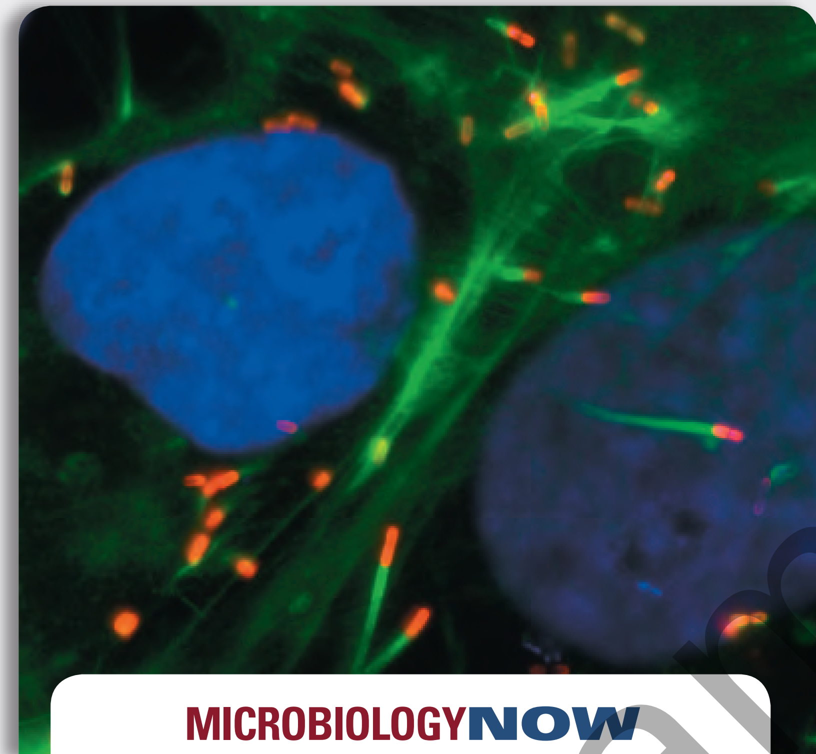
The Microbial World 1



MICROBIOLOGYNOW

Microbiology in Motion

The microbial world is strange and fierce. It is teeming with life, ancient, diverse, and constantly changing. Microorganisms are Earth's life support system, and from our first breath they influence nearly every moment of our lives. Microbes are in our water and our food, and we carry them on us and in us. Indeed, microbes abound in any natural environment that will support life, including many environments too hostile for higher life forms.

While the microbial world is invisible, we can explore it through the science of microbiology. Microbiology evolves at a breathtaking pace. Even the microscope continues to evolve, providing an ever more detailed picture of the microbial world. The image above was made with a fluorescence microscope that uses lasers, guided by a computer, to map the three-dimensional structure of cells. The image shows neighboring human cells with their nuclei stained blue and actin filaments stained green. These cells are infected with the foodborne bacterial pathogen *Listeria monocytogenes*, stained red.

Listeria are soil organisms that sometimes find their way into our food. In soils they infect other microbes such as amoebae. Our cells are similar in many ways to those of microscopic organisms, and so *Listeria* finds itself well adapted to live within us. This bacterium has the unique ability to hijack cellular systems, causing actin to polymerize and propel the cell like a rocket within the host cytoplasm. The force of this propulsion causes *Listeria* to penetrate adjacent cells (image, lower left), spreading the infection. *Listeria* can also invade host vacuoles (not shown), where it hides and survives. This persistent state can prolong infection and promote resistance to antibiotic therapy. Research on *Listeria* has provided new insights on the biology of this pathogen and an ever-changing view of a microbial world in motion.

Source: Kortebi, M., et al. 2017. *Listeria monocytogenes* switches from dissemination to persistence by adopting a vacuolar lifestyle in epithelial cells. *PLoS Pathog. 13*: e1006734.

This chapter launches our journey into the microbial world. Here we will begin to discover what the science of microbiology is all about and what microorganisms are, what they do, and how they can be studied. We also place microbiology in historical context, as a process of scientific discovery driven by simple (yet powerful) experiments and insightful minds.

I • Exploring the Microbial World

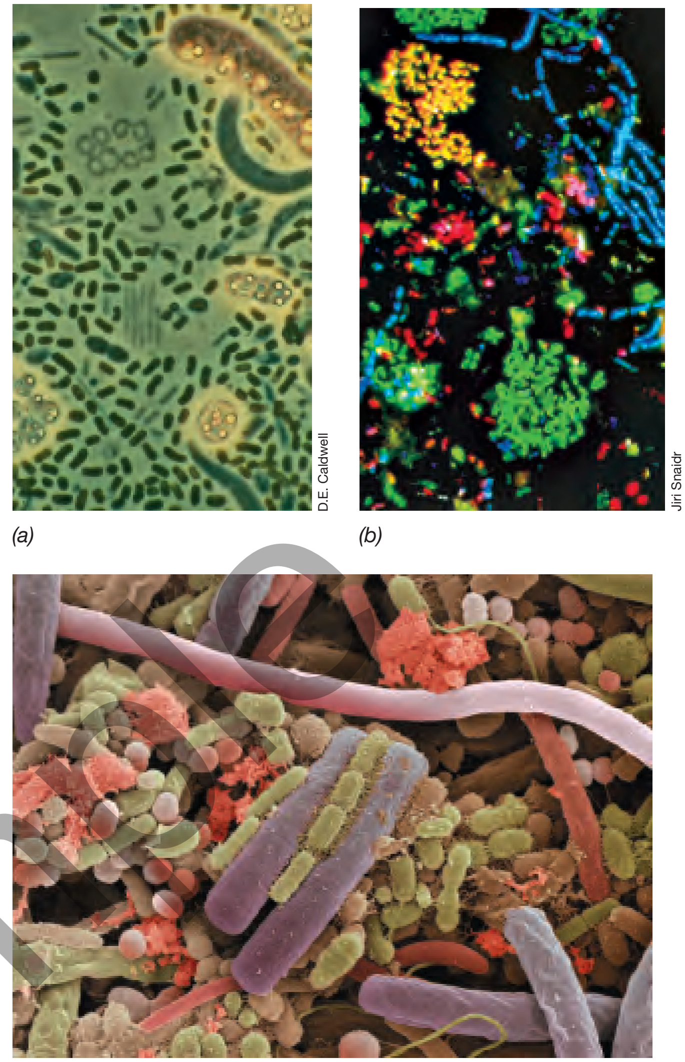
The microbial world consists of microscopic organisms that have defined structures, unique evolutionary histories, and are of enormous importance to the biosphere.

1.1 Microorganisms, Tiny Titans of the Earth

Microorganisms (also called *microbes*) are life forms too small to be seen by the unaided human eye. These microscopic organisms are diverse in form and function, and they inhabit every environment on Earth that supports life. Many microbes are undifferentiated single-celled organisms, but some can form complex structures, and some are even multicellular. Microorganisms typically live in complex **microbial communities** (**Figure 1.1**), and their activities are regulated by interactions with each other, with their environments, and with other organisms. The science of microbiology is all about microorganisms, who they are, how they work, and what they do.

Microorganisms were teeming on the land and in the seas for billions of years before the appearance of plants and animals, and their diversity is staggering. Microorganisms represent a major fraction of Earth's biomass, and their activities are essential to sustaining life. Indeed, the very oxygen (O_2) we breathe is the result of microbial activities. Plants and animals are immersed in a world of microbes, and their evolution and survival are heavily influenced by microbial activities, by microbial symbioses, and by *pathogens*—those microbes that cause disease. Microorganisms are woven into the fabric of human life as well (**Figure 1.2**), from infectious diseases, to the food we eat, the water we drink, the fertility of our soils, the health of our animals, and even the fuel we put in automobiles. Microbiology is the study of the dominant form of life on Earth, and the effect that microbes have on our planet and all of the living things that call it home.

Microbiologists have many tools for studying microorganisms. Microbiology was born of the microscope, and microscopy is foundational to microbiology. Microbiologists have developed an array of methods for visualizing microorganisms, and these microscopic techniques are essential to microbiology. The cultivation of microorganisms is also foundational to microbiology. A microbial **culture** is a collection of cells that have been grown in or on a nutrient medium. A **medium** (plural, media) is a liquid or solid nutrient mixture that contains all of the nutrients required for a microorganism to grow. In microbiology, we use the word **growth** to refer to the increase in cell number as a result of cell division. A single microbial cell placed on a solid nutrient medium can grow and divide into millions or even billions of cells that form a visible **colony** (**Figure 1.3**). The formation of visible colonies makes it easier to see and grow microorganisms. Comprehension of the microbial basis of disease and microbial biochemical diversity has relied on the ability to grow microorganisms in the laboratory.



(a) (b) (c)

Figure 1.1 Microbial communities. *(a)* A bacterial community that developed in the depths of a small Michigan lake, including cells of various phototrophic bacteria. The bacteria were visualized using phase-contrast microscopy. *(b)* A bacterial community in a sewage sludge sample. The sample was stained with a series of dyes, each of which stained a specific bacterial group. From *Journal of Bacteriology 178*: 3496–3500, Fig. 2b. © 1996 American Society for Microbiology. *(c)* Colorized scanning electron micrograph of a microbial community scraped from a human tongue.

The ability to grow microorganisms rapidly under controlled conditions makes them highly useful for experiments that probe the fundamental processes of life. Most discoveries relating to the molecular and biochemical basis of life have been made using microorganisms. The study of molecules and their interactions is essential to defining the workings of microbial cells, and the tools of molecular biology and biochemistry are foundational to microbiology. Molecular biology has also provided a variety of tools to study microorganisms without need for their cultivation in the laboratory. These molecular tools have greatly expanded our knowledge of microbial ecology and diversity. Finally, the tools of genomics and molecular genetics are also cornerstones of modern

Figure 1.2 Microbial applications. Microorganisms have major impacts on the world in which we live. In the chapters that follow we will learn how microorganisms impact our health, the foods we eat, the water we drink, and even the air we breathe. We will learn how microbes can be used to produce valuable products and the many ways in which microorganisms touch our lives.

microbiology and allow microbiologists to study the genetic basis of life, how genes evolve, and how they regulate the activities of cells.

In the next section, we explore the basic elements of microbial cell structure and summarize the major physiological activities that take place in all cells, regardless of their structure.

Check Your Understanding

- In what ways are microorganisms important to humans?
- Why are microbial cells useful for understanding the basis of life?
- What is a microbial colony and how is one formed?

1.2 Structure and Activities of Microbial Cells

Microbial cells are living compartments that interact with their environment and with other cells in dynamic ways. We purposely exclude viruses in most of this discussion because although they resemble cells in many ways, viruses are not cells but instead a special category of microorganism. We consider the structure, diversity, and activities of viruses in Section 1.4 and in Chapters 5 and 11.

Elements of Microbial Structure

All cells have much in common and contain many of the same components (**Figure 1.4**). All cells have a permeability barrier called the **cytoplasmic membrane** that separates the inside of the cell, the **cytoplasm**, from the outside. The cytoplasm is an aqueous mixture of **macromolecules** (for example proteins, lipids, nucleic acids, and polysaccharides), small organic molecules (mostly the precursors of macromolecules), various inorganic ions, and ribosomes. All cells also contain **ribosomes**, which are the structures responsible for protein synthesis. Some cells have a **cell wall** that lends structural strength to a cell. The cell wall is a relatively permeable structure located outside the cytoplasmic membrane and is a much stronger layer than the membrane itself. Cell walls are typically found in plant cells and most microorganisms but are not found in animal cells.

There are two fundamental cell types that differ categorically in cellular organization: those having **prokaryotic** cell structure, and those having **eukaryotic** cell structure (Figure 1.4). Cells having eukaryotic cell structure are found in a group of organisms called the *Eukarya*. This group includes plants and animals as well as diverse microbial eukaryotes such as algae, protozoa, and fungi. Eukaryotic cells contain an assortment of membrane-enclosed cytoplasmic structures called **organelles** (Figure 1.4*b*). These include, most prominently, the DNA-containing nucleus but also mitochondria and chloroplasts, organelles that specialize in supplying the cell with energy, and various other organelles.

Mastering Microbiology
Art Activity: Figure 1.3 Common elements of prokaryotic/eukaryotic cells

Prokaryotic cell structure is found within two different groups of organisms we know as *Bacteria* and *Archaea*. Prokaryotic cells have few internal structures, they lack a nucleus, and they typically lack organelles (Figure 1.4*a*). *Bacteria* and *Archaea* appeared long before the evolution of eukaryotes (Section 1.5). While all *Archaea* and

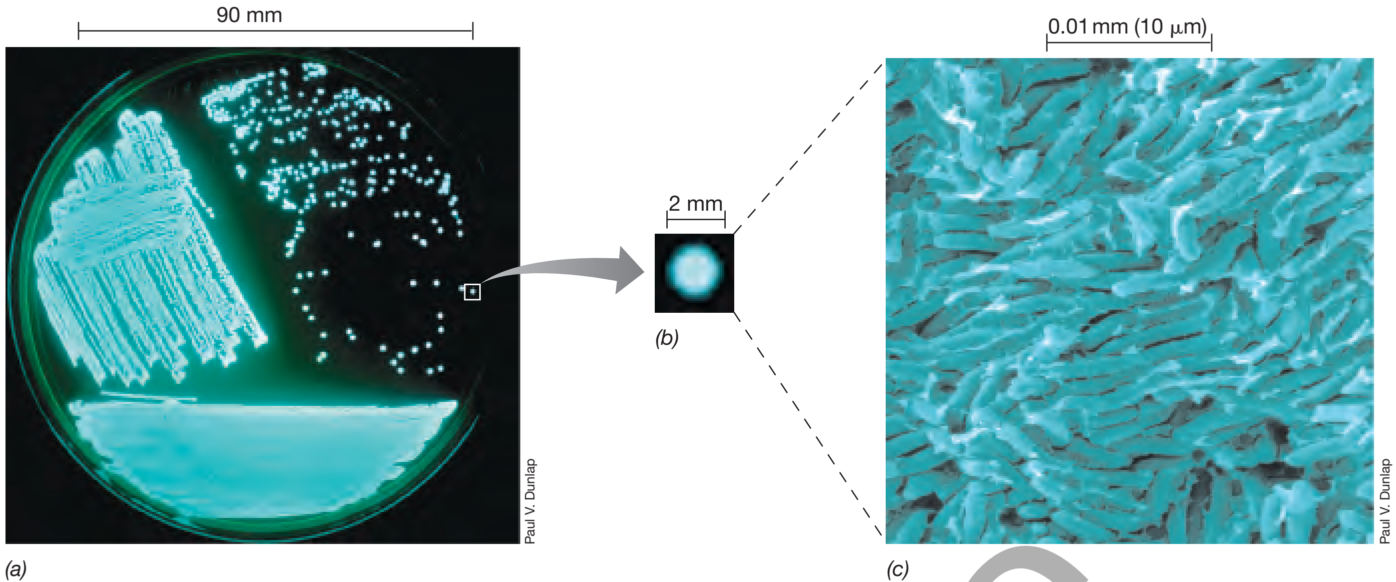


Figure 1.3 Microbial cells. *(a)* Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. *(b)* A single colony can contain more than 10 million (10^7) individual cells. *(c)* Colorized scanning electron micrograph of cells of *Photobacterium*.

