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MICROBIAL ECOLOGY: BEGINNINGS AND THE ROAD FORWARD

1.1 CENTRAL THEMES

- Interdisciplinary studies addressing the origin and evolution of life stimulate many ongoing conversations and research activities.
- Prokaryote classification is based on biochemical and physiological activities as well as structures including cell morphology. Classification within Bacteria and Archaea domains is complicated because the definition for a prokaryotic species is currently under review.
- Our knowledge of the microbial diversity of Earth is growing exponentially with the discovery and implementation of molecular phylogeny to study environmental microbiology.
- Configuration of the "tree of life" has changed since the 1990s with the use of molecular and genomic techniques to evaluate microbial relationships.
- Microbial ecology as a discipline will benefit substantially from the development of a theoretical basis that draws on principles identified in general ecology.

Microbial Ecology, First Edition. Larry L. Barton, Diana E. Northup

1.2 INTRODUCTION

The study of microbial ecology encompasses topics ranging from individual cells to complex systems and includes many different microbial types. Not only is there a visual difference in examining pure cultures and unique microbial environments (see Figure 1.1), but also there is a difference in study approach in each of these images. Microbial ecology has benefited from studies by scientists from many different scientific fields addressing environments throughout the globe. At this time there is considerable interest in understanding microbial community structure in the environment. To achieve this understanding, it is necessary to identify microbes present; this can be

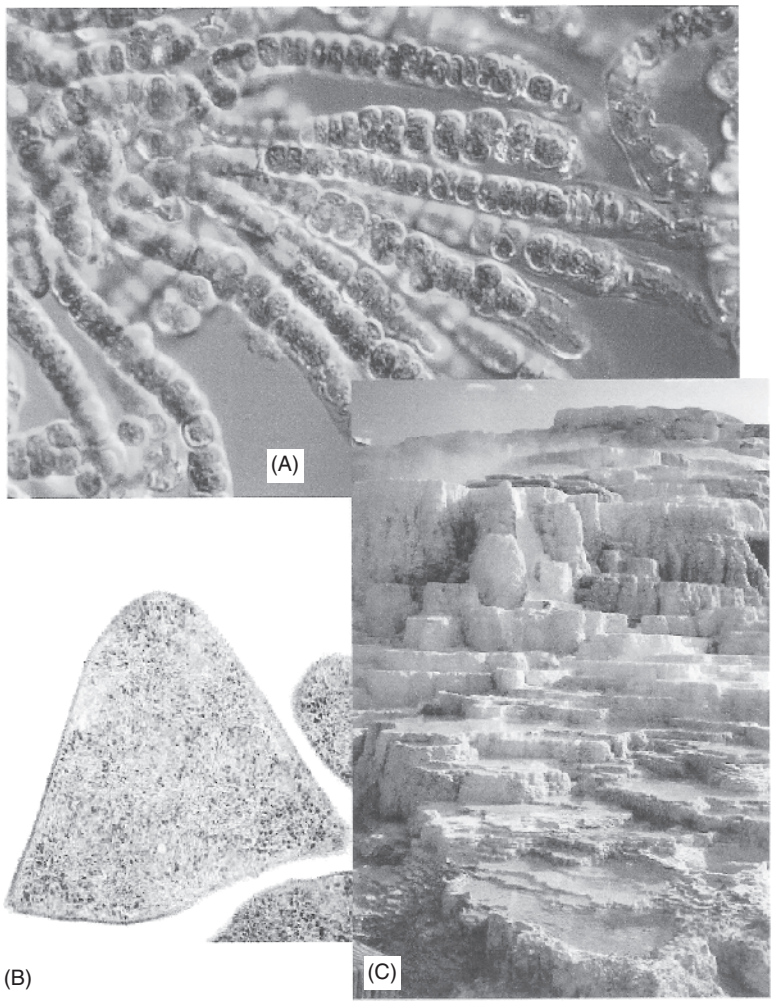


Figure 1.1. Understanding our environment through the study of cells and systems: (A) *Fischerella* sp; (B) electron micrographs of the triangular archaea, *Haloarcula japonica* TR-1 (provided by Yayoi Nishiyama); (C) Mammoth Hot Springs in Yellowstone National Park. (Photos A and B courtesy of Sue Barns). See insert for color representation.

accomplished by using molecular methods even though the microbes have not been cultivated in the laboratory. Enzymatic activities of microorganisms and microbial adaptations to the environment are contributing to our knowledge of the physiological ecology of microorganisms.

Persistent questions about microorganisms in the environment include:

- Which microbes are present?
- What is the role of each species?
- What interactions occur in the microbial environment?
- How do microbes change the environment?

While this book provides some answers to these questions, each discovery brings with it more questions. The objective of this book is to emphasize the basics of microbial ecology and to explain how microorganisms interact in and with the environment.

1.2.1 Roots of Microbial Ecology

For centuries and long before bacteria were known, people from different regions around the world used selective procedures to influence the production of desired foods. Starter cultures were passed throughout a community to make fermented milk, and common procedures were used for fermentation of fruit juices. Pickling procedures involving normal fermentations were customary for food preservation. In various regions of the world, increased production of rice resulted from specific practices that we now understand select for the growth of nitrogen-fixing cyanobacteria. Some consider that microbiology started with the reports by Anton van Leeukenhoek (1632–1723) in 1675 with the description of "very little animacules" that have the shape of bacteria, yeast, and protozoa. The environments that van Leeuwenhoek examined included saliva, dental plaque, and contaminated water. Gradually, information on microorganisms appeared as scientists in various countries explored the environment through direct observations or experimentation (Brock 1961; Lechecalier and Solotorovsky 1965). Early discoveries relevant to microbial ecology are listed in Table 1.1 (Schlegel and Köhler 1999). The contributions of scientists to disprove the "doctrine of spontaneous generation" had a great impact on microbiology, and especially important was the presentation by Louis Pasteur (1822–1895) in 1864 at the Sorbonne in Paris. In addition to studying the role of microorganisms in diseases and their impact on our lives, Pasteur emphasized the importance of microorganisms in fermentation. Many consider that the founders of microbial ecology were Sergei Winogradsky (1845–1916) and Martinus Beijerinck (1851–1931), who were the first to demonstrate the role of bacteria in nutrient cycles and to formulate principles of microbial interactions in soil. Beijerinck worked at the Delft Polytechnic School in The Netherlands, where he developed the enrichment culture technique to isolate several bacterial cultures, including those now known as *Azotobacter, Rhizobium, Desulfovibrio*, and *Lactobacillus*. Also, Beijerinck's early studies contributed to the demonstration of the tobacco mosaic virus and provided insight into the principles of virology. Winogradsky was a Russian soil microbiologist who developed the concept of chemolithotrophy while working with nitrifying bacteria. In addition to demonstrating that bacteria could grow autotrophically with CO_2 as the carbon source, Winogradsky established the concept of nitrogen fixation resulting from his experimentation with *Clostridium pasteuranium*.

TABLE 1.1. Pioneers in the Field of Microbial Ecology

Year	Individual	Contribution
1683	Antonie van Leeuwenhoek	Published drawings of bacteria showing rods, cocci, and spirals
1786	Otto Friedrich Müller	Reported the characteristics of 379 different species in his publication *Animalcules of Infusions, Rivers and the Sea*
1823	Bartholomeo Bizio	Described the "blood" drops in "bleeding" bread used in communion rites as attributed to *Serratia marcescens*
1837	FriedrichTraugott Küzing, Charles Cagniard-Latour, and Theodor Schwann	Independently published papers stating that microorganisms were responsible for ethanol production
1838	Christian Gottfried Ehrenberg	Described *Gallionella ferruginea* as responsible for ocher
1843	Friedrich Traugott Kützing	Described *Leptothrix ochracea*, a filamentous iron-oxidizing bacterium
1852	Maximilian Perty	Described several species of *Chromatium* including *C. vinosum*
1866	Ernst Haeckel	Proposed the term *ecology*
1877	Theophile Schoesing and Achille Muntz	Demonstrated that microorganisms were responsible for nitrification ($NO_3^- \rightarrow NH_3$)
1878	Anton de Berry	Proposed concepts of mutalistic and antagonistic symbiosis
1885	A. B. Frank	Described the fungus–root symbiosis known as *mycorrhiza*
1886	H. Hellriegel and H.Wilfarth	Demonstrated that root nodules on legumes supplied nitrogen to plants
1889	Matrinus W. Beijerinck	Developed enrichment technique that produced pure cultures of many bacteria in nitrogen–sulfur cycle
1889	Sergus N. Winogradsky	Established concept of chemolithotrophy and autotrophic growth of bacteria
1904	L. Hiltner	Studied the biology of the root zone and proposed the term *rhizosphere*
1909	Sigurd Orla-Jensen	Presented a natural system for arrangement of bacteria with lithoautotrophs as the most primitive bacteria

With an increased interest in microbiology, it became apparent that there was a highly dynamic interaction among microorganisms and also between microorganisms with their environment. Today the study of microbial ecology includes many different fields, and these are addressed in subsequent chapters of this book.

