Chapter 2

Microorganisms Found in the Environment

Ian L. Pepper and Terry J. Gentry

Microorganisms other than viruses can be defined as free-living organisms that are so small that they cannot be seen with the naked eye. Generally, this size range is less than 100 μm, but defining microbes just in terms of size can be confusing since some microbes can be seen with the naked eye and are greater than 100 μm in size. Examples of larger microbes include some protozoa, and bacteria such as *Epulopiscium fishelsoni*. Mushrooms are certainly large enough to be seen with the naked eye, and yet are classified as fungi. Viruses also complicate the picture since, although they are certainly small (10−100 nm), they are not free-living and do not metabolize.

Despite these anomalies, microbes found in the environment are generally thought to consist of: Bacteria (including actinomycetes); Archaea; Fungi; Protozoa; Algae; and Viruses. Microbes indigenous to the environment, which includes soil, water and air, are characterized as being able to adapt to variable environmental conditions such as temperature, redox potential, pH, moisture regime and pressure. This differentiates them from microbes found within the human body which exist under much more constant conditions, and which normally do not survive when introduced into the environment. Microorganisms are also capable of existing under oligotrophic (low nutrient) conditions, essentially living under conditions of starvation. These characteristics allow microbes to be found in every habitat imaginable including deserts and jungles, and even under Arctic conditions.

In this chapter we introduce the different types of microbes found in the environment including their structural features and some of their major functions and impacts, not only on human health and welfare, but also on the environment. To put the importance of microbes into perspective, it is interesting to realize that they first appeared on Earth approximately 4 billion years ago, and have been critical to the formation of current global conditions, including the presence of free molecular oxygen which first appeared around 2.5 billion years ago. Additional information and background on these types of microbes can be found in textbooks in the recommended reading listed at the end of this chapter.

2.1 CLASSIFICATION OF ORGANISMS

Until the 1970s, classification of macro- and microorganisms was based primarily on physiological differences with anywhere from two to six major kingdoms proposed for categorizing life as we know it. However, in the 1970s,

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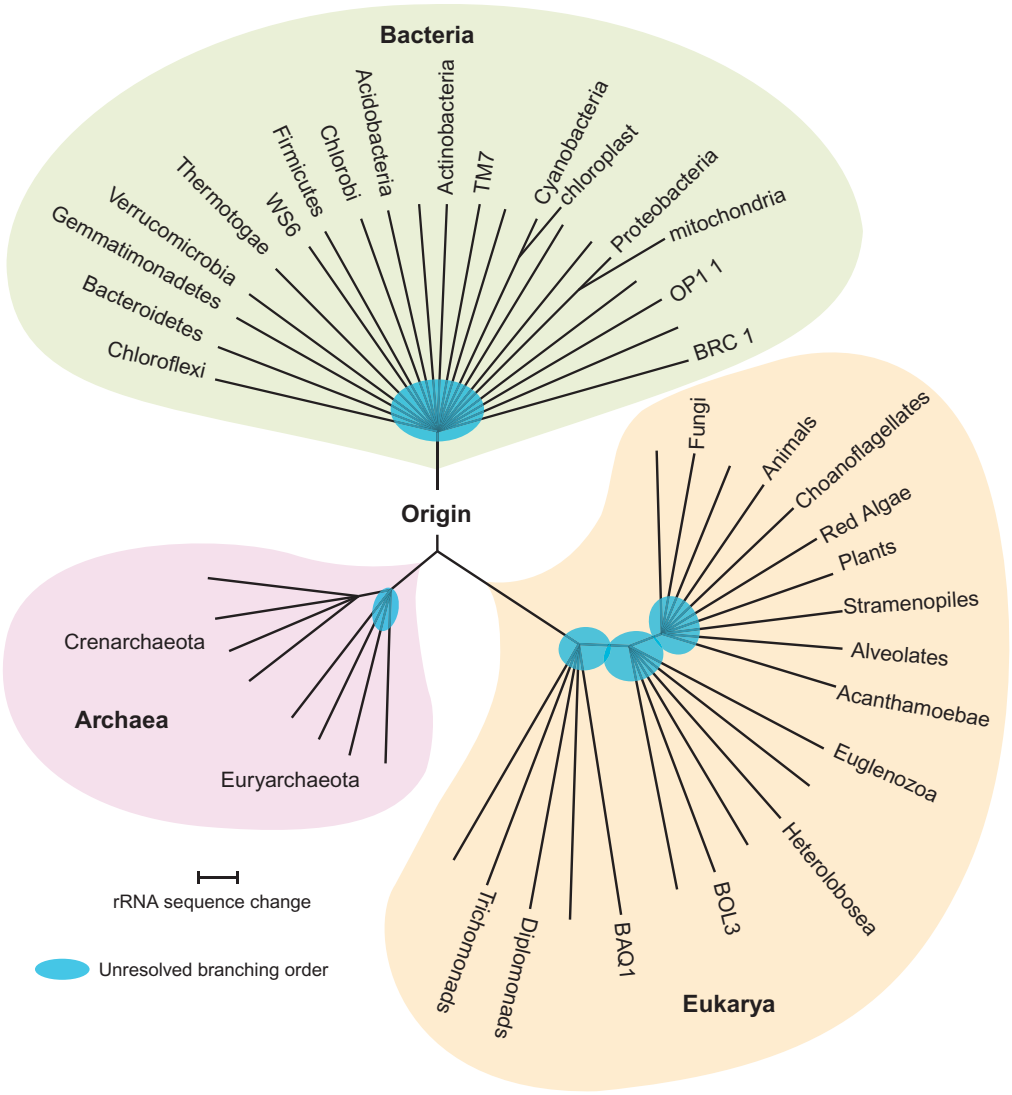

FIGURE 2.1 The three domain tree of life. Classification is based on the ribosomal RNA gene.

techniques became available to allow examination of nucleic acids, including ribosomal RNA (rRNA), which is a highly conserved structure used for synthesis of proteins in living things. Based on analysis of 16S rRNA, Carl Woese identified an entirely new group of organisms—the Archaea (Woese and Fox, 1977)—which eventually led to the modern classification of living organisms into a three domain system consisting of Archaea, Eukarya and Bacteria (Figure 2.1). Of these, the Bacteria and Archaea are termed prokaryotes, and the Eukarya are known as eukaryotes. Eukaryotic microbes other than algae and fungi are collectively called protists. Within the Eukarya are fungi, protozoa, algae, plants, animals and humans.

2.2 PROKARYOTES

Prokaryotes are the simplest of organisms and are characterized by the lack of a true nucleus and membrane-bound cell organelles, such as mitochondria or chloroplasts. The prokaryotes consist of two separate large groups, the Bacteria and the Archaea. The structural features of prokaryotes are shown in Information Box 2.1.

2.2.1 Bacteria

The bacteria are the least complex of the living microorganisms but offer the greatest metabolic flexibility and have the greatest diversity. They dominate numerous environmental processes critical not only to humans, but also to the environment (such as nitrogen fixation); however, they also include some of the most notorious human, animal and plant pathogens. It is estimated that there are more than 50 bacterial phyla based on the analysis of the conserved 16S rRNA sequence (Schloss and Handelsman, 2004). Approximately half of these phyla have not yet been cultured. Thus, we know relatively little about the majority of

Information Box 2.1 Prokaryotic Cell Structure and Function

Location	Structure	Function
Cytoplasm	Chromosome	Information storage and replication (DNA).
	Plasmid	Extrachromosomal DNA that often confers a competitive advantage to the cell, e.g., antibiotic resistance.
	Ribosomes	Protein synthesis (rRNA and protein).
Cell envelope	Cell membrane	Selectively permeable layer found in all bacteria that allows import and export of nutrients, toxins and waste products. Composed of a phospholipid bilayer with proteins that serve as ions channels, proton pumps and receptors.
	Cell wall	Rigid, permeable structure that confers shape and protection to cells.
	Periplasmic space	Involved in nutrient acquisition, electron transport, and alteration of substances toxic to the cell. Especially important in Gram-negative cells.
	Outer membrane	A second semipermeable membrane found only in Gram-negative cells. The outer leaflet of the outer membrane contains lipopolysaccharide (LPS) molecules.
	Lipopolysaccharide (LPS)	Found anchored into the outer leaflet of the outer membrane in Gram-negative cells. This negatively charged molecule helps mediate interactions of the cell with the environment. The molecule is an endotoxin and antigenic.
	Teichoic acids	Found anchored into the peptidoglycan wall of Gram-positive cells. This negatively charged molecule helps mediate interactions of the cell with the environment, e.g., adhesion. Teichoic acids are antigenic.
	S-Layer	Monomolecular protein layer on the exterior of cells that can provide protection against phage, act as a barrier to entry of high molecular weight molecules, help stabilize the cell, and act as an adhesion site for exoproteins. The S-layer is associated with the LPS in gram-negative bacteria, with peptidoglycans in Gram-positive bacteria, with the lipid membrane in Gram-negative Archaea, and with Pseudomurein or Methanochondroitin in Gram-positive Archaea.
Cell exterior	Glycocalyx	A heterogeneous layer of polysaccharides, protein, and DNA that encapsulates the cell and provides protection against predation and desiccation. A diffuse irregular layer is known as a slime layer and a more defined distinct layer is known as a capsule.
Appendages	Flagella	Long appendages that impart motility to a cell.
	Fimbriae or pili	Hollow fine protein structures that aid in adhesion to other cells and surfaces.

environmental bacteria, and the discussion that follows pertains to cells that have been successfully cultured.

Bacteria grown in the laboratory average 0.5−1 μm in diameter and 1−2 μm in length and have the basic composition shown in Table 2.1. They are generally characterized by high rates of replication (*Escherichia coli* can replicate by binary fission in less than 10 minutes), high surface area-to-volume ratio and genetic malleability. They have a single large circular chromosome located in the cytoplasm and there is no compartmentalization of the cell (Figure 2.2). The relative simplicity of the bacterial cell allows it to rapidly respond and adapt to changing environmental conditions.

Actinomycetes are technically classified as bacteria, but are unique enough that they are discussed and frequently cited as an individual group. What distinguishes actinomycetes from other typical bacteria is their tendency to branch into filaments or hyphae that structurally resemble the hyphae of fungi, only smaller in nature. Overall, actinomycetes are Gram-positive organisms that are highly prevalent in soils. Actinomycetes are important antibiotic producers, and are also responsible for the production of geosmin, which can cause odor problems in potable water.

2.2.2 Bacterial Cell Envelope

Those bacteria that have been cultured can be structurally separated into two major groups based on their cell envelope architecture: Gram positive or Gram negative (Figure 2.3). This major architectural difference helps dictate strategies for survival in the environment. For example, the thick cell wall of Gram-positive bacteria, such as in *Bacillus* and *Clostridium*, helps them withstand the harsh physical conditions found in soil environments. On the other hand, the more complex architecture of the cell envelope in Gram-negative bacteria such as *Pseudomonas* and *Shewanella* seems to aid these microbes in interacting with mineral surfaces and solutes in the environment to obtain required nutrients for metabolism.

Starting from the interior side of the cell envelope, both types of bacteria have a cytoplasmic membrane that is impermeable to many of the nutrients the cell needs for growth and energy production (Figure 2.4). Consequently, embedded throughout the cytoplasmic membrane are membrane-spanning proteins specific for the transport of molecules into and out of the cell. These proteins turn the cytoplasmic membrane into a semi-permeable structure that separates the cytoplasm from the exterior of the cell.

TABLE 2.1 Overall Macromolecular Composition of an Average *E. coli* B/r Cell

Macromolecule	Percentage of Total Dry Weight	Weight per Cell (10^{15} × Weight, Grams)	Molecular Weight	Number of Molecules per Cell	Different Kinds of Molecules
Protein	55.0	155.0	4.0×10^4	2,360,000	1050
RNA	20.5	59.0			
23S rRNA		1.0	1.0×10^6	18,700	1
16S rRNA		16.0	5.0×10^5	18,700	1
5S rRNA		1.0	3.9×10^4	18,700	1
Transfer		8.6	2.5×10^4	205,000	60
Messenger		2.4	1.0×10^6	1380	400
DNA	3.1	9.0	2.5×10^9	2.13	1
Lipid	9.1	26.0	705	22,000,000	4
Lipopolysaccharide	3.4	10.0	4346	1,200,000	1
Peptidoglycan	2.5	7.0	$(904)_n$	1	1
Glycogen	2.5	7.0	1.0×10^6	4360	1
Total macromolecules	96.1	273.0			
Soluble pool	2.9	8.0			
Building blocks		7.0			
Metabolites, vitamins		1.0			
Inorganic ions	1.0	3.0			
Total dry weight	100.0	284.0			
Total dry weight/cell		2.8×10^{-13} g			
Water (at 70% of cell)		6.7×10^{-13} g			
Total weight of one cell		9.5×10^{-13} g			

Adapted with permission from Neidhardt et al. (1990).

Other important functions of the cytoplasmic membrane and its embedded proteins are electron transport and energy generation for the cell, as well as biosynthesis of structural molecules and secondary metabolites such as antibiotics that are exported from the cell.