Bacteria have prokaryotic cell structure, these two groups diverged very early in the history of life and as a result many of their molecular and genetic characteristics differ at a fundamental level. Indeed, we will see later that in many ways *Archaea* and *Eukarya* are more similar to each other than either is to *Bacteria*.

Genes, Genomes, Nucleus, and Nucleoid

In addition to a cytoplasmic membrane and ribosomes, all cells also possess a DNA **genome**. The genome is the full set of genes in a cell. A gene is a segment of DNA that encodes a protein or an RNA molecule. The genome is the living blueprint of an organism; the characteristics, activities, and very survival of a cell are governed by its genome.

The genomes of prokaryotic cells and eukaryotic cells are organized into structures called **chromosomes**. In eukaryotic cells, DNA is present as several linear molecules (each one formed into its own chromosome) within the membrane-enclosed **nucleus**. By contrast, the genomes of *Bacteria* and *Archaea* are typically closed circular chromosomes (though some prokaryotic cells have linear chromosomes). The chromosome aggregates within the prokaryotic cell to form the **nucleoid**, a mass that is visible in the electron microscope (Figure 1.4*a*) but which is *not* enclosed by a membrane. Most prokaryotic cells have only a single chromosome, but many also contain one or more small circles of DNA distinct from that of the chromosome, called **plasmids** (Figure 1.4*a*). Plasmids typically contain genes that are not essential but often confer some special property on the cell (such as a unique metabolism, or antibiotic resistance). The genomes of *Bacteria* and *Archaea* are typically small and compact, and most contain between 500 and 10,000 genes encoded by 0.5 to 10 million base pairs of DNA. Eukaryotic cells typically have much larger and much less streamlined genomes than prokaryotic cells. A human cell, for example, contains approximately 3 billion base pairs, which encode about 20,000–25,000 genes.

Mastering Microbiology
Art Activity: Figure 1.4 Basic properties of microbial cells

Activities of Microbial Cells

To be competitive in nature, a microorganism must survive and reproduce. **Figure 1.5** considers structure and some of the activities that are performed by cells to drive survival and reproduction. All cells show some form of **metabolism** through which nutrients are acquired from the environment and transformed into new cellular materials and waste products. During these transformations, energy is used to support synthesis of new structures. Production of these new structures culminates in the division of the cell to form two cells. Microbial growth results from successive rounds of cell division.

Genes contain information that is used by the cell to perform the work of metabolism. Genes are decoded to form proteins that regulate cellular processes. **Enzymes**, those proteins that have catalytic activity, carry out reactions that supply energy and perform biosynthesis within the cell. Enzymes and other proteins are synthesized during *gene expression* in the sequential processes of transcription and translation. **Transcription** is the process by which the information encoded in DNA sequences is copied into an RNA molecule, and **translation** is the process whereby the information in an RNA molecule is used by a ribosome to synthesize a protein (Chapter 6). Gene expression and enzyme activity in a microbial cell are coordinated and highly regulated to ensure that the cell remains optimally tuned to its surroundings. Ultimately, microbial growth requires replication of the genome through the process of **DNA replication**, followed by cell division. All cells carry out the processes of transcription, translation, and DNA replication.

Microorganisms have the ability to sense and respond to changes in their local environment. Many microbial cells are capable of **motility**, typically by self-propulsion (Figure 1.5). Motility allows cells to relocate in response to environmental conditions. Some microbial cells undergo **differentiation**, which may result

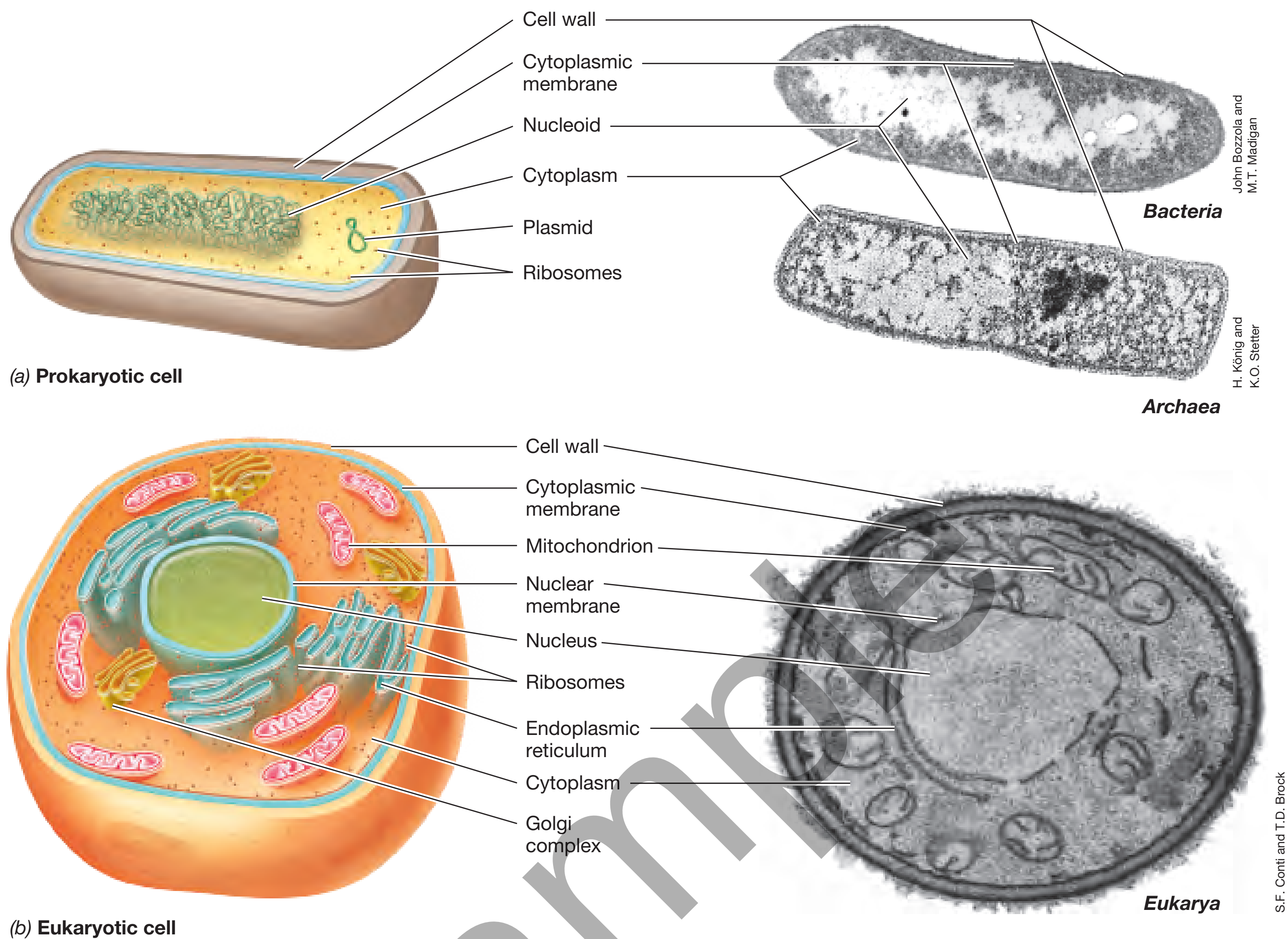


Figure 1.4 Microbial cell structure. *(a)* (Left) Diagram of a prokaryotic cell. (Right) Electron micrograph of *Heliobacterium modesticaldum* (*Bacteria*, cell is about 1 μm in diameter) and *Thermoproteus neutrophilus* (*Archaea*, cell is about 0.5 μm in diameter). *(b)* (Left) Diagram of a eukaryotic cell. (Right) Electron micrograph of a cell of *Saccharomyces cerevisiae* (*Eukarya*, cell is about 8 μm in diameter). In terms of relative scale, the bacterial cell in *a* is about the same size as the mitochondria of *Saccharomyces* in *b*.

in the formation of modified cells specialized for growth, dispersal, or survival. Cells respond to chemical signals in their environment, including those produced by other cells of either the same or different species, and these signals often trigger new cellular activities. Microbial cells thus exhibit **intercellular communication**; that is, they are "aware" of their neighbors and can respond accordingly. Many prokaryotic cells can also exchange genes with neighboring cells, regardless of their species, in the process of **horizontal gene transfer**.

Evolution (Figure 1.5) results when genes in a population of cells change in sequence and frequency over time, leading to descent with modification. The evolution of microorganisms can be very rapid relative to the evolution of plants and animals. For example, the indiscriminate use of antibiotics in human and veterinary medicine has selected for the proliferation of antibiotic resistance in pathogenic bacteria. The rapid pace of microbial evolution can be attributed in part to the ability of microorganisms to grow very quickly and to acquire new genes though the process of horizontal gene transfer. Not all of the processes depicted in Figure 1.5 occur in all cells. Metabolism, growth, and evolution, however, are universal and will be major areas of emphasis throughout this text.

We now move on to consider the diversity of cell shapes and sizes found in the microbial world.

Check Your Understanding

- What structures are universal to all type of cells?
- What processes are universal to all types of cells?
- What structures can be used to distinguish between prokaryotic cells and eukaryotic cells?

1.3 Cell Size and Morphology

Microscopic examination of microorganisms immediately reveals their **morphology**, which is defined by cell size and shape. A variety of cell shapes pervade the microbial world, and although microscopic by their very nature, microbial cells come in a variety of sizes.

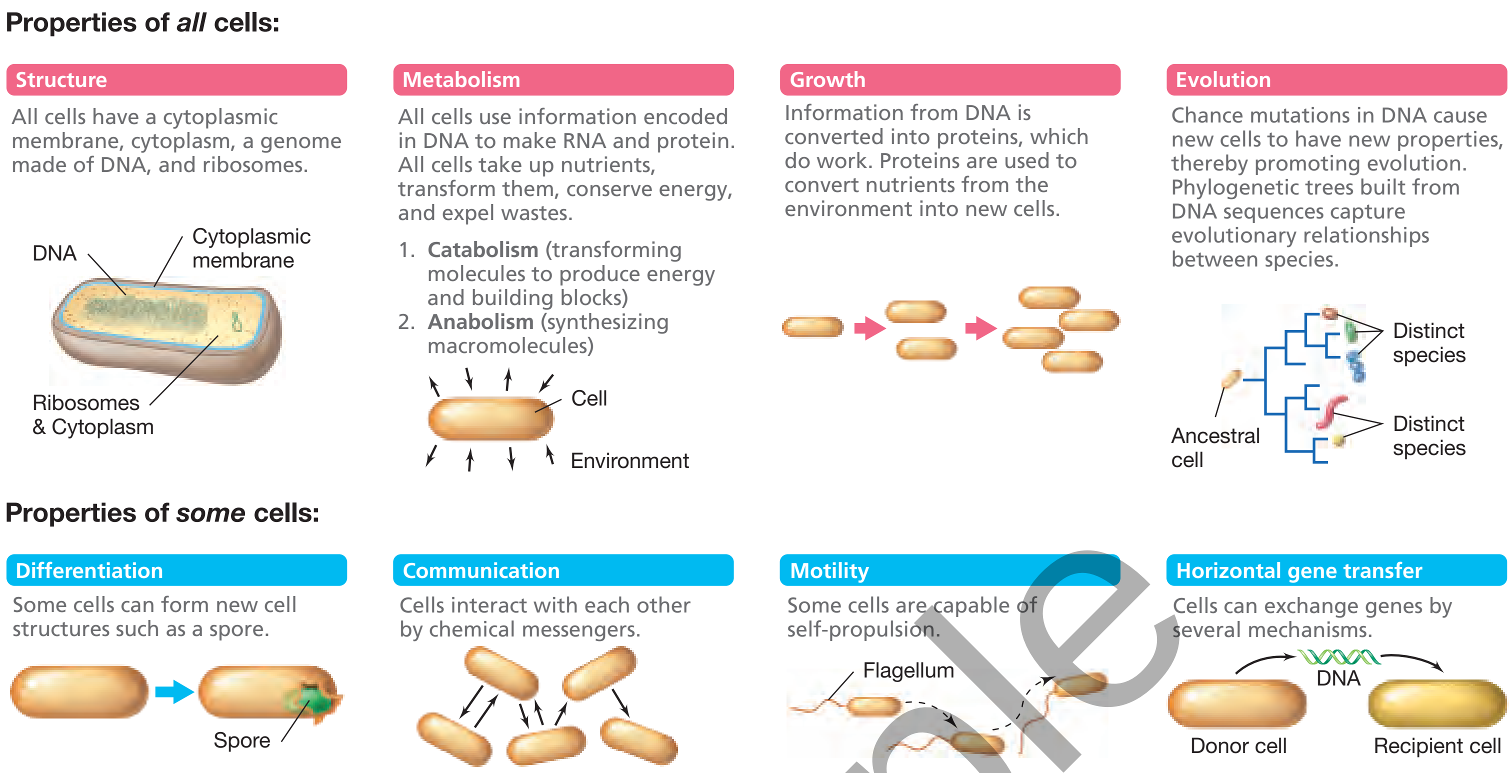


Figure 1.5 The properties of microbial cells. While cells are tremendously diverse in form and function, certain properties are shared by all cells.

Cell shape can be useful for distinguishing different microbial cells and often has ecological significance. Moreover, the very small size of most microbial cells has a profound effect on their ecology and dictates many aspects of their biology. We begin by considering cell size and then consider cell shape.

