Earth, appearing 3.5 to 3.8 billion years ago. These organisms are abundant and ubiquitous; that is, they are present everywhere. In addition to inhabiting moderate environments, they are found in extreme conditions: from boiling springs to permanently frozen environments in Antarctica; from salty environments like the Dead Sea to environments under tremendous pressure, such as the depths of the ocean; and from areas without oxygen, such as a waste management plant, to radioactively contaminated regions, such as Chernobyl. Prokaryotes reside in the human digestive system and on the skin, are responsible for certain illnesses, and serve an important role in the preparation of many foods.

22.1 | Prokaryotic Diversity

By the end of this section, you will be able to do the following:

- Describe the evolutionary history of prokaryotes
- Discuss the distinguishing features of extremophiles
- Explain why it is difficult to culture prokaryotes

Prokaryotes are ubiquitous. They cover every imaginable surface where there is sufficient moisture, and they also live on and inside virtually all other living things. In the typical human body, prokaryotic cells outnumber human body cells by about ten to one. They comprise the majority of living things in all ecosystems. Some prokaryotes thrive in environments that are inhospitable for most living things. Prokaryotes recycle **nutrients**—essential substances (such as carbon and nitrogen)—and they drive the evolution of new ecosystems, some of which are natural and others man-made. Prokaryotes have been on Earth since long before multicellular life appeared. Indeed, eukaryotic cells are thought to be the descendants of ancient prokaryotic communities.

Prokaryotes, the First Inhabitants of Earth

When and where did cellular life begin? What were the conditions on Earth when life began? We now know that prokaryotes were likely the first forms of cellular life on Earth, and they existed for billions of years before plants and animals appeared. The Earth and its moon are dated at about 4.54 billion years in age. This estimate is based on evidence from radiometric dating of meteorite material together with other substrate material from Earth and the moon. Early Earth had a very different atmosphere (contained less molecular oxygen) than it does today and was subjected to strong solar radiation; thus, the first organisms probably would have flourished where they were more protected, such as in the deep ocean or far beneath the surface of the Earth. Strong volcanic activity was common on Earth at this time, so it is likely that these first organisms—the first prokaryotes—were adapted to very high temperatures. Because early Earth was prone to geological upheaval and volcanic eruption, and was subject to bombardment by mutagenic radiation from the sun, the first organisms were prokaryotes that must have withstood these harsh conditions.

Microbial Mats

Microbial mats or large biofilms may represent the earliest forms of prokaryotic life on Earth; there is fossil evidence of their presence starting about 3.5 billion years ago. It is remarkable that cellular life appeared on Earth only a billion years after the Earth itself formed, suggesting that pre-cellular “life” that could replicate itself had evolved much earlier. A **microbial mat** is a multi-layered sheet of prokaryotes (**Figure 22.2**) that includes mostly bacteria, but also archaeans. Microbial mats are only a few centimeters thick, and they typically grow where different types of materials interface, mostly on moist surfaces. The various types of prokaryotes that comprise them carry out different metabolic pathways, and that is the reason for their various colors. Prokaryotes in a microbial mat are held together by a glue-like sticky substance that they secrete called *extracellular matrix*.

The first microbial mats likely obtained their energy from chemicals found near hydrothermal vents. A ***hydrothermal vent*** is a breakage or fissure in the Earth’s surface that releases geothermally heated water. With the evolution of photosynthesis about three billion years ago, some prokaryotes in microbial mats came to use a more widely available energy source—sunlight—whereas others were still dependent on chemicals from hydrothermal vents for energy and food.

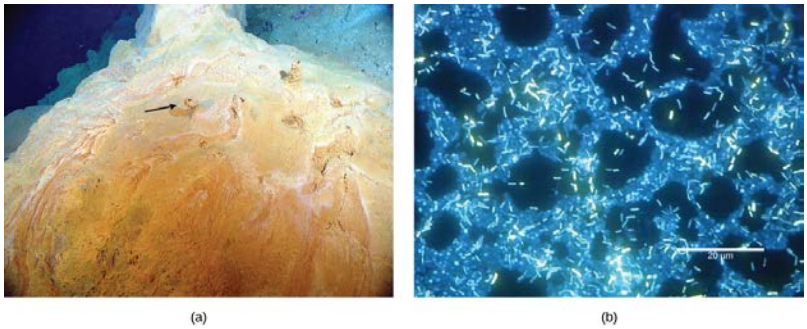


Figure 22.2 A microbial mat. (a) This microbial mat, about one meter in diameter, is growing over a hydrothermal vent in the Pacific Ocean in a region known as the "Pacific Ring of Fire." The mat's colony of bacteria helps retain microbial nutrients. Chimneys such as the one indicated by the arrow allow gases to escape. (b) In this micrograph, bacteria are visualized using fluorescence microscopy. (credit a: modification of work by Dr. Bob Embley, NOAA PMEL, Chief Scientist; credit b: modification of work by Ricardo Murga, Rodney Donlan, CDC; scale-bar data from Matt Russell)

Stromatolites

Fossilized microbial mats represent the earliest record of life on Earth. A **stromatolite** is a sedimentary structure formed when minerals are precipitated out of water by prokaryotes in a microbial mat (**Figure 22.3**). Stromatolites form layered rocks made of carbonate or silicate. Although most stromatolites are artifacts from the past, there are places on Earth where stromatolites are still forming. For example, growing stromatolites have been found in the Anza-Borrego Desert State Park in San Diego County, California.

Figure 22.3 Stromatolites. (a) These living stromatolites are located in Shark Bay, Australia. (b) These fossilized stromatolites, found in Glacier National Park, Montana, are nearly 1.5 billion years old. (credit a: Robert Young; credit b: P. Carrara, NPS)

The Ancient Atmosphere

Evidence indicates that during the first two billion years of Earth's existence, the atmosphere was **anoxic**, meaning that there was no molecular oxygen. Therefore, only those organisms that can grow without oxygen—*anaerobic organisms*—were able to live. Autotrophic organisms that convert solar energy into chemical energy are called **phototrophs**, and they appeared within one billion years of the formation of Earth. Then, **cyanobacteria**, also known as "blue-green algae," evolved from these simple phototrophs at least one billion years later. It was the ancestral cyanobacteria (**Figure 22.4**) that began the "oxygenation" of the atmosphere: Increased atmospheric oxygen allowed the evolution of more efficient O_2-utilizing catabolic pathways. It also opened up the land to increased colonization, because some O_2 is converted into O_3 (ozone) and ozone effectively absorbs the ultraviolet light that could have otherwise caused lethal mutations in DNA. The current evidence suggests that the increase in O_2 concentrations allowed the evolution of other life forms.

Figure 22.4 Cyanobacteria. This hot spring in Yellowstone National Park flows toward the foreground. Cyanobacteria in the spring are green, and as water flows down the gradient, the intensity of the color increases as cell density increases. The water is cooler at the edges of the stream than in the center, causing the edges to appear greener. (credit: Graciela Brelles-Mariño)

Microbes Are Adaptable: Life in Moderate and Extreme Environments

Some organisms have developed strategies that allow them to survive harsh conditions. Almost all prokaryotes have a cell wall, a protective structure that allows them to survive in both hypertonic and hypotonic aqueous conditions. Some soil bacteria are able to form *endospores* that resist heat and drought, thereby allowing the organism to survive until favorable conditions recur. These adaptations, along with others, allow bacteria to remain the most abundant life form in all terrestrial and aquatic ecosystems.

Prokaryotes thrive in a vast array of environments: Some grow in conditions that would seem very normal to us, whereas others are able to thrive and grow under conditions that would kill a plant or an animal. Bacteria and archaea that are adapted to grow under extreme conditions are called **extremophiles**, meaning “lovers of extremes.” Extremophiles have been found in all kinds of environments: the depths of the oceans, hot springs, the Arctic and the Antarctic, in very dry places, deep inside Earth, in harsh chemical environments, and in high radiation environments (**Figure 22.5**), just to mention a few. Because they have specialized adaptations that allow them to live in extreme conditions, many extremophiles cannot survive in moderate environments. There are many different groups of extremophiles: They are identified based on the conditions in which they grow best, and several habitats are extreme in multiple ways. For example, a soda lake is both salty and alkaline, so organisms that live in a soda lake must be both alkaliphiles and halophiles (**Table 22.1**). Other extremophiles, like **radioresistant** organisms, do not prefer an extreme environment (in this case, one with high levels of radiation), but have adapted to survive in it (**Figure 22.5**). Organisms like these give us a better understanding of prokaryotic diversity and open up the possibility of finding new prokaryotic species that may lead to the discovery of new therapeutic drugs or have industrial applications.

Extremophiles and Their Preferred Conditions

Extremophile	Conditions for Optimal Growth
Acidophiles	pH 3 or below
Alkaliphiles	pH 9 or above
Thermophiles	Temperature 60–80 °C (140–176 °F)
Hyperthermophiles	Temperature 80–122 °C (176–250 °F)
Psychrophiles	Temperature of -15-10 °C (5-50 °F) or lower
Halophiles	Salt concentration of at least 0.2 M
Osmophiles	High sugar concentration

Table 22.1

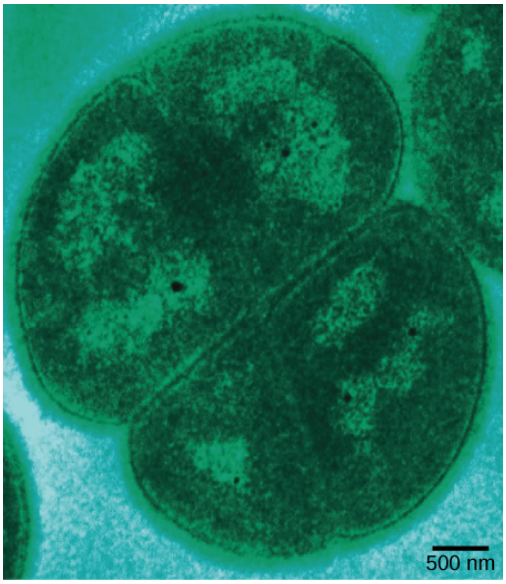


Figure 22.5 Radiation-tolerant prokaryotes. *Deinococcus radiodurans*, visualized in this false color transmission electron micrograph, is a prokaryote that can tolerate very high doses of ionizing radiation. It has developed DNA repair mechanisms that allow it to reconstruct its chromosome even if it has been broken into hundreds of pieces by radiation or heat. (credit: modification of work by Michael Daly; scale-bar data from Matt Russell)

Prokaryotes in the Dead Sea

One example of a very harsh environment is the Dead Sea, a hypersaline basin that is located between Jordan and Israel. Hypersaline environments are essentially concentrated seawater. In the Dead Sea, the sodium concentration is 10 times higher than that of seawater, and the water contains high levels of magnesium (about 40 times higher than in seawater) that would be toxic to most living things. Iron, calcium, and magnesium, elements that form divalent ions (Fe^{2+}, Ca^{2+}, and Mg^{2+}), produce what is commonly referred to as “hard” water. Taken together, the high concentration of divalent cations, the acidic pH (6.0), and the intense solar radiation flux make the Dead Sea a unique, and uniquely hostile, ecosystem[1] (**Figure 22.6**).

What sort of prokaryotes do we find in the Dead Sea? The extremely salt-tolerant bacterial mats include *Halobacterium*, *Haloferax volcanii* (which is found in other locations, not only the Dead Sea), *Halorubrum sodomense*, and *Halobaculum gomorrense*, and the archaean *Haloarcula marismortui*, among others.

Figure 22.6 Halophilic prokaryotes. (a) The Dead Sea is hypersaline. Nevertheless, salt-tolerant bacteria thrive in this sea. (b) These halobacteria cells can form salt-tolerant bacterial mats. (credit a: Julien Menichini; credit b: NASA; scale-bar data from Matt Russell)

Unculturable Prokaryotes and the Viable-but-Non-Culturable State

The process of culturing bacteria is complex and is one of the greatest discoveries of modern science. German physician Robert Koch is credited with discovering the techniques for pure culture, including staining and using

1. Bodaker, I, Itai, S, Suzuki, MT, Feingersch, R, Rosenberg, M, Maguire, ME, Shimshon, B, and others. Comparative community genomics in the Dead Sea: An increasingly extreme environment. *The ISME Journal* 4 (2010): 399–407, doi:10.1038/ismej.2009.141. published online 24 December 2009.

growth media. Microbiologists typically grow prokaryotes in the laboratory using an appropriate culture medium containing all the nutrients needed by the target organism. The medium can be liquid, broth, or solid. After an incubation time at the right temperature, there should be evidence of microbial growth (**Figure 22.7**). Koch's assistant Julius Petri invented the Petri dish, whose use persists in today's laboratories. Koch worked primarily with the *Mycobacterium tuberculosis* bacterium that causes tuberculosis and developed guidelines, called **Koch's postulates**, to identify the organisms responsible for specific diseases. Koch's postulates continue to be widely used in the medical community. Koch's postulates include that an organism can be identified as the cause of disease when it is present in all infected samples and absent in all healthy samples, and it is able to reproduce the infection after being cultured multiple times. Today, cultures remain a primary diagnostic tool in medicine and other areas of molecular biology.

