Introduction to Geomicrobiology

In memory of my mother

Introduction to Geomicrobiology

Kurt Konhauser

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Preface

The past three decades have seen enormous advances in our understanding of how microbial life impacts the Earth. Microorganisms inhabit almost every conceivable environment on the planet's surface, and extend the biosphere to depths of several kilometers into the crust. Significantly, the chemical reactivity and metabolic diversity displayed by those communities makes them integral components of global elemental cycles, from mineral dissolution and precipitation reactions, to aqueous redox processes. In this regard, microorganisms have helped shape our planet over the past 4 billion years and made it habitable for higher forms of life.

Introduction to Geomicrobiology was written in response to the need for a single, comprehensive book that describes our current knowledge of how microbial communities have influenced biogeochemical and mineralogical processes through time. Though other books on geomicrobiology exist, they are either geared more towards the physiological rather than the geological aspects, or they are edited compilations where the individual papers are highly focused on select topics. This has limited the usefulness of those resources in teaching geomicrobiology to my students. Consequently, *Introduction to Geomicrobiology* has taken a different approach. It is process-oriented, with one pervasive theme throughout, that being the geological consequences of microbial activity. I take an interdisciplinary and "global" view on the issues, and although the chapters can stand alone, they are linked through a natural progression of microorganism–environment interactions in modern ecosystems, concluding with a synopsis of how the biosphere evolved during the Precambrian. Designed primarily as a core text for upper undergraduate/graduate students in earth and biological sciences, *Introduction to Geomicrobiology* is also rich in references, with the aim being to provide the reader with a useful starting point for further study. In doing this, particular attention has been paid to making this book a useful resource for researchers from a number of other scientific backgrounds.

Introduction to Geomicrobiology covers the following topics:

- How microorganisms are classified, the physical constraints governing their growth, molecular approaches to studying microbial diversity, and life in extreme environments.
- Bioenergetics, microbial metabolic capabilities, and major biogeochemical pathways.
- Chemical reactivity of the cell surface, metal sorption, and the microbial role in contaminant mobility and bioremediation/biorecovery.
- Microbiological mineral formation and fossilization.
- The function of microorganisms in mineral dissolution and oxidation, and the industrial and environmental ramifications of these processes.
- Elemental cycling in biofilms, formation of microbialites, and sediment diagenesis.
- The events that led to the emergence of life, evolution of metabolic processes, and the diversification of the biosphere.

One feature this book does not have is a methods section. There are many techniques employed in such an interdisciplinary science, and it is my feeling that since they are constantly evolving it is more appropriate for the reader to consult the most recent articles where the methodologies are fully explained.

The preparation of this book was greatly aided by discussions and reviews with a number of colleagues. They include Jill Banfield, Roger Buick, Jeremy Fein, Bill Inskeep, Andreas Kappler, Jon Lloyd, Anna-Louise Reysenbach, Sam Smith, Gordon Southam, and Nathan Yee. Special thanks go out to two of my students, Stefan Lalonde and Larry Amskold, for their help with figures and proofreading. I am also indebted to a number of other colleagues and publishers for making available original photographs or allowing reproduction of previously published material for illustration. Lastly, but most importantly, I owe my wife and son many hours of undivided attention for all the time missed during the writing of this book.

I am grateful to all the individuals and publishers who have given permission to reproduce material in this book.

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Microbial properties and diversity

Since their origin, perhaps some 4 billion years ago, microorganisms have had a profound influence on shaping our planet. From localized niches, that occur on the order of micrometers, to ecosystems as immense as the oceans, microorganisms are intimately involved in transforming inorganic and organic compounds to meet their nutritional and energetic needs. Because the metabolic waste from one type of species nearly always provides substrates for another, there is a continuous recycling of elements throughout the biosphere. This interdependence can exist between species growing in close proximity to one another, where any number of sorption, precipitation, and redox reactions inevitably create unique community-specific biogeochemical and mineralogical signatures. Alternatively, the communities can be spatially separated, and elemental cycling may take on more complex and convoluted pathways, such as the transfer of metabolites across the sediment–water interface or from the ocean water column to the atmosphere. The latter examples are particularly important for global-scale cycling of carbon, nitrogen, sulfur, and oxygen. Given sufficient time, the collective metabolic activities of countless microbial communities can even modify the dynamics of the entire Earth, controlling the composition of the air we breathe, the water we drink, and the soils in which we grow plants. So intimate is this relationship that in order to discover a part of the planet not fundamentally affected by microorganisms, it would be necessary to penetrate deep into the crust where temperatures are outside physiochemical limits for life. From the early recognition that microbial activity affects the environment, and vice versa, was born the field of Geomicrobiology. As this book is primarily concerned with the study of microbially influenced biogeochemical and mineralogical reactions at present and through time, we begin by briefly examining some of the characteristic properties of microorganisms and their overall diversity in the biosphere.

1.1 Classification of life

Since the early eighteenth century, and the first formal taxonomic classification of living organisms by Carolus Linnaeus, a number of attempts have been made to categorize the different life forms according to morphological and nutritional similarities. Traditionally, all organisms were grouped into the kingdoms Animalia or Plantae. However, with the recognition of the immense diversity of microscopic organisms, Whittaker (1969) proposed a five-kingdom classification system that subsequently added Fungi, Protista, and Monera at the kingdom level. The Monera contained the bacteria that, based on their simplistic prokaryotic cell structure, were distinguished from the more complex eukaryotic organisms. The Protista contained all eukaryotes that were neither animals, plants, or fungi – included algae, protozoa, slime molds, and various other primitive microorganisms. According to Whittaker, the Monera were the first organisms on Earth, and the Protista evolved directly from them. Fungi, Plantae, and Animalia evolved from the Protista

via three separate directions of evolution based on differences in how the organisms met their nutritional needs. Fungi evolved as the most complex multicellular organisms that still obtained their nutrients by absorption; animals evolved based on their ability to ingest other organisms; while plants evolved a photosynthetic pathway to synthesize their own organic compounds.

At around the same time as the Whittaker classification scheme was being implemented, others recognized that comparative analyses of specific genes could be used to infer the evolutionary pathways of organisms (e.g., Zuckerkandl and Pauling, 1965). This approach, termed molecular phylogeny, is based on the number and location of differences in the nucleotide sequences of homologous genes from different organisms. This, in turn, provides an indication of the evolutionary relatedness between those organisms because the number of sequence differences identified is proportional to the stable mutational changes that accumulate in a sequence with time. Thus, two closely related species will have accumulated few differences in a sequence shared in common, whereas two more distantly related organisms will have accumulated many more differences in the same sequence.

In order to determine evolutionary relationships it is essential that the gene chosen for sequencing studies: (i) be universally distributed between organisms; (ii) must be properly aligned so regions of homology can be identified; and (iii) have a sequence that changes at a rate commensurate with the evolutionary distance being measured (Olsen et al., 1986). There are several evolutionary conserved genes that are present in most organisms, one of which, however, is particularly important because it encodes a specific component of the protein-synthesizing ribosomes, the ribosomal RNA (rRNA) molecule. In prokaryotic organisms this is known as the 16S ribosomal RNA. In eukaryotes, where the equivalent molecule is slightly larger, it is called 18S ribosomal RNA. Due to the fundamental role they play, rRNA molecules have remained structurally and functionally conserved. However, some portions of the molecule are more important to its function than others, and, as a result, these regions of the molecule are almost identical in sequence from the smallest bacterium to the largest animal. By contrast, regions that have a lesser role to play in the functionality of the molecule have very different sequences in different organisms. This has produced a molecule that consists of a mosaic of sequences that are highly conserved in the rRNAs of all organisms and sequences that can be extremely variable.

When rRNA sequences from different organisms are compared with each other, the number of changes in the sequence can be used to create a phylogenetic tree, with closely spaced "branches" symbolizing species that are genetically similar. There are a number of procedures used to construct phylogenetic trees, but the first stage in these analyses is always the careful alignment of the rRNA nucleotide sequences. This is a relatively straightforward task for regions that have a highly conserved sequence, yet it is considerably more problematic in regions of greater sequence variability. The mutations leading to sequence variability include additions, deletions, and reversions, further complicating the matter. Once the rRNA sequences have been aligned, phylogenetic trees of relatedness can be constructed using a number of different approaches, including distance, parsimony, maximum likelihood, and Bayesian algorithms/methods (see Schleifer and Ludwig, 1994 for details).

In their pioneering work, Woese and Fox (1977) and Fox et al. (1980) used single gene 16S rRNA sequences to determine the phylogenetic relatedness between many different organisms. What they showed was that all life could be grouped into three major domains (a term that supplanted the use of kingdom), the *Bacteria*, *Archaea*, and *Eukarya*, and that these domains diverged from a "root," a graphical representation of a point in evolutionary time when all extant life on Earth had a common ancestor, also known as the Last Universal Common Ancestor, or LUCA (Fig. 1.1). The comparative sequence

analyses further allowed the definition of other major lineages (phyla, class, and order) within the three domains (Woese, 1987). Significantly, the new taxonomic system showed that life is dominated by microbial forms – *Bacteria* and *Archaea* are entirely prokaryotic, while the majority of the phyla in *Eukarya* are also microbial. The "higher" organisms, the plants and animals, are relegated to the peripheral branches of the tree. The degrees of variation in microbial diversity are similarly astounding, with the evolutionary distances between the three domains being much more profound than those that distinguish the traditional kingdoms from each other (Woese et al., 1990). So, in terms of evolutionary distance, humans are only slightly removed from plants or fungi, while the distances between seemingly similar bacteria are considerably greater – in this book the term *bacteria* written with a lowercase "b" is synonymous with the term prokaryote, not the domain.

