Environmental Microbiology

From genomes to biogeochemistry

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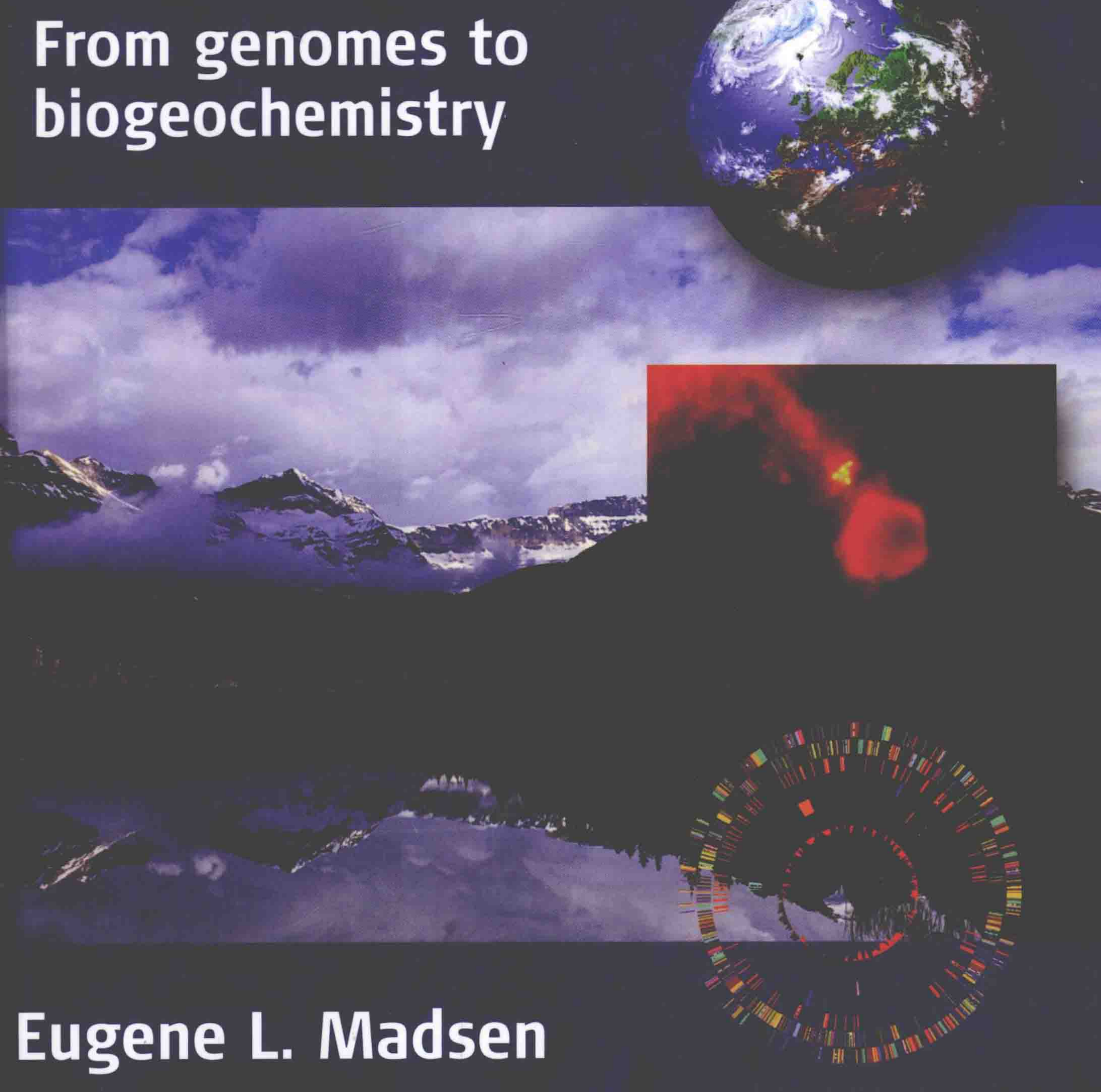