1.2.2 Current Perspectives

The study of microbial ecology includes the influence of environment on microbial growth and development. Not only do physical and chemical changes in the environment select for microorganisms, but biological adaptation enables bacteria and archaea to optimize

the use of nutrients available to support growth. The prokaryotic cell was the perfect system for early life forms because it had the facility for rapid genetic evolution. As we now understand, horizontal gene transfer (Section 4.7.2) between prokaryotes serves as the mechanism for cellular evolution of early life forms to produce progeny with diverse genotypes and phenotypes. While fossils provide evidence of plant and animal evolution, fossils can also provide evidence of early animal forms that have become extinct. It is an irony in biology that the same prokaryotic organisms that evolved to produce eukaryotic organisms also participated in the decomposition of dinosaurs and other prehistoric forms. The prokaryotic form of life not only persists today but thrives and continues to evolve. It has been estimated that there are more living microbial cells in the top one inch of soil than the number of eukaryotic organisms living above ground. William Whitman and colleagues have estimated that there are 5×10^{30} (five million trillion trillion) prokaryotes on Earth, and these cells make up over half of the living protoplasm on Earth (Whitman et al. 1998). The number of bacteria growing in the human body exceeds the number of human cells by a factor of 10 (Curtin 2009). While it is impossible to assess the role of each of these prokaryotic cells, collectively groups of prokaryotic cells can have considerable impact on eukaryotic life. Analysis of the human microbiome reveals that although the microbial flora of the skin is similar, each human has a bacterial biome that is unique for that individual (Curtin 2009). Not only are microorganisms important in cycling of nutrients but they have an important role in community structure and interactions with other life forms. It would be impossible to envision life on Earth without microorganisms. Before addressing important divisions in microbial ecology, it is useful to reflect on the development of microbes on Earth.

1.3 TIMELINE

Formation of Earth occurred about 4.5 billion years ago, and this was followed by development of Earth's crust and oceans. Volcanic and hydrothermal activities of Earth released various gases into the atmosphere. In addition to water vapor, dinitrogen (N_2), carbon dioxide (CO_2), methane (CH_4), and ammonia (NH_3) were the major atmospheric gases, while hydrogen (H_2), carbon monoxide (CO), and hydrogen cyanide (HCN) were present at trace levels. Chemical developments of prebiotic Earth relevant to the evolution of life have been critically reviewed by Williams and Fraústo da Silva (2006). The anaerobic environment on Earth provided the reducing power for the formation of the first organic compounds.

Early life forms were anaerobes that included thermophilic H_2-utilizing chemolithotrophs, methanogens, and various microbes displaying dissimilatory mineral reduction. Hyperthermophilic prokaryotes are proposed to have been one of the earliest life forms, and Karl Stetter has collected over 1500 strains of these organisms from hot terrestrial and submarine environments (Stetter 2006). There is considerable abundance of these microorganisms in the environment, with 10^7 cells of *Thermoproteus* found in a gram of boiling muds near active volcanoes, 10^8 cells of *Methanopyrus* found in a gram of hot vent chimney rock, and 10^7 cells of *Archaeoglobus* and *Pyrococcus* found per milliliter (mL) of deep subterranean fluids under the North Sea (Stetter 2006). While the hyperthermophiles characteristically grow at 80–113°C with a range of pH 0–9.0, one archaeal species, *Pyrolobus fumarii*, withstands one hour in an autoclave that has a temperature of 121°C. Currently, about 90 species

TABLE 1.2. Examples of Hyperthermophilic Prokaryotes

Genera of Archaea	Genera of Bacteria
Acidianus	
Archaeoglobus	*Aquifex*
Ferroglobus	*Desulfurobacterium*
Igniococcus	*Thermocrinis*
Metallosphaera	*Thermotoga*
Methanopyrus	*Thermovibrio*
Methanothermus	
Nanoarchaeum	
Pyrococcus	
Pyrodictium	
Pyrolobus	
Sulfolobus	
Thermococcus	
Thermofilum	
Thermoproteus	

of microorganisms are hyperthermophiles, and some of these species are listed in Table 1.2. Most hyperthermophiles are chemolithotrophic organisms using molecular hydrogen (H_2) as the electron source for energy-yielding reactions. While many of the hyperthermophilic archaea use S^0 as the electron acceptor, some hyperthermophiles can couple growth to the use of Fe^{3+}, SO_4^{2-}, NO_3^-, CO_2, or O_2 as electron acceptors. Molecular oxygen (O_2) is a suitable electron acceptor for a few hyperthermophilic archaea, and in these cases only under microaerophilic conditions. Hyperthermophilic bacteria usually require organic material to support their anaerobic or aerobic growth. Many of the anaerobes have active systems using H_2 as the electron donor.

The biological production of methane is considered to be an ancient process and would have been attributed to prokaryotes catalyzing the following reaction:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

When organic compounds such as acetate accumulated in the environment, methanogens could have produced methane from methanol, formate, or acetate. Only members of the Archaea domain are capable of methane production.

Chemoautotrophic microbes could have evolved to grow on the energy from oxidation of molecular hydrogen and reduction of carbon dioxide according to the following reaction:

$$2H_2 + CO_2 \rightarrow H_2O + [CH_2OH] \text{ (carbohydrate)}$$

In addition to the production of H_2 from geologic formations, ultraviolet radiation could have released H_2 according to the following reaction:

$$2Fe^{2+} + 4H^+ \rightarrow 2H_2 + 2Fe^{3+}$$

Another source of H_2 would be the radiolysis of water attributed to alpha radiation (Landström et al. 1983). With the accumulation of diverse organic compounds in the

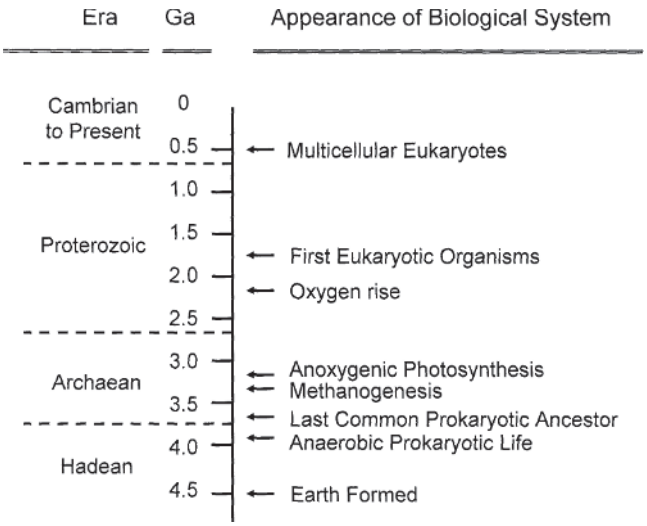


Figure 1.2. Early development of life.

environment, heterotrophic prokaryotes metabolizing organic carbon materials would have appeared sometime after the chemoautotrophs were established.

As presented in Figure 1.2, anaerobic photodriven energy activities may have been present ~3 billion years ago, using light to activate bacteriorhodopsin-like proteins to pump ions across cell membranes. The bacteriorhodopsin type of photodriven energetics would have been followed by chlorophyll-containing anoxygenic bacterial photosynthesis involving purple and green photosynthetic bacteria where H_2S was the electron source. While microbial evolution was initially in the marine environment, microorganisms may have migrated to dry land about 2.75 billion years ago (Rasmussen et al. 2009). Cyanobacteria with oxygenic photosynthesis produced the aerobic atmosphere, and this has been called the "great oxidation event." Since O_2 was produced from water by the photocatalytic process, the rate of O_2 released was not limited by availability of water.

Once molecular oxygen was released into the atmosphere, it reacted with reduced iron and sulfur compounds (i.e., FeS and FeS_2) to produce oxidized inorganic compounds by both microbial and abiotic processes. Gradually the O_2 level in Earth's atmosphere increased and by ~1.78–1.68 billion years ago oxygen respiration could have been used to support the growth of the first single-cell eukaryotes (Rasmussen et al. 2008). Another important development of an aerobic atmosphere was the generation of ozone (O_3) from O_2 due to a reaction with ultraviolet light. Ozone absorbs ultraviolet light and forms a protective layer in the atmosphere to shield Earth from destructive activity of ultraviolet radiation (Madigan et al. 2009). Prior to the development of an ozone layer, microorganisms would have been growing only in subsurface areas or in environments shielded by rocks.