Although it is presently impossible to calibrate sequence changes against geological time because different lines of descent have evolved at different rates (i.e., sequence change is nonlinear), the universal phylogenetic tree does give a relative timing for major evolutionary change. For example, the *Eukarya* are more closely related to *Archaea* than *Bacteria* (Iwabe et al., 1989). This suggests that *Archaea* and *Eukarya* had a common history that excluded the descendants of the bacterial line. What is more, the major organelles of eukaryotes – the mitochondria and chloroplasts – are derived from bacterial partners that had undergone specialization through co-evolution within a host archaeal cell (see section 7.5.2 for details). Because *Eukarya* contain both bacterial and archaeal parts, their origins would appear to be very ancient (for an opposing view see Cavalier-Smith, 2002). This hypothesis, however, creates problems in trying to root the universal phylogenetic tree because we cannot be sure whether the first divergences in the tree separate: (i) *Bacteria* from a line that was to produce *Archaea* and *Eukarya* (as shown in Fig. 1.1); or (ii) a proto-eukaryotic lineage from fully developed bacterial and archaeal lineages (Doolittle and Brown, 1994).

Despite the success of single gene taxonomy, phylogenetic reconstructions based on this technique can lead to ambiguous evolutionary relationships because single gene sequences lack sufficient information to resolve much of the divergence pattern of their major lineages. Additional complexity is also introduced by lateral gene transfer, a process whereby genes from one species are passed onto another, leading to the acquisition of physiological properties or metabolic traits that are not concordant with their 16S rRNA phylogenies (Doolittle, 1999). An example we are all familiar with from the news is how certain bacteria have suddenly developed resistance to antibiotics that used to destroy them. What we now suspect has happened is that they have acquired resistance by a donation of genes from those cells immune to the drug. Lateral gene transfer often results in a confusing picture, where different phylogenies for the same organism are obtained when using different gene sequences because of an unusually high degree of similarity between the donor and recipient genes of otherwise distantly related species. As a result of these incongruities, the entire premise of equating single gene phylogeny with organismal phylogeny has been called into question. These problems can, in part, be addressed by multi-gene- and whole-genome-based systematics (i.e., the study of genomics), with phylogenetic trees being built by either analyzing a large number of conserved genes or by analyzing for the presence or absence of genes in each genome, respectively (e.g., Fitz-Gibbon and House, 1999). Thus far, complete genomic sequences of more than 200 microorganisms have been made available. And, one of the most exciting outcomes of these studies is that regardless of domain, all of the microorganisms share a number of core genes, the so-called "housekeeping" genes that are responsible for critical functions (Koonin, 2003). This suggests that life may indeed have had a common ancestor, with perhaps as few as 500–600 genes.

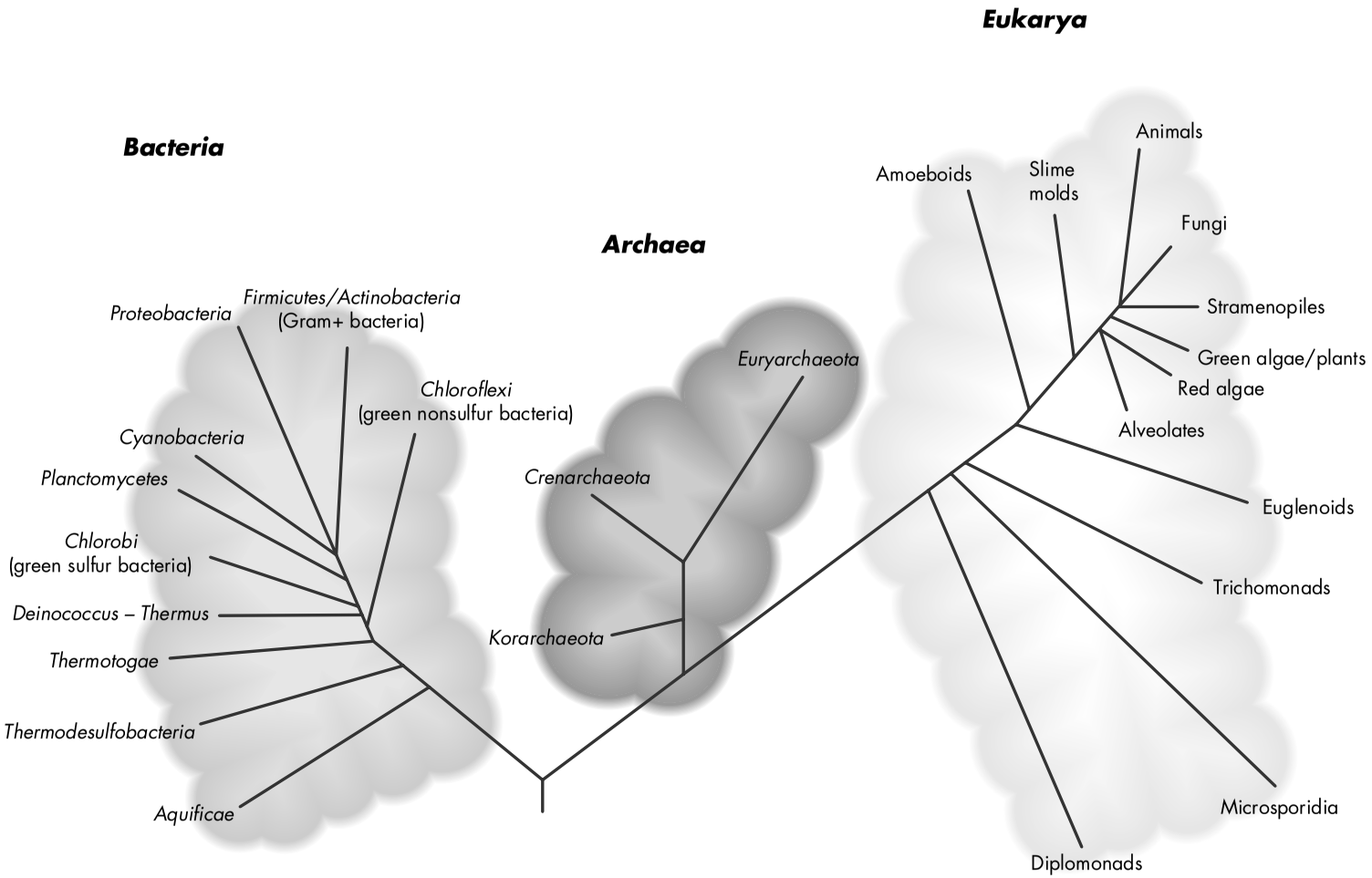


Figure 1.1 The "universal" phylogenetic tree, based on 16S/18S rRNA sequence comparisons, showing the three domains of life: *Bacteria*, *Archaea*, and *Eukarya*. The *Bacteria* and *Archaea* are subdivided by representative phyla, and the *Eukarya* are outlined by familiar grouping for lack of general consensus among taxonomists. Note: all the branch lengths should be considered approximate.

1.2 Physical properties of microorganisms

1.2.1 Prokaryotes

Most *Bacteria* and *Archaea* are between 500 nanometers (nm) and 2 micrometers (μm) in diameter, with a volume between 1 and 3 μm^3 and a wet weight approximating 10^{-12} grams. The only way to observe such small objects is by microscope, hence the term microorganisms or microbes. Individual cells most frequently occur as rods (known as bacilli), spheres (known as cocci), or helical shapes (known as either spirilla or vibrio) (Fig. 1.2). These different shapes in turn affect the relationship between volume and surface area: rods have a surface area to volume ratio of 10:1; spheres, 5.8:1; and helical-shaped, 16:1 (Beveridge, 1988). Individual cells also commonly form together in groups or clusters after division. When they grow end on end, they form what are known as filaments.

The small size of *Bacteria* and *Archaea* has a number of ramifications. First and foremost, they are typically not large enough to differentiate the many cellular processes into compartmentalized organelles. The lack of complex parts, however, benefits them because it means that there is little to fail, perhaps explaining why they are often found in "extreme" environments that preclude the growth of more complex organisms (Nealson and Stahl, 1997). Given the lack of intracellular organelles, many of the important metabolic and biosynthesis reactions take place instead at the cell's periphery where the external sources of nutrition and energy are derived. Hence, establishing an appropriate shape with a high surface area to volume ratio is of considerable importance for optimizing the diffusional properties of the cell (Fig. 1.3). If a cell grows too large in volume, the surface area becomes small in comparison, and the diffusion time becomes excessively long. On the other hand, cells too small would suffer from having insufficient surface area to accommodate the numerous proteins required to transport nutrients into the cell, and not enough volume to house all of the macromolecules and solutes necessary to support normal cell growth (Beveridge, 1989a).