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Preface

Over the past 20 years, environmental microbiology has emerged from a rather obscure, applied niche within microbiology to become a prominent, ground-breaking area of biology. Environmental microbiology's rise in scholarly stature cannot be simply explained. But one factor was certainly pivotal in bringing environmental microbiology into the ranks of other key biological disciplines. That factor was molecular techniques. Thanks largely to Dr. Norman Pace (in conjunction with his many students) and Gary Olson and Carl Woese, nucleic acid analysis procedures began to flow into environmental microbiology in the mid-1980s. Subsequently, a long series of discoveries have flooded out of environmental microbiology. This two-way flow is constantly accelerating and the discoveries increasingly strengthen the links between environmental microbiology and core areas of biology that include evolution, taxonomy, physiology, genetics, environment, genomics, and ecology.

This textbook has grown from a decade of efforts aimed at presenting environmental microbiology as a coherent discipline to both undergraduate and graduate students at Cornell University. The undergraduate course was initially team-taught by Drs. Martin Alexander and William C. Ghiorse. Later, W. C. Ghiorse and I taught the course. Still later I was the sole instructor. Still later I became instructor of an advanced graduate version of the course. The intended audience for this text is upper-level undergraduates, graduate students, and established scientists seeking to expand their areas of expertise.

Environmental microbiology is inherently multidisciplinary. It provides license to learn many things. Students in university courses will rebel if the subject they are learning fails to develop into a coherent body of knowledge. Thus, presenting environmental microbiology to students in a classroom setting becomes a challenge. How can so many disparate areas of science (e.g., analytical chemistry, geochemistry, soil science, limnology, public health, environmental engineering, ecology, physiology, biogeochemistry, evolution, molecular biology, genomics) be presented as a unified body of information?

This textbook is my attempt to answer that question. Perfection is always evasive. But I have used five core concepts (see Section 1.1) that are reiterated throughout the text, as criteria for selecting and organizing the contents of this book.

The majority of figures presented in this book appear as they were prepared by their original authors in their original sources. This approach is designed to illustrate for the reader that advancements in environmental microbiology are a community effort.

A website with downloadable artwork and answers to study questions is available to instructors at www.blackwellpublishing.com/madsen

I hope this book will stimulate new inquiries into what I feel is one of the most fascinating current areas of science. I welcome comments, suggestions, and feedback from readers of this book. I thank the many individuals who provided both direct and indirect sources of information and inspiration. I am particularly grateful to P. D. Butler for assistance in manuscript preparation, to J. Yavitt who guided me to the right destinations in the biogeochemistry literature, and to W. C. Ghiorse for his unbounded enthusiasm for the art and science of microbiology. Constructive comments from several anonymous reviewers are acknowledged. I also apologize for inadvertently failing to include and/or acknowledge scientific contributions from fellow environmental microbiologist friends and colleagues.

Eugene Madsen

Contents

1

Significance, History, and Challenges of Environmental Microbiology

This chapter is designed to instill in the reader a sense of the goals, scope, and excitement that permeate the discipline of environmental microbiology. We begin with five core concepts that unify the field. These are strengthened and expanded throughout the book. Next, an overview of the significance of environmental microbiology is presented, followed by a synopsis of key scholarly events contributing to environmental microbiology's rich heritage. The chapter closes by reminding the reader of the complexity of Earth's biogeochemical systems and that strategies integrating information from many scientific disciplines can improve our understanding of biosphere function.

Chapter 1 Outline

1.1 CORE CONCEPTS CAN UNIFY ENVIRONMENTAL MICROBIOLOGY

Environmental microbiology is inherently multidisciplinary. Its many disparate areas of science need to be presented coherently. To work toward that synthesis, this text uses five recurrent core concepts to bind and organize facts and ideas.

Core concept 1. Environmental microbiology is like a child's picture of a house – it has (at least) five sides (a floor, two vertical sides, and two sloping roof pieces). The floor is evolution. The walls are thermodynamics and habitat diversity. The roof pieces are ecology and physiology. To learn environmental microbiology we must master and unite all sides of the house.