Figure 22.7 Bacteria growing on blood agar plates. In these agar plates, the growth medium is supplemented with red blood cells. Blood agar becomes transparent in the presence of hemolytic *Streptococcus*, which destroys red blood cells and is used to diagnose *Streptococcus* infections. The plate on the left is inoculated with non-hemolytic *Staphylococcus* (large white colonies), and the plate on the right is inoculated with hemolytic *Streptococcus* (tiny clear colonies). If you look closely at the right plate, you can see that the agar surrounding the bacteria has turned clear. (credit: Bill Branson, NCI)

Koch's postulates can be fully applied only to organisms that can be isolated and cultured. Some prokaryotes, however, cannot grow in a laboratory setting. In fact, over 99 percent of bacteria and archaea are *unculturable*. For the most part, this is due to a lack of knowledge as to what to feed these organisms and how to grow them; they may have special requirements for growth that remain unknown to scientists, such as needing specific micronutrients, pH, temperature, pressure, co-factors, or co-metabolites. Some bacteria cannot be cultured because they are obligate intracellular parasites and cannot be grown outside a host cell.

In other cases, *culturable organisms* become unculturable under stressful conditions, even though the same organism could be cultured previously. Those organisms that cannot be cultured but are not dead are in a **viable-but-non-culturable** (VBNC) state. The VBNC state occurs when prokaryotes respond to environmental stressors by entering a dormant state that allows their survival. The criteria for entering into the VBNC state are not completely understood. In a process called **resuscitation**, the prokaryote can go back to "normal" life when environmental conditions improve.

Is the VBNC state an unusual way of living for prokaryotes? In fact, most of the prokaryotes living in the soil or in oceanic waters are non-culturable. It has been said that only a small fraction, perhaps one percent, of prokaryotes can be cultured under laboratory conditions. If these organisms are non-culturable, then how is it known whether they are present and alive? Microbiologists use molecular techniques, such as the polymerase chain reaction (PCR), to amplify selected portions of DNA of prokaryotes, e.g., 16S rRNA genes, demonstrating their existence. (Recall that PCR can make billions of copies of a DNA segment in a process called amplification.)

The Ecology of Biofilms

Some prokaryotes may be unculturable because they require the presence of other prokaryotic species. Until a couple of decades ago, microbiologists used to think of prokaryotes as isolated entities living apart. This model, however, does not reflect the true ecology of prokaryotes, most of which prefer to live in communities where they can interact. As we have seen, a **biofilm** is a microbial community (**Figure 22.8**) held together in a gummy-textured matrix that consists primarily of polysaccharides secreted by the organisms, together with some proteins and nucleic acids. Biofilms typically grow attached to surfaces. Some of the best-studied biofilms are composed of prokaryotes, although fungal biofilms have also been described, as well as some composed of a mixture of

fungi and bacteria.

Biofilms are present almost everywhere: they can cause the clogging of pipes and readily colonize surfaces in industrial settings. In recent, large-scale outbreaks of bacterial contamination of food, biofilms have played a major role. They also colonize household surfaces, such as kitchen counters, cutting boards, sinks, and toilets, as well as places on the human body, such as the surfaces of our teeth.

Interactions among the organisms that populate a biofilm, together with their protective *exopolysaccharidic (EPS)* environment, make these communities more robust than free-living, or planktonic, prokaryotes. The sticky substance that holds bacteria together also excludes most antibiotics and disinfectants, making biofilm bacteria hardier than their planktonic counterparts. Overall, biofilms are very difficult to destroy because they are resistant to many common forms of sterilization.

visual CONNECTION

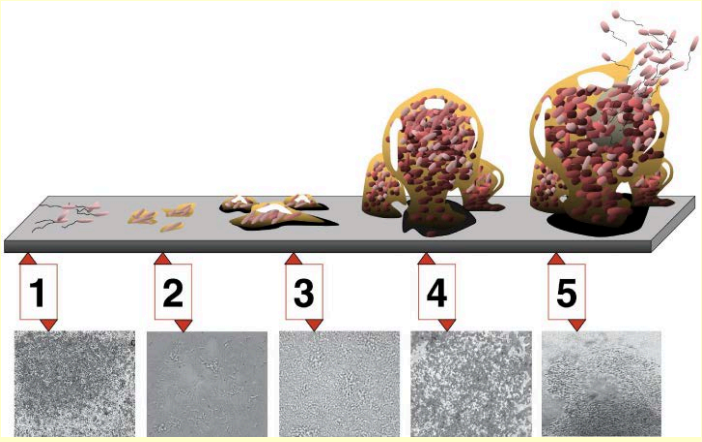


Figure 22.8 Development of a biofilm. Five stages of biofilm development are shown. During stage 1, initial attachment, bacteria adhere to a solid surface via weak *van der Waals interactions* (forces produced by induced electrical interactions between atoms). During stage 2, irreversible attachment, hairlike appendages called *pili* permanently anchor the bacteria to the surface. During stage 3, maturation I, the biofilm grows through cell division and recruitment of other bacteria. An extracellular matrix composed primarily of polysaccharides holds the biofilm together. During stage 4, maturation II, the biofilm continues to grow and takes on a more complex shape. During stage 5, dispersal, the biofilm matrix is partly broken down, allowing some bacteria to escape and colonize another surface. Micrographs of a *Pseudomonas aeruginosa* biofilm in each of the stages of development are shown. (credit: D. Davis, Don Monroe, PLoS)

Compared to free-floating bacteria, bacteria in biofilms often show increased resistance to antibiotics and detergents. Why do you think this might be the case?

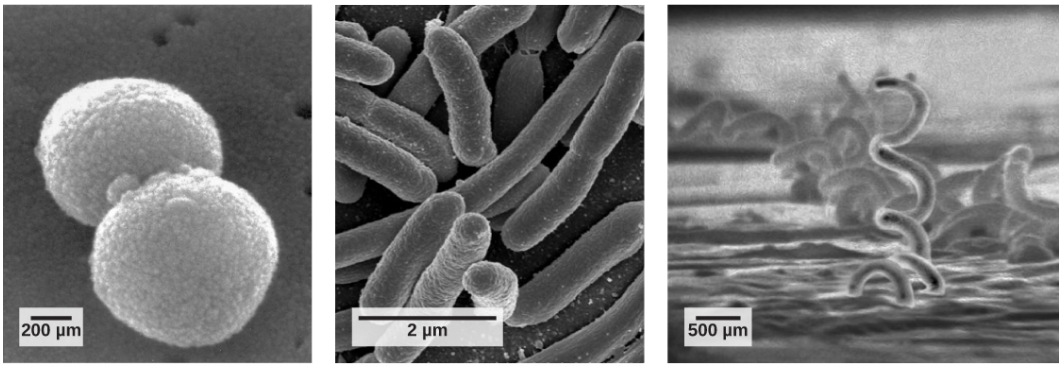
22.2 | Structure of Prokaryotes: Bacteria and Archaea

By the end of this section, you will be able to do the following:

- Describe the basic structure of a typical prokaryote
- Describe important differences in structure between Archaea and Bacteria

There are many differences between prokaryotic and eukaryotic cells. The name "prokaryote" suggests that prokaryotes are defined by exclusion—they are not eukaryotes, or organisms whose cells contain a nucleus and other internal membrane-bound organelles. However, all cells have four common structures: the plasma membrane, which functions as a barrier for the cell and separates the cell from its environment; the cytoplasm, a complex solution of organic molecules and salts inside the cell; a double-stranded DNA genome, the informational archive of the cell; and ribosomes, where protein synthesis takes place. Prokaryotes come in

various shapes, but many fall into three categories: *cocci* (spherical), *bacilli* (rod-shaped), and *spirilli* (spiral-shaped) (**Figure 22.9**).



(a) (b) (c)

Figure 22.9 Common prokaryotic cell types. Prokaryotes fall into three basic categories based on their shape, visualized here using scanning electron microscopy: (a) cocci, or spherical (a pair is shown); (b) bacilli, or rod-shaped; and (c) spirilli, or spiral-shaped. (credit a: modification of work by Janice Haney Carr, Dr. Richard Facklam, CDC; credit c: modification of work by Dr. David Cox; scale-bar data from Matt Russell)

The Prokaryotic Cell

Recall that prokaryotes are unicellular organisms that lack membrane-bound organelles or other internal membrane-bound structures (**Figure 22.10**). Their chromosome—usually single—consists of a piece of circular, double-stranded DNA located in an area of the cell called the **nucleoid**. Most prokaryotes have a cell wall outside the plasma membrane. The **cell wall** functions as a protective layer, and it is responsible for the organism's shape. Some bacterial species have a capsule outside the cell wall. The capsule enables the organism to attach to surfaces, protects it from dehydration and attack by phagocytic cells, and makes pathogens more resistant to our immune responses. Some species also have flagella (singular, flagellum) used for locomotion, and pili (singular, pilus) used for attachment to surfaces including the surfaces of other cells. Plasmids, which consist of extra-chromosomal DNA, are also present in many species of bacteria and archaea.

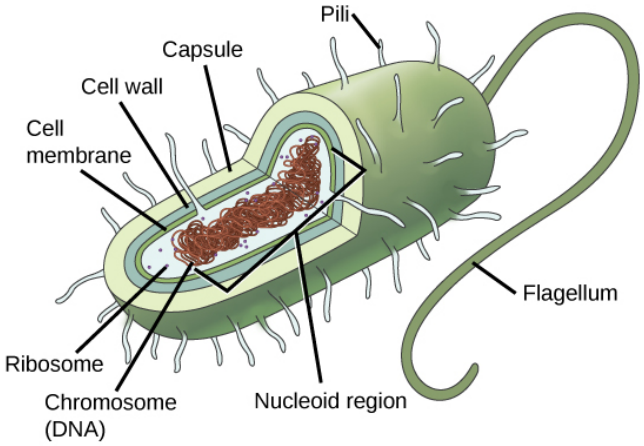


Figure 22.10 The features of a typical prokaryotic cell. Flagella, capsules, and pili are not found in all prokaryotes.

Recall that prokaryotes are divided into two different domains, Bacteria and Archaea, which together with Eukarya, comprise the three domains of life (**Figure 22.11**).

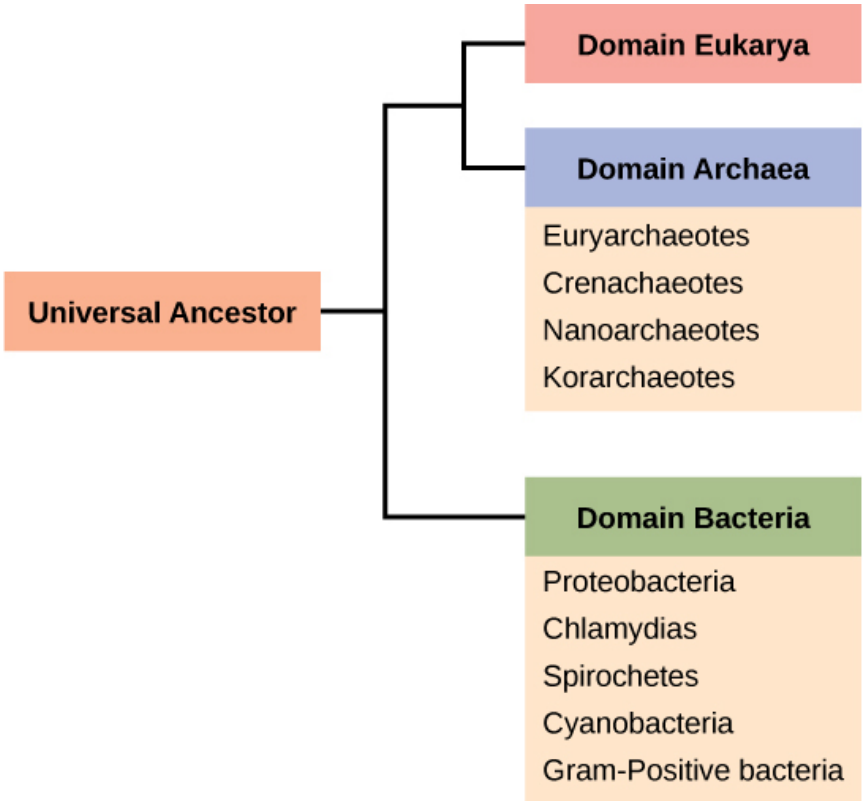


Figure 22.11 The three domains of living organisms. Bacteria and Archaea are both prokaryotes but differ enough to be placed in separate domains. An ancestor of modern Archaea is believed to have given rise to Eukarya, the third domain of life. Major groups of Archaea and Bacteria are shown.

Characteristics of bacterial phyla are described in **Figure 22.12** and **Figure 22.13**. Major bacterial phyla include the Proteobacteria, the Chlamydias, the Spirochaetes, the photosynthetic Cyanobacteria, and the Gram-positive bacteria. The Proteobacteria are in turn subdivided into several classes, from the Alpha- to the Epsilon proteobacteria. Eukaryotic mitochondria are thought to be the descendants of alphaproteobacteria, while eukaryotic chloroplasts are derived from cyanobacteria. Archaeal phyla are described in **Figure 22.14**.

