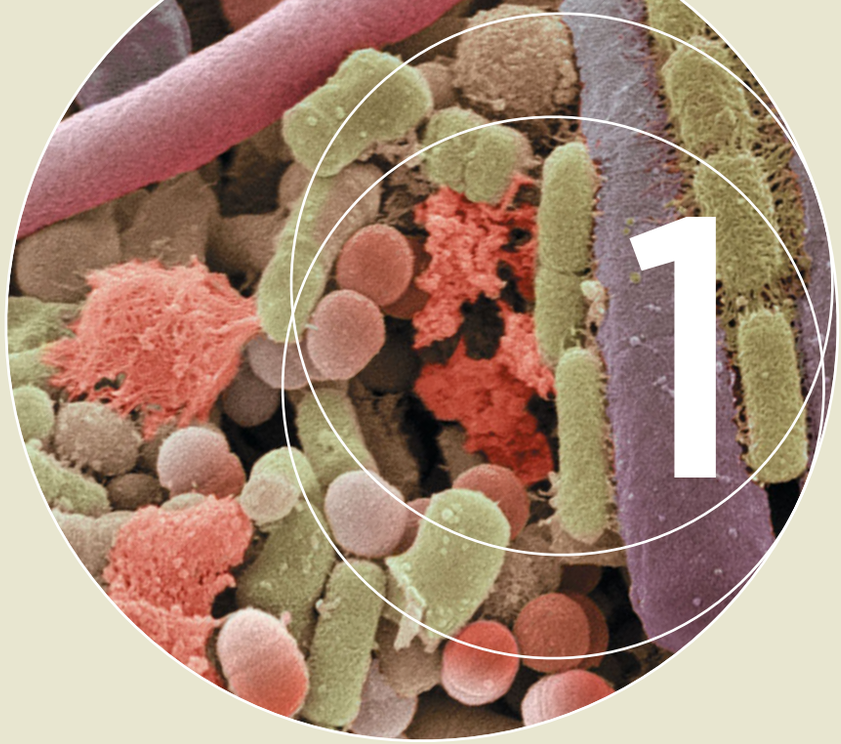

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Microorganisms and Microbiology

Bacteria, such as these scraped from the surface of a human tongue, are independent microorganisms that live and interact with other microorganisms in microbial communities.

Microbiology is the study of microorganisms. **Microorganisms** are all single-celled microscopic organisms and include the viruses, which are microscopic but not cellular. Microbial cells differ in a fundamental way from the cells of plants and animals in that microorganisms are independent entities that carry out their life processes independently of other cells. By contrast, plant and animal cells are unable to live alone in nature and instead exist only as parts of multicellular structures, such as the organ systems of animals or the leaves of plants.

What is the science of microbiology all about? Microbiology is about microbial cells and how they work, especially the bacteria, a very large group of very small cells (**Figure 1.1**) that, collectively, have enormous basic and practical importance. Microbiology is about diversity and evolution of microbial cells, about how different kinds of microorganisms arose and why. It is also about what microorganisms do in the world at large, in soils and waters, in the human body, and in animals and plants. One way or another, microorganisms affect and support all other forms of life, and thus microbiology can be considered the most fundamental of the biological sciences.

This chapter begins our journey into the microbial world. Here we discover what microorganisms are and their impact on planet Earth. We set the stage for consideration of the structure and evolution of microorganisms that will unfold in the next chapter. We also place microbiology in historical perspective, as a process of scientific discovery. From the landmark contributions of both early microbiologists and scientists practicing today, we can see the effects that microorganisms have in medicine, agriculture, the environment, and other aspects of our daily lives.

I Introduction to Microbiology

In the first five sections of this chapter we introduce the field of microbiology, look at microorganisms as cells, examine where and how microorganisms live in nature, survey the evolutionary history of microbial life, and examine the impact that microorganisms have had and continue to have on human affairs.

1.1 The Science of Microbiology

The science of microbiology revolves around two interconnected themes: (1) understanding the living world of microscopic organisms, and (2) applying our understanding of microbial life processes for the benefit of humankind and planet Earth.

As a *basic* biological science, microbiology uses and develops tools for probing the fundamental processes of life. Scientists have obtained a rather sophisticated understanding of the chemical and physical basis of life from studies of microorganisms because microbial cells share many characteristics with cells of multicellular organisms; indeed, *all* cells have much in common. But unlike plants and animals, microbial cells can be grown to extremely high densities in small-scale laboratory cultures (Figure 1.1), making them readily amenable to rapid biochemical and genetic study. Collectively, these features make microorganisms excellent experimental systems for illuminating life processes common to multicellular organisms, including humans.

As an *applied* biological science, microbiology is at the center of many important aspects of human and veterinary medicine, agriculture, and industry. For example, although animal and plant infectious diseases are typically microbial, many microorganisms are absolutely essential to soil fertility and domestic animal welfare. Many large-scale industrial processes, such as the production of antibiotics and human proteins, rely heavily on microorganisms. Thus microorganisms affect the everyday lives of humans in both beneficial and detrimental ways.

Although microorganisms are the smallest forms of life, collectively they constitute the bulk of biomass on Earth and carry out many necessary chemical reactions for higher organisms. In the absence of microorganisms, higher life forms would never have evolved and could not now be sustained. Indeed, the very oxygen we breathe is the result of past microbial activity (as we will see in Figure 1.6). Moreover, humans, plants, and animals are intimately tied to microbial activities for the recycling of key nutrients and for degrading organic matter. It is safe to say that no

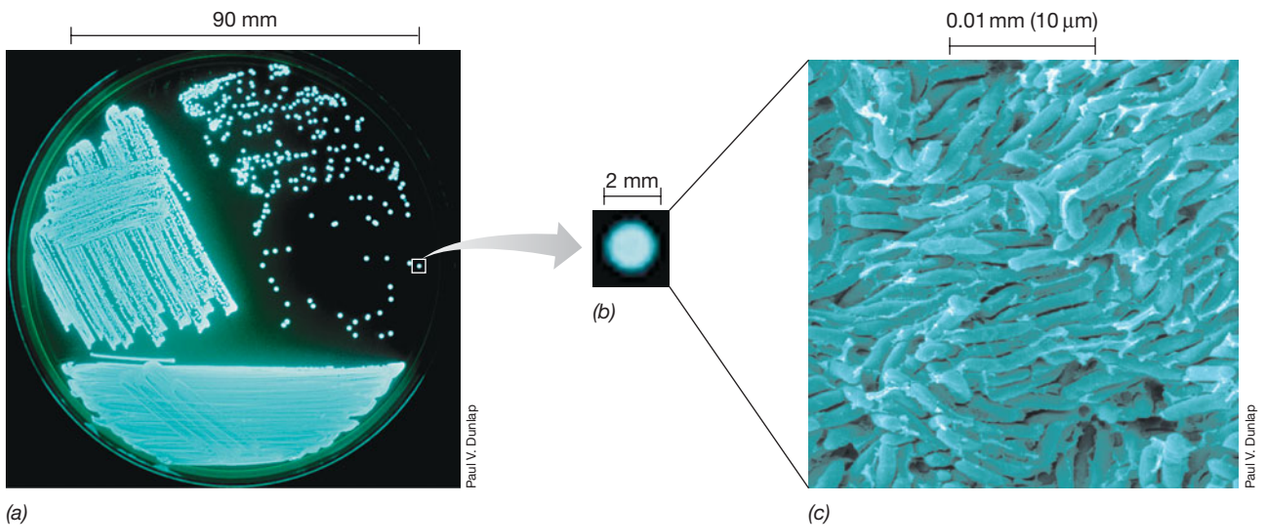


Figure 1.1 Microbial cells. *(a)* Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. *(b)* A single colony can contain more than 10 million (10^7) individual cells. *(c)* Scanning electron micrograph of cells of *Photobacterium*.

other life forms are as important as microorganisms for the support and maintenance of life on Earth.

Microorganisms existed on Earth for billions of years before plants and animals appeared, and we will see later that the genetic and physiological diversity of microbial life greatly exceeds that of the plants and animals. This huge diversity accounts for some of the spectacular properties of microorganisms. For example, we will see how microorganisms can live in places that would kill other organisms and how the diverse physiological capacities of microorganisms rank them as Earth's premier chemists. We will also trace the evolutionary history of microorganisms and see that three groups of cells can be distinguished by their evolutionary relationships. And finally, we will see how microorganisms have established important relationships with other organisms, some beneficial and some harmful.

We begin our study of microbiology with a consideration of the cellular structure of microorganisms.

MiniQuiz

- As they exist in nature, why can it be said that microbial cells differ fundamentally from the cells of higher organisms?
- Why are microbial cells useful tools for basic science?

1.2 Microbial Cells

A basic tenet of biology is that the **cell** is the fundamental unit of life. A single cell is an entity isolated from other such entities by a membrane; many cells also have a cell wall outside the membrane (**Figure 1.2**). The membrane defines the compartment that is the cell, maintains the correct proportions of internal constituents, and prevents leakage, while the wall lends structural strength to the cell. But the fact that a cell is a compartment does not mean that it is a *sealed* compartment. Instead, the membrane is semipermeable and thus the cell is an open, dynamic structure. Cells can communicate, move about, and exchange materials with their environments, and so they are constantly undergoing change.

Properties of Cellular Life

What essential properties characterize cells? **Figure 1.3** summarizes properties shared by all cellular microorganisms and additional properties that characterize only some of them. All cells show some form of **metabolism**. That is, they take up nutrients from the environment and transform them into new cell materials and waste products. During these transformations, energy is conserved in a form that can be drawn upon by the cell to support the synthesis of key structures. Production of the new structures culminates in the division of the cell to form two cells. The metabolic capabilities of cells can differ dramatically, but the final result of any cell's metabolic activities is to form two cells. In microbiology, we typically use the term **growth**, rather than "reproduction," to refer to the increase in cell number from cell division.

All cells undergo **evolution**, the process of descent with modification in which genetic variants are selected based on their reproductive fitness. Evolution is typically a slow process but can occur rapidly in microbial cells when selective pressure is strong. For example, we can witness today the selection for antibiotic resistance in pathogenic (disease-causing) bacteria by the indiscriminate use of antibiotics in human and veterinary medicine. Evolution is *the* overarching theme of biology, and the tenets of evolution—variation and natural selection based on fitness—govern microbial life forms just as they do multicellular life forms.

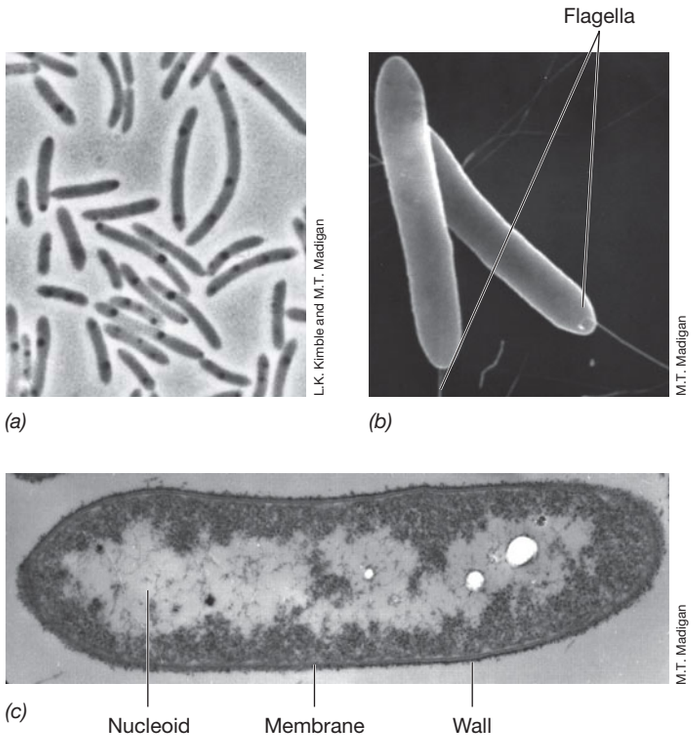


Figure 1.2 Bacterial cells and some cell structures. *(a)* Rod-shaped cells of the bacterium *Heliobacterium modesticaldum* as seen in the light microscope; a single cell is about 1 μm in diameter. *(b)* Scanning electron micrograph of the same cells as in part a showing flagella, structures that rotate like a propeller and allow cells to swim. *(c)* Electron micrograph of a sectioned cell of *H. modesticaldum*. The light area is aggregated DNA, the nucleoid of the cell.

Although all cells metabolize, grow, and evolve, the possession of other common properties varies from one species of cell to another. Many cells are capable of **motility**, typically by self-propulsion (Figure 1.2*b*). Motility allows cells to move away from danger or unfavorable conditions and to exploit new resources or opportunities. Some cells undergo **differentiation**, which may, for example, produce modified cells specialized for growth, dispersal, or survival. Some cells respond to chemical signals in their environment including those produced by other cells of either the same or different species. Responses to these signals may trigger new cellular activities. We can thus say that cells exhibit **communication**. As more is learned about this aspect of microbial life, it is quite possible that cell–cell communication will turn out to be a universal property of microbial cells.

