4 Bacteria and Archaea

Case File 4

You're Outnumbered

They're everywhere, and they outnumber you 10 to 1. They are your microbiota. The thought of bacteria living on and in your body may make you squeamish, but these compatriots are vital to your health. Take the microbiota in your gut, for example: There are approximately 10^{14} bacteria, archaea, and fungi living in your gut, accounting for about 1,100 different species, with about 10^{10} cells per gram of feces. There are also about a billion viruses per gram of feces. The gut microbiota is a complex ecosystem comprised of numerous genera including *Bacteroides, Clostridium, Streptococcus, Lactobacillus, Bifidobacterium,* and others. These organisms play an important role in your health: from helping to digest food, to producing vitamins and amino acids, to stimulating the immune system, to protecting you from harmful pathogens, to even having an influence on your behavior.

Some recent studies have shown that the gut microbiota can have an influence on obesity. Scientists have found that the gut microbiota in obese individuals can extract more energy from the diet that is stored as fat. Other studies have shown that obese individuals have higher levels of *Lactobacillus* species than nonobese populations. In studies with mice, scientists demonstrated that a change in diet from a low-fat, plant-based diet to a high-fat, high-sugar diet shifted the population of gut microbiota within a single day, favoring the microbiota that influence increased fat storage.

- What is the connection between gut microbiota and type II diabetes?
- What other human conditions can be influenced by gut microflora?

Continuing the Case appears on page 91

Outline and Learning Outcomes

4.1 Bacterial Form and Function

1. List the structures all bacteria possess.
2. Identify at least four structures that some, but not all, bacteria possess.
3. Describe the three major shapes of bacteria.
4. Describe other more unusual shapes of bacteria.
5. Provide at least four terms to describe bacterial arrangements.

4.2 External Structures
- **6.** Describe the structure and function of five different types of bacterial external structures.
- **7.** Explain how a flagellum works in the presence of an attractant.

4.3 The Cell Envelope: The Boundary Layer of Bacteria
- **8.** Differentiate between the two main types of bacterial envelope structure.
- **9.** Discuss why gram-positive cell walls are stronger than gram-negative cell walls.
- **10.** Name a substance in the envelope structure of some bacteria that can cause severe symptoms in humans.

4.4 Bacterial Internal Structure
- **11.** Identify five structures that may be contained in bacterial cytoplasm.
- **12.** Detail the causes and mechanisms of sporogenesis and germination.

4.5 The Archaea: The Other "Prokaryotes"
- **13.** List some differences between archaea and bacteria.

4.6 Classification Systems for Bacteria and Archaea
- **14.** Differentiate between *Bergey's Manual of Systematic Bacteriology* and *Bergey's Manual of Determinative Bacteriology*.
- **15.** Name four divisions ending in *–cutes* and describe their characteristics.
- **16.** Define a *species* in terms of bacteria.

In chapter 1, we described bacteria and archaea as being cells with no true nucleus. (Eukaryotes have a membrane around their DNA, and this structure is called the *nucleus*.) Let's look at bacteria and archaea as different from eukaryotes.

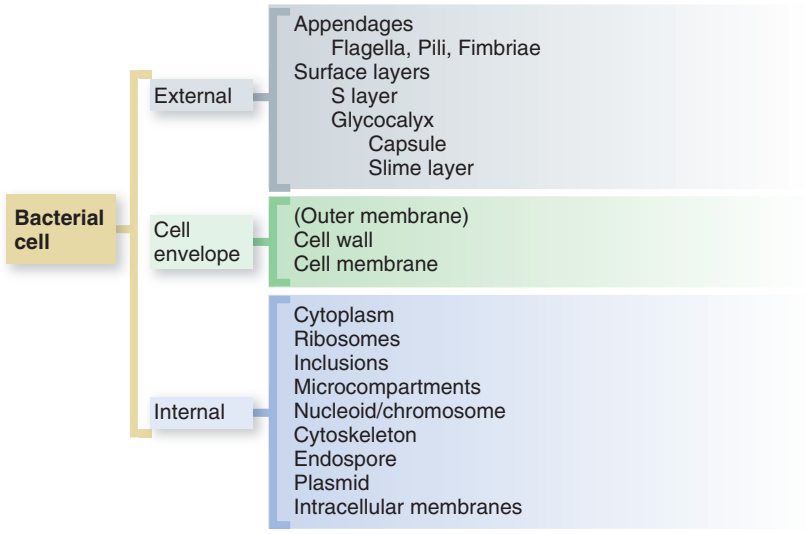
- ***The way their DNA is packaged:*** Bacteria and archaea have nuclear material that is free inside the cytoplasm (i.e., they do not have a nucleus). Eukaryotes have a membrane around their DNA (making up a nucleus). Eukaryotes wind their DNA around proteins called histones and archaea use similar proteins to do the same thing. Bacteria do not wind their DNA around proteins.
- ***The makeup of their cell wall:*** Bacteria and archaea generally have a wall structure that is unique compared to eukaryotes. Bacteria have sturdy walls made of a chemical called peptidoglycan. Archaeal walls are also tough and made of other chemicals, distinct from bacteria and distinct from eukaryotic cells.
- ***Their internal structures:*** Bacteria and archaea do not have complex, membrane-bounded organelles in their cytoplasm (eukaryotes do). A few bacteria and archaea have internal membranes, but they don't surround organelles.

Both non-eukaryotic and eukaryotic microbes are ubiquitous in the world today. Although both can cause infectious disease, drug targeting of bacterial and eukaryotic pathogens will be influenced by their unique cellular characteristics. In this chapter and coming chapters, you will discover why that is.

4.1 Bacterial Form and Function

The evolutionary history of non-eukaryotic cells extends back at least 2.9 billion years. The fact that these organisms have endured for so long in such a variety of habitats indicates a cellular structure and function that are amazingly versatile and adaptable.

The general cellular organization of a bacterial cell can be represented with this flowchart.



All bacterial cells invariably have a cell membrane, cytoplasm, ribosomes, and one (or a few) chromosome(s); the majority have a cell wall and some form of surface coating or glycocalyx. Specific structures that are found in some, but not all, bacteria are flagella, pili, fimbriae, an S layer, a cytoskeleton, inclusions, microcompartments, endospores, and intracellular membranes.

The Structure of a Generalized Bacterial Cell

Bacterial cells appear featureless and two-dimensional when viewed with an ordinary microscope. Not until they are subjected to the scrutiny of the electron microscope and biochemical studies does their intricate and functionally complex nature become evident. **Figure 4.1** presents a three-dimensional anatomical view of a generalized, rod-shaped, bacterial cell. As we survey the principal anatomical features

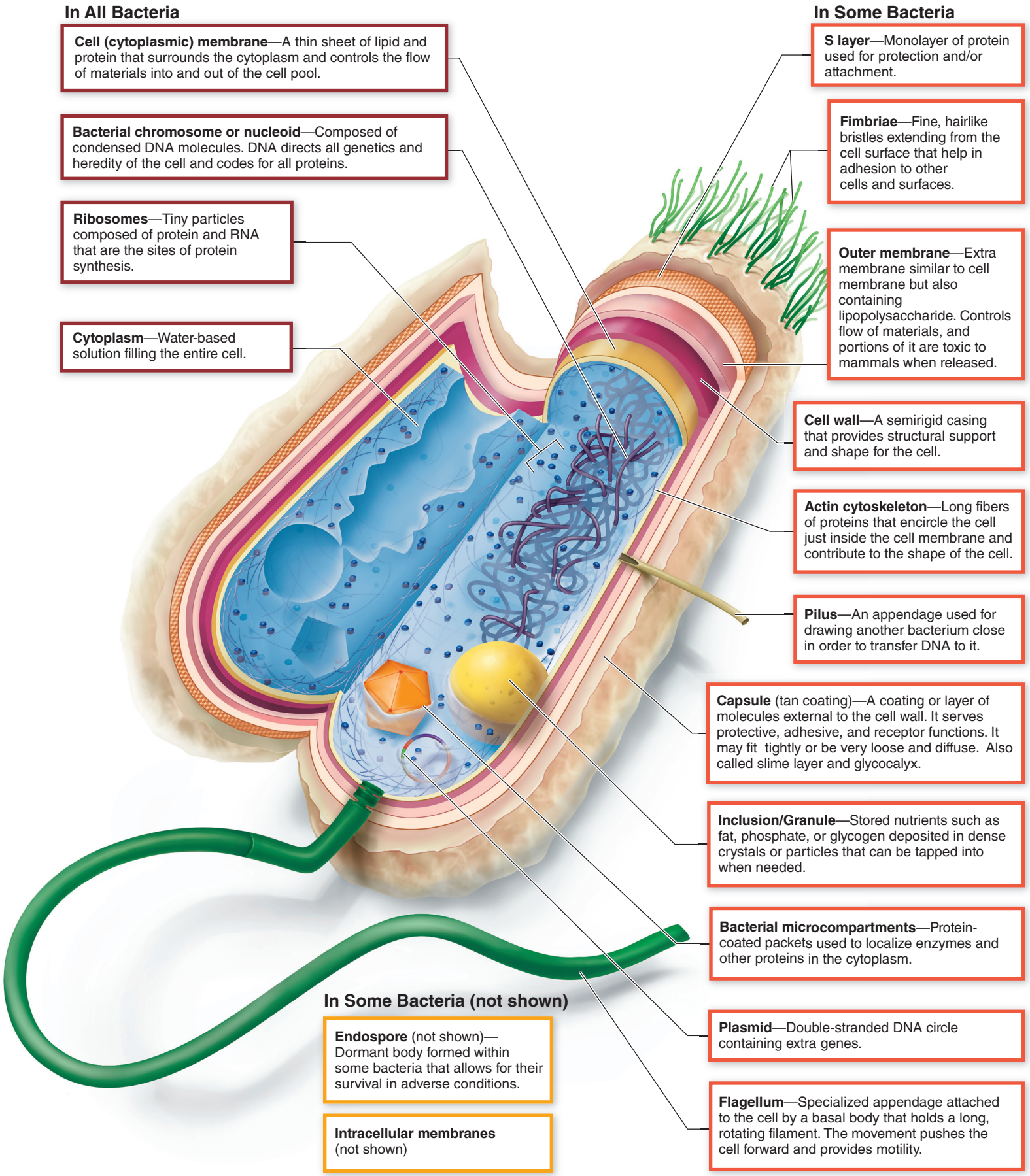


Figure 4.1 Structure of a bacterial cell. Cutaway view of a typical rod-shaped bacterium, showing major structural features.

of this cell, we will perform a microscopic dissection of sorts, beginning with the outer cell structures and proceeding to the internal contents.

Bacterial Arrangements and Sizes

Each individual bacterial cell is fully capable of carrying out all necessary life activities, such as reproduction, metabolism, and nutrient processing, unlike the more specialized cells of a multicellular organism. On the other hand, sometimes bacteria *can* act as a group. When bacteria are close to one another in colonies or in biofilms, they communicate with each other through chemicals that cause them to behave differently than if they were living singly. More surprisingly, some bacteria seem to communicate with each other using structures called *nanowires,* which are appendages that can be many micrometers long and are used for transferring electrons or other substances outside the cell onto metals. The wires also intertwine with the wires of neighboring bacteria. This is not the same as being a multicellular organism, but it represents new findings about microbial cooperation.

Disease Connection

Biofilms can play a major role in infectious diseases. Scientists definitively showed in 2006 that children suffering from chronic ear infections had biofilms of bacteria growing on the mucosa of their middle ears. These biofilms were not eradicated by repeated courses of antibiotics. This discovery gave more support to the procedure of putting tubes in the ears of children with chronic or recurrent ear infections (to drain infected fluids) instead of treating with antibiotics.

Bacteria exhibit considerable variety in shape, size, and colonial arrangement. In terms of size, bacterial cells have an average size of about 1 micron (μm). As with everything in nature, though, there is a great deal of variation in microbial size. The largest non-eukaryote yet discovered is a bacterial species living in ocean sediments near the African country of Namibia. The gigantic individual cocci of *Thiomargarita namibiensis* measure from 100 to 750 μm (3/4 mm), and many are large enough to see with the naked eye **(figure 4.2).** On the other end of the size spectrum, we have *Mycoplasma* cells that generally measure 0.15 to 0.30 μm, which is at the limit of resolution for most light microscopes. A new controversy is brewing over the discovery of tiny cells that look like dwarf bacteria but are 10 times smaller than mycoplasmas and a hundred times smaller than the average bacterial cell. These minute nanobacteria or nanobes (Gr. *nanos,* one-billionth) were first isolated from blood and serum samples, and have a size range of 0.05 to 0.2 μm. They also have been found in sandstone rock deposits in the ocean and deeply embedded in billion-year-old minerals. Not all microbiologists are convinced that they are true microbes, but they expand our view of the size limitations that define life.

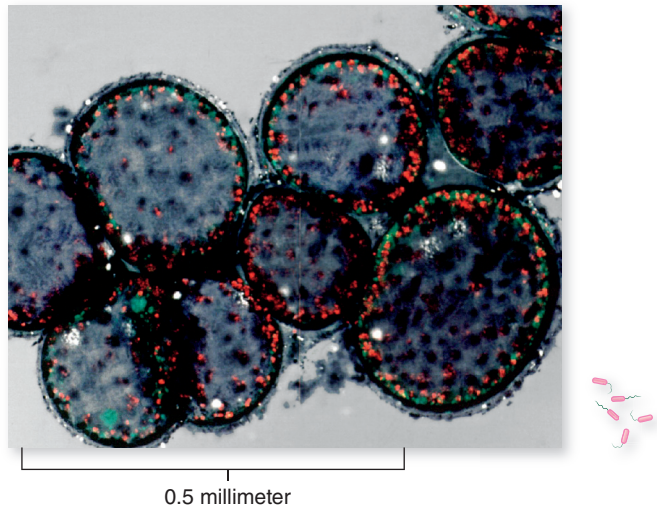
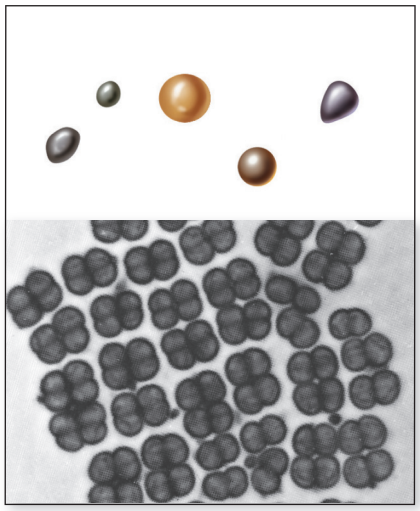


Figure 4.2. *Thiomargarita namibiensis.* A drawing of *E. coli* is included on right for size comparison.

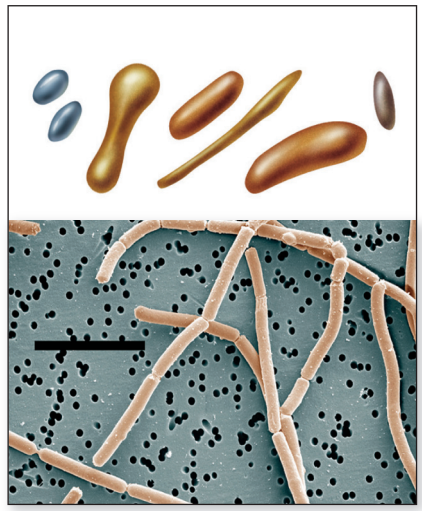
Bacteria come in many different shapes, but the vast majority are one of three general shapes **(figure 4.3).** If the cell is spherical or ball-shaped, the bacterium is described as a **coccus** (kok'-us). Cocci can be perfect spheres, but they also can exist as oval, bean-shaped, or even pointed variants. A cell that is cylindrical (longer than wide) is termed a *rod,* or **bacillus** (bah-sil'-lus). There is also a genus named *Bacillus.* As may be expected, rods are also quite varied in their actual form. Depending on the species, they can be blocky, spindle-shaped, round-ended, long and threadlike (filamentous), or even club-shaped or drumstick-shaped. When a rod is short and plump, it is called a **coccobacillus;** if it is gently curved, it is a **vibrio** (vib'-ree-oh). A bacterium having a slightly curled or spiral-shaped cylinder is called a **spirillum** (spy-ril'-em), a rigid helix, twisted twice or more along its axis (like a corkscrew). Another spiral cell mentioned earlier in the discussion of periplasmic flagella is the **spirochete,** a more flexible form that resembles a spring. Because bacterial cells look two-dimensional and flat with traditional staining and microscope techniques, they are seen to best advantage with a scanning electron microscope, which emphasizes their striking three-dimensional forms **(figure 4.3, micrographs).**

It is also somewhat common for cells of a single species to vary in shape and size. This phenomenon, called *pleomorphism* **(figure 4.4),** is due to individual variations in cell wall structure caused by nutritional or slight genetic differences. For example, although the cells of *Corynebacterium diphtheriae* are generally considered rod-shaped, in culture they display variations such as club-shaped, swollen, curved, filamentous, and coccoid. Pleomorphism reaches an extreme in the mycoplasmas, which entirely lack cell walls and thus display extreme variations in shape.