Moving on to the exterior of the cell envelope, both types of bacteria have a cell wall made of peptidoglycan that is external to the cytoplasmic membrane. One important function of the cell wall is to maintain the shape and integrity of the cell giving rise to various bacterial morphologies ranging from the bacillus (rod) and coccus (round) to the spirillum (twisted), vibrio (comma-shaped) and even stalked bacteria. The cell wall is composed of repeating units of *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) attached to each other through peptide crosslinking (Figure 2.3). This NAM-NAG network forms a rigid porous structure that freely allows molecules of $<$15,000 MW to gain access to or diffuse away from the cytoplasmic membrane. In Gram-negative bacteria, the cell wall is a thin NAM-NAG layer sandwiched between the periplasmic space and the outer membrane (Figure 2.3). The periplasmic space is well defined and contains transport proteins, signaling proteins and degradative enzymes that support growth and metabolism. Continuing the journey toward the exterior of the Gram-negative cell envelope, there is a second membrane called the outer membrane that is attached to the cell wall by lipoproteins. The inner leaflet of the outer membrane is structurally similar to the cytoplasmic membrane, while the outer leaflet contains immunogenic lipopolysaccharides (LPS) that extend out from the cell into the environment. LPS confers a negative charge to the cell, and have both antigenic (causes an immune response) and endotoxic (potentially toxic to humans and animals) properties. The outer membrane has a variety of functions. It acts as a diffusion barrier against large molecules such as

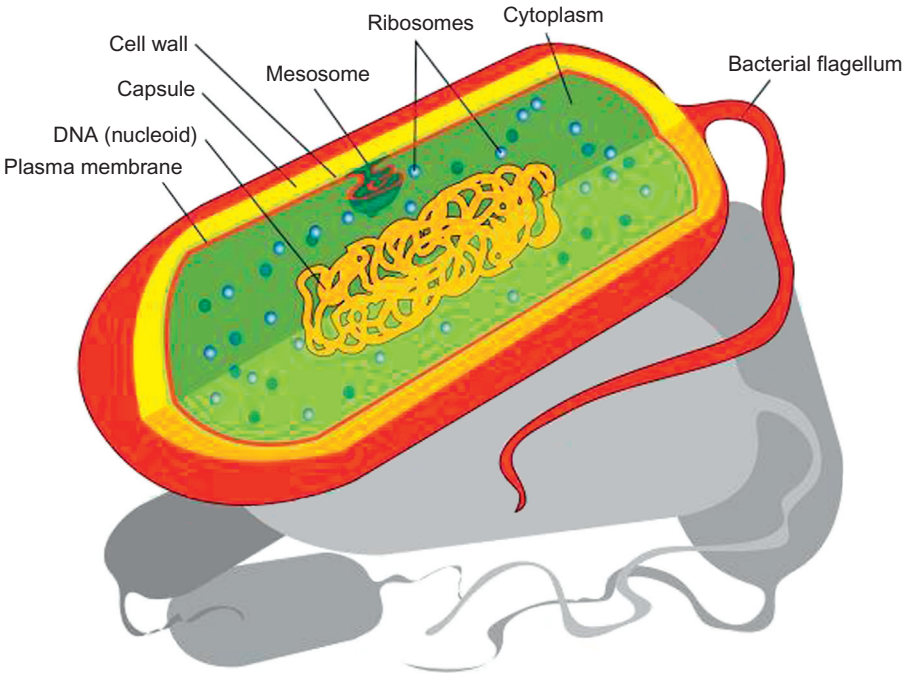

FIGURE 2.2 The components of a bacterial cell are illustrated. All bacteria have the cellular DNA dispersed in the cytoplasm, ribosomes, cell membranes, a cell wall and a capsule. Some bacteria do not have flagella, some have a single flagellum and some have multiple flagella. Not all bacteria have pili, but bacterial pathogens do, and the pili allow them to attach to host cells.

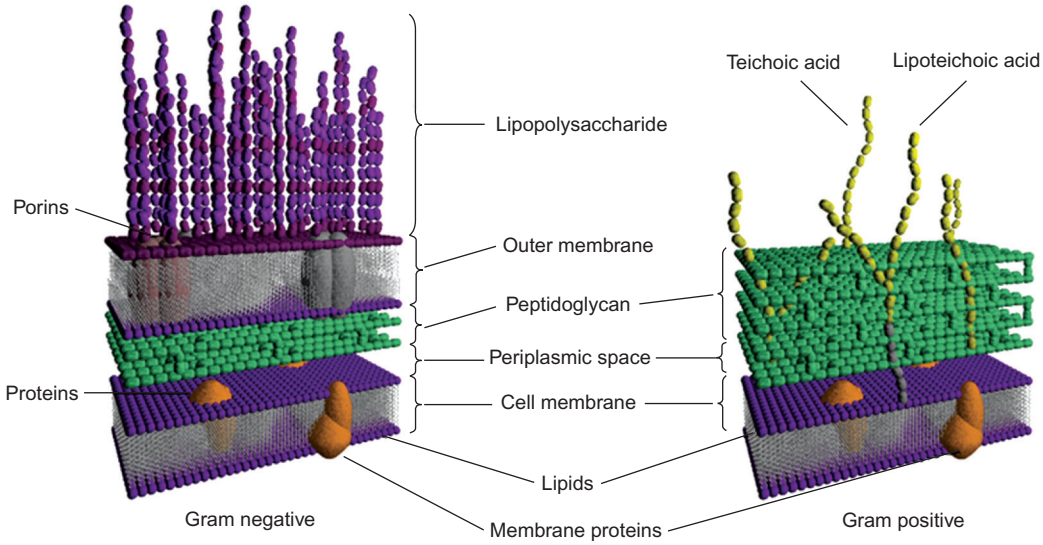

FIGURE 2.3 A comparison of the cell envelope of Gram-negative and Gram-positive bacteria. The Gram-negative cell envelope is characterized by two membranes, the inner membrane and the outer membrane, which are separated by a thin layer of peptidoglycan called the cell wall and the periplasmic space. On the exterior side of the cell wall, lipopolysaccharide molecules stretch out and mediate cell interactions with the environment. The Gram-positive cell wall is characterized by a single cell membrane that is interior to a thick layer of peptidoglycan (cell wall). In this case, teichoic acids stretch out from the cell and mediate interactions with the environment. Drawing courtesy Wayne L. Miller, McGill University.

antibiotics; it contains phage receptors and is involved in the process of conjugation (DNA exchange); it has specific nutrient uptake systems, e.g., for iron, vitamins and sugars; it contains passive diffusion pores that allow entry of low molecular weight substrates; and, finally, it provides protection for periplasmic proteins.

In Gram-positive bacteria, the cell wall is made up of many stacked layers of peptidoglycans to form a thick structure. In addition, there are covalently bound negatively charged teichoic acids, polymers of glycerol or ribitol joined by phosphate groups that extend out from the surface of the cell wall. They are antigenic and help

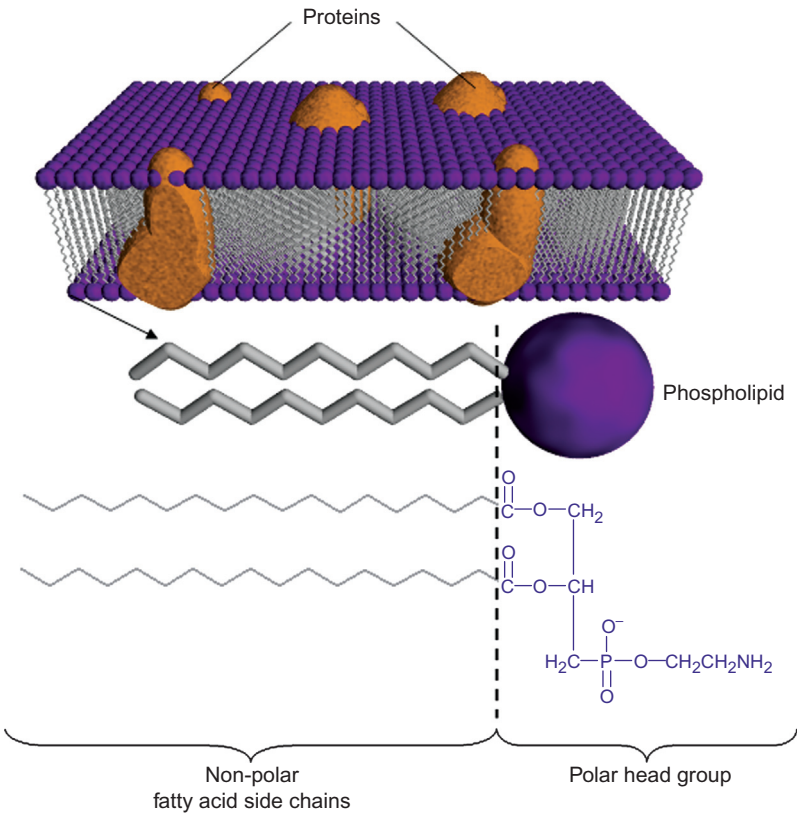

FIGURE 2.4 Structure of a cytoplasmic cell membrane. The membrane is comprised of phospholipids that spontaneously orient themselves so that the polar head groups are directed to the exteriors of the membrane and the nonpolar fatty acid groups are directed toward the interior of the membrane. Proteins are embedded throughout the membrane to aid in transport of molecules into and out of the cell. Drawing courtesy Wayne L. Miller, McGill University.

mediate interactions of the cell, e.g., surface adhesion, with the environment and other microorganisms. To the interior of the cell wall, there is a periplasmic space (much less well defined than for Gram-negative bacteria), which has recently been identified in several Gram-positive microbes, and is thought to be involved in peptidoglycan synthesis (Matias and Beveridge, 2006).

2.2.3 Bacterial Cytoplasm

Cell replication and protein synthesis is centered in the cell cytoplasm, a complex gel-like matrix composed of water, enzymes, nutrients, wastes and gases, as well as ribosomes responsible for protein synthesis, a single circular chromosome and a varied number of small circular auxiliary plasmids ranging up to several thousand base pairs (kbp) (Figure 2.2). The chromosome is localized in the cytoplasm in a region called the nucleoid. Bacterial chromosomes average 4 million base pairs (Mbp) in size and encode for several thousand genes (see Information Box 2.2). It is an amazing feat of packaging that allows the chromosome to fit into the nucleoid region. When stretched out, the chromosome is approximately 1.3 mm in length in comparison to the cell which is 1−2 μm. Thus, the cell uses a number of tightly regulated proteins including small nucleoid-associated ("histone-like") proteins and structural maintenance of chromosomes (SMC) complexes to coil, bend and ultimately condense the chromosome so that it packs into the cell, but yet is available for replication, translation (synthesis of messenger RNA) and recombination (gene rearrangement)

Information Box 2.2 Chromosome Size and Environmental Niche

The environment selects for the fittest microorganisms and thus each bacterium has only the necessary genes for survival (to have additional genes simply requires additional energy to maintain them). Here we compare two bacteria, *Pseudomonas aeruginosa* (a generalist) and *Nitrosomonas europaea* (a specialist). *P. aeruginosa* is a versatile Gram-negative chemoheterotroph found in soil, marshes, and marine habitats as well as on plants and animals, for which it is an opportunistic pathogen. The *P. aeruginosa* genome is 6.3 Mbp with 5570 predicted genes. Approximately 10% of these genes are transcriptional regulators or environmental sensors so that the bacterium can quickly respond to its environment. Compare this to *N. europaea*, which is a Gram-negative chemoautotroph that specializes in the oxidation of ammonia to nitrite. *N. europaea* is found in soil, freshwater, and sewage and in polluted environments with elevated levels of ammonia. The *N. europaea* chromosome is 2.8 Mbp with 2715 predicted genes. Thus, the specialist has a chromosome that is less than half the size of the generalist.

(Thanbichler and Shapiro, 2006). This feat is even more impressive when one considers that in an actively growing cell, there may be from two to four copies of the chromosome being actively replicated at the same time.

Plasmids are DNA sequences that are separate from the chromosome. Normally, plasmids encode genes that are not mandatory for cell growth and division but that often make the cell more competitive in a particular niche in the environment. Plasmids are often only retained if there is a selective pressure, such as the presence of an antibiotic to maintain a plasmid which confers antibiotic resistance. The relationship between plasmids and the chromosome is complex because some plasmids can integrate into the chromosome during replication and function as part of the chromosome. During later replications, this process can be reversed, with the plasmid DNA being excised and allowed to function as a self-replicating entity within the cell. Some of the more important functions of plasmids are shown in Information Box 2.3. Plasmids are autonomous in that the plasmid copy number, or number of identical plasmids per cell, is normally independent of the number of chromosome copies. Plasmids are also expendable, meaning that the accessory genetic element is not essential to the growth of the organism in its normal environment. Plasmids range in size from 10 to 1000 kbp, and a bacterium can harbor a single or several different plasmids with variable copy numbers. Plasmid types are shown in Information Box 2.4.

Ribosomes are the other distinctive feature of the cytoplasm. Ribosomes transcribe messenger RNA into proteins that carry on the basic metabolism of the cell. Ribosomes are composed of a large (50S) and a small (30S) subunit that each contain both ribosomal RNA (rRNA) and proteins. The importance of the ribosomal RNA is illustrated by the highly conserved nature of regions of the gene that encodes for the rRNA. In fact, because it has a combination of both highly conserved and highly variable regions, the 16S rRNA gene that encodes for the 16S rRNA component of the small (30S) subunit of the ribosome is currently used for classification of the Bacteria and the Archaea.

Information Box 2.3 Bacterial Plasmid Functions

Plasmid Type	Function	Reference
Resistance plasmids	Antibiotic resistance	Brooks *et al.*, 2007
	Mercury resistance	Smit *et al.*, 1998
Degradative plasmids	2,4-D degradation	Newby *et al.*, 2000
Plant-interactive plasmids	Tumor induction for crown gall disease nodule formation and nitrogen fixation in rhizobia	Cho and Winans, 2007, Rogel *et al.*, 2001
Fertility plasmids	Conjugative plasmids that contain *tra* genes	Van Biesen and Frost (1992)
Col plasmids	Code for the production of colicins or proteins that kill other bacteria	Riley and Gordon, 1992
Virulence plasmids	Code for toxins in pathogenic bacteria	Sayeed and McClane, 2007
Cryptic plasmids	Unknown	Srivastava *et al.*, 2006

2.2.4 Bacterial Glycocalyx

Finally, the exterior of the cell can have some important features. Some bacteria have an extracellular layer composed primarily of polysaccharide, but which can also contain proteins and even nucleic acids known as extracellular or eDNA. This layer is called a glycocalyx, also known as

Information Box 2.4 Types of Plasmids

Low-copy-number plasmids. Plasmids larger than 10 kbp that have one or two copies per cell.

High-copy-number plasmids. Smaller plasmids (<10 kbp) that have 10 to 100 copies per cell.

Relaxed plasmids. Plasmids whose replication does not depend on initiation of cell replication. Therefore these plasmids can be amplified (i.e., copy number increased) relative to cell number.

Stringent plasmids. These plasmids are dependent on cell replication, and plasmid replication is synchronized with replication of the bacterial chromosome. Thus, stringent plasmids have low copy numbers that cannot be amplified. Since cells growing rapidly may have three or four chromosomes, stringent plasmids can still be present with copy numbers greater than one per cell.