1.4 MICROFOSSILS

Fossils are important for understanding the evolution of plants and animals; however, there are few fossils available for microorganisms. Dating of dinosaur presence can

Figure 1.3. Dinodaur footprints present on surface stone in Texas (A) and Arizona (B). [Photograph (A) by Diana Northup, (B) by Larry Barton]. See insert for color representation.

be derived from bone fragments or footprints left in mud (Figure 1.3). As depicted in Figure 1.4, footprints can provide considerable information about the presence of life; however, the early history of microorganisms is relatively sparse. Electron microscopy of aggregates found in the Archean Apex chert of Western Australia revealed cell-like structures characteristic of cyanobacterial trichomes, and these were reported to be 3.5 billion years old (Schopf 1993). However, the inability to demonstrate appropriate biomarkers in the microfossils has generated concern about the dating of these images (Rasmussen et al. 2008). Fossilized stromatolites (see Section 11.10 for additional information) consisting of mats of cyanobacteria and other microorganisms were reported to be present in rocks from the Warrawoona Group in Western Australia. Images of bacteria are suggested in scanning electron micrographs of rocks that are 3.4 billion years old from the Barberton Greenstone Belt, South Africa. From carbonaceous chert in the Ural Mountains there are structures resembling the bacterium *Gleodiniopsis*, and this has been dated to be 1.5 billion years old. Microfossils of the cyanobacterium *Palaeolyngbya* are 950 million years old and were found in the Khabarousk region in Siberia.

Konhauser (2007) has critiqued the use of Archean microfossils in dating primitive aerobic phototrophs. Some scientists maintain that the mere presence of kerogen in microfossils is not sufficient to indicate biogenic origin. Biomarkers useful in suggesting the presence of prokaryotes would be the lipid soluble hopanes and steranes that would be derivatives of hopanoids and sterols, respectively. Degradation products of these compounds are useful in assessing the biogenic character of microfossils because hopanoids are lipids characteristically found in the plasma membrane of prokaryotes and sterols are typically found in the membranes of eukaryotic cells. An additional significance in finding derivatives of sterols in microfossils is that molecular O_2 is required for one of the final enzyme steps in the biosynthesis of sterols. Of course, definitive proof of life in the microfossils would be the detection of DNA or decomposition products of DNA.

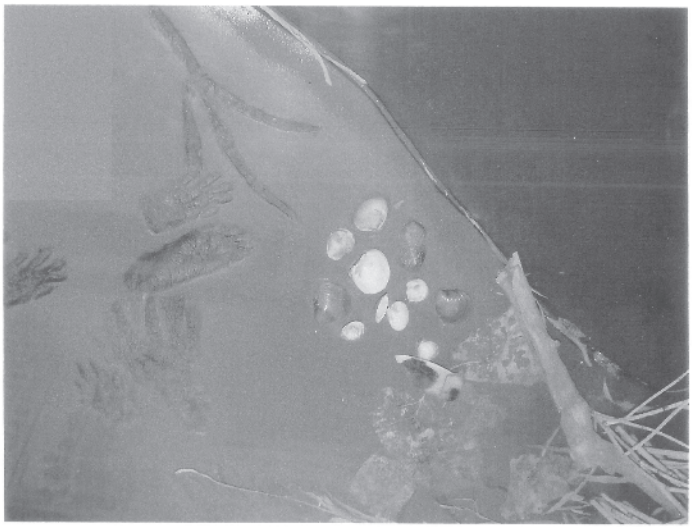


Figure 1.4. Examples of organisms present in a specific environment: footprints of several animals and shell records; exhibit at the educational center in Albuquerque museum (photograph by Larry Barton). See insert for color representation.

1.5 EARLY LIFE

The origin of life on Earth is a topic that has attracted the attention of many scientists and has resulted in publication of numerous fascinating opinions. In a more recent review, Koch and Silver (2005) discuss the stages required in development of chemical processes into a biological unit. The transition from an abiotic environment to a world with microorganisms is summarized in Figure 1.5. Using cellular evolution as a perspective, early development of the evolutionary tree of life could be divided into various phases (Koch and Silver 2005): (1) the *pre-Darwinian phase*, which represents Earth's environment prior to the formation of a cell; (2) the *proto-Darwinian phase*, during which the first cell was formed; and (3) the *Darwinian phase*, which involved selective pressures on cell development that favored diverse forms of prokaryotes and eukaryotes.

1.5.1 The Precellular World

The *precellular phase* would involve astrophysical and geochemical activities at a time before the presence of biological cells. The activities involved in formation of small organic molecules (e.g., sugars, amino acids, lipids, porphyrins, nucleotides, heterocyclic bases) may have been unrelated. There are several different opinions concerning the energy sources and sites or regions where synthesis of organic molecules may have occurred. Wächtershäuser (1990) proposed that the organic macromolecules were produced on clay-like surfaces, while Koch (1985) and Deamer (1997) supported the idea that vesicles enclosed with membrane-like structures were involved in the formation of organic molecules. Some have supported the idea that life arose from a "primordial soup" in a lake on the surface of Earth, while others consider that life arose from a subsurface

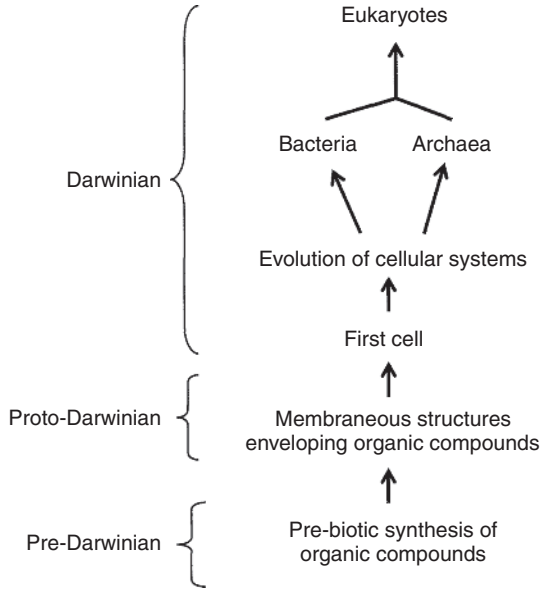


Figure 1.5. Evolutionary development of early life [modified from Koch and Silver (2005)].

spring. All of these theories provide for an interesting interplay of geochemical processes that may have culminated in biological activity.

1.5.2 The First Cell

Prior to the first living cell, various organic compounds presumably accumulated in the environment. Koch and Silver (2005) propose that prebiotic compounds could have included nucleic acid inside a vesicle and that the vesicle had a mechanism for generating an ionic charge across the membrane barrier. It was not necessary for this first cell-like unit to have enzymes for metabolism, nor was there a requirement for ATP, ribosomes, proteins, or DNA. The presence of a self-replicating single-stranded RNA with autocatalytic activity, also known as *ribozyme*, could provide a basis for development of molecular biology in this evolutionary process. The membrane provided a lipid closure for the vesicle, and in terms of structure and composition the early membrane may have been different from current unit membranes.

Energy is paramount for development of life and could have resulted from the following reaction:

$$H_2S + FeS \rightarrow FeS_2 + H_2 \qquad (\Delta G^{\circ\prime} = -42 \text{ kJ})$$

The oxidation of inorganic compounds (see Section 11.4 for additional information), such as given in the reaction above, could have provided the potential for various reactions, including the generation of an ionic gradient across the membrane. This vesicular structure would not yet be a cell but could evolve into a cell after acquiring DNA, proteins for metabolism, ribosomes, ATP, and related components. The presence of RNA in the first membrane vesicle would have been useful because even a small RNA molecule is highly charged and could nonspecifically bind protein and small organic molecules found

in the environment. DNA replaced RNA as the molecule carrying genetic information and was more stable than RNA. Undoubtedly, the time required for development of the first self- replicating unit (cell) was considerable, but once this process was achieved, cellular evolution proceeded at an accelerated rate. The extent of evolution by eukaryotes is apparent when reviewing the diversity of eukaryotic life forms, but it should be recalled that eukaryotes have been on Earth for only one-third as long as prokaryotes.

1.5.3 Development of Cellular Biology

With the presence of DNA and other protein-synthesizing materials inside the membrane vesicle, the cell had the capability for heredity with new phenotypes expressed. Evolution leading to different lifestyles and life forms could follow selection based on the hypothesis of Alfred Wallace and Charles Darwin. The bacterial and archaeal species surviving and reproducing in an environment were the ones capable of dealing with that environment. The evolutionary process was not continuous, but changes in genetic information would have been displayed by periodic environmental changes providing the selective pressure that led to new cell types. Genetic variation in these asexual microorganisms would be attributed to mutations and horizontal (lateral) gene transfer (Section 4.7.2). There is no record suggesting the events responsible for the universal ancestor to produce two lineages of prokaryotes (i.e., Bacteria and Archaea). Many of the biomolecules and biochemical processes found in Bacteria and Archaea are similar, but numerous details in accomplishing certain activities distinguish organisms of these two domains. Since prokaryotes were the only living organisms on Earth for over 2 billion years, it is rather remarkable that only two prokaryotic cell types were produced.

