CHAPTER 1 Microorganisms and Microbiology

Summary

Chapter 1 introduces the study of microbiology for students, most of whom will have had little or no exposure to the subject. Consequently, you have a good opportunity to provide an overview of microbiology that demonstrates the important roles microorganisms play in human activities and in the ecology of the entire biosphere. The ability of microorganisms to exist independently in nature as free-living cells (Figure 1.1) confers enormous adaptive advantages over cells of macroorganisms, which are incapable of an independent existence.

1.1 | What Is Microbiology About and Why Is It Important?

Describe to students ways in which microbiology serves as both a basic and applied biological science. From a basic perspective, the study of microorganisms has been the primary means by which the fundamental genetic and biochemical properties of living cells have been revealed. From an applied perspective, microorganisms directly affect the quality of human life in both detrimental and beneficial ways. Although microorganisms are the causative agents of some of the most important human, animal, and plant diseases, they are also used for the industrial production of antibiotics, pharmaceuticals, and foods. Microorganisms are also increasingly being used for beneficial purposes as diverse as bioremediation of polluted sites, gene therapies for genetic diseases, and the production of biofuels. Microbiology is therefore a science of far-reaching scope, with applications that affect the quality of human life in a variety of ways.

You should also emphasize to students the importance of microorganisms in the emergence and maintenance of higher forms of life. From the production of molecular oxygen (by cyanobacteria) to the biogeochemical cycling of key elements, such as carbon, nitrogen, and sulfur, microorganisms play a major role in sustaining all life on the planet. Point out in your course introduction that for all the reasons summarized in this section, microbiology is the foundation of all biological sciences.

1.2 | Structure and Activities of Microbial Cells

Because microorganisms generally exist as free-living cells, it is important to discuss the characteristics of cells in general. Emphasize that all cells exhibit a nonrandom organization with a semipermeable membrane boundary that encompasses an internal system that is not in equilibrium with its environment. Point out that prokaryotic cells (i.e., all *Bacteria* and *Archaea*) do not contain membrane-bound, internal organelles as traditionally described for eukaryotic cells (the *Eukarya*; Figure 1.2). In particular, the organization of prokaryotic DNA as a *nucleoid*, an aggregated mass of genetic material within the cytoplasm, is in stark contrast to the compartmentalized, multichromosomal configuration typically found in eukaryotes. However, despite the structural and morphological similarities of *Bacteria* and *Archaea*, make sure the students are aware early on that these groups of microorganisms have quite distinct evolutionary lineages and are, therefore, not closely related on a genetic level. This concept is discussed in more detail in Section 1.3.

The ability of cells to maintain a thermodynamic energy flow far from equilibrium defines what we refer to as a *living system*. All living systems display some form of metabolism in which both energy-yielding (catabolic) and energy-consuming (anabolic) biochemical reactions are catalyzed simultaneously. These chemical transformations allow for biosynthesis of new cell structures and, ultimately, cell division (microbial *growth*). Figure 1.3 shows the characteristics that define cellular life, some of which are universal (e.g., metabolism and evolution) and some of which occur only in some cells (e.g., differentiation and motility).

1.3 | Evolution and Diversity of Microbial Cells

Although Earth is believed to be about 4.6 billion years old, several lines of evidence indicate that the appearance of multicellular life did not occur until about 600 million years ago (Figure 1.4). By contrast, microbial cells (single-celled prokaryotes) likely appeared between 3.8 and 3.9 billion years ago and therefore were the *only* inhabitants of the planet for about 80 percent of its history! The appearance of multicellular life was necessarily preceded by the accumulation of significant amounts of molecular oxygen (O_2) in the atmosphere, a phenomenon that resulted from the activity of cyanobacteria and the evolution of a chlorophyll molecule that could use H_2O as electron donor (see Chapter 13). Develop this concept for students in some detail at an early stage in the course to provide proper historical perspective of important events in cellular evolution. You may wish to start with the evidence supporting the hypothesis that the first self-replicating "entities" may have been RNA molecules, and the incorporation of these entities within lipid membranes may have represented the first cell type(s). This topic will be more thoroughly discussed in Chapter 12.