Cells as Biochemical Catalysts and as Genetic Entities

The routine activities of cells can be viewed in two ways. On one hand, cells can be viewed as biochemical catalysts, carrying out the chemical reactions that constitute metabolism (**Figure 1.4**). On the other hand, cells can be viewed as genetic coding devices,

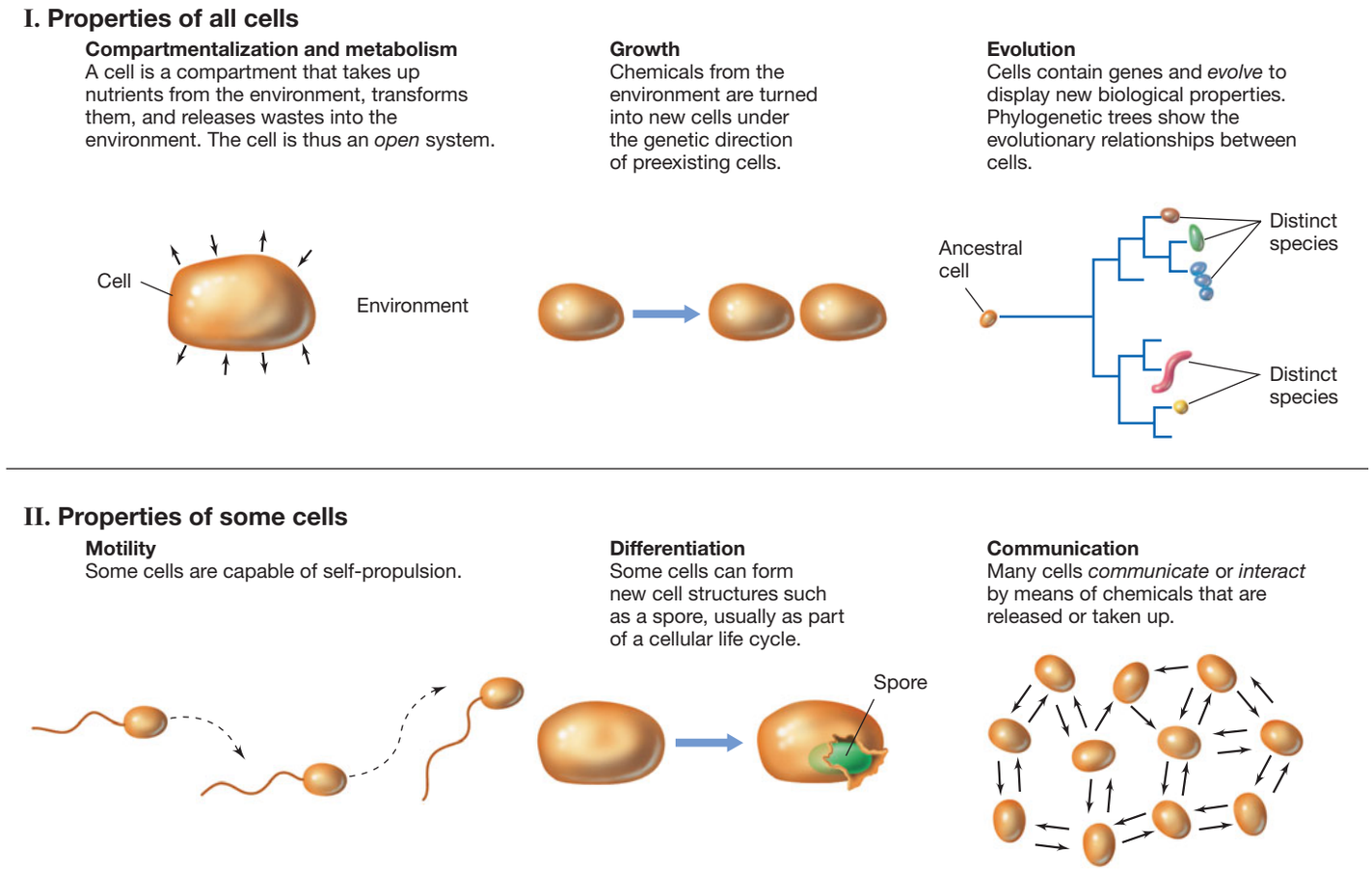


Figure 1.3 The properties of cellular life.

replicating DNA and then processing it to form the RNAs and proteins needed for maintenance and growth under the prevailing conditions. DNA processing includes two main events, the production of RNAs (transcription) and the production of proteins (translation) (Figure 1.4).

Cells coordinate their catalytic and genetic functions to support cell growth. In the events that lead up to cell division, all constituents in the cell double. This requires that a cell's catalytic machinery, its **enzymes**, supply energy and precursors for the biosynthesis of all cell components, and that its entire complement of genes (its **genome**) replicates (Figure 1.4). The catalytic and genetic functions of the cell must therefore be highly coordinated. Also, as we will see later, these functions can be regulated to ensure that new cell materials are made in the proper order and concentrations and that the cell remains optimally tuned to its surroundings.

MiniQuiz

- What does the term "growth" mean in microbiology?
- List the six major properties of cells. Which of these are universal properties of all cells?
- Compare the catalytic and genetic functions of a microbial cell. Why is neither of value to a cell without the other?

1.3 Microorganisms and Their Environments

In nature, microbial cells live in populations in association with populations of cells of other species. A population is a group of cells derived from a single parental cell by successive cell divisions. The immediate environment in which a microbial population lives is called its **habitat**. Populations of cells interact with other populations in **microbial communities** (Figure 1.5). The diversity and abundance of microorganisms in microbial communities is controlled by the resources (foods) and conditions (temperature, pH, oxygen content, and so on) that prevail in their habitat.

Microbial populations interact with each other in beneficial, neutral, or harmful ways. For example, the metabolic waste products of one group of organisms can be nutrients or even poisons to other groups of organisms. Habitats differ markedly in their characteristics, and a habitat that is favorable for the growth of one organism may actually be harmful for another. Collectively, we call all the living organisms, together with the physical and chemical components of their environment, an **ecosystem**. Major microbial ecosystems are aquatic (oceans, ponds, lakes, streams, ice, hot springs), terrestrial (surface soils, deep subsurface), and other organisms, such as plants and animals.

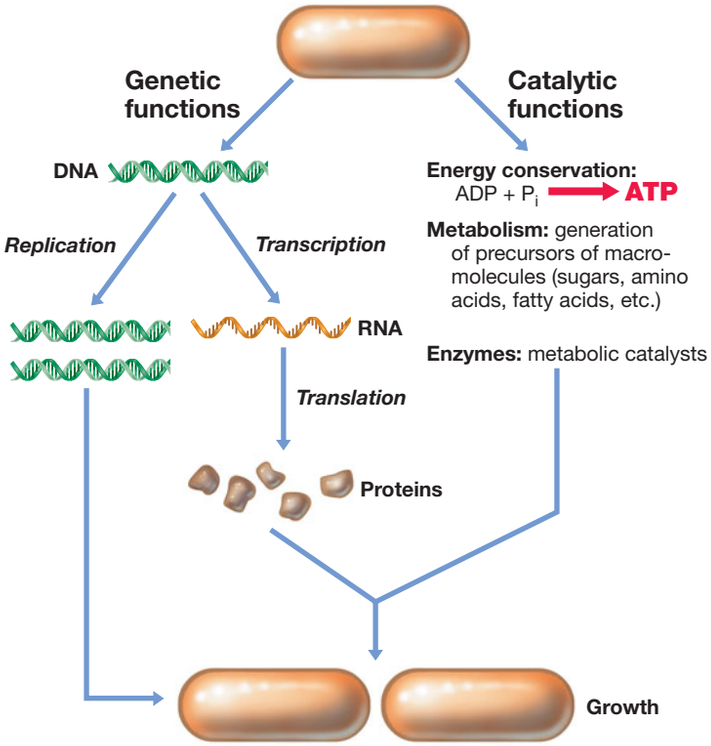
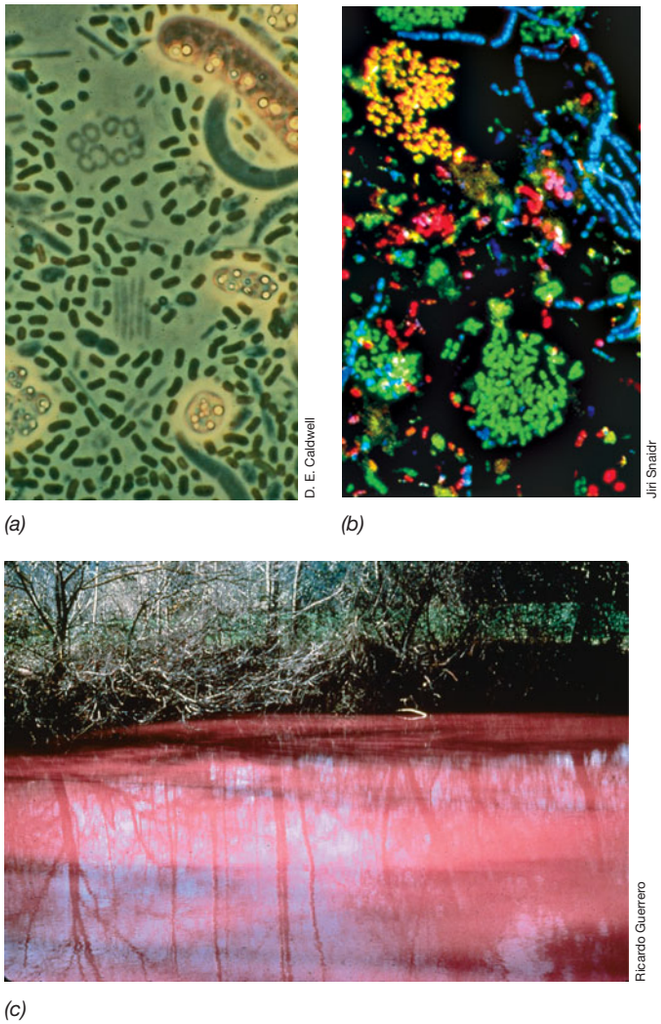


Figure 1.4 **The catalytic and genetic functions of the cell.** For a cell to reproduce itself there must be energy and precursors for the synthesis of new macromolecules, the genetic instructions must be replicated such that upon division each cell receives a copy, and genes must be expressed (transcribed and translated) to produce proteins and other macromolecules. Replication, transcription, and translation are the key molecular processes in cells.

An ecosystem is greatly influenced and in some cases even controlled by microbial activities. Microorganisms carrying out metabolic processes remove nutrients from the ecosystem and use them to build new cells. At the same time, they excrete waste products back into the environment. Thus, microbial ecosystems expand and contract, depending on the resources and conditions available. Over time, the metabolic activities of microorganisms gradually change their ecosystems, both chemically and physically. For example, molecular oxygen (O_2) is a vital nutrient for some microorganisms but a poison to others. If aerobic (oxygen-consuming) microorganisms remove O_2 from a habitat, rendering it anoxic (O_2 free), the changed conditions may favor the growth of anaerobic microorganisms that were formerly present in the habitat but unable to grow. In other words, as resources and conditions change in a microbial habitat, cell populations rise and fall, changing the habitat once again.

In later chapters, after we have learned about microbial structure and function, genetics, evolution, and diversity, we will return to a consideration of the ways in which microorganisms affect animals, plants, and the whole global ecosystem. This is the study of **microbial ecology**, perhaps the most exciting subdiscipline of microbiology today.



(a) *(b)* *(c)*

Figure 1.5 **Microbial communities.** *(a)* A bacterial community that developed in the depths of a small lake (Wintergreen Lake, Michigan), showing cells of various green and purple (large cells with sulfur granules) phototrophic bacteria. *(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)* Purple sulfur bacteria like that shown in part a (see also Figure 1.7*a*) that formed a dense bloom in a small Spanish lake.

MiniQuiz

- How does a microbial community differ from a microbial population?
- What is a habitat? How can microorganisms change the characteristics of their habitats?