Core concept 2. The prime directive for microbial life is survival, maintenance, generation of adenosine triphosphate (ATP), and sporadic growth (generation of new cells). To predict and understand microbial processes in real-world waters, soils, sediments, and other habitats, it is helpful to keep the prime directive in mind.

Core concept 3. There is a mechanistic series of linkages between our planet's habitat diversity and what is recorded in the genomes of microorganisms found in the world today. Diversity in habitats is synonymous with diversity in selective pressures and resources. When operated upon by forces of evolution, the result is molecular, metabolic, and physiological diversity found in extant microorganisms and recorded in their genomes.
Core concept 4. Advancements in environmental microbiology depend upon convergent lines of independent evidence using many measurement procedures. These include microscopy, biomarkers, model cultivated microorganisms, molecular biology, and genomic techniques applied to laboratory- and field-based investigations.
Core concept 5. Environmental microbiology is a dynamic, methods-limited discipline. Each methodology used by environmental microbiologists has its own set of strengths, weaknesses, and potential artifacts. As new methodologies deliver new types of information to environmental microbiology, practitioners need a sound foundation that affords interpretation of the meaning and place of the incoming discoveries.

1.2 SYNOPSIS OF THE SIGNIFICANCE OF ENVIRONMENTAL MICROBIOLOGY

With the formation of planet Earth 4.6×10^9 years ago, an uncharted series of physical, chemical, biochemical, and (later) biological events began to unfold. Many of these events were slow or random or improbable. Regardless of the precise details of how life developed on Earth, (see Sections 2.3–2.7), it is now clear that for ~70% of life's history, prokaryotes were the sole or dominant life forms. Prokaryotes (*Bacteria* and *Archaea*) were (and remain) not just witnesses of geologic, atmospheric, geochemical, and climatic changes that have occurred over the eons. Prokaryotes are also active participants and causative agents of many geochemical reactions found in the geologic record. Admittedly, modern eukaryotes (especially land plants) have been major biogeochemical and ecological players on planet Earth during the most recent 1.4×10^9 years. Nonetheless, today, as always, prokaryotes remain the "hosts" of the planet. Prokaryotes comprise ~60% of the total biomass (Whitman et al., 1998; see Chapter 4), account for as much as 60% of total respiration of some terrestrial habitats (Velvis, 1997; Hanson et al., 2000), and also colonize a variety of Earth's habitats devoid of eukaryotic life due to topographic, climatic and geochemical extremes of elevation, depth, pressure, pH, salinity, heat, or light.

The Earth's habitats present complex gradients of environmental conditions that include variations in temperature, light, pH, pressure, salinity, and both inorganic and organic compounds. The inorganic materials range from elemental sulfur to ammonia, hydrogen gas, and methane and

Table 1.1
Microorganisms' unique combination of traits and their broad impact on the biosphere

Traits of microorganisms	Ecological consequences of traits
Small size	Geochemical cycling of elements
Ubiquitous distribution throughout Earth's habitats	Detoxification of organic pollutants
High specific surface areas	Detoxification of inorganic pollutants
Potentially high rate of metabolic activity	Release of essential limiting nutrients from the biomass in one generation to the next
Physiological responsiveness	Maintaining the chemical composition of soil, sediment, water, and atmosphere required by other forms of life
Genetic malleability	
Potential rapid growth rate	
Unrivaled nutritional diversity	
Unrivaled enzymatic diversity	

the organic materials range from cellulose to lignin, fats, proteins, lipids, nucleic acid, and humic substances (see Chapter 7). Each geochemical setting (e.g., anaerobic peatlands, oceanic hydrothermal vents, soil humus, deep subsurface sediments) features its own set of resources that can be physiologically exploited by microorganisms. The thermodynamically governed interactions between these resources, their settings, microorganisms themselves, and 3.6×10^9 years of evolution are probably the source of metabolic diversity of the microbial world.