- Discuss with your students some of the events that led to "higher" forms of life. The evolution of anaerobic phototrophic metabolisms likely began within 500 million years after the appearance of the first cells, followed nearly a billion years later by the evolution of cyanobacteria from these early phototrophs (Figure 1.5), thus starting the slow process of oxygenating the atmosphere and the evolutionary path to multicellularity.
- Also discuss the methods now routinely used in the development and refinement of the phylogenetic tree of life (Figure 1.6a). Introduce to your students the enormous impact ribosomal RNA gene sequencing has had on our understanding of microbial diversity and on the classification of living organisms into three distinct domains (Figure 1.6b).

1.4 | Microorganisms and Their Environments

The traditional approach to teaching students about microorganisms is from a pure culture perspective (e.g., a bacterial species and its effect on a host). It is more appropriate to introduce microorganisms as populations of cells occupying a particular habitat in which communities of different populations interact in complex ways. These interactions affect both the habitat and its inhabitants, and they have a profound impact on the larger, multicellular inhabitants of an ecosystem. In fact, the very existence of multicellular life depends upon the nutrient cycling activities of microorganisms. Point out to students that pure cultures of microorganisms almost never occur in nature. Many students may have more interest in ecology than in what appears to them a more abstract area, such as microbiology. Therefore, stress the fact that the biochemical conversions and molecular interactions of microorganisms significantly control the balance and community structure of all living organisms in an ecosystem.

Students will likely be unaware of the abundance and ubiquitous nature of microbial life. Microorganisms can be found in even the most inhospitable environments by human standards, and students are often amazed at the extremes in which microorganisms can thrive (Table 1.1). Point out the fact that the total amount of carbon, nitrogen, and phosphorus in microbial cells exceeds that in all plant and animal biomass. Microbial cells represent the major reservoirs of life's essential nutrients.

In this connection, prokaryotes comprise the major portion of Earth's total biomass. Most of this biomass consists of marine and terrestrial subsurface microbial populations (Table 1.2). These communities contain a reservoir of genetic diversity that is largely unexplored and that could benefit humankind in as yet unknown ways. The effect of microorganisms on animals, plants, and global ecosystems is a topic interwoven throughout a number of chapters in this text. These concepts will come as a surprise for most students—who generally consider microorganisms "simple," insignificant creatures that only cause disease—and will set the stage for more detailed study in future chapters.

1.5 | The Impact of Microorganisms on Humans

Students probably understand the importance of microorganisms as agents of infectious disease, and indeed, this has been a driving force in the development of microbiology as a distinct scientific discipline. The effects of microorganisms upon human affairs have been remarkable, particularly when one compares the foremost causes of death in the United States at the beginning of the twentieth century (before the widespread use of antibiotics and vaccines) with the major causes of death in this century (Figure 1.8). While the deleterious effects of pathogenic bacteria to human wellness should be discussed here, it is important to also stress the key beneficial roles microorganisms play in agriculture (Figure 1.9), in human digestive health (Figure 1.10), in the food and beverage industries (Figure 1.11), and in the growing fields of biotechnology and alternative and renewable energy (Figure 1.12). With this discussion, students should become aware of the tremendous influence of microorganisms in human society.

The genomes of microorganisms hold incredible potential for the production of commercial products. The traditional methods of employing microorganisms for human use (e.g., fermentation) have evolved into what students now recognize as "biotech"—the biotechnology industry. The industry arose from the development by microbiologists of tools for genetically manipulating the genome of a bacterial cell to produce a desired product. An excellent example is the production of human insulin by *Escherichia coli*, an innovation that made the treatment of diabetes affordable for people from all economic backgrounds.

In addition to producing a product directly, bacterial cells can be used as a vehicle to introduce specific genes into the genomes of other organisms for commercial production of a desired product. For example, genetic engineering of *Agrobacterium tumefaciens*, a plant pathogen that naturally transfers DNA directly into plant cells upon infection, has led to the use of plants for the production of human antibodies with potential anticancer or antiviral properties.