The Small World

A micrometer (μm or micron) is one-millionth of a meter in length. The unaided human eye has difficulty resolving objects that are less than 100 μm in diameter, but this is the scale of the microbial world. Most prokaryotic cells are small, ranging between 0.5 and 10 μm in length, but prokaryotic cells can vary widely in size. For example, the smallest prokaryotic cells are about 0.2 μm in diameter and the largest can be more than 600 μm long (**Table 1.1**). In contrast, most eukaryotic cells are larger on average than prokaryotic cells, being between 5 and 100 μm in length, but eukaryotic cells can vary widely in size too. For example, the smallest eukaryotic microorganism known is about 0.8 μm in diameter and the largest eukaryotic cells can be many centimeters in length (Section 1.4).

Cell size is influenced fundamentally by cell structure. Eukaryotic cells, owing to their complex intracellular structure and organelles (Figure 1.4), can actively transport molecules and macromolecules within the cytoplasm. Prokaryotic cells, in contrast, rely on diffusion for transport through the cytoplasm and this limits their size. While diffusion is very fast at small distances, the rate of diffusion increases as the square of the distance traveled. Hence, the metabolic rate in a prokaryotic cell varies inversely with the square of its size. This relationship means that, as cell size increases, it becomes advantageous to have cellular structures that facilitate transport and compartmentalize cellular activities as seen in eukaryotic cells. In contrast, since diffusion is rapid at small spatial scales, high metabolic rates can be maintained in small prokaryotic cells without a need for complex cellular structures.

It is possible, though unusual, for prokaryotic cells to be visible to the human eye; the largest are more than 600 μm (0.6 mm) long. To achieve this size, these bacteria must have traits that allow them to overcome diffusional limitation. The bacterium *Epulopiscium fishelsoni* (**Figure 1.6*a***; Figure 1.9), which is found in the gut of the surgeonfish, can be more than 75 μm wide and 600 μm long (Table 1.1). One of the traits that allows this bacterium to get so large is that it can have more than 10,000 copies of its genome distributed throughout its cytoplasm, thereby preventing diffusional limitation between the genome and any region of the cytoplasm. Cells of the largest known bacterium, the sulfur-oxidizing chemolithotroph *Thiomargarita* (Figure 1.6*b*, Table 1.1), are even larger than those of *Epulopiscium*, about 750 μm in diameter. *Thiomargarita* achieves this enormous size by having a large vacuole that fills the center of the cell. Hence, the cytoplasm of *Thiomargarita* occurs as a thin layer squeezed between the cytoplasmic membrane and this central vacuole. In this way, the cytoplasm is never more than 1 μm from the membrane. In addition, *Thiomargarita*, like *Epulopiscium*, also has many copies of its genome, which are distributed throughout its cytoplasm.

TABLE 1.1 Cell size and volume of some cells of *Bacteria*, from the largest to the smallest

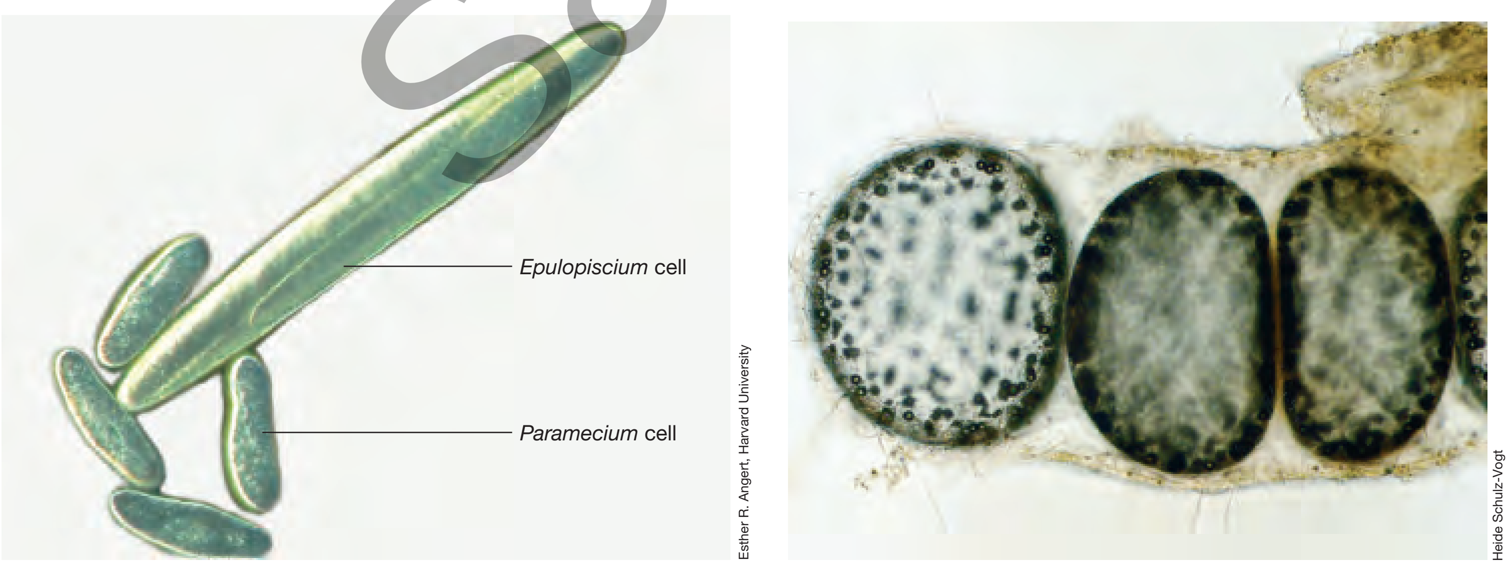
Organism	Characteristics	Morphology	Size[a] (μm^3)	Cell volume (μm^3)	Volumes compared to *E. coli*
Thiomargarita namibiensis	Sulfur chemolithotroph	Cocci in chains	750	200,000,000	100,000,000×
Epulopiscium fishelsoni[a]	Chemoorganotroph	Rods with tapered ends	80 × 600	3,000,000	1,500,000×
Beggiatoa species[a]	Sulfur chemolithotroph	Filaments	50 × 160	1,000,000	500,000×
Achromatium oxaliferum	Sulfur chemolithotroph	Cocci	35 × 95	80,000	40,000×
Lyngbya majuscula	Cyanobacterium	Filaments	8 × 80	40,000	20,000×
Thiovulum majus	Sulfur chemolithotroph	Cocci	18	3,000	1,500×
Staphylothermus marinus[a]	Hyperthermophile	Cocci in irregular clusters	15	1,800	900×
Magnetobacterium bavaricum	Magnetotactic bacterium	Rods	2 × 10	30	15×
Escherichia coli	Chemoorganotroph	Rods	1 × 2	2	1×
Pelagibacter ubique[a]	Marine chemoorganotroph	Rods	0.2 × 0.5	0.014	0.007×
Ultra-small bacteria[a]	Uncultured, from groundwater	Variable	<0.2	0.009	0.0045×
Mycoplasma pneumoniae	Pathogenic bacterium	Pleomorphic[b]	0.2	0.005	0.0025×

[a]Where only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200 μm in diameter. But on occasion, giant cells of 750 μm are observed. Likewise, an average cell of *S. marinus* is about 1 μm in diameter. The species of *Beggiatoa* here is unclear, and *E. fishelsoni*, *M. bavaricum*, and *P. ubique* are not formally recognized names in taxonomy. For more on ultra-small bacteria, see Explore the Microbial World "Tiny Cells."
[b]*Mycoplasma* is a bacterium that lacks a cell wall and can thus take on many shapes (*pleomorphic* means "many shapes").
Source: Data obtained from Schulz, H.N., and B.B. Jørgensen. 2001. *Annu. Rev. Microbiol. 55*: 105–137, and Luef, B., et al. 2015. *Nat. Commun.* doi:10.1038/ncomms7372.

At the opposite end of the spectrum from these large prokaryotic cells are very small prokaryotic cells. Exactly how small a cell can be is not precisely known. However, cells 0.2 μm in diameter exist (see Explore the Microbial World, "Tiny Cells"), and the lower limit is probably only a bit smaller than this. Ultimately, the lower limit to cell size is likely a function of the amount of space needed to house the essential biochemical components—proteins, nucleic acids, ribosomes and so on (Section 1.2)—that all cells need to survive and reproduce.

Surface-to-Volume Ratios, Growth Rates, and Evolution

For a cell, there are advantages to being small. Small cells have more surface area relative to cell volume and thus have a higher *surface-to-volume ratio* than larger cells. To understand this principle, consider a spherical cell. The volume of a sphere is a function of the cube of its radius ($V = \frac{4}{3}\pi r^3$), whereas its surface area is a function of the square of the radius ($S = 4\pi r^2$). Therefore, the S/V ratio of a coccus is $3/r$ (**Figure 1.7**). As cell size *increases*, its S/V ratio *decreases*.



(a) (b)

Figure 1.6 Two very large *Bacteria*. *(a) Epulopiscium fishelsoni.* The rod-shaped cell is about 600 μm (0.6 mm) long and 75 μm wide and is shown with four cells of the protist *Paramecium* (a microbial eukaryote), each of which is about 150 μm long. *(b) Thiomargarita namibiensis*, a large sulfur chemolithotroph and currently the largest known of all prokaryotic cells. Cell widths vary from 400 to 750 μm.

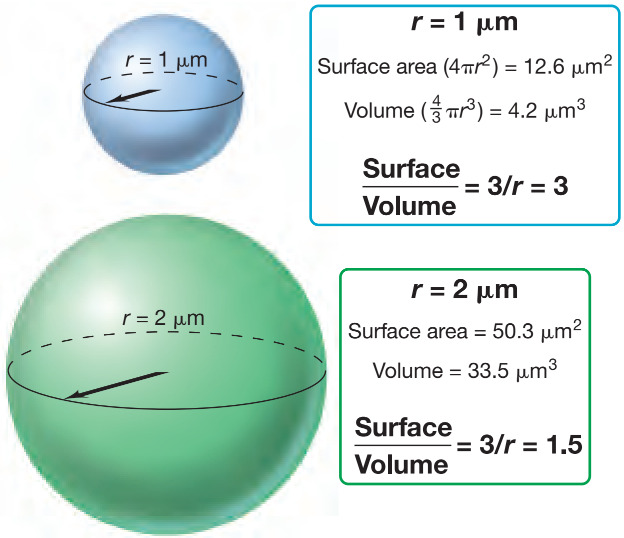


Figure 1.7 Surface area and volume relationships in cells. As a cell increases in size, its *S*/*V* ratio decreases.

To illustrate this, consider the *S*/*V* ratio for some of the cells of different sizes listed in Table 1.1: *Pelagibacter ubique*, 22; *Escherichia coli*, 4.5; and *E. fishelsoni* (Figure 1.6*a*), 0.05. The *S*/*V* of a rod-shaped organism can be estimated as if it were a cylinder; hence, the *S*/*V* of the cell will *decrease* as its radius *increases*.

The *S*/*V* ratio of a cell controls many of its properties, including how fast it grows (its *growth rate*) and shape. Cellular growth rate depends in part on the rate at which cells exchange nutrients and waste products with their environment. As cell size decreases, the *S*/*V* ratio of the cell increases, and this means that small cells can exchange nutrients and wastes more rapidly (per unit cell volume) than can large cells. As a result, free-living cells that are smaller tend to be more efficient than those that are larger, and any given mass of nutrients will support the synthesis of more small cells than large cells. We will see that cell morphology is also often predicated on the effect of cell shape on *S*/*V* ratio. For example, cell shapes that increase the overall membrane area of the cell, such as those having long thin appendages or invaginations, allow bacteria to increase their *S*/*V* ratio for a given mass of cytoplasm. We will see that prokaryotic cell morphology is remarkably diverse and different cell shapes can convey different benefits upon the cell.

Major Morphologies of Prokaryotic Cells

Common morphologies of prokaryotic cells are shown in **Figure 1.8**. A cell that is spherical or ovoid in morphology is called a *coccus* (plural, cocci). A cylindrically shaped cell is called a *rod* or a *bacillus* (plural, bacilli). A spiral-shaped cell is called a *spirillum* (plural, spirilla). A cell that is slightly curved and comma-shaped is called a *vibrio*. A *spirochete* is a special kind of organism (▶ Section 15.17) that has a spiral shape but which differs from spirilla because the cells of spirochetes are flexible, whereas cells of spirilla are rigid. Some bacteria are irregular in shape. Appendages, such as stalks and hyphae, are used by some cells for attachment or to increase surface area. In addition, asymmetrical cell division such as budding can result in irregular and asymmetrical cell shapes.

Cell division has a major impact on morphology because cells that remain attached to each other can form distinctive shapes. For instance, some cocci occur in pairs (diplococci), some form long chains (streptococci), others occur in three-dimensional cubes (tetrads or sarcinae), and still others occur in grapelike clusters (staphylococci). Filamentous bacteria are long, thin, rod-shaped bacteria that divide terminally and then form long filaments composed of many cells attached end to end.