Conjugative plasmids. Self-transmissible plasmids that can be transferred from one bacterial cell to another during conjugation. The cells can be of the same species or of different species. Conjugative plasmids are normally large and contain transfer genes known as ***tra* genes**.

Nonconjugative plasmids. These do not contain *tra* genes and are not self-transmissible. However, some plasmids can transfer to other cells by "mobilization" to other conjugative plasmids, although not all nonconjugative plasmids are mobilizable. In the process of mobilization, transfer of non-conjugative plasmids relies on the *tra* genes of the conjugative plasmids.

Incompatible plasmids. Plasmids vary in their ability to coexist within the same cell. Incompatible plasmids cannot exist together and give rise to incompatibility groups (Inc groups). Compatible plasmids belong to different Inc groups and vice versa.

a slime layer (more diffuse and irregular) or capsule (more defined and distinct) (Figure 2.5). The resulting sticky layer provides protection against desiccation, predation, phagocytosis, and chemical toxicity, such as from antimicrobials, and acts as a means of attachment to surfaces. Glycocalyx-producing bacteria, such as *Pseudomonas* spp., are often found associated with biofilms and microbial mats (Section 6.2.4). This material has been found to bind metals and is being used commercially in the binding and removal of heavy metals from industrial waste streams (Chapter 18).

2.2.5 Bacterial Appendages

Several accessory structures extend from the cell envelope out into the environment surrounding the cell. These appendages are not present in all bacterial types, but they are common, and they typically aid bacteria with either motility or attachment to surfaces. The flagellum (plural flagella) is a complex appendage used for motility (Figure 2.6). Motility is important in aiding a bacterial cell to move short distances (μm) toward nutrients (positive chemotaxis) and away from potentially harmful chemicals (negative chemotaxis). Pili and fimbriae are any surface appendages that are not involved in motility. Fimbriae (singular fimbria) are numerous short surface appendages. The fimbriae aid in attachment of cells to surfaces, and so are important for initial colonization for biofilm formation and also for cell attachment to initiate an infection process. Pili (singular pilus) are normally less numerous than fimbriae but are longer. They are found only on Gram-negative bacteria, and are involved in a mating process between cells known as conjugation (Figure 2.7). In this process the exchange of DNA is facilitated by a pilus forming a connection between two cells. Conjugation in environmental bacteria is important because it enhances microbial diversity, often allowing specific populations to "fit" better in their environmental niche. It has recently been discovered that some bacteria also form extracellular filaments that are conductive (nanowires). During anaerobic respiration (Section 2.2.8),

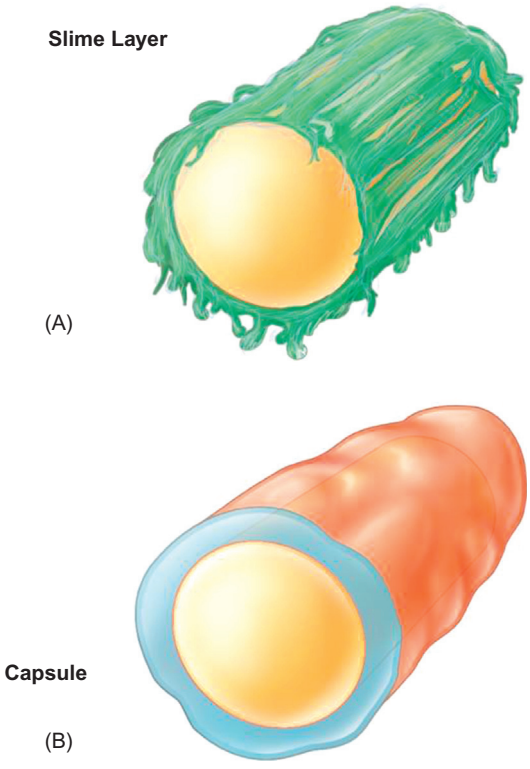

FIGURE 2.5 The surface of bacteria is covered with a sticky coating called a glycocalyx. The glycocalyx is composed of polysaccharides (sugars) and proteins. The bacterial glycocalyx has two forms: (A) a loose slime layer and (B) a rigid capsule. Capsules are found on many pathogenic (disease-causing) bacteria including *Streptococcus pneumoniae*, which causes a respiratory infection of the lungs. The glycocalyx has several functions including: protection, attachment to surfaces and formation of biofilms.

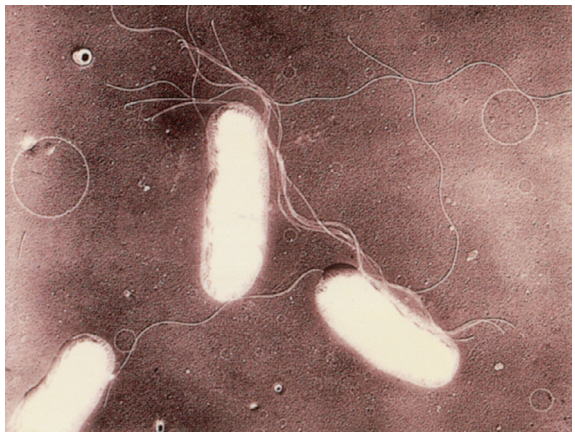

FIGURE 2.6 Scanning electron micrograph of a soil bacterium with multiple flagella. The circles are detached flagella that have spontaneously assumed the shape of a circle.

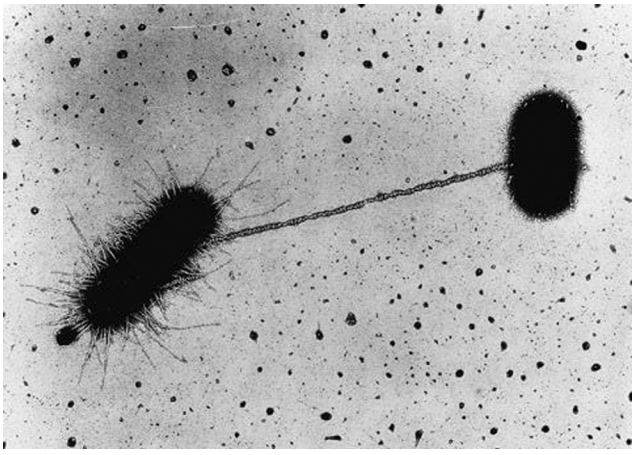

FIGURE 2.7 Bacterial cells exchanging DNA by conjugation using a pilus.

the nanowires transfer electrons directly from the bacterial cells to solid-phase terminal electron acceptors (e.g., iron oxides) in a process termed direct extracellular electron transfer (DEET) (Chapters 3 and 18).

2.2.6 Bacterial Endospores

Some Gram-positive bacteria, such as *Bacillus* and *Clostridium* spp., produce endospores, multi-layered structures capable of withstanding adverse conditions including radiation, UV light, heat, desiccation, low nutrients and chemicals, to ensure the survival of the cell. Endospores are environmentally significant because they can remain in a metabolically dormant state for long periods of time only to germinate and reactivate when conditions become favorable for growth.

2.2.7 Genetic Information Transfer

Perhaps the most unique ability of bacteria is their ability to quickly respond to changing environmental conditions. This can be attributed to their rapid growth and the flexibility of the bacterial chromosome. Bacteria readily incorporate new DNA into their genome through homologous recombination. Homologous recombination involves the alignment of two DNA strands of similar sequence, a crossover between the two DNA strands and a breaking and repair of the DNA at the crossover point to produce an exchange of material between the two strands. The acquisition of new DNA generally occurs via lateral or horizontal gene transfer by one of three mechanisms—conjugation, transduction or transformation—which allow for the exchange of chromosomal and plasmid DNA. The relative importance of these DNA transfer mechanisms is still not known but all have been shown to occur in the environment. Variations of these three methods can be used in the laboratory to genetically modify an organism.

Conjugation relies on direct cell-to-cell transfer of conjugative plasmid DNA through a protein pilus (Figure 2.7). The pilus is extended from a donor cell (containing a conjugative plasmid) to a recipient cell (lacking a conjugative plasmid). The conjugative plasmid is similar to other plasmids in that it can code for a variety of nonessential genes, such as antibiotic resistance or degradation genes. Unlike other plasmids, however, conjugative plasmids also code for the *tra* genes, genes coding for the production of the sex pilus. When a donor cell encounters a recipient cell, the pilus is formed and allows for the replication and transfer of a copy of the conjugative plasmid from donor to recipient. Upon receipt of the plasmid, the recipient now becomes a donor cell capable of spreading the plasmid and its corresponding genes to another recipient cell. Conjugation is thought to require a high cell concentration to increase the odds of encounter between compatible donor and recipient cells. Conjugation, since it is dependent on plasmids, is thought to play a significant role in the rapid transfer of plasmid encoded genes, e.g., antibiotic resistance, among bacterial populations.

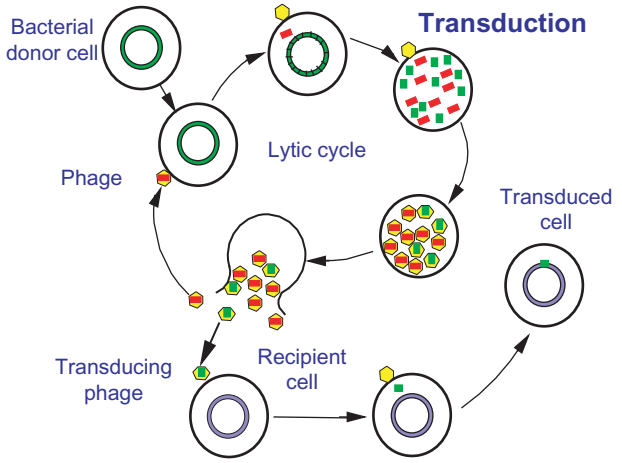

FIGURE 2.8 Transduction is the transfer of host bacterial DNA to a recipient bacterium via a bacteriophage.

Transduction occurs due to the accidental packaging of cellular genetic material during bacteriophage replication inside of its host cell (Figure 2.8). Transducing viruses sacrifice some of their own genome in place of the host's genetic material, resulting in a virus that can still infect a recipient cell but can no longer replicate. When a transducing virus infects a recipient cell, the host's genetic material is incorporated into the recipient's genome. Since the infecting transducing virus is replication defective, the recipient cell continues to grow and metabolize like normal with the acquisition of new genes. The genetic material picked up by transducing viruses reflects a variety of genes, some useful for the recipient organism and others not. Transduction can occur at lower cell concentrations since the process relies on viruses as carriers of genetic information. This process is extensively used in biotechnology for the introduction of genes into cells.

Transformation occurs when a bacterial cell obtains free DNA from its surrounding medium (Figure 2.9). When cells die, they readily lyse releasing cellular contents including chromosomal and plasmid genetic material. Much of this material is rapidly degraded by nucleases in the environment, but some can be adsorbed onto the surfaces of soil particles and organic matter, which can protect the DNA from degradation for long periods of time. Approximately one in every thousand cells is thought to be competent or capable of transporting DNA directly into the cell. Competency is the ability of a cell to transport DNA from its external environment inside the cell and is dependent on the stage of growth and the cell concentration. For example, an exponentially growing culture of 10^7-10^8 cells/mL of *Streptococcus pneumoniae* secretes a competency protein that initiates the production of several other proteins that convert the

cell into one capable of taking up external DNA, e.g., through the production of DNA-specific transport proteins. The uptake of DNA is random; however, when it occurs, the DNA can become incorporated into the genome of the organism increasing genetic diversity.

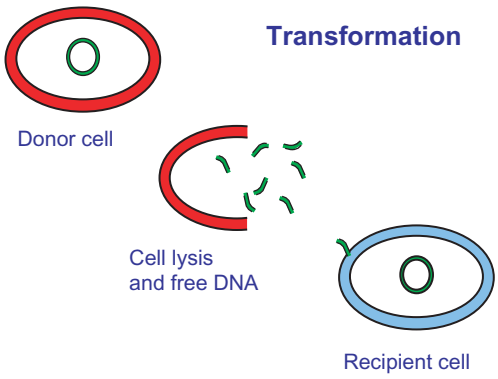

FIGURE 2.9 Transformation is the uptake of free DNA released from a dead or dying cell by a recipient cell.

2.2.8 Bacterial Metabolism

Full consideration of bacterial metabolism is provided in general microbiology textbooks. Here we simply summarize the four major types of metabolism based on the source of energy and carbon used for growth (Table 2.2). Energy can be obtained from light through photosynthesis (phototroph), or from the oxidation of organic or inorganic chemicals (chemotroph). Carbon is obtained either from carbon dioxide (autotroph) or from organic compounds such as glucose (heterotroph or organotroph). Thus, chemoheterotrophs (chemoorganotrophs) use organic compounds both for energy and for carbon, chemoautotrophs (chemolithotrophs) obtain their energy from the oxidation of inorganic compounds and their carbon from carbon dioxide, photoautotrophs obtain energy from light and fix carbon from carbon dioxide and, finally, photoheterotrophs obtain energy from light and carbon from organic compounds.