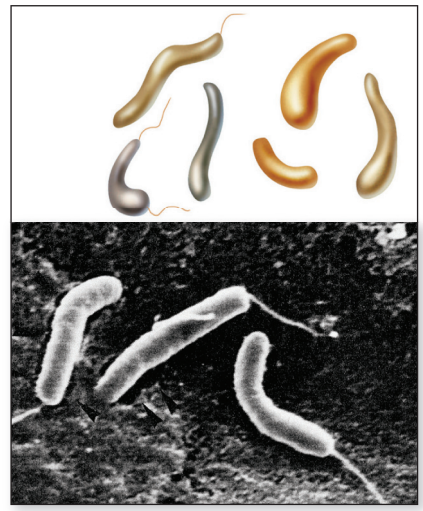
Bacterial cells can also be categorized according to arrangement, or style of grouping (see figure 4.3). The main



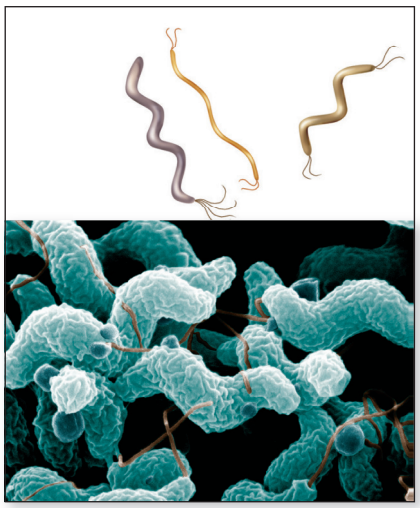
(a) **Coccus**



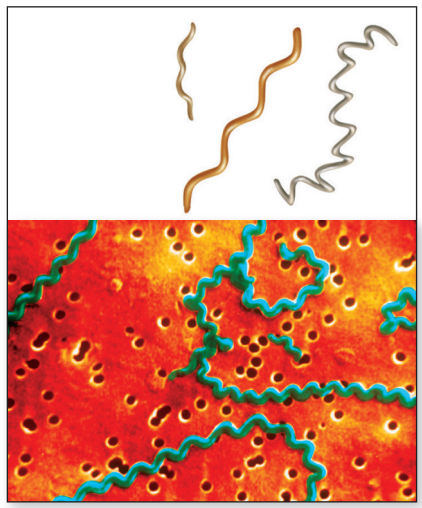
(b) **Rod/Bacillus**



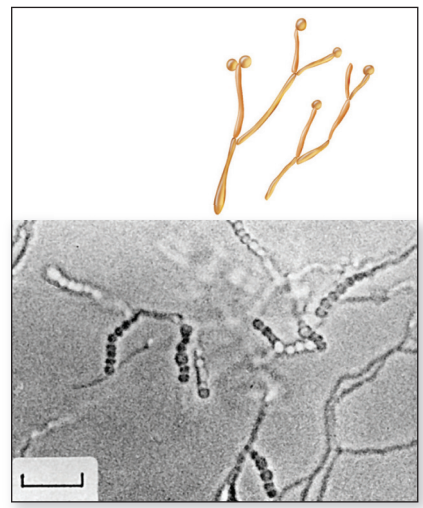
(c) **Vibrio**



(d) **Spirillum**



(e) **Spirochete**



(f) **Branching filaments**

Key to Micrographs
(a) *Deinococcus* (2,000×) (b) *Lactobacillus bulgaricus* (5,000×) (c) *Vibrio cholerae* (13,000×) (d) *Aquaspirillum* (7,500×)
(e) Spirochetes on a filter (14,000×) (f) *Streptomyces* (1,500×)

Figure 4.3 Bacterial shapes and arrangements. Drawings show examples of shape variations for cocci, rods, vibrios, spirilla, spirochetes, and branching filaments. Below each shape is a micrograph of a representative example.

factors influencing the arrangement of a particular cell type are its pattern of division and how the cells remain attached afterward. The greatest variety in arrangement occurs in cocci, which can be single, in pairs (diplococci), in **tetrads** (groups of four), in irregular clusters (as in staphylococci and micrococci), or in chains of a few to hundreds of cells (as in streptococci). An even more complex grouping is a cubical packet of 8, 16, or more cells called a **sarcina** (sar'-sih-nah). These different coccal groupings are the result of the division of a coccus in a single plane, in two perpendicular planes, or in several intersecting planes; after division, the resultant daughter cells remain attached.

Bacilli are less varied in arrangement because they divide only in the transverse plane (perpendicular to the axis). They occur either as single cells, as a pair of cells with their ends attached (diplobacilli), or as a chain of several cells (streptobacilli). A palisades arrangement is formed when

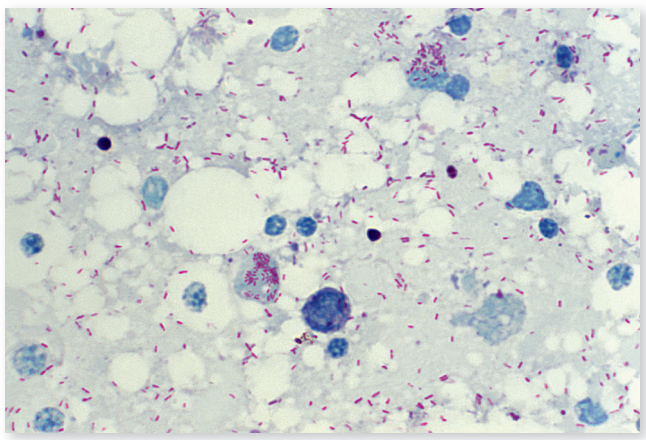


Figure 4.4 Pleomorphic bacteria. If you look closely at this micrograph of stained *Rickettsia rickettsii* bacteria, you will see some coccoid cells, some rod-shaped cells, and some hybrid forms.

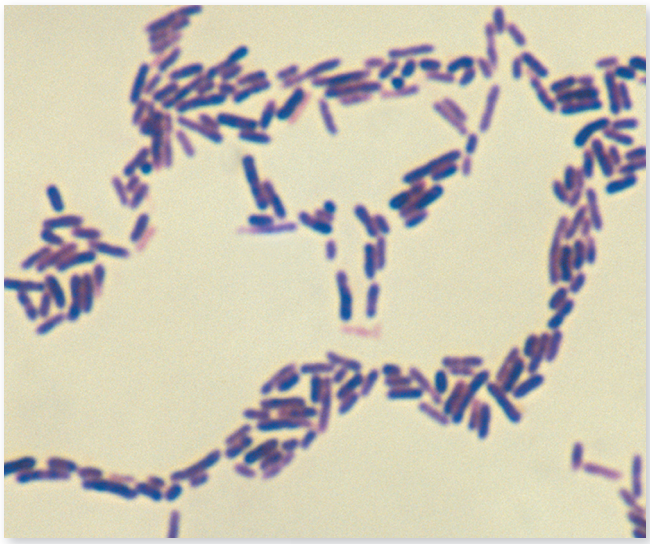


Figure 4.5. ***Corynebacterium*** **cells illustrating the palisades arrangement.**

cells of a chain remain partially attached at the ends; this hinge area can fold back creating a side-by-side row of cells **(figure 4.5).** Spirilla are occasionally found in short chains, but spirochetes rarely remain attached after division.

4.1 Learning Outcomes—Assess Your Progress

1. List the structures all bacteria possess.
2. Identify at least four structures that some, but not all, bacteria possess.
3. Describe the three major shapes of bacteria.
4. Describe other more unusual shapes of bacteria.
5. Provide at least four terms to describe bacterial arrangements.

4.2 External Structures

Appendages: Cell Extensions

Several different types of accessory structures sprout from the surface of bacteria. These long **appendages** are common but are not present on all species. Appendages can be divided into two major groups: those that provide motility (flagella and axial filaments) and those that provide attachment points or channels (fimbriae and pili).

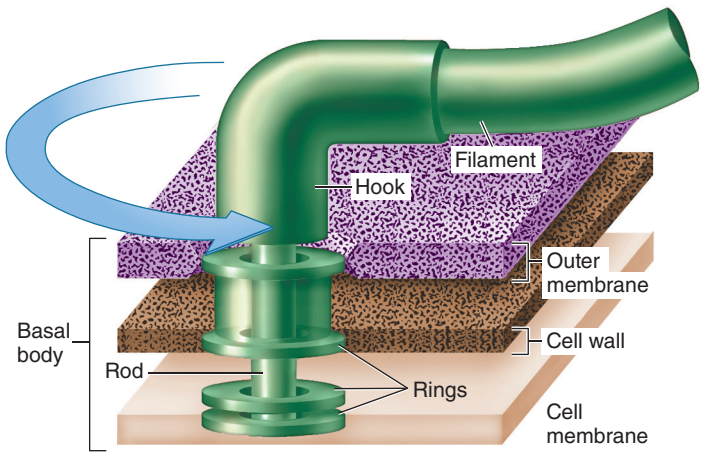
Flagella—Little Propellers

The bacterial **flagellum** (flah-jel'-em), an appendage of truly amazing construction, is certainly unique in the biological world. The primary function of flagella is to confer **motility,** or self-propulsion—that is, the capacity of a cell to swim freely through an aqueous habitat. The extreme thinness of a bacterial flagellum necessitates high magnification to reveal its special architecture, which has three distinct parts: the filament, the hook (sheath), and the basal body **(figure 4.6).** The **filament,** a helical structure composed of proteins, is approximately 20 nanometers in diameter and varies from 1 to 70 microns in length. It is inserted into a curved, tubular hook. The hook is anchored to the cell by the basal body, a stack of rings firmly anchored through the cell wall to the cell membrane and the outer membrane. This arrangement permits the hook with its filament to rotate 360°, rather than undulating back and forth like a whip as was once thought.

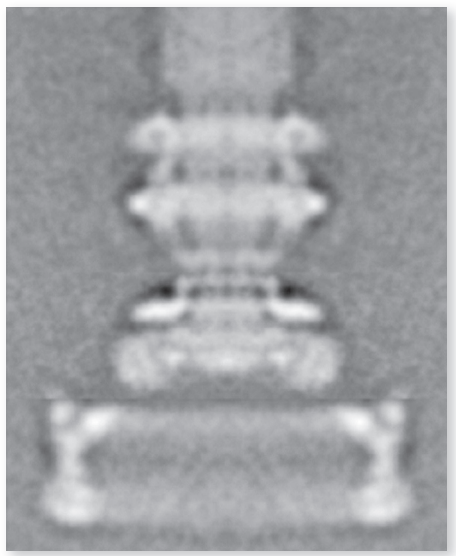
One can generalize that all spirilla, about half of the bacilli, and a small number of cocci have flagella (these bacterial shapes are shown in **figure 4.7**). Flagella vary both in number and arrangement according to two general patterns:

1. In a **polar** arrangement, the flagella are attached at one or both ends of the cell. Three subtypes of this pattern are

Figure 4.6 Details of the basal body of a flagellum in a gram-negative cell. **(a)** The hook, rings, and rod function together as a tiny device that rotates the filament 360°. **(b)** An electron micrograph of the basal body of a bacterial flagellum.

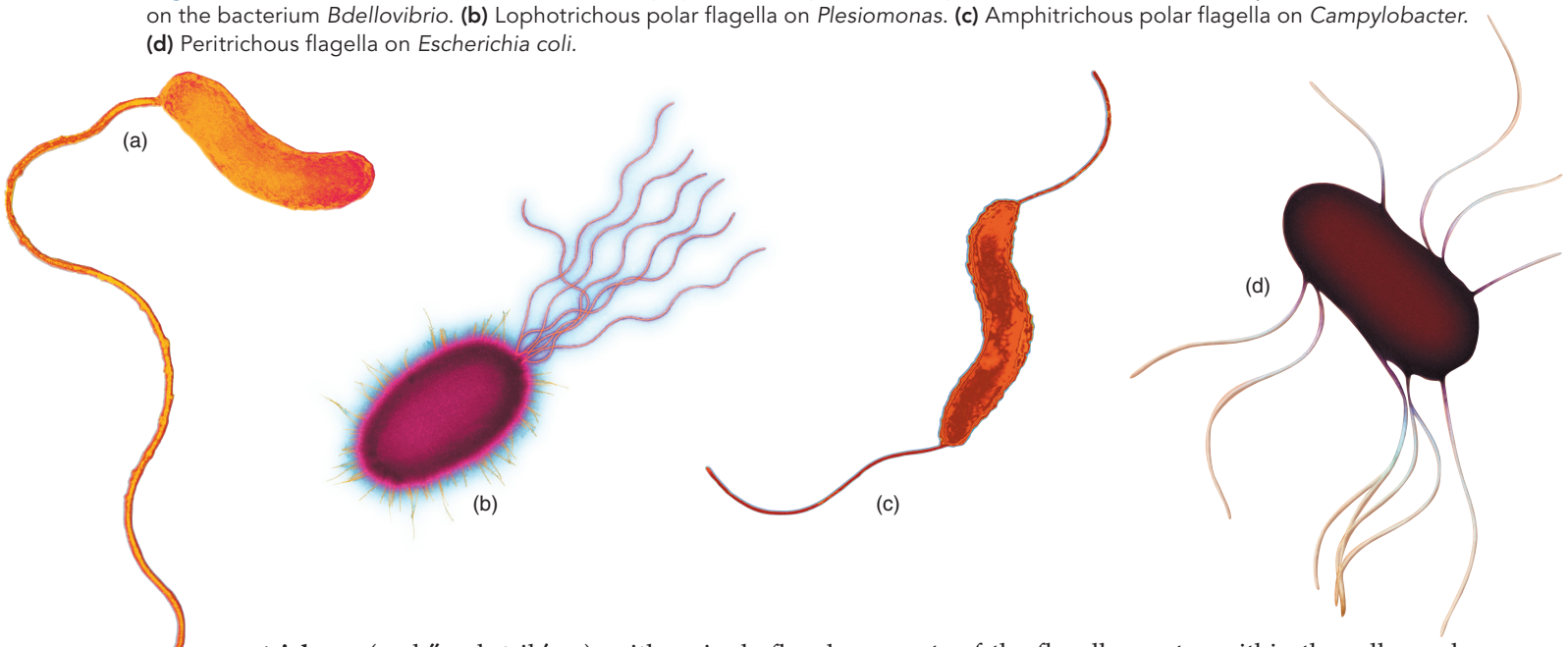


(a)



(b)

Figure 4.7 Electron micrographs depicting types of flagellar arrangements. **(a)** Monotrichous polar flagellum on the bacterium *Bdellovibrio*. **(b)** Lophotrichous polar flagella on *Plesiomonas*. **(c)** Amphitrichous polar flagella on *Campylobacter*. **(d)** Peritrichous flagella on *Escherichia coli*.



monotrichous (mah″-noh-trik′-us), with a single flagellum **(figure 4.7*a*); lophotrichous** (lo″-foh-), with small bunches or tufts of flagella emerging from the same site **(figure 4.7*b*);** and **amphitrichous** (am″-fee-), with flagella at both poles of the cell **(figure 4.7*c*).**

2. In a **peritrichous** (per″-ee-) arrangement, flagella are dispersed randomly over the surface of the cell **(figure 4.7*d*).**

The presence of motility is one piece of information used in the laboratory identification or diagnosis of pathogens. Flagella are hard to visualize in the laboratory, but often it is sufficient to know simply whether a bacterial species is motile. Motility can be assessed using semisolid media or through the hanging drop technique (see chapter 3).

Fine Points of Flagellar Function Flagellated bacteria can perform some rather sophisticated feats. They can detect and move in response to chemical signals—a type of behavior called **chemotaxis** (ke″-moh-tak′-sis). Positive chemotaxis is movement of a cell in the direction of a favorable chemical stimulus (usually a nutrient); negative chemotaxis is movement away from a repellent (potentially harmful) compound.

The flagellum is effective in guiding bacteria through the environment primarily because the system for detecting chemicals is linked to the mechanisms that drive the flagellum. Located in the cell membrane are clusters of receptors[1] that bind specific molecules coming from the immediate environment. The attachment of sufficient numbers of these molecules transmits signals to the flagellum and sets it into rotary motion. The actual "fuel" for the flagellum to turn is a gradient of protons (hydrogen ions) that are generated by the metabolism of the bacterium and that bind to and detach from parts of the flagellar motor within the cell membrane, causing the filament to rotate. If several flagella are present, they become aligned and rotate as a group **(figure 4.8).** As a flagellum rotates counterclockwise, the cell itself swims in a smooth linear direction toward the stimulus; this action is called a **run**. Runs are interrupted at various intervals by **tumbles**, during which the flagellum reverses direction and causes the cell to stop and change its course. It is believed that attractant molecules inhibit tumbles and permit progress toward the stimulus; these appear to play a major role in the process of quorum sensing. Repellents cause numerous tumbles, allowing the

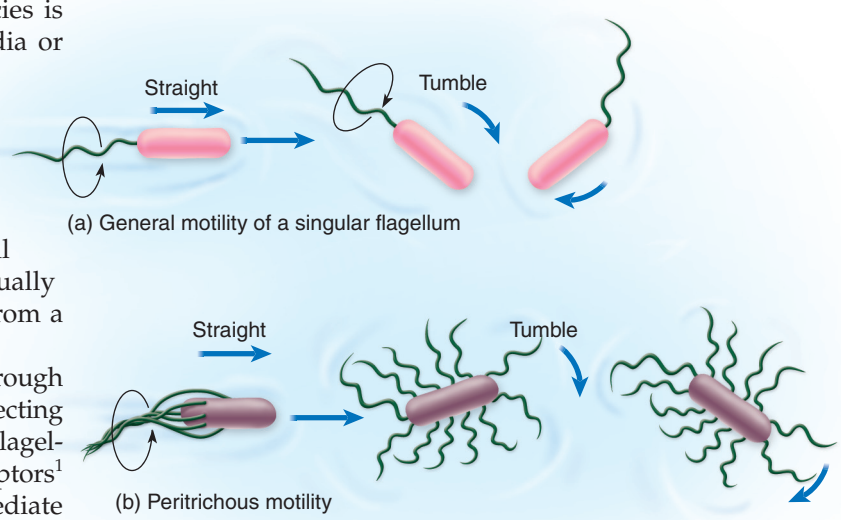
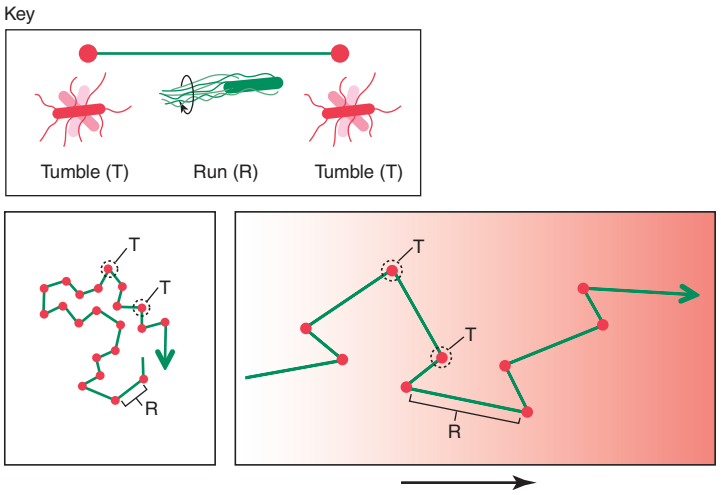


Figure 4.8 The operation of flagella and the mode of locomotion in bacteria with polar and peritrichous flagella. **(a)** In general, when a polar flagellum rotates in a counterclockwise direction, the cell swims forward. When the flagellum reverses direction and rotates clockwise, the cell stops and tumbles. **(b)** In peritrichous forms, all flagella sweep toward one end of the cell and rotate as a single group. During tumbles, the flagella lose coordination.