The cell morphologies described here are representative but certainly not exhaustive; many variations of these morphologies are known. For example, there can be fat rods, thin rods, short rods, and long rods, rods that occur as single cells, as pairs of cells, or rods that

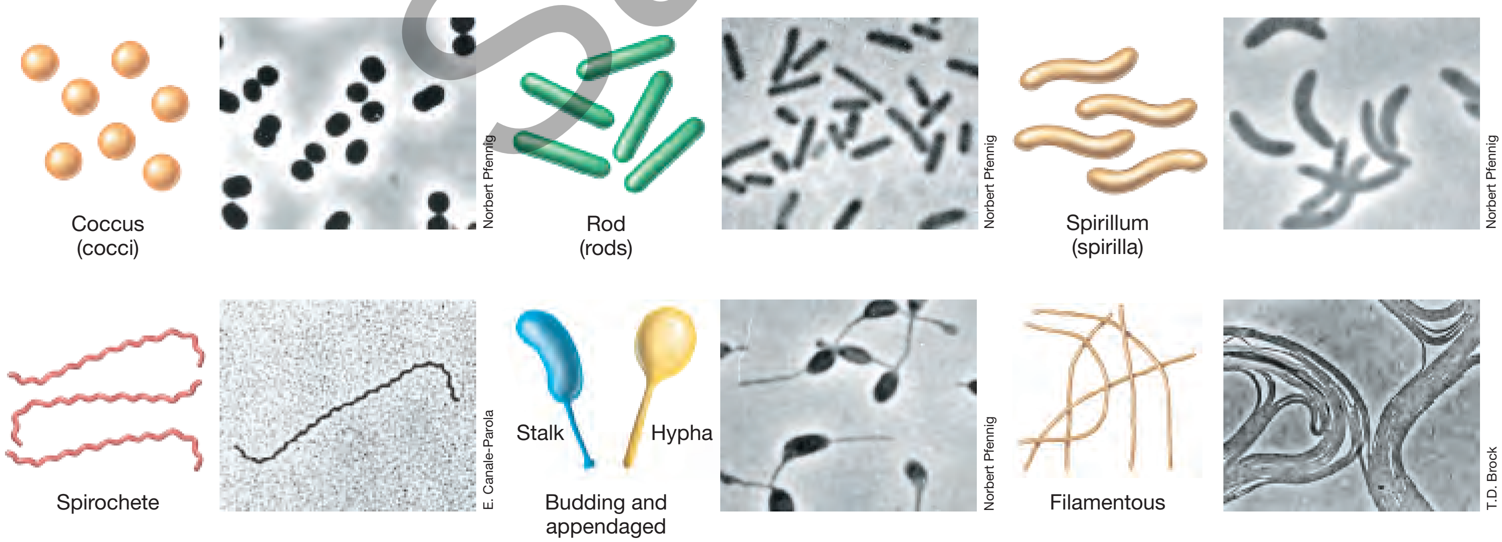


Figure 1.8 Cell morphologies. Beside each drawing is a phase-contrast photomicrograph of cells showing that morphology. Coccus (cell diameter in photomicrograph, 1.5 μm); rod (1 μm); spirillum (1 μm); spirochete (0.25 μm); budding (1.2 μm); filamentous (0.8 μm). All photomicrographs are of species of *Bacteria*. Not all of these morphologies are known among the *Archaea*, but cocci, rods, and spirilla are common.

Explore the Microbial World

Tiny Cells

Viruses are very small microbes and range in diameter from as small as 20 nm to almost 750 nm. Although no cells exist that are as small as most viruses, the recent discovery of ultra-small bacterial cells[1,2] has pushed the lower limits of cell size to what microbiologists feel must be very close to the minimal value. And, because microbiologists today can deduce amazing amounts of information about cells in nature without culturing them, the lack of laboratory cultures of these tiny cells has been only a minor impediment to understanding their biology in detail.

Microbiologists collected groundwater, which travels through Earth's deep subsurface, from a Colorado (USA) aquifer (**Figure 1**) and passed it through a membrane filter whose pores were only 0.2 μm in diameter. The liquid that passed through the filter was then subjected to microbiological analyses. Surprisingly, since filters with 0.2-μm pores have been used for decades to remove bacterial cells from solutions to generate "sterile solutions," prokaryotic cells were present in the groundwater filtrate. In fact, a diverse array of *Bacteria* were present in the filtrate, revealing that the groundwater was inhabited by a microbial community of tiny cells[1] that microbiologists have come to call ultramicrobacteria.

Figure 1 Sampling the anoxic groundwater aquifer that parallels the Colorado River near Rifle, Colorado.

Electron cryotomography, a microscopic technique in which a specimen is examined at extremely cold temperatures without fixation (chemical treatment that can alter a cell's morphology, see Section 1.10), showed the groundwater ultramicrobacteria to consist primarily of oval-shaped cells about 0.2 μm in diameter (**Figure 2**). The volume of these cells was calculated to be about 1/200 that of a cell of the bacterium *Escherichia coli* (see Table 1.1) such that more than 200 of the small cells could fit into one *E. coli* cell! Each of the tiny cells contained about 50 ribosomes, which is also about 1/100 of the number present in a slowly growing (100-min generation time) cell of *E. coli*. The very small size of the groundwater ultramicrobacteria gives them an enormous surface-to-volume ratio, and it is hypothesized that this advantage benefits them in extracting resources from their nutrient-deficient habitat.

Despite the fact that the tiny groundwater bacteria have yet to be cultured in the laboratory, much is already known about them because their small genomes—less than 1 megabase (Mb) in size—were obtained and analyzed.[2] From a phylogenetic perspective, the different species detected were distantly related to major phyla of *Bacteria* known from environmental analyses of diverse environments but which have thus far defied laboratory culture. Further analyses showed that genes encoding the enzymes for several core metabolic pathways widely distributed among microorganisms were absent from the genomes of the groundwater ultramicrobacteria. This suggests a metabolically minimalist lifestyle for these tiny cells and a survival strategy of cross-feeding essential nutrients with neighboring species in their microbial community.

A strategy of obtaining nutrients from other organisms is one widely used in the microbial world. As we will see later in this book, many disease-causing (pathogenic or parasitic) bacteria have very small genomes that are missing many key genes otherwise necessary for a free-living lifestyle. However, the pathogenic or parasitic way of life of these microbes lets them "get away" with a minimal genomic complement because any essential molecules they are unable to biosynthesize are supplied by the host.

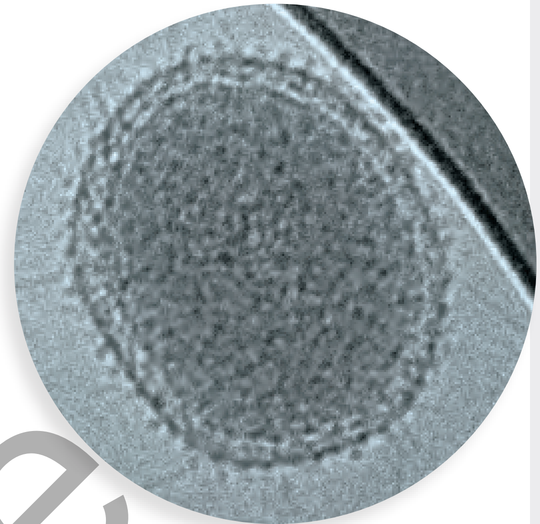


Figure 2 A tiny bacterial cell from anoxic groundwater that passed through a filter with 0.2-μm pores. The cell is not quite 0.2 μm in diameter.

Although we do not yet know exactly how small a microbial cell can be, microbiologists are closing in on this number from environmental analyses such as the Colorado groundwater study. From the same samples that yielded ultra-small *Bacteria* in this study, ultra-small *Archaea* were also detected and found to contain small and highly reduced genomes.[2]

It is thus likely that a large diversity of very small prokaryotic cells occurs in nature, and from the continued study of these tiny cells, more precise values for both the lower limits to cell size and the minimal genomic requirements for life should emerge. Moreover, theoretical considerations of cell size have shown that DNA and proteins dominate the volume of very small cells and that the theoretical lower limit to cell size agrees closely with the smallest bacteria observed in nature thus far.[3]

[1] Luef, B., et al. 2015. *Nat. Commun.* doi:10.1038/ncomms7372.
[2] Castelle, C.J., et al. 2015. *Curr. Biol. 25:* 1–12.
[3] Kempes, C.P., et al. 2016. *ISME J. 10:* 2145–2157.

form into filaments. As we will see, there are even square bacteria, hexagon-shaped bacteria, and star-shaped bacteria! Cell morphologies thus form a continuum, with some shapes, such as rods and cocci, being very common, whereas others, such as spiral, budding, and filamentous shapes, are less common.

Check Your Understanding

- What properties of the cell change as it gets smaller?
- Why is it that eukaryotic cells are typically larger than prokaryotic cells?
- What traits have allowed the bacteria *Epulopiscium* and *Thiomargarita* to have such large cells?

1.4 An Introduction to Microbial Life

As we have seen, microorganisms vary dramatically in size, shape, and structure. In this section we will learn more about different evolutionary (phylogenetic) lineages of cells. All cells fall into one of three major groups: *Bacteria*, *Archaea*, or *Eukarya*. These three major cell lineages are called **domains**, and all known cellular organisms belong to one of these three domains. In addition, while much of our focus in this chapter is on cellular forms of life, not all microbes form cells. In this section, we will also consider viruses, which are a group of microorganisms that lack a cellular structure. All known microorganisms can be classified into one of these four groups.

Bacteria

Bacteria have a prokaryotic cell structure (Figure 1.4*a*). *Bacteria* are often thought of as undifferentiated single cells with a length that ranges from 0.5 to 10 μm. While bacteria that fit this description are common, the *Bacteria* are actually tremendously diverse in appearance, size, and function (**Figure 1.9**). Although most bacteria are unicellular, some bacteria can differentiate to form multiple cell types and others are even multicellular (for example, *Magnetoglobus*, Figure 1.9).

Among the *Bacteria*, 30 major phylogenetic lineages (called *phyla*) have at least one species that has been grown in culture, though many more phyla exist which remain largely uncharacterized. Some of these phyla contain thousands of described species while others contain only a few. More than 90% of cultivated bacteria belong to one of only four phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*. The analyses of environmental DNA sequences provide evidence for the existence of at least 80 bacterial phyla (Section 1.15).

Archaea

Like *Bacteria*, *Archaea* also have a prokaryotic cell structure (Figure 1.4*a*). The domain *Archaea* consists of five described phyla: *Euryarchaeota*, *Crenarchaeota*, *Thaumarchaeota*, *Nanoarchaeota*, and *Korarchaeota*. *Archaea* have historically been associated with extreme environments; the first isolates came from hot, salty, or acidic sites. But not all *Archaea* are extremophiles. *Archaea* are indeed common in

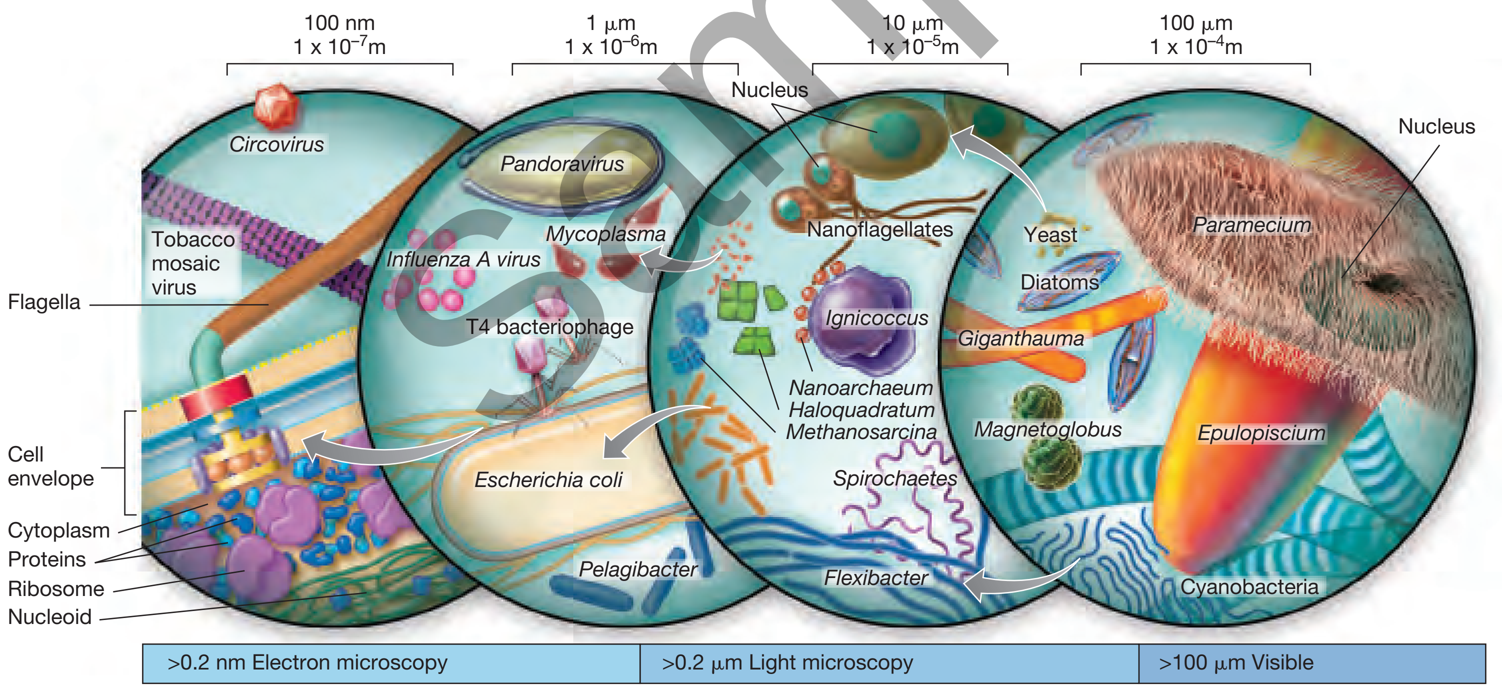


Figure 1.9 Microorganisms vary greatly in size and shape. The smallest known microbe is the circovirus (20 nm) and the largest shown here is the bacterium *Epulopiscium* (700 μm), which represents a 35,000-fold difference in length! Certain protozoa can be even larger than *Epulopiscium* (>2 mm long) and are visible to the unaided eye. Included in the figure are *Eukarya*: *Paramecium* (300 μm × 85 μm), diatoms (*Navicula*, 50 μm × 12 μm), yeast (*Saccharomyces*, 5 μm), and nanoflagellates (*Cafeteria*, 2 μm); *Bacteria*: *Epulopiscium* (700 μm × 80 μm), cyanobacteria (*Oscillatoria*, 10-μm-diameter multicellular filaments), *Magnetoglobus* (multicellular aggregate, 20 μm diameter), *Spirochaetes* (2–10 μm × 0.25 μm), *Flexibacter* (5–100 μm × 0.5 μm filaments), *Escherichia coli* (2 μm × 0.5 μm), *Pelagibacter* (0.4 μm × 0.15 μm), and *Mycoplasma* (0.2 μm); *Archaea*: *Giganthauma* (10-μm-diameter multicellular filament), *Ignicoccus* (6 μm), *Nanoarchaeum* (0.4 μm), *Haloquadratum* (2 μm), *Methanosarcina* (2 μm per cell in packet); and viruses: *Pandoravirus* (1 μm × 0.4 μm), T4 bacteriophage (200 nm × 90 nm), *Influenza A virus* (100 nm), *Tobacco mosaic virus* (300 nm × 20 nm), *Circovirus* (20 nm).

the most extreme environments that support life, such as those associated with volcanic systems, and species of *Archaea* hold many of the records that define the chemical and physical limits of life as we know them. However, in addition to these, *Archaea* are found widely in nature in nonextreme environments. For example, methane-producing *Archaea* (methanogens) are common in wetlands and in the guts of animals (including humans) and have a major impact on the greenhouse gas composition of our atmosphere. In addition, species of *Thaumarchaeota* inhabit soils and oceans worldwide and are important contributors to the global nitrogen cycle.