There are two ways in which bacteria can harvest energy to use for building new cell material, respiration and

TABLE 2.2 Metabolic Classification of Bacteria

Metabolism	Electron Donor (Terminal Electron Acceptor)	Carbon Source	Metabolism Type	Products
			Chemoheterotroph	
Respiration (aerobic)	organic compounds (O_2)	organic compounds	*Pseudomonas, Bacillus*	CO_2, H_2O
(anaerobic)	(e.g., NO_3^-, Fe^{3+}, SO_4^{2-})[a]		*Micrococcus, Geobacter, Desulfovibrio*	CO_2, NO_2^-, N_2O, N_2, Fe^{2+}, S, S^{2-}
Fermentation (anaerobic only)	organic compounds (organic acids)	organic compounds	*Escherichia, Clostridium*	CO_2, organic acids, alcohols
			Chemoautotroph or Chemolithotroph	
Chemolithotrophy (aerobic) (anaerobic)[a]	H_2, S^{2-}, NH_4^+, Fe^{2+} (O_2) (NO_3^-)	CO_2	Hydrogen bacteria, Beggiatoa, Planctomycetes	H_2O, SO_4^{2-}, NO^{2-}, Fe^{3+}
			Photoautotroph	
Photosynthesis (oxygenic)	Light + H_2O ($NADP^+$)	CO_2	*Cyanobacteria*	O_2
(anoxygenic)	Light + H_2S (bacteriochlorophyll)	CO_2	bacteria including: purple sulfur bacteria e.g., *Chromatium*; purple nonsulfur bacteria, e.g., *Rhodospirillum*; green nonsulfur bacteria, e.g., *Chloroflexus*; Heliobacteria, e.g., *Heliobacterium*	S^0
			Photoheterotroph	
Photoheterotrophy	light + H_2S (bacteriochlorophyll)	organic compounds	many purple nonsulfur bacteria purple sulfur bacteria to a limited extent	S^0

[a]*The majority of chemoautotrophs are aerobic. However, there have been several descriptions of anaerobic chemoautotrophs including those that participate in ammonia oxidation (Anammox) using NO_2^- as the terminal electron acceptor (from the order* Planctomycetes, *e.g., Brocadia anammoxidans) and those that participate in sulfur oxidation using NO_3^- as a terminal electron acceptor (Thiobacillus denitrificans).*

fermentation. In respiration, the cell uses a combination of substrate level phosphorylation (provides a small amount of ATP) and oxidative phosphorylation, which combines the tricarboxylic acid cycle (TCA cycle) and electron transport chain (provides a large amount of ATP) to generate ATP energy and reducing power. Key for respiration is the terminal electron acceptor (TEA) that is used to receive electrons from the electron transport chain. Under aerobic conditions, the TEA is oxygen which maximizes the amount of energy produced; a net total of 38 moles of ATP per mole glucose metabolized. Under anaerobic conditions, an alternate TEA such as NO_3^-, Fe^{3+}, SO_4^{2-} or CO_2 is used. However, the relative amount of energy that can be derived from the reduction of alternate terminal electron acceptors is less than for oxygen. Thus, respiration under anaerobic conditions is always less efficient than under aerobic conditions (less energy is produced). It should be noted that some alternate TEAs are very close to oxygen in the amount of energy produced, most notably nitrate. Thus, many bacteria are facultative anaerobes in that if oxygen is present they use it as a TEA, but if it is not present, they can use NO_3^- instead. A good example of a facultative genus is *Pseudomonas*. Although facultative anaerobes can use either oxygen or nitrate, the range of TEAs they can use is limited. For example, the sulfate-reducers are obligate anaerobes that specialize in using sulfate as a TEA.

Fermentation is an anaerobic process that uses only substrate-level phosphorylation with a net generation of 2 moles of ATP per mole glucose (thus there is no use of the electron transport chain or need for an external electron acceptor). Instead, electrons are shunted among organic compounds usually ending in the production of organic acids or alcohols resulting in very small amounts of energy. Thus, in fermentation, the end products include a combination of CO_2 and organic acids and alcohols. Fermentation is a process that has been taken advantage of in the manufacture of alcoholic beverages and a variety of other food products (vinegar, olives, yogurt, bread, cheese).

In considering metabolism in terms of this textbook, it is important to consider the environment and which type of respiration or photosynthesis will occur given the local availability of oxygen. In fact, it must be recognized that there is not simply a presence or absence of oxygen in the environment but rather a continuum of concentrations. As a result, within a single tiny niche, there may be both aerobic and anaerobic respiration occurring (as well as fermentation). Each of these types of metabolism has energy requirements and energy outputs that can be calculated. These calculations are shown in Chapter 3.

Overall, because of the ubiquitous nature of bacteria and their impact on human health and welfare, the habitats, functions and importance of bacteria are described in detail throughout this textbook.

2.2.9 The Archaea

The archaeans are microbes that look somewhat similar to bacteria in size and shape under the light microscope, but they are actually genetically and biochemically quite different. They appear to be a simpler form of life, and may in fact be the oldest form of life on Earth. Archaeans were originally thought only to inhabit extreme environments, leading to their being described as extremophiles, but more recently they have been shown to exist in a variety of normal or nonextreme environments. For example, Fan *et al*. (2006) identified similar archaeans from nonextreme environments in four Chinese and American pristine soils. The significance of the presence of archaeans in nonextreme environments has yet to be determined.

Interestingly, some aspects of archaean cell structure and metabolism are similar to those of bacterial cells. However, there are key differences in genetic transcription and translation that are actually more similar to those of the eukaryotes than to bacteria. Some of the key structural differences between archaeans and bacteria are identified in Table 2.3. Also of interest is the fact that the archaean lipids are based on the isoprenoid side chain, which is a 5-carbon unit that is also present in rubber. Archaeans have tough outer cell walls that contain different kinds of amino acids and sugars than those found in bacteria. The cell membranes are also different with glycerol-ether lipids

TABLE 2.3 Structural Comparison of Archaea and Bacteria

Structure	Archaea	Bacteria
Lipid	Glycerol-based phospholipid but stereochemistry of the glycerol is opposite that of bacteria and eukaryotes	Glycerol-based phospholipid
Membranes	Composed of glycerol-ether lipids	Composed of glycerol-ester lipids
Cell wall	Lacks peptidoglycan, contains surface layer proteins or S-layer	Peptidoglycan and S-layer
Flagella	Resembles Type IV pili	Resembles Type III pili
Chromosome	One circular chromosome	One circular chromosome

rather than the glycerol-ester lipids found in bacteria. The ether bonds are chemically more resistant than ester bonds and may help archaeans survive extreme environments.

2.2.9.1 Archaean Habitats

Many archaeans are extremophiles that can survive either hot or cold temperatures, or extreme salinity, alkalinity or acidity (Pikuta *et al*., 2007). Nonextreme archaeans have been found in a variety of environments including soil, seawater or even sewage. Originally it was thought that no pathogenic archaea existed (Cavicchioli *et al*., 2003). However, archaeans were later linked to clinical infections (Vianna *et al*., 2006). Archaeans are usually placed into three groups based on habitat. The two major divisions of archaea are the Crenarchaeota (mostly thermophiles) and the Euryarchaeota (mostly haloarchaeans and methanogens). The halophiles or haloarchaeans exist in saline environments. In contrast, methanogens live in anaerobic environments and produce methane. Methanogens can be found in low temperature environments, which is in contrast to thermophiles which are located in high temperature environments such as hot springs in Yellowstone Park (Information Box 2.5). Archaeans are also found in high numbers in cold marine environments (Giovannoni and Stingl, 2005).

2.2.9.2 Archaean Function

Many archaeans remain nonculturable, and this, coupled to the relatively short period of time since the discovery of many archaeans, means that information is limited on archaean physiology, function and the impact on global biochemical cycles. Despite this their widespread presence and major roles in extreme environments suggest that they are likely to be critically important to nonextreme ecosystems also. For example, it has recently been demonstrated that archaeans capable of nitrification are widely distributed (Santoro *et al*., 2010), and may actually control nitrification processes (instead of bacteria) in some ecosystems. Archaea have also been implicated as mediators of horizontal gene transfer between archaeans and bacteria (Nelson *et al*., 1999). Clearly, information on the archaea will increase dramatically in the near future, particularly information on archaeans found in nonextreme environments.

2.2.10 The Planctomycetes, Verrucomicrobia and Chlamydiae Superphylum

Planctomycetes, Verrucomicrobia and Chlamydiae (PVC) form a discrete phylum of the domain Bacteria, and possess very distinctive features that set them apart from other phyla. Structurally, the cell walls do not contain peptidoglycan, a universal component of most prokaryotic microbes. Also, they are capable of intracellular compartmentalization of cells, utilizing internal membranes and even membrane-bound nucleoids (Fuerst and Sagulenko 2011). Further, utilizing a process associated with internal vesicle formation, they have the ability to take up proteins from external solutions, similar to eukaryotic endocytosis (Lonhienne Thierry *et al*., 2010). Overall, the origin of these eukaryotic characteristics and traits are currently being debated, including the concept that the Planctomycetes may have evolved from a eukaryotic-like last universal common ancestor (LUCA) (Fuerst and Sagulenko, 2011). Thus Planctomycetes could be evolutionary intermediates between prokaryotes and eukaryotes.

Planctomycetes have been identified from all corners of the world, but are particularly prevalent in soils, fresh waters and marine environments (Buckley *et al*., 2006).

Information Box 2.5 Thermophilic Archaeans—How Do They Survive?

Hyperthermophilic archaeans are those that can grow in temperatures above 80°C. *Pyrococcus furiosus* can grow in temperatures from 73 to 103°C, with an optimum growth temperature of 100°C. Originally isolated from heated marine sediments on Vulcano Island, Italy, *P. furiosus* is an anaerobic bacterium capable of fermenting proteins and carbohydrates and has one of the fastest growth rates of 40 minutes observed among the hyperthermophiles. In order to survive in such extreme temperatures, structural modifications of the cell are required. Heat stabilization of proteins occurs through key amino acid substitutions that prevent protein denaturation under high temperatures. Membranes have increased concentrations of saturated fatty acids, which increase the hydrophobicity and stability of the membrane. Many hyperthermophiles have no fatty acids in their membranes, substituting them with long chain (e.g., C_{40}) hydrocarbons bonded together by ether linkages. These hydrocarbon membranes, conversely, form very temperature stable monolayers instead of the bilayers observed with fatty acid-based membranes.

Hyperthermophiles also have to prevent their DNA chromosome from melting under the high temperature. This is thought to be accomplished through the use of a reverse DNA gyrase enzyme that introduces positive supercoils into DNA. Nonhyperthermophilic bacteria use DNA gyrase to introduce the negative supercoils found in their DNA. Positive supercoiling prevents DNA denaturation. Additionally, some hyperthermophiles use heat-stable DNA binding proteins that can increase the temperature required to melt DNA by several-fold. Obviously, other structural modifications exist to stabilize lipids and small molecules in the cell. What is the upper temperature limit such modifications can afford? Because of the instability of ATP beyond 150°C, scientists think this is the upper limit for life. Only future research will tell if this is the case.

In addition, they have been identified as dominating the biofilm microbial communities associated with the surfaces of brown kelp seaweed in marine waters (Bengtsson and Øvreås, 2010). Specifically, depending on the time of year, 24–53% of all bacteria within the kelp biofilm were identified as Planctomycetes. Two potential roles for Planctomycetes within biofilms include C and N nutrient cycling. Planctomycetes possess genes that encode enzymes for C_1 transfer, but the role of these genes is currently under debate (Chistoserdova *et al.*, 2004). Some Planctomycetes are capable of carrying out anammox reactions, which involves the anaerobic oxidation of ammonium to dinitrogen using nitrite as an electron acceptor as carbon dioxide is reduced. This chemoautotrophic metabolism occurs within a membrane-bound cell compartment called the anammoxosome, in some ways similar to eukaryotic mitochondria. Thus, biofilms with Planctomycetes could have potential for the removal of nitrogen from wastewaters (Kartal *et al.*, 2010). "Overall Planctomycetes challenge our concept of the bacterial cell and of a prokaryote as a structural cell type, as well as our ideas about origins of the eukaryotic nucleus" (Fuerst, 2010).

2.3 EUKARYOTES

Eukaryotes are more complex than prokaryotes and contain a true nucleus and membrane-bound cell organelles. Important groups of environmental eukaryotic microorganisms include fungi, protozoa and algae.

2.3.1 Fungi

While bacteria may represent the most abundant microorganisms in terms of numbers of individuals, the fungi, which are a physically larger group of eukaryotic microorganisms, have the greatest biomass. In a landmark paper, 1.5 million fungal species were estimated to exist (Hawksworth, 2001) with only 7% of them identified so far (Crous *et al.*, 2006). Traditionally, the identification of fungi has been based on morphology, spore structure and membrane fatty acid composition. However, more recent estimates using high-throughput sequencing methods suggest that as many as 5.1 million fungal species exist (Blackwell, 2011).

Fungi are ubiquitous and primarily found in the soil environment where they can adapt to a variety of conditions and have a primary role as decomposers. As with bacteria, some fungi are pathogenic to both humans and plants (in fact, economically, fungi are the most important plant pathogens). Other fungi are important in industrial processes involving fermentation, and in biotechnology for the production of antimicrobial compounds (Table 2.4). Metabolically, fungi are chemoheterotrophs. Most fungi are obligately aerobic, but yeasts are facultative anaerobes and the zoosporic fungi found in ruminants are obligately anaerobic. These anaerobic fungi generally ferment sugars and in doing so produce a variety of useful by-products, such as ethanol, acetic acid and lactic acid, making them important commercially for production of many staple foods (e.g., yogurt, cheese, bread, pickles) and alcoholic products such as beer and wine.

In addition to their primary metabolism which supports biosynthesis and energy production, fungi are known for producing secondary metabolites (compounds produced during the stationary phase of growth). These secondary metabolites have revolutionized medicine, biotechnology and agriculture. For example, fungi are responsible for such antimicrobials as penicillin produced by *Penicillium notatum*, cephalosporin produced by *Cephalosporium acremonium* and griseofulvin produced by *Penicillium griseofulvum*. While the fungal production of antimicrobials under *in situ* conditions is not well

TABLE 2.4 Examples of Fungi and their Role in the Environment

Fungus	Common Environments	Role
Molds		
Rhizopus spp.	Spoiled food; soil; crops	Degradation; plant diseases, e.g., rice seedling blight
Penicillium spp.	Spoiled food; soil	Degradation; antibiotic (penicillin) production
Mushrooms		
Polyporus squamosus	Dead trees and plant material	Decomposition
Cryptococcus neoformans	Soil; can be airborne	Degradation; causes *Cryptococcus* (infection of the lungs and central nervous system in humans)
Yeasts		
Saccharomyces cerevisiae	Fruits; soils; aquatic environments	Fermentation; degradation
Candida albicans	Normal microbiota of animals	Causes candidiasis (yeast infections) of the skin or mucous membranes

understood, it is hypothesized that they help reduce competition from other microorganisms for nutrients.

2.3.1.1 Fungal Structure

Fungal membranes and cell walls are complex structures that act as selectively permeable barriers and protective outer barriers, respectively. The composition of these two structures varies somewhat among genera, in part due to the large variation in behaviors and life cycles, habitats and physiologies seen in the fungi. As eukaryotes, fungi have membrane-bound organelles in addition to a cytoplasmic membrane composed of a phospholipid bilayer with interspersed proteins for transport and degradation. Fungal membranes can be quite complex with structural and compositional differences observed in organelle membranes and in the life cycle stages. In addition to phospholipids, fungal membranes can include sterols, glycolipids and sphingolipids, which can be used for fungal identification as the ratio, type and amount of lipids can be species specific.