1.4 Evolution and the Extent of Microbial Life

Microorganisms were the first entities on Earth with the properties of living systems (Figure 1.3), and we will see that a particular group of microorganisms called the *cyanobacteria* were pivotal

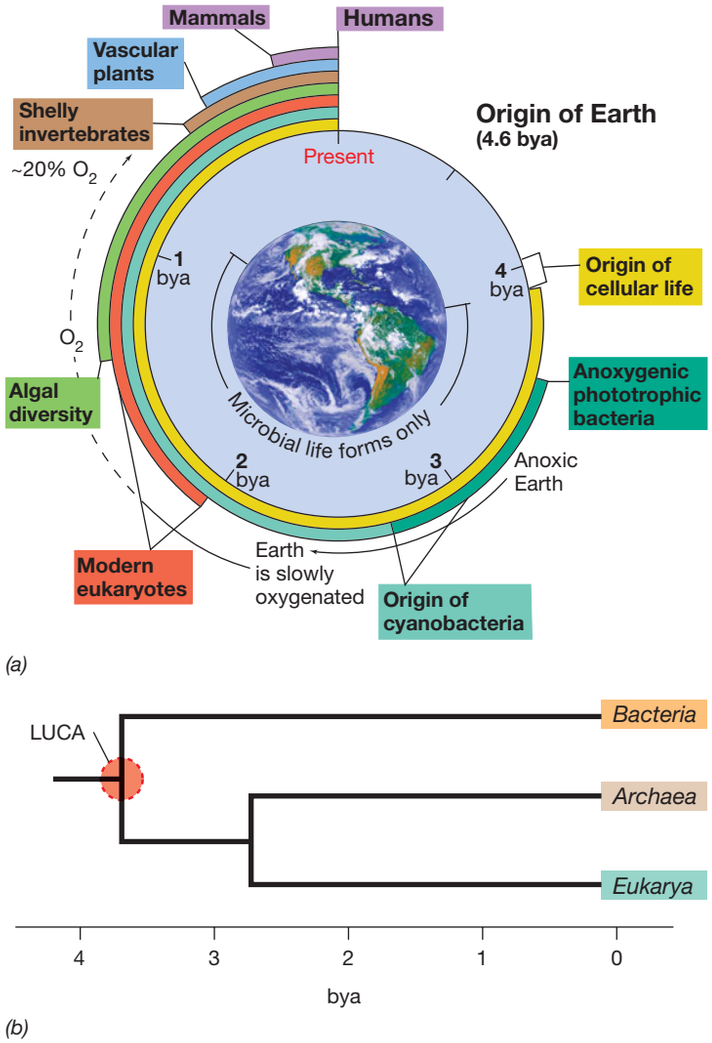


Figure 1.6 **A summary of life on Earth through time and origin of the cellular domains.** *(a)* Cellular life was present on Earth about 3.8 billion years ago (bya). Cyanobacteria began the slow oxygenation of Earth about 3 bya, but current levels of O_2 in the atmosphere were not achieved until 500–800 million years ago. Eukaryotes are nucleated cells and include both microbial and multicellular organisms. (Shelly invertebrates have shells or shell-like parts.) *(b)* The three domains of cellular organisms are *Bacteria*, *Archaea*, and *Eukarya*. The latter two lineages diverged long before nucleated cells with organelles (labeled as "modern eukaryotes" in part a) appear in the fossil record. LUCA, last universal common ancestor. Note that 80% of Earth's history was exclusively microbial.

in biological evolution because oxygen (O_2)—a waste product of their metabolism—prepared planet Earth for more complex life forms.

The First Cells and the Onset of Biological Evolution

How did cells originate? Were cells as we know them today the first self-replicating structures on Earth? Because all cells are constructed in similar ways, it is thought that all cells have descended from a common ancestral cell, the *last universal common ancestor* (LUCA). After the first cells arose from nonliving materials, a process that occurred over hundreds of millions of years, their subsequent growth formed cell populations, and these then began to interact with other populations in microbial communities. Evolution selected for improvements and diversification of these early cells to eventually yield the highly complex and diverse cells we see today. We will consider this complexity and diversity in Chapters 2 and 17–21. We consider the topic of how life originated from nonliving materials in Chapter 16.

Life on Earth through the Ages

Earth is 4.6 billion years old. Scientists have evidence that cells first appeared on Earth between 3.8 and 3.9 billion years ago, and these organisms were exclusively microbial. In fact, microorganisms were the only life on Earth for most of its history (**Figure 1.6**). Gradually, and over enormous periods of time, more complex organisms appeared. What were some of the highlights along the way?

During the first 2 billion years or so of Earth's existence, its atmosphere was anoxic; O_2 was absent, and nitrogen (N_2), carbon dioxide (CO_2), and a few other gases were present. Only microorganisms capable of anaerobic metabolisms could survive under these conditions, but these included many different types of cells, including those that produce methane, called *methanogens.* The evolution of phototrophic microorganisms—organisms that harvest energy from sunlight—occurred within a billion years of the formation of Earth. The first phototrophs were relatively simple ones, such as purple bacteria and other anoxygenic (non-oxygen-evolving) phototrophs (**Figure 1.7*a***; see also Figure 1.5), which are still widespread in anoxic habitats today. Cyanobacteria (oxygenic, or oxygen-evolving, phototrophs) (Figure 1.7*b*) evolved from anoxygenic phototrophs nearly a billion years later and began the slow process of oxygenating the atmosphere. Triggered by increases in O_2 in the atmosphere, multicellular life forms eventually evolved and continued to increase in complexity, culminating in the plants and animals we know today (Figure 1.6). We will

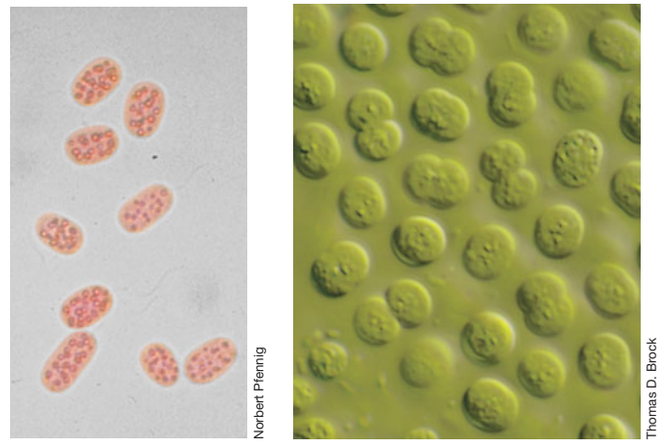


Figure 1.7 **Phototrophic microorganisms.** *(a)* Purple sulfur bacteria (anoxygenic phototrophs). *(b)* Cyanobacteria (oxygenic phototrophs). Purple bacteria appeared on Earth long before oxygenic phototrophs evolved (see Figure 1.6a).

explore the evolutionary history of life later, but note here that the events that unfolded beyond LUCA led to the evolution of three major lineages of microbial cells, the *Bacteria*, the *Archaea*, and the *Eukarya* (Figure 1.6*b*); microbial *Eukarya* were the ancestors of the plants and animals.

How do we know that evolutionary events unfolded as summarized in Figure 1.6? The answer is that we may never know that all details in our description are correct. However, scientists can reconstruct evolutionary transitions by using *biomarkers*, specific molecules that are unique to particular groups in present-day microorganisms. The presence or absence of a given biomarker in ancient rocks of a known age therefore reveals whether that particular group was present at that time.

One way or the other and over enormous periods of time (Figure 1.6), natural selection filled every suitable habitat on Earth with one or more populations of microorganisms. This brings us to the question of the current distribution of microbial life on Earth. What do we know about this important topic?

The Extent of Microbial Life

Microbial life is all around us. Examination of natural materials such as soil or water invariably reveals microbial cells. But unusual habitats such as boiling hot springs and glacial ice are also teeming with microorganisms. Although widespread on Earth, such tiny cells may seem inconsequential. But if we could count them all, what number would we reach?

Estimates of total microbial cell numbers on Earth are on the order of 2.5×10^{30} cells. The total amount of carbon present in this very large number of very small cells equals that of all plants on Earth (and plant carbon far exceeds animal carbon). But in addition, the collective contents of nitrogen and phosphorus in microbial cells is more than 10 times that in all plant biomass.

Thus, microbial cells, small as they are, constitute the major fraction of biomass on Earth and are key reservoirs of essential nutrients for life. Most microbial cells are found in just a few very large habitats. For example, most microbial cells do not reside on Earth's *surface* but instead lie underground in the oceanic and terrestrial *subsurface* (**Table 1.1**). Depths up to about 10 km under Earth's surface are clearly suitable for microbial life. We will see later that subsurface microbial habitats support diverse populations of microbial cells that make their livings in unusual ways and grow extremely slowly. By comparison to the subsurface, surface soils and waters contain a relatively small percentage of the total microbial cell numbers, and animals (including humans), which can be heavily colonized with microorganisms (see Figure 1.10), collectively contain only a tiny fraction of the total microbial cells on Earth (Table 1.1).

Because most of what we know about microbial life has come from the study of surface-dwelling organisms, there is obviously much left for future generations of microbiologists to discover and understand about the life forms that dominate Earth's biology. And when we consider the fact that surface-dwelling organisms already show enormous diversity, the hunt for new microorganisms in Earth's unexplored habitats should yield some exciting surprises.

Table 1.1 *Distribution of microorganisms in and on Earth*[a]

Habitat	*Percent of total*
Marine subsurface	66
Terrestrial subsurface	26
Surface soil	4.8
Oceans	2.2
All other habitats[b]	1.0

[a]Data compiled by William Whitman, University of Georgia, USA; refer to total numbers (estimated to be about 2.5×10^{30} cells) of *Bacteria* and *Archaea*. This enormous number of cells contain, collectively, about 5×10^{17} grams of carbon.
[b]Includes, in order of decreasing numbers: freshwater and salt lakes, domesticated animals, sea ice, termites, humans, and domesticated birds.

MiniQuiz

- What is LUCA and what major lineages of cells evolved from LUCA? Why were cyanobacteria so important in the evolution of life on Earth?
- How old is Earth, and when did cellular life forms first appear? How can we use science to reconstruct the sequence of organisms that appeared on Earth?
- Where are most microbial cells located on Earth?

1.5 The Impact of Microorganisms on Humans

Through the years microbiologists have had great success in discovering how microorganisms work, and application of this knowledge has greatly increased the beneficial effects of microorganisms and curtailed many of their harmful effects. Microbiology has thus greatly advanced human health and welfare. Besides understanding microorganisms as agents of disease, microbiology has made great advances in understanding the role of microorganisms in food and agriculture, and in exploiting microbial activities for producing valuable human products, generating energy, and cleaning up the environment.

Microorganisms as Agents of Disease

The statistics summarized in **Figure 1.8** show microbiologists' success in preventing infectious diseases since the beginning of the twentieth century. These data compare today's leading causes of death in the United States with those of 100 years ago. At the beginning of the twentieth century, the major causes of death in humans were infectious diseases caused by microorganisms called **pathogens**. Children and the aged in particular succumbed in large numbers to microbial diseases. Today, however, infectious diseases are much less deadly, at least in developed countries. Control of infectious disease has come from an increased understanding of disease processes, improved sanitary and public health practices, and the use of antimicrobial agents, such as antibiotics. As we will see from the next sections, the development of microbiology as a science can trace important aspects of its roots to studies of infectious disease.

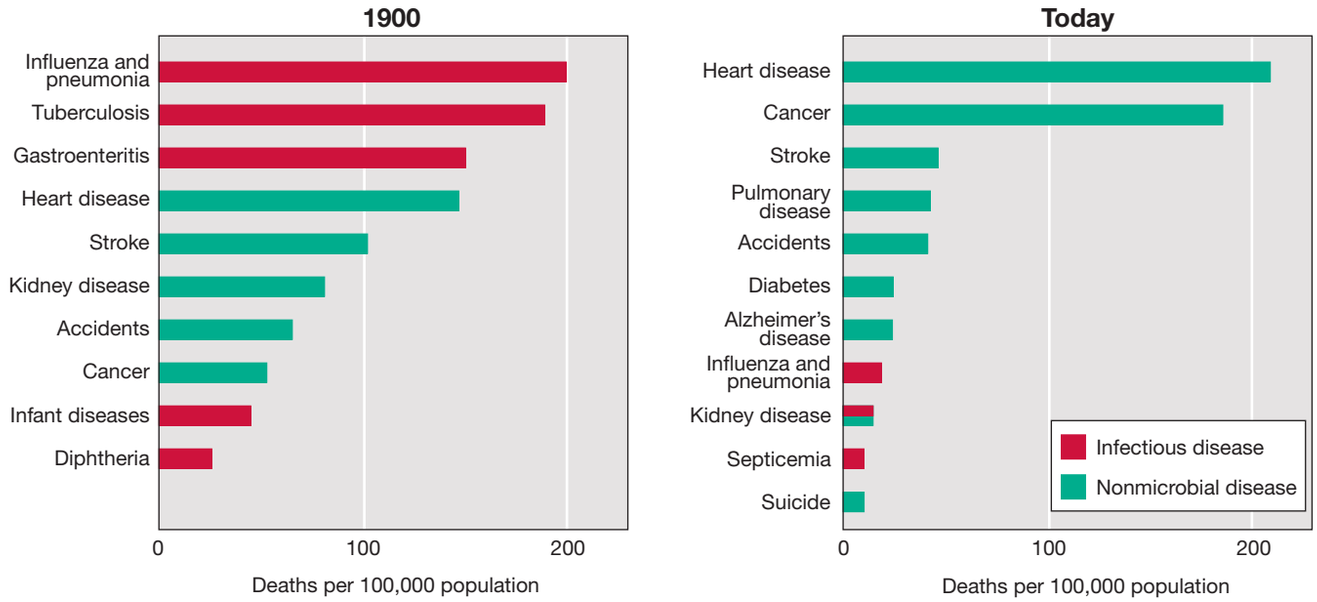


Figure 1.8 **Death rates for the leading causes of death in the United States: 1900 and today.**
Infectious diseases were the leading causes of death in 1900, whereas today they account for relatively few deaths. Kidney diseases can be the result of microbial infections or systemic sources (diabetes, certain cancers, toxicities, metabolic diseases, etc.). Data are from the United States National Center for Health Statistics and the Centers for Disease Control and Prevention and are typical of recent years.