Archaea are also notable in that this domain lacks any known disease-causing (pathogenic or parasitic) species of plants or animals. Most described species of *Archaea* fall within the phyla *Crenarchaeota* and *Euryarchaeota* while only a handful of species have been described for the *Nanoarchaeota*, *Korarchaeota*, and *Thaumarchaeota*. Analysis of environmental DNA sequences indicate more than 12 archaeal phyla likely exist. We discuss *Archaea* in detail in Chapter 17.

Eukarya

Plants, animals, and fungi are the most well-known groups of *Eukarya*. These groups are phylogenetically relatively young compared with *Bacteria* and *Archaea*, originating during an evolutionary burst called the *Cambrian explosion*, which began about 600 million years ago. The first eukaryotes, however, were unicellular microbes. Microbial eukaryotes, which include diverse algae and protozoa, may have first appeared as early as 2 billion years ago, well before the origin of plants, animals, and fungi (Section 1.5). The major lineages of *Eukarya* are traditionally called *kingdoms* instead of phyla. There are at least six kingdoms of *Eukarya*, and this diverse domain contains microorganisms as well as the plants and animals.

Microbial eukaryotes vary dramatically in size, shape, and physiology (Figure 1.9). Among the smallest are the nanoflagellates, which are microbial predators that can be as small as 2 μm long. In addition, *Ostreococcus*, a genus of green algae that contains species whose cells are only 0.8 μm in diameter, are smaller than many bacteria. The largest single-celled organisms are eukaryotes, but they are hardly microbial. Xenophyophores are amoeba-like, single-celled organisms that live exclusively in the deep oceans and can be up to 10 *centimeters* in length. In addition, plasmodial slime molds consisting of a single cytoplasmic compartment can be up to 30 cm in diameter. In Chapter 18 we consider microbial eukaryotes in detail.

Viruses

Viruses are not found on the tree of life, and for a variety of reasons, it can be argued that they are not truly alive. Although viruses can replicate—a hallmark of cells—viruses are obligate parasites that can only replicate within the cytoplasm of a host cell. Viruses are not cells, and they lack the cytoplasmic membrane, cytoplasm, and ribosomes found in all forms of cellular life. Viruses do not carry out metabolic processes; instead, they take over the metabolic systems of infected cells and turn them into vessels for producing more viruses. Unlike cells, which all have genomes composed of double-stranded DNA, viruses have genomes composed of DNA *or* RNA that can be either double- or single-stranded. Viral genomes are often quite small, with the smallest having only three genes. The small size of most viral genomes means that no genes are conserved among all viruses, or between all viruses and all cells.

Although they are not cells, viruses are as diverse as the cells they infect, and different viruses are known to infect cells from all three domains of life. Viruses are often classified on the basis of their structure, genome composition, and host specificity. Viruses that infect bacteria are called *bacteriophages* (or *phages*, for short). Bacteriophages have been used as model systems to explore many aspects of viral biology. While most viruses are considerably smaller than bacterial cells (Figure 1.9), there are also unusually large viruses such as the Pandoraviruses, which can be more than 1 micrometer long and have a genome that contains as many as 2500 genes, larger than that of many bacteria! We will learn much more about viruses in Chapters 5 and 11.

Check Your Understanding

- How are viruses different from *Bacteria*, *Archaea*, and *Eukarya*?
- What four bacterial phyla contain the largest number of well-characterized species?
- What phylum of *Archaea* is common worldwide in soils and in the oceans?

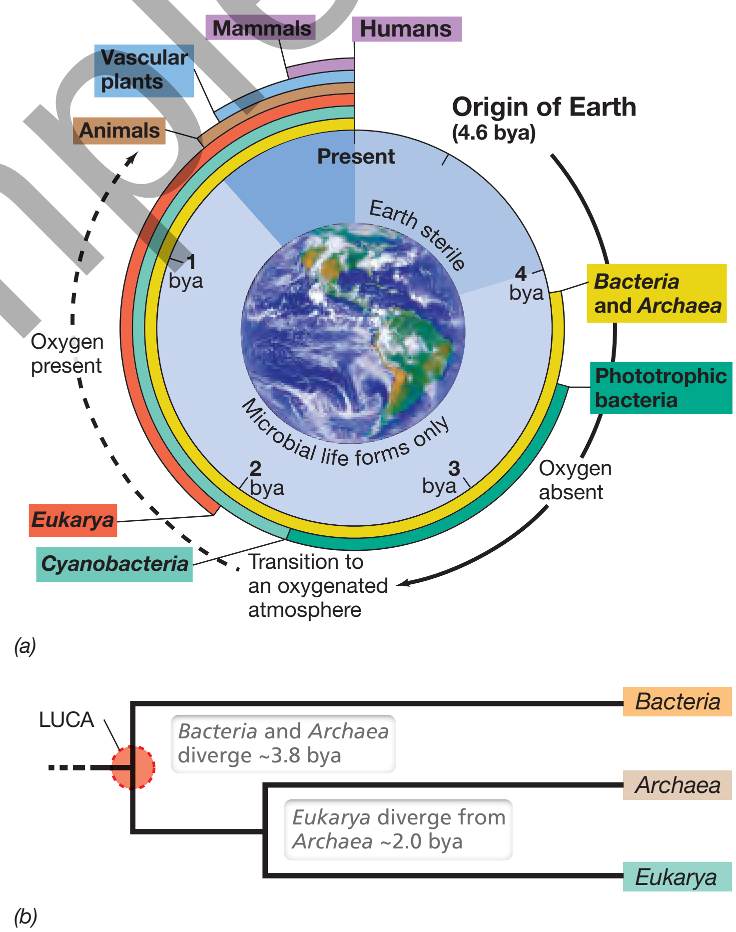


Figure 1.10 A summary of life on Earth through time and origin of the cellular domains. *(a)* At its origin, Earth was sterile and anoxic. Cellular life, in the form of *Bacteria* and *Archaea*, was present on Earth by 3.8 billion years ago (bya). The evolution of phototrophic bacteria called *Cyanobacteria* caused Earth's atmosphere to become oxygenated over time. While the first evidence for oxygen in Earth's atmosphere appears 2.4 bya, current levels of atmospheric O_2 were not achieved until 500–800 million years ago. *(b)* The three domains of cellular organisms are *Bacteria, Archaea*, and *Eukarya. Bacteria* and *Archaea* appeared first and *Eukarya* evolved later, diverging from the *Archaea*. LUCA, last universal common ancestor.

1.5 Microorganisms and the Biosphere

Microbes are the oldest form of life on Earth, and they have evolved to perform critical functions that sustain the biosphere. In this section we will learn how microbes have changed our planet and how they continue to do so.

A Brief History of Life on Earth

Earth is about 4.6 billion years old, and microbial cells first appeared between 3.8 and 4.3 billion years ago (**Figure 1.10**). During the first 2 billion years of Earth's existence, its atmosphere was anoxic (O_2 was absent), and only nitrogen (N_2), carbon dioxide (CO_2), and a few other gases were present. Only microorganisms capable of anaerobic metabolism (that is, metabolisms that do not require O_2) could survive under these conditions.

The evolution of phototrophic microorganisms—organisms that harvest energy from sunlight—occurred within 1 billion years of the formation of Earth (Figure 1.10*a*). The first phototrophs were anoxygenic (non-oxygen-producing), such as the purple sulfur bacteria and green sulfur bacteria we know today (**Figure 1.11**). *Cyanobacteria*—oxygen-producing (oxygenic) phototrophs (Figure 1.11*f*)—evolved nearly a billion years later (Figure 1.10*a*) and began the slow process of oxygenating Earth's atmosphere. These early phototrophs lived in structures called *microbial mats*, which are still found on Earth today (Figure 1.11*a–c*). After the oxygenation of Earth's atmosphere, multicellular life forms eventually evolved, culminating in the plants and animals we know today. But plants and animals have only existed for about half a billion years. The timeline of life on Earth (Figure 1.10*a*) shows that *80% of life's history was exclusively microbial*, and thus in many ways, Earth can be considered a microbial planet.

As evolutionary events unfolded, three major lineages of microbial cells—the *Bacteria*, the *Archaea*, and the *Eukarya* (Figure 1.10*b*)—were distinguished. All cellular organisms share certain characteristics (Figure 1.5) and as a result, certain genes are found in all cells. For example, approximately 60 genes are universally present in cells of all three domains. Examination of these genes reveals that all three domains have descended from a common ancestor, the *last universal common ancestor* (LUCA, Figure 1.10*b*). Over enormous periods of time, microorganisms derived from these three domains have evolved to fill every habitable environment on Earth.

Microbial Abundance and Activity in the Biosphere

Microorganisms are present everywhere on Earth that will support life. They constitute a major fraction of global biomass and are key reservoirs of nutrients essential for life. There are an estimated 2×10^{30} microbial cells on Earth. To put this number in context, the universe in all its vast extent is estimated to contain merely 7×10^{22} stars. The total amount of carbon present in all microbial cells is a significant fraction of Earth's biomass (**Figure 1.12**). Moreover, the total amount of nitrogen and phosphorus (essential nutrients for life) within microbial cells is almost four times that in all plant and animal cells combined. Microbes also represent a major fraction of the total DNA in the biosphere (about 31%), and their genetic diversity far exceeds that of plants and animals.

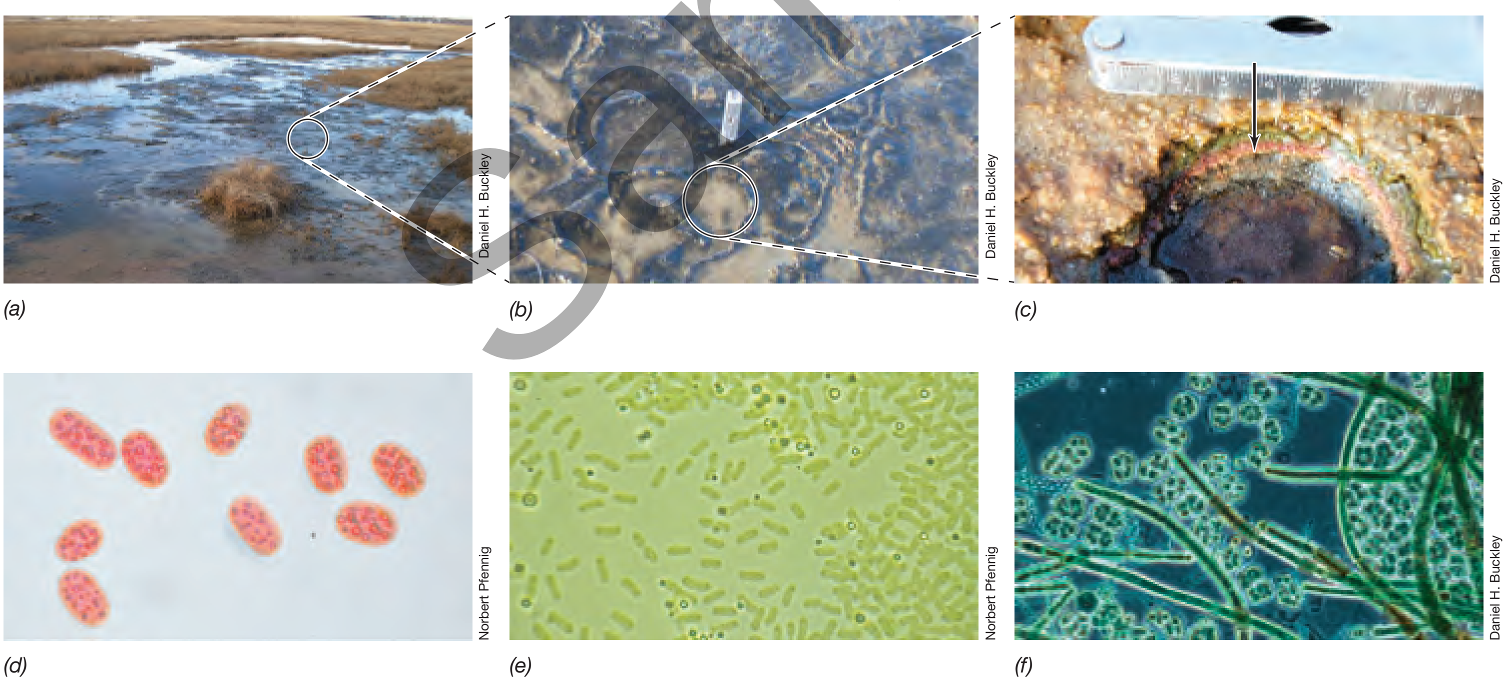


Figure 1.11 Phototrophic microorganisms. The earliest phototrophs lived in microbial mats. *(a)* Microbial mats in the Great Sippewissett Marsh, a salt marsh in Massachusetts, USA. *(b)* Mats develop a cohesive structure that forms at the sediment surface. *(c)* A slice through the mat shows colored layers that form due to the presence of photopigments. Cyanobacteria form the green layer nearest the surface, purple sulfur bacteria form the purple and yellow layers below, and green sulfur bacteria form the bottommost green layer. The scale on the knife is in cm. *(d)* Purple sulfur bacteria, *(e)* green sulfur bacteria, and *(f)* cyanobacteria imaged by bright-field and phase-contrast microscopy. Purple and green sulfur bacteria are anoxygenic phototrophs that appeared on Earth long before oxygenic phototrophs (that is, *Cyanobacteria*) evolved (see Figure 1.10*a*).

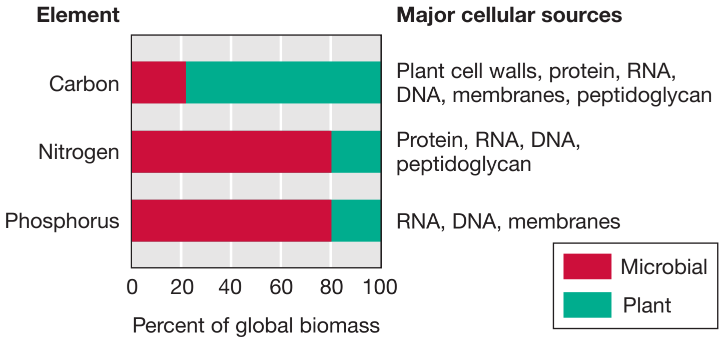


Figure 1.12 Contribution of microbial cells to global biomass. Microorganisms comprise a significant fraction of the carbon (C) and a majority of the nitrogen (N) and phosphorus (P) in the biomass of all organisms on Earth. C, N, and P are the macronutrients required in the greatest quantity by living organisms. Animal biomass is a minor fraction ($<0.1\%$) of total global biomass and is not shown.