1.6 | The Discovery of Microorganisms

It is important to emphasize the historical roots of microbiology, particularly considering its rather recent beginnings as a systematic field of science in the nineteenth century and the fact that several new fields have arisen from this discipline (e.g., molecular biology and biotechnology; see Table 1.3). Lead the students on a chronological journey, beginning with Robert Hooke's first description of a microorganism (a fungal mold) in 1665 (Figure 1.13) and Antoni van Leeuwenhoek's microscope and publication of the first observation of bacteria in 1684 (Figure 1.14). Perhaps the most important early figure in general microbiology is Ferdinand Cohn (a contemporary of Louis Pasteur and Robert Koch) who is widely regarded as the founder of bacteriology. Several important events followed the early observations of van Leeuwenhoek, a few of which are listed below:

- Nineteenth-century scientists tested the issues of spontaneous generation and the nature of infectious disease, leading to the genesis of the field of medical microbiology.
- Improvements in microscopy allowed for Cohn's observations of bacteria, which included a preliminary characterization of the sulfur-oxidizing species *Beggiatoa mirabilis* (Figure 1.15) and his discovery of heat-resistant endospores in the genus *Bacillus.* Cohn discovered that boiling killed the vegetative form of *Bacillus* but not the endospore stage.
- Cohn laid the foundation for classification of bacteria and designed the first methods for preventing contamination of culture media; these methods were later adopted and improved upon by German physician Robert Koch.

1.7 | Pasteur and Spontaneous Generation

As a young researcher, Louis Pasteur was formally trained as a chemist with expertise in the field of crystallography. His knowledge of the optical properties of crystals provided the basis for his early investigations into the biological sciences. Begin the discussion of his celebrated scientific achievements by pointing out that it was his observation that living organisms discriminate between optical isomers of metabolites during growth (Figure 1.16) that led him to begin a series of experiments that would result in the defeat of the centuries old theory of spontaneous generation. Pasteur's experimental design that finally disproved this theory introduced the now famous swan-necked flask (Figure 1.17). His work is a classic example of the effectiveness of using the scientific method and controlled experimentation to resolve questions rather than personal observation and interpretation, which often lead to unsubstantiated (and often erroneous) conclusions. Point out to students that disproving spontaneous generation resulted in the development of effective procedures for controlling microbial growth, including the familiar process of pasteurization. Outline the major career accomplishments of Pasteur, pointing out the fact that although his professional training pertained to chemistry rather than biology, his laboratory experimentation led him to the study of microorganisms.

- As a chemist, Pasteur's work with optical isomers of amyl alcohol, a byproduct of alcohol fermentation by beets, led him to confirm that fermentation was not a strictly chemical phenomenon but that living yeast cells were required for the process. The outcome of his fermentation studies led him to the classic experiments that defeated spontaneous generation (Figure 1.17).

- Discuss the many other accomplishments of Pasteur, such as the development of vaccines for anthrax, fowl cholera, and rabies. The dramatic results of his rabies vaccine, which saved a young boy, startled the scientific world and led the French government to build the Pasteur Institute in Paris in 1888 (Figure 1.18). Together with Robert Koch, whose scientific achievements are discussed next, Pasteur played a major role in establishing the field of medical microbiology.

1.8 | Koch, Infectious Disease, and Pure Cultures

Explain to your students that for centuries many thought that some unknown agent from a diseased person could be transmitted to healthy people, but no real proof of this existed until the work of Robert Koch (Figure 1.19). Although Koch was 21 years younger than Pasteur, several of their greatest achievements were carried out around the same time during the late 1800s. The following list serves as an introduction to the works of Koch for your students.