Microorganisms are the primary agents of geochemical change. Their unique combination of traits (Table 1.1) cast microorganisms in the role of recycling agents for the biosphere. Enzymes accelerate reaction rates between thermodynamically unstable substances. Perhaps the most ecologically important types of enzymatic reactions are those that catalyze oxidation/reduction reactions between electron donors and electron acceptors. These allow microorganisms to generate metabolic energy, survive, and grow. Microorganisms procreate by carrying out complex, genetically regulated sequences of biosynthetic and assimilative intracellular processes. Each daughter cell has essentially the same macromolecular and elemental composition as its parent. Thus, integrated metabolism of all nutrients (e.g., carbon, nitrogen, phosphorus, sulfur, oxygen, hydrogen, etc.) is implicit in microbial growth. This growth and survival of microorganisms drives the geochemical cycling of the elements, detoxifies many contaminant organic and inorganic compounds, makes essential nutrients present in the biomass of one generation available to the next, and maintains the conditions required by other inhabitants of the biosphere (Table 1.1). Processes carried out by microorganisms in soils, sediments, oceans, lakes, and groundwaters have a major impact on environmental quality, agriculture, and global climate change. These processes are also the basis for current and emerging biotechnologies with industrial and environmental applications (see Chapter 8). Table 1.2 presents

Table 1.2

Examples of nutrient cycling and physiological processes catalyzed by microorganisms in biosphere habitats (reproduced with permission from *Nature Reviews Microbiology* from Madsen, E.L. 2005. Identifying microorganisms responsible for ecologically significant biogeochemical processes. *Nature Rev. Microbiol.* **3**:439–446. Macmillan Magazines, www.nature.com/reviews)

Nutrient cycle	Process	Nature of process	Typical habitat	References
Carbon	Photosynthesis	Light-driven CO_2 fixation into biomass	FwS, Os, Ow	Pichard et al., 1997; Partensky et al., 1999; Ting et al., 2002
	Carbon respiration	Oxidation of organic C to CO_2	Sl	Heemsbergen, 2004
	Cellulose decomposition	Depolymerization, respiration	Sl	Jones et al., 1998
	Methanogenesis	Methane production	FwS, Os, Sw	Conrad, 1996; Schink, 1997
	Aerobic methane oxidation	Methane becomes CO_2	Fw, Ow, Sl	Segers, 1998; Bull et al., 2000
	Anaerobic methane oxidation	Methane becomes CO_2	Os	Boetius et al., 2000
Biodegradation	Synthetic organic compounds	Decomposition, CO_2 formation	All habitats	Alexander, 1999; Boxall et al., 2004
	Petroleum hydrocarbons	Decomposition, CO_2 formation	All habitats	Van Hamme et al., 2003
	Fuel additives (MTBE)	Decomposition, CO_2 formation	Gw, Sl, Sw	Deeb et al., 2003
	Nitroaromatics	Decomposition	Gw, Sl, Sw	Spain et al., 2000, Esteve-Núñez et al., 2001
	Pharmaceuticals, personal care products	Decomposition	Gw, Sl, Sw	Alexander, 1999; Ternes et al., 2004
	Chlorinated solvents	Compounds are dechlorinated via respiration in anaerobic habitats	Gw, Sl, Sw	Maymo-Gatell et al., 1997; Adrian et al., 2000

Nitrogen	Nitrogen fixation	N_2 gas becomes ammonia	Ow, Sl	Karl et al., 2002
	Ammonium oxidation	Ammonia becomes nitrite and nitrate	Sl, Sw	Stark and Hart, 1997; Kowalchuk and Stephen, 2001
	Anaerobic ammonium oxidation	Nitrite and ammonia become N_2 gas	Os, Sw	Dalsgaard et al., 2003; van Niftrik et al., 2004
	Denitrification	Nitrate is used as an electron acceptor and converted to N_2 gas	Sl, Sw	Zumft, 1997; van Breemen et al., 2002
Sulfur	Sulfur oxidation	Sulfide and sulfur become sulfate	Os	Taylor and Wirsen, 1997
	Sulfate reduction	Sulfate is used as an electron acceptor and converted to sulfur and sulfide	Os	Habicht and Canfield, 1996
Other elements	Hydrogen oxidation	Hydrogen is oxidized to H^+, electrons reduce other substances	Sl, Os, Sw,	Schink, 1997
	Mercury methylation and reduction	Organic mercury is formed and mercury ion is converted to metallic mercury	FwS, Os	Morel et al., 1998; Sigel et al., 2005
	(Per)chlorate reduction	Oxidants in rocket fuel and other sources are converted to chloride	Gw	Coates and Achenbach, 2004
	Uranium reduction	Uranium oxyanion is used as an electron acceptor; hence immobilized	Gw	Lovley, 2003
	Arsenate reduction	Arsenic oxyanion is used as an electron acceptor; hence toxicity is diminished	FwS, Gw	Oremland and Stolz, 2003
	Iron oxidation, acid mine drainage	Iron sulfide ores are oxidized, strong acidity is generated	FwS, Gw	Edwards et al., 2000