Although many infectious diseases can now be controlled, microorganisms can still be a major threat, particularly in developing countries. In the latter, microbial diseases are still the major causes of death, and millions still die yearly from other microbial diseases such as malaria, tuberculosis, cholera, African sleeping sickness, measles, pneumonia and other respiratory diseases, and diarrheal syndromes. In addition to these, humans worldwide are under threat from diseases that could emerge suddenly, such as bird or swine flu, or Ebola hemorrhagic fever, which are primarily animal diseases that under certain circumstances can be transmitted to humans and spread quickly through a population. And if this were not enough, consider the threat to humans worldwide from those who would deploy microbial bioterrorism agents! Clearly, microorganisms are still serious health threats to humans in all parts of the world.

Although we should obviously appreciate the powerful threat posed by pathogenic microorganisms, in reality, most microorganisms are not harmful to humans. In fact, most microorganisms cause no harm but instead are beneficial—and in many cases even essential—to human welfare and the functioning of the planet. We turn our attention to these microorganisms now.

Microorganisms, Digestive Processes, and Agriculture

Agriculture benefits from the cycling of nutrients by microorganisms. For example, a number of major crop plants are legumes. Legumes live in close association with bacteria that form structures called *nodules* on their roots. In the root nodules, these bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) that the plants use as a nitrogen source for growth (**Figure 1.9**). Thanks to the activities of these nitrogen-fixing bacteria, the legumes have no need for costly and polluting nitrogen fertilizers. Other bacteria cycle sulfur compounds, oxidizing toxic sulfur species such as hydrogen sulfide (H_2S) into sulfate (SO_4^{2-}), which is an essential plant nutrient (Figure 1.9*c*).

Also of major agricultural importance are the microorganisms that inhabit ruminant animals, such as cattle and sheep. These important domesticated animals have a characteristic digestive vessel called the *rumen* in which large populations of microorganisms digest and ferment cellulose, the major component of plant cell walls, at neutral pH (Figure 1.9*d*). Without these symbiotic microorganisms, cattle and sheep could not thrive on cellulose-rich (but otherwise nutrient-poor) food, such as grass and hay. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats—are also ruminants.

The ruminant digestive system contrasts sharply with that of humans and most other animals. In humans, food enters a highly acidic stomach where major digestive processes are chemical rather than microbial. In the human digestive tract, large microbial populations occur only in the colon (large intestine), a structure that comes after the stomach and small intestine and which lacks significant numbers of cellulose-degrading bacteria. However, other parts of the human body can be loaded with bacteria. In addition to the large intestine, the skin and oral cavity (**Figure 1.10**) contain a significant normal microbial flora, most of which benefits the host or at least does no harm.

In addition to benefiting plants and animals, microorganisms can also, of course, have negative effects on them. Microbial diseases of plants and animals used for human food cause major

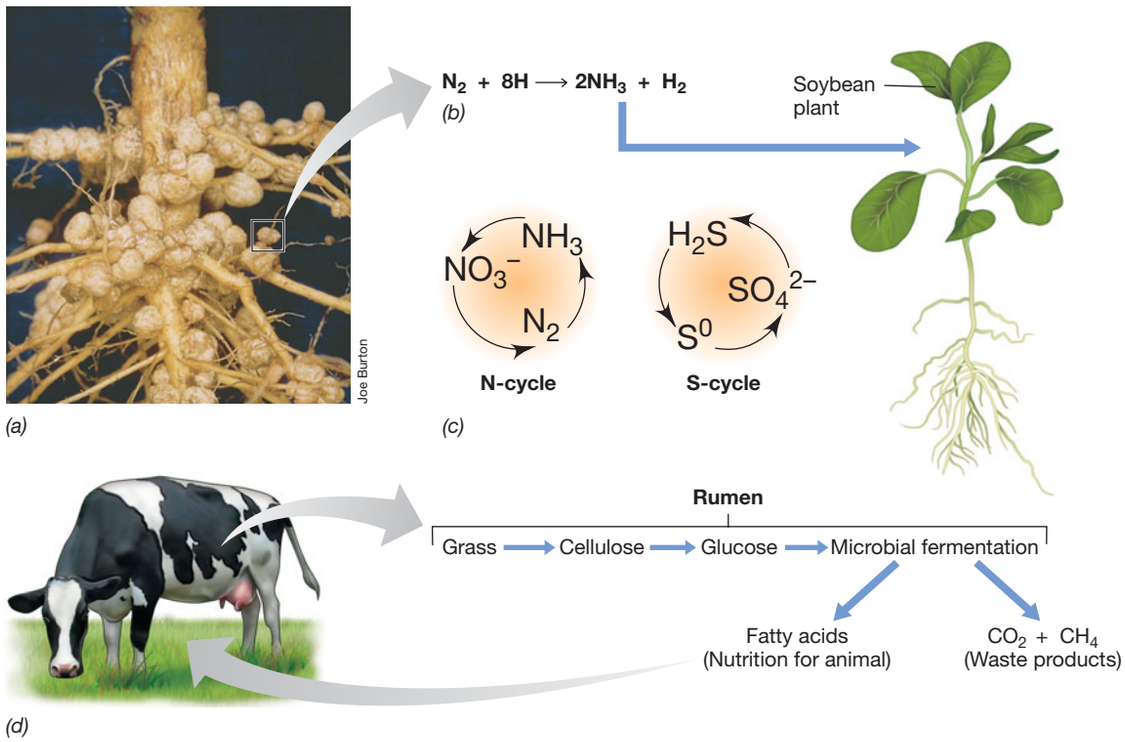


Figure 1.9 **Microorganisms in modern agriculture.** *(a, b)* Root nodules on this soybean plant contain bacteria that fix molecular nitrogen (N_2) for use by the plant. *(c)* The nitrogen and sulfur cycles, key nutrient cycles in nature. *(d)* Ruminant animals. Microorganisms in the rumen of the cow convert cellulose from grass into fatty acids that can be used by the animal.

economic losses in the agricultural industry every year. In some cases a food product can cause serious human disease, such as when pathogenic *Escherichia coli* or *Salmonella* is transmitted from infected meat, or when microbial pathogens are ingested with contaminated fresh fruits and vegetables. Thus microorganisms significantly impact the agriculture industry both positively and negatively.

Microorganisms and Food, Energy, and the Environment

Microorganisms play important roles in the food industry, including in the areas of spoilage, safety, and production. After plants and animals are produced for human consumption, the products must be delivered to consumers in a wholesome form. Food spoilage alone results in huge economic losses each year. Indeed, the canning, frozen food, and dried-food industries were founded as means to preserve foods that would otherwise easily undergo microbial spoilage. Food safety requires constant monitoring of food products to ensure they are free of pathogenic microorganisms and to track disease outbreaks to identify the source(s).

However, not all microorganisms in foods have harmful effects on food products or those who eat them. For example, many dairy products depend on the activities of microorganisms, including the fermentations that yield cheeses, yogurt, and buttermilk. Sauerkraut, pickles, and some sausages are also products of microbial fermentations. Moreover, baked goods and alcoholic beverages rely on the fermentative activities of yeast, which generate carbon dioxide (CO_2) to raise the dough and alcohol as a key ingredient, respectively. Many of these fermentations are discussed in Chapter 14.

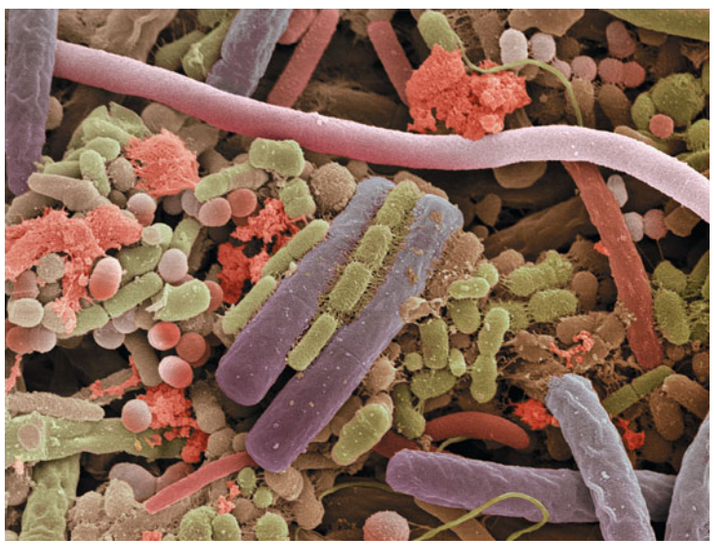


Figure 1.10 **Human oral bacterial community.** The oral cavity of warm-blooded animals contains high numbers of various bacteria, as shown in this electron micrograph (false color) of cells scraped from a human tongue.

(a)

(b)

Figure 1.11 **Biofuels.** *(a)* Natural gas (methane) is collected in a funnel from swamp sediments where it was produced by methanogens and then ignited as a demonstration experiment. *(b)* An ethanol plant in the United States. Sugars obtained from corn or other crops are fermented to ethanol for use as a motor fuel extender.

Some microorganisms produce *biofuels*. Natural gas (methane) is a product of the anaerobic degradation of organic matter by methanogenic microorganisms (**Figure 1.11**). Ethyl alcohol (ethanol), which is produced by the microbial fermentation of glucose from feedstocks such as sugarcane or cornstarch, is a major motor fuel in some countries (Figure 1.11*b*). Waste materials such as domestic refuse, animal wastes, and cellulose can also be converted to biofuels by microbial activities and are more efficient feedstocks for ethanol production than is corn. Soybeans are also used as biofuel feedstocks, as soybean oils can be converted into biodiesel to fuel diesel engines. As global oil production is waning, it is likely that various biofuels will take on a greater and greater part of the global energy picture.

Microorganisms are used to clean up human pollution, a process called *microbial bioremediation*, and to produce commercially valuable products by *industrial microbiology* and *biotechnology*. For example, microorganisms can be used to consume spilled oil, solvents, pesticides, and other environmentally toxic pollutants. Bioremediation accelerates cleanup in either of two ways: (1) by introducing specific microorganisms to a polluted environment, or (2) by adding nutrients that stimulate preexisting microorganisms to degrade the pollutants. In both cases the goal is to accelerate metabolism of the pollutant.

In industrial microbiology, microorganisms are grown on a large scale to make products of relatively low commercial value, such as antibiotics, enzymes, and various chemicals. By contrast, the related field of biotechnology employs *genetically engineered* microorganisms to synthesize products of high commercial value, such as human proteins. **Genomics** is the science of the identification and analysis of genomes and has greatly enhanced biotechnology. Using genomic methods, biotechnologists can access the genome of virtually any organism and search in it for genes encoding proteins of commercial interest.

At this point the influence of microorganisms on humans should be apparent. Microorganisms are essential for life and their activities can cause significant benefit or harm to humans. As the eminent French scientist Louis Pasteur, one of the founders of microbiology, expressed it: "The role of the infinitely small in nature is infinitely large." We continue our introduction to the microbial world in the next section with an historical overview of the contributions of Pasteur and a few other key scientists.

MiniQuiz

- List two ways in which microorganisms are important in the food and agricultural industries.
- Which biofuel is widely used in many countries as a motor fuel?
- What is biotechnology and how might it improve the lives of humans?

II Pathways of Discovery in Microbiology

The future of any science is rooted in its past accomplishments. Although microbiology claims very early roots, the science did not really develop in a systematic way until the nineteenth century. Since that time, microbiology has expanded in a way

Table 1.2 *Giants of the early days of microbiology and their major contributions*

Investigator	*Nationality*	*Dates*[a]	*Contributions*
Robert Hooke	English	1664	Discovery of microorganisms (fungi)
Antoni van Leeuwenhoek	Dutch	1684	Discovery of bacteria
Edward Jenner	English	1798	Vaccination (smallpox)
Louis Pasteur	French	Mid- to late 1800s	Mechanism of fermentation, defeat of spontaneous generation, rabies and other vaccines, principles of immunization
Joseph Lister	English	1867	Methods for preventing infections during surgeries
Ferdinand Cohn	German	1876	Discovery of endospores
Robert Koch	German	Late 1800s	Koch's postulates, pure culture microbiology, discovery of agents of tuberculosis and cholera
Sergei Winogradsky	Russian	Late 1800s to mid-1900s	Chemolithotrophy and chemoautotrophy, nitrogen fixation, sulfur bacteria
Martinus Beijerinck	Dutch	Late 1800s to 1920	Enrichment culture technique, discovery of many metabolic groups of bacteria, concept of a virus

[a]The year in which the key paper describing the contribution was published, or the date range in which the investigator was most scientifically active.

unprecedented by any of the other biological sciences and has spawned several new but related fields. We retrace these pathways of discovery now and discuss a few of the major contributors (**Table 1.2**).

1.6 The Historical Roots of Microbiology: Hooke, van Leeuwenhoek, and Cohn

Although the existence of creatures too small to be seen with the naked eye had long been suspected, their discovery was linked to the invention of the microscope. Robert Hooke (1635–1703), an English mathematician and natural historian, was also an excellent microscopist. In his famous book *Micrographia* (1665), the first book devoted to microscopic observations, Hooke illustrated, among many other things, the fruiting structures of molds (**Figure 1.12**). This was the first known description of microorganisms. The first person to see bacteria was the Dutch draper and amateur microscope builder Antoni van Leeuwenhoek (1632–1723). In 1684, van Leeuwenhoek, who was well aware of the work of Hooke, used extremely simple microscopes of his own construction (**Figure 1.13**) to examine the microbial content of natural substances.