Microbes are even abundant in habitats that are much too harsh for other forms of life, such as volcanic hot springs, glaciers and ice-covered regions, high-salt environments, extremely acidic or alkaline habitats, and deep in the sea or deep in the earth at extremely high pressure. Such microorganisms are called **extremophiles** and their properties define the physiochemical limits to life as we know it (**Table 1.2**). We will revisit many of these organisms in later chapters and discover the special structural and biochemical properties that allow them to thrive under extreme conditions.

All ecosystems are influenced to one extent or another by microbial activities. The metabolic activities of microorganisms can change the habitats in which they live, both chemically and physically, and these changes can affect other organisms. For example, excess nutrients added to a habitat can cause aerobic (O_2-consuming) microorganisms to grow rapidly and consume O_2, rendering the habitat anoxic (O_2-free). Many human activities release nutrients into the coastal oceans, thereby stimulating excessive microbial growth, which can cause enormous anoxic zones in these waters. These "dead zones" cause massive mortality of fish and shellfish in coastal oceans worldwide, because most aquatic animals require O_2 and die if it is not available. Only by understanding microorganisms and microbiology can we predict and minimize the effects of human activity on the biosphere that sustains us.

Though diverse habitats are influenced strongly by microorganisms, their contributions are easy to overlook because of their small sizes. Within the human body, for example, more microbial cells can be present than human cells, and more than 200 microbial genes are present for every human gene. These microbes provide benefits and services that are essential to human health. In later chapters, we will return to a consideration of the ways in which microorganisms affect animals, plants, and the entire global ecosystem. This is the science of **microbial ecology**, perhaps the most exciting subdiscipline of microbiology today. We will see that microbes are important to myriad issues of global importance to humans including climate change, agricultural productivity, and even energy policy.

We focus now on the effects of microbes on humans and human activities.

Check Your Understanding

- How old is Earth and when did cells first appear on Earth?
- Name the three domains of life. Which of these contain eukaryotic life forms?
- Why were cyanobacteria so important in the evolution of life on Earth?

1.6 The Impact of Microorganisms on Human Society

Microbiologists have made great strides in discovering how microorganisms function, and application of this knowledge has greatly advanced human health and welfare. Besides understanding microorganisms as agents of disease, microbiology has made great

TABLE 1.2 Classes and examples of extremophiles[a]

Extreme	Descriptive term	Genus, species	Domain	Habitat	Minimum	Optimum	Maximum
Temperature							
High	Hyperthermophile	*Methanopyrus kandleri*	*Archaea*	Undersea hydrothermal vents	90°C	106°C	122°C[b]
Low	Psychrophile	*Psychromonas ingrahamii*	*Bacteria*	Sea ice	−12°C[c]	5°C	10°C
pH							
Low	Acidophile	*Picrophilus oshimae*	*Archaea*	Acidic hot springs	−0.06	0.7[d]	4
High	Alkaliphile	*Natronobacterium gregoryi*	*Archaea*	Soda lakes	8.5	10[e]	12
Pressure	Barophile (piezophile)	*Moritella yayanosii*	*Bacteria*	Deep ocean sediments	500 atm	700 atm[f]	>1000 atm
Salt (NaCl)	Halophile	*Halobacterium salinarum*	*Archaea*	Salterns	15%	25%	32% (saturation)

[a]The organisms listed are the current "record holders" for growth in laboratory culture at the extreme condition listed.
[b]Anaerobe showing growth at 122°C only under several atmospheres of pressure.
[c]The permafrost bacterium *Planococcus halocryophilus* can grow at −15°C and metabolize at −25°C. However, the organism grows optimally at 25°C and grows up to 37°C and thus is not a true psychrophile.
[d]*P. oshimae* is also a thermophile, growing optimally at 60°C.
[e]*N. gregoryi* is also an extreme halophile, growing optimally at 20% NaCl.
[f]*M. yayanosii* is also a psychrophile, growing optimally near 4°C.

advances in understanding the important roles microorganisms play in food and agriculture, and microbiologists have exploited microbial activities to produce valuable human products, generate energy, and clean up the environment.

Microorganisms as Agents of Disease

The statistics summarized in **Figure 1.13** show how microbiologists and clinical medicine have combined to conquer infectious diseases in the past 120 years. At the beginning of the twentieth century, more than half of all humans died from infectious diseases caused by bacterial and viral **pathogens**. Today, however, infectious diseases are largely preventable due to advances in our understanding of microbiology. Microbiology has fueled advances in medicine such as vaccination and antibiotic therapy, advances in engineering such as water and wastewater treatment, advances in food safety such as pasteurization, and a better understanding of how microorganisms are transmitted. Infectious diseases now cause fewer than 5% of all deaths in countries where these interventions, made possible by microbiology, are readily available. However, while infectious diseases are preventable, the World Health Organization has documented that they still account for more than a third of all deaths in countries where microbial interventions are less available, such as those having low-income economies. As we will see later in this chapter, the development of microbiology as a science can be traced to pioneering studies of infectious disease.

While pathogens and infectious disease remain a major threat to humanity, and combating these harmful organisms remains a major focus of microbiology, most microorganisms are not harmful to humans. In fact, most microorganisms are beneficial, and in many cases are even essential to human welfare and the functioning of the planet. We turn our attention to these microorganisms and microbial activities now.

Microorganisms, Agriculture, and Human Nutrition

Agriculture benefits from nutrient cycling performed by microorganisms, in particular, the cycling of nitrogen, sulfur, and carbon compounds. For example, legumes are a diverse family of plants that include major crop species such as soybeans, peas, and lentils, among others. Legumes live in close association with bacteria that form structures called *nodules* on their roots. In the nodules, these bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) through the process of *nitrogen fixation*. NH_3 is the major nutrient found in fertilizer and is used as a nitrogen source for plant growth (**Figure 1.14**). In this way bacteria allow legumes to make their own fertilizer, thereby reducing the need for farmers to apply fertilizers produced industrially. When plants die they are decomposed by bacteria in the soil, and this process produces the nutrients that form the basis of soil fertility. Bacteria regulate nutrient cycles (Figure 1.14), in soils and throughout the biosphere, transforming and recycling the nutrients required by plants and animals.

Also of major agricultural importance are microorganisms that inhabit the rumen of ruminant animals, such as cattle and sheep. Ruminants, like most animals, lack enzymes for breaking down the polysaccharide cellulose, the major component of plant cell walls. The digestive tract of ruminants has a large specialized chamber called the *rumen* in which cellulose is digested. The rumen contains a dense and diverse community of microorganisms that digest and ferment cellulose. Without these symbiotic microorganisms, ruminants could not digest plant matter like grass and hay, most of which consists of cellulose. Ruminants ultimately get their nutrition by metabolizing the waste products of microbial fermentation and by digesting dead microbial cells. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats—are also ruminants.

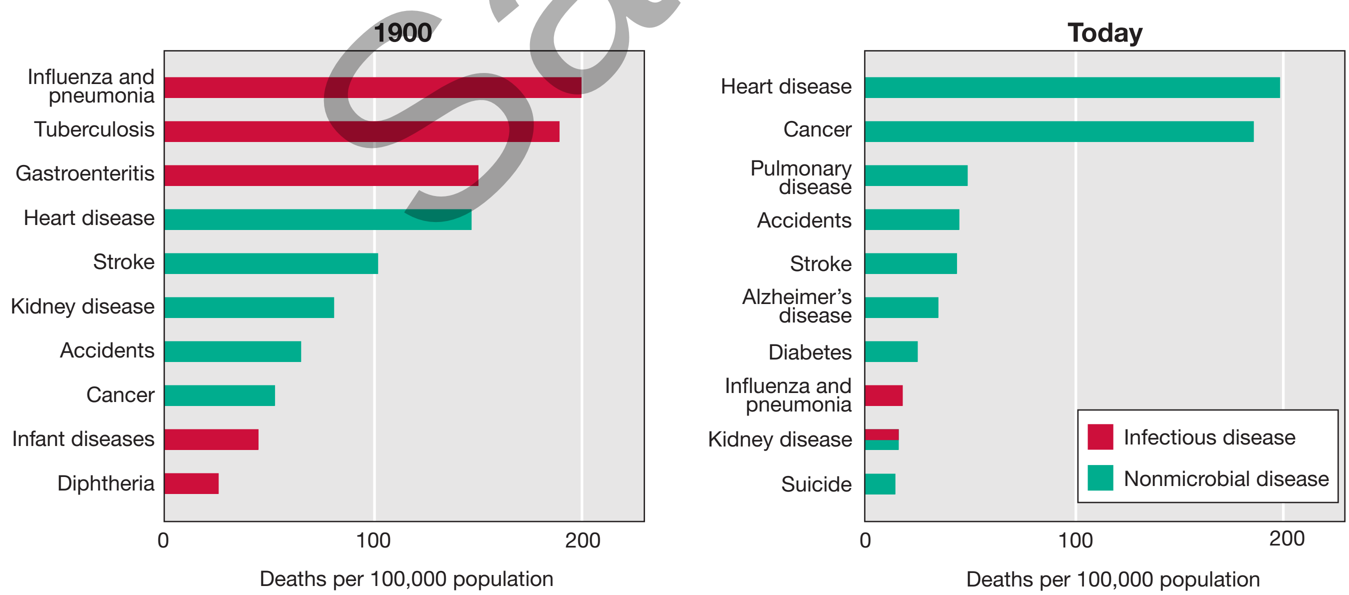


Figure 1.13 Death rates for the leading causes of death in the United States: 1900 and 2016. Infectious diseases were the leading causes of death in 1900, whereas today they account for relatively few deaths. Kidney diseases can be caused by microbial infections or systemic sources (diabetes, cancers, toxicities, metabolic diseases, etc.). Data are from the United States National Center for Health Statistics and the Centers for Disease Control and Prevention.

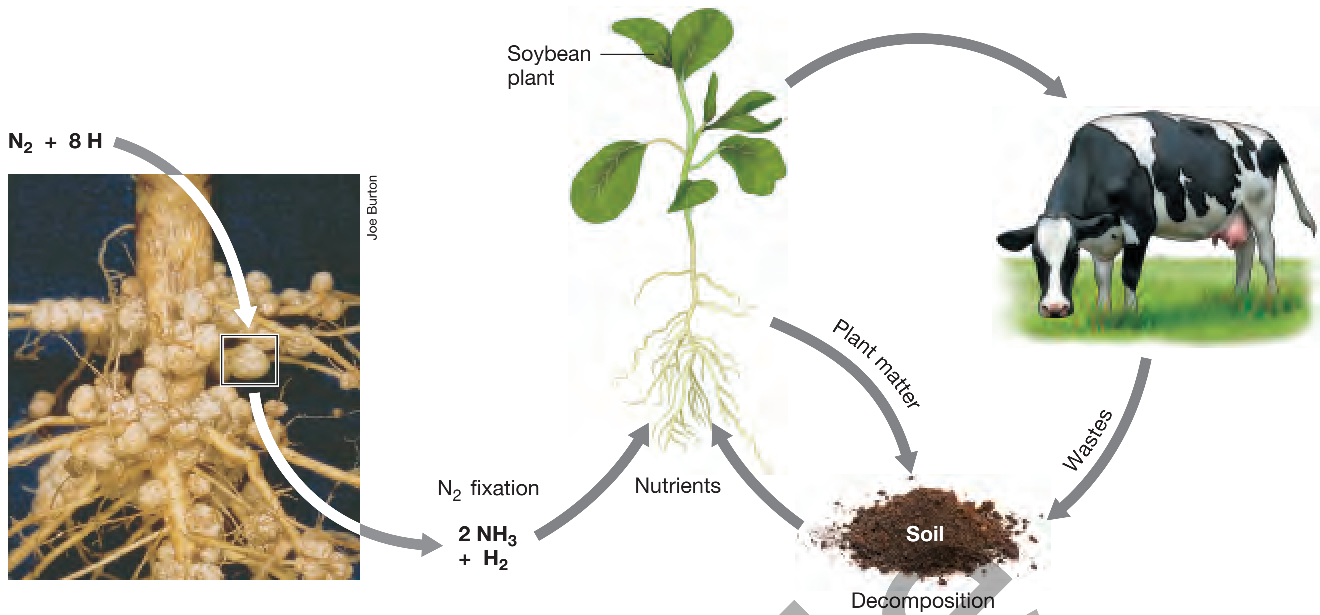


Figure 1.14 Microorganisms in modern agriculture. Root nodules on this soybean plant contain bacteria that fix atmospheric nitrogen (N_2) to form nitrogenous compounds used by the plant. Ruminant animals such as cows and sheep require rumen microbes to digest cellulose from plants. Plant matter and animal wastes are decomposed in soil to produce nutrients that are the basis of soil fertility and which are required for plant growth.

The human gastrointestinal (GI) tract lacks a rumen, but we too rely on microbial partners for our nutrition. Human enzymes lack the ability to break down complex carbohydrates (which can represent 10–30% of food energy) and so we rely on our **gut microbiome** for this purpose. The colon, or large intestine (**Figure 1.15**), follows the stomach and small intestine in the human digestive tract, and it contains about 10^{11} microbial cells per gram of colonic contents. Microbial cell numbers are low in the very acidic (pH 2) stomach (about 10^4 per gram) but increase to about 10^8 per gram near the end of the small intestine (pH 4–5) and then reach maximal numbers in the colon (pH 7) (Figure 1.15). The colon contains diverse microbial species that assist in the digestion of complex carbohydrates, and that synthesize vitamins and other nutrients essential to host nutrition. The gut microbiome develops from birth, but it can change over time with the human host. The composition of the gut microbiome has major effects on GI function and human health as we will see in Chapter 24.

Microorganisms and Food

Microbes are intimately associated with the foods we eat. Microbial growth in food can cause food spoilage and foodborne disease. The manner in which we harvest and store food (for example, canning, refrigeration, drying, salting, etc.), the ways in which we cook it, and even the spices we use, have all been fundamentally influenced by the goal of eliminating harmful organisms from our food. Microbial food safety and prevention of food spoilage is a major focus of the food industry and a major cause of economic loss every year.