1. Cell surface molecules that bind specifically with other molecules.



(a) No attractant or repellent results in a "random walk" form of motion.

(b) In presence of attractant, the walk becomes "biased" toward more runs and fewer tumbles, directing the cell towards the attractant.

Figure 4.9 Chemotaxis in bacteria. **(a)** A bacterium moves via a random series of short runs and tumbles when there is no attractant or repellent. **(b)** The cell spends more time on runs as it gets closer to the attractant.

bacterium to redirect itself away from the stimulus **(figure 4.9).** Some photosynthetic bacteria exhibit **phototaxis**, movement in response to light rather than chemicals.

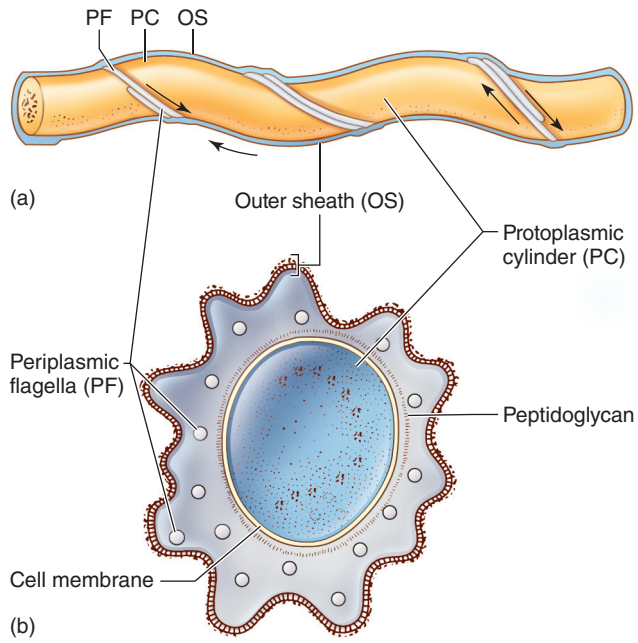
Periplasmic Flagella

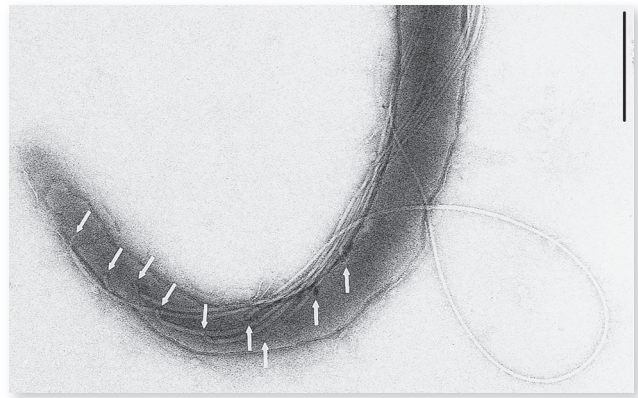
Corkscrew-shaped bacteria called *spirochetes* (spy'-roh-keets) show an unusual, wriggly mode of locomotion caused by two or more long, coiled threads, the periplasmic flagella or **axial filaments**. A periplasmic flagellum is a type of internal flagellum that is enclosed in the space between the cell wall and the cell membrane **(figure 4.10).** The filaments curl closely around the spirochete coils yet are free to contract and impart a twisting or flexing motion to the cell.

Appendages for Attachment and Mating

Although their main function is motility, bacterial flagella can be used for attachment to surfaces in some species. Two other structures, the **pilus** (pil-us; plural, *pili*) and the **fimbria** (fim'-bree-ah), are bacterial surface appendages that provide some type of adhesion, but not locomotion. As we think about all three structures, we must remember that attachment can enhance pathogenicity or the ability to cause disease; thus, targeting these structures could drive the development of new antibiotics.

Fimbriae are small, bristlelike fibers sprouting off the surface of many bacterial cells **(figure 4.11).** Their exact composition varies, but most of them contain protein. Fimbriae have an inherent tendency to stick to each other and to surfaces. They may be responsible for the mutual clinging of cells that leads to biofilms and other thick aggregates of cells on the surface of liquids and for the microbial colonization of inanimate solids such as rocks and glass **(Insight 4.1).** Some





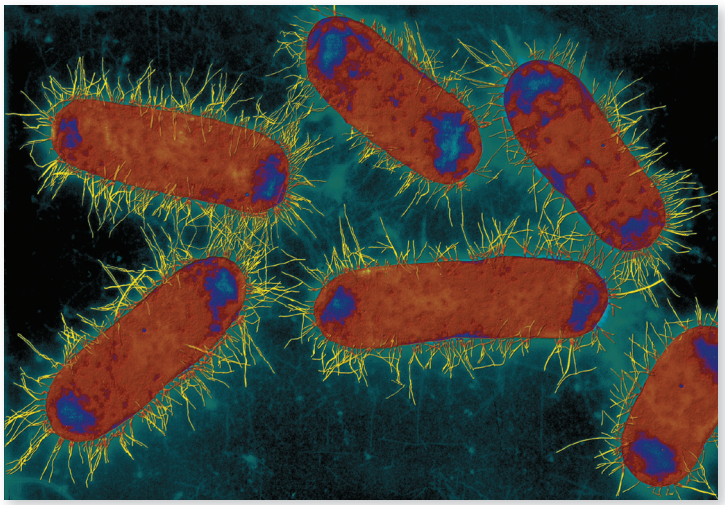
(c)

Figure 4.10 The orientation of periplasmic flagella on the spirochete cell. **(a)** Longitudinal section. **(b)** Cross section (end-on view). Contraction of the filaments imparts a spinning and undulating pattern of locomotion. **(c)** Electron micrograph captures the details of periplasmic flagella and their insertion points (arrows) in *Borrelia burgdorferi*, the cause of Lyme disease. One flagellum has escaped the outer sheath, probably during preparation for EM. (Bar = 0.2 microns)

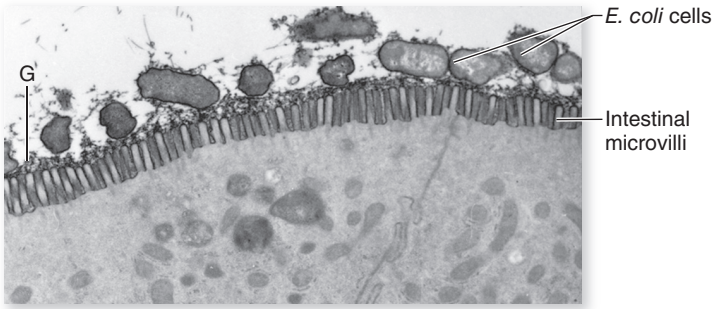
pathogens, such as the gonococcus and *Escherichia coli*, can colonize and infect host tissues because of a tight adhesion between their fimbriae and epithelial cells **(figure 4.11*b*).** Mutant forms of these pathogens that lack a fimbriae, however, are unable to cause infections.

A pilus is a long, rigid tubular structure made of a special protein, **pilin.** So far, true pili have been found only on gram-negative bacteria, where they are utilized in a "mating" process between cells called **conjugation,**[2] which involves partial transfer of DNA from one cell to another **(figure 4.12).** A pilus from the donor cell unites with a recipient cell, bring-

2. Although the term *mating* is sometimes used for this process, it is not a form of sexual reproduction.



(a)



(b)

Figure 4.11 Form and function of bacterial fimbriae.
(a) Several cells of pathogenic *Escherichia coli* covered with numerous stiff fibers called fimbriae (30,000×). Note also the dark-blue granules, which are the chromosomes. **(b)** A row of *E. coli* cells tightly adheres by their fimbriae to the surface of intestinal cells (12,000×). This is how the bacterium clings to the body during an infection. (G = Glycocalyx)

ing it close enough for DNA transfer. Production of pili is controlled genetically, and conjugation takes place only between compatible gram-negative cells. Conjugation in gram-positive bacteria does occur but involves aggregation proteins rather than pili. There is a special type of structure in some bacteria called a Type IV pilus. Like the pili described here, it can transfer genetic material. In addition, it can act like fimbriae and assist in attachment, and act like flagella and make a bacterium motile. The roles of pili and conjugation are further explored in chapter 9.

Surface Coatings: The S Layer and the Glycocalyx

The bacterial cell surface is frequently exposed to severe environmental conditions. Bacterial cells protect themselves with either an **S layer** or a **glycocalyx,** or both. S layers are single layers of thousands of copies of a single protein linked together like tiny chain mail. They are often called "the armor" of a bacterial cell. It took scientists a long time to discover them because bacteria only produce them when they are in a hostile environment. The nonthreatening conditions

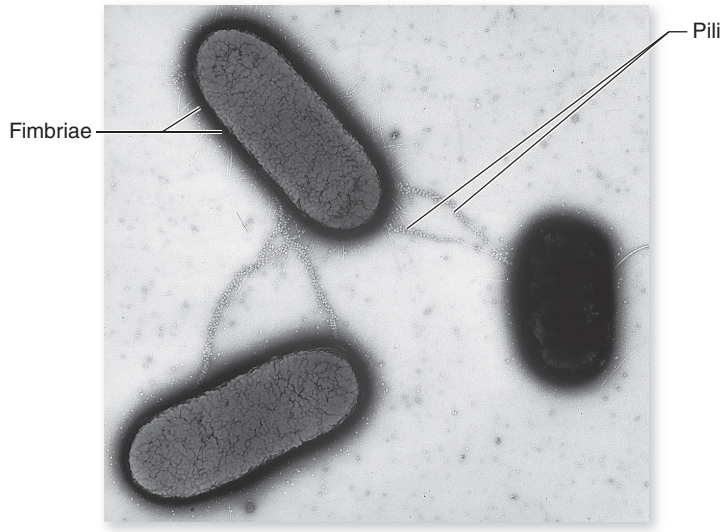


Figure 4.12 Three bacteria in the process of conjugating.
Clearly evident are the sex pili forming mutual conjugation bridges between a donor (middle cell) and two recipients (cells on left side). Fimbriae can also be seen on the two left-hand cells.

of growing in a lab in a nutritious broth with no competitors around ensured that bacteria did not produce the layer. We now know that many different species have the ability to produce an S layer, including pathogens such as *Clostridium difficile* and *Bacillus anthracis*. Some bacteria use S layers to aid in attachment, as well.

The glycocalyx develops as a coating of repeating polysaccharide units that may or may not include protein. This protects the cell and, in some cases, helps it adhere to surfaces in its environment. Glycocalyces differ among bacteria in thickness, organization, and chemical composition. Some bacteria are covered with a loose shield called a **slime layer** that evidently protects them from loss of water and nutrients **(figure 4.13*a*).** A glycocalyx is called a **capsule** when it is

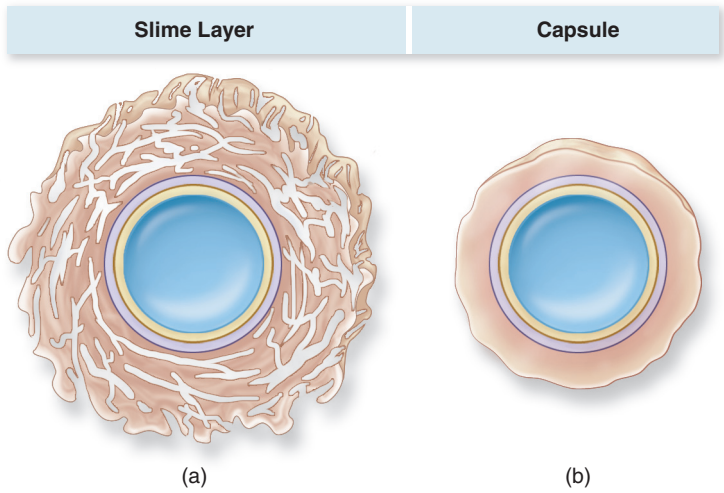
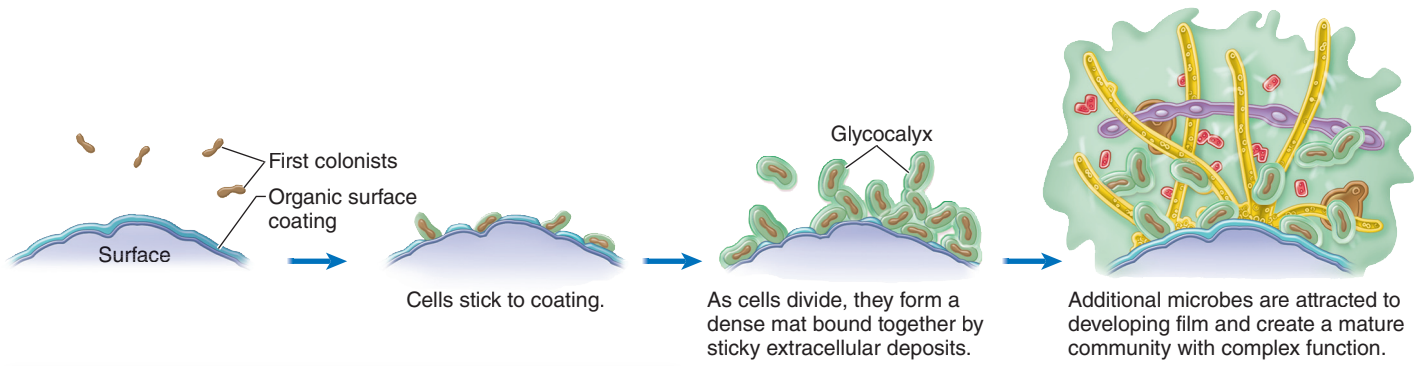
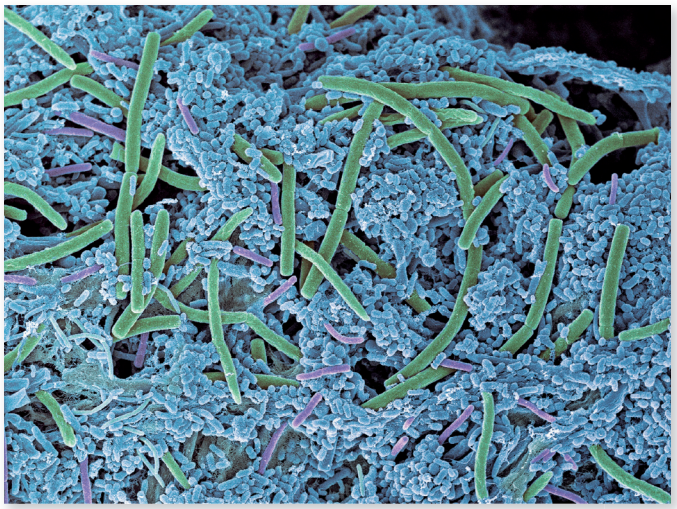


Figure 4.13 Drawings of sectioned bacterial cells to show the types of glycocalyces. **(a)** The slime layer is a loose structure that is easily washed off. **(b)** The capsule is a thick, structured layer that is not readily removed.

INSIGHT 4.1 Biofilms: Biological Glue





You've seen it before—the scum that builds up on the inside of your toilet, in your shower, or even on your teeth. This slimy gunk isn't merely evidence that you haven't cleaned in a while; it is a community of microbes called a **biofilm.** Scientists are discovering that bacteria often do not exist in a **planktonic** or single-cell form but rather live in cooperative associations that can include other organisms of the same species as well as other species of bacteria, archaea, fungi, and algae. These biofilms are microbial habitats with adequate access to food, water, atmosphere, and other environmental factors that are beneficial to each type of organism living there. Often, the biofilm is stratified, with the aerobic microbes near the surface where the oxygen levels are high and the anaerobic microbes near the bottom where oxygen levels are low. Each member of the biofilm community finds its niche.

