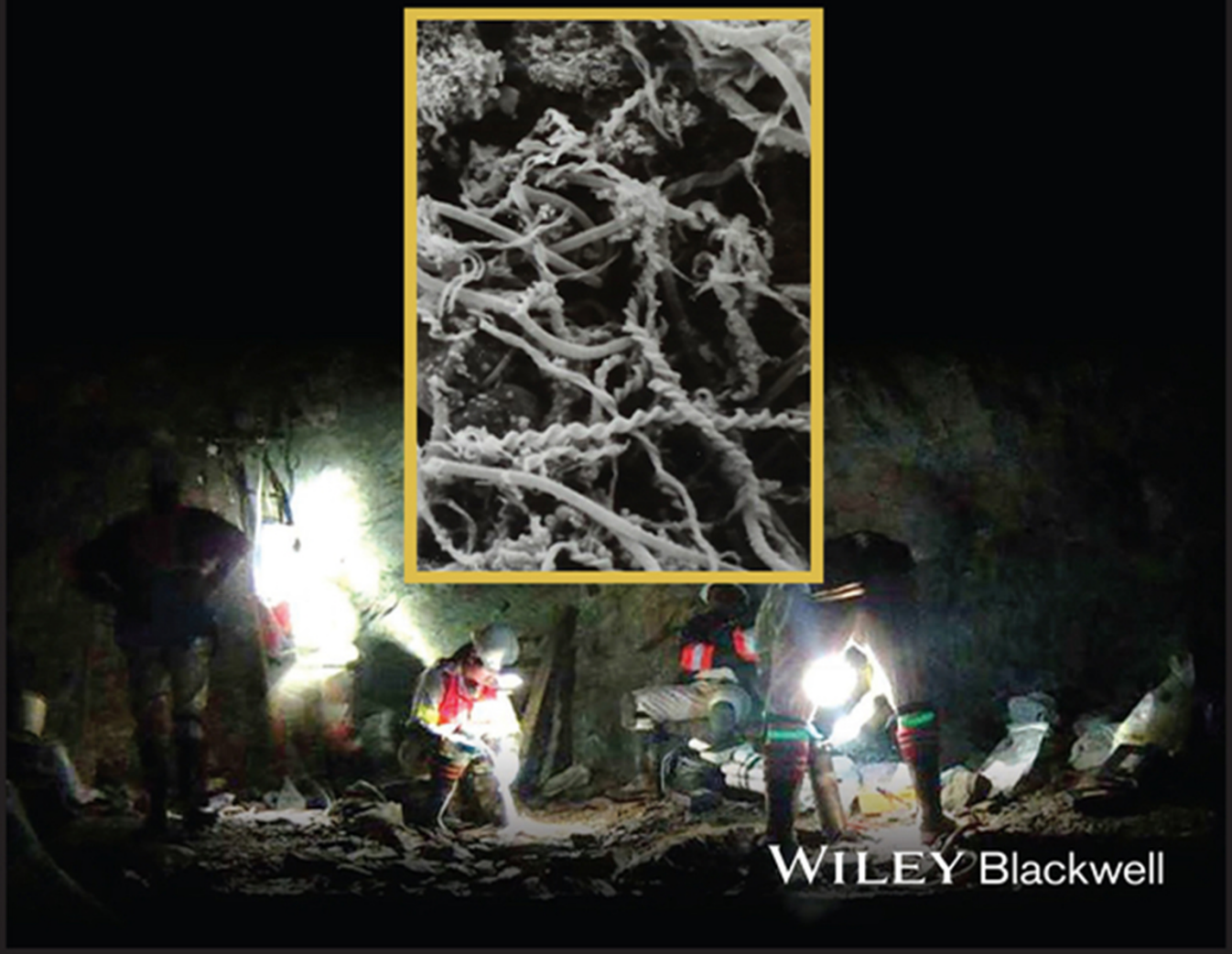
LARRY L. BARTON • ROBERT J.C. McLEAN

ENVIRONMENTAL MICROBIOLOGY AND MICROBIAL ECOLOGY



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Environmental Microbiology
and Microbial Ecology

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Deep subsurface sampling for bacteria in the Beatrix Gold Mine in South Africa. See Figure 5.5 and supporting narrative for details. Image provided by Gaetan Borgonie and Tullis C. Onstott.

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Contents

Preface

Environmental Microbiology and Microbial Ecology provides an overview and discussion of the presence of microorganisms and the significance of their interactions in numerous environments from the perspective of microbiology, environmental science, and biogeochemistry, using current publications as a resource. Diverse topics are organized and discussed in eleven chapters that include current knowledge concerning the bacteria, archaea, fungi, and viruses present in the biosphere. Concepts, results, and conclusions are presented and referenced to enable the reader to examine the original publications on which the various topics are based, with most references readily available in the Open Access literature. The extensive use of cited literature distinguishes *Environmental Microbiology and Microbial Ecology* from introductory text books.

The presentation of recent microbial studies presented in this book builds on the success of the previous text *Microbial Ecology* by Larry L. Barton and Diana E. Northup published by Wiley-Blackwell in 2011. This is an exciting time in environmental microbiology and microbial ecology, where numerous microbial interactions are being evaluated and associations are being proposed. The topics covered in this book include the following:

- Microbial formation of biofilms
- Microbial response to stress and adaption to extreme environments
- Identification of environmental sites and microbial communities relevant to exploration for life on Mars and other extraterrestrial habitats
- Structure and activities of microbial communities
- Discussion of the prokaryote–eukaryote dichotomy and the Tree of Life
- The role of viruses, lysogeny, gene transfer agents, and the CRISPR–*cas* system in horizontal gene transfer
- Microbial presence and activities in extreme environments, including deep subsurface, deserts, cold environments, and hydrothermal vents
- Extracellular electron transfer, biocorrosion, biomineralization, and bioremediation
- Biogeochemical cycles with contributions by microorganisms
- Mutualism and communication between bacteria and plants
- Mutualism between microorganisms and animals, insects, and humans, with an emphasis on intestinal microbiology.