While some microbes can cause foodborne disease and food spoilage, not all microorganisms in foods are harmful. Indeed, beneficial microbes have been used for thousands of years to improve food safety and to preserve foods (**Figure 1.16**). For example, cheeses, yogurt, and buttermilk are all produced by microbial fermentation of dairy products. Microbial production of lactic acid in these foods improves their shelf life and prevents the growth of foodborne pathogens. Lactic acid–producing bacteria are used to produce a variety of sour-tasting foods, including sauerkraut, kimchi, pickles, and even certain sausages. Even the production of chocolate and coffee rely on microbial fermentation. Moreover, the fermentative activities of yeast are essential for baking (by generating carbon dioxide—CO_2—to raise the dough), and for the production of alcoholic beverages (by generating alcohol). The products of microbial fermentation affect the flavor and taste of foods and can prevent spoilage as well as the growth of deleterious organisms.

Microorganisms and Industry

Microorganisms play important roles in all manner of human activity. The field of *industrial microbiology* is focused on the use of microorganisms as tools for major industries such as pharmaceuticals and brewing (**Figure 1.17**). For example, in large industrial settings, naturally occurring microorganisms are grown on a massive scale in bioreactors called *fermentors* to make large amounts of products, such as antibiotics, enzymes, alcohol, and certain other chemicals, at relatively low cost. By contrast, *biotechnology* employs genetically engineered microorganisms to synthesize products of high commercial value, such as insulin or other human proteins, usually on a small scale.

Microorganisms can also be used to produce *biofuels* (▶ Section 12.19 and Figure 12.33). For example, as previously discussed,

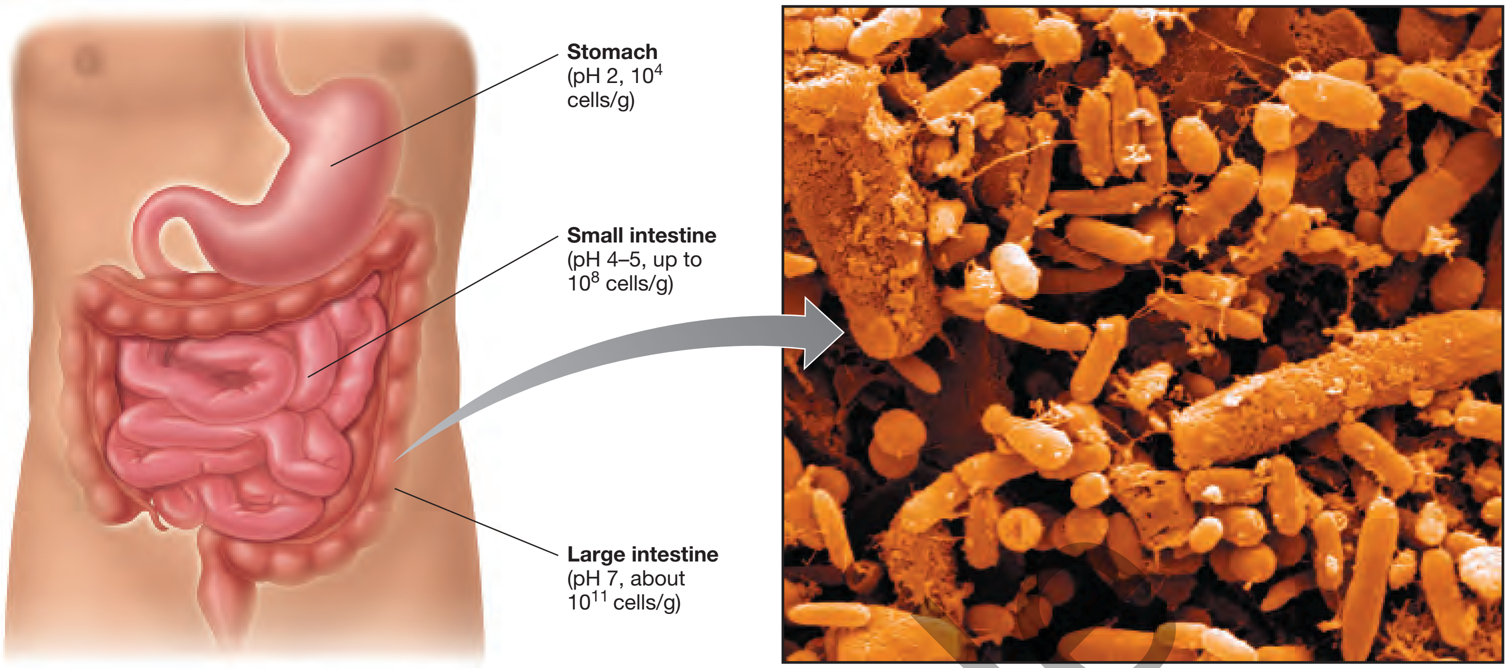


Figure 1.15 The human gastrointestinal tract. *(a)* Diagram of the human GI tract showing the major organs. *(b)* Scanning electron micrograph of microbial cells in the human colon (large intestine). Cell numbers in the colon can reach as high as 10^{11} per gram. As well as high *numbers* of cells, the microbial *diversity* in the colon is also quite high.

natural gas (methane, CH_4) is a product of the anaerobic metabolism of methanogenic *Archaea*. Ethyl alcohol (ethanol) is a major fuel supplement, which is produced by the microbial fermentation of glucose obtained from carbon-rich feedstocks such as sugarcane, corn, or rapidly growing grasses. Microorganisms can even convert waste materials, such as domestic refuse, animal wastes, and cellulose, into ethanol and methane. In producing these biofuels, humans are simply exploiting the metabolic features of particular microbes, but at the same time, are reducing the use of fossil fuels. As we will document in Chapter 21, CO_2 levels have been rising

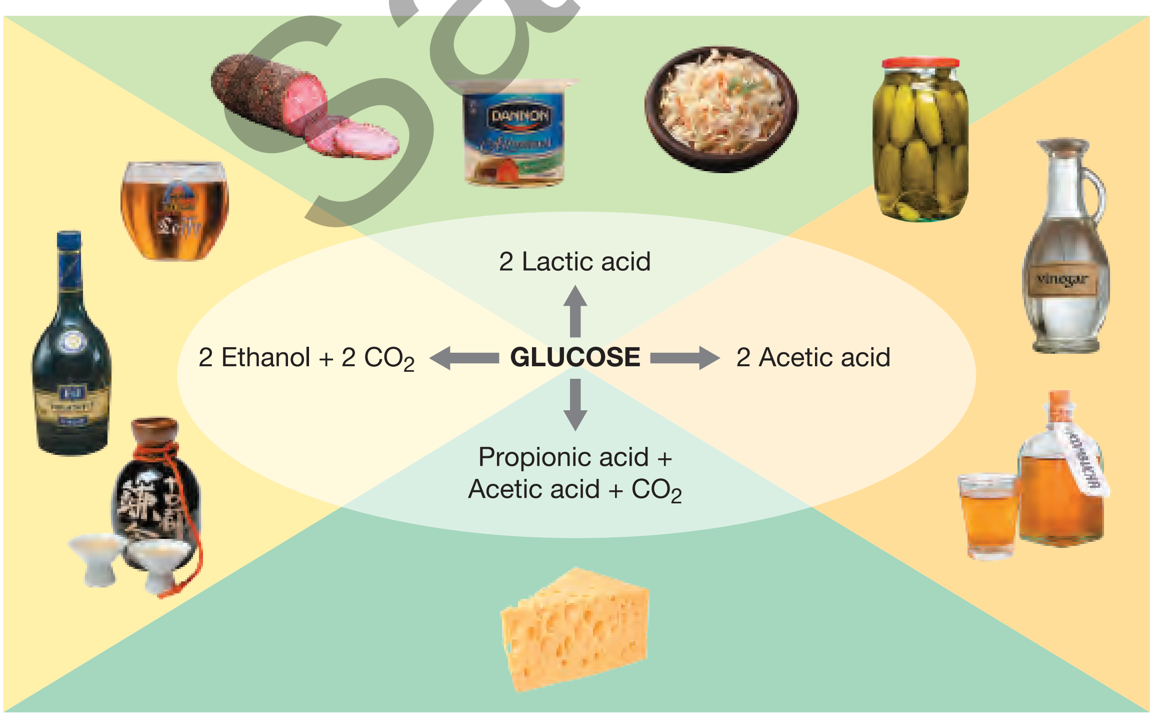


Figure 1.16 Fermented foods. Major fermentations in various fermented foods. It is the fermentation product (ethanol, or lactic, propionic, or acetic acids) that both preserves the food and renders in it a characteristic flavor.

Wastewater Treatment: Microbes are used to clean wastewater.

Bioremediation: Microbes are used to clean contaminated environments.

Biofilms: Microbes grow on surfaces and can foul pipes and pipelines.

Biotechnology: Microbes can be genetically modified to produce high-value products such as pharmaceuticals and enzymes.

Fermentation: Microbes are used at industrial scale to make chemicals, solvents, enzymes, and pharmaceuticals.

Biofuels: Microbes are used to convert biomass into ethanol and wastes into natural gas (methane).

Figure 1.17 Industrial microbiology. Microbes have major impacts on human industry. Microbes can be used to produce valuable products and biofuels and they can also be used to clean up our wastes. Microbial biofilms have major impacts on industry because biofilms can clog and corrode pipelines and holding tanks in factories, in ships, and in the oil industry.

rapidly on Earth in the industrial era, and the link between this "greenhouse gas" and Earth's rising temperatures is firm. Thus, as a sustainable fuel source, biofuels should help cool our planet and are one facet of the "green revolution" many countries support today.

Microorganisms are also used to clean up wastes. Wastewater treatment is essential to sanitation and human health. *Wastewater treatment* relies on microbes to treat water contaminated with human waste so that it can be reused or returned safely to the environment. Waterborne diseases such as cholera and typhoid (major killers before the blossoming of microbiology: see gastroenteritis, Figure 1.13) can proliferate in the absence of proper wastewater treatment. Microbes can also be used to clean up industrial pollution in a process called *bioremediation*. In bioremediation, microorganisms are used to transform spilled oil, solvents, pesticides, heavy metals, and other environmentally toxic pollutants into nontoxic forms. Bioremediation accelerates the cleanup process either by adding special microorganisms to a polluted environment or by adding nutrients that stimulate indigenous microorganisms to degrade the pollutants. In either case the goal is to accelerate disappearance of the pollutant.

Microbes can grow in almost any environment containing liquid water, including structures made by humans. For example, microbes often grow on submerged surfaces, forming *biofilms*. Biofilms that grow in pipes and drains can cause fouling and blockages in factory settings and pipelines, in sewers, and even in water distribution systems. In addition, biofilms that grow on ships' hulls can cause marked reductions in speed and efficiency. Biofilms can even grow in tanks that store oil and fuel, leading to spoilage of these products. We will learn that biofilms are also of great importance in medicine, as biofilms that form on implanted medical devices (▶ Section 4.9) can cause infections that are extremely difficult to treat.

As these examples show, the influence of microorganisms on humans is great and their activities are essential for the functioning of the planet. Or, as the famous French chemist and early microbiologist Louis Pasteur so aptly put it: "The role of the infinitely small in nature is infinitely large." Microscopes provide an essential portal through which microbiologists such as Pasteur gazed into the world of microbes. We therefore continue our introduction to the microbial world with an overview of microscopy.

Check Your Understanding

- How do microbes contribute to the nutrition of animals such as humans and cows?
- Describe several ways in which microorganisms are important in the food and agricultural industries.
- What is wastewater treatment and why is it important?

II • Microscopy and the Origins of Microbiology

The microscope first revealed the microbial world, and the several different types of microscopes available today remain among the microbiologist's foremost tools.

Historically, the science of microbiology has taken its greatest leaps forward as new tools are developed and old tools improve. The microscope is the microbiologist's oldest and most fundamental tool for studying the microbial world. Indeed, microbiology did not exist before the invention of the microscope. Many forms of microscopy are available, and some are extremely powerful. Throughout this text you will see images of microorganisms that were taken through the microscope using a variety of different techniques. So let's take a moment to explore how microscopy can be used to visualize microbial cells, starting at the very beginning with the invention of the microscope.

1.7 Light Microscopy and the Discovery of Microorganisms

Although the existence of creatures too small to be seen with the naked eye had been suspected for centuries, their discovery had to await invention of the microscope. The English mathematician and natural historian Robert Hooke (1635–1703) was an excellent microscopist. In his famous book *Micrographia* (1665), the first book devoted to microscopic observations, Hooke illustrated many microscopic images including the fruiting structures of molds (**Figure 1.18**). This was the first known description of microorganisms.

The first person to see bacteria, the smallest microbial cells, was the Dutch draper and amateur microscopist Antoni van Leeuwenhoek (1632–1723). Van Leeuwenhoek constructed extremely simple microscopes containing a single lens to examine various natural substances for microorganisms (**Figure 1.19**). These microscopes were crude by today's standards, but by careful manipulation and focusing, van Leeuwenhoek was able to see bacteria. He discovered bacteria in 1676 while studying pepper–water infusions and reported his observations in a series of letters to the prestigious Royal Society of London, which published them in English translation in 1684. Drawings of some of van Leeuwenhoek's "wee animalcules," as he referred to them, are shown in Figure 1.19*b*, and a photo taken through a van Leeuwenhoek microscope is shown in Figure 1.19*c*.

Van Leeuwenhoek's microscope was a *light* microscope, and his design used a simple lens that could magnify an image

Figure 1.18 Robert Hooke and early microscopy. A drawing of the microscope used by Robert Hooke in 1664. The lens was fitted at the end of an adjustable bellows (G) and light focused on the specimen by a separate lens (1). Inset: Hooke's drawing of a bluish mold he found degrading a leather surface; the round structures contain spores of the mold.