One theory for the formation of a eukaryotic cell is the establishment of a nucleus prior to the development of mitochondria and chloroplasts by endosymbiosis (see Section 8.2 for additional information). The *genome fusion hypothesis* has been developed to explain the formation of the eukaryotic nucleus where the eukaryotic genome arose from a combination of archaeal and bacterial genes. An examination of energy production and chemistry of lipids in the cell membrane reveals that eukaryotic cells are more similar to Bacteria than to Archaea. However, when examining transcription and translation processes, eukaryotes have characteristics of the Archaea. As the genome of the ancestral eukaryote increased in size, chromosomes were developed to enhance organization of DNA, and it has been proposed that the nuclear membrane arose spontaneously to segregate DNA from the cytoplasm. More recently it has been discovered that one bacterial species has a "primitive" nuclear membrane (see Section 3.8.3), and the function of this internal membrane is unresolved.

The *endosymbiotic hypothesis* (see Section 8.2) addresses the origin of chloroplasts and mitochondria where both of these organelles developed from bacteria. Lynn Margulis (see "Microbial spotlight" in Chapter 8) suggests that the formation of the eukaryotic cell is a product of several sequential endosymbiotic steps. Spirochete bacteria were an early surface symbiont with an anaerobic organism resulting in motility of the eukaryotic cell. Endosymbiotic activity contributed to the development of mitochondria and chloroplasts. The endosymbyote provided the host with a capability useful to the host cell, while the endosymbiont benefited from nutrients and a safe environment provided by the host. Some have proposed that the primitive eukaryotic cell receiving the endosymbiont was derived from the archaeal cell line. Genes for the synthesis of bacterial-like membranes may have been transferred to the host archaeal cell and may have promoted the early

development of cytoplasmic membranes. The genome of *Rickettsia prowazekii*, a member of the Alphaproteobacteria, is remarkably similar to the mitochondrial genome, and additional inspection is required to determine whether it was the source of the mitochondria or if both the mitochondria and rickettsia evolved from a common ancestor. Most likely chloroplasts developed in the cell line producing higher plants. Chloroplasts in green algae and higher plants could have evolved from *Prochloron*, a cyanobacterium, because it is the only aerobic photosynthetic cell that has both chlorophyll *a* and *b*.

An alternate idea pertaining to development of organelles in eukaryotes is the *hydrogen hypothesis*. The endosymbiont in this situation is proposed to be an anaerobic member of the Alphaproteobacteria that releases CO_2 and H_2 as end products. This endosymbiont is proposed to evolve along two distinct lines to produce a hydrogenosome for anaerobic metabolism and a mitochondrion for aerobic respiration. The hydrogenosome (Figure 1.6) would obtain ATP from pyruvate metabolism with the release of CO_2 and H_2. From genome analysis, it appears that there is considerable similarity between the genomes of hydrogenosomes and mitochondria.

1.5.4 Evolution of Metabolic Pathways

The origin and evolution of metabolic pathways were important for molecular evolution and are attracting considerable attention (Canfield et al. 2006; Falkowski et al. 2008; Fani and Fondi 2009; Fondi et al. 2009). Many consider that ancestral cells, in comparison to current prokaryotic cells, had relatively few genes, no gene regulation, and no mobile genetic elements. While early cells may have had only a few hundred genes, the expansion of the genome to several thousand genes per cell could be explained by the "patchwork" hypothesis (Jensen 1976; Ycas 1974), in which genes encoding for enzymes of low specificity were duplicated, and through selective pressures evolved into genes encoding for enzymes of considerable specificity. In terms of gene duplication there could be duplication of the entire gene, a part of a gene, or several genes from the same or different

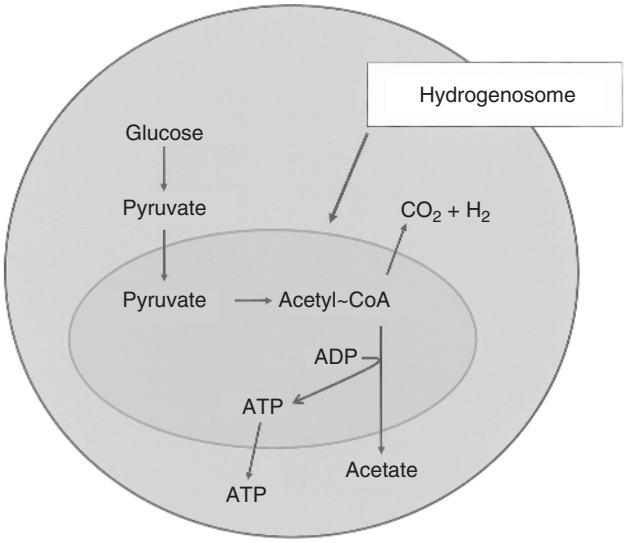


Figure 1.6. Hydrogenosome in the eukaryotic cell.

metabolic pathway. Gradually the primordial cells expanded their metabolic capabilities and established regulatory mechanisms. Cells with efficient metabolic pathways were selected through pressures of population growth. Genetic exchange between cells involving horizontal gene transfer and fusion of protoplasmic prokaryotic cells would have been important in early evolutionary processes. Initially it may have been more important for transfer of operational genes than for transfer of genes involved in information processing (transcription, translation, etc.). Abiotic geochemical cycles were replaced (or supplemented) by biotic processes, resulting in interconnection of biogeochemical cycles.

1.6 CHARACTERISTICS OF MICROBIAL LIFE

The characteristics of life that have become associated with microorganisms are similar to those of higher plants and animals. A distinguishing feature is that for microorganisms a cell constitutes the individual while with higher forms of life the individual is multicellular and even contains numerous tissues. The biochemical and physiological processes seen in microorganisms are compared in Table 1.3. Introductory courses in biology include a listing of the characteristics defining life, and it is important to reflect on these characteristics of life since they also pertain to prokaryotes. The following discussion addresses how bacteria and archaea conform to the requirements of a defined structure, metabolism, growth, reproduction, and response to stimulus.

1.6.1 Structure and Evolution of Cell Shape

Cells of microorganisms have a precise organization and their structure is continuous with their progeny. While crystals of minerals show organization due to alignment of inorganic atoms, differences in crystal organization occur as seen in the differences in the structure of snowflakes. Structural organization in microbial cells reflects the molecular alignment in membranes, ribosomes, protein cell walls, DNA, and other macromolecules. The molecular architecture in the cell walls of microorganisms is reproduced in the progeny of each species. An example of this structural organization is seen in the mosaic arrangement seen on the surface of bacteria and archaea that have been designated as the S layer. Glycoproteins form a lattice with the precision of crystalline minerals, and models of the lattice are shown in Figure 1.7.

TABLE 1.3. Selected Phenotypic Characteristics of Bacteria, Archaea, and Eukarya

Characteristic	Bacteria	Archaea	Eukarya
Dissimilatory reduction of SO_4^{2-} or Fe^{3+}	Yes	Yes	No
Nitrification	Yes	Yes	No
Denitrification	Yes	Yes	No
Nitrogen fixation	Yes	Yes	No
Chemolithotrophy	Yes	Yes	No
Methanogenesis	No	Yes	No
Oxygenic photosynthesis (chlorophyll-based)	Yes	No	Yes
Anaerobic photosynthesis (chlorophyll-based)	Yes	No	No
Rhodopsin-based energy metabolism	Yes	Yes	No

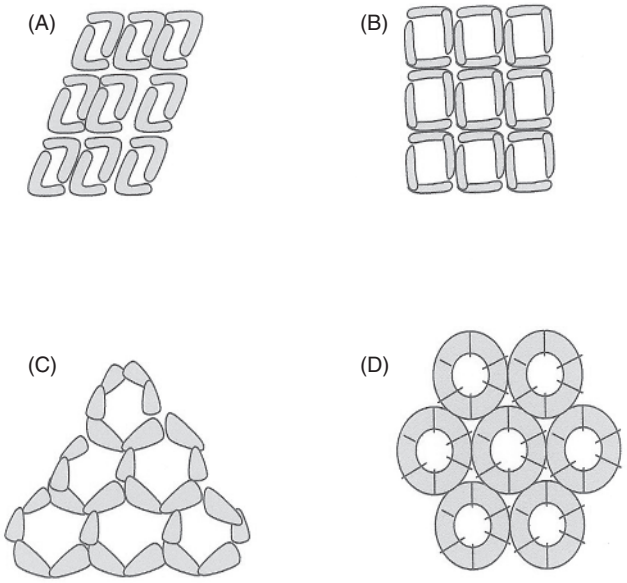


Figure 1.7. S layer of microorganisms as examined with freeze-etched preparations or atomic force microscopy displays a surface composed of proteins in four different lattice formations: (A) oblique lattice; (B) square lattice; (C) hexagonal–triangular lattice; (D) hexagonal rosette lattice [modifed from Sleytr et al. (1996)].