This book is an appropriate resource for instructors of Microbial Ecology or Environmental Microbiology courses as it includes the following special features:

- Discussion questions are provided for each chapter to promote critical thinking and to instigate class debate.
- Lists of references and further reading provide students with numerous Open Access reviews and primary literature.

- Numerous tables and boxes provide extra information on specific topics related to the chapter.
- The text is supported by numerous figures that act as visual models.
- A broad range of specific environments are discussed with numerous presentations on specific sites, hosts, and interactions.

The authors have attempted to provide specific documentation for the data presented and for the generalizations made throughout the book. The extensive citation of publications enables readers to gain an insight into how the research was conducted and how any scientific conclusions were established. Currently, there is a wealth of scientific information available in the Open Access literature, which can be readily accessed online and we have identified numerous publications related to topics covered in this book. To draw attention to specific topics or clarify complex issues, numerous illustrations are presented throughout the book. The authors greatly appreciate the contributions of photographs provided by the following individuals:

Gaeton Borgonie, Ghent University, Belgium
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We appreciate the numerous pictures used from the Public Domain or similar sources because they provide expertly presented colorful details. In the process of compiling this book, we received numerous encouraging comments from colleagues and for this we are most appreciative. This book is written for upper-level undergraduate and graduate students in microbiology, ecology, biology, and environmental science. Also, it is a valuable reference for professionals working in the area of microbial ecology and environmental science. This area encompasses both the microorganisms and their interactions in the environment and is rapidly evolving. It is the hope of the authors that this book will stimulate future investigations into the role of microorganisms in the biosphere.

Finally, we are indebted to the staff at Wiley for their support throughout preparation of this text and for their skill in the final presentation of this material.

Larry L. Barton
Robert J.C. McLean

1

Introduction to Microorganisms and Their Activities

1.1 Central Themes of Environmental Microbiology and Microbial Ecology

The terms "environmental microbiology" and "microbial ecology" are often used interchangeably but there are some subtle distinctions. Environmental microbiology is the study of processes in the environment mediated by microorganisms whereas microbial ecology addresses the interactions between microorganisms as well as between microorganisms and higher life forms. However, many microorganism interactions are dependent on chemicals from the environment or from other biological systems and so microbial ecology overlaps with environmental microbiology where abiotic chemistry occurs. This first chapter provides an overview of the components involved in environmental microbiology and provides a perspective on the breadth of the microbial relationships in the biosphere. The central themes of this chapter include the following:

- Discussion on the continued use of the terms prokaryote and eukaryote and on the Tree of Life
- Horizontal gene transfer and the role of viruses and gene transfer agents
- Perspective of cell size and cell shape
- Bacterial production of dormant cells

1.2 Are the Terms Prokaryotes or Eukaryotes Relevant?

Traditional microbiology classifies microorganisms into two groups: prokaryotes and eukaryotes. Several structural distinctions may be drawn between these groups of microorganisms and the major differences are listed in Table 1.1. This distinction between prokaryotic and eukaryotic life evolved from a publication by Stanier and van Niel (1962), which proved to be the stimulus to include "blue-green algae" as cyanobacteria. As stated by Stanier and van Niel, bacterial cells were unlike eukaryotic cells in that they lacked true membranes to localize the cell "nucleus" and bacteria used nuclear division by fission and not mitosis. However, after several decades of microbial phylogeny, the term "prokaryote" has become controversial because the designation of a prokaryote is based on the absence of certain characteristics (Sapp 2005; Pace 2009; Whitman 2009). It has been proposed that bacteria and archaea, unlike eukaryotes, display coupled transcription and translation where translation starts before transcription is finished (Martin and Koonin 2006; French et al. 2007). There is a concern that

Environmental Microbiology and Microbial Ecology, First Edition. Larry L. Barton and Robert J.C. McLean.

Table 1.1 Differences between prokaryotes and eukaryotes.

- Prokaryotic cells lack a true nucleus with a nuclear membrane.
- Prokaryotic cells lack histones to provide condensation of DNA into chromosomes.
- DNA in prokaryotes is circular whereas DNA in eukaryotes is linear.
- Prokaryotic cells lack organelles in the cytoplasm and cytoplasmic membranes.
- Prokaryotic cells lack carbohydrates and sterols in the plasma membrane whereas both are found in eukaryotic cells.
- In prokaryotic cells, ribosomes are 70S; in eukaryotic cells, ribosomes are 80S with 70S found in organelles.
- Cell division in prokaryotic cells is by binary fission whereas in eukaryotic cells it is by mitosis.
- Sexual recombination does not occur in prokaryotic cells except for DNA transfer. Sexual recombination in eukaryotic cells involves meiosis.
- Prokaryotic cells are 0.2–2.0 μm wide whereas eukaryotic cells are 10–100 μm wide.

bacteria and archaea themselves are sufficiently distinct and should not be united into the single group prokaryotes. The contrast between the nuclear organization and the presence of a nuclear membrane in prokaryotic and eukaryotic organisms has become blurred. Bacteria have long been considered to lack nuclear organization; however, in the bacterial phylum *Planctomycetes*, *Gemmata obscuriglobus* has a nucleoid enveloped in a membrane that forms a structure analogous to the eukaryotic nucleus (Fuerst 2005). The giant bacterium *Epulopiscium fishelsoni* has DNA highly condensed into chromosome-like structures that are physically separated from the cytoplasm (Bresler et al. 1998). In contrast, dinoflagellates, eukaryotic algae, lack histones for the condensation of DNA and lack nucleosomes (Rizzo 2003). Histone proteins are found in mesophilic, thermophilic, and hyperthermophilic archaea and the DNA interactions of archaeal histones is like that found in eukaryotes (Reeve et al. 2004).