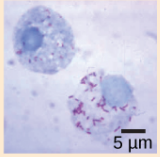
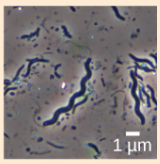
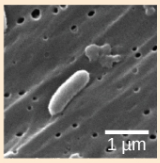
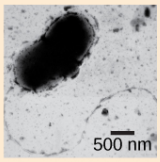
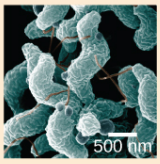
Bacteria of Phylum Proteobacteria		
Class	**Representative organisms**	**Representative micrograph**
Alpha Proteobacteria Some species are photoautotrophic but some are symbionts of plants and animals and others are pathogens. Eukaryotic mitochondria are thought be derived from bacteria in this group.	*Rhizobium* Nitrogen-fixing endosymbiont associated with the roots of legumes *Rickettsia* Obligate intracellular parasite that causes typhus and Rocky Mountain Spotted Fever (but not rickets, which is caused by Vitamin C deficiency)	 *Rickettsia rickettsia,* stained red, grow inside a host cell.
Beta Proteobacteria This group of bacteria is diverse. Some species play an important role in the nitrogen cycle.	*Nitrosomas* Species from this group oxidize ammonia into nitrite. *Spirillum minus* Causes rat-bite fever	 *Spirillum minus*
Gamma Proteobacteria Many are beneficial symbionts that populate the human gut, but others are familiar human pathogens. Some species from this subgroup oxidize sulfur compounds.	*Escherichia coli* Normally beneficial microbe of the human gut, but some strains cause disease *Salmonella* Certain strains cause food poisoning or typhoid fever *Yersinia pestis* Causative agent of Bubonic plague *Psuedomonas aeruginosa* Causes lung infections *Vibrio cholera* Causative agent of cholera *Chromatium* Sulfur-producing bacteria that oxidize sulfur, producing H_2S	 *Vibrio cholera*
Delta Proteobacteria Some species generate a spore-forming fruiting body in adverse conditions. Others reduce sulfate and sulfur.	*Myxobacteria* Generate spore-forming fruiting bodies in adverse conditions *Desulfovibrio vulgaris* Aneorobic, sulfate-reducing bacterium	 *Desulfovibrio vulgaris*
Epsilon Proteobacteria Many species inhabit the digestive tract of animals as symbionts or pathogens. Bacteria from this group have been found in deep-sea hydrothermal vents and cold seep habitats.	*Campylobacter* Causes blood poisoning and intestinal inflammation *Heliobacter pylori* Causes stomach ulcers	 *Campylobacter*

Figure 22.12 The Proteobacteria. Phylum *Proteobacteria* is one of up to 52 bacteria phyla. *Proteobacteria* is further subdivided into five classes, Alpha through Epsilon. (credit “Rickettsia rickettsia”: modification of work by CDC; credit “Spirillum minus”: modification of work by Wolframm Adlassnig; credit “Vibrio cholera”: modification of work by Janice Haney Carr, CDC; credit “Desulfovibrio vulgaris”: modification of work by Graham Bradley; credit “Campylobacter”: modification of work by De Wood, Pooley, USDA, ARS, EMU; scale-bar data from Matt Russell)

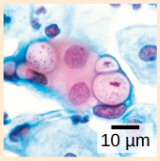
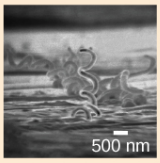
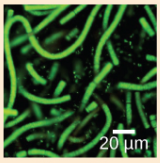
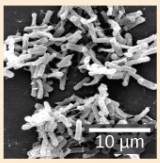
Bacteria: Chlamydia, Spirochaetae, Cyanobacteria, and Gram-positive		
Phylum	**Representative organisms**	**Representative micrograph**
Chlamydias All members of this group are obligate intracellular parasites of animal cells. Cells walls lack peptidoglycan.	*Chlamydia trachomatis* Common sexually transmitted disease that can lead to blindness	 In this pap smear, *Chlamydia trachomatis* appear as pink inclusions inside cells.
Spirochetes Most members of this species, which has spiral-shaped cells, are free-living aneaerobes, but some are pathogenic. Flagella run lengthwise in the periplasmic space between the inner and outer membrane.	*Treponema pallidum* Causative agent of syphilis *Borrelia burgdorferi* Causative agent of Lyme disease	 *Treponema pallidum*
Cyanobacteria Also known as blue-green algae, these bacteria obtain their energy through photosynthesis. They are ubiquitous, found in terrestrial, marine, and freshwater environments. Eukaryotic chloroplasts are thought be derived from bacteria in this group.	*Prochlorococcus* Believed to be the most abundant photosynthetic organism on earth; responsible for generating half the world's oxygen	 *Phormidium*
Gram-positive Bacteria Soil-dwelling members of this subgroup decompose organic matter. Some species cause disease. They have a thick cell wall and lack an outer membrane.	*Bacillus anthracis* Causes anthrax *Clostridium botulinum* Causes Botulism *Clostridium difficile* Causes diarrhea during antibiotic therapy *Streptomyces* Many antibiotics, including streptomyocin, are derived from these bacteria. *Mycoplasmas* These tiny bacteria, the smallest known, lack a cell wall. Some are free-living, and some are pathogenic.	 *Clostridium difficile*

Figure 22.13 Other bacterial phyla. Chlamydia, Spirochetes, Cyanobacteria, and Gram-positive bacteria are described in this table. Note that bacterial shape is not phylum-dependent; bacteria within a phylum may be cocci, rod-shaped, or spiral. (credit *"Chlamydia trachomatis"*: modification of work by Dr. Lance Liotta Laboratory, NCI; credit *"Treponema pallidum"*: modification of work by Dr. David Cox, CDC; credit *"Phormidium"*: modification of work by USGS; credit *"Clostridium difficile"*: modification of work by Lois S. Wiggs, CDC; scale-bar data from Matt Russell)

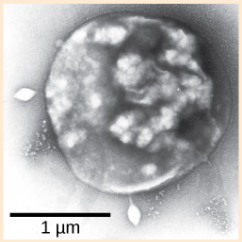
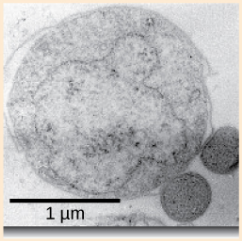
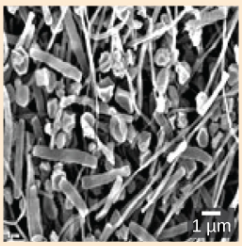
Archaea		
Phylum	**Representative organisms**	**Representative micrograph**
Euryarchaeota This phylum includes methanogens, which produce methane as a metabolic waste product, and halobacteria, which live in an extreme saline environment.	*Methanogens* Methane production causes flatulence in humans and other animals. *Halobacteria* Large blooms of this salt-loving archaea appear reddish due to the presence of bacterirhodopsin in the membrane. Bacteriorhodopsin is related to the retinal pigment rhodopsin.	 *Halobacterium* strain NRC-1
Crenarchaeota Members of the ubiquitous phylum play an important role in the fixation of carbon. Many members of this group are sulfur-dependent extremophiles. Some are thermophilic or hyperthermophilic.	*Sulfolobus* Members of this genus grow in volcanic springs at temperatures between 75° and 80°C and at a pH between 2 and 3.	 *Sulfolobus* being infected by bacteriophage
Nanoarchaeota This group currently contains only one species, *Nanoarchaeum equitans*.	*Nanoarchaeum equitans* This species was isolated from the bottom of the Atlantic Ocean and from a hydrothermal vent at Yellowstone National Park. It is an obligate symbiont with *Ignicoccus*, another species of archaea.	 *Nanoarchaeum equitans* (small dark spheres) are in contact with their larger host, *Ignicoccus.*
Korarchaeota Members of this phylum, considered to be one of the most primitive forms of life, have only been found in the Obsidian Pool, a hot spring at Yellowstone National Park.	No members of this species have been cultivated.	 This image shows a variety of korarchaeota species from the Obsidian Pool at Yellowstone National Park.

Figure 22.14 Archaeal phyla. Archaea are separated into four phyla: the Korarchaeota, Euryarchaeota, Crenarchaeota, and Nanoarchaeota. (credit *"Halobacterium"*: modification of work by NASA; credit *"Nanoarchaeotum equitans"*: modification of work by Karl O. Stetter; credit "Korarchaeota": modification of work by Office of Science of the U.S. Dept. of Energy; scale-bar data from Matt Russell)

The Plasma Membrane of Prokaryotes

The prokaryotic plasma membrane is a thin lipid bilayer (6 to 8 nanometers) that completely surrounds the cell and separates the inside from the outside. Its selectively permeable nature keeps ions, proteins, and other molecules within the cell and prevents them from diffusing into the extracellular environment, while other molecules may move through the membrane. Recall that the general structure of a cell membrane is a phospholipid bilayer composed of two layers of lipid molecules. In archaeal cell membranes, *isoprene (phytanyl) chains* linked to glycerol replace the fatty acids linked to glycerol in bacterial membranes. Some archaeal membranes are lipid monolayers instead of bilayers (**Figure 22.15**).

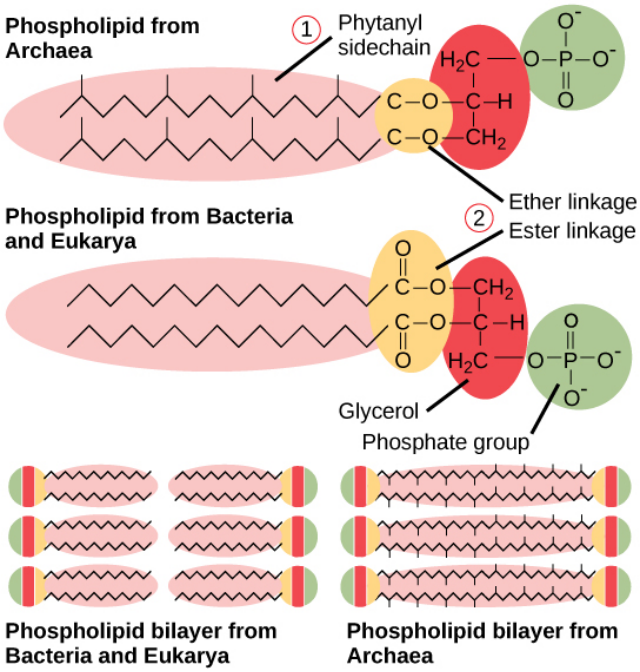


Figure 22.15 Bacterial and archaeal phospholipids. Archaeal phospholipids differ from those found in Bacteria and Eukarya in two ways. First, they have branched phytanyl sidechains instead of linear ones. Second, an ether bond instead of an ester bond connects the lipid to the glycerol.

The Cell Wall of Prokaryotes

The cytoplasm of prokaryotic cells has a high concentration of dissolved solutes. Therefore, the osmotic pressure within the cell is relatively high. The cell wall is a protective layer that surrounds some cells and gives them shape and rigidity. It is located outside the cell membrane and prevents *osmotic lysis* (bursting due to increasing volume). The chemical composition of the cell wall varies between Archaea and Bacteria, and also varies between bacterial species.

Bacterial cell walls contain **peptidoglycan**, composed of polysaccharide chains that are cross-linked by unusual peptides containing both L- and D-amino acids including D-glutamic acid and D-alanine. (Proteins normally have only L-amino acids; as a consequence, many of our antibiotics work by mimicking D-amino acids and therefore have specific effects on bacterial cell-wall development.) There are more than 100 different forms of peptidoglycan. *S-layer (surface layer) proteins* are also present on the outside of cell walls of both Archaea and Bacteria.

Bacteria are divided into two major groups: **Gram positive** and **Gram negative**, based on their reaction to Gram staining. Note that all Gram-positive bacteria belong to one phylum; bacteria in the other phyla (Proteobacteria, Chlamydias, Spirochetes, Cyanobacteria, and others) are Gram-negative. The Gram staining method is named after its inventor, Danish scientist Hans Christian Gram (1853–1938). The different bacterial responses to the staining procedure are ultimately due to cell wall structure. *Gram-positive organisms typically lack the outer membrane found in Gram-negative organisms* (**Figure 22.16**). Up to 90 percent of the cell-wall in Gram-positive bacteria is composed of peptidoglycan, and most of the rest is composed of acidic substances called *teichoic acids*. Teichoic acids may be covalently linked to lipids in the plasma membrane to form *lipoteichoic acids*. Lipoteichoic acids anchor the cell wall to the cell membrane. Gram-negative bacteria have a relatively thin cell wall composed of a few layers of peptidoglycan (only 10 percent of the total cell wall), surrounded by an outer envelope containing lipopolysaccharides (LPS) and lipoproteins. This outer envelope is sometimes referred to as a second lipid bilayer. The chemistry of this outer envelope is very different, however, from that of the typical lipid bilayer that forms plasma membranes.

visual CONNECTION

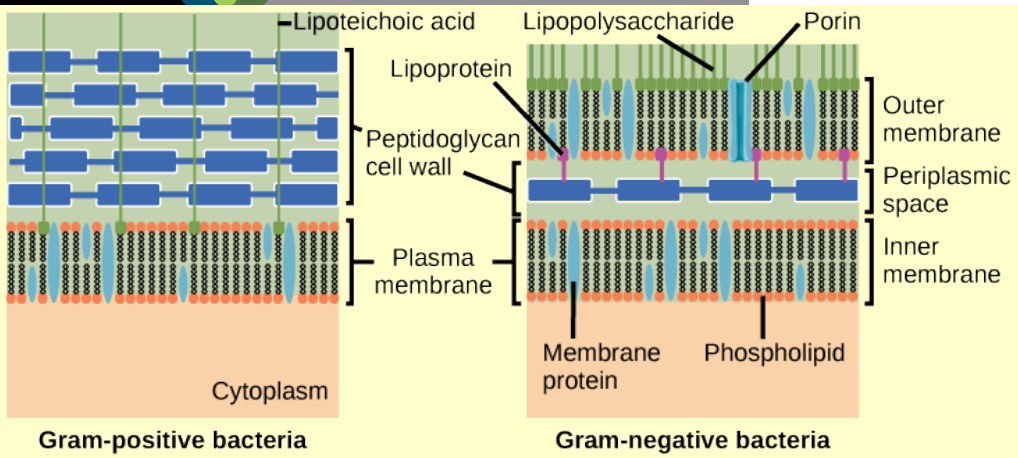


Figure 22.16 Cell walls in Gram-positive and Gram-negative bacteria. Bacteria are divided into two major groups: Gram positive and Gram negative. Both groups have a cell wall composed of peptidoglycan: in Gram-positive bacteria, the wall is thick, whereas in Gram-negative bacteria, the wall is thin. In Gram-negative bacteria, the cell wall is surrounded by an outer membrane that contains lipopolysaccharides and lipoproteins. Porins are proteins in this cell membrane that allow substances to pass through the outer membrane of Gram-negative bacteria. In Gram-positive bacteria, lipoteichoic acid anchors the cell wall to the cell membrane. (credit: modification of work by "Franciscosp2"/Wikimedia Commons)

Which of the following statements is true?

a. Gram-positive bacteria have a single cell wall anchored to the cell membrane by lipoteichoic acid.
b. Porins allow entry of substances into both Gram-positive and Gram-negative bacteria.
c. The cell wall of Gram-negative bacteria is thick, and the cell wall of Gram-positive bacteria is thin.
d. Gram-negative bacteria have a cell wall made of peptidoglycan, whereas Gram-positive bacteria have a cell wall made of lipoteichoic acid.