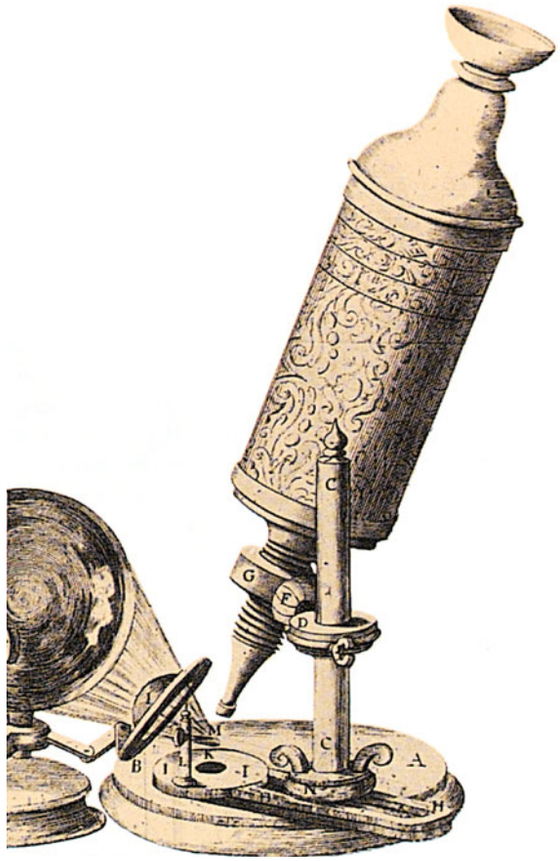
Van Leeuwenhoek's microscopes were crude by today's standards, but by careful manipulation and focusing he was able to see bacteria, microorganisms considerably smaller than molds (molds are fungi). He discovered bacteria in 1676 while studying pepper–water infusions. He reported his observations in a series of letters to the prestigious Royal Society of London, which published them in 1684 in English translation. Drawings of some of van Leeuwenhoek's "wee animalcules," as he referred to them, are shown in Figure 1.13*b*, and a photo taken through such a microscope is shown in Figure 1.13*c*.

As years went by, van Leeuwenhoek's observations were confirmed by many others. However, primarily because of the lack of experimental tools, little progress in understanding the nature and importance of the tiny creatures was made for almost 150 years. Only in the nineteenth century did improved microscopes and some simple tools for growing microoorganisms in the laboratory become available, and using these, the extent and nature of microbial life became more apparent.

In the mid- to late nineteenth century major advances in the science of microbiology were made because of the attention given to two major questions that pervaded biology and medicine at the time: (1) Does spontaneous generation occur? and (2) What is the nature of infectious disease? Answers to these seminal questions emerged from the work of two giants in the fledgling field of microbiology: the French chemist Louis Pasteur and the German physician Robert Koch. But before we explore their work, let us briefly consider the groundbreaking efforts of a German botanist, Ferdinand Cohn, a contemporary of Pasteur and Koch, and the founder of the field we now call *bacteriology*.

Ferdinand Cohn (1828–1898) was born in Breslau (now in Poland). He was trained as a botanist and became an excellent microscopist. His interests in microscopy led him to the study of unicellular algae and later to bacteria, including the large sulfur bacterium *Beggiatoa* (**Figure 1.14**). Cohn was particularly interested in heat resistance in bacteria, which led to his discovery that some bacteria form endospores. We now know that bacterial endospores are formed by differentiation from the mother (vegetative) cell (Figure 1.3) and that endospores are extremely heat-resistant. Cohn described the life cycle of the endospore-forming bacterium *Bacillus* (vegetative cell → endospore → vegetative cell) and showed that vegetative cells but not endospores were killed by boiling.

Cohn is credited with many other accomplishments. He laid the groundwork for a system of bacterial classification, including an early attempt to define a bacterial species, an issue still unresolved today, and founded a major scientific journal of plant and microbial biology. He strongly advocated use of the techniques and research of Robert Koch, the first medical microbiologist. Cohn devised simple but effective methods for preventing the contamination of culture media, such as the use



(a)

(b)

Figure 1.12 **Robert Hooke and early microscopy.** *(a)* 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). *(b)* This drawing of a mold that was growing on the surface of leather, together with other drawings and accompanying text published by Robert Hooke in *Micrographia* in 1665, were the first descriptions of microorganisms. The round structures contain spores of the mold. Compare Hooke's microscope with that of van Leeuwenhoek's shown in Figure 1.13.

of cotton for closing flasks and tubes. These methods were later used by Koch and allowed him to make rapid progress in the isolation and characterization of several disease-causing bacteria (Section 1.8).

MiniQuiz

- What prevented the science of microbiology from developing before the era of Hooke and van Leeuwenhoek?
- What major discovery emerged from Cohn's study of heat resistance in microorganisms?

1.7 Pasteur and the Defeat of Spontaneous Generation

The late nineteenth century saw the science of microbiology blossom. The theory of spontaneous generation was crushed by the brilliant work of the Frenchman Louis Pasteur (1822–1895).

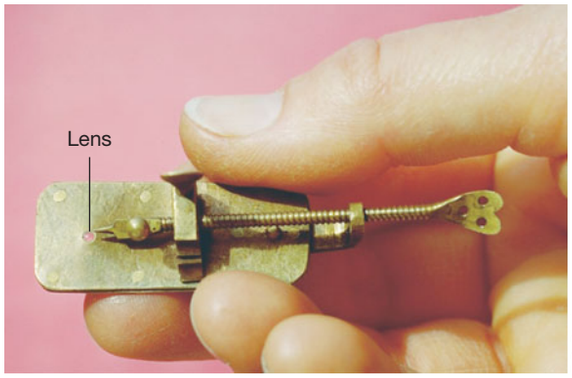
Optical Isomers and Fermentations

Pasteur was a chemist by training and was one of the first to recognize the significance of *optical isomers*. A molecule is optically active if a pure solution or crystal diffracts light in only one direction. Pasteur studied crystals of tartaric acid that he separated by hand into those that bent a beam of polarized light to the left and those that bent the beam to the right (**Figure 1.15**). Pasteur found that the mold *Aspergillus* metabolized D-tartrate, which bent light to the right, but did not metabolize its optical isomer, L-tartrate. The fact that a living organism could discriminate between optical isomers was of profound significance to Pasteur, and he began to see living organisms as inherently asymmetric entities.

Pasteur's thinking on the asymmetry of life carried over into his work on fermentations and, eventually, spontaneous generation. At the invitation of a local industrialist who was having problems making alcohol from the fermentation of beets, Pasteur studied the mechanism of the alcoholic fermentation, at that time thought to be a strictly chemical process. The yeast cells in the fermenting broth were thought to be a complex chemical substance formed by the fermentation. Although ethyl alcohol does not form optical isomers, one of the side products of beet fermentation is amyl alcohol, which does, and Pasteur tested the fermenting juice and found the amyl alcohol to be of only one optical isomer. From his work on tartrate metabolism this suggested to Pasteur that the beet fermentation was a biological process. Microscopic observations and other simple but rigorous experiments convinced Pasteur that the alcoholic fermentation was catalyzed by living organisms, the yeast cells. Indeed, in Pasteur's own words: ". . . fermentation is associated with the life and structural integrity of the cells and not with their death and decay." From this foundation, Pasteur began a series of classic experiments on spontaneous generation, experiments that are forever linked to his name and to the science of microbiology.

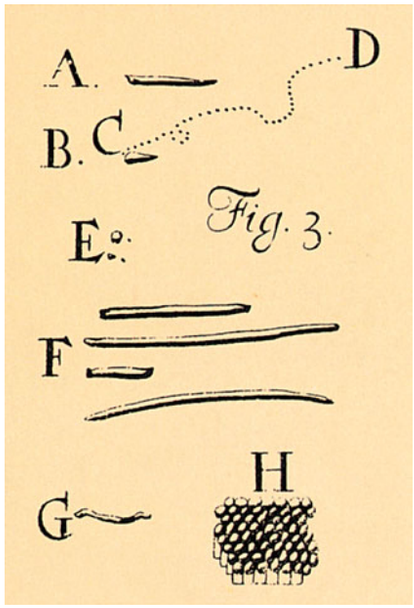
Spontaneous Generation

The concept of **spontaneous generation** had existed since biblical times and its basic tenet can be easily grasped. For example, if



T. D. Brock

(a)



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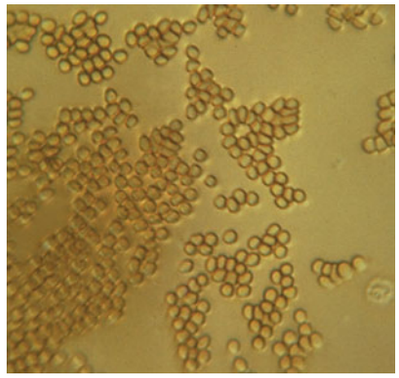


Brian J. Ford

(c)

Figure 1.13 **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.

food is allowed to stand for some time, it putrefies. When examined microscopically, the putrefied food is seen to be teeming with bacteria and perhaps even maggots and worms. From where do these organisms not apparent in the fresh food originate? Some people said they developed from seeds or germs that entered the food from air. Others said they arose spontaneously from nonliving materials, that is, by *spontaneous generation*. Who was right? Keen insight was necessary to solve this controversy, and this was exactly the kind of problem that appealed to Louis Pasteur.

Pasteur became a powerful opponent of spontaneous generation. Following his discoveries about fermentation, Pasteur predicted that microorganisms observed in putrefying materials are also present in air and that putrefaction resulted from the activities of microorganisms that entered from the air or that had been present on the surfaces of the containers holding the decaying materials. Pasteur further reasoned that if food were treated in such a way as to destroy all living organisms contaminating it, that is, if it were rendered **sterile** and then protected from further contamination, it should not putrefy.

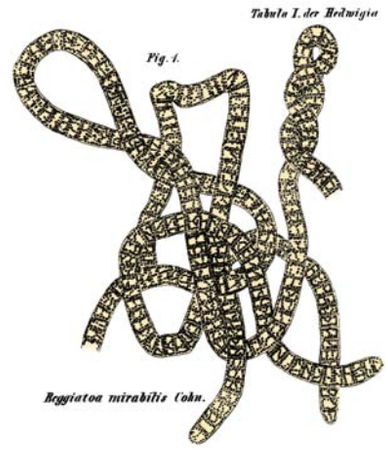
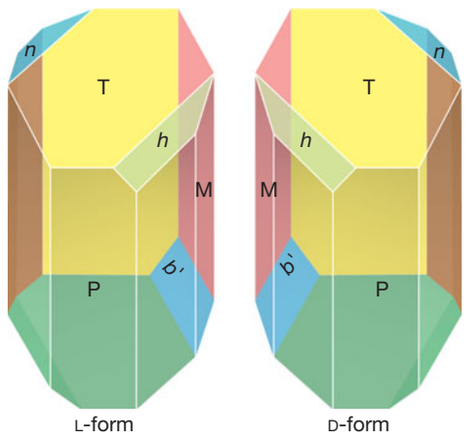


Figure 1.14 **Drawing by Ferdinand Cohn of large filamentous sulfur-oxidizing bacteria *Beggiatoa mirabilis*.** The small granules inside the cells consist of elemental sulfur, produced from the oxidation of hydrogen sulfide (H_2S). Cohn was the first to identify the granules as sulfur in 1866. A cell of *B. mirabilis* is about 15 μm in diameter. Compare with Figure 1.22*b*. *Beggiatoa* moves on solid surfaces by a gliding mechanism and in so doing, cells often twist about one another.



(a) (b)

Figure 1.15 **Louis Pasteur's drawings of tartaric acid crystals from his famous paper on optical activity.** *(a)* Left-handed crystal (bends light to the left). *(b)* Right-handed crystal (bends light to the right). Note that the two crystals are mirror images of one another, a hallmark of optical isomers.