The physical makeup of *Bacteria* and *Archaea* is remarkably simple (see Madigan et al., 2003 for details). All possess a plasma membrane that completely envelopes the cell and separates the inside of the microorganism from the external environment. Its primary function is as a selective and semipermeable barrier that regulates the flow of material into and out of the cell. The plasma membrane is 7–8 nm in thickness (Fig. 1.4). In *Bacteria*, it consists of a lipid bilayer with a fatty acid portion sandwiched in the middle of two glycerol layers, while in *Archaea* the fatty acid is replaced by repeating units of the hydrocarbon molecule isoprene. The placement of ionized phosphate compounds at the outer surface of the glycerol imparts a negative charge that makes the phospholipid hydrophilic, that is, attracted to water (see Box 1.1). Therefore, the phosphate portions of one layer interact with the water outside the cell and those of the other layer interact with the fluids within the cell. The fatty acids, on the other hand, are made up of nonpolar hydrocarbon chains (with H–C bonds) that are hydrophobic. Accordingly, they cluster together and point inwards towards each other in an attempt to minimize their exposure to the aqueous environment. Despite the exclusion of water from their interior, most plasma membranes are actually quite fluid, with a viscosity similar to light grade oil. As a result, the phospholipids have the capacity to move about with a certain degree of flexibility.

The plasma membrane also houses a number of different proteins, making up to 60% of the membrane weight. Some of these proteins are found on the outer surfaces where they bind and process substrates for transport into the cell. Within the plasma membrane, various proteins

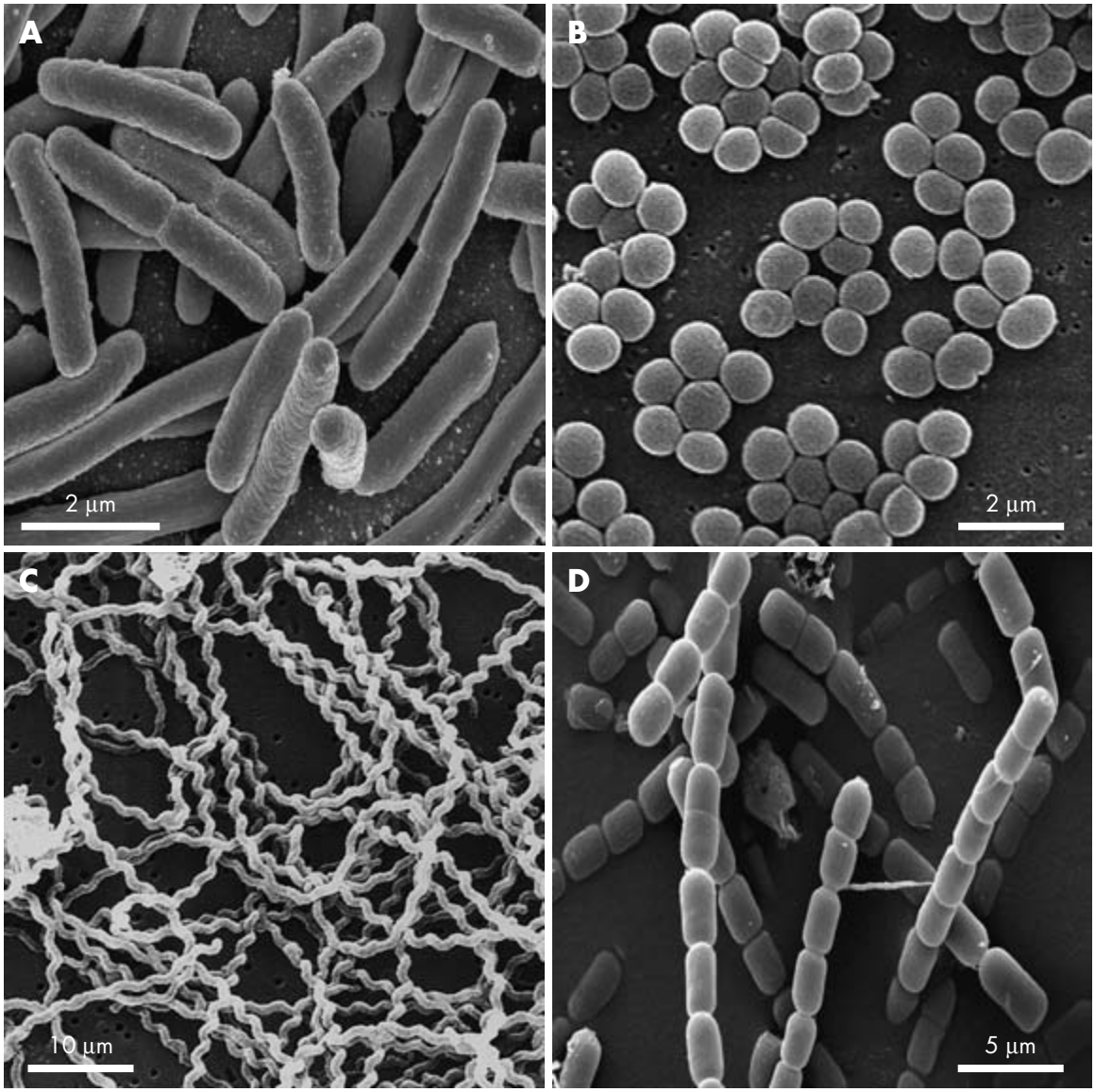


Figure 1.2 Basic shapes of bacteria as seen under the scanning electron microscope (SEM). (A) Rods, *Escherichia coli* (courtesy of Rocky Mountain Laboratories, NIAID and NIH). (B) Cocci, *Staphylococcus aureus* (courtesy of Janice Carr and CDC). (C) Spirilla, *Leptospira* sp. (courtesy of Janice Carr and CDC). (D) Filaments, *Anabaena* sp. (courtesy of James Ehrman).

facilitate energy production in respiration and photosynthesis (see Chapter 2). There are also proteins that completely span the membrane, having portions exposed to both the interior of the cell and the external environment. Some of these proteins, called membrane transport proteins or permeases, provide passageways through which specific molecules can pass.

Enclosed within the plasma membrane is the cell's interior or cytoplasm, which is over 70%

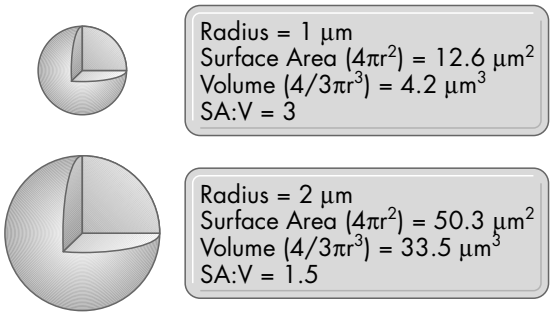


Figure 1.3 The relationship between cell surface area and volume.

water and has a pH maintained near neutrality. Within the cytoplasm, raw materials from the external environment are enzymatically degraded and new organic macromolecules are biosynthesized. It contains a particulate fraction, consisting of: (i) its genome, which is in the form of a large double-stranded molecule (the bacterial chromosome) that aggregates to form a visible mass called the nucleoid, as well as some extrachromosomal DNA called plasmids that confer special properties on the cell and are amenable to lateral gene transfer; (ii) ribosomes, the machinery needed in the manufacture of proteins; (iii) carboxysomes, polyhydroxybutyrate bodies, and various other inclusions and granules, that serve as specific storage sites; (iv) gas vacuoles, that confer buoyancy on the cells; and (v) magnetosomes, the magnetic particles found in some cells. The soluble portion (the cytosol) contains a variety of small organic molecules and dissolved inorganic ions (Fig. 1.5).

Most cells have internal solute concentrations that greatly exceed their external environment. As a result, there is a constant tendency throughout the life of the cell for external water to enter into the cytoplasm to dilute the salt content. This creates an internal hydrostatic pressure that may reach up to 50 atmospheres. External hydrostatic pressures are usually significantly lower. This pressure differential causes the delicate and deformable plasma membrane to stretch near the breaking point, and were it the sole structural support, it would likely rupture and the cell would die (known as lysis). *Bacteria* and most *Archaea* have, however, evolved an additional layer, the cell wall, which superimposes the plasma membrane, providing extra rigidity and support for the cell, as well as governing cell morphogenesis (Beveridge, 1981). It also functions as an exterior armor that protects the cell from physical

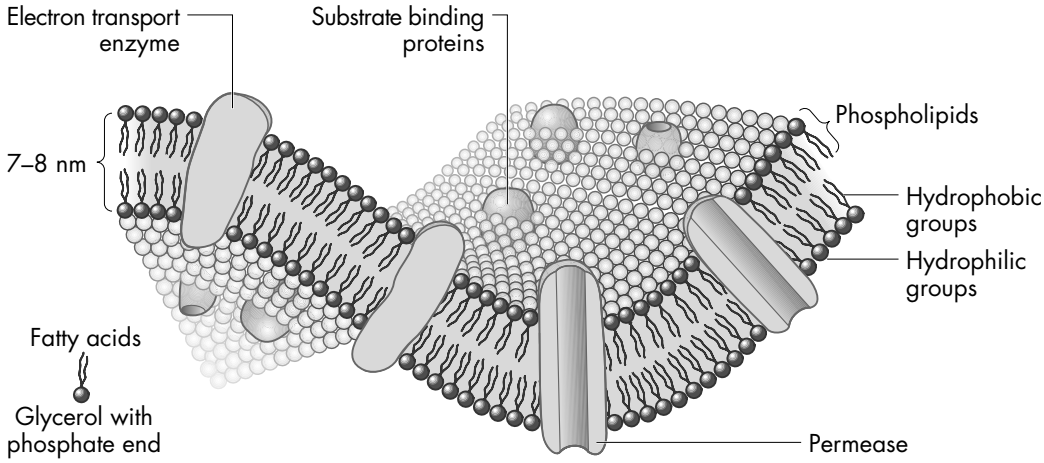


Figure 1.4 Structure of a bacterial plasma membrane, showing the phospholipid molecules with their hydrophilic groups pointing outwards and their hydrophobic groups pointing inwards. Embedded within the membrane are various proteins, each of which serves a specific cellular function.