Fw, freshwater; FwS, freshwater sediment; Gw, groundwater; Os, ocean sediments; Ow, ocean waters; Sl, soil; Sw, sewage.

a sampling of the ecological and biogeochemical processes that microorganisms catalyze in aquatic or terrestrial habitats. Additional details of biogeochemical processes and ways to recognize and understand them are presented in Chapters 3 and 7.

1.3 A BRIEF HISTORY OF ENVIRONMENTAL MICROBIOLOGY

Early foundations of microbiology rest with microscopic observations of fungal sporulation (by Robert Hooke in 1665) and "wee animalcules" – true bacterial structures (by Antonie van Leeuwenhoek in 1684). In the latter half of the nineteenth century, Ferdinand Cohn, Louis Pasteur, and Robert Koch were responsible for methodological innovations in aseptic technique and isolation of microorganisms (Madigan and Martinko, 2006). These, in turn, allowed major advances pertinent to spontaneous generation, disease causation, and germ theory.

Environmental microbiology also experienced major advancements in the nineteenth century; these extend through to the present. Environmental microbiology's roots span many continents and countries (Russia, Japan, Europe, and England) and a complex tapestry of contributions has developed. To a large degree, the challenges and discoveries in environmental microbiology have been habitat-specific. Thus, one approach for grasping the history and traditions of environmental microbiology is to recognize subdisciplines such as marine microbiology, soil microbiology, rumen microbiology, sediment microbiology, geomicrobiology, and subsurface microbiology. In addition, the contributions from various centers of training can also sometimes be easily discerned. These necessarily revolved around various investigators and the institutions where they were based.

As early as 1838 in Germany, C. G. Ehrenberg was developing theories about the influence of the bacterium, *Gallionella ferruginea*, on the generation of iron deposits in bogs (Ehrlich, 2002). Furthermore, early forays into marine microbiology by A. Certes (in 1882), H. L. Russell, P. Regnard, B. Fischer, and P. and G. C. Frankland allowed the completion of preliminary surveys of microorganisms from far-ranging oceanic waters and sediments (Litchfield, 1976).

At the University of Delft (the Netherlands) near the end of the nineteenth century, M. W. Beijerinck (Figure 1.1) founded the Delft School traditions of elective enrichment techniques (see Section 6.2) that allowed Beijerinck's crucial discoveries including microbiological transformations of nitrogen and carbon, and also other elements such as manganese (van Niel, 1967; Atlas and Bartha, 1998; Madigan and Martinko, 2006). The helm of the Delft School changed hands from Beijerinck to A. J. Kluyver, and the traditions have been continued in the Netherlands, Germany, and other parts of Europe through to the present. After training in Delft with Beijerinck and Kluyver, C. B. van Niel was asked by L. G. M. Baas Becking to established a research program at Stanford University's Hopkins Marine Station (done in 1929), where R. Y. Stainer, R. Hungate, M. Doudoroff and many others were trained, later establishing their own research programs at other institutions in the United States (van Niel, 1967).

S. Winogradsky (Figure 1.2) is regarded by many as the founder of soil microbiology (Atlas and Bartha, 1998). Working in the latter part of the nineteenth and early decades of the twentieth centuries, Winogradsky's career contributed immensely to our knowledge of soil and environmental microbiology, especially regarding microbial metabolism of sulfur, iron, nitrogen, and manganese. In 1949, much of Winogradsky's work was published as a major treatise entitled, *Microbiologie du sol, problémes et methods: cinquante ans de recherches. Oeuvres Complétes* (Winogradsky, 1949).