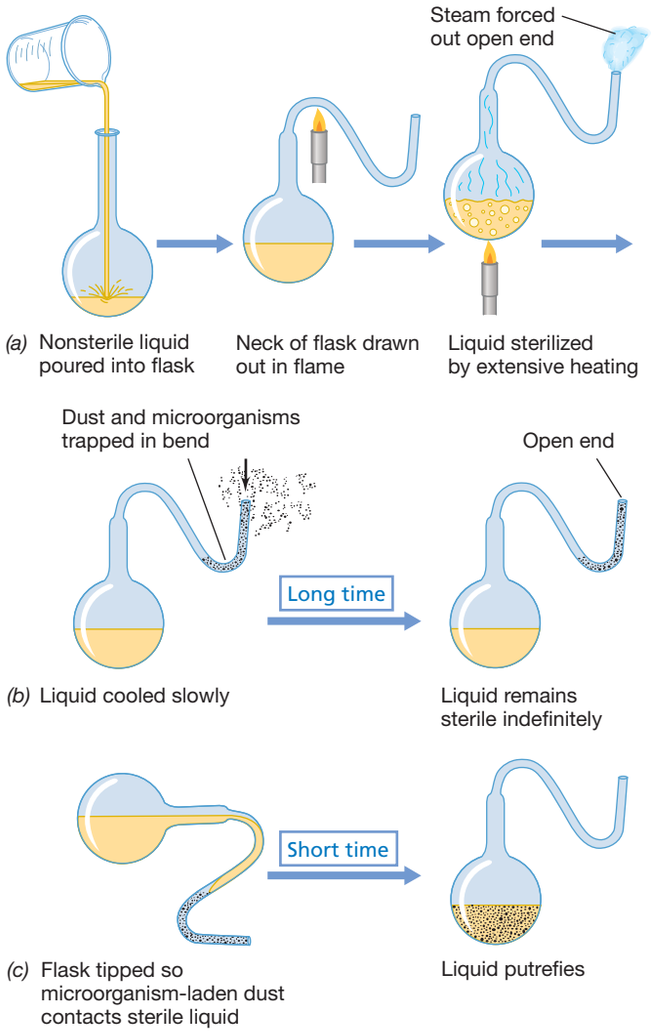


Figure 1.16 The defeat of spontaneous generation: Pasteur's swan-necked flask experiment. In *(c)* the liquid putrefies because microorganisms enter with the dust.

Pasteur used heat to eliminate contaminants. Killing all the bacteria or other microorganisms in or on objects is a process we now call *sterilization*. Proponents of spontaneous generation criticized such experiments by declaring that "fresh air" was necessary for the phenomenon to occur. In 1864 Pasteur countered this objection simply and brilliantly by constructing a swan-necked flask, now called a *Pasteur flask* (**Figure 1.16**). In such a flask nutrient solutions could be heated to boiling and sterilized. However, after the flask was cooled, air was allowed to reenter, but the bend in the neck prevented particulate matter (including microorganisms) from entering the nutrient solution and causing putrefaction.

The teeming microorganisms observed after particulate matter was allowed to enter at the end of this simple experiment (Figure 1.16*c*) effectively settled the controversy, and microbiology was able to bury the idea of spontaneous generation for good and move ahead on firm footing. Incidentally, Pasteur's work also led to the development of effective sterilization procedures that were eventually refined and carried over into both basic and applied microbiological research. Food science also owes a debt to Pasteur, as his principles are applied today in the preservation of milk and many other foods by heat treatment (pasteurization). www.microbiologyplace.com **Online Tutorial 1.1: Pasteur's Experiment**

Other Accomplishments of Louis Pasteur

Pasteur went on to many other triumphs in microbiology and medicine. Some highlights include his development of vaccines for the diseases anthrax, fowl cholera, and rabies during a very scientifically productive period from 1880 to 1890. Pasteur's work on rabies was his most famous success, culminating in July 1885 with the first administration of a rabies vaccine to a human, a young French boy named Joseph Meister who had been bitten by a rabid dog. In those days, a bite from a rabid animal was invariably fatal. News spread quickly of the success of Meister's vaccination, and of one administered shortly thereafter to a young shepherd boy, Jean Baptiste Jupille (**Figure 1.17**). Within a

(a)

M.T. Madigan

(b)

Figure 1.17 Louis Pasteur and some symbols of his contributions to microbiology. *(a)* A French 5-franc note honoring Pasteur. The shepherd boy Jean Baptiste Jupille is shown killing a rabid dog that had attacked children. Pasteur's rabies vaccine saved Jupille's life. In France, the franc preceded the euro as a currency. *(b)* The Pasteur Institute, Paris, France. Today this structure, built for Pasteur by the French government, houses a museum that displays some of the original swan-necked flasks used in his experiments.

year several thousand people bitten by rabid animals had traveled to Paris to be treated with Pasteur's rabies vaccine.

Pasteur's fame from his rabies research was legendary and led the French government to establish the Pasteur Institute in Paris in 1888 (Figure 1.17*b*). Originally established as a clinical center for the treatment of rabies and other contagious diseases, the Pasteur Institute today is a major biomedical research center focused on antiserum and vaccine research and production. The medical and veterinary breakthroughs of Pasteur were not only highly significant in their own right but helped solidify the concept of the germ theory of disease, whose principles were being developed at about the same time by a second giant of this era, Robert Koch.

MiniQuiz

- Define the term sterile. How did Pasteur's experiments using swan-necked flasks defeat the theory of spontaneous generation?
- Besides ending the controversy over spontaneous generation, what other accomplishments do we credit to Pasteur?

1.8 Koch, Infectious Disease, and Pure Culture Microbiology

Proof that some microorganisms cause disease provided the greatest impetus for the development of microbiology as an independent biological science. Even as early as the sixteenth century it was thought that something that induced disease could be transmitted from a diseased person to a healthy person. After the discovery of microorganisms, it was widely believed that they were responsible, but definitive proof was lacking. Improvements in sanitation by Ignaz Semmelweis and Joseph Lister provided indirect evidence for the importance of microorganisms in causing human diseases, but it was not until the work of a German physician, Robert Koch (1843–1910) (**Figure 1.18**), that the concept of infectious disease was given experimental support.

The Germ Theory of Disease and Koch's Postulates

In his early work Koch studied anthrax, a disease of cattle and occasionally of humans. Anthrax is caused by an endospore-forming bacterium called *Bacillus anthracis.* By careful microscopy and by using special stains, Koch established that the bacteria were always present in the blood of an animal that was succumbing to the disease. However, Koch reasoned that the mere association of the bacterium with the disease was not proof of cause and effect. He sensed an opportunity to study cause and effect experimentally using anthrax. The results of this study formed the standard by which infectious diseases have been studied ever since.

Koch used mice as experimental animals. Using appropriate controls, Koch demonstrated that when a small amount of blood from a diseased mouse was injected into a healthy mouse, the latter quickly developed anthrax. He took blood from this second animal, injected it into another, and again observed the characteristic disease symptoms. However, Koch carried this experiment a critically important step further. He discovered that the anthrax bacteria could be grown in nutrient fluids *outside the host* and that even after many transfers in laboratory culture, the bacteria still caused the disease when inoculated into a healthy animal.

Figure 1.18 **Robert Koch.** The German physician and microbiologist is credited with founding medical microbiology and formulating his famous postulates.

On the basis of these experiments and others on the causative agent of tuberculosis, Koch formulated a set of rigorous criteria, now known as **Koch's postulates**, for definitively linking a specific microorganism to a specific disease. Koch's postulates state the following:

1. The disease-causing organism must always be present in animals suffering from the disease but not in healthy animals.
2. The organism must be cultivated in a pure culture away from the animal body.
3. The isolated organism must cause the disease when inoculated into healthy susceptible animals.
4. The organism must be isolated from the newly infected animals and cultured again in the laboratory, after which it should be seen to be the same as the original organism.

Koch's postulates, summarized in **Figure 1.19**, were a monumental step forward in the study of infectious diseases. The postulates not only offered a means for linking the cause and effect of an infectious disease, but also stressed the importance of *laboratory culture* of the putative infectious agent. With these postulates as a guide, Koch, his students, and those that followed them discovered the causative agents of most of the important

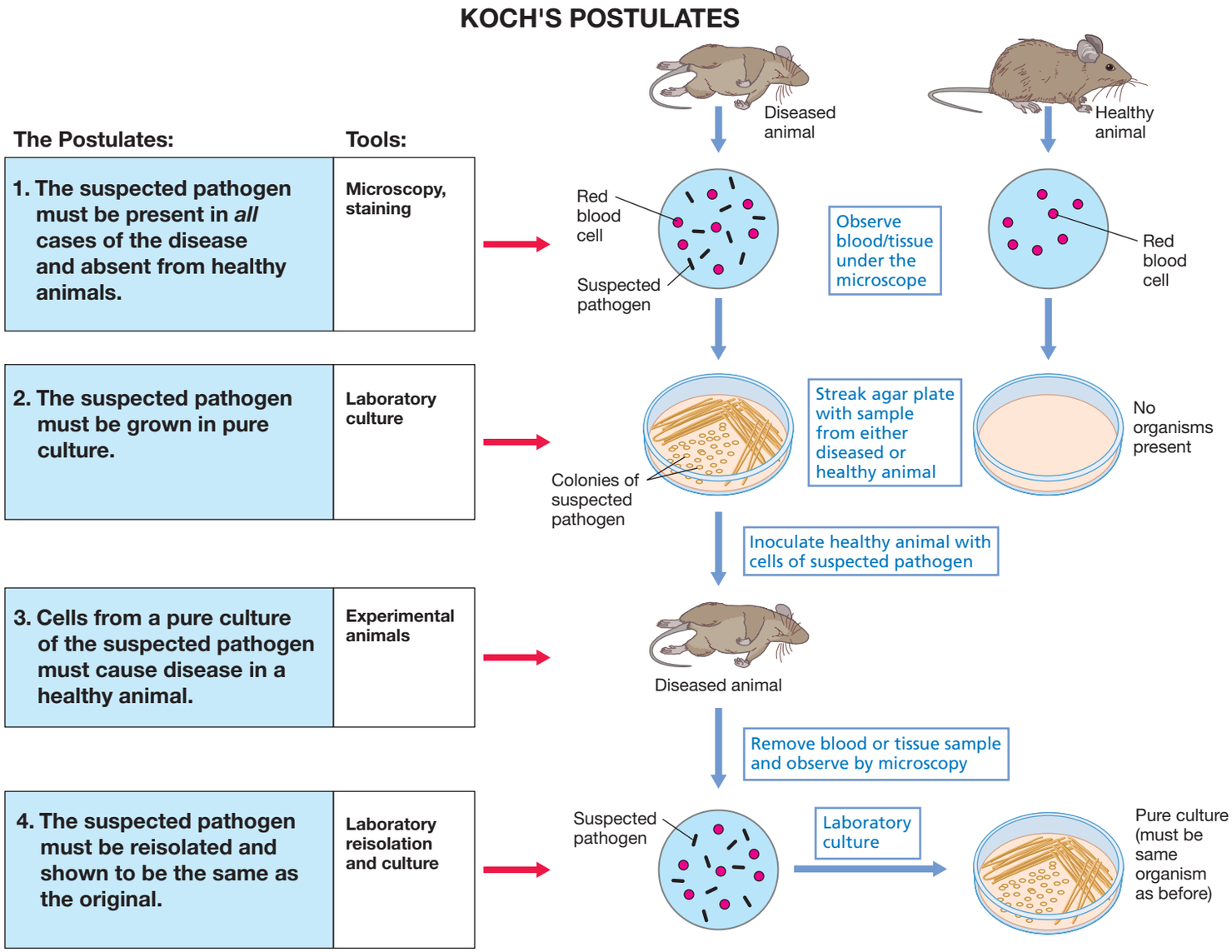


Figure 1.19 **Koch's postulates for proving cause and effect in infectious diseases.** Note that following isolation of a pure culture of the suspected pathogen, the cultured organism must both initiate the disease and be recovered from the diseased animal. Establishing the correct conditions for growing the pathogen is essential; otherwise it will be missed.

infectious diseases of humans and domestic animals. These discoveries led to the development of successful treatments for the prevention and cure of many of these diseases, thereby greatly improving the scientific basis of clinical medicine and human health and welfare (Figure 1.8).

Koch and Pure Cultures

To satisfy the second of Koch's postulates, the suspected pathogen must be isolated and grown away from other microorganisms in laboratory culture; in microbiology we say that such a culture is *pure*. The importance of this was not lost on Robert Koch in formulating his famous postulates, and to accomplish this goal, he and his associates developed several simple but ingenious methods of obtaining and growing bacteria in **pure culture**.

Koch started by using solid nutrients such as a potato slice to culture bacteria, but quickly developed more reliable methods, many of which are still in use today. Koch observed that when a solid surface was incubated in air, bacterial colonies developed, each having a characteristic shape and color. He inferred that each colony had arisen from a single bacterial cell that had fallen on the surface, found suitable nutrients, and multiplied. Each colony was a population of identical cells, or in other words, a pure culture, and Koch quickly realized that solid media provided an easy way to obtain pure cultures. However, because not all organisms grow on potato slices, Koch devised more exacting and reproducible nutrient solutions solidified with gelatin and, later, with agar—laboratory techniques that remain with us to this day (see the Microbial Sidebar, "Solid Media, Pure Cultures, and the Birth of Microbial Systematics").

Solid Media, Pure Cultures, and the Birth of Microbial Systematics

Robert Koch was the first to grow bacteria on solid culture media. Koch's early use of potato slices as solid media was fraught with problems. Besides the problem that not all bacteria can grow on potatoes, the slices were frequently overgrown with molds. Koch thus needed a more reliable and reproducible means of growing bacteria on solid media, and he found the answer for solidifying his nutrient solutions in agar.