Fungal cell walls are multilayered structures composed of chitin, the glucose derivative *N*-acetylglucosamine (Figure 2.10). Fungal cell walls may also contain cellulose, galactosans, chitosans and mannans. Other cell wall components include proteins and lipids. Similarly to bacteria, the fungal cell wall lies outside of the cytoplasmic membrane, protecting the membrane from damage. The cell wall additionally provides the scaffolding for the fairly complex three-dimensional structures characteristic of some fungi, e.g., mushrooms.

2.3.1.2 Fungal Diversity

Fungi can be divided into three general groups based on morphological descriptions: molds, mushrooms and yeasts (Figure 2.11). Molds, such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Pilobolus*, are filamentous fungi which are found in many fungal phyla. Each filamentous fungal cell is called a hypha (pl. hyphae), which grows in mass to form tufts of hyphae or mycelia. Some hyphae extend out from the mycelium to form aerial hyphae responsible for the formation of asexual spores or conidia ranging from 1 to 50 μm in diameter. The fuzzy appearance of mold colonies is due to the aerial hyphae and the color of fungal colonies is the result of the coloration of the spores. Some molds produce sexual spores as the result of sexual reproduction. While not as resistant as bacterial spores, both asexual and sexual spores can be resistant to extreme temperatures, desiccation and chemicals, and are in large part responsible for the widespread occurrence of molds.

The mushrooms are part of the Basidiomycota, which are filamentous fungi that form the large fruiting bodies referred to as mushrooms. Aerial mycelia come together to form the macroscopic mushroom, whose main purpose is dispersal of the sexual basidiospores found underneath the cap. The rest of the mushroom fungus is below ground as a mycelium that extends outward for nutrient absorption. Both molds and mushrooms are important decomposers of natural products, such as wood, paper and cloth, as discussed below. However, both groups of fungi can additionally produce sticky extracellular substances that bind soil particles to each other to form stable soil aggregates that reduce soil erosion (Chapter 4). In some cases, fungi are thought to play a more important role in controlling erosion than plants.

The yeasts are unicellular fungi that are able to ferment under anaerobic conditions. Most important are *Saccharomyces* and *Candida*, which are members of the Ascomycota. While the yeasts do not produce spores, they are prolific in sugary environments, and are particularly associated with fruits, flowers and sap from trees. With a few exceptions where sexual reproduction occurs, yeasts

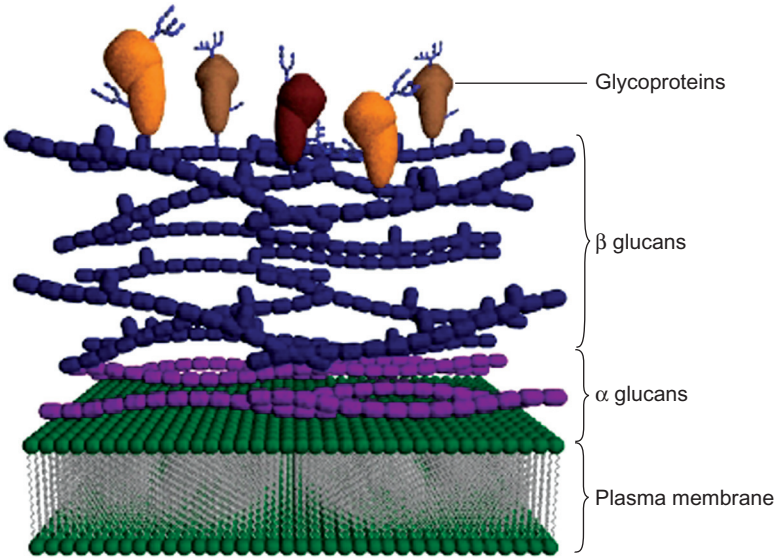

FIGURE 2.10 Structure of a fungal cell wall.

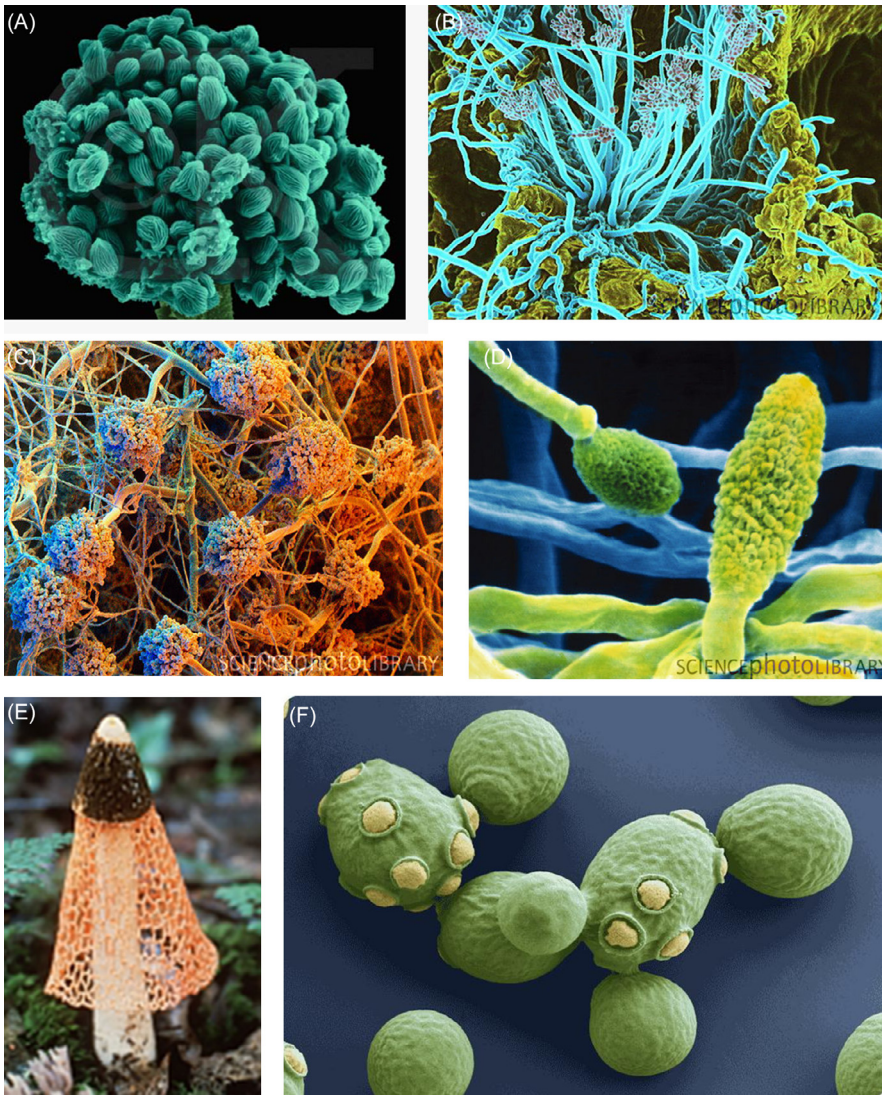

FIGURE 2.11 Various types of fungi. (A)–(D) show the fruiting bodies of various molds. In (E) is illustrated the Maiden Veil fungus or *Dictophora indusiata* within the *Basidiomycota* (mushroom). In (F) shown the budding yeast *Saccharomyces cerevisiae*.

reproduce by budding where the daughter cell forms as an outgrowth from the mother cell, eventually pinching off as a single cell. The number of bud scars left behind with each budding event can be used to estimate the number of replication cycles a particular yeast cell has undergone. While commonly found in the environment, yeasts, especially *Saccharomyces*, play a significant role in commercial applications, including the production of food, alcoholic and medicinal products. Some yeasts, e.g., *Candida*, can cause vaginal, oral and respiratory infections, and can experience a filamentous stage during pathogenesis.

Some fungi, usually members of the Ascomycota, establish symbiotic relationships with algae and cyanobacteria to form lichens. These are extremely important because lichens encourage mineral weathering through the secretion of organic acids that degrade rocks and other inorganic surfaces. The fungus:phototroph relationship actually occurs when fungal haustoria (hyphal projections) penetrate the algal cell wall. Both organisms have to be nutritionally deprived to establish their relationship. In exchange for the oxygen and organic carbon provided by the alga, the fungus provides water and minerals in addition to protection from harsh environmental conditions.

2.3.1.3 Fungal Environmental Aspects

Fungi are chemoheterotrophic microorganisms that rely on simple sugars for carbon and energy. However, simple sugars are limiting in many environments due to intense competition. Consequently, many fungi secrete extracellular

enzymes (exoenzymes) to break down complex polymers to simple carbon compounds for cell utilization. Often referred to as saprophytic, fungi are extremely important in the degradation and recycling of dead plant, insect and animal biomass, especially the complex polymers associated with these organisms, e.g., cellulose and lignin found in plants, and chitin found in insects. The filamentous fungi and mushrooms are especially adapted to a saprophytic lifestyle due to the large surface area provided by their hyphae. Because of their unique ability to degrade complex polymers, fungi have been found to have the ability to degrade a variety of environmental contaminants making them important in waste degradation and recycling (Chapters 16 and 17). For example, the yeast-like fungus *Aureobasidium pullulans* has been found to degrade polyvinyl chloride (PVC) containing plastics (Webb *et al*., 2000), and filamentous fungi such as *Penicillium*, *Stachybotrys*, *Allescheriella* and *Phlebia* can degrade the aromatic hydrocarbons associated with petroleum products and agricultural pesticides (Boonchan *et al*., 2000; D'Annibale *et al*., 2006). One special fungus is *Phanerochaete chrysosporium*, which is described further in Information Box 2.6.

A second important environmental group of fungi are the mycorrhizae. Mycorrhizae form symbiotic relationships with many plants. Through their increased surface area, mycorrhizae can increase the absorptive area of a plant's roots by hundreds of thousands of times, help to prevent desiccation of the roots and increase uptake of nutrients, especially phosphates. In return, the plant provides the fungus with sugars made during photosynthesis. Mycorrhizal fungi are present in 92% of all plant families studied and include ectomycorrhizal fungi (Wang and Qiu, 2006) and endomycorrhizal fungi (Rinaldi *et al*., 2008).

Endophytes are defined as microbes that live within plants and include fungal species; they are frequently a source of natural products. For example, recently a novel endophytic fungus was isolated from wild pineapples in the Bolivian Amazon basin. This endophyte produces volatile organic compounds with antibiotic properties capable of killing plant pathogens such as *Pythium ultimum* and human pathogens such as *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Mitchell *et al*., 2010) (see also Section 19.4).

Phenotypically similar to both fungi and protozoa, slime molds produce spores but move with amoeba-like gliding motility. Phylogenetically, slime molds are more related to the amoeboid protozoa than the fungi. There are two types of slime molds. The cellular slime molds are composed of single amoeboid cells during their vegetative stage, while the vegetative acellular slime molds are comprised of plasmodia, amorphic masses of protoplasm. Both forms can be found in moist environments on decaying organic matter where they consume bacteria and other microorganisms via phagocytosis. Environmental factors, such as nutrients or stress, can trigger cell accumulation and differentiation into fruiting bodies for the production and dispersal of spores. The spores can later germinate into vegetative amoeboid cells. The consensus of individual vegetative cells coming together and forming fruiting bodies, implying cell-to-cell communication involving chemical signals, is of much interest to scientists (see also Chapter 20).

2.3.2 Protozoa

The 18S rRNA-based classification being used for eukaryotic microorganisms has revealed fundamental

Information Box 2.6 The White-Rot Fungi: The Ultimate Fungus?

Fungi, in general, are known for their degradative abilities, and much of the conversion of recognizable organic matter into unrecognizable organic matter is attributed to fungal activity. One group of fungi, however, far exceeds other groups of fungi in their ability to degrade recalcitrant and xenobiotic compounds. These fungi are known as the white-rot fungi. The majority of white-rot fungi are basidiomycetes and include members such as *Phanerochaete chrysosporium* and *Trametes versicolor*. The white-rot fungi are especially known for their ability to degrade lignin, a structurally complex component of wood, as a result of a secondary metabolic process that yields no energy for the fungus. Lignin seems to be accidentally degraded as white-rot fungi release extracellular enzymes to access the polysaccharides tied up in the lignin. Because these enzymes are oxidases, the process of lignin degradation is aerobic. White-rot fungi are the only identified organisms capable of significant lignin degradation. The three primary enzymes responsible for degradation include lignin peroxidase, manganese peroxidase, and laccase. The nonspecificity of these enzymes for their substrate target allows them to act on other substrates, including pollutants, to facilitate their degradation. Consequently, white-rot fungi are being actively pursued in bioremediation. To date, white-rot fungi have been noted to degrade munitions waste, for example, TNT (2,4,6-trinitrotoluene); pesticides, for example, DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) and lindane; polychlorinated biphenyls used as plasticizers and in hydraulic fluids; polyaromatic hydrocarbons, benzene homologues found in fossil fuels; synthetic dyes such as azo dyes and triphenylmethane used in textiles and plastic; synthetic polymers such as plastics; and toxic wood preservatives such as creosote and pentachlorophenol (PCP) (Kullman and Matsumura, 1996; Pointing, 2001). Widespread in the environment and able to withstand harsh environmental conditions, white-rot fungi are being examined for *in situ* and *ex situ* remediation applications.

genetic differences among the protozoa. Consequently, it has emerged that the single-celled protozoa are a polyphyletic (developed from more than one ancestral type) group of eukaryotic microorganisms that are grouped together based on similar morphological, physiological, reproductive and ecological characteristics. All protozoa rely on water, and as such they are most commonly observed in freshwater and marine habitats, although some are terrestrial in moist soils and others are exclusively found in the gastrointestinal tracts of animals. However, some protozoa are saprophytic, some are parasitic and some are photosynthetic. Protozoa are important environmentally because they serve as the foundation of the food chain in many aquatic ecosystems, making up a large part of the plankton fed on by aquatic animals (see also Chapter 6).