1.2.1 Intracellular Membranes in Prokaryotes

Models of some of the intracellular membrane structures found in bacteria are presented in Figure 1.1. Bacteria that obtain energy from methane oxidization often use particulate methane monooxygenase (pMMO). which is localized in the membrane. For the greatest efficiency of methane gas oxidation, multiple membrane structures are present in the cytoplasm and, based on internal membrane structure, methanotrophic bacteria may have either stacked (type I) or paired concentric (type II) cytoplasmic membranes (Davies and Whittenbury 1970). A recently isolated filamentous bacterium *Crenothrix fusca* Roze 1896 also has stacked membranes; however, they only extend part way across the diameter of the cell (Vigliotta et al. 2007). *Nitrosomonas*, *Nitrosococcus*, and *Nitrosocystis* are genera of nitrifying bacteria that have internal membranes to achieve the oxidation of ammonia (NH_3) to nitrite (NO_2^-). The configuration of the internal membranes appears to be specific for each genus: *Nitrosomonas* has membranes along the periphery of the cell, *Nitrosococcus* has laminar membranes through the central region of the cell, and *Nitrosocystis* has a complex membrane structure of small vesicles along the exterior of the cell (Murray and Watson 1965). For almost 100 years, acidocalcisomes have been known to occur in bacteria; however, they have only been investigated recently. In bacteria and protists, acidocalcisomes store inorganic phosphates and calcium ions, and participate in maintaining intracellular pH and in osmoregulation (Docampo and Moreno 2011). Members of the *Planctomycetes* have a structure called the anammoxosome, which is used for the anaerobic oxidation of ammonia (annamox) (van Nifrik et al. 2004). Magnetotactic bacteria produce cytoplasmic magnetosomes with a surrounding membrane that originates at the plasma membrane (Lefèvre and Bazylinski 2013). In addition, phototrophic bacteria

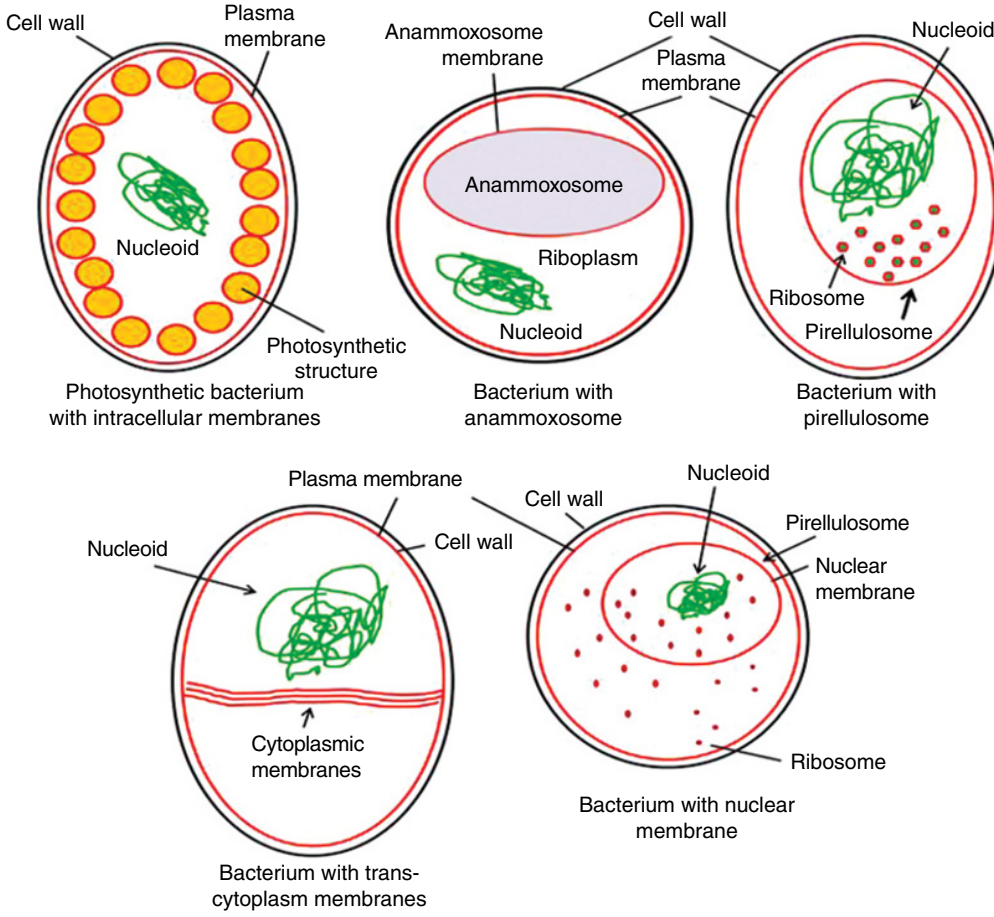

Figure 1.1 Examples of internal structures observed in bacteria.

contain photosynthetic units within cytoplasmic membrane structures (chromatophores) (Willey et al. 2014; Saier 2014). Thus, in this book the term prokaryotes is used sparingly and includes both bacteria and archaea, with considerations as stated earlier.

1.2.2 Compartmentalized Heterotrophic Bacterial Cells

The presence of a compartment inside a heterotrophic bacterium was reported initially for cells of *G. obscuriglobus* (Fuerst 2005). However, recent developments indicate that the compartmentalized bacteria, members of the *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) superphylum, have a homolog of a eukaryotic protein that occurs in eukaryotic membranes (Santarella-Mellwig et al. 2010). Ladderanes (Figure 1.2) are unusual lipids found in the anammoxosome membrane and in *Kuenenia stuttgartiensis* and *Borcadia anammoxidans* the ladderanes account for over half of the lipids present (Damsté et al. 2002). The ladderane lipids produce a dense membrane with low permeability, which is needed to retain potentially toxic intermediates in the anammox reaction. Unlike the anaerobic ammonia-oxidizing *Planctomycetes*, members of the phylum *Verrucomicrobia* (i.e. *Verrucomicrobium spinosum*,

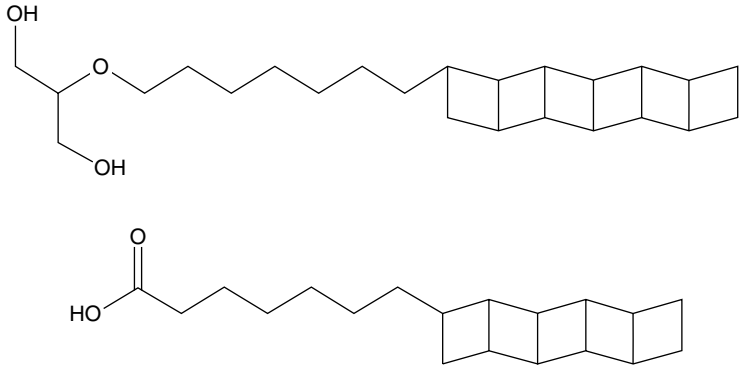

Figure 1.2 Ladderane lipids present in anammoxosome. About half of the lipid in the anammoxosome membrane consists of ladderane lipids; the fused five cyclobutane groups enable the lipids to be closely packed, which contributes to the impermeable character of this membrane.