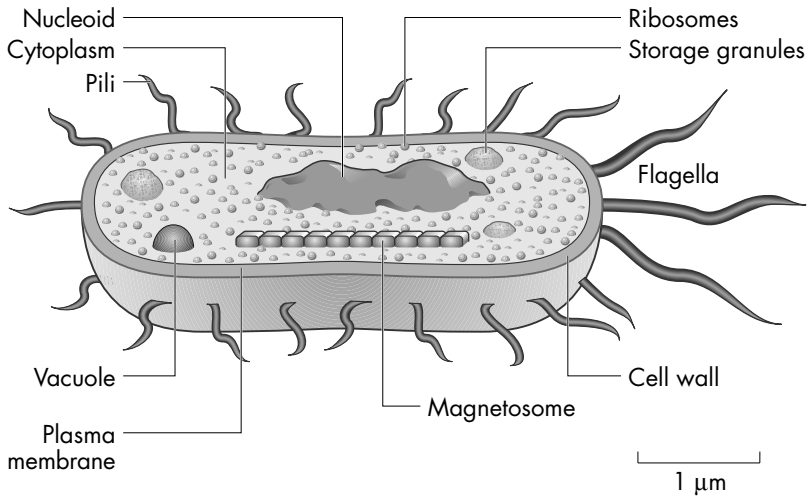


Figure 1.5 Idealized drawing of a prokaryotic cell, showing some of its internal structure.

stresses, such as collision with other particles, and chemical perturbants, such as pH changes, dissolved inorganic ions, and organic solvents (Koch, 1983). Furthermore, the cell wall acts as a filter controlling the passage of dissolved molecules into the cell (see Chapter 3). Without going into detail here, it suffices to mention that there is variation in wall types based on morphology and composition. *Bacteria* are typically classified according to how they react with what is known as the Gram stain. Gram-positive cells stain purple, and this reflects their thick, single-layer walls made up of a material called peptidoglycan. Gram-negative cells stain pink, a reflection of their more complex structure, with a thin peptidoglycan layer, overlain by gel-like space known as the periplasm, on top of which lies the outer membrane. *Archaea* are much more diverse, and have several wall types.

1.2.2 Eukaryotes

Eukaryotes include microorganisms, such as protists and fungi, as well as multicellular life, such as the plants and animals. Of the various protists, two will be covered in the following chapters, the algae and protozoans. Algae are a diverse group of photosynthetic organisms that produce O_2 as a byproduct of their metabolism. They are typically found in fresh and sea water, but they can also grow on rocks and trees, or in soils when water is available to them. Algae are broadly classified into green, brown, golden, and red based on the biochemical characteristics of their chlorophyll molecules and accessory pigments. Collectively they display a wide variety of morphologies, ranging from unicellular forms to macroscopic seaweeds that can grow tens of meters in length (see van den Hoek et al., 1996 for details). Others can grow symbiotically with animals, such as the dinoflagellates associated with coral reefs. Despite being relatively primitive, green algae are classified in the same clade as the plants because of their shared use of photosynthetic pigments. Some algae even form mineralized shells (or exoskeletons), including the silica-precipitating diatoms and the calcite-precipitating coccolithophores (see Chapter 4). By contrast, protozoans are colorless and unicellular animal-like organisms that obtain food by ingesting other organisms or particulate organic matter (see Sleigh, 1992 for details). They are classified according to their ability to move in a concerted manner (known as motility); some use flagella, which are whip-like appendages that arise from the cell surface, others have shorter appendages called cilia that

move in a wave-like manner, while some use extensions of their cytoplasm called pseudopods. Many protozoans live as parasites, absorbing or ingesting organic compounds from their host cell; one well known example is *Plasmodium* sp., a parasite that causes malaria. Some protozoans form mineralized shells as well, such as the calcite-forming foraminifera and the silica-forming radiolarians.

Fungi also have a range of morphologies, from unicellular yeasts, to filamentous molds that clump together to form what is known as mycelia, to macroscopic forms such as mushrooms. Their most important roles are: (i) as decomposers of organic material, leading to nutrient cycling and spoilage of natural and synthetic materials, such as wood or food; (ii) as symbionts of algae and cyanobacteria (e.g., as lichens); and (iii) as producers of economically important substances, such as ethanol, citric acid, antibiotics, and vitamins. They are often dominant in acidic conditions and, in soil, represent the largest fraction of biomass (see Moore-Landecker, 1996 for details).

Eukarya are larger and much more complex in structure than either *Bacteria* or *Archaea*, typically 5–100 μm in diameter. However, this demarcation has been clouded by the discovery of marine bacteria on the seafloor that can reach sizes as large as 750 μm (Schulz et al., 1999) and the recent recognition that a diversity of 0.5–5 μm sized "picoeukaryotes," with compact organization and limited organelles, exist throughout the ocean waters (Moreira and López-García, 2002). Most eukaryotes have a rigid cytoskeleton that varies in type amongst the different phyla. They also have a plasma membrane that contains complex lipids known as sterols. These molecules stabilize the cell structure, making them less flexible than their prokaryotic counterparts, and, depending on the type of cell, sterols can comprise up to 25% of the total lipids in the membrane (Madigan et al., 2003). Eukaryotes also contain a membrane-enclosed nucleus that accommodates its genome in the form of DNA-containing chromosomes, as well as a number of membrane-bound organelles that are used to segregate the various cellular functions from one another. These include, amongst many, the mitochondria for energy production and the chloroplasts, the chlorophyll-containing sites used by algae (and plants) for photosynthesis (Fig. 1.6). This compartmentalization is necessary for localizing the various metabolic processes,

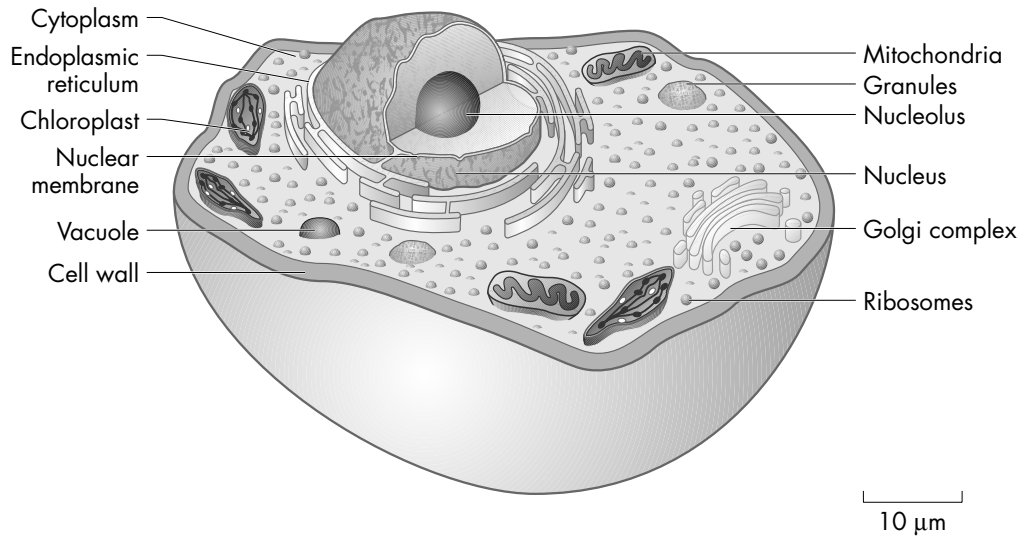


Figure 1.6 Idealized drawing of an eukaryotic cell, showing some of its internal structure.

and thereby minimizing the inherent problems that their large size would face in obtaining nutrients and excreting waste products simply by diffusional processes (Beveridge, 1988).

1.3 Requirements for growth

Although microorganisms are ubiquitous in the surface environment, each particular species is subject to a number of variables that affect their rates of growth. We can divide these requirements into two categories, physical and chemical. Physical aspects include temperature, pH, and osmotic pressure, while the chemical aspects include sources of nutrition and energy.