Archaean cell walls do not have peptidoglycan. There are four different types of archaean cell walls. One type is composed of **pseudopeptidoglycan**, which is similar to peptidoglycan in morphology but contains different sugars in the polysaccharide chain. The other three types of cell walls are composed of polysaccharides, glycoproteins, or pure protein. Other differences between Bacteria and Archaea are seen in Table 22.2. Note that features related to DNA replication, transcription and translation in Archaea are similar to those seen in eukaryotes.

Differences and Similarities between Bacteria and Archaea

Structural Characteristic	Bacteria	Archaea
Cell type	Prokaryotic	Prokaryotic
Cell morphology	Variable	Variable
Cell wall	Contains peptidoglycan	Does not contain peptidoglycan
Cell membrane type	Lipid bilayer	Lipid bilayer or lipid monolayer
Plasma membrane lipids	Fatty acids-glycerol ester	Phytanyl-glycerol ethers
Chromosome	Typically circular	Typically circular
Replication origins	Single	Multiple

Table 22.2

Differences and Similarities between Bacteria and Archaea

Structural Characteristic	Bacteria	Archaea
RNA polymerase	Single	Multiple
Initiator tRNA	Formyl-methionine	Methionine
Streptomycin inhibition	Sensitive	Resistant
Calvin cycle	Yes	No

Table 22.2

Reproduction

Reproduction in prokaryotes is *asexual* and usually takes place by binary fission. (Recall that the DNA of a prokaryote is a single, circular chromosome.) Prokaryotes do not undergo mitosis; instead, the chromosome is replicated and the two resulting copies separate from one another, due to the growth of the cell. The prokaryote, now enlarged, is pinched inward at its equator and the two resulting cells, which are *clones*, separate. Binary fission does not provide an opportunity for genetic recombination or genetic diversity, but prokaryotes can share genes by three other mechanisms.

In **transformation**, the prokaryote takes in DNA shed by other prokaryotes into its environment. If a nonpathogenic bacterium takes up DNA for a toxin gene from a pathogen and incorporates the new DNA into its own chromosome, it too may become pathogenic. In **transduction**, bacteriophages, the viruses that infect bacteria, may move short pieces of chromosomal DNA from one bacterium to another. Transduction results in a *recombinant organism*. Archaea also have viruses that may translocate genetic material from one individual to another. In **conjugation**, DNA is transferred from one prokaryote to another by means of a *pilus*, which brings the organisms into contact with one another, and provides a channel for transfer of DNA. The DNA transferred can be in the form of a plasmid or as a composite molecule, containing both plasmid and chromosomal DNA. These three processes of DNA exchange are shown in **Figure 22.17**.

Reproduction can be very rapid: a few minutes for some species. This short generation time coupled with mechanisms of genetic recombination and high rates of mutation result in the rapid evolution of prokaryotes, allowing them to respond to environmental changes (such as the introduction of an antibiotic) very quickly.

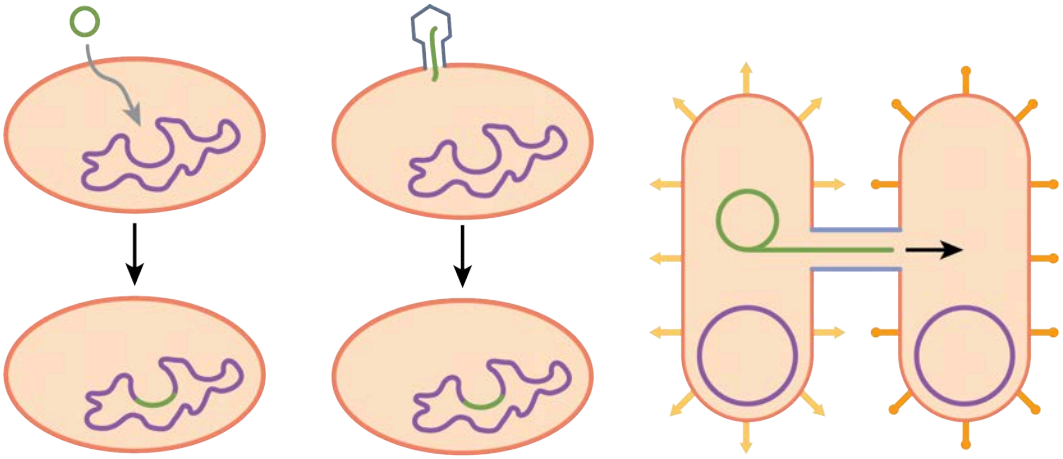


Figure 22.17 Gene transfer mechanisms in prokaryotes. There are three mechanisms by which prokaryotes can exchange DNA. In (a) transformation, the cell takes up prokaryotic DNA directly from the environment. The DNA may remain separate as plasmid DNA or be incorporated into the host genome. In (b) transduction, a bacteriophage injects DNA into the cell that contains a small fragment of DNA from a different prokaryote. In (c) conjugation, DNA is transferred from one cell to another via a mating bridge, or pilus, that connects the two cells after the sex pilus draws the two bacteria close enough to form the bridge.

evolution CONNECTION

The Evolution of Prokaryotes

How do scientists answer questions about the evolution of prokaryotes? Unlike with animals, artifacts in the fossil record of prokaryotes offer very little information. Fossils of ancient prokaryotes look like tiny bubbles in rock. Some scientists turn to genetics and to the principle of the molecular clock, which holds that the more recently two species have diverged, the more similar their genes (and thus proteins) will be. Conversely, species that diverged long ago will have more genes that are dissimilar.

Scientists at the NASA Astrobiology Institute and at the European Molecular Biology Laboratory collaborated to analyze the molecular evolution of 32 specific proteins common to 72 species of prokaryotes.[2] The model they derived from their data indicates that three important groups of bacteria—Actinobacteria, *Deinococcus*, and Cyanobacteria (collectively called *Terrabacteria* by the authors)—were the first to colonize land. Actinobacteria are a group of very common Gram-positive bacteria that produce branched structures like fungal mycelia, and include species important in decomposition of organic wastes. You will recall that *Deinococcus* is a genus of bacterium that is highly resistant to ionizing radiation. It has a thick peptidoglycan layer in addition to a second external membrane, so it has features of both Gram-positive and Gram-negative bacteria.

Cyanobacteria are photosynthesizers, and were probably responsible for the production of oxygen on the ancient earth. The timelines of divergence suggest that bacteria (members of the domain Bacteria) diverged from common ancestral species between 2.5 and 3.2 billion years ago, whereas the Archaea diverged earlier: between 3.1 and 4.1 billion years ago. Eukarya later diverged from the archaean line. The work further suggests that stromatolites that formed prior to the advent of cyanobacteria (about 2.6 billion years ago) photosynthesized in an anoxic environment and that because of the modifications of the Terrabacteria for land (resistance to drying and the possession of compounds that protect the organism from excess light), photosynthesis using oxygen may be closely linked to adaptations to survive on land.

22.3 | Prokaryotic Metabolism

By the end of this section, you will be able to do the following:

- Identify the macronutrients needed by prokaryotes, and explain their importance
- Describe the ways in which prokaryotes get energy and carbon for life processes
- Describe the roles of prokaryotes in the carbon and nitrogen cycles

Prokaryotes are metabolically diverse organisms. In many cases, a prokaryote may be placed into a species clade by its defining metabolic features: Can it metabolize lactose? Can it grow on citrate? Does it produce H_2S? Does it ferment carbohydrates to produce acid and gas? Can it grow under anaerobic conditions? Since metabolism and metabolites are the product of enzyme pathways, and enzymes are encoded in genes, the metabolic capabilities of a prokaryote are a reflection of its genome. There are many different environments on Earth with various energy and carbon sources, and variable conditions to which prokaryotes may be able to adapt. Prokaryotes have been able to live in every environment from deep-water volcanic vents to Antarctic ice by using whatever energy and carbon sources are available. Prokaryotes fill many niches on Earth, including involvement in nitrogen and carbon cycles, photosynthetic production of oxygen, decomposition of dead organisms, and thriving as parasitic, commensal, or mutualistic organisms inside multicellular organisms, including humans. The very broad range of environments that prokaryotes occupy is possible because they have diverse metabolic processes.

2. Battistuzzi, FU, Feijao, A, and Hedges, SB. A genomic timescale of prokaryote evolution: Insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BioMed Central: Evolutionary Biology* 4 (2004): 44, doi:10.1186/1471-2148-4-44.

Needs of Prokaryotes

The diverse environments and ecosystems on Earth have a wide range of conditions in terms of temperature, available nutrients, acidity, salinity, oxygen availability, and energy sources. Prokaryotes are very well equipped to make their living out of a vast array of nutrients and environmental conditions. To live, prokaryotes need a source of energy, a source of carbon, and some additional nutrients.

Macronutrients

Cells are essentially a well-organized assemblage of macromolecules and water. Recall that macromolecules are produced by the polymerization of smaller units called monomers. For cells to build all of the molecules required to sustain life, they need certain substances, collectively called **nutrients**. When prokaryotes grow in nature, they must obtain their nutrients from the environment. Nutrients that are required in large amounts are called *macronutrients*, whereas those required in smaller or trace amounts are called *micronutrients*. Just a handful of elements are considered macronutrients—carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. (A mnemonic for remembering these elements is the acronym *CHONPS*.)

Why are these macronutrients needed in large amounts? They are the components of organic compounds in cells, including water. Carbon is the major element in all macromolecules: carbohydrates, proteins, nucleic acids, lipids, and many other compounds. Carbon accounts for about 50 percent of the composition of the cell. In contrast, nitrogen represents only 12 percent of the total dry weight of a typical cell. Nitrogen is a component of proteins, nucleic acids, and other cell constituents. Most of the nitrogen available in nature is either atmospheric nitrogen (N_2) or another inorganic form. Diatomic (N_2) nitrogen, however, can be converted into an organic form only by certain microorganisms, called nitrogen-fixing organisms. Both hydrogen and oxygen are part of many organic compounds and of water. Phosphorus is required by all organisms for the synthesis of nucleotides and phospholipids. Sulfur is part of the structure of some amino acids such as cysteine and methionine, and is also present in several vitamins and coenzymes. Other important macronutrients are potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). Although these elements are required in smaller amounts, they are very important for the structure and function of the prokaryotic cell.

Micronutrients

In addition to these macronutrients, prokaryotes require various metallic elements in small amounts. These are referred to as micronutrients or trace elements. For example, iron is necessary for the function of the cytochromes involved in electron-transport reactions. Some prokaryotes require other elements—such as boron (B), chromium (Cr), and manganese (Mn)—primarily as enzyme cofactors.

The Ways in Which Prokaryotes Obtain Energy

Prokaryotes are classified both by the way they obtain energy, and by the carbon source they use for producing organic molecules. These categories are summarized in **Table 22.3**. Prokaryotes can use different sources of energy to generate the ATP needed for biosynthesis and other cellular activities. **Phototrophs** (or phototrophic organisms) obtain their energy from sunlight. Phototrophs trap the energy of light using chlorophylls, or in a few cases, bacterial rhodopsin. (Rhodopsin-using phototrophs, oddly, are phototrophic, but not photosynthetic, since they do not fix carbon.) **Chemotrophs** (or chemosynthetic organisms) obtain their energy from chemical compounds. Chemotrophs that can use organic compounds as energy sources are called chemoorganotrophs. Those that can use inorganic compounds, like sulfur or iron compounds, as energy sources are called chemolithotrophs.

Energy-producing pathways may be either **aerobic**, using oxygen as the terminal electron acceptor, or anaerobic, using either simple inorganic compounds or organic molecules as the *terminal electron acceptor*. Since prokaryotes lived on Earth for nearly a billion years before photosynthesis produced significant amounts of oxygen for aerobic respiration, many species of both Bacteria and Archaea are anaerobic and their metabolic activities are important in the carbon and nitrogen cycles discussed below.