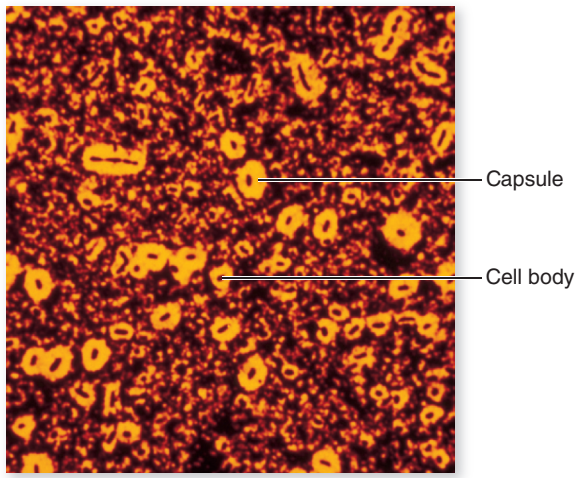
Biofilms can form on numerous inert substances, usually when the surface is moist and has developed a thin layer of organic material such as polysaccharides or glycoproteins. This slightly sticky texture attracts the first single-celled "colonists" that attach and begin to multiply on the surface. As the first colonizing organisms grow, they secrete substances such as cell signal receptors, fimbriae, slime layers, capsules, and even DNA molecules that attract other microbes to the surface as well. This cell-to-cell communication, including a process called *quorum sensing* (see chapter 7), allows for microbes of various species to grow together and secrete more extracellular matrix (shown in green in part *a* of the diagram). The biofilm can vary in thickness, depending on where it begins growing and how long it has been growing there (or how long it has been since you brushed and flossed your teeth).

Biofilms also have serious medical implications. Often, microbes will accumulate on damaged tissue such as heart valves or hard surfaces such as teeth. Bacteria also have an affinity for implanted medical devices such as IUDs, catheters, shunts, gastrostomy tubes, and urinary catheters, and readily form biofilms on these surfaces. Treating these types of infections is extremely difficult, and it has always been assumed that this was due to antibiotics being unable to penetrate the thick glycocalyx of the biofilm. Recent studies have shown that in biofilm form, microbes turn on different genes, allowing them to be impervious to antibiotic treatment. Finding novel ways to treat biofilm infections is an ongoing battle, and it is estimated that treating biofilm infections costs more than 1 billion dollars a year in the United States alone.

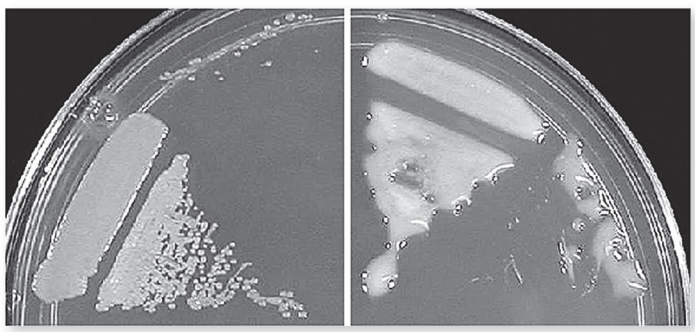
bound more tightly to the cell than a slime layer is and it is denser and thicker (**figure 4.13*b*).** Capsules can be viewed after a special staining technique **(figure 4.14*a*).** They are also often visible on agar because they give their colonies a sticky (mucoid) appearance **(figure 4.14*b*).**

Specialized Functions of the Glycocalyx

Capsules are formed by many pathogenic bacteria, such as *Streptococcus pneumoniae* (a cause of pneumonia, an infection of the lung), *Haemophilus influenzae* (one cause of meningitis), and *Bacillus anthracis* (the cause of anthrax). Encapsulated bacterial cells generally have greater pathogenicity because capsules protect the bacteria against white blood cells called phagocytes. Phagocytes are a natural body defense that can engulf and destroy foreign cells through phagocytosis, thus preventing infection. A capsular coating blocks the mechanisms that phagocytes use to attach to and engulf bacteria. By escaping phagocytosis, the bacteria are free to multiply



(a)



(b)

Figure 4.14 Encapsulated bacteria. **(a)** Negative staining reveals the microscopic appearance of a large, well-developed capsule. **(b)** Colony appearance of a nonencapsulated (left) and encapsulated (right) version of a soil bacterium called *Sinorhizobium*.

Case File 4 *Continuing the Case*

In a recent study, the fecal composition of normal, healthy adult males was compared with that of adult males with type II diabetes. Scientists used PCR to analyze the 16S rRNA of the gut microbiota, and found that patients with type II diabetes also had elevated levels of bacteria from the phyla *Bacteroidetes* and *Proteobacteria*. Bacteria from these phyla are gram-negative, and this study suggests that the lipopolysaccharide outer membrane of these organisms may induce inflammation in the gut that could play a role in the development of type II diabetes.

Yet another study suggests that multiple sclerosis (MS), an autoimmune disease that attacks the myelin sheath of nerve cells, may be triggered by gut microbiota. Scientists found that mice raised without gut microbiota and then colonized with certain intestinal bacteria developed MS-like symptoms. Researchers still need to determine if a faulty immune system overreacting to gut bacteria is the cause of MS or if a specific organism triggers the autoimmunity.

- What happens when your gut microflora is disrupted?

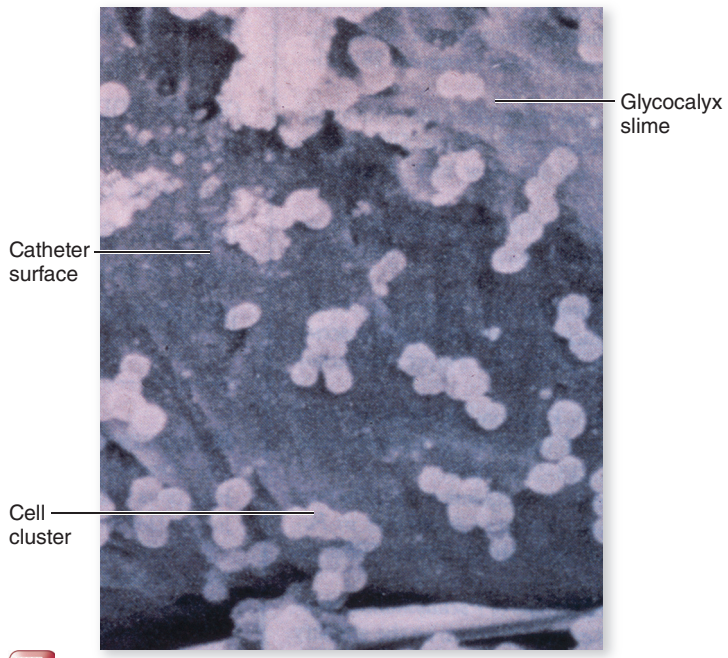


Figure 4.15 Biofilm formation. Scanning electron micrograph of *Staphylococcus aureus* cells attached to a catheter by a slime secretion.

and infect body tissues. Encapsulated bacteria that mutate to nonencapsulated forms usually lose their ability to cause disease.

Other types of glycocalyces can be important in the formation of biofilms. The thick, white plaque that forms on teeth comes in part from the surface slimes produced by certain streptococci in the oral cavity. This slime protects them from being dislodged from the teeth and provides a niche for other oral bacteria that, in time, can lead to dental disease. The glycocalyx of some bacteria is so highly adherent that it is responsible for persistent colonization of nonliving materials such as plastic catheters, intrauterine devices, and metal pacemakers that are in common medical use **(figure 4.15).**

4.2 Learning Outcomes—Assess Your Progress

6. Describe the structure and function of five different types of bacterial external structures.
7. Explain how a flagellum works in the presence of an attractant.

4.3 The Cell Envelope: The Boundary Layer of Bacteria

The majority of bacteria have a chemically complex external covering, termed the *cell envelope,* that lies outside of the cytoplasm. It is composed of two or three basic layers: the cell wall; the cell membrane; and, in some bacteria, the outer membrane. The layers of the envelope are stacked one upon

another and are often tightly bonded together like the outer husk and casings of a coconut. Although each envelope layer performs a distinct function, together they act as a single protective unit.

Differences in Cell Envelope Structure

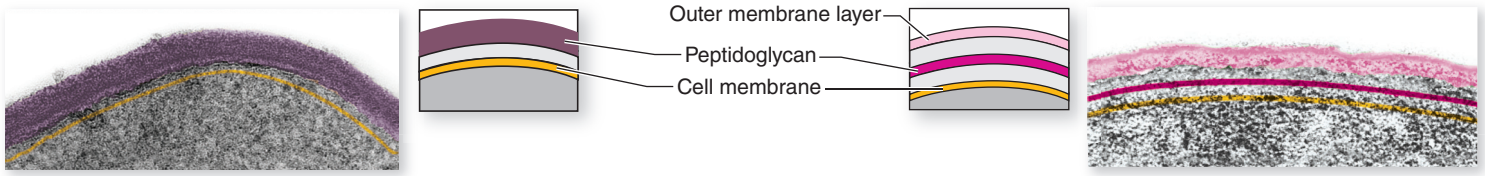
More than a hundred years ago, long before the detailed anatomy of bacteria was even remotely known, a Danish physician named Hans Christian Gram developed a staining technique, the **Gram stain,** that delineates two generally different groups of bacteria **(Insight 4.2).** The two major groups shown by this technique are the **gram-positive** bacteria and the **gram-negative** bacteria.

The structural differences denoted by the designations *gram-positive* and *gram-negative* lie in the cell envelope **(figure 4.16).** In gram-positive cells, a microscopic section reveals two layers: the thick cell wall, composed primarily of peptidoglycan (defined in the next section), and the cytoplasmic membrane. A similar section of a gram-negative cell envelope shows three layers: an outer membrane, a thin cell wall, and the cytoplasmic membrane.

Moving from outside to in, the outer membrane (if present) lies just under the glycocalyx. Next comes the cell wall. Finally, the innermost layer is always the cytoplasmic membrane. Because only some bacteria have an outer membrane, we discuss the cell wall first.

Structure of the Cell Wall

The **cell wall** accounts for a number of important bacterial characteristics. In general, it helps determine the shape of a bacterium, and it also provides the kind of strong structural support necessary to keep a bacterium from bursting or collapsing because of changes in osmotic pressure. In this way, the cell wall functions like a bicycle tire that maintains the necessary shape and prevents the more delicate inner tube (the cytoplasmic membrane) from bursting when it is expanded.



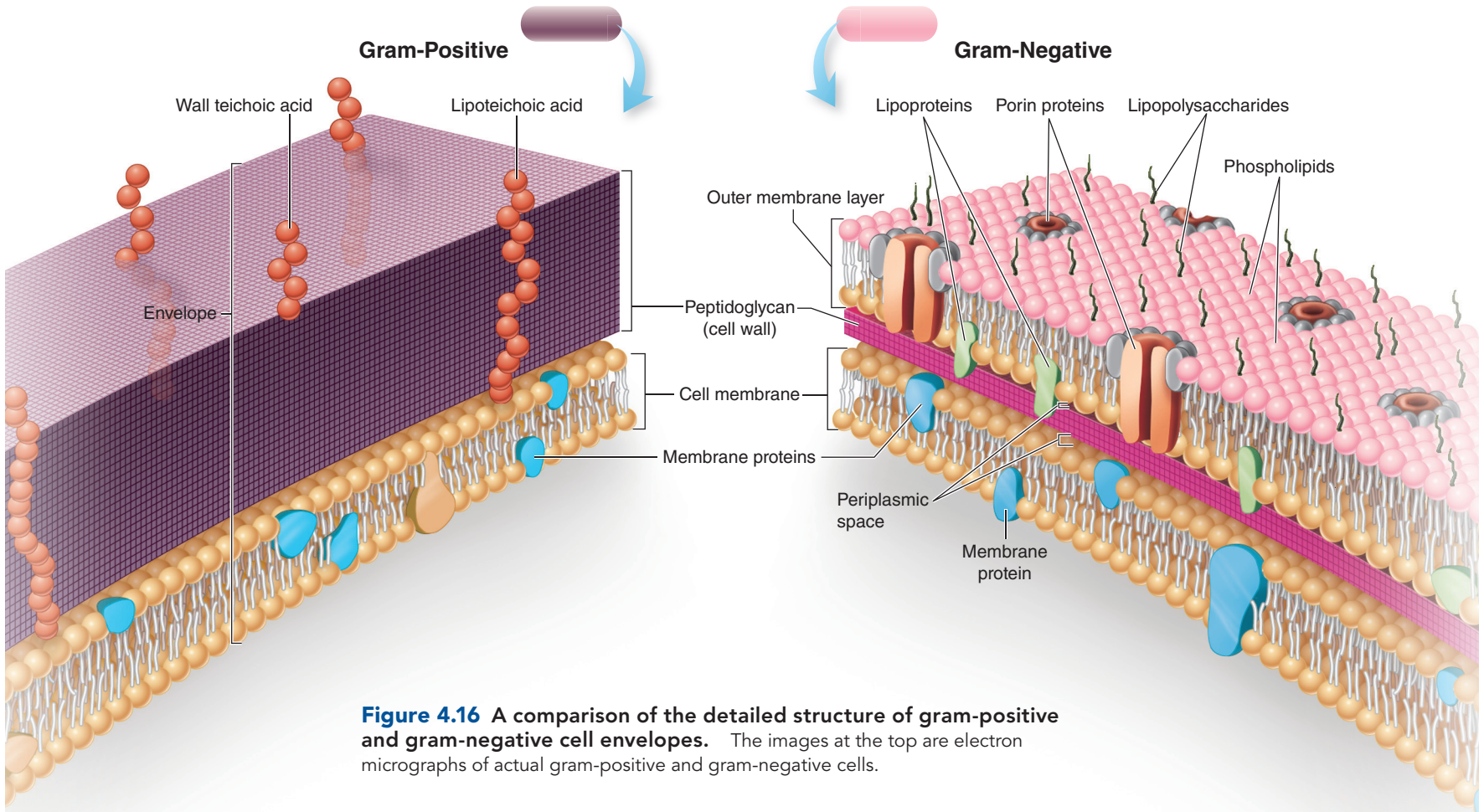
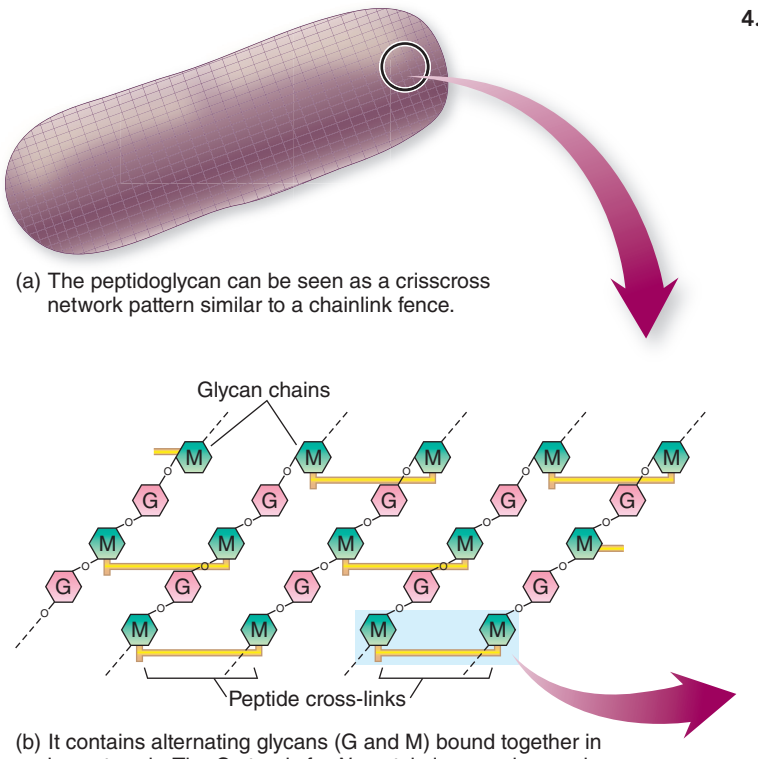


Figure 4.16 A comparison of the detailed structure of gram-positive and gram-negative cell envelopes. The images at the top are electron micrographs of actual gram-positive and gram-negative cells.



(a) The peptidoglycan can be seen as a crisscross network pattern similar to a chainlink fence.

(b) It contains alternating glycans (G and M) bound together in long strands. The G stands for *N*-acetyl glucosamine, and the M stands for *N*-acetyl muramic acid.

(c) A detailed view of the links between the muramic acids. Tetrapeptide chains branching off the muramic acids connect by interbridges also composed of amino acids. It is this linkage that provides rigid yet flexible support to the cell and that may be targeted by drugs like penicillin.

Figure 4.17 Structure of peptidoglycan in the cell wall.

The cell walls of most bacteria gain their relatively rigid quality from a unique macromolecule called **peptidoglycan** (PG). This compound is composed of a repeating framework of long **glycan** (sugar) chains cross-linked by short peptide (protein) fragments to provide a strong but flexible support framework **(figure 4.17).** The amount and exact composition of peptidoglycan vary among the major bacterial groups.

Because many bacteria live in aqueous habitats with a low concentration of dissolved substances, they are constantly absorbing excess water by osmosis. Were it not for the strength and relative rigidity of the peptidoglycan in the cell wall, they would rupture from internal pressure. This function of the cell wall has been a tremendous boon to the drug industry. Several types of drugs used to treat infection (penicillin, cephalosporins) are effective because they target the peptide cross-links in the peptidoglycan, thereby disrupting its integrity. With their cell walls incomplete or missing, such cells have very little protection from **lysis** (ly′-sis), which is the disintegration or rupture of the cell. Lysozyme, an enzyme contained in tears and saliva, provides a natural defense against certain bacteria by hydrolyzing the bonds in the glycan chains and causing the wall to break down. (Chapter 11 discusses the actions of antimicrobial chemical agents.)

The Gram-Positive Cell Wall

The bulk of the gram-positive cell wall is a thick, homogeneous sheath of peptidoglycan ranging from 20 to 80 nm in thickness. It also contains tightly bound acidic polysaccharides, including **teichoic acid** and **lipoteichoic acid** (see figure 4.16). Teichoic acid is a polymer of ribitol or glycerol (alcohols) and phosphate that is embedded in the peptidoglycan sheath. Lipoteichoic acid is similar in structure but is attached to the lipids in the plasma membrane. These molecules probably function in cell wall maintenance and enlargement during cell division, and they also contribute to the acidic charge on the cell surface.