Another example of an important structure in prokaryotes is the plasma membrane or cell membrane, which functions as a barrier to segregate molecules essential for cellular growth from the extracellular environment. The chemical structure of the plasma membrane includes lipids that form a hydrophobic barrier and proteins that contribute to solute transport, metabolic processes, and communication between the cytoplasm and the environment. Lipids found in prokaryotes consist of phospholipids and fatty acids or fatty acyl groups attached to the glycerol backbone. Although there is a molecular distinction in the lipids found in archaeal and bacterial cells, lipophilic affinity of these molecules functions to stabilize the plasma membrane (Madigan et al. 2009). Phosphate moieties and other charged groups on the surface of the membranes are important for carrying the charge on the membrane. Integrity of the membrane structure is required for cell viability, and disruption of this organization results in cell death.

The cell wall is an important structure for bacterial and archaeal cells in that it prevents osmotic disruption of the cell and contributes to cell shape. For bacteria, rigidity of the cell wall is attributed to a macromolecule called *peptidoglycan* that consists of a sugar polymer with a covalent crossbridge to peptides. Even after disruption of the bacterial cell, the structure of the peptidoglycan is evident (see Figure 1.8). *N*-Acetylglucosamine and *N*-acetylmuramic acid make up the dimer that contributes to the linear strength of the peptidoglycan molecule. As discussed in general texts (Madigan et al. 2009), the crossbridge peptide in the peptidoglycan contains alternating D and L forms of amino acids. Considerable similarity of cell wall composition is found in all of the various types of bacteria; the quantity of peptidoglycan surrounding Gram-positive bacteria is greater than that found with Gram-negative cells. While the cell wall in archaea does not

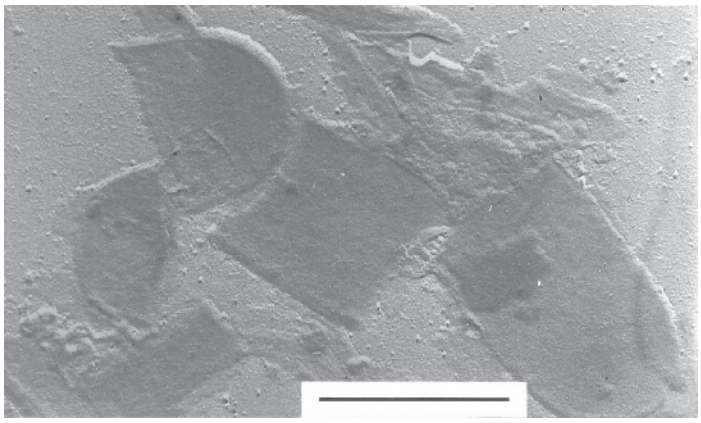


Figure 1.8. Remnants of the peptidoglycan structure of *Bacillus stearothermophilus* after distrution of bacterial cell by high-pressure treatment (electron micrograph provided by Sandra Barton).

contain peptidoglycan, the covalent bonds attributed to polymers of L forms of amino acids and sugars provide for structural stability of the archaeal cell.

Specific proteins account for cell division and cellular form for prokaryotic cells. For cell division, there are a series of proteins located on the inner side of the cell membrane, and prior to binary fission many of these proteins polymerize to form the FtsZ ring located at the midpoint of the cell. The FtsZ ring recruits other proteins for the division process and is present in both archaea and bacteria. To underscore the evolutionary relationship between prokaryotes and eukaryotes, FtsZ-like proteins are also found in chloroplasts, mitochondria, and cell division proteins in eukaryotes. Additional proteins on the inner side of the cell membrane in bacteria and archaea are the MreB proteins (Figure 1.9). The MreB proteins influence the localized synthesis of the cell wall and account for the rod-shaped cell form. Bacteria without the genes for the production of MreB proteins are of the coccus form. Scientists speculate that the ancestral cell was spherical and the rod form appeared with the development of the specific gene for MreB synthesis. Some

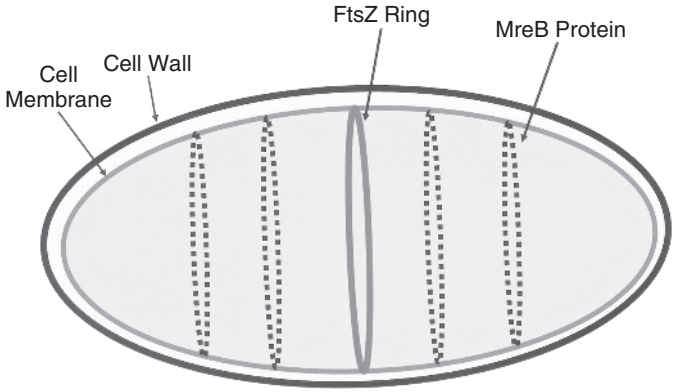


Figure 1.9. Localization of FtsZ and MreB proteins in a bacterial cell.

bacteria have a curved rod shape also known as a *vibrio form*. In one vibrio-shaped bacterium, *Caulobacter crescentus* (Section 3.7), the cell shape is attributed to crescentin in addition to MreB. The crescentin proteins accumulate on the concave face of the vibrio cell and contribute to the curvature of the cell. Since proteins similar to crescentin have been found in another vibrio, *Helicobacter*, some have suggested that unique proteins are needed to produce a curved bacterial cell.

1.6.2 Metabolism and Use of Energy

Microbial cells use chemical energy from organic compounds, minerals, and light-driven reactions. While solar energy is restricted to microorganisms at Earth's surface, the use of reduced organic compounds or inorganic materials provides energy for metabolic reactions in anaerobic and aerobic environments. A hallmark characteristic of living systems is the flow of electrons from electron donors to electron acceptors, and this characteristic is observed in both aerobic and anaerobic cultures (see discussion on energetics in Chapter 3). The generation of ATP and establishment of a charge on the cell membrane are coupled to this electron flow. As indicated in the model in Figure 1.10, energy from cell metabolism is also used for motility and nutrient transport. As with other life forms, metabolism in microorganisms is the summation of incremental changes. Additionally, there is a similarity in all forms of life in that electron transfer is mediated by cytochromes, quinones, and proteins with iron–sulfur centers; however, considerable variability of these electron carriers distinguishes prokaryotes from mitochondria-containing life forms. In terms of transmembrane movement, nutrient transport is driven by chemiosmotic or ion gradients in all living cells with prokaryotes commonly relying on H^+- or Na^+-driven transporters.

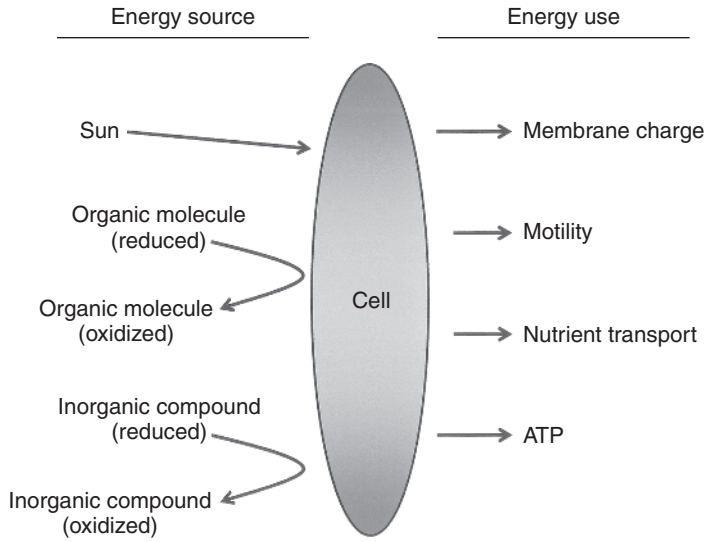


Figure 1.10. Energy flow in microorganisms.