The Ways in Which Prokaryotes Obtain Carbon

Prokaryotes not only can use different sources of energy, but also different sources of carbon compounds. Autotrophic prokaryotes synthesize organic molecules from carbon dioxide. In contrast, heterotrophic prokaryotes obtain carbon from organic compounds. To make the picture more complex, the terms that describe how prokaryotes obtain energy and carbon can be combined. Thus, photoautotrophs use energy from sunlight, and carbon from carbon dioxide and water, whereas chemoheterotrophs obtain both energy and carbon from an organic chemical source. Chemolithoautotrophs obtain their energy from inorganic compounds, and they build their complex molecules from carbon dioxide. Finally, prokaryotes that get their energy from light, but their carbon from organic compounds, are photoheterotrophs. The table below (**Table 22.3**) summarizes carbon and

energy sources in prokaryotes.

Carbon and Energy Sources in Prokaryotes

Energy Sources			Carbon Sources	
Light	Chemicals		Carbon dioxide	Organic compounds
Phototrophs	Chemotrophs		Autotrophs	Heterotrophs
	Organic chemicals	Inorganic chemicals		
	Chemo-organotrophs	Chemolithotrophs		

Table 22.3

Role of Prokaryotes in Ecosystems

Prokaryotes are ubiquitous: There is no niche or ecosystem in which they are not present. Prokaryotes play many roles in the environments they occupy. The roles they play in the carbon and nitrogen cycles are vital to life on Earth. In addition, the current scientific consensus suggests that metabolically interactive prokaryotic communities may have been the basis for the emergence of eukaryotic cells.

Prokaryotes and the Carbon Cycle

Carbon is one of the most important macronutrients, and prokaryotes play an important role in the carbon cycle (**Figure 22.18**). The carbon cycle traces the movement of carbon from inorganic to organic compounds and back again. Carbon is cycled through Earth's major reservoirs: land, the atmosphere, aquatic environments, sediments and rocks, and biomass. In a way, the carbon cycle echoes the role of the "four elements" first proposed by the ancient Greek philosopher, Empedocles: fire, water, earth, and air. Carbon dioxide is removed from the atmosphere by land plants and marine prokaryotes, and is returned to the atmosphere via the respiration of chemoorganotrophic organisms, including prokaryotes, fungi, and animals. Although the largest carbon reservoir in terrestrial ecosystems is in rocks and sediments, that carbon is not readily available.

Participants in the carbon cycle are roughly divided among producers, consumers, and decomposers of organic carbon compounds. The *primary producers* of organic carbon compounds from CO_2 are land plants and photosynthetic bacteria. A large amount of available carbon is found in living land plants. A related source of carbon compounds is *humus*, which is a mixture of organic materials from dead plants and prokaryotes that have resisted decomposition. (The term "humus," by the way, is the root of the word "human.") Consumers such as animals and other heterotrophs use organic compounds generated by producers and release carbon dioxide to the atmosphere. Other bacteria and fungi, collectively called **decomposers**, carry out the breakdown (decomposition) of plants and animals and their organic compounds. Most carbon dioxide in the atmosphere is derived from the respiration of microorganisms that decompose dead animals, plants, and humus.

In aqueous environments and their anoxic sediments, there is another carbon cycle taking place. In this case, the cycle is based on one-carbon compounds. In anoxic sediments, prokaryotes, mostly archaea, produce methane (CH_4). This methane moves into the zone above the sediment, which is richer in oxygen and supports bacteria called *methane oxidizers* that oxidize methane to carbon dioxide, which then returns to the atmosphere.

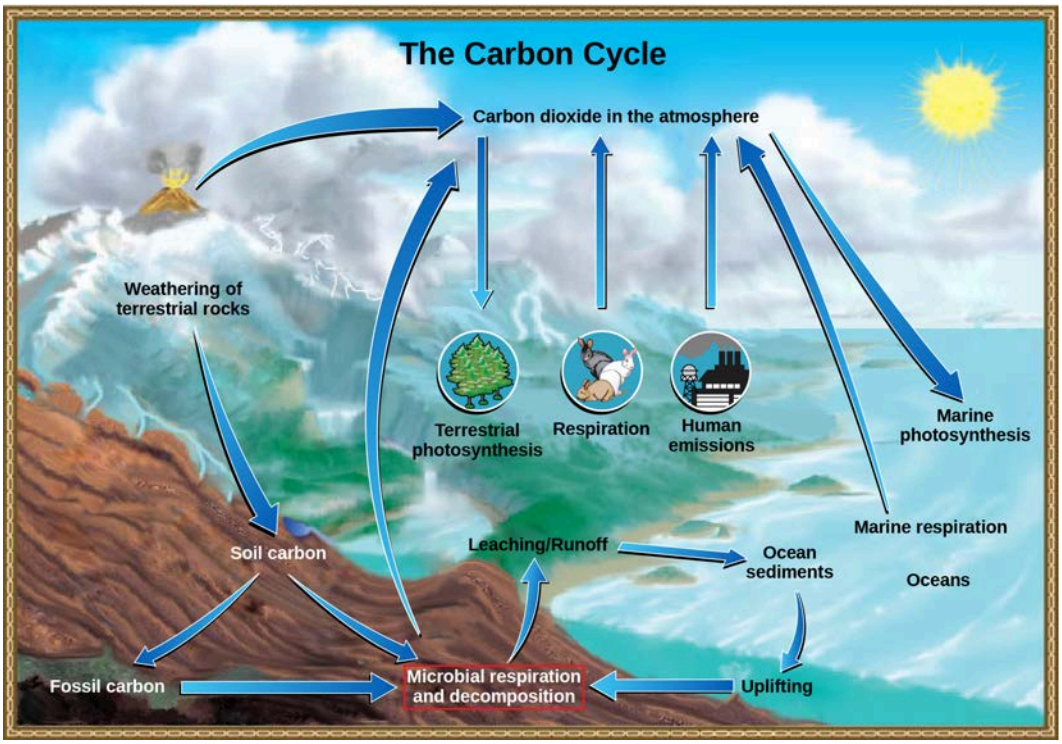


Figure 22.18 The carbon cycle. Prokaryotes play a significant role in continuously moving carbon through the biosphere. (credit: modification of work by John M. Evans and Howard Perlman, USGS)

Prokaryotes and the Nitrogen Cycle

Nitrogen is a very important element for life because it is a major constituent of proteins and nucleic acids. It is a macronutrient, and in nature, it is recycled from organic compounds to ammonia, ammonium ions, nitrate, nitrite, and nitrogen gas by many processes, many of which are carried out only by prokaryotes. As illustrated in **Figure 22.19**, prokaryotes are key to the nitrogen cycle. The largest pool of nitrogen available in the terrestrial ecosystem is *gaseous nitrogen* (N_2) from the air, but this nitrogen is not usable by plants, which are primary producers. Gaseous nitrogen is transformed, or "fixed" into more readily available forms, such as ammonia (NH_3), through the process of **nitrogen fixation**. Nitrogen-fixing bacteria include *Azotobacter* in soil and the ubiquitous photosynthetic cyanobacteria. Some nitrogen fixing bacteria, like *Rhizobium*, live in symbiotic relationships in the roots of legumes. Another source of ammonia is **ammonification**, the process by which ammonia is released during the decomposition of nitrogen-containing organic compounds. The ammonium ion is progressively oxidized by different species of bacteria in a process called nitrification. The nitrification process begins with the conversion of ammonium to nitrite (NO_2^-), and continues with the conversion of nitrite to nitrate. Nitrification in soils is carried out by bacteria belonging to the genera *Nitrosomas*, *Nitrobacter*, and *Nitrospira*. Most nitrogen in soil is in the form of ammonium (NH_4^+) or nitrate (NO_3^-). Ammonia and nitrate can be used by plants or converted to other forms.

Ammonia released into the atmosphere, however, represents only 15 percent of the total nitrogen released; the rest is as N_2 and N_2O (nitrous oxide). Ammonia is catabolized anaerobically by some prokaryotes, yielding N_2 as the final product. Denitrifying bacteria reverse the process of nitrification, reducing the nitrate from soils to gaseous compounds such as N_2O, NO, and N_2.

visual CONNECTION

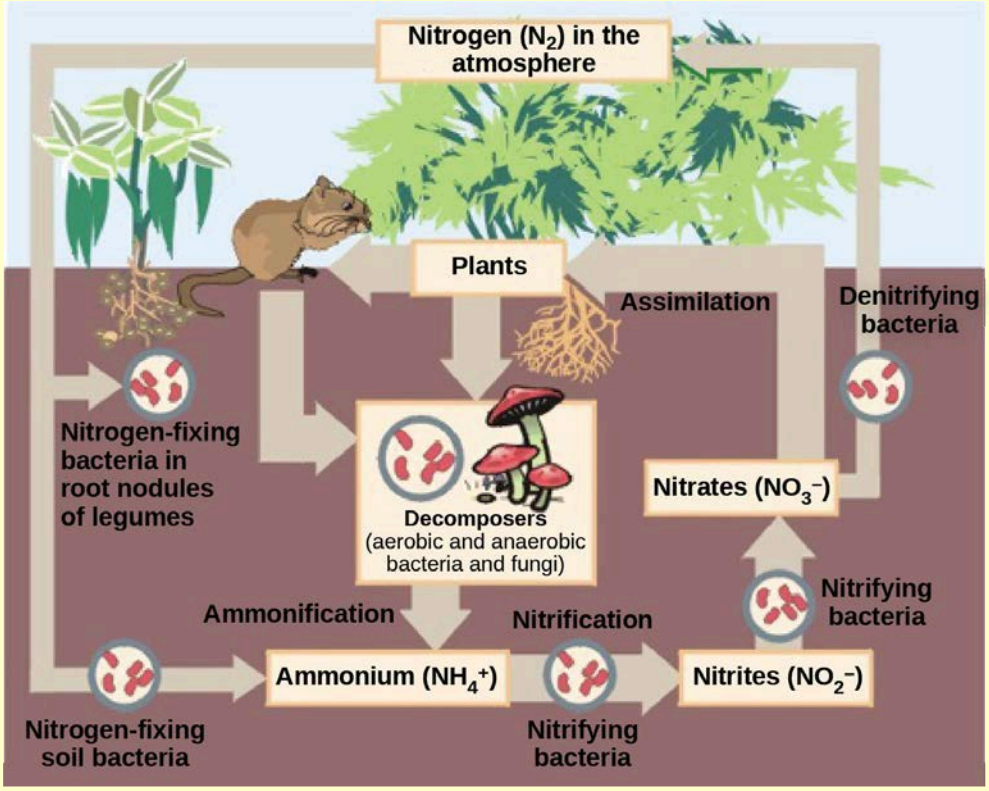


Figure 22.19 The nitrogen cycle. Prokaryotes play a key role in the nitrogen cycle. (credit: Environmental Protection Agency)

Which of the following statements about the nitrogen cycle is false?

a. Nitrogen-fixing bacteria exist on the root nodules of legumes and in the soil.

b. Denitrifying bacteria convert nitrates (NO_3^-) into nitrogen gas (N_2).

c. Ammonification is the process by which ammonium ion (NH_4^+) is released from decomposing organic compounds.

d. Nitrification is the process by which nitrites (NO_2^-) are converted to ammonium ion (NH_4^+).

22.4 | Bacterial Diseases in Humans

By the end of this section, you will be able to do the following:

- Identify bacterial diseases that caused historically important plagues and epidemics
- Describe the link between biofilms and foodborne diseases
- Explain how overuse of antibiotics may be creating “super bugs”
- Explain the importance of MRSA with respect to the problems of antibiotic resistance

To a prokaryote, humans may be just another housing opportunity. Unfortunately, the tenancy of some species can have harmful effects and cause disease. Bacteria or other infectious agents that cause harm to their human hosts are called **pathogens**. Devastating pathogen-borne diseases and plagues, both viral and bacterial in

nature, have affected humans and their ancestors for millions of years. The true cause of these diseases was not understood until modern scientific thought developed, and many people thought that diseases were a “spiritual punishment.” Only within the past several centuries have people understood that staying away from afflicted persons, disposing of the corpses and personal belongings of victims of illness, and sanitation practices reduced their own chances of getting sick.

Epidemiologists study how diseases are transmitted and how they affect a population. Often, they must following the course of an **epidemic**—a disease that occurs in an unusually high number of individuals in a population at the same time. In contrast, a **pandemic** is a widespread, and usually worldwide, epidemic. An **endemic disease** is a disease that is always present, usually at low incidence, in a population.

Long History of Bacterial Disease

There are records about infectious diseases as far back as 3000 B.C. A number of significant pandemics caused by bacteria have been documented over several hundred years. Some of the most memorable pandemics led to the decline of cities and entire nations.

In the 21st century, infectious diseases remain among the leading causes of death worldwide, despite advances made in medical research and treatments in recent decades. A disease *spreads* when the pathogen that causes it is passed from one person to another. For a pathogen to cause disease, it must be able to reproduce in the host’s body and damage the host in some way.