2.3.2.1 Structure and Function

Cell morphology among the protozoa is extremely diverse, perhaps due the absence of a cell wall and wide range in size (5 μm to 1 mm) (Figure 2.12). In protozoa, cell rigidity is provided, in part, by a gelatinous cytoplasmic material called the ectoplasm located just inside the cell membrane. The ectoplasm is where the cilia and flagella are anchored in motile protozoans. The cell membrane and the ectoplasm together are known as the pellicle. Finally, inside the pellicle is the fluid endoplasm which contains organelles. Similar to other microorganisms, the cell membrane is a phospholipid bilayer with interspersed proteins for nutrient and waste transport.

Within the protozoa cytoplasm, vacuoles serve a variety of functions. Phagocytic vacuoles participate in the digestion of food; contractile vacuoles maintain osmolarity for protozoa living in hypotonic environments; and secretory vacuoles contain enzymes for various cell functions. Also apparent in the cytoplasm are multiple nuclei. In some protozoa, the nuclei are identical, while in others, such as the *Ciliophora*, there is a macronucleus and a micronucleus. The larger macronucleus is associated with cell growth and metabolism. The smaller micronucleus is diploid and is involved in genetic recombination during reproduction and regeneration of the macronucleus. Many genetically identical copies of each nucleus can exist in a cell.

Some protozoa form cysts or oocysts as part of a complex life cycle. Similar to spores in bacteria and fungi, cysts can increase the survival of the organism. *Giardia*, for example, produces cysts that persist under environmental conditions until transmission to an animal host occurs, at which point the cyst will undergo excystation to produce a vegetative cell resulting in giardiasis, a diarrheal disease. The ability to form resilient cysts substantially increases the likelihood of exposure, and thus the possibility of disease. In the last few decades, *Giardia* and another cyst-forming protozoan *Cryptosporidium* have been linked to outbreaks of waterborne illness.

Many protozoa are distinguished based on their structural morphology and mechanism of motility. Morphological characterizations are based on colony formation (single existence or in colonies), swimming style (sedentary or motile), external structures (naked, shelled or scaled) and pigmentation. Most protozoa are motile and are divided into taxonomic groups based on their mechanism of motility. For example, the *Mastigophora* use flagella; the *Ciliophora* use cilia (hair-like structures that extend outward from the cell membrane); the *Sarcodina* use ameboid motility; and the *Apicomplexa* are nonmotile.

2.3.2.2 Protozoan Environmental Aspects

Protozoa have a number of important ecological roles (Information Box 2.7). Many protozoa are chemoheterotrophic using either aerobic respiration or fermentation.

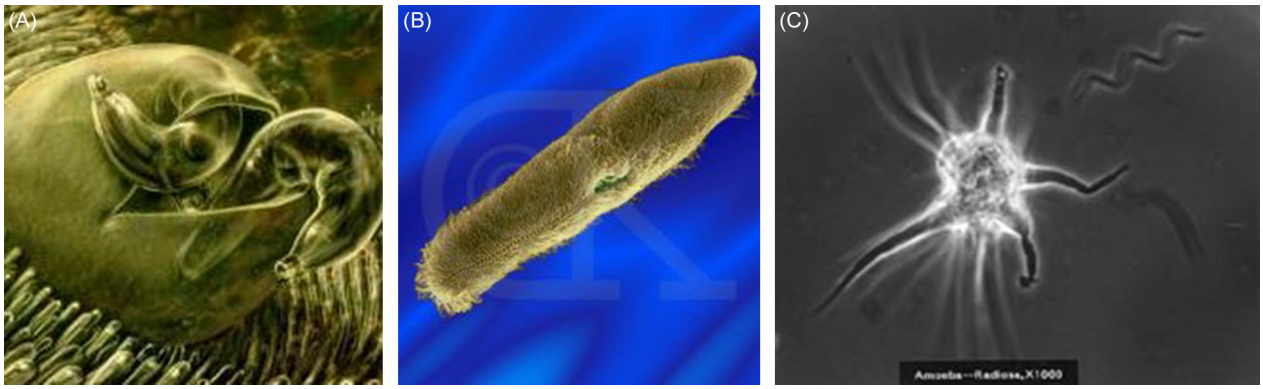

FIGURE 2.12 Morphological diversity seen among the protozoa. (A) Infection of an intestinal cell with *Cryptosporidium* (an Apicomplexan), specifically a sporozoite is attached to an intestinal epithelial cell with several merozoites emerging. For details on the life cycle see Chapter 22. (B) Paramecia are members of the Ciliophora. They are commonly found in freshwater environments where they feed on bacterial cells. (C) *Amoeba radiosa* is a member of the Sarcodina. It is found in many different environments and obtains its food by surrounding and engulfing it.

Information Box 2.7 Roles of Protozoa in Environmental Microbiology

- Serve as the base of the food chain in aquatic systems
- Population control through the predation of bacteria, algae, and even other protozoa
- Human and vertebrate parasites, food-borne and water-borne disease
- Degradation of complex organic materials, such as cellulose
- Symbioses with some animals, such as termites and ruminants

Interestingly, anaerobic and microaerophilic protozoa do not contain true mitochondria (as found in other eukaryotes). Instead, they rely on membrane-bound structures called hydrogenosomes for energy production. Hydrogenosomes lack many of the citric acid cycle enzymes normally associated with mitochondria and use protons as terminal electron acceptors forming molecular hydrogen instead of water.

Protozoa have an important role in the degradation and cycling of organic matter in the environment. These protozoa make and release a variety of extracellular enzymes for the degradation of polymers such as cellulose from plants and peptidoglycan from bacterial cell walls. Some bacteriovorous protozoa release such enzymes to aid in feeding on bacteria. Other protozoa engulf their nutrients via phagocytosis (engulfment) which are then degraded by digestive enzymes stored in phagocytic vacuoles. The ability to degrade large molecules contributes to the complex relationships these organisms have with animals. In fact, protozoa are responsible for up to one-third of fiber digestion in ruminants and contribute half the microbial mass in the rumen. In the anaerobic environment of the rumen, protozoa carry out fermentation producing organic acids and alcohols.

Water quality is an area where protozoa are having an increasingly important impact. Outbreaks of disease have been attributed to protozoa in drinking and recreational waters. The three most commonly reported protozoa affecting water quality are *Giardia* (*Mastigophora*), *Cryptosporidium* (*Apicomplexa*) and *Toxoplasma* (*Apicomplexa*). Originating from infected humans and animals, the cysts and oocysts of these organisms can withstand the water temperatures and salinities encountered to survive long periods of time in the environment (Fayer *et al.*, 2004). They can also withstand chlorine disinfection (see also Chapters 22 and 25). Other important protozoa include the so-called brain-eating amoeba such as *Naegleria fowleri* and *Balamuthia mandrillaris*. These organisms can be found in water or soil, and result in brain encephalitis which is often fatal in humans (Niyyati *et al.*, 2009) (see also Chapter 22).

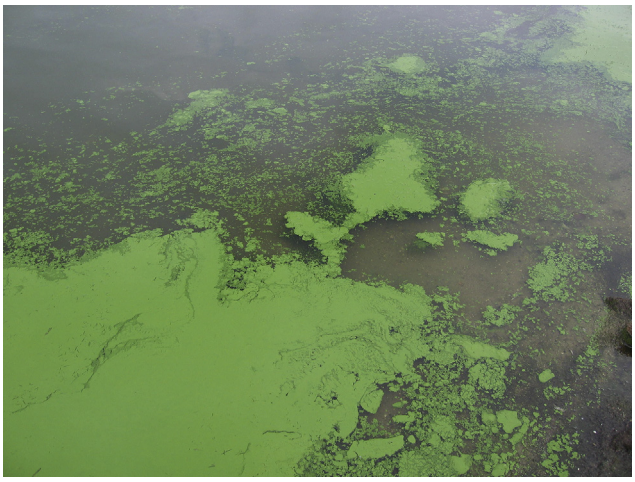

FIGURE 2.13 An algal bloom leaves a green scum-like substance on the waters of Lake Zurich, Switzerland. Courtesy Dr. Jennifer L. Graham, U.S. Geological Survey.

2.3.3 Algae

Algae are a group of eukaryotic oxygenic photosynthetic microorganisms that contain chlorophyll *a* (as seen in plants). Algae range from single-celled organisms to complex multicellular organisms like seaweeds (Figure 2.13). Inhabiting a wide range of habitats from aquatic environments (freshwater, marine and brackish) to soils and rocks, only inadequate light or water seems to limit the presence of algae. Algae are most commonly found in saturated environments either suspended (planktonic), attached to surfaces or at the air−water interface (neustonic). Endolithic algae can be found in porous rock or as surface crusts on desert soils. Algae are often the predominant microorganisms in acidic (<pH 4) habitats, as seen with the red alga *Cyanidium* that can grow at <pH 2. Generally free-living, some algae have symbiotic relationships with fungi (lichens), mollusks, corals and plants, and some algae can be parasitic. Classification of algae is complex involving numerous cellular properties. For example, algae can be grouped based on cell wall chemistry, cell morphology, chlorophyll molecules and accessory pigments, flagella number and type of insertion in the cell wall, reproductive structures, life cycle and habitat. Based on cell properties, algae include the green algae (*Chlorophyta*), the euglenoids (*Euglenophyta*), the dinoflagellates (*Dinoflagellata*), the golden-brown algae, diatoms (*Chrysophyta*), the brown algae (*Phaeophyta*) and the red algae (*Rhodophyta*). However, similar to the protozoa, 18S rRNA criteria reveal a phylogenetically diverse group.

2.3.3.1 Cell Structure

Algae can be unicellular, colonial (occurring as cell aggregates) or filamentous, resulting in great diversity in overall

cell morphology. Algal cell walls surround cytoplasmic membranes and are thin and rigid but vary in their composition. They generally contain cellulose with a variety of other polysaccharides including pectin, xylans and alginic acid. Some walls are calcareous containing calcium carbonate deposits. Chitin (a polymer of *N*-acetylglucosamine) may also be present in some algae. The euglenoids, however, differ from other algae by lacking cell walls. For diatoms, the cell wall is composed of silica giving rise to fossils. Other cell wall-associated structures include gelatinous capsules outside the cell wall for adhesion and protection, and flagella arranged in different patterns on the cell for motility.

All algae also contain membrane-bound chloroplasts containing chlorophyll *a* and other chlorophylls, such as chlorophyll *b*, *c* or *d*. Some contain differently colored pigments called xanthophylls, which can give rise to differently colored algae. Many algae also contain pyrenoids, which serve as sites for storage and synthesis of starch. Starch is one of the many types of carbohydrate storage that algae use to support respiration in the absence of photosynthesis. Other types of storage molecules include paramylon (β-1,2-glucan), lipids and lammarin (β-1,3-glucan).

2.3.3.2 Algal Environmental Aspects

Algae are primarily oxygenic photoautotrophs although a few are chemoheterotrophic using simple organic compounds, for example acetate, to help support cell metabolism. Oxygenic photosynthesis produces oxygen as a waste product obtaining energy from the breakdown of water. The production of oxygen is one of the desirable effects of algal growth in some aquatic systems. Oxygenic photosynthesis by algae is also responsible for primary production (production of organic matter) in many aquatic habitats. Thus, primary production by algae sustains the food web in many aquatic environments, and is equivalent to the role plants play in terrestrial systems.

Found in a variety of disparate habitats, the versatility of algal reproduction is partially responsible for their success. Algae can reproduce sexually and asexually with sexual reproduction involving the formation of eggs within structures called oogonia and sperm within antheridia. The egg and sperm fuse forming a diploid zygote resulting in a vegetative algal cell. Asexually, algae reproduce through binary fission or fragmentation, where fragments of filamentous algae break off and continue to grow. Binary fission is especially prevalent among the single-celled algae. Finally, some algae can produce spores (e.g., zoospores or aplanospores) that can germinate into fully functioning vegetative cells.

An interesting characteristic of some algae, especially the coastal dinoflagellates, is the production of secondary metabolites. Many of these metabolites are in the form of toxins released extracellularly. For example, the dinoflagellates *Gymnodinium* and *Gonyaulax* species can produce the neurotoxin saxitoxin that paralyzes muscles of the respiratory system in vertebrates. The toxin itself, potent at nanogram (ng) concentrations, does not harm shellfish; however, shellfish accumulate the toxin making them dangerous for consumption. Ciguatera is a disease resulting from the consumption of fish that have ingested or have accumulated the toxin of the dinoflagellate *Gambierdiscus toxicus*. The toxin itself survives cooking and can cause diarrhea and central nervous system disorders. Toxin accumulation in fish and shellfish generally occurs during blooms of dinoflagellates (algal blooms). Unfortunately, the occurrence of toxic algal blooms is on the rise nationally and internationally. This is due to a number of contributing factors, the most prevalent of which is the increasing nutrients, especially nitrogen and phosphorus, that are being released into coastal waters from sewage and agricultural runoff.

2.4 VIRUSES

Viruses are a unique group of biological entities that can infect eukaryotic or prokaryotic organisms. Although some viruses do contain a few enzymes, they are obligate parasites that have no metabolic capacity and rely on host metabolism to produce viral parts that self-assemble. Viruses consist of nucleic acid encapsulated within a protein coat known as the capsid of variable size (Figure 2.14) and morphology (Figure 2.15). Viral nucleic acids can consist of single- or double-stranded DNA or RNA. The replication of a virus can be described in five steps: (1) adsorption; (2) penetration; (3) replication; (4) maturation; and (5) release (Figure 2.16). All viruses share a common mechanism of replication at the molecular level, but different viruses replicate at varying rates. For example, prokaryotic viruses known as bacteriophage or phage infect bacteria and often replicate rapidly, in minutes, whereas a typical animal virus replicates in hours to days. Coliphages are viruses that infect coliform bacteria such as *Escherichia coli* (Figure 2.17). All viruses begin infection by adsorption to the host via specific receptors and injection of the nucleic acid or uptake of the total virus particle into the cell. The cycle then goes into what is known as the eclipse phase, a period of time during which no virus particles can be detected because of release and incorporation of the nucleic acid in the host cell machinery. Finally, new viral components are produced, assembled and released from the host by disruption of the cell or budding at the cell membrane surface. The latter release mechanism is less destructive to the host cell and may support a symbiotic condition between the virus and the host.