- Koch's work on the germ theory of disease (1876) was perhaps the most important stimulus for the development of medical microbiology. Given the popular focus on bioterrorism in recent years, students may find it interesting that Koch's work leading to this important theory involved the study of the endospore-forming bacterium *Bacillus anthracis*, the causative agent of anthrax. Carefully discuss Koch's postulates, the use of which has since led to discoveries for the prevention and cure of many infectious diseases (Figure 1.20). However, be sure to note that while Koch's postulates are the "gold standard" for determining the causative agent of a disease, it is not always possible to carry them out in full, especially considering that many pathogenic bacteria resist laboratory culture. It is in these cases that genomics is proving useful in linking a causative agent to a particular disease in the absence of cultivable microorganisms (see feature box *Explore the Microbial World: The Black Death Decoded*).
- Koch's postulates require that the suspected organism be obtained in pure culture. Koch developed clever ways of obtaining pure cultures, including the use of solidifying agents such as gelatin and agar to obtain single colonies. Point out to students that a colony is a population of cells arising from a single cell, and thus each colony contains a single type of microorganism, an observation that Koch himself was quick to realize.
- Ferdinand Cohn had also been active in his research during this time, and he and Koch regarded each other's work with mutual respect. Several of Cohn's innovations for preventing contamination of pure cultures were adopted by Koch and his associate Walter Hesse (Figure 1.21), thereby aiding many of Koch's studies of pathogenic bacteria.
- Koch was the first to isolate *Mycobacterium tuberculosis* and demonstrate that it was the causative agent of tuberculosis (Figure 1.22). Considering the huge number of individuals that succumbed to this disease during Koch's time (about 15% of all reported deaths), the significance of this achievement could hardly be exaggerated. In addition to this, Koch also discovered and characterized *Vibrio cholera*, the causative agent of the severe diarrheal disease cholera

1.9 | The Rise of Microbial Diversity

Continue your discussion of the historical roots of microbiology by introducing the work of Martinus Beijerinck and Sergei Winogradsky, both of whom are known for their study of microbial diversity. In the same way that Pasteur and Koch propelled the field of medical microbiology to the scientific mainstream, Beijerinck and Winogradsky became the chief figures of environmental microbiology. Both of these researchers were interested in the biogeochemical cycling of nitrogen and sulfur in the environment, including the reduction of atmospheric nitrogen to ammonia by nitrogen-fixing bacteria (Figure. 1.23). In addition, Beijerinck was the first to describe the existence of subbacterial infectious agents of disease (viruses) through his study of tobacco mosaic disease.

- *Martinus Beijerinck*, a Dutchman, developed the concept of *enrichment culture technique* for the selective culture of specific types of microorganisms from natural samples. The use of selective culture media and incubation conditions was a major innovation for the isolation of novel strains of microorganisms, and the technique is still used extensively today. For example, enrichment cultures are routinely used to isolate novel bacteria for industrial and medical use, such as antibiotic-producing microorganisms from soil (see Chapter 15). It is important to describe the principle of this important technique to students, since this concept will be especially relevant for certain laboratory exercises that usually accompany courses in general microbiology.
- *Sergei Winogradsky* of Russia used enrichment culture to study bacteria in soil, particularly those that play a role in the cycling of nitrogen and sulfur (Figure 1.22). He isolated the first N_2-fixing bacterium, *Clostridium pasteurianum*, and developed the concept of bacterial fixation—an important ecological discovery. Winogradsky was also interested in bacteria that *oxidize* inorganic compounds for energy conservation, including the nitrifying and sulfur-oxidizing bacteria, and he showed that these organisms are autotrophic, using CO_2 as their sole source of carbon. Winogradsky was perhaps the first scientist to recognize the *metabolic significance* of biogeochemical processes. Briefly discuss Winogradsky's major discoveries: chemolithotrophy and nitrogen fixation.

1.10 | Modern Microbiology and Genomics

Conclude your discussion of the history of microbiology by summarizing the major subdisciplines that have arisen from the basic and applied directions that the discipline took in the twentieth century (Table 1.3). It is important to note that the distinction between *basic* and *applied* is not subdiscipline-specific in a strict sense, as might be implied in the table; it is defined by the questions being asked by the researcher.

- Basic: microbial systematics, microbial physiology, microbial biochemistry, virology, microbial genetics, microbial ecology, molecular biology, and genomics. With the use of molecular tools, microbial ecology has now become an important discipline for identifying novel molecules from microorganisms for biotechnological applications. These tools are allowing scientists to probe the immense and untapped genetic diversity of the most efficient bioengineers—microorganisms. The development of microbial genetics has given rise to the era of molecular biology, genomics, and biotechnology. This is an important point to emphasize to students, many of whom will work in the biotechnology industry and/or use the very products that have developed through discoveries that have come out of this field.