Prosthecobacter dejongeii, and *Chthonibacter flavus*) have a compartmentalized unit in the cell but do not have the enzymes to carry out the anammox reaction (Lee et al. 2009). The membrane enclosure contains nucleoid and ribosome-like particles and is like the pirellulosome in some *Planctomycetes* (i.e. *Pirellula staleyi* and *Blastopirellula marina*) (Lindsay et al. 2001).

1.2.3 The Universal Tree of Life: Rooted or Unrooted

Microorganisms addressed here are associated with the phylogenetic domains of Bacteria, Archaea, and Eukarya. Although there have been several other systems used to arrange life forms, the classification system dealing with phylogenetic relationships or evolutionary aspects of cells or individuals that has been favored by microbiologists involves the domains of Bacteria, Archaea, and Eukarya. Salient distinctions between these groups of organisms are indicated in Table 1.2.

Tree configurations have long been used to organize physical characteristics into different categories and the Tree of Life, as proposed by Woese et al. (1990), relies on the DNA segment encoding for ribosomal RNA (rRNA). While numerous other genes could be used to express relationships between microorganisms, the genes encoding for rRNA seem to be stable and not subject to evolutionary changes. The hope was that through evaluation of the many genes found in microorganisms, a universal common ancestor for life could be determined. However, it has been difficult to construct a valid Tree of Life for organisms on Earth due to horizontal (lateral) gene transfer throughout the life span of microorganisms. Doolittle (2015) has summarized the various concerns in construction of a Tree of Life and suggested organizing the three domains using an unrooted tree (see Figure 1.3). Even though gene trees have stimulated a great amount of research, it is worth recalling Whitman's (2009) statement that "gene trees are not equivalent to organismal trees." For a thoughtful presentation of the status of the universal Tree of Life the reader is encouraged to consult the review by Forterre (2015).

1.2.4 What About the Giant Viruses?

In the past few years there have been many double stranded DNA (dsDNA) viruses isolated that are gigantic. These viruses make up the Nucleocytoplasmic Large DNA Viruses (NCLDV). which infect protozoa, algae, and other aquatic eukaryotes. The family *Phycodnaviridae* are

Table 1.2 Distinctions between members of the Tree of Life.

Characteristic	Bacteria domain	Archaea domain	Eukarya domain
Histones associated with DNA	Absent	Present in some	Present
Chromosome or nucleoid	Most circular, few linear	Circular	Linear
Nuclear membrane	Absent[a]	Absent	Present
Introns	Absent	Present in a few	Present
RNA polymerase	One type	Several types	Several types
Peptidoglycan in cell wall	Present	Absent	Absent
Amino acid initiating protein synthesis	*N*-formyl methionine	Methionine	Methionine
Ribosomes sensitive to antibiotics	70S inhibited	70S not inhibited	80S not inhibited
Cholesterol in membrane	Absent[b]	Absent	Present
Membrane lipids	Fatty acids esterified to glycerol	Phytanols ether linked to glycerol	Fatty acids esterified to glycerol
Heat-resistant endospores	Present	Absent	Absent

[a] Present in only one bacterium.
[b] An exception is that cholesterol is found in a few species of bacteria of the genus *Mycoplasma*.

large icosahedral dsDNA viruses with genomes of 160–560 kb that infect eukaryotic algae (Wilson et al. 2009). Mimiviruses are large dsDNA viruses that infect amoeba and have been associated with many human pneumonia cases; due to their size, they were initially considered to be gram-positive intracellular bacteria (Raoult et al. 2007). Faustavirus is an icosahedral virus ~200 nm that was isolated from *Acanthamoeba* spp. and appears closely related to the pathogenic African swine fever virus (Reteno et al. 2015). *Pithovirus sibericum*, an ancestral large virus that infects amoeba, was isolated from a 30 000-year-old Siberian permafrost sample (Legendre et al. 2014). The giant virus *Pandoravirus salinus*, which attacks amoeba, was isolated from seawater near the coast of central Chile (Philippe et al. 2013). Several giant viruses have genome sizes greater than some bacteria and the genomes of mimiviruses, pithoviruses, pandoraviruses, and Faustovirus are 1.2, 0.6, 2.5, and 0.466 mbp, respectively. When these viruses were discovered, some microbiologists proposed that the giant viruses represented a fourth form of cellular life or a fourth branch on the Tree of Life (Sharma et al. 2015). Although the discussion concerning the origin of these viruses continues, a strong argument has been presented that suggests giant viruses arose from smaller DNA viruses that have acquired genes from their eukaryotic hosts (Yutin et al. 2014).

1.3 Major Approach to Study Microorganisms

There are several major approaches for the study of microorganisms, especially bacteria, from an environmental or ecological perspective. Analysis of specific environmental sites includes traditional cultivation procedures as well as the use of molecular techniques to identify bacteria or archaea and to characterize their response to environmental conditions. The molecular study of environmental microorganisms is discussed in further detail in Chapter 2.