1.3.1 Physical requirements

(a) Temperature

Each microorganism has a temperature range over which it can grow. Psychrophiles are capable of growth up to 20°C. In nature, they are commonly found in deep ocean waters or in polar regions, but they also include those that grow within our refrigerators. Mesophiles grow between 15 and 45°C. These are the most common types of microorganisms in nature, and include most of the pathogenic species. Thermophiles grow between 45 and 80°C and hyperthermophiles grow above 80°C. The last two groups of microorganisms are associated with terrestrial geothermal regions or seafloor hydrothermal vent systems.

The temperature limits of life are constrained in one of two ways. At high temperatures, subtle changes occur in the configuration of the proteins and nucleic acids causing them to become irreversibly altered. As temperatures approach the surface boiling point of water, the monomers that make up the cells also begin to hydrolyse (bonds break by reacting with water); the half-life of adenosine triphosphate (ATP) is less than 30 minutes at 100°C (Miller and Bada, 1988). Also, as reactions generally proceed faster at higher temperatures, solute transfer across the plasma membrane may become excessively quick, thereby inhibiting the cells' ability to generate energy through what is known as the proton motive force (see section 2.1.4(b)). To date, the uppermost survival temperature measured is 121°C for an archaeal species most similar to *Pyrodictium occultum* (Kashefi and Lovley, 2003). Hyperthermophiles compensate for the high temperatures in a number of ways, including altering some of their protein structures and by possessing plasma membranes composed of hydrocarbons with repeating units of isoprene (instead of fatty acids) that are nearly impermeable to ions and protons (van de Vossenberg et al., 1998).

One principal factor that governs a microorganism's minimum temperatures is whether the cell membrane retains its fluid state, so that its capability for nutrient transport and energy generation still exists. Experiments with psychrophiles have shown that as temperature is decreased, lipids in their plasma membrane change composition by adding an increasing proportion of unsaturated fatty acids that help maintain an optimal degree of fluidity (Gounot, 1986). Other adaptations include producing cold-acclimation enzymes that allow the cells to metabolize at rates comparable to mesophiles (Feller and Gerday, 2003) and formation of extracellular layers (i.e., outside the cell wall) containing compounds that increase the viscosity of the immediate fluid phase, in essence acting as a natural antifreeze agent (Raymond and Fritsen, 2001). Thus far, the minimum temperature recorded for actively growing bacteria is –20°C in Siberian permafrost (Rivkina et al., 2000).

(b) pH

Each microorganism has an external pH range within which growth is possible. For most microorganisms (e.g., the neutrophiles), this is within pH 5–9, although the intracellular – within the cell – pH must remain neutral in order to prevent destruction of its cytoplasmic macromolecules.

Only a few species are tolerant of pH values below 2, these are the so called acidophiles. They are generally restricted to mine drainage and geothermal environments, where acid is generated from the oxidation of reduced sulfur-containing compounds. Other microorganisms, the alkaliphiles, can grow at pH 10–11. Such extremely alkaline environments are often associated with soda lakes and carbonate-rich soils.

(c) Water availability

The primary impediment for microbial life is the availability of water. Water is crucial because it facilitates biochemical reactions by serving as the transport agent for reactants and products. When a substance cannot move across the plasma membrane in response to a chemical gradient, water will move across instead. This occurs because the presence of solutes changes the concentration of water. Such movement of water is called osmosis, and the osmotic pressure is the force with which water moves from low to high solute concentrations when the solutions are separated by a semipermeable barrier. In a hypotonic medium, the solute concentration is higher in the cell. As a consequence, water will move into the cell to attain positive water balance. If the flow into the cell was unrestricted, the plasma membrane would burst. The reverse occurs in a hypertonic medium, where the solute concentration is higher outside the cell than inside. In brines, a cell would inadvertently shrink through plasmolysis if it did not naturally possess the means to deal with osmotic stresses. Thus, microorganisms living in evaporitic environments generally have some specific requirements for sodium ions (Na^+) to maintain osmotic equilibrium. Such organisms are called halophiles.

1.3.2 Chemical requirements

Life is made up of a few basic organic ingredients. Besides water, carbon is the primary requirement for growth, making up 50% of an organism's dry weight. It serves as the structural backbone of living matter, to which carbon and a number of other elements, such as hydrogen, oxygen, nitrogen, sulfur, and phosphorous, can bind. Covalently linked carbon atoms can form linear chains, branched chains (aliphatics), benzene rings (aromatics), or ring structures containing one or more noncarbon atoms (heterocyclic). The other elements usually take the form of anions or neutral species that have a definite geometrical arrangement around the central carbon atom. The resultant molecules form what are known as functional groups, each of which possesses characteristic chemical and physical properties that can be observed in a range of organic compounds. Many organic macromolecules are polyfunctional, containing two or more different kinds of functional groups. Figure 1.7 shows the common functional groups associated with microbial cell surfaces, some of which play an important role in metal sorption processes (see Chapter 3).

The various functional groups are bonded together to form low molecular weight compounds called monomers, which in turn combine to form much larger macromolecules (Box 1.1). Monomers are covalently bonded together, often through reaction of a hydrogen atom from one monomer with the hydroxyl group from another, liberating a molecule of water. These are referred to as dehydration or condensation reactions. By contrast, macromolecules can be broken down into their precursor monomers through the addition of water. These chemical reactions are known as digestion or hydrolysis. When several of the same monomers are repetitively bonded together, the macromolecule is called a polymer. The important macromolecules in living systems, in order of their dry cell percentage, are proteins (55%), nucleic acids (24%), lipids (9%), and carbohydrates (10%).

(a) Nutrition

Organisms obtain their carbon in one of two ways. Those that convert CO_2 to organic carbon, such as glucose ($C_6H_{12}O_6$), function autotrophically,

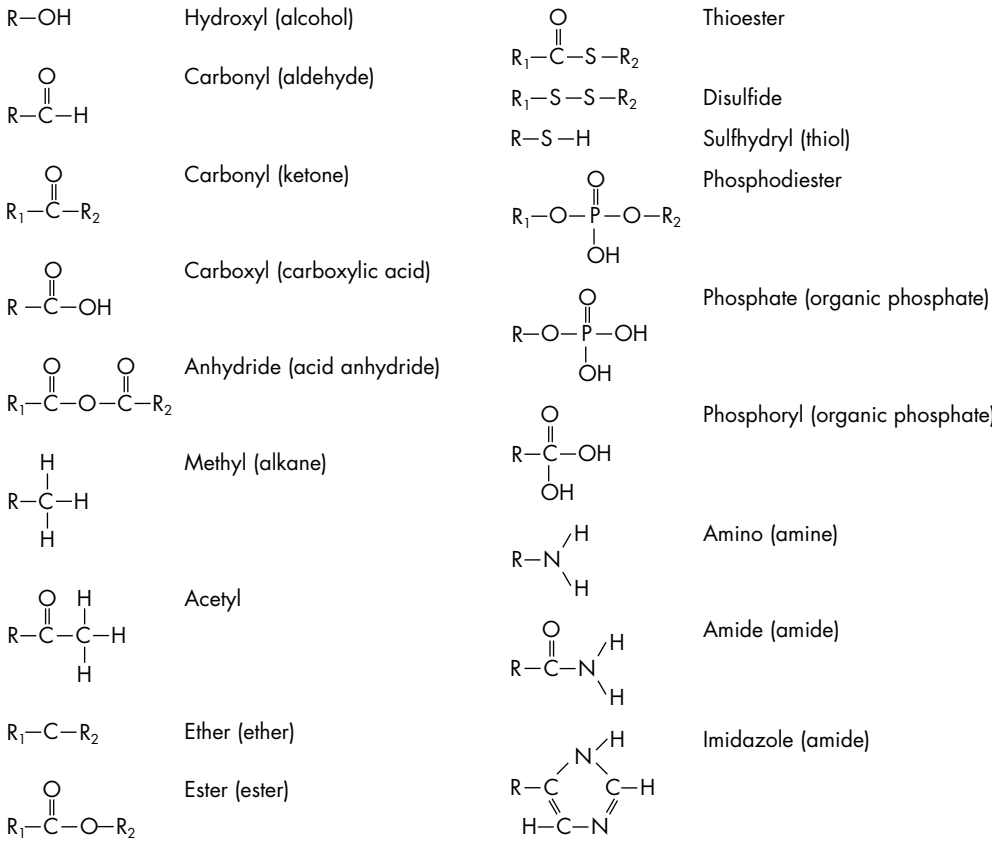


Figure 1.7 Some organic functional groups, shown in their protonated state. The symbol "R" is used to represent any substituents, but typically it is a carbon-containing moiety. When two substituents are shown in a molecule, they are designated "R_1" and "R_2".

whereas those that consume pre-existing organic materials act heterotrophically.