- Applied: immunology, medical microbiology, agricultural microbiology, industrial microbiology, aquatic microbiology, and biotechnology. The subdisciplines of applied microbiology arose directly from the works discussed in previous sections (Pasteur and Koch—medical microbiology and immunology; Beijerinck and Winogradsky—agricultural microbiology, aquatic microbiology, etc.).
- Molecular microbiology: Lastly, discuss the era of molecular microbiology that rapidly emerged during the 1970s, leading to the subdisciplines of *biotechnology*, *genomics, transcriptomics, proteomics*, and *metabolomics*. These topics will be discussed further in Chapter 6.

Answers to Review Questions

1. Microbiology is both a *basic* biological science (a science of discovery) and an *applied* biological science (a science of solving problems). Knowledge of the world around us is gained through basic research, and this knowledge can often be applied to resolve challenges that face our society or threaten human welfare.
2. The *Bacteria* and *Archaea* consist of prokaryotic, generally free-living cells. The *Eukarya* are eukaryotic cells (or multicellular organisms consisting of eukaryotic cells) that are generally significantly larger than prokaryotes and have a greater degree of complexity with a defined nucleus and membrane-bound organelles. Cells function as machines in a chemical sense, carrying out metabolic reactions essential for their integrity and survival. However, information from coding devices is also required so that metabolic events may be initiated or deferred at the appropriate time. Translation of the genetic information encoded in DNA requires ribosomes to coordinate the interaction of messenger RNA with the other components required to assemble the protein product, transfer RNA, and amino acids. Though catalytic and coding functions of cells differ (one specifies *what* can occur, the other specifies *when*), neither can function without the other within the confines of the cell boundary. Thus, order is maintained against a thermodynamically unfavorable gradient. A living cell must possess both.
3. The evolution of cyanobacteria had a profound effect on the biology of the planet, as it resulted in the oxygenation of the atmosphere. For the 2 billion years before this event, only single-celled organisms capable of anaerobic metabolisms inhabited the planet. The evolution of a metabolism that could transfer electrons extracted from reduced organic carbon molecules to O_2 as an electron acceptor (i.e., aerobic respiration) permitted the generation of much larger amounts of ATP (due to the highly electropositive reduction potential of O_2, described in Chapter 3) and the eventual evolution of the eukaryotic cell and multicellularity. Three domains of life exist today: the *Bacteria*, *Archaea*, and *Eukarya*. It is generally believed that all three domains are derived from a single ancestral cell, the *last universal common ancestor*.
4. An ecosystem consists of the biotic factors (living organisms) together with the abiotic factors (physical and chemical nonliving constituents) of a habitat in the environment. In nature, microorganisms almost never occur in pure culture; they occur as mixed populations composing a larger microbial community. The microorganisms in an ecosystem use nutrients from the environment and excrete waste products of their metabolisms into the

environment. The life processes of various microorganisms significantly contribute to primary production, decomposition, and nutrient cycling in an ecosystem.