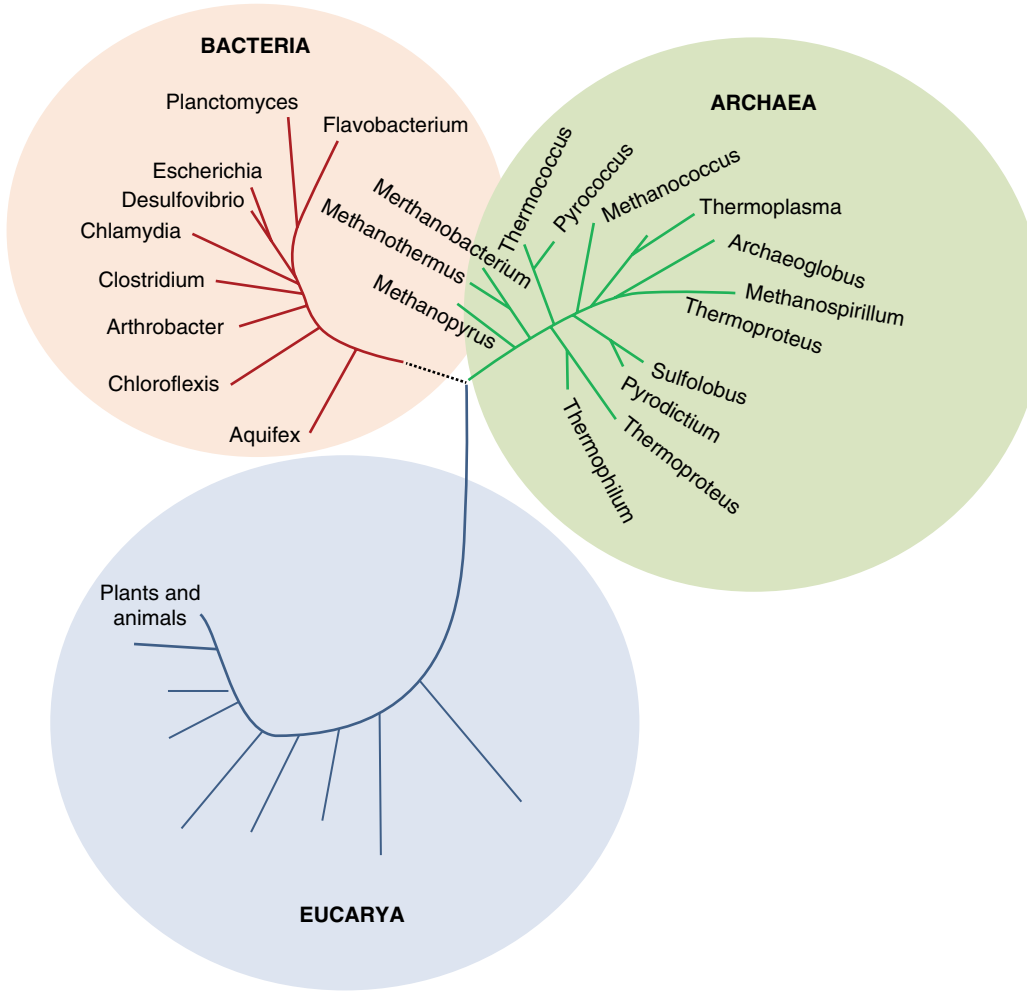

Figure 1.3 An unrooted tree showing the relationship of the three main branches of life based on the small subunit rRNA sequences.

1.3.1 Application of Genomics, Metagenomics, and Proteomics

Scientists may ask "What are these microorganisms and what specific genes do they possess?" To answer this question, scientists may use genomics, metagenomics, or proteomics. Microbiologists examining a specific site may use 16S rRNA from bacteria that were isolated and grown in the laboratory and this use of the bacterial genome to identify the bacteria present is genomics. Since not all prokaryotes at a site can be grown in the laboratory, extraction and analysis of all DNA at a site (metagenomics) provides the identity of the bacteria and archaea present, whether or not they can be cultivated. It is generally considered that less than 1% of bacteria and archaea in an environmental sample can be grown in the laboratory. The inability to count colonies of bacteria from diluted soil samples as a means of enumerating the bacteria present is referred to as the "great plate count anomaly." With the number of bacteria on Earth estimated to be 1.7×10^{30} cells (Table 1.3). it would be a daunting task to work out cultivation schemes for all bacteria and then analyze their genes. The identification of genes to

Table 1.3 Estimation of prokaryotes distributed throughout the biosphere.

Location	Number of prokaryotic cells[a]
Aquatic environment	1.2×10^{29}
Subsurface of oceans	3.5×10^{29}
Terrestrial subsurface	2.5×10^{29}
Soil	2.6×10^{27}
Plant surfaces	1.0×10^{26}

[a] Estimated number of prokaryotic cells produced each year is 1.7×10^{30} cells (Whitman et al. 1998).
Source: Reproduced with permission from Whitman et al. (1998), Lindow and Brandl (2003), and Kallmeyer et al. (2012).

explain specific activities may be obtained by sequencing DNA isolated from a site or by in situ hybridization techniques using probes targeting specific genes. Proteomics can also provide an excellent analysis of bacteria present (Dworzanski and Snyder 2005). For proteomics, protein produced by bacteria is isolated and nuclear magnetic resonance (NMR) analysis is used to match it to protein from a known gene, so the specific gene can be identified. Using genomic analysis, bacterial diversity and community structure at a site can be established. From this molecular analysis, relatively sound proposals can be made concerning the contribution of specific bacteria to the overall biological activity at a specific site.

1.3.2 Biochemical and Physiological Analysis

Another approach is to ask "How do microorganisms function in a specific process?" In this case, the activities of specific bacteria are examined by transcriptomics. The bacteria involved in the active synthesis of a specific protein can be identified by isolating mRNA from a site and its subsequent conversion to DNA by reverse transcriptase,. This is important to demonstrate which gene is being expressed because the presence of a gene in bacteria does not mean that gene is being decoded. For example, the presence of a gene for nitrogen fixation can be determined by genomic analysis but RNA isolation would indicate the actual expression of the nitrogen-fixing gene. With bacteria isolated from a site, the biochemical processes can be studied to understand how bacteria are capable of accomplishing a given activity. Included in this type of investigation would be chemical analysis to assess biotic and abiotic activities as well as processes resulting from mixed populations. This is an important approach for the study of processes such as biogeochemical cycles, bacterial symbiosis, and bioremediation.