The Plague of Athens

In 430 B.C., the Plague of Athens killed one-quarter of the Athenian troops who were fighting in the great Peloponnesian War and weakened Athens’s dominance and power. The plague impacted people living in overcrowded Athens as well as troops aboard ships that had to return to Athens. The source of the plague may have been identified recently when researchers from the University of Athens were able to use DNA from teeth recovered from a mass grave. The scientists identified nucleotide sequences from a pathogenic bacterium, *Salmonella enterica* serovar Typhi (**Figure 22.20**), which causes *typhoid fever*.[3] This disease is commonly seen in overcrowded areas and has caused epidemics throughout recorded history.

3. Papagrigorakis MJ, Synodinos PN, and Yapijakis C. Ancient typhoid epidemic reveals possible ancestral strain of *Salmonella enterica* serovar Typhi. *Infect Genet Evol* 7 (2007): 126–7, Epub 2006 Jun.

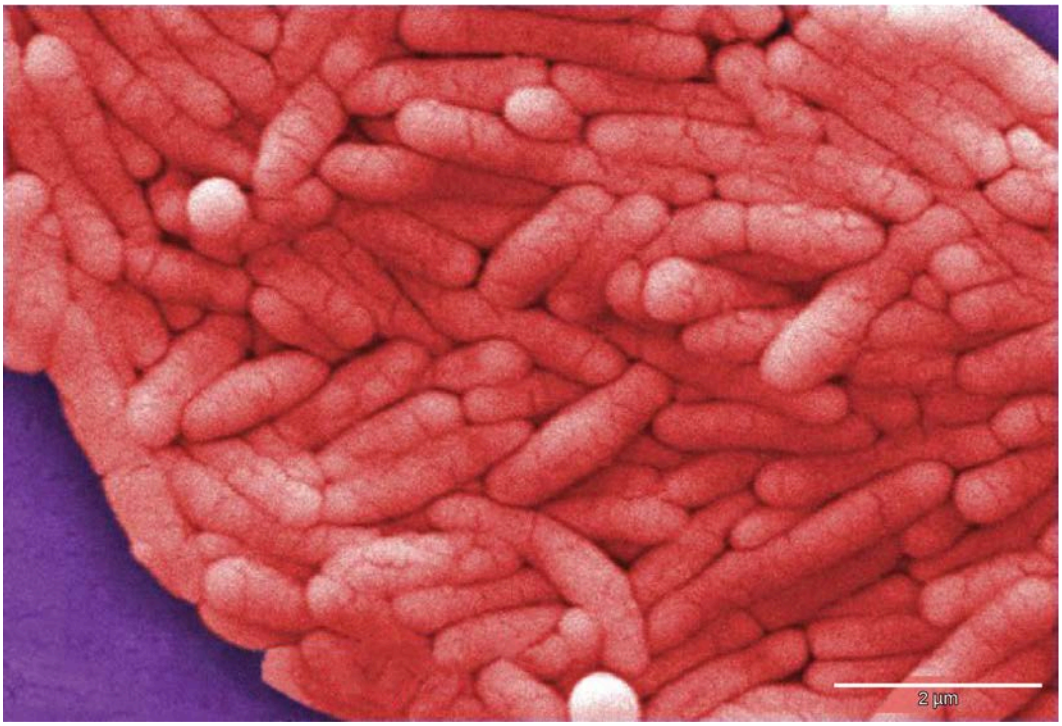
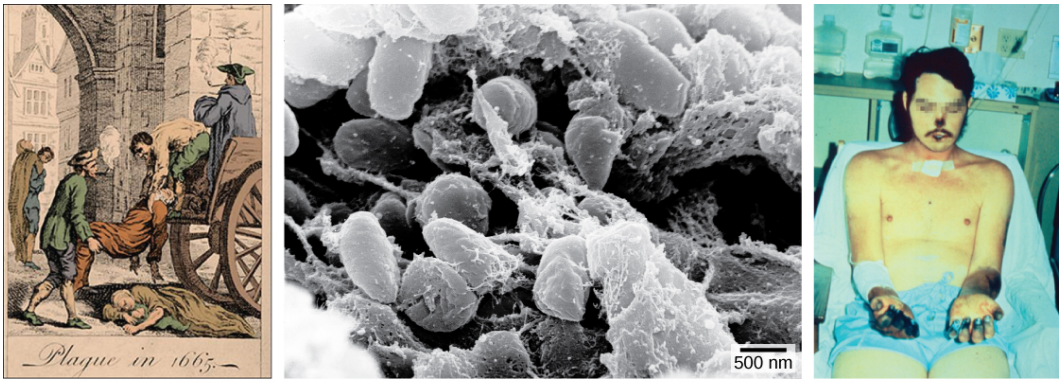


Figure 22.20 *Salmonella enterica. Salmonella enterica* serovar Typhi, the causative agent of Typhoid fever, is a Gram-negative, rod-shaped gamma proteobacterium. Typhoid fever, which is spread through feces, causes intestinal hemorrhage, high fever, delirium, and dehydration. Today, between 16 and 33 million cases of this re-emerging disease occur annually, resulting in over 200,000 deaths. Carriers of the disease can be asymptomatic. In a famous case in the early 1900s, a cook named Mary Mallon ("Typhoid Mary") unknowingly spread the disease to over fifty people, three of whom died. Other serotypes of *Salmonella* cause food poisoning. (credit: modification of work by NCI, CDC)

Bubonic Plagues

From 541 to 750, the Plague of Justinian, an outbreak of what was likely *bubonic plague*, eliminated one-quarter to one-half of the human population in the eastern Mediterranean region. The population in Europe dropped by 50 percent during this outbreak. Astoundingly, bubonic plague would strike Europe more than once!

Bubonic plague is caused by the bacterium *Yersinia pestis*. One of the most devastating pandemics attributed to bubonic plague was the **Black Death** (1346 to 1361). It is thought to have originated in China and spread along the Silk Road, a network of land and sea trade routes, to the Mediterranean region and Europe, carried by fleas living on black rats that were always present on ships. The Black Death was probably named for the tissue necrosis (**Figure 22.21**c) that can be one of the symptoms. The "buboes" of bubonic plague were painfully swollen areas of lymphatic tissue. A *pneumonic form* of the plague, spread by the coughing and sneezing of infected individuals, spreads directly from human to human and can cause death within a week. The pneumonic form was responsible for the rapid spread of the Black Death in Europe. The Black Death reduced the world's population from an estimated 450 million to about 350 to 375 million. Bubonic plague struck London yet again in the mid-1600s (**Figure 22.21**). In modern times, approximately 1,000 to 3,000 cases of plague arise globally each year, and a "sylvatic" form of plague, carried by fleas living on rodents such as prairie dogs and black footed ferrets, infects 10 to 20 people annually in the American Southwest. Although contracting bubonic plague before antibiotics meant almost certain death, the bacterium responds to several types of modern antibiotics, and mortality rates from plague are now very low.



(a) (b) (c)

Figure 22.21 The Black Death. The (a) Great Plague of London killed an estimated 200,000 people, or about 20 percent of the city's population. The causative agent, the (b) bacterium *Yersinia pestis*, is a Gram-negative, rod-shaped bacterium from the class Gammaproteobacteria. The disease is transmitted through the bite of an infected flea, which is carried on a rodent. Symptoms include swollen lymph nodes, fever, seizure, vomiting of blood, and (c) gangrene. (credit b: Rocky Mountain Laboratories, NIAID, NIH; scale-bar data from Matt Russell; credit c: Textbook of Military Medicine, Washington, D.C., U.S. Dept. of the Army, Office of the Surgeon General, Borden Institute)

LINK TO LEARNING

Watch a **video (http://openstaxcollege.org/l/black_death)** on the modern understanding of the Black Death—bubonic plague in Europe during the 14th century.

Migration of Diseases to New Populations

One of the negative consequences of human exploration was the accidental "biological warfare" that resulted from the transport of a pathogen into a population that had not previously been exposed to it. Over the centuries, Europeans tended to develop genetic immunity to endemic infectious diseases, but when European conquerors reached the western hemisphere, they brought with them disease-causing bacteria and viruses, which triggered epidemics that completely devastated many diverse populations of Native Americans, who had no natural resistance to many European diseases. It has been estimated that up to 90 percent of Native Americans died from infectious diseases after the arrival of Europeans, making conquest of the New World a foregone conclusion.

Emerging and Re-emerging Diseases

The distribution of a particular disease is *dynamic*. Changes in the environment, the pathogen, or the host population can dramatically impact the spread of a disease. According to the World Health Organization (WHO), an **emerging disease** (**Figure 22.22**) is one that has appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range. This definition also includes *re-emerging diseases* that were previously under control. Approximately 75 percent of recently emerging infectious diseases affecting humans are zoonotic diseases. **Zoonoses** are diseases that primarily infect animals but can be transmitted to humans; some are of viral origin and some are of bacterial origin. Brucellosis is an example of a prokaryotic zoonosis that is re-emerging in some regions, and *necrotizing fasciitis* (commonly known as flesh-eating bacteria) has been increasing in virulence for the last 80 years for unknown reasons.

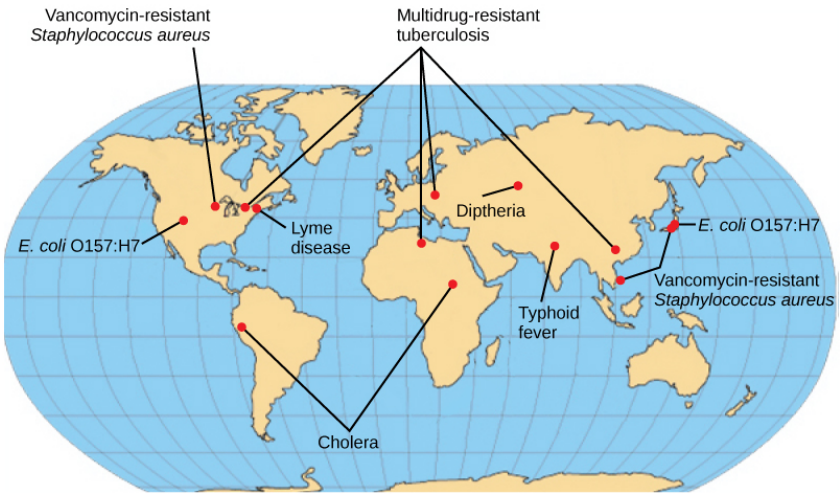


Figure 22.22 Emerging diseases. The map shows regions where bacterial diseases are emerging or re-emerging. (credit: modification of work by NIH)

Some of the present emerging diseases are not actually new, but are diseases that were catastrophic in the past (**Figure 22.23**). They devastated populations and became dormant for a while, just to come back, sometimes more virulent than before, as was the case with bubonic plague. Other diseases, like tuberculosis, were never eradicated but were under control in some regions of the world until coming back, mostly in urban centers with high concentrations of immunocompromised people. WHO has identified certain diseases whose worldwide re-emergence should be monitored. Among these are three viral diseases (dengue fever, yellow fever, and zika), and three bacterial diseases (diphtheria, cholera, and bubonic plague). The war against infectious diseases has no foreseeable end.

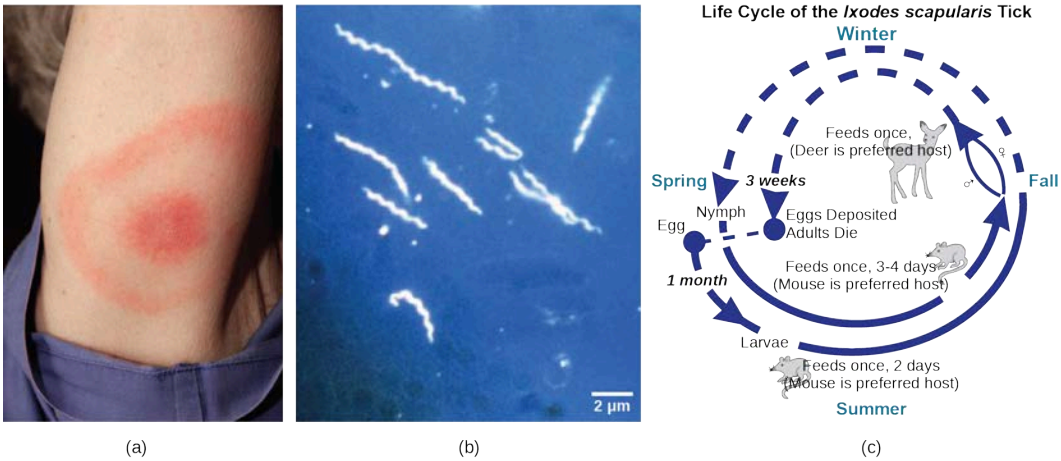


Figure 22.23 Lyme Disease. Lyme disease often, but not always, results in (a) a characteristic bullseye rash. The disease is caused by a (b) Gram-negative spirochete bacterium of the genus *Borrelia*. The bacteria (c) infect ticks, which in turn infect mice. Deer are the preferred secondary host, but the ticks also may feed on humans. Untreated, the disease causes chronic disorders in the nervous system, eyes, joints, and heart. The disease is named after Lyme, Connecticut, where an outbreak occurred in 1995 and has subsequently spread. The disease is not new, however. Genetic evidence suggests that Ötzi the Iceman, a 5,300-year-old mummy found in the Alps, was infected with *Borrelia*. (credit a: James Gathany, CDC; credit b: CDC; scale-bar data from Matt Russell)

Foodborne Diseases

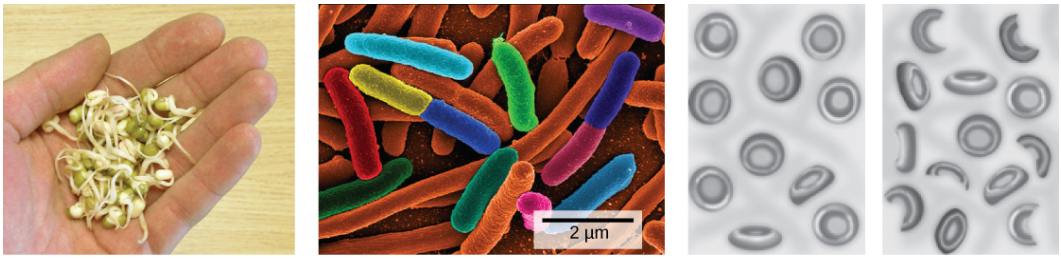
Prokaryotes are everywhere: They readily colonize the surface of any type of material, and food is not an exception. Most of the time, prokaryotes colonize food and food-processing equipment in the form of a *biofilm*, as we have discussed earlier. Outbreaks of bacterial infection related to food consumption are common. A foodborne disease (commonly called “food poisoning”) is an illness resulting from the consumption the pathogenic bacteria, viruses, or other parasites that contaminate food. Although the United States has one of the safest food supplies in the world, the U.S. Centers for Disease Control and Prevention (CDC) has reported

that “76 million people get sick, more than 300,000 are hospitalized, and 5,000 Americans die each year from foodborne illness.”