Koch initially employed gelatin as a solidifying agent for the various nutrient fluids he used to culture bacteria, and he kept horizontal slabs of solid gelatin free of contamination under a bell jar or in a glass box (see Figure 1.20c). Nutrient-supplemented gelatin was a good culture medium for the isolation and study of various bacteria, but it had several drawbacks, the most important of which was that it did not remain solid at 37°C, the optimum temperature for growth of most human pathogens. Thus, a different solidifying agent was needed.

Agar is a polysaccharide derived from red algae. It was widely used in the nineteenth century as a gelling agent. Walter Hesse, an associate of Koch, first used agar as a solidifying agent for bacteriological culture media (Figure 1). The actual suggestion that agar be used instead of gelatin was made by Hesse's wife, Fannie. She had used agar to solidify fruit jellies. When it was tried as a solidifying agent for microbial media, its superior gelling qualities were immediately evident. Hesse wrote to Koch about this discovery, and Koch quickly adapted agar to his own studies, including his classic studies on the isolation of the bacterium *Mycobacterium tuberculosis,* the cause of the disease tuberculosis (see text and Figure 1.20).

Agar has many other properties that make it desirable as a gelling agent for microbial culture media. In particular, agar remains solid at 37°C and, after melting during the sterilization process, remains liquid to about 45°C, at which time it can be poured into sterile vessels. In addition, unlike gelatin, agar is not degraded by most bacteria and typically yields a transparent medium, making it easier to differentiate bacterial colonies from inanimate particulate matter. For these reasons, agar found its place early in the annals of microbiology and is still used today for obtaining and maintaining pure cultures.

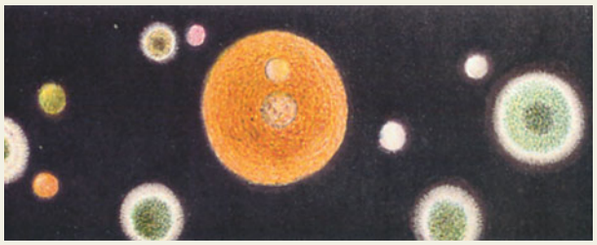
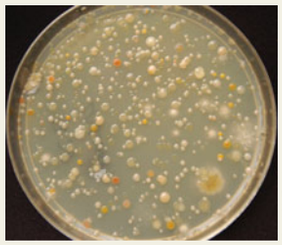


Figure 1 A hand-colored photograph taken by Walter Hesse of colonies formed on agar. The colonies include those of molds and bacteria obtained during Hesse's studies of the microbial content of air in Berlin, Germany, in 1882. From Hesse, W. 1884. "Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen," in Struck, H. (ed.), *Mittheilungen aus dem Kaiserlichen Gesundheitsamte.* August Hirschwald.



Paul V. Dunlap

Figure 2 Photo of a Petri dish containing colonies of marine bacteria. Each colony contains millions of bacterial cells descended from a single cell.

In 1887 Richard Petri, a German bacteriologist, published a brief paper describing a modification of Koch's flat plate technique (Figure 1.20c). Petri's enhancement, which turned out to be amazingly useful, was the development of the transparent double-sided dishes that bear his name (Figure 2). The advantages of Petri dishes were immediately apparent. They could easily be stacked and sterilized separately from the medium, and, following the addition of molten culture medium to the smaller of the two dishes, the larger dish could be used as a cover to prevent contamination. Colonies that formed on the surface of the agar in the Petri dish retained access to air without direct exposure to air and could easily be manipulated for further study. The original idea of Petri has not been improved on to this day, and the Petri dish, constructed of either glass or plastic, is a mainstay of the microbiology laboratory.

Koch quickly grasped the significance of pure cultures and was keenly aware of the implications his pure culture methods had for classifying microorganisms. He observed that colonies that differed in color, morphology, size, and the like (see Figure 2) bred true and could be distinguished from one another. Cells from different colonies typically differed in size and shape and often in their temperature or nutrient requirements as well. Koch realized that these differences among microorganisms met all the requirements that biological taxonomists had established for the classification of larger organisms, such as plant and animal species. In Koch's own words (translated from the German): "All bacteria which maintain the characteristics which differentiate one from another when they are cultured on the same medium and under the same conditions, should be designated as species, varieties, forms, or other suitable designation." Such insightful thinking was important for the rapid acceptance of microbiology as a new biological science, rooted as biology was in classification at the time of Koch. It has since had a profound impact on the diagnosis of infectious diseases and the field of microbial diversity.

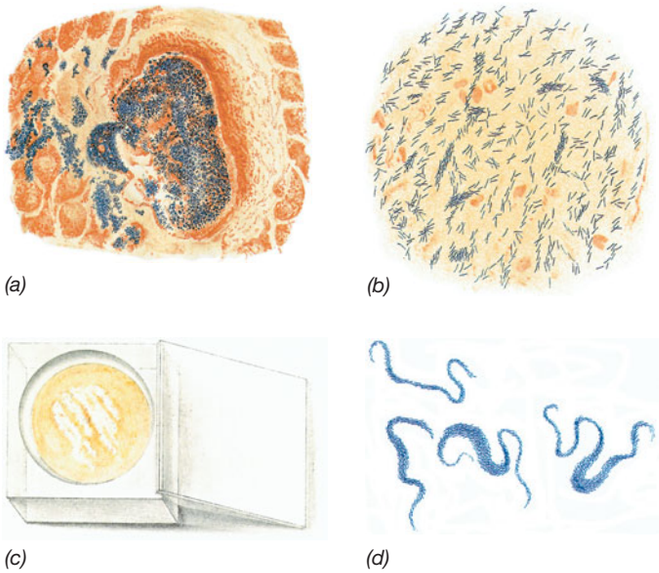


Figure 1.20 Robert Koch's drawings of *Mycobacterium tuberculosis.* *(a)* Section through infected lung tissue showing cells of *M. tuberculosis* (blue). *(b) M. tuberculosis* cells in a sputum sample from a tubercular patient. *(c)* Growth of *M. tuberculosis* on a glass plate of coagulated blood serum stored inside a glass box to prevent contamination. *(d) M. tuberculosis* cells taken from the plate in part c and observed microscopically; cells appear as long cordlike forms. Original drawings from Koch, R. 1884. "Die Aetiologie der Tuberkulose." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte* 2:1–88.

Tuberculosis: The Ultimate Test of Koch's Postulates

Koch's crowning accomplishment in medical bacteriology was his discovery of the causative agent of tuberculosis. At the time Koch began this work (1881), one-seventh of all reported human deaths were caused by tuberculosis (Figure 1.8). There was a strong suspicion that tuberculosis was a contagious disease, but the suspected agent had never been seen, either in diseased tissues or in culture. Koch was determined to demonstrate the cause of tuberculosis, and to this end he brought together all of the methods he had so carefully developed in his previous studies with anthrax: microscopy, staining, pure culture isolation, and an animal model system.

As is now well known, the bacterium that causes tuberculosis, *Mycobacterium tuberculosis,* is very difficult to stain because of the large amounts of a waxy lipid present in its cell wall. But Koch devised a staining procedure for *M. tuberculosis* cells in tissue samples; using this method, he observed blue, rod-shaped cells of *M. tuberculosis* in tubercular tissues but not in healthy tissues (**Figure 1.20**). However, from his previous work on anthrax, Koch realized that he must *culture* this organism in order to prove that it was the cause of tuberculosis.

Obtaining cultures of *M. tuberculosis* was not easy, but eventually Koch was successful in growing colonies of this organism on a medium containing coagulated blood serum. Later he used agar, which had just been introduced as a solidifying agent (see the Microbial Sidebar). Under the best of conditions, *M. tuberculosis* grows slowly in culture, but Koch's persistence and patience eventually led to pure cultures of this organism from human and animal sources.

From this point it was relatively easy for Koch to use his postulates (Figure 1.19) to obtain definitive proof that the organism he had isolated was the cause of the disease tuberculosis. Guinea pigs can be readily infected with *M. tuberculosis* and eventually succumb to systemic tuberculosis. Koch showed that diseased guinea pigs contained masses of *M. tuberculosis* cells in their lungs and that pure cultures obtained from such animals transmitted the disease to uninfected animals. Thus, Koch successfully satisfied all four of his postulates, and the cause of tuberculosis was understood. Koch announced his discovery of the cause of tuberculosis in 1882 and published a paper on the subject in 1884 in which his postulates are most clearly stated. For his contributions on tuberculosis, Robert Koch was awarded the 1905 Nobel Prize for Physiology or Medicine. Koch had many other triumphs in medicine, including discovering the organism responsible for the disease cholera and developing methods to diagnose exposure to *M. tuberculosis* (the tuberculin test).

Koch's Postulates Today

For human diseases in which an animal model is available, it is relatively easy to use Koch's postulates. In modern clinical medicine, however, this is not always so easy. For instance, the causative agents of several human diseases do not cause disease in any known experimental animals. These include many of the diseases associated with bacteria that live only *within* cells, such as the rickettsias and chlamydias, and diseases caused by some viruses and protozoan parasites. So for most of these diseases cause and effect cannot be unequivocally proven. However, the clinical and epidemiological (disease tracking) evidence for virtually every infectious disease of humans lends all but certain proof of the specific cause of the disease. Thus, although Koch's postulates remain the "gold standard" in medical microbiology, it has been impossible to satisfy all of his postulates for every human infectious disease.

MiniQuiz

- How do Koch's postulates ensure that cause and effect of a given disease are clearly differentiated?
- What advantages do solid media offer for the isolation of microorganisms?
- What is a pure culture?

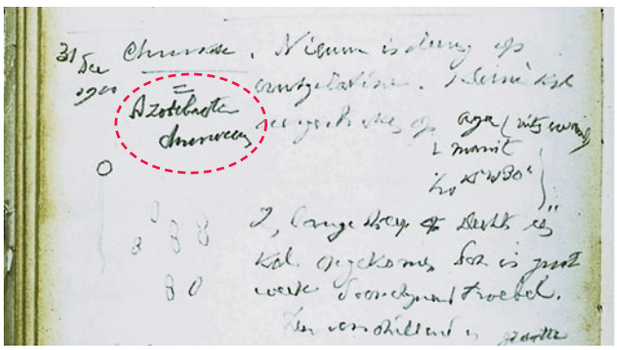
1.9 The Rise of Microbial Diversity

As microbiology moved into the twentieth century, its initial focus on basic principles, methods, and medical aspects broadened to include studies of the microbial diversity of soil and water and the metabolic processes that organisms in these habitats carried out. Two giants of this era included the Dutchman Martinus Beijerinck and the Russian Sergei Winogradsky.

Martinus Beijerinck and the Enrichment Culture Technique

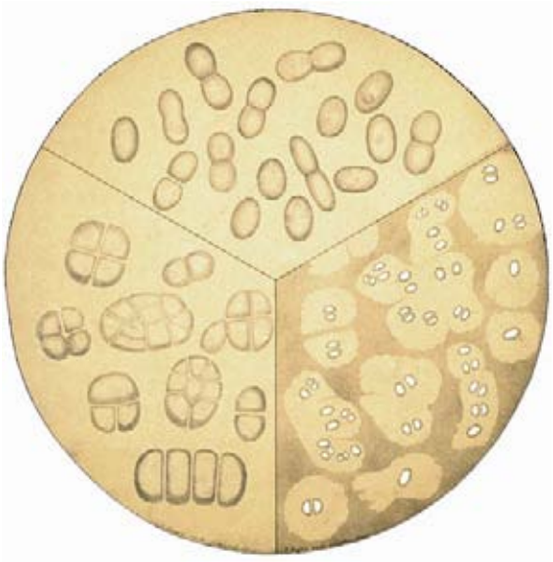
Martinus Beijerinck (1851–1931), a professor at the Delft Polytechnic School in Holland, was originally trained in botany, so he began his career in microbiology studying plants. Beijerinck's greatest contribution to the field of microbiology was his clear formulation of the **enrichment culture technique**. In enrichment cultures microorganisms are isolated from natural samples using highly selective techniques of adjusting nutrient and incubation conditions to favor a particular metabolic group of organisms. Beijerinck's skill with the enrichment method was readily apparent when, following Winogradsky's discovery of the process of nitrogen fixation, he isolated the aerobic nitrogen-fixing bacterium *Azotobacter* from soil (**Figure 1.21**).

Using the enrichment culture technique, Beijerinck isolated the first pure cultures of many soil and aquatic microorganisms,



Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

(a)



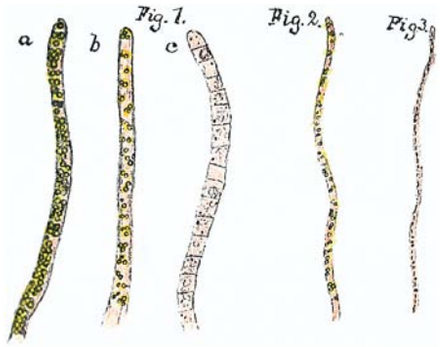
Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

(b)

Figure 1.21 **Martinus Beijerinck and *Azotobacter*.** *(a)* A page from the laboratory notebook of M. Beijerinck dated 31 December 1900 describing the aerobic nitrogen-fixing bacterium *Azotobacter chroococcum* (name circled in red). Compare Beijerinck's drawings of pairs of *A. chroococcum* cells with the photomicrograph of cells of *Azotobacter* in Figure 17.18*a*. *(b)* A painting by M. Beijerinck's sister, Henriëtte Beijerinck, showing cells of *Azotobacter chroococcum*. Beijerinck used such paintings to illustrate his lectures.