1.4 The Impact of Horizontal Gene Transfer Between Microorganisms

The vertical transmission of genes refers to the transfer of DNA from the parent to the offspring, whereas the horizontal transmission of genes describes the movement of DNA between biological systems. The distinction between horizontal gene transfer and vertical inheritance (Andam et al. 2010) and the frequency at which DNA transfer occurs between phylogenic groups has been reviewed elsewhere (Kloesges et al. 2011). The movement of genes between bacteria has been extensively studied and DNA is transferred by conjugation, transduction,

Box 1.1 Characteristics of Bacterial DNA Exchange

Conjugation – DNA exchange between bacteria that requires cell-to-cell contact is referred to as conjugation. As described in most introductory microbiology text books (Willey et al. 2014). DNA is moved from a donor cell to a recipient cell as a plasmid or as "chromosomal" genetic material. In *Escherichia coli*, a special plasmid referred to as the F-factor enhances this horizontal gene transfer. Conjugation is assumed to occur in most bacteria and new examples are being discovered as is the case with *Mycobacterium smegmatis* (Gray et al. 2013). Often the DNA transferred to the recipient cell provides new catabolic genes, resistance to toxic metals or resistance to antibiotics. Conjugation between bacteria and plant cells accounts for tumor induction in plants by introduction of Ti plasmids from *Agrobacterium tumefaciens* or Ri plasmids from *Agrobacterium rhizogenes* (Pan et al. 1995).

Transduction – In bacteria this is the transfer of DNA into a recipient host by a bacteriophage (bacterial virus). While generalized transduction carries any gene from a donor, specialized transduction moves only a gene near the provirus in the "chromosome" of the donor. Both processes are described in introductory microbiology text books such as Willey et al. (2014).

Transformation – Bacteria have the capability of taking up DNA from outside of the cell and DNA becomes part of its genome (Chen and Dubnau 2004). The DNA acquired may be as a plasmid or a disrupted bacterial chromosome. Cells of a specific competent state are capable of acquiring exogenous DNA and there is the potential for acquiring DNA from species different from the host cell.

Gene transfer agents – The mechanism of gene transfer between prokaryotes involves a particle that is similar to a small bacterial virus. These gene transfer agents (GTAs) are released from prokaryotes by cell lysis and transduce random genomic segments to a recipient prokaryote. Enhanced gene transfer is attributed to the GTA particle (Maxmen 2010). In addition to the microorganisms listed in the text, bacteria with GTAs receiving attention include *Rhodovulum sulfidophilum*, *Bartonella* spp., *and Bacillus* spp. (Nagao et al. 2015; Lang et al. 2012).

transformation, or gene transfer agents (GTAs). Characteristics of these four processes for genetic exchange are summarized in Box 1.1 and while DNA exchanges are observed in the environment, the frequency that each mechanism is used may not be apparent. It is well documented from several different environments that considerable movement of DNA between microorganisms occurs. It has been estimated that there may be 10^{13} prokaryotic gene transfers per year in the Mediterranean Sea (McDaniel et al. 2010). 1.3×10^{14} transduction events each year in Tampa Bay (Jiang and Paul 1998). and 10^{24} genes transferred by transduction each year in the oceans of the world (Rohwer and Vega Thurber 2009). The extensive horizontal exchange of DNA between microorganisms contributes to the difficulties of finding the Last Universal Common Ancestor and the rooting of the Tree of Life. Note that once DNA is transferred to a bacterial or archaeal cell, coevolution processes will determine if the host cell incorporates the DNA into its genome and if the newly acquired DNA is expressed.

Since bacteria and archaea do not have "sexual stages" in their growth processes, the mixing of the gene pool is accomplished by asexual horizontal gene transfer. There are many examples of horizontal gene transfer between microorganisms and higher biological forms and a few documented cases are given in Table 1.4. Potentially any gene in the donor cell can be mobilized but at least three classes of genes transferred between bacteria have been identified. The most common genes associated with horizontal transfer include those associated with the replication, translocation, and integration of mobile genetic elements and viruses. Additionally, genes encoding for antibiotic resistance, pathogenicity, and host–pathogen interaction are

Table 1.4 Examples of interspecies horizontal gene transfer involving microorganisms.

- From bacteria to the yeast *Saccharomyces cerevisiae* (Hall et al. 2005).
- Adzuki bean beetle, filarial nematodes, and arthropods have acquired DNA from their endosymbiont *Wolbachia* (Kondo et al. 2002).
- Pea aphids (*Acyrthosiphon pisum*) contain multiple genes from fungi (Moran and Jarvik 2010).
- *Plasmodium vivax*, a malaria pathogen, has acquired DNA from its human host and this enables it to escape defenses of host (Bar 2011).
- Genes for Shiga toxin moved from *Shigella sonnei* to *Escherichia coli* (Strauch et al. 2001).
- Genes for dissimilatory sulfite reductase have been transferred between bacteria and from bacteria to archaea (Klein et al. 2001).
- Several genes transferred from relatives of endosymbionts *Buchnera* and *Wolbachia* to pea aphid, *Acyrthosiphon pisum* (Nikoh et al. 2010).

moved between bacteria at the highest rate. At the intermediate level of mobilization are genes encoding for metabolic or structural development. Genes transferred at the lowest frequency include those dealing with transcription, translocation, or other informational processes (Keese 2008). Bacteria that have received genes enabling them to survive and grow in the presence of antibiotics or to engage in catabolism of toxic materials in the environment are often referred to as "super bugs." Horizontal gene exchanges occur between different bacteria, bacteria and archaea, and most likely between archaeal cells.