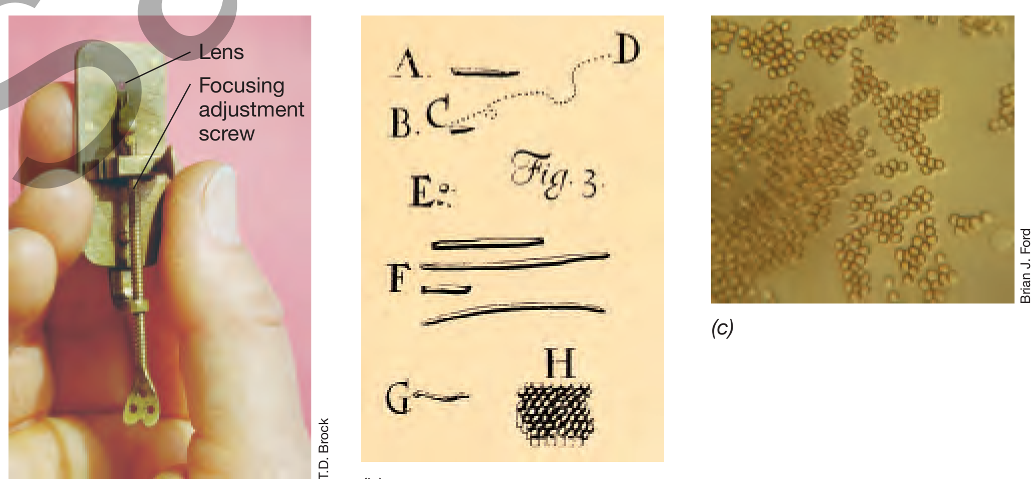


Figure 1.19 The van Leeuwenhoek microscope. *(a)* A replica of Antoni van Leeuwenhoek's microscope. *(b)* Van Leeuwenhoek's drawings of bacteria, published in 1684. Even from these simple drawings we can recognize several shapes of common bacteria: A, C, F, and G, rods; E, cocci; H, packets of cocci. *(c)* Photomicrograph of a human blood smear taken through a van Leeuwenhoek microscope. Red blood cells are clearly apparent.

at least 266 times. In a light microscope the sample is illuminated with visible light. **Magnification** describes the capacity of a microscope to enlarge an image. All microscopes employ lenses that provide magnification. Magnification, however, is not the limiting factor in our ability to see small objects. It is *resolution* that governs our ability to see the very small. **Resolution** is the ability to distinguish two adjacent objects as distinct and separate. The limit of resolution for a light microscope is about 0.2 μm (μm is the abbreviation for micrometer, 10^{-6} m). What this means is that two objects that are closer together than 0.2 μm cannot be resolved as distinct and separate.

Microscopy has improved remarkably since the days of van Leeuwenhoek. Several types of light microscopy are now available, including *bright-field, phase-contrast, differential interference contrast, dark-field*, and *fluorescence*. With the modern compound light microscope, light is focused on the specimen by the condenser (**Figure 1.20**) and this light passes through the sample and is collected by the lenses. The modern compound light microscope contains two types of lenses, *objective* and *ocular*, that function in combination to magnify the image. Microscopes used in microbiology have ocular lenses that magnify 10–30× and objective lenses that magnify 10–100× (Figure 1.20*b*). The total magnification of a compound light microscope is the *product* of the magnification of its objective and ocular lenses (Figure 1.20*b*). Magnification of 1000× is required to resolve objects 0.2 μm in diameter, which is the limit of resolution for most light microscopes (increasing magnification beyond 1000× provides little improvement in the resolution of a light microscope).

In addition to magnification, the limit of resolution for a light microscope is a function of the wavelength of light used and the light-gathering ability of the objective lens, a property known as its *numerical aperture*. There is a correlation between the magnification of a lens and its numerical aperture; lenses with higher magnification typically have higher numerical apertures. The diameter of the smallest object resolvable by any lens is equal to 0.5λ/numerical aperture, where λ is the wavelength of light used. With objectives that have a very high numerical aperture (such as the 100× objective), an optical-grade oil is placed between the microscope slide and the objective. Lenses on which oil is used are called *oil-immersion* lenses. Immersion oil increases the light-gathering ability of a lens, that is, it increases the amount of light that is collected and viewed by the lens.

Mastering Microbiology
Microbiology Animation: Light Microscopy

In light microscopy, specimens are visualized because of differences in **contrast** that exist between them and their surroundings (**Figure 1.21**). In bright-field microscopy, contrast results when cells absorb or scatter light differently from their surroundings. Bacterial cells typically lack contrast, that is, their optical properties are similar to the surrounding liquid, and hence they are difficult to see well with the bright-field microscope. Pigmented microorganisms are an exception because the color of the organism adds contrast, thus improving visualization by bright-field optics (Figure 1.21). For cells lacking pigments there are several ways to boost contrast, and we consider these methods in the next section.

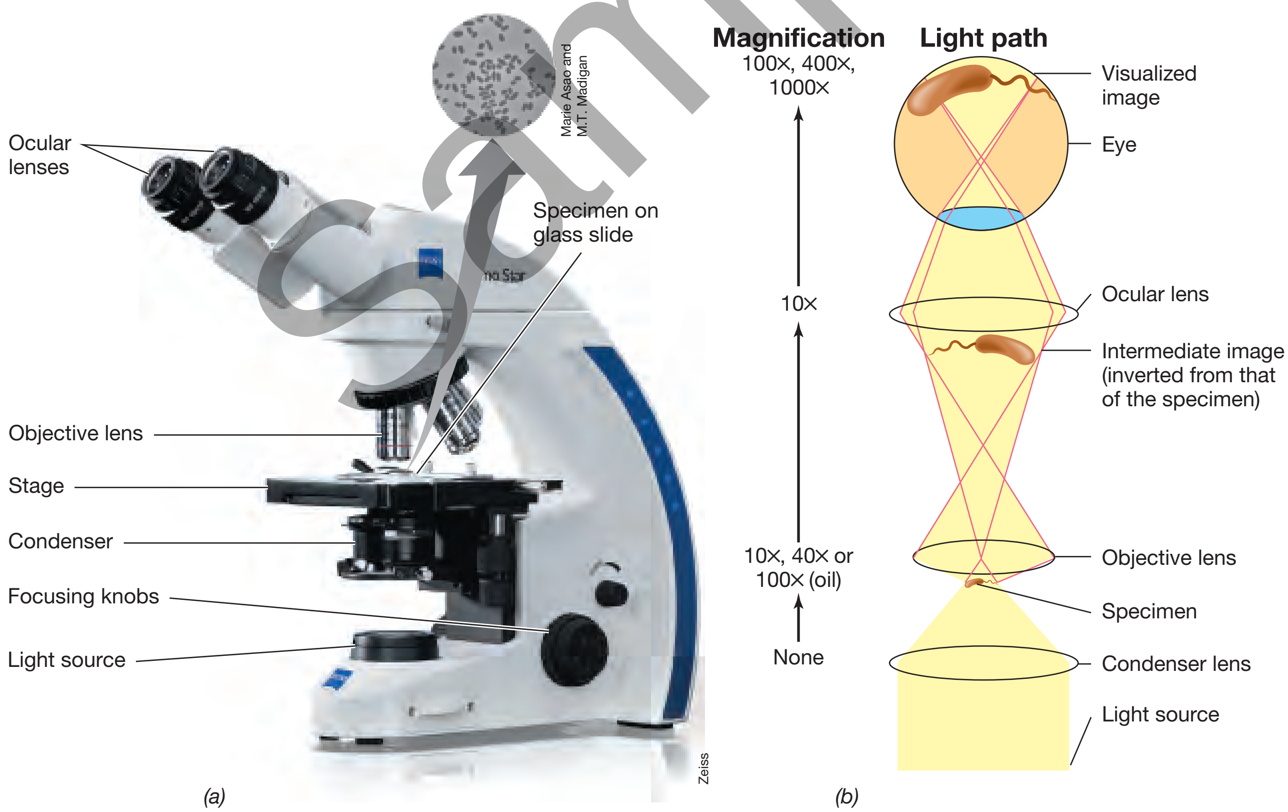


Figure 1.20 Microscopy. *(a)* A compound light microscope (inset photomicrograph of unstained cells taken through a phase-contrast light microscope). *(b)* Path of light through a compound light microscope. Figure 1.24 compares cells visualized by bright field with those visualized by phase contrast.

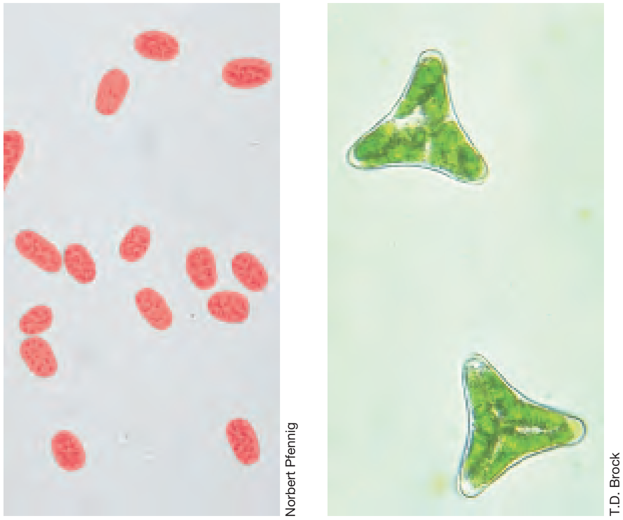


Figure 1.21 **Bright-field photomicrographs of pigmented microorganisms.** *(a)* Purple phototrophic bacteria (*Bacteria*). The bacterial cells are about 5 μm wide. *(b)* A green alga (eukaryote). The green structures are chloroplasts. The algal cells are about 15 μm wide. Purple bacteria are anoxygenic phototrophs, whereas algae are oxygenic phototrophs. Both groups contain photosynthetic pigments, but only oxygenic phototrophs produce O_2 (Section 1.5 and Figure 1.10*a*).

Check Your Understanding

- Define the terms magnification and resolution.
- What is the limit of resolution for a bright-field microscope? What defines this limit?

1.8 Improving Contrast in Light Microscopy

Contrast is necessary in light microscopy to distinguish microorganisms from their surroundings. Cells can be stained to improve contrast, and staining is commonly used to visualize bacteria with bright-field microscopy. In addition to staining, other methods of light microscopy have been developed to improve contrast with or without staining, and we consider all of these methods here.

Staining: Increasing Contrast for Bright-Field Microscopy

Dyes can be used to stain cells and increase their contrast so that they can be more easily seen in the bright-field microscope. Each class of dye has an affinity for specific cellular materials. Many dyes used in microbiology are positively charged, and for this reason, they are called *basic dyes*. Examples of basic dyes include methylene blue, crystal violet, and safranin. Basic dyes bind strongly to negatively charged cell components, such as nucleic acids and acidic polysaccharides. These dyes also stain the surfaces of cells because cell surfaces tend to be negatively charged. These properties make basic dyes useful general-purpose stains that nonspecifically stain most bacterial cells.

To perform a *simple* stain, one begins with dried preparations of cells (**Figure 1.22**). A clean glass slide containing a dried suspension of cells is flooded for a minute or two with a dilute solution of a basic dye, rinsed several times in water, and blotted dry. Because bacterial cells are so small, it is common to observe dried, stained preparations of those cells with a high-power (oil-immersion) lens.

Differential Stains: The Gram Stain

Stains that render different kinds of cells different colors are called *differential* stains. An important differential-staining procedure used in microbiology is the **Gram stain** (**Figure 1.23**). On the basis of their reaction in the Gram stain, bacteria can be divided into two major groups: **gram-positive** and **gram-negative**. After Gram staining, gram-positive bacteria appear purple-violet and gram-negative bacteria appear pink (Figure 1.23*b*). The color difference in the Gram stain arises because of differences in the cell wall structure of gram-positive and gram-negative cells (▶ Section 2.3). Staining with a basic dye such as crystal violet renders cells purple in color. Cells are then treated with ethanol, which decolorizes gram-negative cells but

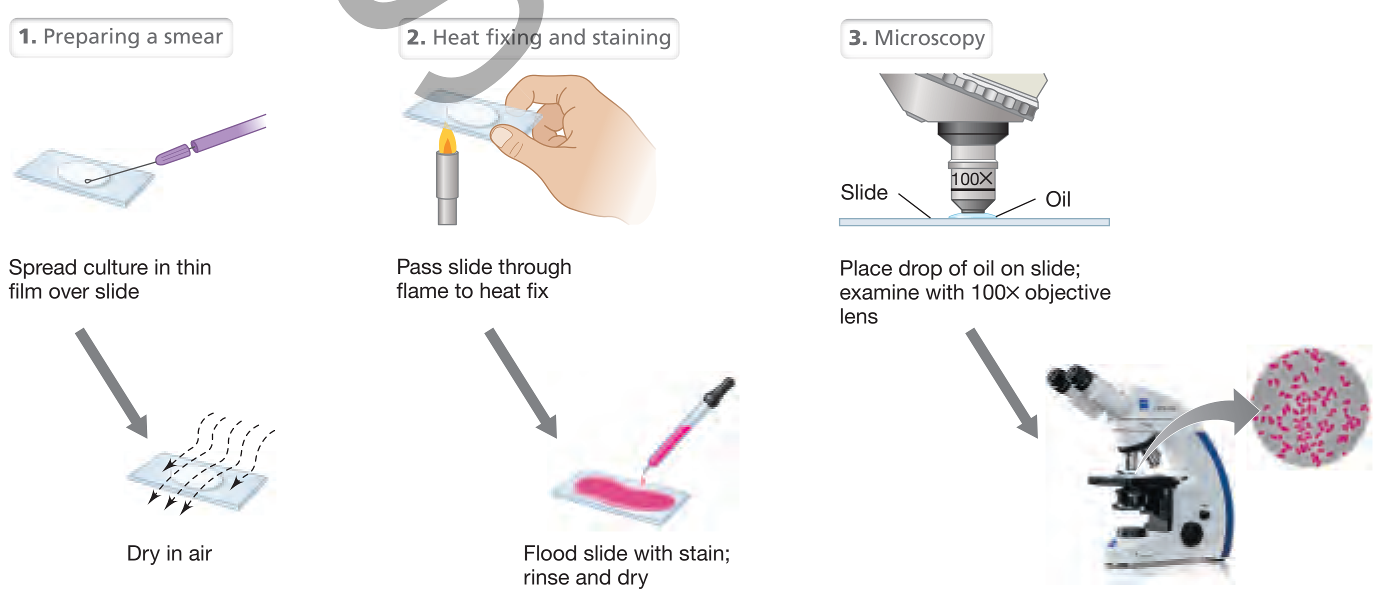


Figure 1.22 **Staining cells for microscopic observation.** Stains improve the contrast between cells and their background. Step 3 lower right: Same cells as shown in Figure 1.20*a* inset but stained with a basic dye.