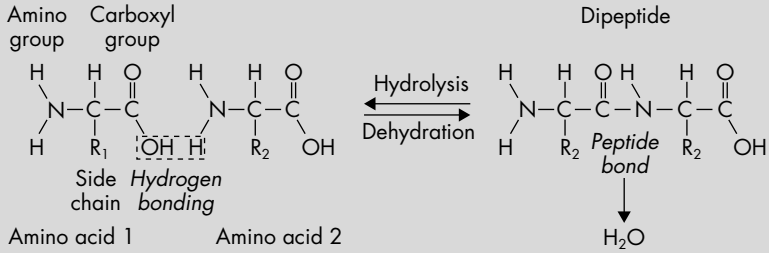
A living cell further requires a number of trace metals to fulfill essential biochemical roles in cell metabolism. For example, iron, nickel, copper, manganese, cobalt, molybdenum, and zinc are all important components of different metalloenzymes. Other trace metals, however, are toxic to the cell (e.g., arsenic, cadmium, copper, and mercury), and in order for a cell to survive when they are present in excess, they must actively prevent their intracellular transport. As such, microorganisms possess a suite of genes that serve to activate specific proteins designed either to facilitate metal transport into the cell or, alternatively, help rid the cells of them through their efflux, biomethylation, volatilization, or immobilization (e.g., Nies, 2000).

Because of the unique physicochemical properties of each individual ion or compound, a cell requires more than one transport mechanisms to obtain the full spectrum of solutes and compounds required. There are a wide variety of mechanisms utilized by microorganisms, of which four will be covered here. These include: (i) passive diffusion; (ii) facilitated diffusion; (iii) active transport; and (iv) cytosis.

Box 1.1 Organic macromolecules

Proteins

The importance of proteins to living organisms stems from their roles as enzymes, as well as their involvement in solute transport and cell structure. A single cell of *Escherichia coli*, a typical bacterium, contains about 1900 different kinds of proteins and 2.4 million total protein molecules (Madigan et al., 2003). The basic building blocks of proteins are amino acids, which in themselves comprise both amino and carboxyl functional groups, as well as an additional side group that distinguishes the various amino acids (there are 20 different kinds of amino acids commonly found in proteins). The side group may be a hydrogen atom, or a more complex organic molecule, such as a sulfhydryl (–SH) group or an aromatic ringed structure. The side chains also impact the surface characteristics of the amino acid because insertion of a carboxyl group makes it anionic, while insertion of methyl groups (CH_3) makes them more hydrophobic. Dipeptides result from the covalent bonding of the carboxyl group of one amino acid to the amino group of another, releasing water via dehydration. Further addition of amino acids would result in the formation of a long chain-like molecule called a polypeptide. Polypeptides can take on any number of more complex structures resulting from the way they fold in accordance with the positioning of the side groups and the way different polypeptides interact with one another in the same protein. This gives rise to the incredible diversity in protein structures.

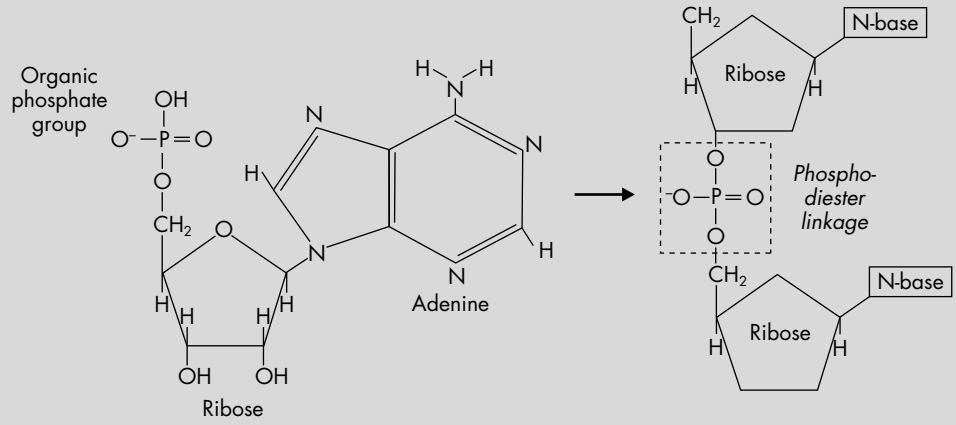


Nucleic acids

Nucleic acids consist of basic structural monomers called nucleotides, where each nucleotide contains a nitrogen-containing base, a pentose sugar, and an organic phosphate group. Two principal types of polynucleotides are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA contains the genetic information of the cell. RNA plays three crucial roles: (i) messenger RNA carries select genetic information from DNA in a single-stranded molecule; (ii) transfer RNA are adaptor molecules that translate mRNA during protein synthesis; and (iii) ribosomal RNA, of which several types exist, are catalytic components of the ribosome that carry out the synthesis of proteins. The nitrogen-containing bases are all ring structures, with either a single ring (e.g., the pyrimidines: thyamine, cytosine, and uracil) or a double ring (e.g., the purines: adenine and guanine). The pentose in DNA is deoxyribose, while the pentose in RNA is ribose. In a nucleotide, a nitrogenous base is attached to a pentose sugar by a glycosidic bond, while the sugars are held together by phosphodiester bonds. Polynucleotides, such as DNA and RNA, then form by covalent bonding between the phosphate of one nucleotide with the pentose of another. An extremely important nucleotide, aside from being a constituent in nucleic acids, is adenosine triphosphate (ATP). It functions as a carrier of chemical energy.

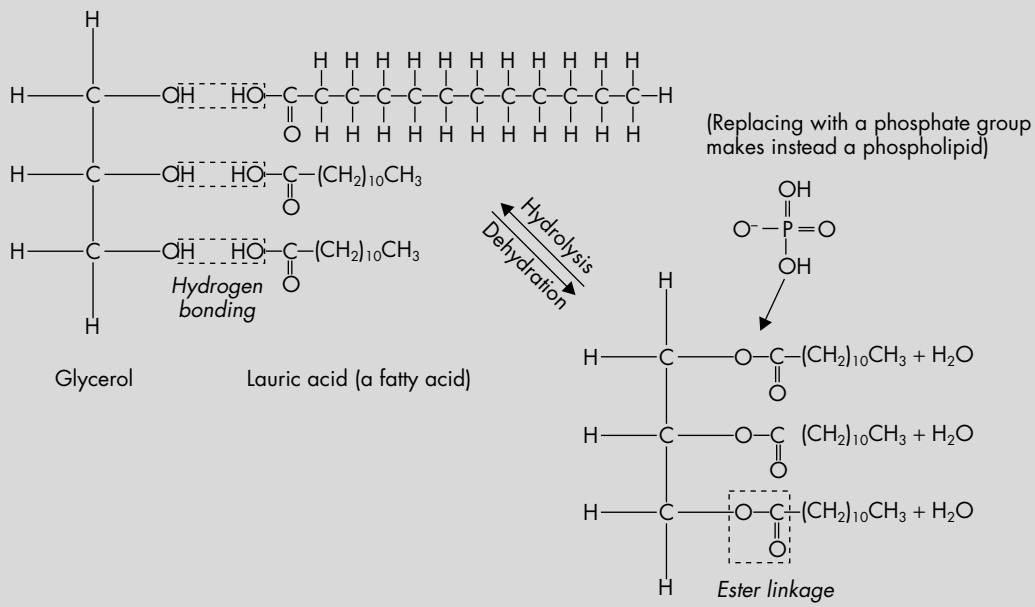
continued

Box 1.1 *continued*



Lipids

Lipids are a diverse group of organic compounds that are insoluble in water, but dissolve readily in nonpolar solvents such as alcohol. They are essential to the structure and function of cell membranes, as well as serving as fuel reserves. Simple lipids contain two monomers; an alcohol called glycerol and a group of hydrocarbons known as fatty acids. A fat molecule is formed when a molecule of glycerol combines with one, two, or three fatty acid molecules to form a monoglyceride, diglyceride, or triglyceride, respectively. The chemical bond that forms between a fatty acid and a glycerol molecule is called an ester linkage. Complex lipids form when phosphate, carboxylate (COO^-), or amino groups replace a fatty acid molecule – recall the



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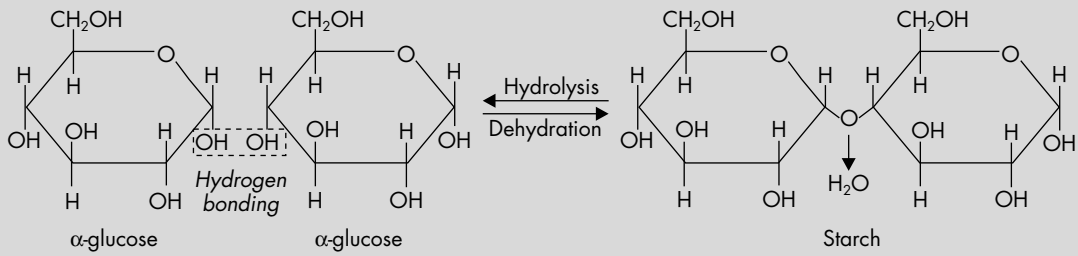
Box 1.1 *continued*

phospholipids in cell membranes. This can make the macromolecule amphipathic in nature, with a hydrophilic "head" that interacts with water and a hydrophobic "tail" that avoids water.

