacent cells share a common sheath?

b. What do you suppose is the function of the sheath?

c. Do clusters of *Gloeocapsa* represent multicellular organisms? Why or why not?

d. What is the best stain for *Gloeocapsa,* India ink or methylene blue?

6. Examine some living *Merismopedia* (fig. 24.12*d*), which also form colonies. Sketch these cyanobacteria in the following space.

Question 8

a. How is the shape of *Merismopedia* different from other cyanobacteria you studied in this exercise?

b. How would a colony attain this shape?

INVESTIGATION

Bacterial Sensitivity to Inhibitors

Observations: Bacteria grow virtually everywhere. Some common household products effectively inhibit bacterial growth on floors and tabletops but may leave behind resistant species. Some microenvironments, however, are not exposed to the selection pressure of disinfectants.

Question: Do some places in our surroundings harbor bacteria that resist household disinfectants especially well?

a. Establish a working lab group and obtain Investigation Worksheet 24 from your instructor.
b. Discuss with your group a well-defined question relevant to the preceding observation and question. Record your question on Worksheet 24.
c. Translate your question into a testable hypothesis and record it.
d. Review the discussion of sensitivity plates and procedure 24.7. Outline on Worksheet 24 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What is meant by Gram positive? Explain the mechanism of Gram-positive staining.

2. What happens when milk is pasteurized?

3. What causes milk to sour?

4. What ecological roles are performed by cyanobacteria?

5. How do antibiotics kill bacteria? Why do they not affect viruses?

6. How could bacteria become resistant to an antibiotic?

7. Antibiotic resistance is promoted by overprescription of antibiotics. How do we stop this trend? What problems are involved? Is antibiotic resistance inevitable?

DOING BIOLOGY YOURSELF

Obtain two nutrient agar plates. Touch and drag the tip of your finger on the agar surface of one plate. Wash your hand and repeat the procedure on the other plate. Incubate the plates for 24–48 h. Then compare colony appearances and number. What can you conclude about the presence and diversity of bacteria on your plates? Is the appearance of a colony a good way to distinguish species of bacteria? Why or why not?

WRITING TO LEARN BIOLOGY

There is a great diversity of roles in a typical ecosystem, some shared by a variety of organisms. What ecological roles are performed by cyanobacteria?

DOING BIOLOGY YOURSELF

Doorknobs, sinks, tables, and so on are often laden with bacteria. Devise a protocol to swab areas of your workplace to determine which microenvironments harbor the most bacteria.