The magnitude of horizontal gene exchange between bacteria can be evaluated by comparing the core genome to the Pan-genome for bacteria (Willey et al. 2014). The core genome refers to the genes present in a species that would reflect the minimum number of genes required to enable that species to grow. Included in the core genome are the genes for transcription, translation, and replication. The Pan-genome refers the total of all genes found among all taxa and not limited to a single taxon. If the number of core genes is subtracted from the number of Pan-genes in a strain, the difference would represent the number of genes acquired to enable bacteria to colonize new niches. The examples provided by Willey et al. (2014) include the following: *Bacillus anthracis* has 3600 and 3800 in the core genome and Pan-genome, respectively. *Streptococcus agalactiae* has 1800 and 2700 in the core genome and 2700 in the Pan-genome. *Escherichia coli* has 2800 in the core genome and 6000 in the Pan-genome. This would indicate that *B. anthracis* would be limited to a few habitats whereas *E. coli* may expand into numerous habitats. Although horizontal gene exchange is a reality for bacteria, the amount of DNA exchanged varies considerably with the individual species.

1.4.1 Genetic Islands

The genes associated with horizontal gene transfer include a large segment of DNA that is 10–200 kb and which is often referred to as a genetic island (Juhas et al. 2009). Encoded in this gene cluster there are usually insertion elements or plasmid conjugation factors that mobilize this genetic information. Antibiotic resistance and virulence bacterial genes are some of the best-known genes moved by horizontal gene transfer and these discrete DNA elements are referred to as pathogenicity islands. Other discrete DNA segments may be symbiosis islands, which enhance bacterial interactions with nodulation in plants or associations with animals. Metabolic islands may describe genes for catabolism or mineral metabolism. Geochemical or geomicrobiology islands refer to a unit of genes that enable mineral transformations. Resistance or fitness islands describe those discrete DNA segments that enable bacteria to grow in toxic environments or under chemical stress.

1.4.2 Risks from Genetically Modified Organisms

With the reality that horizontal gene transfer is an ongoing process in nature, there is concern that new segments of DNA will be introduced into the environment by genetically modified organisms (GMOs). In agriculture, transgenic plants are grown and seeds from these plants are used for human consumption. The transfer of genes from these transgenic plants to humans or bacteria in the human gut has been of concern but it is highly likely that the only genes that could be transferred would be the genes of bacterial origin that were used to construct the genes for the development of GMOs. The bacterial genes of interest would be the antibiotic resistance genes used to insert the genes into the plant cells. The most common antibiotic resistance genes used for the wide transgenic development of plants are the genes encoding for kanamycin and hygromycin B. Both antibiotics are rarely used in therapeutic situations. Additionally, although there may be reports of gene transfer from transgenic plants to environmental bacteria, these are carefully controlled laboratory evaluations that have not been demonstrated in field settings (Keese 2008). Recently, transgenic salmon have been approved for human consumption and studies are needed to evaluate the potential for gene transfer from GMOs to humans. With transgenic animals, the greatest potential for gene transfer is from the viruses that were used to construct the donor DNA (Keese 2008).

1.4.3 Microbial Viruses and Gene Transfer Agents

Bacterial viruses, also known as "bacteriophages" or simply "phages," are dispersed throughout the environment and it has been estimated that there are about 10^{31-32} bacterial viruses in the biosphere. The presence of microbial viruses in various environments is indicated in Table 1.5.

Each bacterial and archaeal species is subject to phage attack and excellent reviews by Weinbauer (2004) and Suttle (2007) characterize bacterial viruses in the environment. The abundance of microbial viruses in aquatic and soil environments is determined by electron microscopy and epifluorescence microscopy using uranyl acetate staining and fluorochromes for staining nucleic acid, respectively. Electron microscopy has revealed the presence of virus-like structures in the environment with the most common structure being viruses with a head and a tail. Some bacterial phages have a long flexible tail, some have a short tail, and most

Table 1.5 Abundance of bacterial virus in the environment.

Estimates of viruses	Site of collection	Reference
10^6–10^9 ml^{-1}	Seawater	Suttle et al. 1990
10^5–10^7 ml^{-1}	Sewage effluent	Bitton 1987
10^8 mg^{-1}	Soil	Ashelford et al. 2003; Williamson et al. 2007
10^8 mg^{-1}	Cold deserts Ice-free region of Antarctica	Zablocki et al. 2016
10^5 ml^{-1}	117–292 m beneath ocean Juan de Fuca Ridge, Pacific Ocean	Nigro et al. 2017
10^4–10^9 ml^{-1}	320 m below sea floor South Pacific Gyre	Engelhardt et al. 2016
10^6–10^9 ml^{-1}	Arctic sea ice	Maranger et al. 1994
6.5×10^{10} ml^{-1}	Algal floc (marine snow)	Peduzzi and Weinbauer 1993

marine phages have a contractile tail. The phage head or capsid size is 30–60 nm with DNA-containing phages significantly more abundant than those containing RNA. As transformation and conjugation are not effective in natural settings, the process of transduction is a major mechanism for genetic transfer in most environments.

In addition to bacterial viruses, the virosphere also includes infection particles targeting cells of the Archaea domain and algal cells of the Eukarya domain (Prangishvili 2013). It has been suggested that viruses against archaeal cells should not be called "phages", the term "phage" should be reserved for bacterial viruses (Abedon and Murray 2013) due to the differences between bacterial and archaeal cells, and the domain Akamara (Greek for without chamber) should be used for archaeal viruses (Hurst 2000). As reviewed by Pietilä et al. (2013, 2014), there are over 6000 known phages but only about 100 archaeal viruses have been reported. Archaeal cells targeted by viruses include methanogens, hyperthermophiles, and extreme halophiles of the Crenarchaeota and Euryarchaeota phyla. The shapes of these archaeal viruses range from lemon, bottle, droplet to icosahedral heads, and many of the forms have tails.

Eukaryotic algae are important to provide nutrients to aquatic systems and, therefore, algae constitute an important segment of the food chain. In an unbalanced aquatic system, algal blooms occur and the decline of these blooms may be attributed to algal viruses. The virus-mediated lysis of algal cells releases nutrients into the water for use by bacteria, and the algal viruses join the phages in nutrient cycling known as the viral loop. A historical review of algal viruses is available (Van Etten et al. 1991). Recently, considerable interest has been given to algal viruses because one of these DNA-containing viruses, *Chlorovirus ATCV-1*, has been found to be part of the human virome and has been implicated in causing changes in cognitive functions in humans and mice (Yolken et al. 2014).