1.6.3 Growth, Reproduction, and Development

The goal of microbial metabolism is to provide microorganisms with sufficient biosynthetic material in an organized fashion that enables them to reproduce. With bacteria, archaea, and single-cell protists, growth generally implies an increase in the number of individual cells. The idealized growth curve is commonly used to describe bacterial or archaeal growth (Figure 1.11); however, logarithmic growth of these microorganisms is only transiently seen in the environment. As discussed in Box 1.1, logarithmic growth of bacteria or archaea has the potential of quick production of biomass. While there may be bursts of rapid growth by individual species of prokaryotes due to nutrient flux, growth of bacteria or archaea in many stable environments is similar to stationary-phase growth. Although reproduction in bacteria and archaea is asexual, the acquisition of new heredity information from horizontal gene transfer (Section 4.7.2) provides for mixing of the gene pool. The production of spores by bacteria is an asexual process of cell differentiation, with one cell producing one spore. Bacterial spores are produced to enable a species to

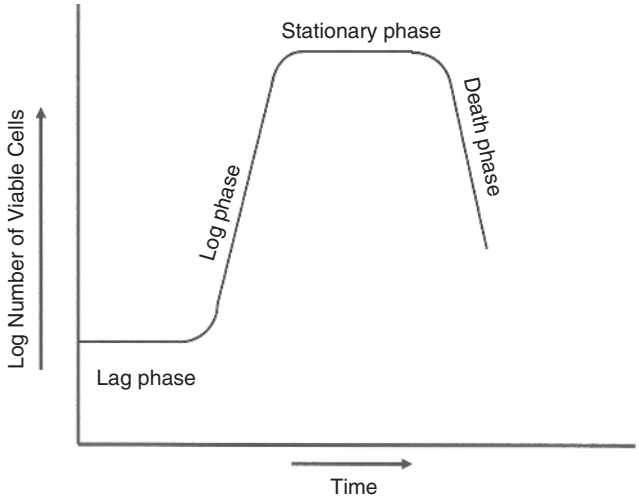


Figure 1.11. Idealized growth curve for bacteria indicating that the rate of rapidly dividing cells is a logarithmic function.

Box 1.1 The Power of Log Growth

Bacteria and archaea grow by binary fission where one cell divides to give two cells, these two cells divide to give four, the four cells divide to give eight, and so the progression of log growth proceeds. If a bacterial species grows with cell division occurring every 60 min, at the end of 96 h there would be 10^{29} cells. If the weight of one cell equals 2.5×10^{-13} g, then at the end of 96 h the mass of bacteria would be 2.5×10^{13} kg. Fast-growing bacteria like *Escherichia coli* divide every 20 min, and at the end of 48 h, *E. coli* would produce a mass of about 2.2×10^{24} kg. It is inappropriate to consider that bacteria in the environment display log growth for any extended time because the mass of Earth is 5.97×10^{24} kg and bacteria could quickly exceed this value.

persist through periods that are detrimental to cells and are not a form of reproduction as is the case of asexual spore formation in fungi. A few bacteria and archaea display cellular differentiation or development, and some bacteria display a simple lifecycle.

1.6.4 Adaptations and Response to Stimuli

It is desirable for microorganisms to respond to environmental changes so that growth and physiological processes can be maintained at near-optimal conditions. When physical or chemical changes are extreme, selection favors the cell line that has a genetic content that enables cells to grow at low pH, high temperature, high salt content, or other permanent environmental changes. These adaptations can become fixed in a population, resulting in new species with special traits. However, many environmental changes are transient where the duration of the new stimulus is not long but may be relatively frequent. Bacteria and archaea display stress response to many different transient stimuli, including temperature, toxic metals, desiccation, oxygen content, and many other environmental situations. In many instances the response to stimuli may be to promote bacterial movement toward a useful nutrient or desirable environment. Chemotactic movement may be attributed to flagellar or gliding activity and is regulated by a complex sensory process. Bacteria have the capability of transferring a physical or chemical signal across the cell membrane to elicit an appropriate response.

Additionally, numerous metabolic changes occur in microorganisms as they respond to changes in the chemical environment. Induction or repression of gene expression occurs in bacteria in a few minutes, and this enables cells to synthesize only those enzymes needed for catabolism or biosynthesis. Furthermore, microbial cells can modulate gene expression instantaneously as chemical changes occur in the environment. This highly regulated production of enzymes ensures that energy is conserved through the synthesis of only those enzymes that are needed for that environment. This physiological and metabolic adaptability by bacteria enables them to persist in the environment and to successfully compete with eukaryotic life forms.

Bacteria and archaea have made considerable adjustments as Earth's environment has changed over the years. Major changes in Earth's temperature occurred, and microbial life forms responded appropriately. Both microorganisms and hosts were required to adapt for the continuation of parasitic or mutualistic interactions. Ocean temperature has been calculated using oxygen isotope ratio in fossil plankton found in marine sediments and is illustrated in Figure 1.12. Over the past 800,000 years there have been cycles of temperature change with fluctuations of $\pm$ 4°C; however, these changes were extremely slow. We are on a global warming cycle and this will have an effect on microbial activities and especially on microbe-host interactions.

1.7 CLASSIFICATION AND TAXONOMY: THE SPECIES CONCEPT

The classical definition of a species, as applied to the animal world in particular, includes shared morphological traits and the ability of a group of individuals to interbreed and produce fertile offspring through sexual reproduction. Because reproduction in bacteria, archaea, and some other microorganisms is primarily asexual, this definition immediately runs into trouble with prokaryotic organisms, which exchange DNA through conjugation, transduction, and transformation. To solve this problem, microbiologists used phenotypic

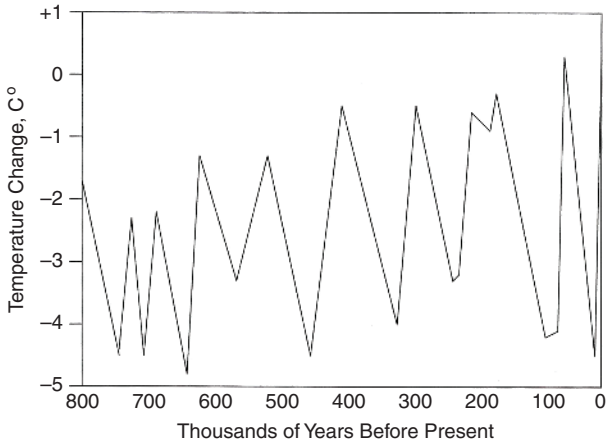


Figure 1.12. Marine temperature from measurement of oxygen isotope ratios in fossil plankton [based on data from Imbrie et al. (1984)].

characteristics, metabolism in particular, to discern closely related species. However, the growing realization that we have not figured out how to grow many species in the environment, as revealed by 16S rDNA studies of diversity, has eroded confidence in relying on this method. Microbiologists then proposed the use of a $\geq$70% level of whole genome DNA-DNA reassociation and similar G-C ratios (the percentage of guanine and cytosine in a genome) to define a species (Staley et al. 2007); however, individuals of a species so defined may share only 80–90% of genes. Some authors use the phylogenetic species concept that specifies that sequence identity of the 16S rRNA gene must be 97% or greater (Madigan et al. 2009) or even 99% sequence identity (Cohan and Perry 2007). The latter is not a good marker for resolving closely related species, however. New ways of approaching the definition of a bacterial/archaeal species are being developed that incorporate an ecological and evolutionary perspective and put the species concept for microorganisms on a more theoretical basis (Cohan and Perry 2007).

Within a bacterial species, there exists what are now termed *ecotypes* that have adapted to their environment in different ways, such as using different carbon sources or mineral nutrients, or using different levels of light energy. Additionally, some species can have strains that are pathogenic, while others within the same species are not pathogenic. Because of the importance of such distinctions, medical microbiologists have separated some of these organisms into distinctly named species, such as *Bacillus anthracis* (pathogenic) and *Bacillus cereus*, while other named species, such as *Escherichia coli*, provide an umbrella for both pathogenic and nonpathogenic strains. Some researchers advocate moving to ecotype-based systematics, in which ecologically distinct species are named and existing species that harbor ecologically distinct strains are given trinomials that include an ecovar epithet to distinguish the different ecotypes contained within a species (Cohan and Perry 2007).

1.8 THE THREE DOMAINS: TREE OF LIFE

At one time it was taught that there were five kingdoms of life: Animalia, Plantae, Fungi, Monera, and Protista (Margulis and Schwartz 1998). In the 1970s, this view of

life was challenged by Woese and Fox (1977), who proposed a new division of life, the Archaea (Sections 2.5 and 2.6), as one of the three major lines of descent. This was followed in 1990 by the theory of Woese et al. (1990), that all of life could be classified into three domains: *Bacteria*, which they called *Eubacteria, Archaea*, which they called *Archaebacteria*, and *Eukarya*, which they called *Eucarya*. The methods employed by Carl Woese and Norman Pace [the sequencing of the small subunit (SSU) of the ribosome] touched off studies that revealed that eukaryotes are not the most diverse organisms on Earth, but are far surpassed by the diversity present in the bacteria and archaea (Pace 1997). In proposing this new scheme for the tree of life, Woese et al. noted the following in 1990:

> Our present view of the basic organization of life is still largely steeped in the ancient notion that all living things are either plant or animal in nature. Unfortunately, this comfortable traditional dichotomy does not represent the true state of affairs.

