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.

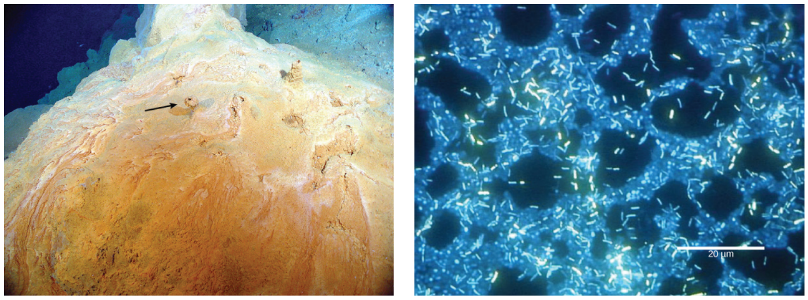
Prokaryotes, the First Inhabitants of Earth

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.



(a) (b)

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.

(a) (b)

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

Table 22.1

Extremophile	Conditions for Optimal Growth
Halophiles	Salt concentration of at least 0.2 M
Osmophiles	High sugar concentration

Table 22.1

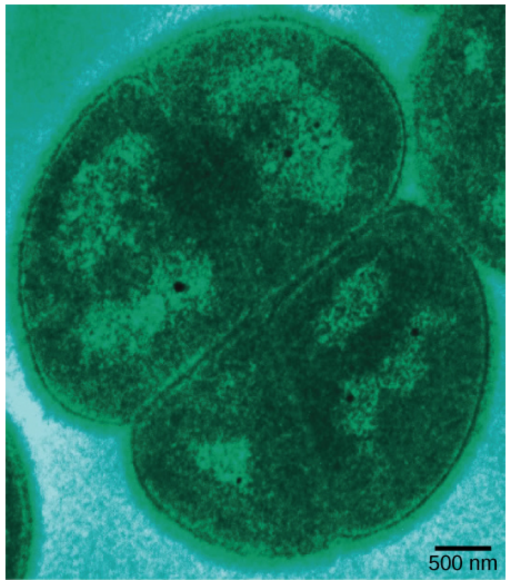


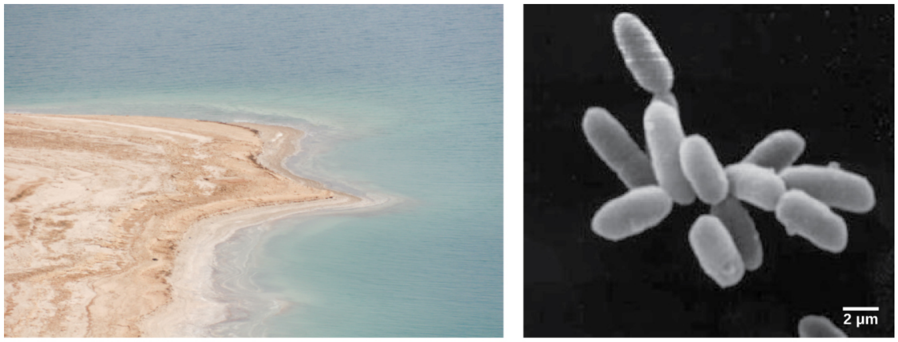
Figure 22.5 Radiation-tolerant prokaryotes. *Deinococcus radiodurans*, visualized in this false color transmission electron micrograph, is a prokaryote that can tolerate very high doses of ionizing radiation. It has developed DNA repair mechanisms that allow it to reconstruct its chromosome even if it 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.

1Bodaker, 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.



(a) (b)

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 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

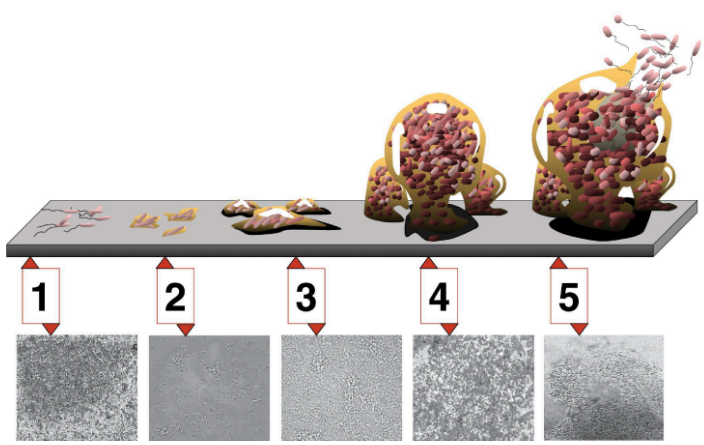


Figure 22.8 Development of a biofilm. Five stages of biofilm development are shown. During stage 1, initial attachment, bacteria adhere to a solid surface via weak *van der Waals interactions* (forces produced by induced electrical interactions between atoms). During stage 2, irreversible attachment, 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?