Viruses associated with fungi are called mycoviruses and were discovered in mushrooms over 50 years ago. Mycoviruses are broadly distributed and have been detected in Ascomycota, Basidiomycota, Chytridiomycota, Deuteromycota, and Zymomycota phyla of fungi (Son et al. 2015). Currently, most of the mycoviruses are characterized as containing dsRNA, about 30% of the mycoviruses contain positive-sense, single stranded RNA (ssRNA) and a few mycoviruses contain negative-sense ssRNA and ssDNA. Most mycoviruses do not have an extracellular route for infection and transmission of a mycovirus is dependent on cell fusion, cell division, and spore production. Therefore, to detect and identify mycoviruses, scientists rely on purification of dsRNA from fungal cultures because some mycoviruses have a genome of dsRNA but other mycoviruses produce dsRNA intermediates in their hosts. Fungi infected with mycoviruses display changes in cell pigmentation, reduced growth rate, altered sexual reproduction, and most importantly, a reduction of virulence with reduced production of mycotoxin. Considerable success has been obtained in using specific mycoviruses to control the chestnut blight fungus, *Cryphonectria parasitica*, and rapeseed stem rot by *Sclerotinia sclerotiorum*. Not all mycoviruses reduce fungal virulence but some are latent mycoviruses that have a unique effect on the host. There is one report where the symbiotic activity of an endophytic mycorrhizal fungus, mycovirus, and a grass plant enables survival at elevated temperatures when the three partners are present (Son et al. 2015). In some instances, the mycovirus is destructive to cells, as in yeast where host death occurs when the mycovirus unites with a DNA virus-like particle. Additional research is needed to clarify the role of mycoviruses in the biosphere.

The gene transfer agent (GTA) was discovered by Barry Marrs in 1994 using *Rhodobacter capsulatus*. The GTA particle consists of protein that is encoded on 15–17 genes located on the bacterial chromosome and moves dsDNA randomly acquired from the host to a recipient bacterial cell (see reviews by Lang et al. 2012; McDaniel et al. 2012). Unlike phages, GTAs do not contain DNA that encodes for GTA production but the GTA uses genes from the host cell.

Reported GTAs have a head size of about 40 nm and tail length varies from 7 to 190 nm. Unlike bacterial viruses, which carry their own DNA in the head of the infectious particle, GTAs carry 4.4–14 kb of DNA randomly collected from the host cell (McDaniel et al. 2010, 2012). GTAs have been found in several microbial strains: RcGTA from *R. capsulatus* (formerly *Rhodopseudomonas capsulatus*). Dd1 from *Desulfovibrio desulfuricans*, VSH-1 from *Brachyspira hyodysenteriae* (formerly *Serpulina hyodysenteriae*). and VAT from the archaeon *Methanococcus voltae* (Lang et al. 2012). *Roseovarius nubinhibens* ISM and *Nitratireductor* 44B9s isolated from a costal environment near Georgia, USA, were mutagenized with Tn5 (which encodes for both kanamycin and streptomycin resistance) and were found to effectively transfer dual antibiotic resistance to bacteria in environmental samples. Additional studies indicated that *R. nubinhibens*, *R. capsulatus*, *Oceanicola granulosus*, and *Ruegeria* (formerly *Silicibacter*) *pomeroyi* carried genes for GTA proteins and these were correlated with the production of 10^5–10^9 virus-like particles per milliliter (McDaniel et al. 2012). Examination of genome reports indicates that genes similar to those for the production of RcGAT are broadly distributed throughout the *Alphaproteobacteria* and that the enhanced exchange of DNA between bacteria of different species occurs (McDaniel et al. 2010, 2012; Biers et al. 2008; Lang and Beatty 2007). Using the estimated abundance of bacteria in marine waters as 1.6×10^6 ml^{-1} and 30% of the marine bacteria as *Alphaproteobacteria* with the potential for GTA production, suggests that the GTAs could represent 0.05% of the marine viruses. As a point of reference, the bacterial viruses in the oceans are estimated to be 4.4×10^7 ml^{-1} (McDaniel et al. 2012).

1.5 What Determines Which Microorganisms are Present?

When microbiologists sample different environments, the microorganisms isolated reflect the physical and chemical nature of the environment. That is, agricultural soil has different microorganisms than hot water springs and acid runoff from mines has different microbes than fresh water. However, when bacteria from agricultural soil at different sites around the world are isolated, similar types of bacteria would be expected just as bacteria from different marine settings may have similar phenotypes. The question arises "What determines which microorganisms are present in the environment?" In response to this question, Lourens Gerhard Marinus Bass Becking in 1934 proposed that "Everything is everywhere *but* the environment selects" (de Wit and Bouvier 2006). This hypothesis has been a basic component of environmental microbiology for many years. The idea is that microorganisms become airborne due to some type of physical activity and winds distribute the suspended cells over the entire Earth. Also, in fresh water or marine aquatic environments, the aquatic currents contribute to the mixing of suspended microorganisms. The physical and chemical environments where the cells are deposited select for organisms that are able to grow at those sites. However, Bass Becking's hypothesis does not explain the extent of genetic variation between the *Synechococcus* spp., a cyanobacterium, in hot springs (Pake et al. 2003) or the archaeon *Sulfolobus* spp. (Whitaker et al. 2003) that are geographically separated. Although the phenotypic characteristics of microbial communities in surface ocean waters are correlated with environmental features and not physical distance (Raes et al. 2011; Jiang et al. 2012). microbial biogeochemistry in extreme environments may be influenced by microbial dispersion with genetic selection as well as geographical isolation (Lau et al. 2014).

There are many chemical and physical environments that are highly restrictive and select for a unique type of microorganism. In environments containing high levels of acid, alkali, or salt, or areas containing toxic metals, the absence of one or more nutrients selects for a specific type of microorganism. At the bottom of oceans, the hydrostatic pressures are extreme and only barophilic bacteria can grow. Recognition sites and molecular interactions select for