The genes that encode the16S and 18S SSU of the ribosome have been used by many studies of a wide range of environments over recent decades. The universal nature of ribosomal DNA, its highly conserved regions, and the relative ease of sequencing made this an ideal candidate for exploring the natural world. Researchers have found a wealth of microbial sequences that represented new groups of microorganisms, never before cultivated. The diversity revealed in these studies is truly stunning and intriguing. As our knowledge of this diversity grew, we have constructed a "tree of life" that encompasses these three domains of life (Figure 1.13).

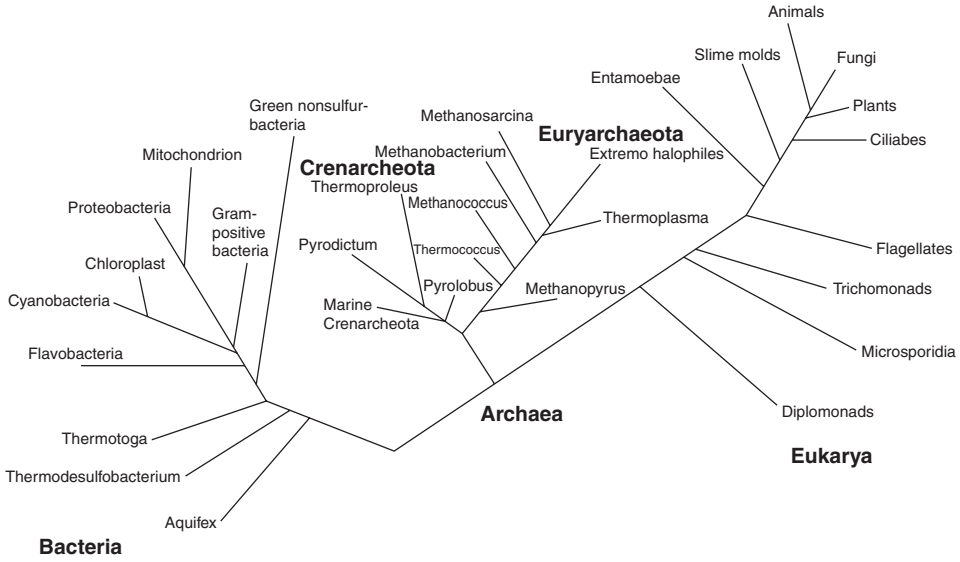
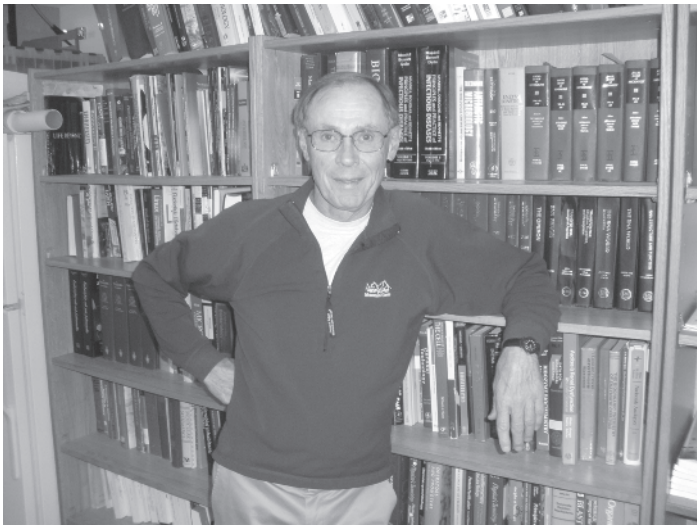


Figure 1.13. Three domains of life—Bacteria, Archaea, and Eukarya—are depicted in a phylogenic tree [modified from Madigan et al. (2009)].

Microbial Spotlight

NORMAN R. PACE



Norm Pace has long been the proponent and architect of the SEARCH for microbial diversity in the natural world. His route to this passion is a fascinating one that shows the power of early experiences:

I first became aware of microorganisms when I was young, about the age of 12 and my parents bought me a pretty cheap microscope; the microscope didn't work very well, but I could look through the microscope at hay infusions and stuff like that. The thing that I found most remarkable about it was that I could look through the microscope and see all these things in there, but I couldn't find any information about what the hell they were. I tucked away that note that the microbial world was a pretty interesting place, but there didn't seem to be a way to get a handle on it.

I ended up being an RNA jock at the U of I [University of Illinois—UC] . . . and became friends with Carl Woese. [My former wife, Bernadette Pace, did the] first [DNA] hybridization experiments for taxonomy. [From these experiments] Carl got really interested in the residual homology. He argued that that highly conserved regions were ''the essence of the RNA molecule.'' One of my first postdocs, Mitch Sogin, did a 5S catalog from *B*[*acillus*] *subtilus*—took 2 years for 120 nucleotides. [Then] the game became how to understand how the 5S rRNA processing enzyme, ribonuclease M5, how it recognized the 5S precursor. We needed the atomic structure, which meant crystallography. [This required a thermophilic molecule that would crystallize well, but it was hard to get the high-temperature RNAs in the quantity needed for crystallography.] I was sitting in my office reading Tom Brock's book *Thermophilic Microorganisms and Life at High Temperatures* and read about Octopus Springs with literally kilogram quantities of pink filaments. ''Wow, kilogram quantities, near boiling! 93°C! So I went running out into the lab, and said, guys, look at this! High

> temperature microbiology in kilogram quantities. Let's take a bucket of phenol up to Yellowstone and get all the 5S we need . . . and somebody, I forget who, said, ''But you don't even know what the organism is. I said, that's okay, we can sequence for it.'' Intake of breath, ''My God!'' That was it—I knew immediately what we had. The others knew what we had, they just didn't know what we didn't have, namely a whole understanding of the natural world. So, Octopus Springs became [one of] the first targets of the SEARCH.

To add robustness to the tree of life described by these 16S and 18S rDNA studies, researchers are now mining whole genomes to identify universal protein gene sequences, which have been concatenated to construct phylogenic trees. Ciccarelli et al. (2006) have used such data to refine the relationships among and within the three domains. In addition to the microbial diversity seen in phylogenic trees, microorganisms exhibit much greater metabolic diversity than do nonmicrobial eukaryotes and have evolved complex interactions with other microorganisms, plants, and animals.

1.9 RELATIONSHIP OF MICROBIAL ECOLOGY TO GENERAL ECOLOGY

The wealth of microbial diversity and the vigorous debate about what constitutes a microbial species highlight one of the major challenges of the evolving field of microbial ecology: the need to provide a theoretical foundation for the organization of the large amounts of new data on microbial diversity. Theories provide explanations of phenomena that have been tested and substantiated and allow us to predict future occurrences. The application of theory into the foundation of microbial ecology allows us to incorporate our predominately quantitative data into an overall framework that provides understanding of the microbial world. An excellent example of the advantages this provides is seen in the application of ecological and epidemiological theory to the study of emerging diseases (Smith et al. 2005). As different scientific disciplines examine the emerging pathogens, they come from different frameworks. The work of early microbiologists and medical scholars (Koch, Pasteur, and Ehrlich) has led to a focus on the individual patient and their interaction with a given pathogen, while epidemiologists focus on populations of pathogens and their interactions with hosts. A third approach involves modeling of host–pathogen interactions using the ecological and evolutionary perspective. The application of ecological theories to this problem have been especially successful in predicting the spread of such diseases as the Ebola virus and rabies, which then allows the application of control measures in the most useful locations. The melding of these three approaches, and the theories underlying them, can provide the best means of controlling emerging diseases (Smith et al. 2005). This is just one example of the many compelling reasons to develop a theoretical basis for microbial ecology.

Prosser et al. (2007) suggest that two factors limit the theory development in microbial ecology:

1. The lack of distinguishing microbial morphological characteristics and our inability to culture many organisms, which have led to a scarcity of data and insights
2. The slow progress in incorporating general ecological theory and quantitative reasoning into microbial ecology education and research

Some scientists also protest that microorganisms are very different than plants and animals because of their small size, diversity, reproductive methods and rates, dispersal means, and metabolic diversity. Does this preclude our application of existing ecological theory to microbial ecology? In actuality, some ecological theory is derived from microbial model systems, which provide a simplified version of interactions that occur in nature (Jessup et al. 2004). The study of such model systems allows us to better understand natural systems and to predict future interactions in nature by testing hypotheses about how ecological processes work. Model microbial systems can allow us to explore several key questions in ecology, such as:

- How do local interactions influence the patterns of diversity seen at larger scales (e.g., landscape)?
- How does the energy available or the productivity of an ecosystem affect the temporal and spatial distribution of organisms?
- What is the relationship between community diversity/complexity and stability?
- Does productivity determine food chain length?

Microorganisms can be quite useful in testing these and other fundamental ecological concepts.