The Gram-Negative Cell Wall

The gram-negative wall is a single, thin (1–3 nm) sheet of peptidoglycan. Although it acts as a somewhat rigid protective structure as previously described, its thinness gives gram-negative bacteria a relatively greater flexibility and sensitivity to lysis.

Nontypical Cell Walls

Several bacterial groups lack the cell wall structure of gram-positive or gram-negative bacteria, and some bacteria have

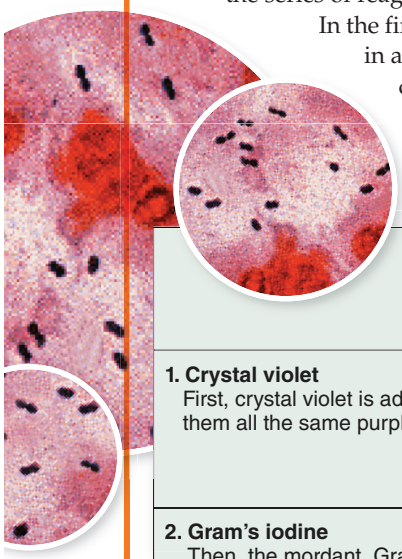
INSIGHT 4.2 The Gram Stain: A Grand Stain

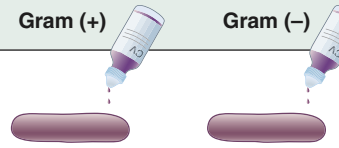
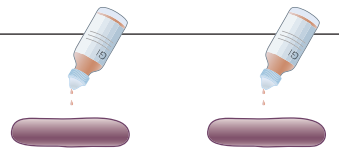
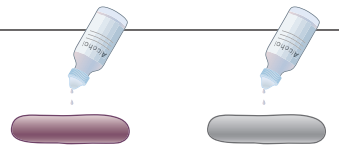
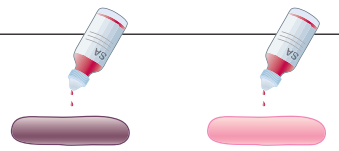
In 1884, Hans Christian Gram discovered a staining technique that could be used to make bacteria in infectious specimens more visible. His technique consisted of timed, sequential applications of crystal violet (the primary dye), Gram's iodine (the mordant), an alcohol rinse (decolorizer), and a contrasting counterstain. The initial counterstain used was yellow or brown and was later replaced by the red dye safranin. Bacteria that stain purple are called gram-positive, and those that stain red are called gramnegative.

Although these staining reactions involve an attraction of the cell to a charged dye (see chapter 3), it is important to note that the terms **gram-positive** and **gram-negative** are not used to indicate the electrical charge of cells or dyes but whether or not a cell retains the primary dye-iodine complex after decolorization. There is nothing specific in the reaction of gram-positive cells to the primary dye or in the reaction of gram-negative cells to the counterstain. The different results in the Gram stain are due to differences in the structure of the cell wall and how it reacts to the series of reagents applied to the cells.

In the first step, crystal violet is added to the cells in a smear. It stains them all the same purple color. The second and key differentiating step is the addition of the mordant—Gram's iodine. The mordant is a stabilizer that causes the dye to form large complexes in the peptidoglycan meshwork of the cell wall. Because the peptidoglycan layer in gram-positive cells is thicker, the entrapment of the dye is far more extensive in them than in gram-negative cells. Application of alcohol in the third step dissolves lipids in the outer membrane and removes the dye from the peptidoglycan layer and the gramnegative cells. By contrast, the crystals of dye tightly embedded in the peptidoglycan of gram- positive bacteria are relatively inaccessible and resistant to removal. Because gram-negative bacteria are colorless after decolorization, their presence is demonstrated by applying the counterstain safranin in the final step.

This century-old staining method remains the universal basis for bacterial classification and identification. It permits differentiation of four major categories based upon color reaction and shape: gram-positive rods, gram-positive cocci, gram-negative rods, and gram-negative cocci (see table 4.2). The Gram stain can also be a practical aid in diagnosing infection and in guiding drug treatment. For example, Gram staining a fresh urine or throat specimen can help pinpoint the possible cause of infection, and in some cases it is possible to begin drug therapy on the basis of this stain. Even in this day of elaborate and expensive medical technology, the Gram stain remains an important and unbeatable first tool in diagnosis.



Step	Microscopic Appearance of Cell		Chemical Reaction in Cell Wall (very magnified view)	
	Gram (+)	Gram (–)	Gram (+)	Gram (–)
1. Crystal violet First, crystal violet is added to the cells in a smear. It stains them all the same purple color.			 Both cell walls affix the dye	
2. Gram's iodine Then, the mordant, Gram's iodine, is added. This is a stabilizer that causes the dye to form large complexes in the peptidoglycan meshwork of the cell wall. The thicker gram-positive cell walls are able to more firmly trap the large complexes than those of the gram-negative cells.			 Dye complex trapped in wall	No effect of iodine
3. Alcohol Application of alcohol dissolves lipids in the outer membrane and removes the dye from the peptidoglycan layer—only in the gram-negative cells.			 Crystals remain in cell wall	Outer membrane weakened; wall loses dye
4. Safranin (red dye) Because gram-negative bacteria are colorless after decolorization, their presence is demonstrated by applying the counterstain safranin in the final step.			 Red dye masked by violet	Red dye stains the colorless cell

no cell wall at all. Although these exceptional forms can stain positive or negative in the Gram stain, examination of their fine structure and chemistry shows that they do not really fit the descriptions for typical gram-negative or -positive cells. For example, the cells of *Mycobacterium* and *Nocardia* contain peptidoglycan and stain gram-positive, but the bulk of their cell wall is composed of unique types of lipids. One of these is a very-long-chain fatty acid called **mycolic acid**, or cord factor, that contributes to the pathogenicity of this group (see chapter 21). The thick, waxy nature imparted to the cell wall by these lipids is also responsible for a high degree of resistance to certain chemicals and dyes. Such resistance is the basis for the **acid-fast stain** used to diagnose tuberculosis and leprosy. In this stain, hot carbol fuchsin dye becomes tenaciously attached (is held fast) to these cells so that an acid-alcohol solution will not remove the dye (see chapter 3).

Mycoplasmas and Other Cell-Wall-Deficient Bacteria

Mycoplasmas are bacteria that naturally lack a cell wall. Although other bacteria require an intact cell wall to prevent the bursting of the cell, the mycoplasma cell membrane is stabilized by sterols and is resistant to lysis. These extremely tiny, **pleomorphic** cells are very small bacteria, ranging from 0.1 to 0.5 µm in size. They range in shape from filamentous to coccus or doughnut-shaped. They are *not* obligate parasites and can be grown on artificial media, although added sterols are required for the cell membranes of some species. Mycoplasmas are found in many habitats, including plants, soil, and animals. The most important medical species is *Mycoplasma pneumoniae* **(figure 4.18),** which adheres to the epithelial cells in the lung and causes an atypical form of pneumonia in humans (described in chapter 21).

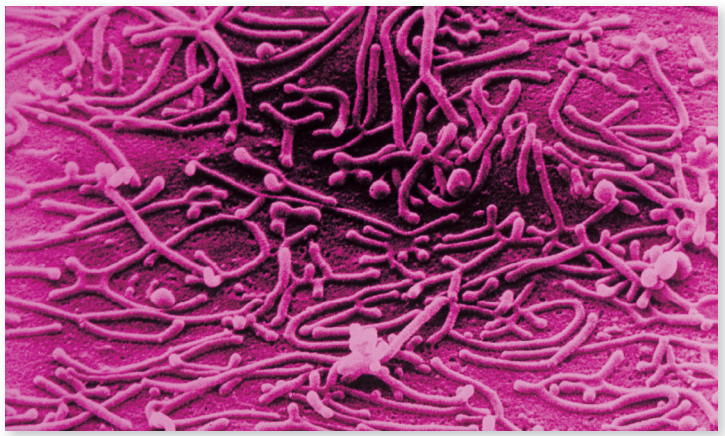


Figure 4.18 Scanning electron micrograph of *Mycoplasma pneumoniae* (magnified 62,000×). Cells like these that naturally lack a cell wall exhibit extreme variation in shape.

The Gram-Negative Outer Membrane

The **outer membrane** (OM) is somewhat similar in construction to the cell membrane, except that it contains specialized types of polysaccharides and proteins. The uppermost layer of the OM "sandwich" contains **lipopolysaccharide** (LPS). The polysaccharide chains extending off the surface function as cell markers and receptors. The lipid portion of LPS has been referred to as **endotoxin** because it stimulates fever and shock reactions in gram-negative infections such as meningitis and typhoid fever. The innermost layer of the OM is a phospholipid layer anchored by means of lipoproteins to the peptidoglycan layer below. The outer membrane serves as a partial chemical sieve by allowing only relatively small molecules to penetrate. Access is provided by special membrane channels formed by **porin proteins** that completely span the outer membrane. The size of these porins can be altered so as to block the entrance of harmful chemicals, making them one defense of gram-negative bacteria against certain antibiotics (see figure 4.16).

Cell Membrane Structure

Appearing just beneath the cell wall is the **cell membrane,** which is often called the **cytoplasmic membrane.** It is a very thin (5–10 nm), flexible sheet molded completely around the cytoplasm. Its general composition was described in chapter 2 as a lipid bilayer with proteins embedded to varying degrees (see Insight 2.3). Bacterial cell membranes have this typical structure, containing primarily phospholipids (making up about 30%–40% of the membrane mass) and proteins (contributing 60%–70%). Major exceptions to this description are the membranes of mycoplasmas, which contain high amounts of sterols—rigid lipids that stabilize and reinforce the membrane; and the membranes of archaea, which contain unique branched hydrocarbons rather than fatty acids.

Some environmental bacteria, including photosynthesizers and ammonia oxidizers, contain dense stacks of internal membranes that are studded with enzymes or photosynthetic pigments. The inner membranes allow a higher concentration of these enzymes and pigments and also accomplish a compartmentalization that allows for higher energy production.

Functions of the Cell Membrane

Because bacteria have none of the eukaryotic organelles, the cell membrane provides a site for functions such as energy reactions, nutrient processing, and synthesis. A major action of the cell membrane is to regulate **transport,** the passage of nutrients into the cell and the discharge of wastes. Although water and small uncharged molecules can diffuse across the membrane unaided, the membrane is a **selectively permeable** structure with special carrier mechanisms for passage of most molecules (see chapter 7). The cell membrane is

also involved in **secretion,** or the discharge of a metabolic product into the extracellular environment.

The membranes of bacteria are an important site for a number of metabolic activities. Many enzymes of respiration and ATP synthesis reside in the cell membrane because these cells lack mitochondria (see chapter 8). Enzyme structures located in the cell membrane also help synthesize structural macromolecules to be incorporated into the cell envelope and appendages. Other products (enzymes and toxins) are secreted by the membrane into the extracellular environment.

Practical Considerations of Differences in Cell Envelope Structure

Variations in cell envelope anatomy contribute to several other differences between the two cell types. The outer membrane contributes an extra barrier in gram-negative bacteria that makes them impervious to some antimicrobial chemicals such as dyes and disinfectants, so they are generally more difficult to inhibit or kill than are gram-positive bacteria. One exception is alcohol-based compounds, which can dissolve the lipids in the outer membrane and disturb its integrity. Treating infections caused by gram-negative bacteria often requires different drugs from gram-positive infections, especially drugs that can cross the outer membrane.

The cell envelope or its parts can interact with human tissues and contribute to disease. Proteins attached to the outer portion of the cell wall of several gram-positive species, including *Corynebacterium diphtheriae* (the agent of diphtheria) and *Streptococcus pyogenes* (the cause of strep throat), also have toxic properties. The lipids in the cell walls of certain *Mycobacterium* species are harmful to human cells as well. Because most macromolecules in the cell walls are foreign to humans, they stimulate antibody production by the immune system (see chapter 15).

Looking at the unique structures within both gram-negative and gram-positive cell envelopes, we gain insight into the potential targets for new drug development by researchers today.

4.3 Learning Outcomes—Assess Your Progress

8. Differentiate between the two main types of bacterial envelope structure.
9. Discuss why gram-positive cell walls are stronger than gram-negative cell walls.
10. Name a substance in the envelope structure of some bacteria that can cause severe symptoms in humans.

4.4 Bacterial Internal Structure

Contents of the Cell Cytoplasm

Cytoplasm is a gelatinous solution encased by the cell membrane. It is another prominent site for many of the cell's biochemical and synthetic activities. Its major component is water (70%–80%), which serves as a solvent for the cell pool, a complex mixture of nutrients including sugars, amino acids, and salts. The components of this pool serve as building blocks for cell synthesis or as sources of energy. The cytoplasm also contains larger, discrete cell masses such as the chromatin body, ribosomes, granules, and fibers resembling actin and tubulin strands that act as a cytoskeleton in bacteria that have them.

Bacterial Chromosomes and Plasmids: The Sources of Genetic Information

The hereditary material of most bacteria exists in the form of a single circular strand of DNA designated as the **bacterial chromosome.** Some bacteria have multiple chromosomes. By definition, bacteria do not have a nucleus; that is, their DNA is not enclosed by a nuclear membrane but instead is aggregated in a dense area of the cell called the **nucleoid (figure 4.19).** (Note that a very few species of bacteria have been found to have a nucleus-like structure, but these remain the exception.) The chromosome is actually an extremely long molecule of double-stranded DNA that is tightly coiled around special basic protein molecules so as to fit inside the cell compartment. Arranged along its length are genetic units (genes) that carry information required for bacterial maintenance and growth.

Although the chromosome is the minimal genetic requirement for bacterial survival, many bacteria contain other nonessential pieces of DNA called **plasmids** (refer to figure 4.1 for a representation of the nuclear material)**.** Plasmids exist as separate double-stranded circles of DNA, although at times they can become integrated into the chromosome. During conjugation, they may be duplicated and passed on to related nearby bacteria. During bacterial reproduction, they are duplicated and passed on to offspring. They are not essential to bacterial growth and metabolism, but they often confer protective traits such as the ability to resist drugs and to produce toxins and enzymes (see chapter 9). Because they can be readily manipulated in the laboratory and transferred from one bacterial cell to another, plasmids are an important agent in genetic engineering techniques.

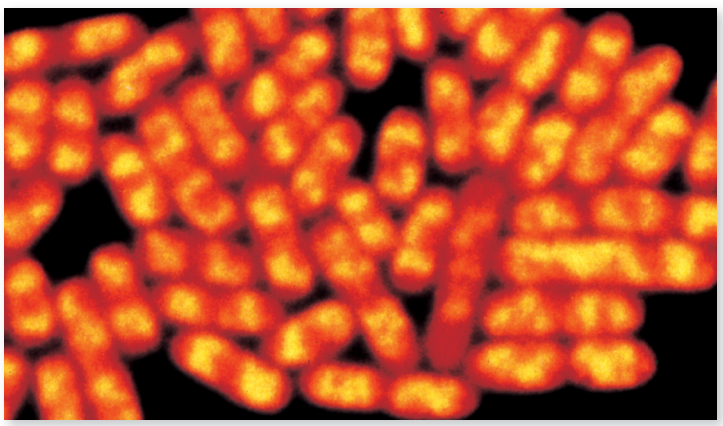


Figure 4.19 Chromosome structure. Fluorescent staining highlights the chromosomes of the bacterial pathogen *Salmonella enteritidis*. The cytoplasm is orange, and the chromosome(s) fluoresce(s) bright yellow.

Disease Connection

Typhoid fever is a major disease in the developing world. It is caused by *Salmonella enterica* serovar Typhi. Before 1995, these bacteria carried a variety of different plasmids carrying a variety of different genes. Ninety-eight percent of the isolates collected after 1995, however, carry a single type of plasmid, which confers resistance to multiple drugs and also allows the bacterium to survive in high-salt environments. Researchers speculate that these abilities give the bacterium a competitive advantage over bacteria that had the other plasmid types.

Ribosomes: Sites of Protein Synthesis

All cells contain thousands of tiny **ribosomes,** which are made of RNA and protein. When viewed even by very high magnification, ribosomes show up as fine, spherical specks dispersed throughout the cytoplasm and often occur in chains called polysomes. Many are also attached to the cell membrane. Chemically, a ribosome is a combination of a special type of RNA called ribosomal RNA, or rRNA (about 60%), and protein (40%). One method of characterizing ribosomes is by S, or Svedberg,[3] units, which rate the molecular sizes of various cell parts that have been spun down and separated by molecular weight and shape in a centrifuge. Heavier, more compact structures sediment faster and are assigned a higher S rating. Combining this method of analysis with high-resolution electron microscopy has revealed that the ribosome in bacteria, which has an overall rating of 70S, is actually composed of two smaller subunits **(figure 4.20).** They fit together to form a miniature platform upon which protein synthesis is performed. Note that eukaryotic ribosomes have a rating of 80S, making bacterial ribosomes a unique target for therapeutic drugs. We examine the more detailed functions of ribosomes in chapter 9 and drug targeting in chapter 12.