Carbohydrates

The basic building blocks of carbohydrates consist of simple sugars called monosaccharides, with each molecule containing three to seven carbon atoms. Pentoses (five-carbon sugars) and hexoses (six-carbon sugars) are extremely important to life because they include ribose (found in RNA and ATP), deoxyribose (found in DNA), and glucose (the main constituent of cell walls and an important energy reserve). Derivatives of monosaccharides can be formed by replacing one or more of the hydroxyl groups by another chemical species, such as through N acetylation (N replacing O in sugar and adding $-CH_3CO$ group) to form one of the sugar derivatives in peptidoglycan. Polysaccharides consist of eight or more monosaccharides covalently bonded to one another via glycosidic bonds. A high proportion of polysaccharides have simple repetitive structures of the same monosaccharide (e.g., starch, cellulose, chitin) or disaccharides (e.g., peptidoglycan). More complicated structures (e.g., heteropolysaccharides) may involve three or more sugars with perhaps a side-branch, or the repeating sequences can be interrupted (i.e., as in alginates). Polysaccharides can even combine with other macromolecules, such as protein or lipids, to form glycoproteins and glycolipids, respectively.



1 *Passive* – As discussed above, prokaryotes have evolved cell shapes that maximize their diffusional properties. The process of passive diffusion involves the movement of solutes across the plasma membrane due to a concentration gradient, i.e., from an area of high concentration to one that is lower, in order to achieve a state of equilibrium. During the process there is no consumption of energy. The rate of diffusion, in turn, is proportional to the difference in concentration between the external and internal environments, the permeability, and the total surface area. Cells commonly rely on this process to transport nonpolar, fatty-acid-soluble molecules, such as alcohols, or those molecules sufficiently uncharged, such as gases and water.

2 *Facilitated* – The hydrophobic nature of the inside membrane prevents the passive movement of soluble ions and simple organic molecules. Even a substance as small as a proton (H^+) is restricted because it is always hydrated as the charged hydronium ion (H_3O^+). If the ions or organic molecules have external concentrations in excess of the cytosol, then they can diffuse into the cell along a concentration gradient with the aid of specialized membrane transport proteins called permeases. Three kinds of permeases exist (Booth, 1988). Uniporters transfer single ions from one side of the plasma membrane to the other. Symporters move ions or organic molecules, with a coupling ion required for transport of the first, in the same direction. Antiporters move the ions one way, and

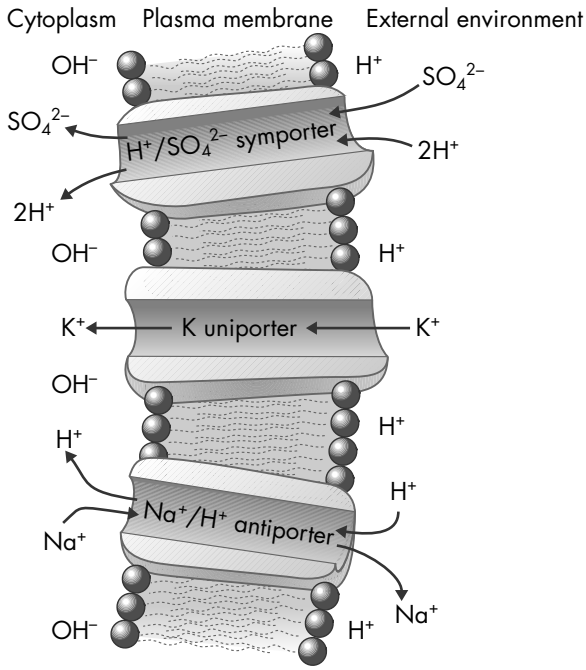


Figure 1.8 The three types of permeases; uniporters, symporters, and antiporters. Energy to drive the transport of ions (or simple organic molecules) towards the cytoplasm comes from the pH gradient between the outside and inside of the cell.

a secondary ion the other. Each permease is highly specific to the uptake of certain ions or simple organic molecules, and the cell has the means to regulate which permeases are active depending on its nutritional needs (Fig. 1.8).

3 *Active* – The concentration of most chemicals within the cytosol is generally higher than those outside the cell. Therefore, neither passive or facilitated diffusion allows the cells to acquire their necessary concentrations. As an alternative, active transport is an energy-dependent process that moves solutes or simple organic molecules against the concentration gradient. This process similarly uses permeases, with the energy derived from either ATP or an electrochemical gradient (see section 2.1.4(b)).

4 *Cytosis* – As a consequence of their more rigid cytoskeleton, protozoans use an additional mechanism called cytosis, in which organic molecules or solutes are moved in and out of a cell without passing through the plasma membrane. It essentially involves the plasma membrane wrapping around a substance, engulfing it to form a membrane-bound sphere called a vesicle, which can then open inside the cell and release the substance. Alternatively, it can separate from the plasma membrane and remain intact as a vesicle.

(b) Energy

All life forms require a source of energy to drive cellular biosynthesis and function. The ways in which organisms metabolize are covered in detail in Chapter 2, but at this point it is sufficient to point out that the energy can come either from the conversion of radiant energy into chemical energy via the process of photosynthesis or from oxidation-reduction reactions using organic or inorganic molecules. The former behave phototrophically, while the latter behave chemotrophically. The nutritional patterns among all organisms can thus be distinguished on the basis of both the carbon and energy sources (Table 1.1). Those that use light as an energy source and carbon dioxide as their chief source of carbon are called photoautotrophs. Those phototrophs that cannot convert CO_2 to organic carbon, but instead use organic compounds, are known as photoheterotrophs. Organisms that use inorganic compounds for energy and CO_2 as their carbon source are referred to as chemolithoautotrophs (or simply chemoautotrophs), while

Table 1.1 Nutritional classification of organisms.

Nutritional pattern	Carbon source	Energy source
Photoautotroph	CO_2	Sunlight
Photoheterotroph	Organic compounds	Sunlight
Chemolithoautotroph	CO_2	Inorganic compounds
Chemoheterotroph	Organic compounds	Organic compounds

those that use organic compounds for their carbon source are called chemolithoheterotrophs (or mixotrophs). When organic compounds are used for both energy and carbon the organism is a chemoorganoheterotroph (or simply chemoheterotroph). As we will see in later chapters, chemoheterotrophy is very important for element cycling because it couples the oxidation of organic carbon to the reduction of an oxidized compound, referred to as the terminal electron acceptor (TEA). The major TEAs are O_2, nitrate (NO_3^-), Mn(IV) oxides, Fe(III) oxyhydroxides, sulfate (SO_4^{2-}), or CO_2.

1.3.3 Growth rates

As a microbial population colonizes a new environment (whether in a laboratory experiment or in a natural setting) the microorganisms will collectively undergo a growth cycle that consists of the lag, exponential (or log), stationary, and death phases (Fig. 1.9). The lag phase marks the initial period of time when the cells adjust to their new surroundings, perhaps requiring the synthesis of new enzymes. Once acclimatized, the cells begin to reproduce very rapidly during the exponential phase, with, for instance, the bacteria *Escherichia coli* dividing on the order of every 20–30 minutes under typical laboratory batch culture conditions. Not only is this phase marked by significant increases in biomass, but it is also at this stage that the cells exhibit their most visible characteristics: the shape, color, density, and the way their colonies aggregate (Madigan et al., 2003). If exponential phase were to continue unchecked, an astronomical number of cells would result. In the example above, *E. coli* would produce a population of 2^{144} cells in just 48 hours, a population weighing about 4000 times the weight of the Earth! This of course does not happen because the cells would soon run out of required nutrients, their wastes would build up to toxic levels, and there may even be significant changes in the localized aqueous composition that would impact negatively on the cells. In reality, growth rates in nature are considerably slower than estimated in the lab, often less than 1% of the maximal experimental rates, because physiochemical conditions in natural environments are rarely ideal and indigenous populations must contend with competition for a limited suite of nutrients.

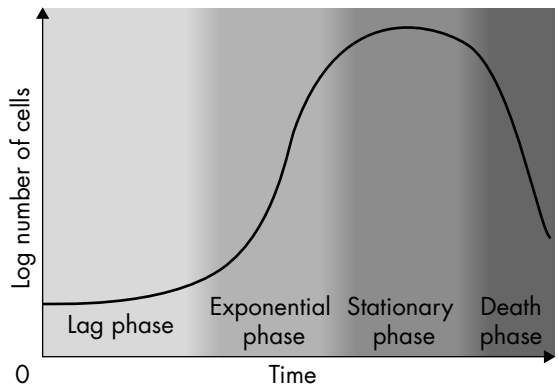


Figure 1.9 Typical growth curve for a bacterial population based on batch culture. Time (0) represents inoculation.

In the stationary phase, there is no net increase or decrease in overall cell number – some cells in the group actively grow while others die. But, if conditions continue to deteriorate for the entire population, a large number of cells enter the final death phase. Microorganisms are, however, extremely adaptable to adverse conditions, and if the supply of nutrients becomes diminished, they will employ a variety of compensatory strategies (Sunda, 2000). One typical response is for the cells to become smaller, thereby increasing their surface area to volume ratios to maximize their diffusional capabilities. This is observed in the surface waters of the oceans where there is a shift in species dominance from large cells to those that are extremely small (<2 μm), such as the so-called picoplankton. Cells also respond by growing at a slower rate, which increases cellular concentrations at a given uptake rate and decreases the metabolic demand for metalloenzymes. Some species also decrease their requirements for limiting metals by altering metabolic pathways or by changing the type of metal-containing enzyme in key pathways. For instance, under iron-limiting conditions, many marine species are able to replace ferrodoxin, an iron-containing protein, with flavodoxin, a nonmetalloprotein (e.g., La Roche et al., 1996).