Significant changes lie ahead in bringing a stronger theoretical basis to microbial ecology (Prosser et al. 2007). As discussed in Section 1.7, the development of the ecological species concept versus the traditional biological species concept is a key need in microbial ecology. Because of the stunning amount of microbial diversity in many environments, we currently lack the ability to accurately measure diversity, except in less complex ecosystems. Species abundance curves allow us to theoretically estimate diversity, but accurately measuring diversity remains a challenge. Limited work has been done on microbial species–area relationships, in which microbial diversity is correlated with spatial scales. This is the fertile ground for the development of a theoretical basis for microbial ecology, in which macroecology theory can be linked to a molecular characterization of microbial communities. Along similar lines, much remains to be discovered about the theoretical basis of the relationship between energy available in the ecosystem and microbial diversity (richness and abundance).

1.10 CHANGING FACE OF MICROBIAL ECOLOGY

1.10.1 Change in Focus

The first reports describing the presence of bacteria were important in terms of natural history of the microorganisms, and some of the current interest includes the impact of microorganisms on global activities. When Anton van Leeuwenhoek described the shapes of the bacteria present in scrapings from his teeth and when Martinus Beijerinck reported nitrogen-fixing root nodule bacteria, they contributed to an interest in the types of bacteria in the environment. The isolation of physiologically unique bacteria from every region on Earth provided a wealth of information concerning the ecological role of microorganisms. Over time there has been an expansion of interests to include the contribution of microorganisms to global nutrient cycling, bioremediation, greenhouse gases, and climate change.

More recently, considerable interest has been placed on system-based technologies to evaluate microbial ecology, and these are collectively included as the "omic" technologies. These technologies are heavily dependent on sensitive analytical instrumentation with high flow through capabilities. Several applications to microbial ecology are listed in Table 1.4. and some of these technologies are discussed in Chapter 5. With the availability of DNA and protein sequences, gene content, and sequence structures of many microorganisms, new approaches are being developed to evaluate microbial relationships. One of these studies has raised the possibility that early microbial evolution was influenced by microbes migrating to a terrestrial ecosystem from the marine environment. Battistuzzi and Hedges (2009) have separated bacterial evolution into two major groups: Hydrobacteria and Terrabacteria (see Figure 1.14). Evolution of the Terrabacteria would reflect adaptations to life on land with the development of spore producing *Bacillus*, soil based actinomycetes, and phototrophic cyanobacteria. Another dimension of current research impacting microbial ecology is the more recent report that an engineered genome can be transferred into a bacterial cell (Lartigue et al. 2009). While this will have considerable value for synthetic biotechnology, it may also lead to the *de novo* creation of new bacteria and provide insight into evolution. One could conclude that while analytical techniques will be continued in microbial ecology, systems ecology and synthetic approaches will become important in the future.

1.10.2 Diversity: From Culturing to Molecular Phylogeny

For many decades microbiology and the emerging field of microbial ecology relied on cultivation (Section 5.5) to identify microorganisms in the environment. This eventually led to the elucidation of what was called "the great plate count anomaly" (Section 5.2), in which researchers noticed the discrepancy in numbers between what they observed

TABLE 1.4. "Omic" Technologies with Applications to Microbial Ecology

Terms	Characteristics
Genomics	Analysis of gene content of an organism by sequencing and mapping of genomes (chromosomes of eukaryotes or nucleoid of prokaryotes)
Metagenomics	Analysis of gene content of all organisms in a specific environment
Transcriptomics	Study evaluating the production of mRNA produced at a specific time by a cultured organism
Proteomics	Study of protein structure and protein regulation of an organism
Metaproteomics	Analysis of all proteins produced by all the organisms in a specific environment
Metabiomics	Study of small molecules and intermediate compounds produced from metabolism; frequently this includes the end products of metabolism
Metallomics	Study of the various metal ions and their activities in a biological cell
Biolomics	Study of all the biological systems and biochemical components of cellular system
Microbiomics	Study including all the microorganisms and their interactions with the immediate environment.

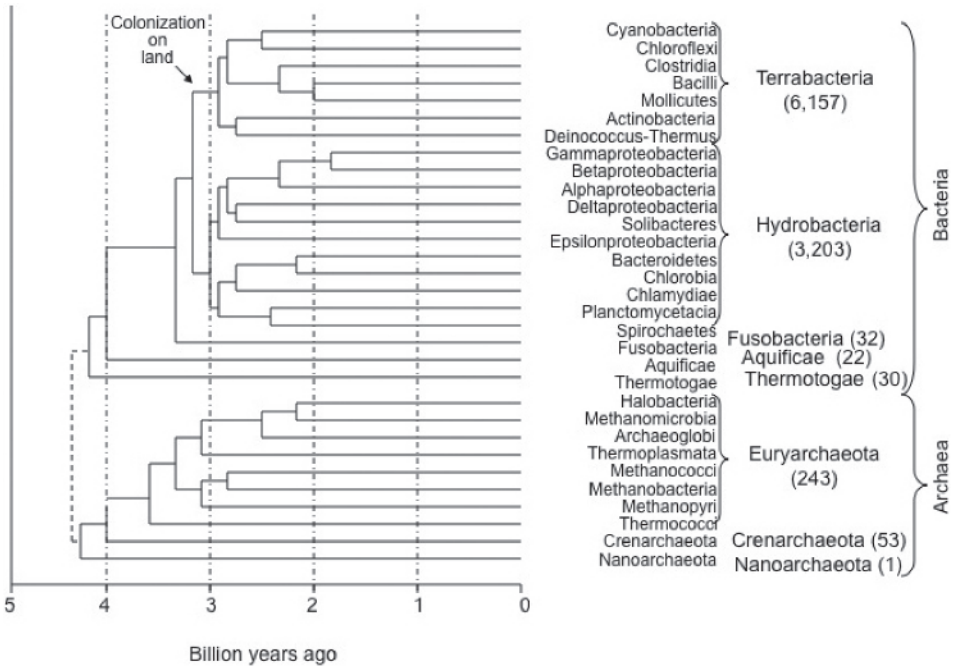


Figure 1.14. Analysis of nucleic acid and protein sequences suggests importance of bacterial adaptation to life on land; numbers in parentheses indicate number of species of organisms in that group [modified from Battistuzzi and Hedges (2009)].

in the microscope versus what they could grow using standard media (less than 1% of microorganisms present in the environmental sample) (Staley and Konopka 1985). At about the same time, Carl Woese and Norm Pace were investigating the ability to identify and compare environmental organisms by their small-subunit ribosomal RNA genes, which provided a measure of evolutionary distance between organisms. This development revolutionized our view of the natural world and eventually, the classification of bacteria/archaea in the main reference work, *Bergey's Manual of Systematic Bacteriology*. Their efforts and subsequent studies revealed an amazing level of diversity in the microbial world.

1.11 SUMMARY

With respect to early life on Earth, there are two distinct activities: genesis of life and evolution of organisms. While scientists provide insightful discussions on these activities, new theories and past observations continue to attract the attention of microbiologists. The fossil record for bacteria is limited primarily to cyanobacteria and related microorganisms found in fossilized stromatolites. As an alternate to evaluation of available fossils, microbiologists rely on life-related processes consistent with the geologic record of Earth. Physiological process of O_2 release from photosynthesis was an important activity of early life, and it can be concluded that bacteria with these metabolic capabilities were

present early in cellular evolution. Once cellular life was achieved, evolutionary development proceeded along several avenues, including the survival strategies as outlined by Darwin. The genetic design of prokaryotic organisms enabled bacteria and archaea to use horizontal gene flow in the generation of new species. Eukaryotic cells evolved with internal organelles developed from endosymbiosis of bacteria and genes from both bacteria and archaea. The tree of life as described by Woese serves to provide a structure for cellular evolution and establishes the dominant presence of bacteria and archaea in evolution of life on Earth. As new species of bacteria and archaea present in the environment are discovered, the picture becomes more complete with respect to prokaryote life, and with new computer programs (software) developed to evaluate molecular trees, the tree of life in the future is sure to become more detailed and will change to reflect newer information.

1.12 DELVING DEEPER: CRITICAL THINKING QUESTIONS

1. What is the evidence that the first forms of life were prokaryotes?
2. Describe some of the hypothesis for evolution of eukaryotes.
3. What evidence is there that bacteria are evolving today?
4. What evidence is there to suggest that bacteria did not evolve from archaea?
5. Why is it difficult to describe a species in microbiological terms?
6. What are some benefits in studying microbial ecology along with general ecology?
7. What are some limitations of the molecular techniques in evaluating microbial ecology? What are some areas for future development of new techniques for studying microbial ecology?
8. What is an ecotype? Compare and contrast this concept with that of a microbial species.
9. Describe several situations where it is desirable for microorganisms to respond to stimuli in the environment.
10. Why does the traditional biological species concept not work for defining a bacterial species?
11. In what ways can the identification of a theoretical basis for how microorganisms interact with each other and their environment help human society?

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