5. Microorganisms carry out many important functions, with only a small percentage of bacteria (<1%) capable of causing disease. In fact, the human body has far more bacterial cells on and in it than human cells, and while a small fraction of these microorganisms are potentially pathogenic, the vast majority are commensal species essential for maintaining proper human health. In addition, some bacteria fix nitrogen (N_2) and are thus important in agriculture. Others are important in food and beverage production. Most microorganisms play crucial ecological roles in the environment.
6. Robert Hooke was the first to observe and describe microorganisms while studying the fruiting structures of molds (fungi). Antoni van Leeuwenhoek was the first person to observe bacteria using microscopes of his own design and build. Both researchers were active and accomplished their most influential work in the late 1600s.
7. Pasteur's experiments using swan-necked flasks silenced the objection of vitalists who claimed that sealing the mouth of a flask blocked the availability of open air, which they viewed as being essential for spontaneous generation (they were entirely unaware of the existence of anaerobes). Pasteur definitively showed that, even in open air, putrefaction of the heated broth did not occur unless the flask was tipped such that the broth came in contact with dust particles trapped in the neck of the flask, thus proving that the microorganisms that contaminated the broth were carried in the air.
8. A pure culture is a culture that consists of only one type of microorganism. The most common method of obtaining pure cultures is by the isolation of single colonies on solid growth media. Pure cultures may be used to determine the specific effect an organism has on its environment. For example, Koch and others used pure cultures to determine the causative agents for many diseases.
9. Koch's postulates are a set of criteria for proving that a specific type of microorganism causes a specific disease. The main points are as follows: (1) The suspected microorganism must be present in all cases of disease and not present in healthy individuals; (2) the organism must be cultivated from diseased individuals and isolated in pure culture; (3) inoculation of a healthy individual with organisms from the pure culture must initiate disease in susceptible animals; and (4) the organism should be re-isolated from these experimental animals in pure culture and be identified as the same infecting organism as that found in the original diseased animal. Investigators still use these postulates as a guide to determine the causative agents of diseases and for developing successful treatments for the prevention and cure of many infectious diseases.
10. While Robert Koch was an eminent early medical microbiologist, Beijerinck and Winogradsky were responsible for developing the field of microbial ecology as it relates to the environment rather than the human body. Their primary contributions to microbiology were in the design and implementation of the enrichment culture technique and the isolation and characterization of bacteria responsible for cycling nitrogen and sulfur, including those having chemolithoautotrophic metabolisms. Before Winogradsky's work, the use of CO_2 as the sole source of carbon by nonphotosynthetic organisms was unknown. Winogradsky isolated and described the first nitrogen-fixing bacterium, the anaerobe *Clostridium pasteurianum*. Later, Beijerinck described the first aerobic nitrogen-fixing bacterium, *Azotobacter chroococcum*, from a soil sample.

11. Subdisciplines with an emphasis in *basic* science are those that primarily pertain to the advancement of knowledge in the area of the fundamental processes of life. Areas such as biochemistry, physiology, genetics, virology, and microbial ecology are all related to the molecular biology of cells and therefore fall largely under this category. Subdisciplines with an emphasis in *applied* science are those directly linked to important practical problems that affect human welfare, and these include areas of microbiology related to medicine, agriculture, industry, technology, and sanitation.

Answers to Application Questions

1. Before Pasteur's experiments, and despite some evidence to the contrary, it was thought that microorganisms could arise spontaneously in or on the surface of a nutrient-containing medium and, by extension, in any environment. Pasteur showed that microorganisms must be carried into the environment from the outside. A medium that was initially sterile remained so until a living cell from outside was introduced. Pasteur's work provided some of the basic concepts for the development of aseptic technique, as well as methods of food preservation, such as canning and pasteurization. Moreover, with this development, it became possible to think of microorganisms as the causative agents of disease.

2. Koch used the following methodology, which became known as Koch's postulates, to determine the causative agent of a disease:

   a. The organism must be isolated from the diseased animal and grown in pure culture.

   b. The organism must not be present in healthy animals.

   c. When the microorganism is injected into a healthy animal, the disease must occur.

   d. The pathogenic agent must be re-isolated from the diseased animal.

   Koch succeeded in isolating the organism we now know as *Mycobacterium tuberculosis* from every animal that died from the disease (postulates *a, b*). He injected cells of a pure culture of *M. tuberculosis* into healthy guinea pigs, and these guinea pigs developed systemic tuberculosis (postulate *c*). These microorganisms were then re-isolated from the newly diseased animals and were shown to be *M. tuberculosis* (postulate *d*). Thus, all four criteria were met.

3. The sudden elimination of all microorganisms from Earth would result in the rapid disappearance of animal and plant life for a number of reasons. Nitrogen-fixing bacteria provide the reduced nitrogen source for the nucleic acids and proteins found in plants and animals, and cessation of that process alone would eliminate higher organisms. In addition, photosynthesis by cyanobacteria would cease, causing a huge deficiency in both primary production (the conversion of inorganic forms of carbon, such as CO_2, to organic forms that can be catabolized by animals) and the release of O_2 to the atmosphere via photosynthesis. By contrast, the elimination of plants and animals would affect only a small percentage of microorganisms. Prokaryotes carry out a variety of aerobic and anaerobic metabolisms and molecular interconversions that are independent of higher organisms; therefore, prokaryotic life would persist.