3. Named in honor of T. Svedberg, the Swedish chemist who developed the ultracentrifuge in 1926.

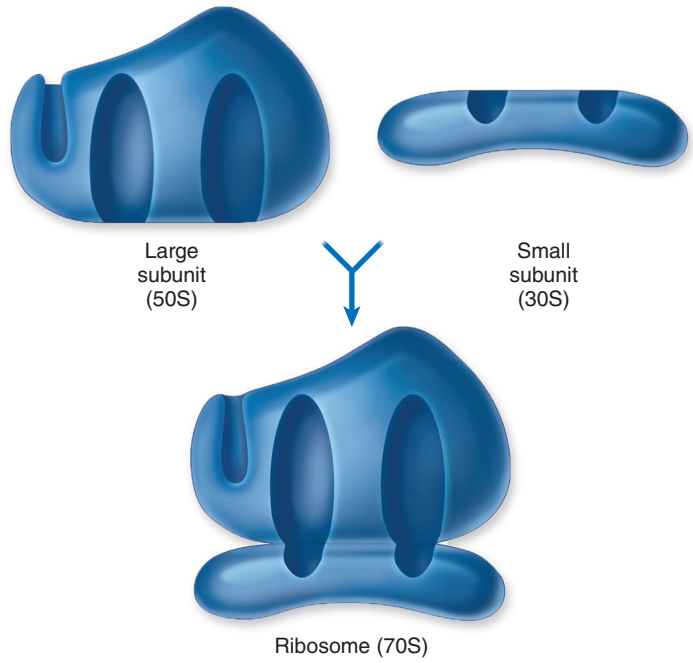
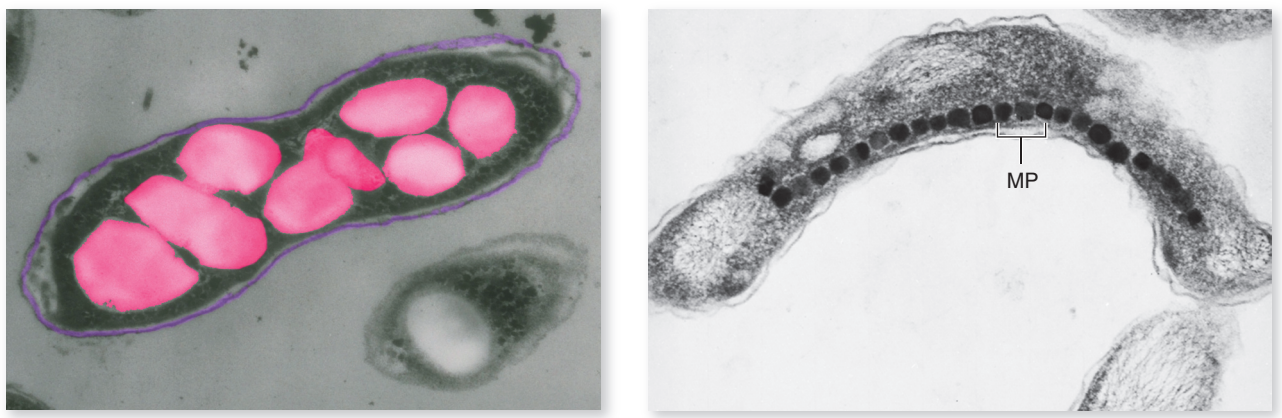


Figure 4.20 **A model of a bacterial ribosome, showing the small (30S) and large (50S) subunits, both separate and joined.**

Inclusion Bodies and Microcompartments

Most bacteria are exposed to severe shifts in the availability of food. During periods of nutrient abundance, some can compensate by laying down nutrients intracellularly in **inclusion bodies,** or **inclusions,** of varying size, number, and content. As the environmental source of these nutrients becomes depleted, the bacterial cell can mobilize its own storehouse as required. Some inclusion bodies carry condensed, energy-rich organic substances, such as glycogen and polyhydroxybutyrate (PHB), within special single-layered membranes **(figure 4.21).** A unique type of inclusion found in some aquatic bacteria is gas vesicles that



(a) (b)

Figure 4.21 **Bacterial inclusion bodies.** **(a)** Large particles (pink) of polyhydroxybutyrate are deposited in a concentrated form that provides an ample long-term supply of that nutrient (32,500×). **(b)** A section through *Aquaspirillum* reveals a chain of tiny iron magnets, or magnetosomes (MP). These unusual bacteria use these inclusions to orient themselves within their habitat (123,000×).

provide buoyancy and flotation. Other inclusions, also called granules, are crystals of inorganic compounds and are not enclosed by membranes. Sulfur granules of photosynthetic bacteria and polyphosphate granules of *Corynebacterium* and *Mycobacterium*, described later, are of this type. The latter represent an important source of building blocks for nucleic acid and ATP synthesis. They have been termed **metachromatic granules** because they stain a contrasting color (red, purple) in the presence of methylene blue dye.

Perhaps the most unique cell granule is involved not in cell nutrition but rather in cell orientation. Magnetotactic bacteria contain crystalline particles of iron oxide (magnetosomes) that have magnetic properties. The bacteria use these granules to be pulled by the polar and gravitational fields into deeper habitats with a lower oxygen content.

In the early 2000s, new compartments inside bacterial cells were discovered. These were named bacterial microcompartments, or BMCs. Their outer shells are made of protein, arranged geometrically, and are packed full of enzymes that are designed to work together in pathways, thereby ensuring that they are in close proximity to one another.

The Cytoskeleton

Until very recently, scientists thought that the shape of all bacteria was completely determined by the peptidoglycan layer (cell wall). Although this is true of many bacteria, particularly the cocci, other bacteria produce long polymers of proteins that are very similar to eukaryotic **actin.** In bacteria, these are arranged in helical ribbons around the cell just under the cell membrane **(figure 4.22).** Fibers contribute to cell shape, perhaps by influencing the way peptidoglycan is manufactured, and also function in cell division. The fibers have been found in rod-shaped and spiral bacteria. They are composed in part of proteins unique to bacterial cells, making them a potentially powerful target for future antibiotic development.

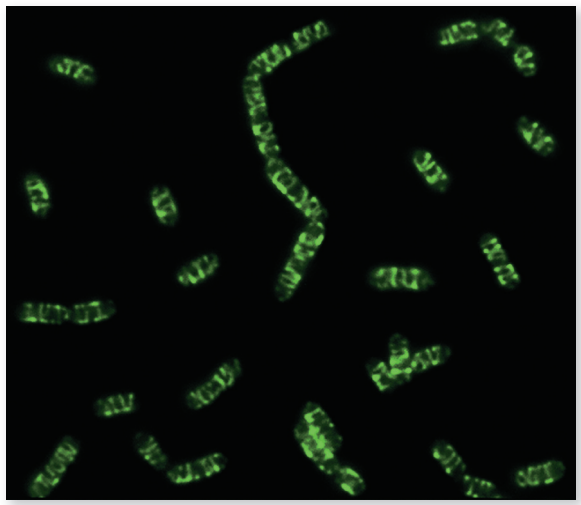


Figure 4.22 Bacterial cytoskeleton. The fibers in these rod-shaped bacteria are fluorescently stained.

Bacterial Endospores: An Extremely Resistant Stage

Ample evidence indicates that the anatomy of bacteria helps them adjust rather well to adverse habitats. But of all microbial structures, nothing can compare to the bacterial **endospore** (or, simply, *spore*) for withstanding hostile conditions and facilitating survival.

Endospores are dormant bodies produced by bacteria of the genera *Bacillus*, *Clostridium*, and *Sporosarcina*. These bacteria have a two-phase life cycle—a vegetative cell and an endospore **(figure 4.23).** The vegetative cell is a metabolically active and growing entity that can be induced by environmental conditions to undergo spore formation, or **sporulation.** Once formed, the spore exists in an inert, resting condition that shows up prominently in a spore or Gram stain (figure 4.23). Features of spores, including size, shape, and position in the vegetative cell, are somewhat useful in identifying some species. Both gram-positive and gram-negative bacteria can form endospores, but the medically relevant ones are all gram-positive. Most bacteria form only one endospore; therefore, this is not a reproductive function for them.

Bacterial endospores are the hardiest of all life forms, capable of withstanding extremes in heat, drying, freezing, radiation, and chemicals that would readily kill vegetative cells. Their survival under such harsh conditions is due to several factors. The heat resistance of spores has been linked to their high content of calcium and **dipicolinic acid.** We know, for instance, that heat destroys cells by inactivating proteins and DNA and that this process requires a certain amount of water in the protoplasm. Because the deposition of calcium dipicolinate in the endospore removes water and leaves the endospore very dehydrated, it is less vulnerable to the effects of heat. It is also metabolically inactive and highly resistant to damage from further drying. The thick, impervious cortex and spore coats also protect against radiation and chemicals. The longevity of bacterial spores verges on immortality. One record describes the isolation of

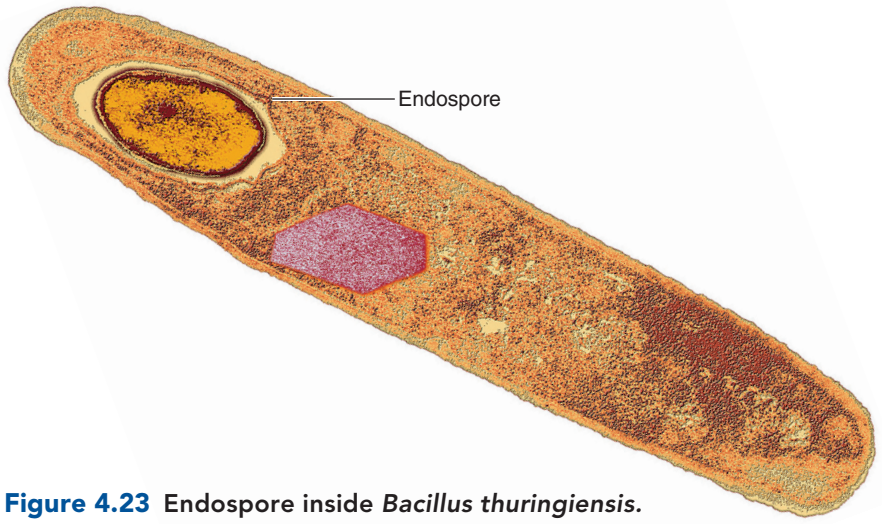


Figure 4.23 Endospore inside *Bacillus thuringiensis*. The genus *Bacillus* forms endospores. *B. thuringiensis* additionally forms crystalline bodies (pink) that are used as insecticides.

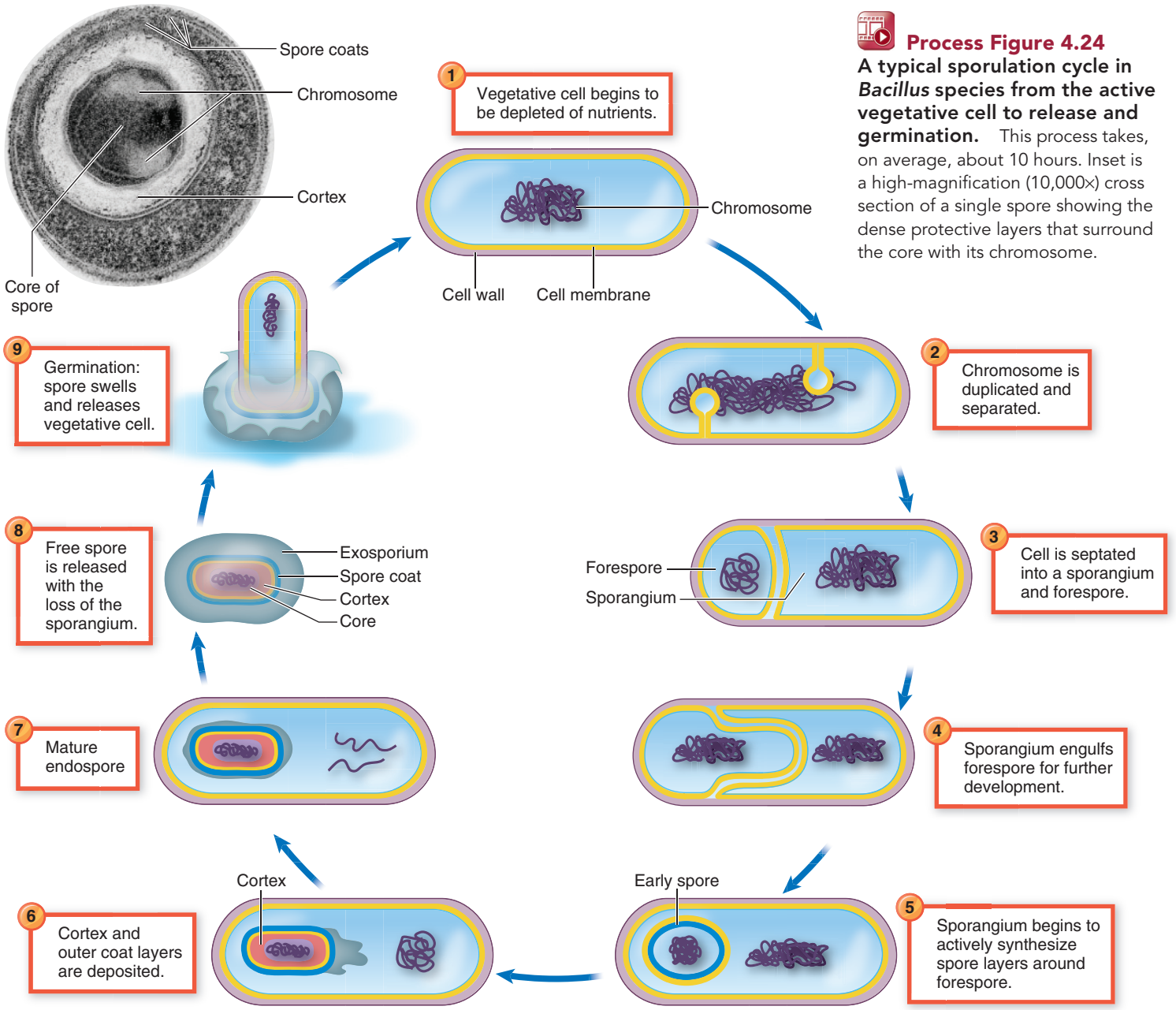
viable endospores from a fossilized bee that was 25 million years old. More recently, microbiologists unearthed a viable endospore from a 250-million-year-old salt crystal. Initial analysis of this ancient microbe indicates it is a species of *Bacillus* that is genetically different from known species.

Endospore Formation and Resistance

The depletion of nutrients, especially an adequate carbon or nitrogen source, is the stimulus for a vegetative cell to begin endospore formation. Once this stimulus has been received by the vegetative cell, it undergoes a conversion to become a sporulating cell called a **sporangium.** Complete transformation of a vegetative cell into a sporangium and then into an endospore requires 6 to 8 hours in most spore-forming species. **Process Figure 4.24** illustrates some major physical and chemical events in this process.

The Germination of Endospores

After lying in a state of inactivity for an indefinite time, endospores can be revitalized when favorable conditions arise. The breaking of dormancy, or germination, happens in the presence of water and a specific chemical or environmental stimulus (germination agent). Once initiated, it proceeds to completion quite rapidly (1½ hours). Although the specific germination agent varies among species, it is generally a small organic molecule such as an amino acid or an inorganic salt. This agent stimulates the formation of hydrolytic (digestive) enzymes by the endospore membranes. These enzymes digest the cortex and expose the core to water. As the core rehydrates and takes up nutrients, it begins to grow out of the endospore coats. In time, it reverts to a fully active vegetative cell, resuming the vegetative cycle.



Process Figure 4.24
A typical sporulation cycle in *Bacillus* species from the active vegetative cell to release and germination. This process takes, on average, about 10 hours. Inset is a high-magnification (10,000×) cross section of a single spore showing the dense protective layers that surround the core with its chromosome.

A Note on Terminology

The word *spore* can have more than one usage in microbiology. It is a generic term that refers to any tiny compact cell that is produced by vegetative or reproductive structures of microorganisms. Fungi have spores that serve as reproductive structures. The bacterial type discussed here is most accurately called an **endospore,** because it is produced inside a cell. They function in *survival*, not in reproduction, because no increase in cell numbers is involved in their formation. In contrast, the fungi produce many different types of spores for both survival and reproduction (see chapter 5).

Medical Significance of Bacterial Spores

Although the majority of endospore-forming bacteria are relatively harmless, several bacterial pathogens are endospore-formers. In fact, some aspects of the diseases they cause are related to the persistence and resistance of their endospores. *Bacillus anthracis* is the agent of anthrax; its persistence in endospore form makes it an ideal candidate for bioterrorism. The genus *Clostridium* includes even more pathogens, such as *C. tetani,* the cause of tetanus (lockjaw); *C. difficile,* the cause of pseudomembranous colitis; and *C. perfringens,* the cause of gas gangrene. When the endospores of these species are embedded in a wound that contains dead tissue, they can germinate, grow, and release potent toxins. Another toxin-forming species, *C. botulinum,* is the agent of botulism, a deadly form of food poisoning. (Each of these disease conditions is discussed in the infectious disease chapters, according to the organ systems it affects.)

Because they inhabit the soil and dust, endospores are constant intruders where sterility and cleanliness are important. They resist ordinary cleaning methods that use boiling water, soaps, and disinfectants; and they frequently contaminate cultures and media. Hospitals and clinics must take precautions to guard against the potential harmful effects of endospores in wounds, especially those of *Clostridium difficile,* the causative agent of a gastrointestinal disease commonly known as *C. diff.* Endospore destruction is a particular concern of the food-canning industry. Several endospore-forming species cause food spoilage or poisoning. Ordinary boiling (100°C) will usually not destroy such endospores, so canning is carried out in pressurized steam at 120°C for 20 to 30 minutes. Such rigorous conditions ensure that the food is sterile and free from viable bacteria.

4.4 Learning Outcomes—Assess Your Progress

11. Identify five structures that may be contained in bacterial cytoplasm.

12. Detail the causes and mechanisms of sporogenesis and germination.

4.5 The Archaea: The Other "Prokaryotes"

The discovery and characterization of novel cells resembling bacteria that have unusual anatomy, physiology, and genetics changed our views of microbial taxonomy and classification (see chapter 1). These single-celled, simple organisms, called **archaea,** are now considered a third cell type in a separate superkingdom (the Domain Archaea). We include them in this chapter because they share many bacterial characteristics. But it has become clear that they are actually more closely related to Domain Eukarya than to Bacteria. For example, archaea and eukaryotes share a number of ribosomal RNA sequences that are not found in bacteria, and their protein synthesis and ribosomal subunit structures are similar. **Table 4.1** outlines selected points of comparison of the three domains.