The characteristics of foodborne illnesses have changed over time. In the past, it was relatively common to hear about sporadic cases of botulism, the potentially fatal disease produced by a toxin from the anaerobic bacterium *Clostridium botulinum*. Some of the most common sources for this bacterium were non-acidic canned foods, homemade pickles, and processed meat and sausages. The can, jar, or package created a suitable anaerobic environment where *Clostridium* could grow. Proper sterilization and canning procedures have reduced the incidence of this disease.

While people may tend to think of foodborne illnesses as associated with animal-based foods, most cases are now linked to produce. There have been serious, produce-related outbreaks associated with raw spinach in the United States and with vegetable sprouts in Germany, and these types of outbreaks have become more common. The raw spinach outbreak in 2006 was produced by the bacterium *E. coli* serotype O157:H7. A serotype is a strain of bacteria that carries a set of similar antigens on its cell surface, and there are often many different serotypes of a bacterial species. Most *E. coli* are not particularly dangerous to humans, but serotype O157:H7 can cause bloody diarrhea and is potentially fatal.

All types of food can potentially be contaminated with bacteria. Recent outbreaks of *Salmonella* reported by the CDC occurred in foods as diverse as peanut butter, alfalfa sprouts, and eggs. A deadly outbreak in Germany in 2010 was caused by *E. coli* contamination of vegetable sprouts (**Figure 22.24**). The strain that caused the outbreak was found to be a new serotype not previously involved in other outbreaks, which indicates that *E. coli* is continuously evolving. Outbreaks of listeriosis, due to contamination of meats, raw cheeses, and frozen or fresh vegetables with *Listeria monocytogenes*, are becoming more frequent.



(a) (b) (c)

Figure 22.24 Foodborne pathogens. (a) Vegetable sprouts grown at an organic farm were the cause of an (b) *E. coli* outbreak that killed 32 people and sickened 3,800 in Germany in 2011. The strain responsible, *E. coli* O104:H4, produces Shiga toxin, a substance that inhibits protein synthesis in the host cell. The toxin (c) destroys red blood cells resulting in bloody diarrhea. Deformed red blood cells clog the capillaries of the kidney, which can lead to kidney failure, as happened to 845 patients in the 2011 outbreak. Kidney failure is usually reversible, but some patients experience kidney problems years later. (credit c: NIDDK, NIH)

Biofilms and Disease

Recall that biofilms are microbial communities that are very difficult to destroy. They are responsible for diseases such as Legionnaires’ disease, otitis media (ear infections), and various infections in patients with cystic fibrosis. They produce dental plaque and colonize catheters, prostheses, transcutaneous and orthopedic devices, contact lenses, and internal devices such as pacemakers. They also form in open wounds and burned tissue. In healthcare environments, biofilms grow on hemodialysis machines, mechanical ventilators, shunts, and other medical equipment. In fact, 65 percent of all infections acquired in the hospital (nosocomial infections) are attributed to biofilms. Biofilms are also related to diseases contracted from food because they colonize the surfaces of vegetable leaves and meat, as well as food-processing equipment that isn’t adequately cleaned.

Biofilm infections develop gradually and may not cause immediate symptoms. They are rarely resolved by host defense mechanisms. Once an infection by a biofilm is established, it is very difficult to eradicate, because biofilms tend to be resistant to most methods used to control microbial growth, including antibiotics. The matrix that attaches the cells to a substrate and to other another protects the cells from antibiotics or drugs. In addition, since biofilms grow slowly, they are less responsive to agents that interfere with cell growth. It has been reported that biofilms can resist up to 1,000 times the antibiotic concentrations used to kill the same bacteria when they are free-living or planktonic. An antibiotic dose that large would harm the patient; therefore, scientists are working on new ways to get rid of biofilms.

Antibiotics: Are We Facing a Crisis?

The word *antibiotic* comes from the Greek *anti* meaning "against" and *bios* meaning "life." An **antibiotic** is a chemical, produced either by microbes or synthetically, that is hostile to or prevents the growth of other organisms. Today's media often address concerns about an antibiotic crisis. Are the antibiotics that easily treated bacterial infections in the past becoming obsolete? Are there new "superbugs"—bacteria that have evolved to become more resistant to our arsenal of antibiotics? Is this the beginning of the end of antibiotics? All these questions challenge the healthcare community.

One of the main causes of antibiotic resistance in bacteria is overexposure to antibiotics. The imprudent and excessive use of antibiotics has resulted in the natural selection of resistant forms of bacteria. The antibiotic kills most of the infecting bacteria, and therefore only the resistant forms remain. These resistant forms reproduce, resulting in an increase in the proportion of resistant forms over non-resistant ones. In addition to transmission of resistance genes to progeny, lateral transfer of resistance genes on plasmids can rapidly spread these genes through a bacterial population. A major misuse of antibiotics is in patients with viral infections like colds or the flu, against which antibiotics are useless. Another problem is the excessive use of antibiotics in livestock. The routine use of antibiotics in animal feed promotes bacterial resistance as well. In the United States, 70 percent of the antibiotics produced are fed to animals. These antibiotics are given to livestock in low doses, which maximize the probability of resistance developing, and these resistant bacteria are readily transferred to humans.

LINK TO LEARNING

Watch a recent news **report (http://openstaxcollege.org/l/antibiotics)** on the problem of routine antibiotic administration to livestock and antibiotic-resistant bacteria.

One of the Superbugs: MRSA

The imprudent use of antibiotics has paved the way for the expansion of resistant bacterial populations. For example, *Staphylococcus aureu*s, often called "staph," is a common bacterium that can live in the human body and is usually easily treated with antibiotics. However, a very dangerous strain, **methicillin-resistant *Staphylococcus aureus* (MRSA)** has made the news over the past few years (**Figure 22.25**). This strain is resistant to many commonly used antibiotics, including methicillin, amoxicillin, penicillin, and oxacillin. MRSA can cause infections of the skin, but it can also infect the bloodstream, lungs, urinary tract, or sites of injury. While MRSA infections are common among people in healthcare facilities, they have also appeared in healthy people who haven't been hospitalized, but who live or work in tight populations (like military personnel and prisoners). Researchers have expressed concern about the way this latter source of MRSA targets a much younger population than those residing in care facilities. *The Journal of the American Medical Association* reported that, among MRSA-afflicted persons in healthcare facilities, the average age is 68, whereas people with "community-associated MRSA" (**CA-MRSA**) have an average age of 23.[4]

4. Naimi, TS, LeDell, KH, Como-Sabetti, K, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 290 (2003): 2976–84, doi: 10.1001/jama.290.22.2976.

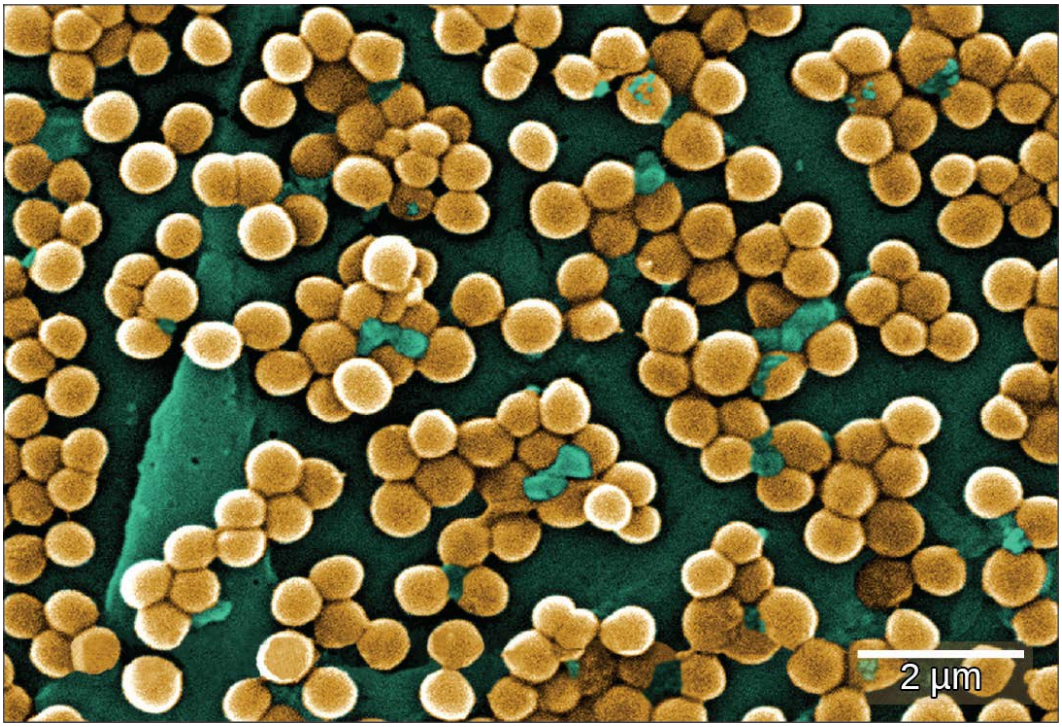


Figure 22.25 MRSA. This scanning electron micrograph shows methicillin-resistant *Staphylococcus aureus* bacteria, commonly known as MRSA. *S. aureus* is not always pathogenic, but can cause diseases such as food poisoning and skin and respiratory infections. (credit: modification of work by Janice Haney Carr; scale-bar data from Matt Russell)

In summary, the medical community is facing an antibiotic crisis. Some scientists believe that after years of being protected from bacterial infections by antibiotics, we may be returning to a time in which a simple bacterial infection could again devastate the human population. Researchers are developing new antibiotics, but it takes many years of research and clinical trials, plus financial investments in the millions of dollars, to generate an effective and approved drug.

career CONNECTION

Epidemiologist

Epidemiology is the study of the occurrence, distribution, and determinants of health and disease in a population. It is, therefore, part of public health. An epidemiologist studies the frequency and distribution of diseases within human populations and environments.

Epidemiologists collect data about a particular disease and track its spread to identify the original mode of transmission. They sometimes work in close collaboration with historians to try to understand the way a disease evolved geographically and over time, tracking the natural history of pathogens. They gather information from clinical records, patient interviews, surveillance, and any other available means. That information is used to develop strategies, such as vaccinations (**Figure 22.26**), and design public health policies to reduce the incidence of a disease or to prevent its spread. Epidemiologists also conduct rapid investigations in case of an outbreak to recommend immediate measures to control it.

An epidemiologist has a bachelor's degree, plus a master's degree in public health (MPH). Many epidemiologists are also physicians (and have an M.D. or D.O degree), or they have a Ph.D. in an associated field, such as biology or microbiology.

Figure 22.26 Vaccination. Vaccinations can slow the spread of communicable diseases. (credit: modification of work by Daniel Paquet)

22.5 | Beneficial Prokaryotes

By the end of this section, you will be able to do the following:

- Explain the need for nitrogen fixation and how it is accomplished
- Describe the beneficial effects of bacteria that colonize our skin and digestive tracts
- Identify prokaryotes used during the processing of food
- Describe the use of prokaryotes in bioremediation

Fortunately, only a few species of prokaryotes are pathogenic! Prokaryotes also interact with humans and other organisms in a number of ways that are beneficial. For example, prokaryotes are major participants in the carbon and nitrogen cycles. They produce or process nutrients in the digestive tracts of humans and other animals. Prokaryotes are used in the production of some human foods, and also have been recruited for the degradation of hazardous materials. In fact, our life would not be possible without prokaryotes!

Cooperation between Bacteria and Eukaryotes: Nitrogen Fixation

Nitrogen is a very important element to living things, because it is part of nucleotides and amino acids that are the building blocks of nucleic acids and proteins, respectively. Nitrogen is usually the most limiting element in terrestrial ecosystems, with atmospheric nitrogen, N_2, providing the largest pool of available nitrogen. However, eukaryotes cannot use atmospheric, gaseous nitrogen to synthesize macromolecules. Fortunately, nitrogen can be "fixed," meaning it is converted into a more accessible form—ammonia (NH_3)—either biologically or abiotically.