From *Microbiologie du Sol*, used with permission

(a)



From Winogradsky, S. 1949. *Microbiologie du Sol*. Masson, Paris.

(b)

Figure 1.22 **Sulfur bacteria.** The original drawings were made by Sergei Winogradsky in the late 1880s and then copied and hand-colored by his wife Hèlène. *(a)* Purple sulfur phototrophic bacteria. Figures 3 and 4 show cells of *Chromatium okenii* (compare with photomicrographs of *C. okenii* in Figures 1.5*a* and 1.7*a*). *(b) Beggiatoa*, a sulfur chemolithotroph (compare with Figure 1.14).

including sulfate-reducing and sulfur-oxidizing bacteria, nitrogen-fixing root nodule bacteria (Figure 1.9), *Lactobacillus* species, green algae, various anaerobic bacteria, and many others. In his studies of tobacco mosaic disease, Beijerinck used selective filtering techniques to show that the infectious agent (a virus) was smaller than a bacterium and that it somehow became incorporated into cells of the living host plant. In this insightful work, Beijerinck not only described the first virus, but also the basic principles of virology, which we present in Chapters 9 and 21.

Sergei Winogradsky, Chemolithotrophy, and Nitrogen Fixation

Sergei Winogradsky (1856–1953) had interests similar to Beijerinck's—the diversity of bacteria in soils and waters—and was highly successful in isolating several key bacteria from natural samples. Winogradsky was particularly interested in bacteria that cycle nitrogen and sulfur compounds, such as the nitrifying bacteria and the sulfur bacteria (**Figure 1.22**). He showed that these bacteria catalyze specific chemical transformations in nature and

proposed the important concept of **chemolithotrophy**, the oxidation of *inorganic* compounds to yield energy. Winogradsky further showed that these organisms, which he called *chemolithotrophs*, obtained their carbon from CO_2. Winogradsky thus revealed that, like phototrophic organisms, chemolithotrophic bacteria were *autotrophs*.

Winogradsky performed the first isolation of a nitrogen-fixing bacterium, the anaerobe *Clostridium pasteurianum,* and as just mentioned, Beijerinck used this discovery to guide his isolation of aerobic nitrogen-fixing bacteria years later (Figure 1.21). Winogradsky lived to be almost 100, publishing many scientific papers and a major monograph, *Microbiologie du Sol* (*Soil Microbiology*). This work, a milestone in microbiology, contains drawings of many of the organisms Winogradsky studied during his lengthy career (Figure 1.22).

MiniQuiz

- What is meant by the term "enrichment culture"?
- What is meant by the term "chemolithotrophy"? In what way are chemolithotrophs like plants?

1.10 The Modern Era of Microbiology

In the twentieth century, the field of microbiology developed rapidly in two different yet complementary directions—*applied* and *basic*. During this period a host of new laboratory tools became available, and the science of microbiology began to mature and spawn new subdisciplines. Few of these subdisciplines were purely applied or purely basic. Instead, most had both discovery (basic) and problem-solving (applied) components. **Table 1.3** summarizes these major subdisciplines of microbiology that arose in the twentieth century.

Several microbiologists are remembered for their key contributions during this period. In the early twentieth century many remained focused on medical aspects of microbiology, and even today, many dedicated microbiologists grapple with the impacts of microorganisms on human, animal, and plant disease. But following World War II, an exciting new emphasis began to take hold with studies of the genetic properties of microorganisms. From roots in microbial genetics has emerged "modern biology," driven by molecular biology, genetic engineering, and genomics. This molecular approach has revolutionized scientific thinking in the life sciences and has driven experimental approaches to the most compelling problems in biology. Some key Nobel laureates and their contributions to the molecular era of microbiology are listed in **Table 1.4**.

Many of the advances in microbiology today are fueled by the genomics revolution; that is, we are clearly in the era of "molecular microbiology." Rapid progress in DNA sequencing technology and improved computational power have yielded huge amounts of genomic information that have supported major advances in medicine, agriculture, biotechnology, and microbial ecology. For example, to obtain the sequence of the entire genome of a bacterium takes only a few hours (although sequence analysis is a much more time-consuming process). The fast-paced field of genomics has itself spawned highly focused new subdisciplines, such as *transcriptomics, proteomics,* and *metabolomics,* which explore, respectively, the patterns of RNA, protein, and metabolic expression in cells. The concepts of genomics, transcriptomics, proteomics, and metabolomics are all developed in Chapter 12.

All signs point to a continued maturation of molecular microbiology as we enter a period where technology is almost ahead of our ability to formulate exciting scientific questions. In fact, microbial research today is very close to defining the *minimalist genome*—the minimum complement of genes necessary for a living cell. When such a genetic blueprint is available, microbiologists should be able to define, at least in biochemical terms, the prerequisites for life. When that day arrives, can the laboratory creation of an actual living cell from nonliving components, that is, spontaneous generation under controlled laboratory conditions, be far off? Almost certainly not. Stay tuned, as much exciting science is on the way!

Table 1.3 *The major subdisciplines of microbiology*

Subdiscipline	*Focus*
I. Basic emphases[a]	
Microbial physiology	Nutrition, metabolism
Microbial genetics	Genes, heredity, and genetic variation
Microbial biochemistry	Enzymes and chemical reactions in cells
Microbial systematics	Classification and nomenclature
Virology	Viruses and subviral particles
Molecular biology	Nucleic acids and protein
Microbial ecology	Microbial diversity and activity in natural habitats; biogeochemistry
II. Applied emphases[a]	
Medical microbiology	Infectious disease
Immunology	Immune systems
Agricultural/soil microbiology	Microbial diversity and processes in soil
Industrial microbiology	Large-scale production of antibiotics, alcohol, and other chemicals
Biotechnology	Production of human proteins by genetically engineered microorganisms
Aquatic microbiology	Microbial processes in waters and wastewaters, drinking water safety

[a]None of these subdisciplines are devoted entirely to basic science or applied science. However, the subdisciplines listed in I tend to be more focused on discovery and those in II more focused on solving specific problems or synthesizing commercial products from microbial sources.

MiniQuiz

- For each of the following topics, name the subdiscipline of microbiology that focuses on it: metabolism, enzymology, nucleic acid and protein synthesis, microorganisms and their natural environments, microbial classification, inheritance of characteristics.

Table 1.4 *Some Nobel laureates in the era of molecular microbiology*[a]

Investigator(s)	*Nationality*	*Discovery/Year*[b]
George Beadle, Edward Tatum	American	One gene–one enzyme hypothesis/1941
Max Delbrück, Salvador Luria	German/Italian	Inheritance of characteristics in bacteria/1943
Joshua Lederberg	American	Conjugation and transduction in bacteria/1946/1952
James Watson, Francis Crick, Maurice Wilkins	American/British	Structure of DNA/1953
François Jacob, Jacques Monod, Andre Lwoff	French	Gene regulation by repressor proteins, operon concept/1959
Sydney Brenner	British	Messenger RNA, ribosomes as site of protein synthesis/1961
Marshall Nirenberg, Robert Holley, H. Gobind Khorana	American/Indian	Genetic code/1966
Howard Temin, David Baltimore, and Renato Dulbecco	American/Italian	Retroviruses and reverse transcriptase/1969
Hamilton Smith, Daniel Nathans, Werner Arber	American/Swiss	Restriction enzymes/1970
J. Michael Bishop, Harold Varmus	American	Cancer genes (oncogenes) in retroviruses/1972
Paul Berg	American	Recombinant DNA technology/1973
Roger Kornberg	American	Mechanism of transcription in eukaryotes/1974
Fred Sanger	British	Structure and sequencing of proteins, DNA sequencing 1958/1977
Carl Woese[c]	American	Discovery of *Archaea*/1977
Stanley Prusiner	American	Discovery and characterization of prions/1981
Sidney Altman, Thomas Cech	American	Catalytic properties of RNA/1981
Barry Marshall, Robin Warren	Australian	*Helicobacter pylori* as cause of peptic ulcers/1982
Luc Montagnier, Françoise Barré-Sinoussi, Harald zur Hausen	French/German	Discovery of human immunodeficiency virus as cause of AIDS/1983
Kary Mullis	American	Polymerase chain reaction/1985
Andrew Fire, Craig Mello	American	RNA interference/1998

[a]This select list covers major accomplishments since 1941. In virtually every case, the laureates listed had important coworkers that did not receive the Nobel Prize.
[b]Year indicates the year in which the discovery awarded with the Nobel Prize was published.
[c]Recipient of the 2003 Crafoord Prize in Biosciences, equivalent in scientific stature to the Nobel Prize.

Big Ideas

1.1
Microorganisms, which include all single-celled microscopic organisms and the viruses, are essential for the well-being of the planet and its plants and animals.

1.2
Metabolism, growth, and evolution are necessary properties of living systems. Cells must coordinate energy production and consumption with the flow of genetic information during cellular events leading up to cell division.

1.3
Microorganisms exist in nature in populations that interact with other populations in microbial communities. The activities of microorganisms in microbial communities can greatly affect and rapidly change the chemical and physical properties of their habitats.

1.4
Diverse microbial populations were widespread on Earth for billions of years before higher organisms appeared, and cyanobacteria in particular were important because they oxygenated the atmosphere. The cumulative microbial biomass on Earth exceeds that of higher organisms, and most microorganisms reside in the deep subsurface. *Bacteria*, *Archaea*, and *Eukarya* are the major phylogenetic lineages of cells.

1.5
Microorganisms can be both beneficial and harmful to humans, although many more microorganisms are beneficial or even essential than are harmful.

1.6
Robert Hooke was the first to describe microorganisms, and Antoni van Leeuwenhoek was the first to describe bacteria. Ferdinand Cohn founded the field of bacteriology and discovered bacterial endospores.

1.7
Louis Pasteur is best remembered for his ingenious experiments showing that living organisms do not arise spontaneously from nonliving matter. He developed many concepts and techniques central to the science of microbiology, including sterilization.

1.8
Robert Koch developed a set of criteria anchored in experimentation—Koch's postulates—for the study of infectious diseases and developed the first methods for growth of pure cultures of microorganisms.

1.9
Beijerinck and Winogradsky studied bacteria that inhabit soil and water. Out of their work came the enrichment culture technique and the concepts of chemolithotrophy and nitrogen fixation.

1.10
In the middle to latter part of the twentieth century, basic and applied subdisciplines of microbiology emerged; these have led to the current era of molecular microbiology.

Review of Key Terms

Cell the fundamental unit of living matter
Chemolithotrophy a form of metabolism in which energy is generated from inorganic compounds
Communication interactions between cells using chemical signals
Differentiation modification of cellular components to form a new structure, such as a spore
Ecosystem organisms plus their nonliving environment
Enrichment culture technique a method for isolating specific microorganisms from nature using specific culture media and incubation conditions
Enzyme a protein (or in some cases an RNA) catalyst that functions to speed up chemical reactions
Evolution descent with modification leading to new forms or species
Genome an organism's full complement of genes
Genomics the identification and analysis of genomes
Growth in microbiology, an increase in cell number with time
Habitat the environment in which a microbial population resides
Koch's postulates a set of criteria for proving that a given microorganism causes a given disease
Metabolism all biochemical reactions in a cell
Microbial community two or more populations of cells that coexist and interact in a habitat
Microbial ecology the study of microorganisms in their natural environments
Microorganism a microscopic organism consisting of a single cell or cell cluster or a virus
Motility the movement of cells by some form of self-propulsion
Pathogen a disease-causing microorganism
Pure culture a culture containing a single kind of microorganism
Spontaneous generation the hypothesis that living organisms can originate from nonliving matter
Sterile free of all living organisms (cells) and viruses

Review Questions

1. List six key properties associated with the living state. Which of these are characteristics of all cells? Which are characteristics of only some types of cells (Sections 1.1 and 1.2)?
2. Cells can be thought of as both catalysts and genetic entities. Explain how these two attributes of a cell differ (Section 1.2).
3. What is an ecosystem? What effects can microorganisms have on their ecosystems (Section 1.3)?
4. Why did the evolution of cyanobacteria change Earth forever (Section 1.4)?
5. How would you convince a friend that microorganisms are much more than just agents of disease (Section 1.5)?
6. For what contributions are Hooke, van Leeuwenhoek, and Ferdinand Cohn most remembered in microbiology (Section 1.6)?
7. Explain the principle behind the use of the Pasteur flask in studies on spontaneous generation (Section 1.7).
8. What is a pure culture and how can one be obtained? Why was knowledge of how to obtain a pure culture important for development of the science of microbiology (Section 1.8)?
9. What are Koch's postulates and how did they influence the development of microbiology? Why are Koch's postulates still relevant today (Section 1.8)?
10. In contrast to those of Robert Koch, what were the major microbiological interests of Martinus Beijerinck and Sergei Winogradsky (Section 1.9)?
11. Select one major subdiscipline of microbiology from each of the two major categories of Table 1.3. Why do you think the subdiscipline is "basic" or "applied" (Section 1.10)?