Among the ways that the archaea differ significantly from other cell types are that certain genetic sequences are found only in their rRNA, and that they exhibit a unique

Table 4.1 Comparison of Three Cellular Domains

Characteristic	Bacteria	Archaea	Eukarya
Cell type	Prokaryotic	Prokaryotic	Eukaryotic
Chromosomes	Single, or few, circular	Single, circular	Several, linear
Types of ribosomes	70S	70S but structure is similar to 80S	80S
Contains unique ribosomal RNA signature sequences	+	+	+
Number of sequences shared with Eukarya	1	3	All
Protein synthesis similar to Eukarya	–	+	
Presence of peptidoglycan in cell wall	+	–	–
Cell membrane lipids	Fatty acids with ester linkages	Long-chain, branched hydrocarbons with ether linkages	Fatty acids with ester linkages
Sterols in membrane	– (Some exceptions)	–	+
Pili	N-linked glycans	O-linked glycans	None

method of DNA compaction. They also have unique membrane lipids, cell wall composition, and pilin proteins.

The archaea exhibit unusual and chemically distinct cell walls. In some, the walls are composed almost entirely of polysaccharides, and in others, the walls are pure protein; but as a group, they all lack the true peptidoglycan structure described previously. Because a few archaea lack a cell wall entirely, their cell membrane must serve the dual functions of support and transport.

It is clear that the archaea are the most primitive of all current life forms and are most closely related to the first cells that originated on the earth 4 billion years ago. The early earth is thought to have contained a hot, anaerobic "soup" with sulfuric gases and salts in abundance. The modern archaea still live in the remaining habitats on the earth that have these same ancient conditions—the most extreme habitats in nature. It is for this reason that they are often called extremophiles, meaning that they "love" extreme conditions in the environment.

Metabolically, the archaea exhibit incredible adaptations to what would be deadly conditions for other organisms. These hardy microbes have adapted to multiple combinations of heat, salt, acid, pH, pressure, and atmosphere. Included in this group are methane producers, hyperthermophiles, extreme halophiles, and sulfur reducers.

Members of the group called **methanogens** can convert CO_2 and H_2 into methane gas (CH_4) through unusual and complex pathways. These archaea are common inhabitants of anaerobic swamp mud, the bottom sediments of lakes and oceans, and even the digestive systems of animals. The gas they produce collects in swamps and may become a source of fuel. Methane may also contribute to the "greenhouse effect," which maintains the earth's temperature and can contribute to global warming (see chapter 24).

Other types of archaea—the extreme halophiles—require salt to grow, and some have such a high salt tolerance that they can multiply in sodium chloride solutions (36% NaCl) that would destroy most cells. They exist in the saltiest places

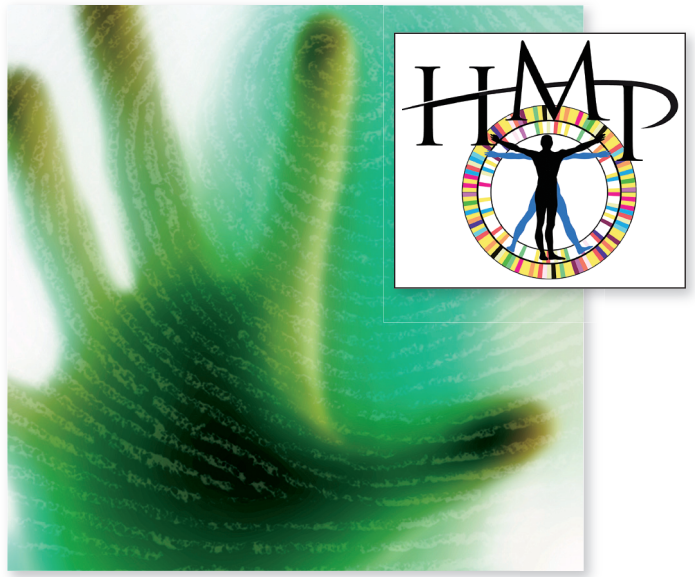
INSIGHT 4.3 CSI: Bacteria?

What if, instead of fingerprints, crime scene investigators looked for patterns of specific bacteria left behind by suspects? Noah Fierer at the University of Colorado, Boulder, studied the variability in bacterial communities on human fingertips and found that this scenario isn't as unlikely as you may think.

Fierer and his colleagues took samples from computer keyboards and computer mice, analyzed the bacterial DNA from the samples, and came up with a bacterial "fingerprint" that could be matched to the individual that had used the keyboard or mouse. His analysis showed that there is only about a 13% correspondence of bacterial species between any two individuals and that these communities of bacteria on the skin are stable over time, recovering themselves within a few hours after washing. Additionally, because skin bacteria are resistant to varying environmental conditions, these bacterial fingerprints can persist on surfaces for up to 2 weeks.

Current forensic analysis requires enough DNA from a crime scene (blood, semen, tissue, or saliva) to amplify, analyze, and match to a suspect. Bacterial cells are much more abundant on skin surfaces, and their DNA is much easier to recover and amplify. Further testing is needed to determine if these methods could actually be feasible for use in forensics, but Fierer brings up an excellent question: "Could our microbial fingerprint be more personally identifying than our human genome?"

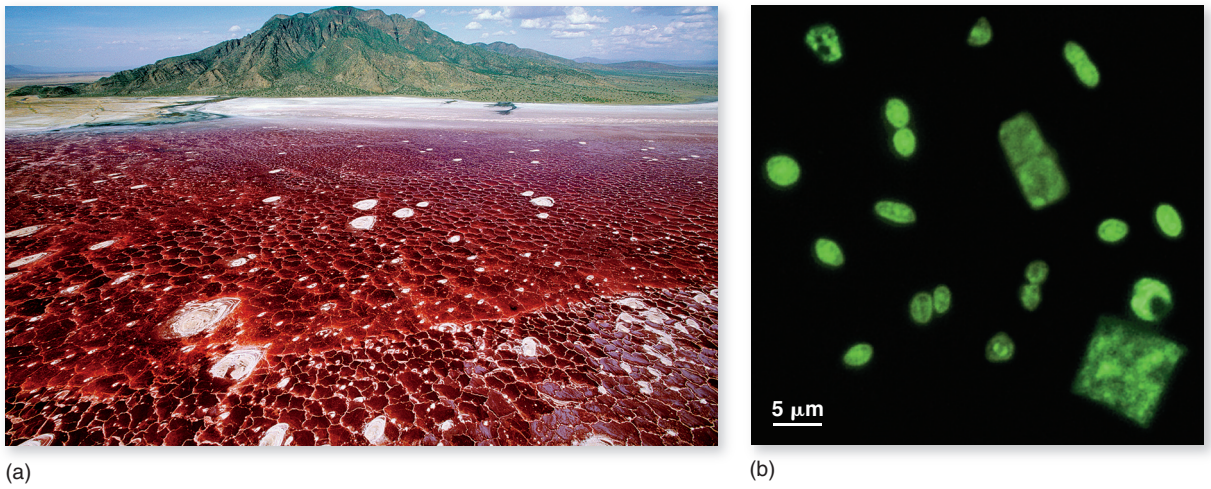
Fierer's work is part of a much larger project designed to identify and study the microbes that live on and in our bodies: the Human Microbiome Project (HMP). Using metagenomic techniques developed for the Human Genome Project and J. Craig Venter's quest to discover novel microbes in the oceans, the HMP has been launched to study all of the microbes colonizing the human body. The goal of the HMP is to sequence as many as 3,000 genomes from cultured and uncultured



bacteria as well as other microbes such as archaea, fungi, and protozoa that make up the human microbiome. This preliminary genome collection will serve as a reference to determine whether there is a core microbiome common to all human beings or the degree to which there are commonalities. The main body sites that are being studied are the oral cavity, skin, vagina, gut, and nasal/lung cavities. The HMP will also study the relationship between diseases and changes in the human microbiome.

Could the next clues at a crime scene be bacterial?

Source: 2010. *Proc. Natl. Acad. Sci. U.S.A.* vol. 107, no. 14, p. 6477.



(a) (b)

Figure 4.25 Halophiles around the world. **(a)** Lake Natron in the Great Rift Valley on the border of Tanzania and Kenya. Halophilic algae give the lake its color. **(b)** A sample taken from a saltern in Australia viewed by fluorescent microscopy (1,000×). Note the range of cell shapes (cocci, rods, and squares) found in this community.

on the earth—inland seas, salt lakes, salt mines, and in salted fish. They are not particularly common in the ocean because the salt content is not high enough. Many of the "halobacteria" use a red pigment to synthesize ATP in the presence of light. These pigments are responsible for the color of the Red Sea, and the red color of salt ponds **(figure 4.25).**

Archaea that are adapted to growth at very low temperatures are called **psychrophilic** (loving cold temperatures); those growing at very high temperatures are **hyperthermophilic** (loving high temperatures). Hyperthermophiles flourish at temperatures between 80°C and 113°C and cannot grow at 50°C. They live in volcanic waters and soils and submarine vents and are also often salt- and acid-tolerant as well. One member, *Thermoplasma,* lives in hot, acidic habitats in the waste piles around coal mines that regularly sustain a pH of 1 and a temperature of nearly 60°C. Because many archaea are unculturable, the technology of rRNA sequencing has been invaluable in the identification of these microbes (see section 4.6 and chapter 17). Analysis of these unique sequences has advanced not only the process of identification but also our knowledge of transcription, translation, and cellular evolution.

Archaea are not just environmental microbes. They have been isolated from human tissues such as the colon, the mouth, and the vagina. Recently, an association was found between the degree of severity of periodontal disease and the presence of archaeal RNA sequences in the gingiva, suggesting—but not proving—that archaea may be capable of causing human disease.

4.5 Learning Outcomes—Assess Your Progress

13. List some differences between archaea and bacteria.

4.6 Classification Systems for Bacteria and Archaea

Classification systems serve both practical and academic purposes. They aid in differentiating and identifying unknown species in medical and applied microbiology. They are also useful in organizing microbes and as a means of studying their relationships and origins. Since classification was started around 200 years ago, several thousand species of bacteria and archaea have been identified, named, and cataloged.

For years, scientists have had intense interest in tracing the origins of and evolutionary relationships among bacteria and archaea, but doing so has not been an easy task. One of the questions that has plagued taxonomists is, What characteristics are the most indicative of closeness in ancestry? Early bacteriologists found it convenient to classify bacteria according to shape, variations in arrangement, growth characteristics, and habitat. However, as more species were discovered and as techniques for studying their biochemistry were developed, it soon became clear that similarities in cell shape, arrangement, and staining reactions do not automatically indicate relatedness. Even though the gram-negative rods look alike, there are hundreds of different species, with highly significant differences in biochemistry and genetics. If we attempted to classify them on the basis of Gram stain and shape alone, we could not assign them to a more specific level than class. Increasingly, classification schemes are turning to genetic and molecular traits that cannot be visualized under a microscope or in culture.

One of the most viable indicators of evolutionary relatedness and affiliation is comparison of the sequence of nitrogen bases in ribosomal RNA, a major component of ribosomes.

Ribosomes have the same function (protein synthesis) in all cells, and they tend to remain more or less stable in their nucleic acid content over long periods. Thus, any major differences in the sequence, or "signature," of the rRNA is likely to indicate some distance in ancestry. This technique is powerful at two levels: It is effective for differentiating general group differences, allowing for the creation of branching tree diagrams showing evolutionary relatedness among microbes (see figure 1.14); and it can be fine-tuned for bacterial identification at the species level (for example in *Mycobacterium* and *Legionella*). Elements of these and other identification methods are presented in more detail in chapter 17.

The definitive published source for bacterial and archaea classification, called *Bergey's Manual,* has been in print continuously since 1923. The basis for the early classification in *Bergey's* was the **phenotypic** traits of bacteria, such as their shape, cultural behavior, and biochemical reactions. These traits are still used extensively by clinical microbiologists or researchers who need to quickly identify unknown bacteria. As methods for RNA and DNA analysis became available, this information was used to supplement the phenotypic information. The current version of the publication, called *Bergey's Manual of Systematic Bacteriology,* presents a comprehensive view of bacterial and archaea relatedness, combining phenotypic information with rRNA sequencing information to classify them; it is a huge, five-volume set. (We need to remember that all classification systems are in a state of constant flux; no system is ever finished.)

With the explosion of information about evolutionary relatedness among bacteria, the need for a *Bergey's Manual* that contained easily accessible information for identifying unknown bacteria became apparent. Now there is a separate book, called *Bergey's Manual of Determinative Bacteriology,* based entirely on phenotypic characteristics. It is utilitarian in focus, categorizing bacteria by traits commonly assayed in clinical, teaching, and research labs. It is widely used by microbiologists who need to identify bacteria but need not know their evolutionary backgrounds. This phenotypic classification is more useful for students of medical microbiology, as well.

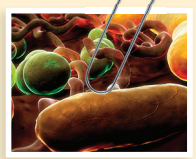
Taxonomic Scheme

Bergey's Manual of Determinative Bacteriology organizes the bacteria and archaea into four major divisions. These somewhat natural divisions are based on the nature of the cell wall. The **Gracilicutes** (gras″-ih-lik′-yoo-teez) have gram-negative cell walls and thus are thin-skinned; the **Firmicutes** have gram-positive cell walls that are thick and strong; the **Tenericutes** (ten″-er-ik′-yoo-teez) lack a cell wall and thus are soft; and the **Mendosicutes** (men-doh-sik′-yoo-teez) are the archaea. The first two divisions contain the greatest number of species. The 200 or so species that are so-far known to cause human and animal diseases can be found in four classes: the Scotobacteria, Firmibacteria, Thallobacteria, and Mollicutes. The system used in *Bergey's Manual* organizes bacteria and archaea into subcategories such as classes, orders, and families, but these are not available for all groups.

Diagnostic Scheme

As mentioned earlier, many medical microbiologists prefer an informal working system that outlines the major families and genera. **Table 4.2** is an example of an adaptation of the phenotypic method of classification that may be used in clinical microbiology. This system is more applicable for diagnosis because it is restricted to bacterial disease agents, depends less on nomenclature, and is based on readily accessible morphological and physiological tests rather than on phylogenetic relationships. It also divides the bacteria into gram-positive, gram-negative, and those without cell walls and then subgroups them according to cell shape, arrangement, and certain physiological traits such as oxygen usage. **Aerobic** bacteria use oxygen in metabolism; **anaerobic** bacteria do not use oxygen in metabolism; and **facultative** bacteria may or may not use oxygen. Further tests not listed in the

Case File 4 *Wrap-Up*



One important function of the gut microbiota is protection; the mere presence of billions of different bacteria prevents harmful bacteria from colonizing and causing infection. However, what if these helpful bacteria are removed? Often, individuals taking antibiotics for long periods of time lose their protective gut microbiota and develop ulcerative colitis caused by *Clostridium difficile*, also known as "*C-diff.*" This infection causes severe diarrhea, nausea, vomiting, and abdominal pain, and is very difficult to treat, because the organism has already been exposed to multiple antibiotics. The cure for some? Fecal transplants. Recently, gastroenterologists have suggested that a fecal "donation" from a healthy donor given via enema or through a nasogastric tube can re-establish a healthy gut microbiota and eliminate *C. difficile*. Even though the practice has been documented since 1958, few doctors are willing to admit performing fecal transplants, even though the success rate is 90% or higher. The practice is gaining acceptance in the United States as *C. difficile* infection rates increase.

What is your poop worth? Apparently more than you thought.

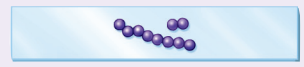
Sources: 2010. *PLoS ONE.* Vol. 1, no. 2. p. 2126.

Table 4.2 Medically Important Families and Genera of Bacteria, with Notes on Some Diseases*

I. Bacteria with Gram-Positive Cell Wall Structure

Cocci in clusters or packets
Family *Micrococcaceae: Staphylococcus* (members cause boils, skin infections)

Cocci in pairs and chains
Family *Streptococcaceae: Streptococcus* (species cause strep throat, dental caries)



Anaerobic cocci in pairs, tetrads, irregular clusters
Family *Peptococcaceae: Peptococcus, Peptostreptococcus* (involved in wound infections)

Spore-forming rods
Family *Bacillaceae: Bacillus* (anthrax), *Clostridium* (tetanus, gas gangrene, botulism)

Non-spore-forming rods
Family *Lactobacillaceae: Lactobacillus, Listeria, Erysipelothrix* (erysipeloid)
Family *Propionibacteriaceae: Propionibacterium* (involved in acne)

Family *Corynebacteriaceae: Corynebacterium* (diphtheria)

Family *Mycobacteriaceae: Mycobacterium* (tuberculosis, leprosy)

Family *Nocardiaceae: Nocardia* (lung abscesses)

Family *Actinomycetaceae: Actinomyces* (lumpy jaw), *Bifidobacterium*

Family *Streptomycetaceae: Streptomyces* (important source of antibiotics)

II. Bacteria with Gram-Negative Cell Wall Structure

Aerobic cocci
Neisseria (gonorrhea, meningitis), *Branhamella*

Aerobic coccobacilli
Moraxella, Acinetobacter

Anaerobic cocci
Family *Veillonellaceae*
Veillonella (dental disease)

Miscellaneous rods
Brucella (undulant fever), *Bordetella* (whooping cough), *Francisella* (tularemia)

Aerobic rods
Family *Pseudomonadaceae: Pseudomonas* (pneumonia, burn infections)
Miscellaneous: *Legionella* (Legionnaires' disease)

Facultative or anaerobic rods and vibrios
Family *Enterobacteriaceae: Escherichia, Edwardsiella, Citrobacter, Salmonella* (typhoid fever), *Shigella* (dysentery), *Klebsiella, Enterobacter, Serratia, Proteus, Yersinia* (one species causes plague)
Family *Vibrionaceae: Vibrio* (cholera, food infection), *Campylobacter, Aeromonas*

Miscellaneous genera: *Chromobacterium, Flavobacterium, Haemophilus* (meningitis), *Pasteurella, Cardiobacterium, Streptobacillus*

Anaerobic rods
Family *Bacteroidaceae: Bacteroides, Fusobacterium* (anaerobic wound and dental infections)

Helical and curviform bacteria
Family *Spirochaetaceae: Treponema* (syphilis), *Borrelia* (Lyme disease), *Leptospira* (kidney infection)

Obligate intracellular bacteria
Family *Rickettsiaceae: Rickettsia* (Rocky Mountain spotted fever), *Coxiella* (Q fever)
Family *Bartonellaceae: Bartonella* (trench fever, cat scratch disease)
Family *Chlamydiaceae: Chlamydia* (sexually transmitted infection)

III. Bacteria with No Cell Walls

Family *Mycoplasmataceae: Mycoplasma* (pneumonia), *Ureaplasma* (urinary infection)

*Details of pathogens and diseases appear in chapters 18 through 23.

table would be required to separate closely related genera and species. Many of these are included in later chapters on specific bacterial groups.