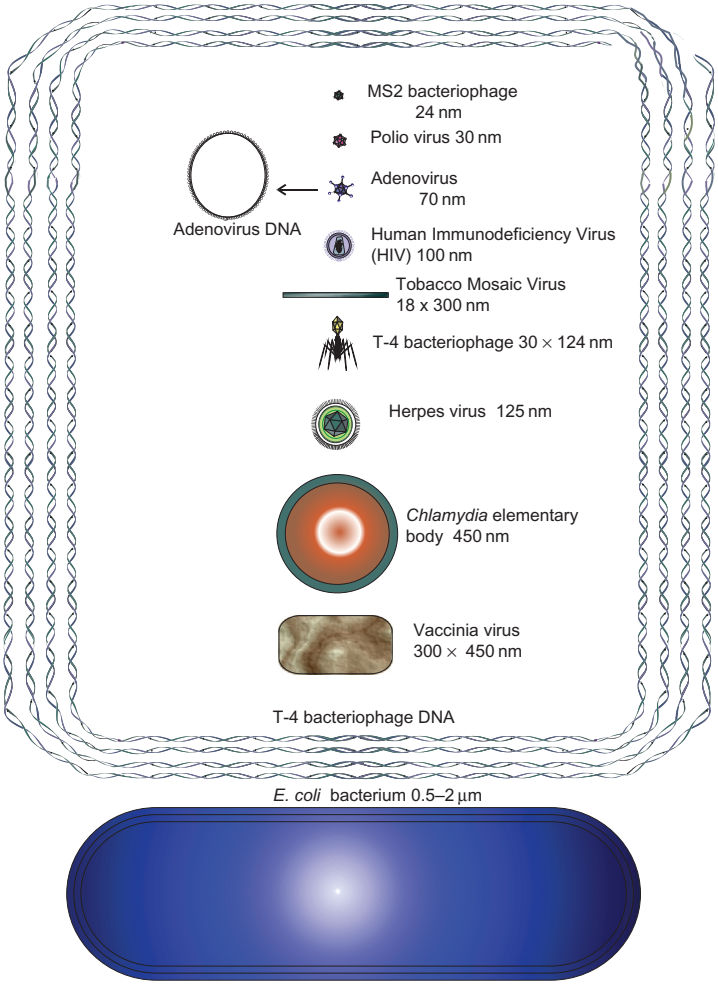

FIGURE 2.14 Comparative sizes of selected viruses in comparison to a bacterial cell and nucleic acids.

2.4.1 Infective Nature of Viruses

Outside their hosts, viruses are inert objects, incapable of movement. Thus, these tiny infectious agents require a vehicle, such as air or water, for transport. Once in contact with a potential host, viruses find their way into target cells using specific receptor sites on their capsid or envelope surfaces. This is why viruses of bacteria or plants do not normally infect humans and vice versa. Once viruses invade host cells and replicate, they can invade neighboring cells to continue the infection process. Infection of a cell by a virus is often but not always debilitating to the cell's regular functions. Thus, viral infection may be asymptomatic or may cause acute, chronic, latent or slow infections, or may cause cell death.

In bacteria, some viral infections appear to cause no immediate harm to the host cell. Therefore, the phage is carried by the host. These carrier hosts, however, may still be sensitive to other phage populations. This condition is known as lysogeny. In a lysogenic phage, also known as a temperate phage, the nucleic acid is integrated with the chromosome of the host, persisting indefinitely, and is transmitted to host descendants or daughter cells. This stable, noninfectious form of the virus is known as a prophage. The phage may remain latent for many generations and then suddenly be mobilized and initiate replication and eventually cause host lysis. Typically, only a portion of temperate phage becomes lysogenic, while other members of the population remain virulent, multiplying and lysing host cells. Similar to lysogenic bacteriophage, animal retroviruses integrate their nucleic acid into the cell chromosome, producing persistent infections. Such a cycle is typical of herpesvirus infections in humans. In fact, the herpesvirus may be passed from grandparent to grandchild, remaining dormant through two generations. Half of the human population is estimated to be infected by the age of 1 year, and up to 85% of the population is

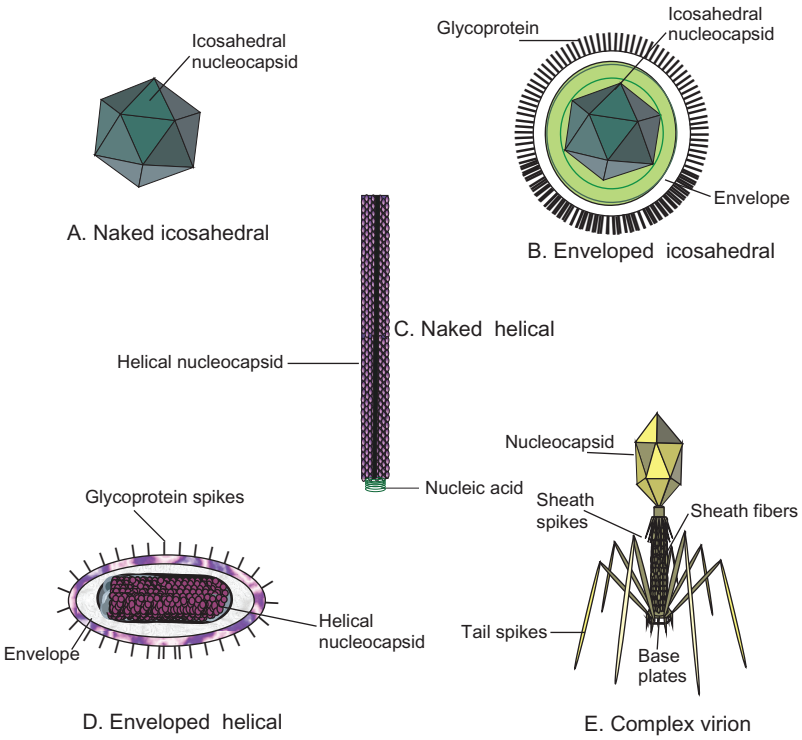

FIGURE 2.15 Simple forms of viruses and their components: (A) Naked icosahedral viruses resemble small crystals; (B) the enveloped icosahedral viruses are made up of icosahedral nucleocapsids surrounded by the envelope; (C) naked helical viruses resemble rods with a fine regular helical pattern in their surface; (D) enveloped helical viruses are helical nucleocapsids surrounded by the envelope; and (E) complex viruses are mixtures of helical, icosahedral and other structural shapes.

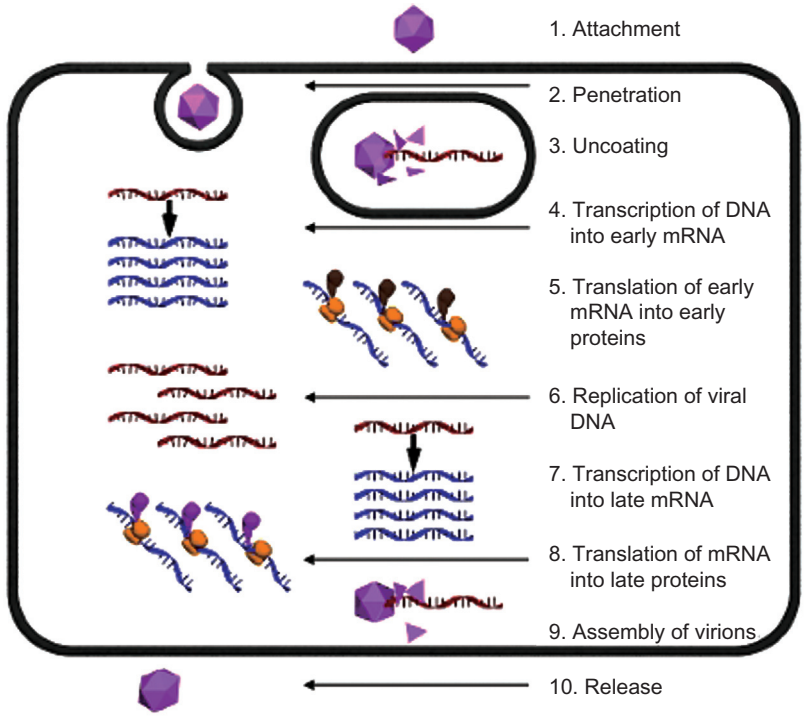

FIGURE 2.16 The basic steps of virus multiplication. Representation of an icosahedral DNA virus showing the main steps including adsorption, penetration, replication, maturation and release.

seropositive by puberty. Most latent animal viruses, such as mumps and measles viruses, lack the ability to lyse the host cell or prevent host cell division. Infection is therefore ensured by the production of infected daughter host cells. In latent infections, an equilibrium is reached between host and parasite until a nonspecific stimulus, such as compromised host immunity, evokes active infection.

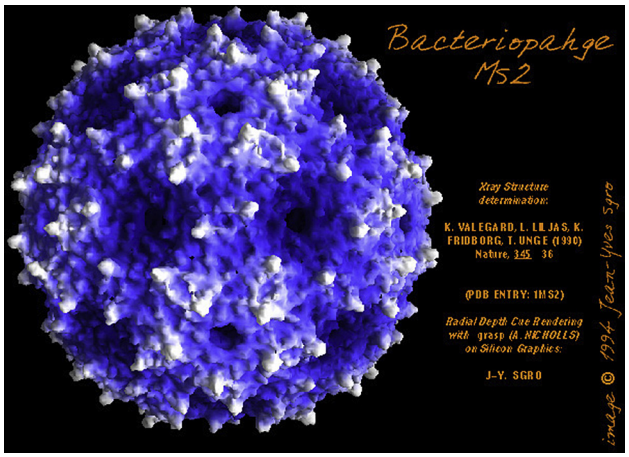

FIGURE 2.17 The bacteriophage MS2 infects the *E. coli* bacterium. Courtesy of Dr. Karin Valegard.

2.4.2 Prokaryotic Viruses

2.4.2.1 Structure and Life Cycle of Prokaryotic Viruses

The interactions of bacteriophages with a prokaryotic host are diverse and outlined in Information Box 2.8. Lytic phage are predators of prokaryotes. In contrast, lysogenic and chronic infections are actually a parasitic interaction that could be described as mutualism (Weinbauer, 2004). In the lytic cycle, the number of virions released per cell is known as the burst size. Figure 2.18 shows a bacterial cell visibly infected with phage. Overall, the size of phage usually ranges from 30 to 60 nm. The morphology of environmental phage varies considerably. Typically, phage consist of a head and a tail held together by a connector, but other forms can be cubic, spindle, filamentous or pleomorphic. Further on the metabolic state of phage and methods of viral infection and replication are given in Section 2.4.3 on eukaryotic viruses.

2.4.2.2 Prokaryotic Virus Environmental Aspects

Prokaryotic phage are abundant in a variety of environments (Table 2.5), and phage populations appear to be correlated with bacterial populations. Interestingly, estimates of phage populations vary depending on the methodology utilized in a manner similar to that for bacteria. When direct microscopic counts are made utilizing transmission electron microscopy, counts are two to three orders of magnitude higher than when traditional viable plaque counts are used (Ashelford *et al.*, 2003). Virus to bacteria ratios (VBR) are often used to illustrate the large number of phage in a sample. For example, large diverse populations of phage exist in marine environments (Sogin *et al.*, 2006) resulting in VBRs in aquatic systems that often average around 10 but sometimes exceed 100. Parada *et al.* (2007) reported marine virus to picoplankton ratios ranging from 9 at 100 m depth to 110 at a depth of 3500 to 5000 m (see Chapter 6). Picoplankton are aquatic microorganisms found in the size range 0.2 to 2 μm including small protozoa and bacteria. In comparison to aquatic systems, VBRs in terrestrial systems appear to be far more variable—ranging from <1 to several thousand (Ashelford *et al.*, 2003; Srinivasiah *et al.*, 2008).

Information Box 2.8 Host/Phage Interactions

Lytic infection. Lytic or virulent phages redirect the host metabolism toward the production of new phages which are released as the host cell lyses.

Lysogenic infection. Phages nucleic acid material of the temperate or lysogenic phage remains dormant within the host as **prophage**. Prophage are replicated along with the host until the lytic cycle is induced.

Chronic infection. Infected host cells constantly release phage progeny by budding or extrusion without lysing the host cell.

Pseudolysogenic infection. Phages multiply in only a fraction of the infected host cells. Also known as the phagecarrier state.

Mode of Infection	Phage Type
Infection via pili or flagella	F-specific phage
Infection via recognition of outer host layer or polysaccharide capsule	Capsule phage
Infection via cell wall	Somatic phage

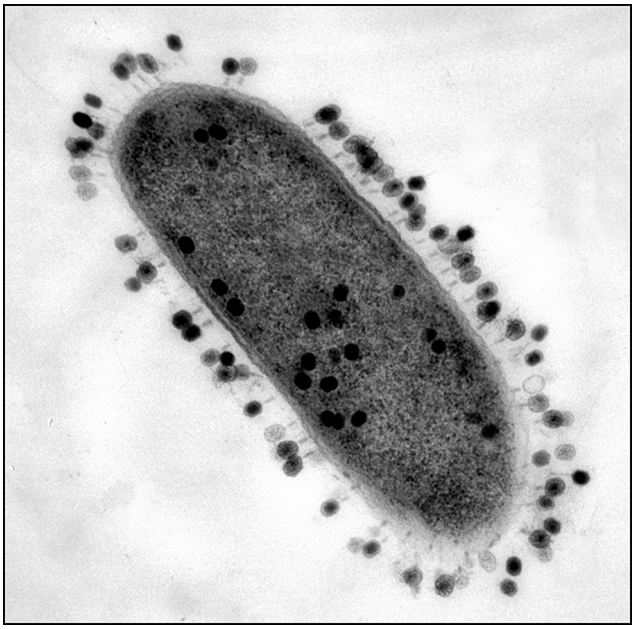

FIGURE 2.18 Transmission electron micrograph of an *E. coli* cell being infected by several T4 phages. Courtesy of J. Wertz.

TABLE 2.5 Incidence of Phages in Different Environments

Environment	Number of Phages (g^{-1} or mL^{-1})	Virus to Bacteria Ratio (VBR)
Marine		
Oligotrophic	1.4×10^6–1.6×10^7	3–110
Mesotrophic and eutrophic	2.8×10^6–4.0×10^7	
Freshwater		
Oligotrophic	4.2×10^6–4×10^7	3–9
Mesotrophic	5.3×10^6–1.4×10^8	
Eutrophic	5.6×10^6–1.2×10^9	0.1–72
Terrestrial		
Forest soil	1.3–4.2×10^9	5–12
Agricultural soil	1.5×10^8–1.1×10^9	0.04–3346

Data compiled from Srinivasiah et al. (2008) and Williamson et al. (2005).