Abiotic nitrogen fixation occurs as a result of physical processes such as lightning or by industrial processes. **Biological nitrogen fixation** (BNF) is exclusively carried out by prokaryotes: soil bacteria, cyanobacteria, and *Frankia* spp. (filamentous bacteria interacting with actinorhizal plants such as alder, bayberry, and sweet fern). After photosynthesis, BNF is the most important biological process on Earth. The overall nitrogen fixation equation below represents a series of *redox reactions* (Pi stands for inorganic phosphate).

$$N_2 + 16ATP + 8e^- + 8H^+ \rightarrow 2NH_3 + 16ADP + 16Pi + H_2$$

The total fixed nitrogen through BNF is about 100 to 180 million metric tons per year, which contributes about 65 percent of the nitrogen used in agriculture.

Cyanobacteria are the most important nitrogen fixers in aquatic environments. In soil, members of the genera *Clostridium* and *Azotobacter* are examples of free-living, nitrogen-fixing bacteria. Other bacteria live symbiotically with legume plants, providing the most important source of fixed nitrogen. Symbionts may fix more nitrogen in soils than free-living organisms by a factor of 10. Soil bacteria, collectively called rhizobia, are able to symbiotically interact with legumes to form **nodules**, specialized structures where nitrogen fixation occurs (**Figure 22.27**). *Nitrogenase*, the enzyme that fixes nitrogen, is inactivated by oxygen, so the nodule provides an oxygen-free area for nitrogen fixation to take place. The oxygen is sequestered by a form of plant hemoglobin called *leghemoglobin*, which protects the *nitrogenase*, but releases enough oxygen to support respiratory activity.

Symbiotic nitrogen fixation provides a natural and inexpensive plant fertilizer: It reduces atmospheric nitrogen to ammonia, which is easily usable by plants. The use of legumes is an excellent alternative to chemical fertilization and is of special interest to *sustainable agriculture*, which seeks to minimize the use of chemicals and conserve natural resources. Through symbiotic nitrogen fixation, the plant benefits from using an endless source of nitrogen: the atmosphere. The bacteria benefit from using photosynthates (carbohydrates produced during photosynthesis) from the plant and having a protected niche. In addition, the soil benefits from being naturally fertilized. Therefore, the use of rhizobia as biofertilizers is a sustainable practice.

Why are legumes so important? Some, like soybeans, are key sources of agricultural protein. Some of the most important legumes consumed by humans are soybeans, peanuts, peas, chickpeas, and beans. Other legumes, such as alfalfa, are used to feed cattle.

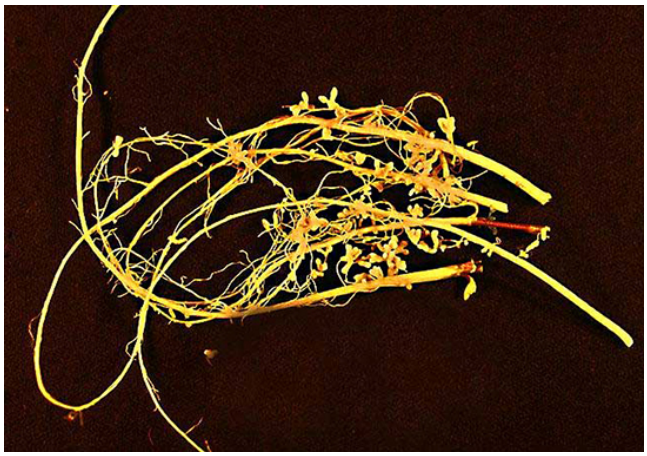


Figure 22.27 Nitrogen-fixation nodules on soybean roots. Soybean (*Glycine max*) is a legume that interacts symbiotically with the soil bacterium *Bradyrhizobium japonicum* to form specialized structures on the roots called *nodules* where nitrogen fixation occurs. (credit: USDA)

everyday CONNECTION

Microbes on the Human Body

The commensal bacteria that inhabit our skin and gastrointestinal tract do a host of good things for us. They protect us from pathogens, help us digest our food, and produce some of our vitamins and other nutrients. These activities have been known for a long time. More recently, scientists have gathered evidence that these bacteria may also help regulate our moods, influence our activity levels, and even help control weight by affecting our food choices and absorption patterns. The Human Microbiome Project has begun the process of cataloging our normal bacteria (and archaea) so we can better understand these functions.

A particularly fascinating example of our normal flora relates to our digestive systems. People who take high doses of antibiotics tend to lose many of their normal gut bacteria, allowing a naturally antibiotic-resistant species called *Clostridium difficile* to overgrow and cause severe gastric problems, especially chronic diarrhea (**Figure 22.28**). Obviously, trying to treat this problem with antibiotics only makes it worse. However, it has been successfully treated by giving the patients fecal transplants from healthy donors to reestablish the normal intestinal microbial community. Clinical trials are underway to ensure the safety and effectiveness of this technique.

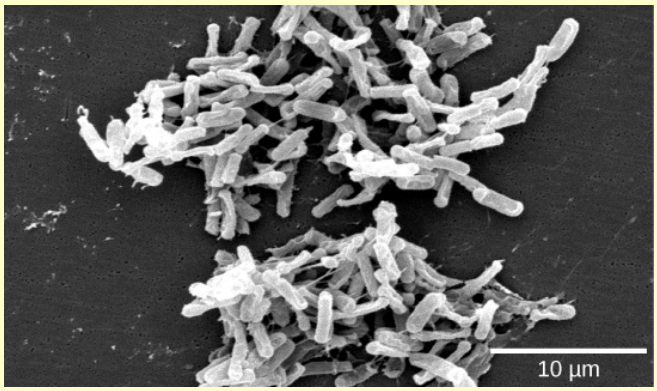


Figure 22.28 *Clostridium difficile*. This scanning electron micrograph shows *Clostridium difficile*, a Gram-positive, rod-shaped bacterium that causes severe diarrhea. Infection commonly occurs after the normal gut fauna are eradicated by antibiotics, and in the hospital can be deadly to seriously ill patients. (credit: modification of work by CDC, HHS; scale-bar data from Matt Russell)

Scientists are also discovering that the absence of certain key microbes from our intestinal tract may set us up for a variety of problems. This seems to be particularly true regarding the appropriate functioning of the immune system. There are intriguing findings that suggest that the absence of these microbes is an important contributor to the development of allergies and some autoimmune disorders. Research is currently underway to test whether adding certain microbes to our internal ecosystem may help in the treatment of these problems, as well as in treating some forms of autism.

Early Biotechnology: Cheese, Bread, Wine, Beer, and Yogurt

According to the United Nations Convention on Biological Diversity, **biotechnology** is “any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."[5] The concept of “specific use” involves some sort of commercial application. Genetic engineering, artificial selection, antibiotic production, and cell culture are current topics of study in biotechnology and will be described in later chapters. However, humans were using prokaryotes before the term biotechnology was even coined. Some of the products of this early biotechnology are as familiar as cheese, bread, wine, beer, and yogurt, which employ both bacteria and other microbes, such as yeast, a fungus (**Figure 22.29**).

5. http://www.cbd.int/convention/articles/?a=cbd-02 (http://openstax.org/l/UN_convention) , United Nations Convention on Biological Diversity: Article 2: Use of Terms.

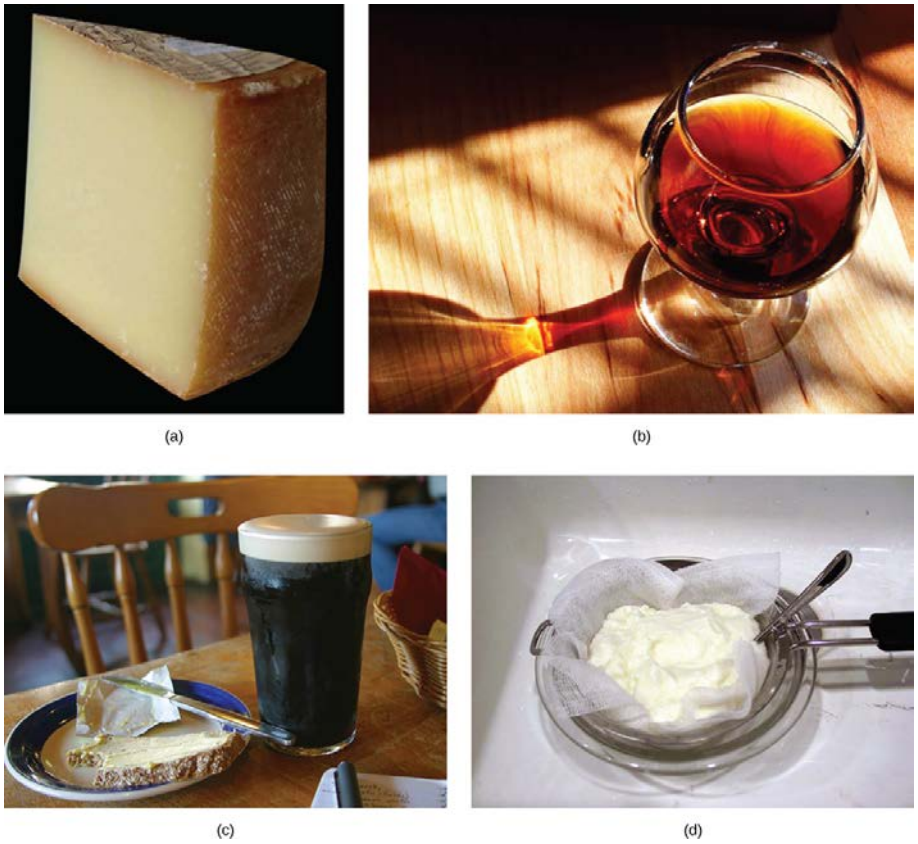


Figure 22.29 Some foods produced by microorganisms. Some of the products derived from the use of prokaryotes in early biotechnology include (a) cheese, (b) wine, (c) beer and bread, and (d) yogurt. (credit bread: modification of work by F. Rodrigo/Wikimedia Commons; credit wine: modification of work by Jon Sullivan; credit beer and bread: modification of work by Kris Miller; credit yogurt: modification of work by Jon Sullivan)

Cheese production began around 4,000 to 7,000 years ago when humans began to breed animals and process their milk. Fermentation in this case preserves nutrients: Milk will spoil relatively quickly, but when processed as cheese, it is more stable. As for beer, the oldest records of brewing are about 6,000 years old and were an integral part of the Sumerian culture. Evidence indicates that the Sumerians discovered fermentation by chance. Wine has been produced for about 4,500 years, and evidence suggests that cultured milk products, like yogurt, have existed for at least 4,000 years.

Using Prokaryotes to Clean up Our Planet: Bioremediation

Microbial **bioremediation** is the use of prokaryotes (or microbial metabolism) to remove pollutants. Bioremediation has been used to remove agricultural chemicals (e.g., pesticides, fertilizers) that leach from soil into groundwater and the subsurface. Certain toxic metals and oxides, such as selenium and arsenic compounds, can also be removed from water by bioremediation. The reduction of SeO_4^{-2} to SeO_3^{-2} and to Se^0 (metallic selenium) is a method used to remove selenium ions from water. Mercury (Hg) is an example of a toxic metal that can be removed from an environment by bioremediation. As an active ingredient of some pesticides, mercury is used in industry and is also a by-product of certain processes, such as battery production. Methyl mercury is usually present in very low concentrations in natural environments, but it is highly toxic because it accumulates in living tissues. Several species of bacteria can carry out the biotransformation of toxic mercury into nontoxic forms. These bacteria, such as *Pseudomonas aeruginosa*, can convert Hg^{+2} into Hg^0, which is nontoxic to humans.

One of the most useful and interesting examples of the use of prokaryotes for bioremediation purposes is the cleanup of oil spills. The significance of prokaryotes to petroleum bioremediation has been demonstrated in several oil spills in recent years, such as the Exxon Valdez spill in Alaska (1989) (**Figure 22.30**), the Prestige oil spill in Spain (2002), the spill into the Mediterranean from a Lebanon power plant (2006), and more recently, the BP oil spill in the Gulf of Mexico (2010). In the case of oil spills in the ocean, ongoing natural bioremediation tends to occur, since there are oil-consuming bacteria in the ocean prior to the spill. In addition to these naturally

occurring oil-degrading bacteria, humans select and engineer bacteria that possess the same capability with increased efficacy and spectrum of hydrocarbon compounds that can be processed. Bioremediation is enhanced by the addition of inorganic nutrients that help bacteria to grow.

Some hydrocarbon-degrading bacteria feed on hydrocarbons in the oil droplet, breaking down the hydrocarbons into smaller subunits. Some species, such as *Alcanivorax borkumensis*, produce surfactants that *solubilize* the oil (making it soluble in water), whereas other bacteria degrade the oil into carbon dioxide. Under ideal conditions, it has been reported that up to 80 percent of the nonvolatile components in oil can be degraded within one year of the spill. Other oil fractions containing aromatic and highly branched hydrocarbon chains are more difficult to remove and remain in the environment for longer periods of time.

(a) (b)

Figure 22.30 Prokaryotes and bioremediation. (a) Cleaning up oil after the Exxon Valdez spill in Alaska, workers hosed oil from beaches and then used a floating boom to corral the oil, which was finally skimmed from the water surface. Some species of bacteria are able to solubilize and degrade the oil. (b) One of the most catastrophic consequences of oil spills is the damage to fauna. (credit a: modification of work by NOAA; credit b: modification of work by GOLUBENKOV, NGO: Saving Taman)