Species and Subspecies in Bacteria and Archaea

Among most organisms, the species level is a distinct, readily defined, and natural taxonomic category. In animals, for instance, a species is a distinct type of organism that can produce viable offspring only when it mates with others of its own kind. This definition does not work for bacteria and archaea primarily because they do not exhibit a typical mode of sexual reproduction. They can accept genetic information from unrelated forms, and they can also alter their genetic makeup by a variety of mechanisms. Thus, it is necessary to hedge a bit when we define a bacterial species. Theoretically, it is a collection of bacterial cells, all of which share an overall similar pattern of traits, in contrast to other groups whose patterns differ significantly. Although the boundaries that separate two closely related species in a genus are in some cases arbitrary, this definition still serves as a method to separate the bacteria into various kinds that can be cultured and studied.

Individual members of a given species can show variations, as well. Therefore, more categories within species exist, but they are not well defined. Microbiologists use terms like **subspecies**, **strain**, or **type** to designate bacteria of the same species that have differing characteristics. **Serotype** refers to representatives of a species that stimulate a distinct pattern of antibody (serum) responses in their hosts because of distinct surface molecules.

4.6 Learning Outcomes—Assess Your Progress

14. Differentiate between *Bergey's Manual of Systematic Bacteriology* and *Bergey's Manual of Determinative Bacteriology.*

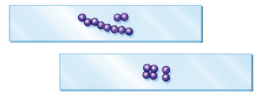
15. Name four divisions ending in *–cutes* and describe their characteristics.

16. Define a *species* in terms of bacteria.

Chapter Summary

4.1 Bacterial Form and Function (ASM Guideline* 2.4)

- Bacteria and archaea are ancient forms of cellular life. They are also the most widely dispersed, occupying every conceivable niche on the planet.
- Most bacteria and archaea have one of three general shapes: coccus (round), bacillus (rod), or spiral, based on the configuration of the cell wall. Two types of spiral cells are the spirochetes and the spirilla.
- Shape and arrangement of cells are key means of describing bacteria and archaea. Arrangements of cells are based on the number of planes in which a given species divides.



- Cocci can divide in many planes to form pairs, chains, packets, or clusters. Bacilli divide only in the transverse plane. If they remain attached, they form chains or palisades.

4.2 External Structures (ASM Guidelines 2.1, 2.2, 2.4)

- The external structures of bacteria include appendages (flagella, fimbriae, and pili) and surface coatings (the S layer and the glycocalyx).
- Flagella vary in number and arrangement as well as in the type and rate of motion they produce.

4.3 The Cell Envelope: The Boundary Layer of Bacteria (ASM Guidelines 2.1, 2.4, 3.4, 8.1)

- The cell envelope is the complex boundary structure surrounding a bacterial cell. In gram-negative bacteria, the envelope consists of an outer membrane, the cell wall, and the cell membrane. Gram-positive bacteria have only the cell wall and cell membrane.

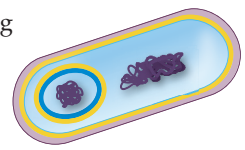
- In a Gram stain, gram-positive bacteria retain the crystal violet and stain purple. Gram-negative bacteria lose the crystal violet and stain red from the safranin counterstain.
- Gram-positive bacteria have thick cell walls of peptidoglycan and acidic polysaccharides such as teichoic acid. The cell walls of gram-negative bacteria are thinner and have a wide periplasmic space.
- The outer membrane of gram-negative cells contains lipopolysaccharide (LPS). LPS is toxic to mammalian hosts.
- The bacterial cell membrane is typically composed of phospholipids and proteins, and it performs many metabolic functions as well as transport activities.

4.4 Bacterial Internal Structure (ASM Guidelines 1.1, 2.1, 2.2, 2.4, 3.4, 4.2, 5.4)

- The cytoplasm of bacterial cells serves as a solvent for materials used in all cell functions.
- The genetic material of bacteria is DNA. Genes are arranged on large, circular chromosomes. Additional genes are carried on plasmids.
- Bacterial ribosomes are dispersed in the cytoplasm in chains (polysomes) and are also embedded in the cell membrane.
- Bacteria may store nutrients in their cytoplasm in structures called *inclusions*. Inclusions vary in structure and the materials that are stored.
- Packets in the cytoplasm called *bacterial microcompartments* are shells of protein packed with enzymes.

**ASM Curriculum Guidelines* (American Society for Microbiology, 2012). Complete guidelines in appendix B of this book.

- Some bacteria manufacture long actin- and tubulin-like filaments that help determine their cellular shape.
- A few families of bacteria produce dormant bodies called *endospores,* which are the hardiest of all life forms, surviving for hundreds or thousands of years.
- The genera *Bacillus* and *Clostridium* are endospore formers, and both contain deadly pathogens.

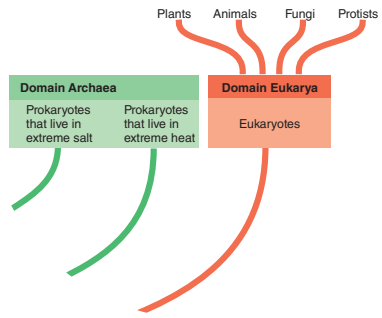


4.5 The Archaea: The Other "Prokaryotes" (ASM Guidelines 1.1, 1.4, 1.5, 4.2)

- Archaea constitute the third domain of life. They exhibit unusual biochemistry and genetics that make them different from bacteria. Many members are adapted to extreme habitats with low or high temperature, salt, pressure, or acid.

4.6 Classification Systems for Bacteria and Archaea (ASM Guidelines 1.1, 1.4, 1.5)

- Bacteria and archaea are formally classified by phylogenetic relationships and phenotypic characteristics.



- Medical identification of pathogens uses an informal system of classification based on Gram stain, morphology, biochemical reactions, and metabolic requirements.
- A *bacterial species* is loosely defined as a collection of bacterial cells that share an overall similar pattern of traits different from other groups of bacteria.
- Variant forms within a species (subspecies) include strains and types.

Multiple-Choice and True-False Questions | Bloom's Levels 1 and 2: Remember and Understand

Multiple-Choice Questions. Select the correct answer from the options provided.

1. Which of the following is not found in all bacterial cells?
   a. cell membrane
   b. a nucleoid
   c. ribosomes
   d. actinlike cytoskeleton
2. Pili are tubular shafts in _____ bacteria that serve as a means of _____.
   a. gram-positive, genetic exchange
   b. gram-positive, attachment
   c. gram-negative, genetic exchange
   d. gram-negative, protection
3. An example of a glycocalyx is
   a. a capsule.
   b. a pilus.
   c. an outer membrane.
   d. a cell wall.
4. Which of the following is a primary bacterial cell wall function?
   a. transport
   b. motility
   c. support
   d. adhesion
5. Which of the following is present in both gram-positive and gram-negative cell walls?
   a. an outer membrane
   b. peptidoglycan
   c. teichoic acid
   d. lipopolysaccharides
6. Darkly stained granules are concentrated crystals of _____ that are found in _____.
   a. fat, *Mycobacterium*
   b. dipicolinic acid, *Bacillus*
   c. sulfur, *Thiobacillus*
   d. PO_4, *Corynebacterium*
7. Bacterial endospores usually function in
   a. reproduction.
   b. survival.
   c. protein synthesis.
   d. storage.
8. A bacterial arrangement in packets of eight cells is described as a _____.
   a. micrococcus
   b. diplococcus
   c. tetrad
   d. sarcina
9. To which division of bacteria does *E. coli* belong?
   a. Tenericutes
   b. Gracilicutes
   c. Firmicutes
   d. Mendosicutes
10. Which stain is used to distinguish differences between the cell walls of medically important bacteria?
    a. simple stain
    b. acridine orange stain
    c. Gram stain
    d. negative stain

True-False Questions. If the statement is true, leave as is. If it is false, correct it by rewriting the sentence.

11. One major difference in the envelope structure between gram-positive bacteria and gram-negative bacteria is the presence or absence of a cytoplasmic membrane.
12. A research microbiologist looking at evolutionary relatedness between two bacterial species is more likely to use *Bergey's Manual of Determinative Bacteriology* than *Bergey's Manual of Systematic Bacteriology.*
13. Nanobes may or may not actually be bacteria.
14. Both bacteria and archaea used to be known as prokaryotes.
15. A collection of bacteria that share an overall similar pattern of traits is called a *species.*

Critical Thinking Questions | Bloom's Levels 3, 4, and 5: Apply, Analyze, and Evaluate

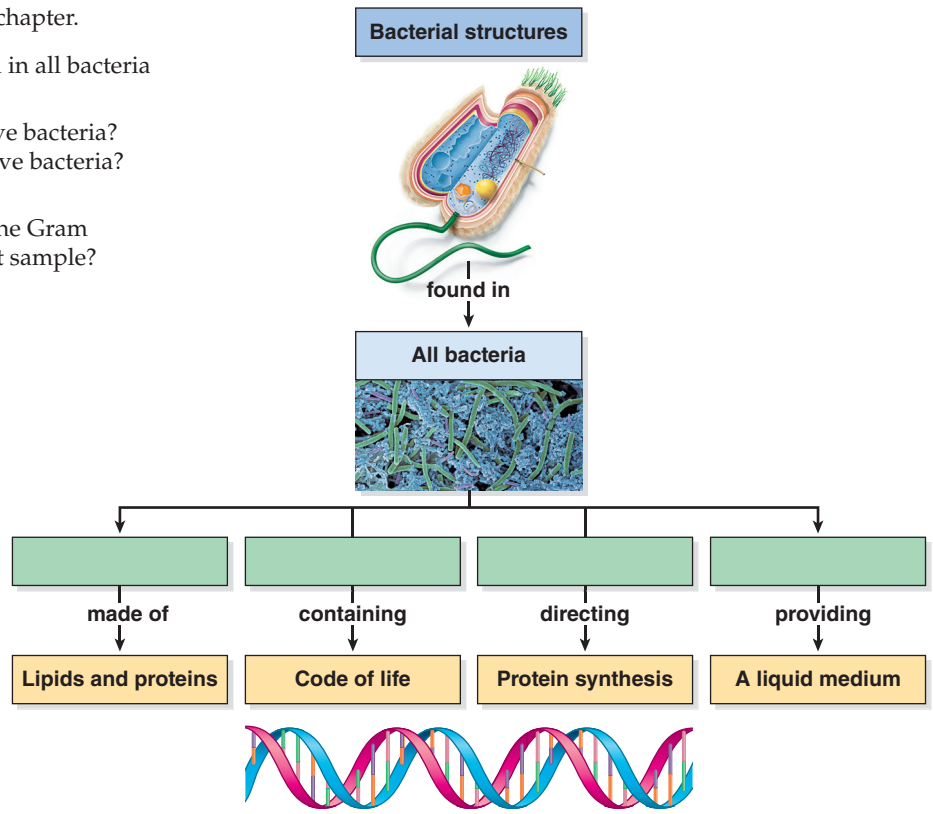
Critical thinking is the ability to reason and solve problems using facts and concepts. These questions can be approached from a number of angles and, in most cases, they do not have a single correct answer.

1. Define the term *ubiquitous* and explain whether this term can be used appropriately to describe bacteria and archaea.
2. Draw a picture to illustrate how bacteria and archaea can be distinguished from eukaryotic cells and to provide details on the general characteristics of these cell types.
3. Quorum sensing is a process used by many bacteria for communication. It involves the production of molecules called *autoinducers,* which act as bacterial chemoattractants. Describe how a motile bacterium would use its flagellum to respond to such a stimulus in its environment.
4. Provide examples of at least three different structures used for bacterial attachment and explain which are useful in the formation of biofilms.
5. You are a scientist viewing a Gram-stained slide of a bacterial culture obtained from a patient. You note purple spherical cells that are attached to one another in groups of four. Make a sketch of this bacterium; using proper terminology from this chapter, describe the morphology and arrangement of this bacterium as well as its cell wall composition.
6. Based upon your knowledge of cell wall structure, explain how the microbes causing meningitis and typhoid fever can induce fever and systemic shock in an infected patient.
7. Provide evidence in support of or refuting the following statement: The cell, or cytoplasmic, membrane is a nonessential structure in bacteria because its function is replaced by the cell wall in these microbes.
8. a. Describe the characteristics of an endospore-producing bacterium that make it an ideal candidate for bioterrorism but an undesirable intruder in a hospital setting.
   b. Explain why the production of endospores is not considered a method of reproduction in most bacterial species.
9. Compare and contrast main characteristics of the three domains of life: Bacteria, Archaea, and Eukarya.
   a. Create a branched-tree diagram showing the evolutionary relationship among the three domains of life, and list the characteristics that have led microbiologists to believe the archaea are more closely related to eukaryotes than to bacteria.
   b. Which archaeal adaptations make these microbes most suited to extreme habitats?
10. A microbe has been found in the boiling hot waters of a deep ocean hydrothermal vent. It cannot be readily stained or cultured in the laboratory, but its rRNA has just been sequenced and analyzed.
    a. Make a hypothesis regarding the domain of life to which this microbe most likely belongs.
    b. Explain which edition of *Bergey's Manual* should be used in this case to determine the identity of this microbe.
    c. Explain whether the rRNA sequence information is enough to define the species of a microbe.

Concept Connections | Bloom's Levels 4 and 6: Analyze and Create

This activity ties together multiple concepts in the chapter.

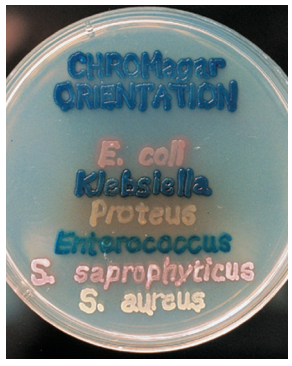
1. Name at least five structures that are not found in all bacteria but are important in some.
2. What characteristics are unique to gram-positive bacteria? What characteristics are unique to gram-negative bacteria? What characteristics are common to both?
3. What is the clinical importance of identifying the Gram reaction of an organism cultured from a patient sample?



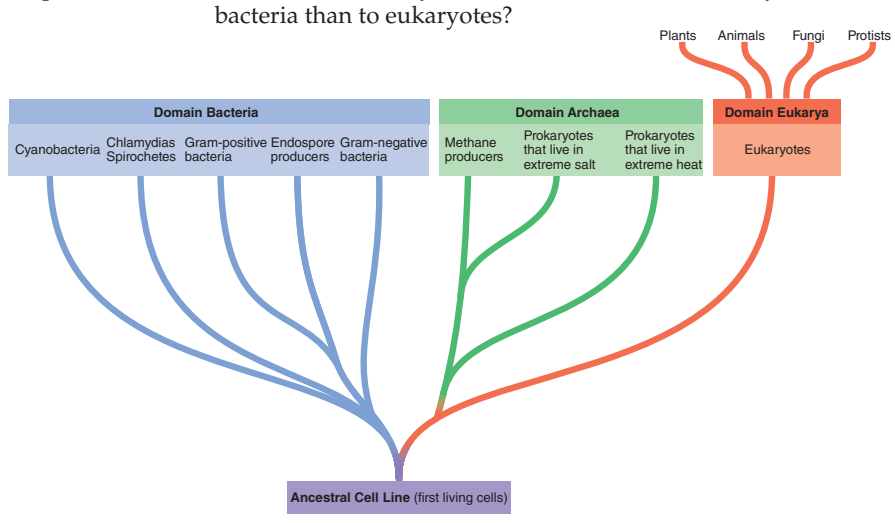
Visual Connections | Bloom's Level 5: Evaluate

These questions use visual images or previous content to make connections to this chapter's concepts.

1. **From chapter 3, figure 3.6*b*.** Do you believe that the bacteria spelling *"Klebsiella"* or the bacteria spelling *"S. aureus"* possess the larger capsule? Defend your answer.



2. **From chapter 1, figure 1.14.** Study this figure. How would it be drawn differently if the archaea were more closely related to bacteria than to eukaryotes?



Concept Mapping | Bloom's Level 6: Create

Appendix D provides guidance for working with concept maps.

1. Using the words that follow, please create a concept map illustrating the relationships among these key terms from chapter 4.

genus	serotype	*Borrelia burgdorferi*
species	domain	spirochete