Information Box 2.9 Role of Phage in Environmental Microbiology

- Control of bacterial populations
- Control of specific bacterial pathogens
- Control of marine cyanobacteria
- Interactions with food web processes
- Interactions with biogeochemical cycles
- Enhanced prokaryotic diversity via horizontal gene transfer

Given these large numbers, phages play critical roles in the environment (Information Box 2.9). The direct role of phages is to control bacterial populations by induction of the lytic cycle, which causes bacterial cell lysis. Without phage as a controlling factor, bacterial populations could increase significantly. Controlled studies by Wommack and Colwell (2000) showed that additions of phage to bacterial populations resulted in a 20–40% decrease in bacterial numbers. Overall, phage and protozoa (nanoflagellates) are the two major predators of prokaryotes in marine waters. Control of bacterial populations occurs in both marine and soil environments and can be general or very specific. For example, bacteriophage SF-9 is known to specifically lyse *Shigella dysenteriae* Type 1 (Faruque *et al.*, 2003). Phage have also been explored for use in the biological control of fish disease. In this case, two different phage were used to control the bacterium *Pseudomonas plecoglossicida*, the causative agent of bacterial hemorrhagic ascites disease in cultured ayu fish (*Pleoglossus altiuelis*) (Park *et al.*, 2000).

Viruses are also influential in controlling marine cyanobacteria. Such viruses are known as cyanophage, which infect numerically dominant primary producers such as the marine cyanobacteria *Prochlorococcus* and *Synechococcus* (Sullivan *et al.*, 2006). Due to the large numbers of bacteria that are lysed daily in marine waters, phages are important in both food web processes and biogeochemical cycles (Figure 2.19). Significant amounts (6–26%) of the carbon fixed by primary producers enter the dissolved organic matter (DOM) pool via virus-induced lysis at different trophic levels. In addition, it has been estimated that phage contribute from 1 to 12.3% of the total dissolved DNA in samples from freshwater, estuarine and offshore oligotrophic environments (Jiang and Paul, 1995).

Phages also influence bacterial diversity by mediating horizontal gene transfer through the process of transduction. Two types of transduction are known to be undertaken by phage. For generalized transduction, bacterial host genetic material is packed in error into the capsids of virulent phages, which is subsequently transferred into a new recipient host following infection. In specialized transduction, a host sequence is excised along with the prophage and subsequently transferred to a host. Information on transduction rates in natural ecosystems is limited but it is believed that generalized transduction may be more important, because phage-encapsulated DNA is protected from degradation (Weinbauer, 2004).

2.4.3 Eukaryotic Viruses

Eukaryotic viruses infect humans and other animals, plants and other eukaryotic microorganisms including algae and fungi. Eukaryotic viruses are also ubiquitous and are readily found in marine and soil environments. Most interest in eukaryotic viruses has centered on human and plant pathogens. Human viruses cause ailments to almost every part of the human body and include smallpox, mumps, measles, meningitis, hepatitis, encephalitis, colds, influenza and diarrhea. Information on fate and transport of some the human viruses is given in Chapter 22; however, a detailed discussion of infectious disease is beyond the scope of this book. Examples of important animal viruses include the Rhabdoviridae, which causes rabies in dogs, and the Apthovirus, which causes foot and mouth diseases in cows. Studies on plant pathogenic viruses have focused on those affecting major agricultural crops including tobacco, potatoes and tomatoes, but yet again a discussion of plant pathology is not warranted here.

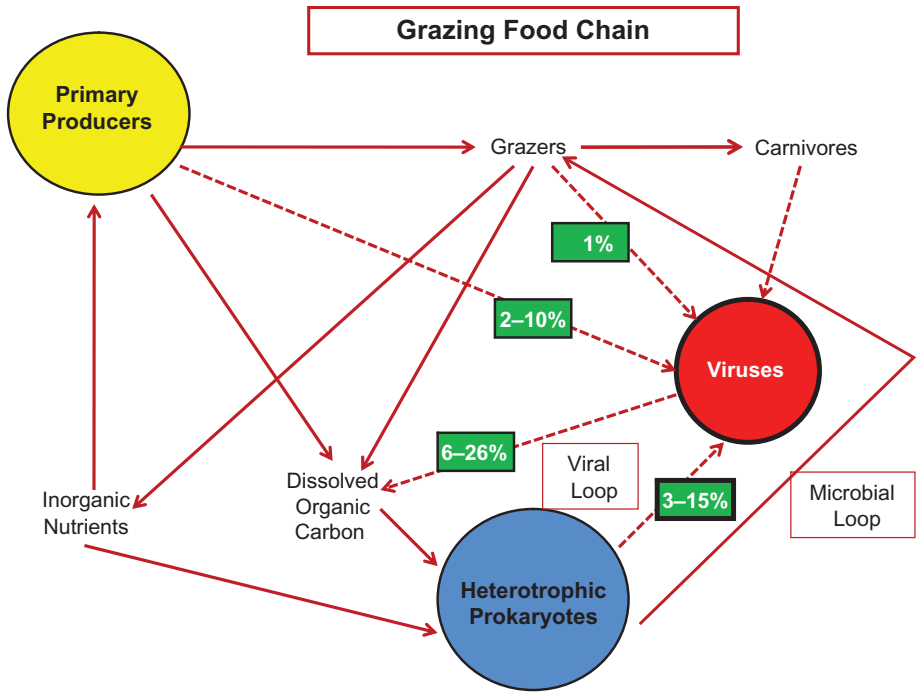

FIGURE 2.19 Pelagic food chain model and virus-mediated carbon flow. The dotted lines point to virus-mediated pathways. All values are in terms of the flux of carbon fixed by primary producers (100%). Only data for viruses are shown. The data indicate that between 6% and 26% of the carbon fixed by primary producers enters the DOC pool via virus-induced lysis at different trophic levels. Adapted from Weinbauer (2004).

Studies on eukaryotic viruses that affect algae have been mostly limited to the marine environment. Here, viruses have been identified as important agents in controlling phytoplankton populations (Brussard, 2004). Eukaryotic viruses not only aid in the control of algal blooms, but are also now being recognized as important agents that affect fluxes of energy, nutrients and dissolved organic matter in marine waters. Similar to the prokaryotic viruses, interest in eukaryotic viruses is now increasing rapidly as it becomes clear that algal viruses are important in the biogeochemical cycles. In recent years, 13 viral infections of marine microalgae have been reported (Brussard, 2004). As an example, diatoms are the major phytoplankton group that play a role in maintaining oxygen levels in the atmosphere and in the carbon cycle that sustains primary production in aquatic environments. The most abundant diatom is *Chaetocerus*, which was recently reported to have been infected by a previously unknown virus (Nagasaki *et al.*, 2005).

Eukaryotic viruses that infect fungi are known as mycoviruses. Although mycoviruses have been identified in all major fungal families, information on environmental aspects of these viruses is limited. An example of a mycovirus with economic repercussions is the causative agent of La France Disease, which affects the edible common mushroom *Agaricus bisporus* (Romaine and Schlagnhaufer, 1995). Viral infections of yeasts have also been reported (Schmitt and Neuhausen, 1994). Undoubtedly, numerous mycoviruses are present in soil environments that most likely influence control of fungal populations and biogeochemical cycling, but currently data on such incidence are limited. One intriguing recent example from Yellowstone National Park involves a fungus, *Curvularia protuberata*, and a plant, *Dichanthelium lanuginosum*, that only grow at high temperature (65°C) when in a mutualistic association. Research has shown that this association involves a third partner, a mycovirus. In the absence of viral infection, the fungus does not confer thermal tolerance on the plant (Márquez *et al.*, 2007).

2.5 OTHER BIOLOGICAL ENTITIES

2.5.1 Viroids

Viroids are sub-viral particles that have virus-like properties but are not viruses. Viroids are infectious circular single-stranded RNAs from 250 to 400 nucleotides long that replicate in their plant host and are transmitted from host to host through mechanical means, e.g., through a wound or through transmission of contaminated pollen or in ovules. Viroids have been linked to over 16 plant diseases, including potato spindle-tuber disease and citrus exocortis disease, and appear to be highly homologous to each other indicating a common evolutionary origin. Viroids lack a protein capsid and the RNA of the viroid contains no protein-coding genes. As a result, the mechanism of disease for viroids remains unclear. In a given infected cell, however, hundreds to thousands of viroid copies may be present.

2.5.2 Prions

2.5.2.1 Structure of Prions

Prions are found in all humans and consist totally of protein (Figure 2.20). Abnormal prions are infectious protein that can destroy brain tissue, giving it a spongy appearance. Diseases caused by prions are termed transmissible spongiform encephalopathy (TSE) diseases. TSE diseases are thought to be the active agent of prion disease TSEs including: the agent of "mad cow disease" (or bovine spongiform encephalopathy—BSE) in cattle; scrapie in sheep; Creutzfeldt−Jakob disease in humans; kuru in humans; and chronic wasting disease (CWD) in wild deer and elk.

TSE diseases are transmissible from host to host of a single species or from one species to another, e.g., cow to humans. Normal prions are found in the human body and are known as PrP^c where:

PrP = prion protein
c = cellular

PrP^c have three-dimensional configurations that are easily digested by proteases. The secondary structure of PrP^c is dominated by α helices (Figure 2.21). The abnormal prions are known as PrP^{sc} where:

SC = scrapie

The primary structure of PrP^{sc} is similar to that of PrP^c (amino acid sequences) but the secondary structure is dominated by β sheet conformations (Figure 2.21). PrP^{sc} are not easily degraded by proteases. Of critical importance is the fact that when PrP^{sc} comes into contact with PrP^c it converts the PrP^c into PrP^{sc}. Therefore, although abnormal prions do not replicate, there exists a mechanism to increase the numbers of the abnormal form. When numbers of PrP^{sc} in the brain exceed a critical threshold, a TSE illness results.

2.5.2.2 Prion Environmental Aspects

BSE gained notoriety in Britain where almost 200,000 cases in cattle were detected by 2005. BSE has also been confirmed in cattle in many European countries as well as Canada and the United States (Sreevatsan and Michel, 2002). However, of greater concern to the United States is chronic wasting disease (CWD) that infects deer and elk. To date, there is no evidence of transmission of CWD from elk to humans, but there is concern about the potential for such transfer. However, a recent publication has stated that the risk if any of transmission of CWD to humans is low (Belay *et al*., 2004).

Recently, studies have demonstrated that prions are sorbed by mica, montmorillonite and other natural soils (Rigou *et al*., 2006). There is evidence both for the eventual degradation of prions in the environment (Gale and Stanfield, 2001) and that prions can remain infectious even after sorption to soil minerals (Johnson *et al*., 2006), so the question of prion survival is still an open one. Other concerns were that prions were considered to be capable of surviving conventional wastewater treatment, especially treatment that utilized mesophilic (normal temperature) digestion instead of thermophilic (high temperature) digestion (Kirchmayr *et al*., 2006). However, early studies utilized technologies for detection that did not distinguish between infectious and noninfectious prions. More recent studies utilizing a standard scrapie cell assay that only detects infectious prions show that prions do not survive meosphilic or thermophilic wastewater treatment. Further, lime treatment of Class B biosolids to produce Class A biosolids is very effective in eliminating infectious prions (Miles *et al*., 2013).

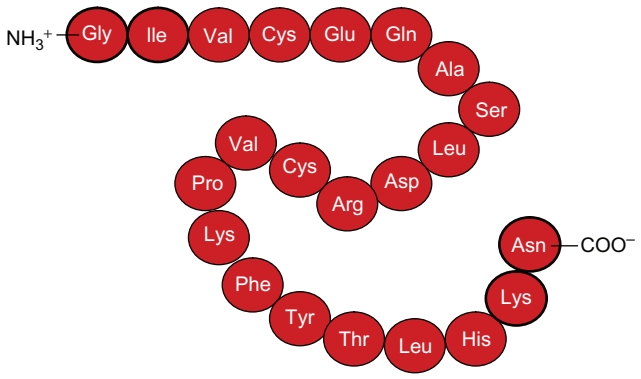

FIGURE 2.20 Primary amino acid sequence of PrP^c (a normal prion).

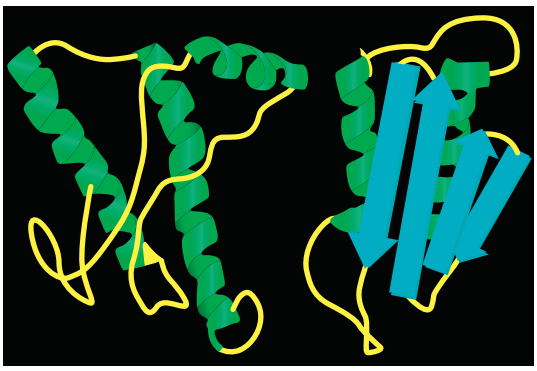

FIGURE 2.21 Secondary structures of PrP^c and PrP^{sc}.

QUESTIONS AND PROBLEMS

1. For the following groups of microorganisms—viruses, bacteria, actinomycetes, archaea, fungi, protozoa and algae—identify:
   a. Which can be pathogenic to humans?
   b. Which can be pathogenic to plants?
   c. Which can degrade organic compounds?
   d. Which can be autotrophic and heterotrophic? Give samples at the genus or species level.
2. What are some similarities and differences between:
   a. Fungi and protozoa?
   b. Archaeans and bacteria?
   c. Viruses and prions?

REFERENCES AND RECOMMENDED READING

Recommended General Microbiology Texts

Madigan, M. T., and Martinko, J. M. (2006) "Brock Biology of Microorganisms," Prentice Hall, New Jersey.

Prescott, L. M., Harley, J. P., and Klein, D. A. (2005) "Microbiology," McGraw Hill, New York.

Recommended Texts on Archaea

Garrett, R. A., and Klenk, H.-P. (2007) "Archaea: Evolution, Physiology, and Molecular Biology," Blackwell Publishing, MA.

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Gadd, G. M. (2006) "Fungi in Biogeochemical Cycles," Cambridge University Press, New York.

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