If conditions worsen still, and the microorganism is potentially faced with starvation, it may respond by forming protective structures. Certain bacterial genera, such as *Bacillus* and *Clostridium*, form endospores (spores formed intracellularly) that are released into the environment once the parent cell decays. Then, as conditions improve, the endospore converts back to a vegetative cell. Other bacteria, such as *Methylosinus* species, form exospores (spores formed outside the cell – extracellularly) by growing or budding out from one end of the cell. Both types of spores are extremely durable to heat and chemicals, so much so that they can even survive conditions in space, leading to the hypothesis that life may have been transported to Earth from elsewhere in the solar system (see section 1.5.7). What is truly amazing is that some *Bacillus* spores apparently have been resuscitated after preservation in amber for 25–40 million years (Cano and Borucki, 1995) and in brine inclusions within salt crystals that are some 250 million years old (Vreeland et al., 2000), although these claims are controversial. Species of *Azotobacter*, *Bdellovibrio*, *Myxococcus* and some cyanobacteria instead form protective structures called cysts. These are thick-walled structures that, like spores, protect the microorganism from harm, but they are somewhat less durable than spores.

1.4 Microbial diversity

Microorganisms are the most ubiquitous life forms. Unlike multicellular life that is restricted to the Earth's surface, the adaptable nature of microorganisms has allowed them to inhabit the most diverse environments imaginable, often representing the only life forms. In most aquatic systems, microbial cell densities are remarkably similar, ranging from 10^5 to 10^6 cells ml^{-1}. Such consistency reflects less the overall microbial productivity than it does control of numbers by grazing (Fenchel et al., 2000). Much higher cell densities can, however, exist, wherever abundant nutrients and energy are available, and often where predation is minimized. One such setting is in the waters immediately surrounding warm, deep-sea hydrothermal vents, where cell densities up to 10^9 cells ml^{-1} have been reported (e.g., Corliss et al., 1979). The high microbial densities, in general, have important implications for global dispersal because microorganisms are unlikely to be restricted by any geographical barriers. Yet ubiquitous dispersal also means relatively low global species richness when compared with more complex organisms that have geographically restricted ranges (Finlay, 2002). In terms of global biomass, the total amount of microbial carbon is 60–100% of the estimated total carbon found in plants, with their total biomass estimated to be roughly $4–6 \times 10^{30}$ cells or 350–550 Pg of C, where 1 Pg = 10^{15} g

(Whitman et al., 1998). In addition, microorganisms contain 85–130 Pg of N and 9–14 Pg of P, which amounts to roughly ten times more nutrients than those stored in plants.

The vast majority of microorganisms in aquatic systems grow in microcolonies attached to submerged surfaces in the form of biofilms. The biofilms consist mainly of highly hydrated extracellular polymers (EPS) that are secreted by the microorganisms embedded within it. Benthic strategies can include growing on suspended sediment particles, plants and mineral surfaces (epilithic mode), the latter often leads to mineral weathering and metal corrosion (see Chapter 5). Biofilms are remarkably resilient communities where the cells live and are retained within protected adherent microcolonies, while taking advantage of the inorganic and organic compounds that preferentially accumulate at interfaces. As microbial populations grow, they eventually enshroud available surfaces, while at the same time dispatching mobile "swarmer" cells to reconnoitre neighboring niches and to establish new microcolonies in the most favorable of them (Costerton et al., 1994). Under ideal conditions, the biofilms thicken into what is commonly referred to as a microbial mat (see Chapter 6). These natural ecosystems contain many types of microbial species, and in any given part of the mat there exists a highly organized community where nutrients and metabolites are continuously recycled between cells in close proximity.

One of the primary goals of microbial ecologists has been the isolation and culture of microorganisms of interest. During enrichment culture techniques, specific media and growth conditions are chosen to duplicate as closely as possible the natural conditions of the desired microorganism. Unfortunately, the enrichment cultures favor some species over others, and a frequent outcome is that the microorganisms that thrive and dominate the culture conditions may only have comprised a small component of the natural population. Indeed, estimates suggest that some 99% of the microorganisms visualized microscopically in environmental samples are not cultivated by routine techniques (Amann et al., 1995). This has led to one of the greatest obstacles to understanding the diversity of natural microbial communities – our inability to make sound ecological inferences based on the metabolic properties of a few cultivatable species.

In order to circumvent the inherent culture-based biases, it was suggested that, by extraction of nucleic acids directly from environmental samples, genes that were present in all microorganisms (e.g., rRNA) could be isolated, sequenced, and compared with pre-existing cultures (e.g., Pace et al., 1986). Such a phylogenetic approach is now routinely used to study microbial diversity, and is often published in the form of a tree that strictly highlights the evolutionary relationship between the microorganisms, without actually inferring an evolutionary path beyond the species of interest (e.g., Fig. 1.10). Consequently, rRNA studies have provided a much more comprehensive view of the microbial world, particularly in the sense that novel species are continuously being reported to exist in environments where we previously thought we had a firm understanding of the microbial ecology (e.g., Dojka et al., 2000).

Ribosomal RNA genes are obtained from DNA isolated directly from natural samples. However, these genes need to be separated from all the other genes in the genomic DNA, and the quickest way to this is to specifically amplify rRNA using the polymerase chain reaction or PCR, a method that multiplies DNA by up to a billion-fold in a test tube – only very small amounts of DNA are initially required to obtain workable amounts of rRNA. Once the different 16S rRNA genes have been amplified from environmental DNA, they have to be sorted or separated so their sequences can be identified. There are numerous approaches to separate this mixture of 16S rRNA genes, including cloning, which separates individual genes into cloning vectors that are then assimilated by a large number of laboratory cultures (also known as a clone library), or more rapid approaches

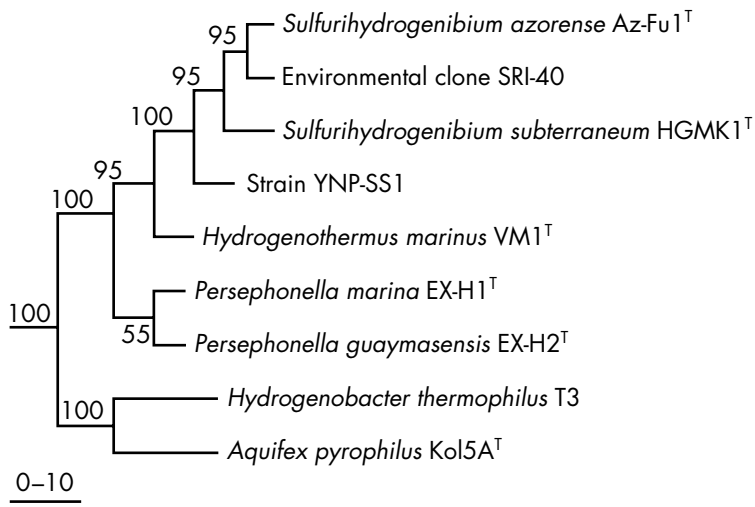


Figure 1.10 Maximum-likelihood phylogenetic tree showing the position of various members of the *Aquificales*, the purported deepest branching lineage within the domain *Bacteria*. The length of the scale bar represents evolutionary distance by the number of fixed mutations per nucleotide position. The numbers associated with the branches are a statistical measure of the confidence of divergence between two lineages (expressed in percentages) based on an algorithmic re-sampling known as bootstrapping. (From Aguiar et al., 2004. Reproduced with permission from the Society for General Microbiology.)

such as denaturing gradient gel electrophoresis (DGGE), which separates different genes by sequence in the form of individual bands on an acrylamide gradient gel. In both cases, the environmental sequences can then be compared with sequences from known cultured isolates found in reference databases such as GenBank (http://www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu). Molecular phylogenetic trees are then constructed by placing the unknown environmental sequences within a phylogenetic framework with their closest cultured relatives (see Theron and Cloete, 2000 for details).

At this stage, we still have very little idea of what the relative abundance of some of these environmental sequences are. To determine this, one can use the sequence obtained from the environmental analysis to design a probe that will identify the cell from which the sequence was originally isolated (e.g., Devereux et al., 1992). These probes are short fluorescently tagged oligonucleotides that bind specifically to the ribosomal RNAs of the probe-target population. This method, known as fluorescence in situ hybridization (FISH), provides a way to visualize the community spatial distribution, and the ability to design these probes to detect groups of related microorganisms means that they can be used to identify and enumerate similar types of microorganisms even if they cannot be grown in culture (e.g., Fig. 1.11). However, the amount of rRNA present in a microorganism is proportional to its metabolic activity. Inactive cells have too few rRNA molecules within their cells to be detected using these techniques and thus only active members of a microbial community contain enough ribosomes to yield a detectable signal (e.g., DeLong et al., 1989). Natural samples can also be subjected to multiple FISH probing, where a suite of probes, each deigned to react with a specific microorganism or group, can lead to phylogenetically characterizing the diversity of an entire population.