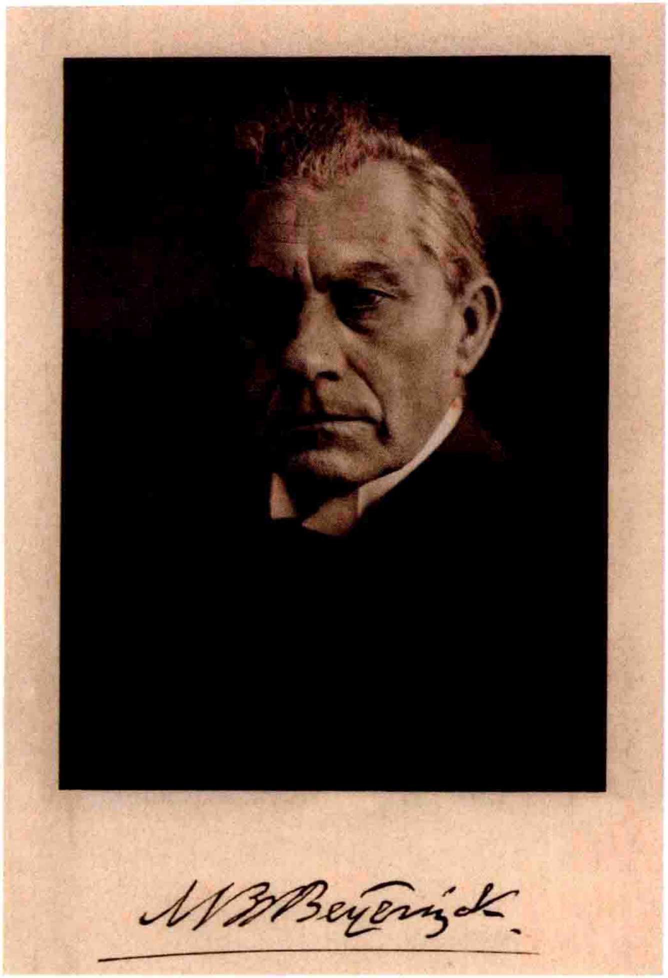

Figure 1.1 Martinus Beijerinck (1851–1931). Founder of the Delft School of Microbiology, M. Beijerinck worked until the age of 70 at the University of Delft, the Netherlands. He made major discoveries in elective enrichment techniques and used them to advance the understanding of how microorganisms transform nitrogen, sulfur, and other elements. (Reproduced with permission from the American Society for Microbiology Archives, USA.)

Many of the marine microbiologists in the early twentieth century focused their attention on photoluminescent bacteria (E. Pluger, E. W. Harvey, H. Molisch, W. Beneche, G. H. Drew, and J. W. Hastings). Later, transformation by marine microorganisms of carbon and nitrogen were explored, as well as adaptation to low-temperature habitats (S. A. Waksman, C. E. ZoBell, S. J. Niskin, O. Holm-Hansen, and N. V. and V. S. Butkevich). The mid-twentieth century marine studies continued exploration of the physiological and structural responses of microorganisms to salt, low temperature, and pressure (J. M. Shewan, H. W. Jannasch, R. Y. Morita, R. R. Colwell, E. Wada, A. Hattori, and N. Taga). Also, studies of nutrient uptake (J. E. Hobbie) and food chains constituting the "microbial loop" were conducted (L. R. Pomeroy).

Figure 1.2 Sergei Winogradsky (1856–1953). A major contributor to knowledge of soil microbiology, S. Winogradsy described microbial cycling of sulfur and nitrogen compounds. He developed the "Winogradsky column" for growing diverse physiological types of aerobic and anaerobic, heterotrophic and photosynthetic bacteria across gradients of oxygen, sulfur, and light. (Reproduced with permission from the Smith College Archives, Smith College.)

At Rutgers University, Selman A. Waksman was perhaps the foremost American scholar in the discipline of soil microbiology. Many of the Rutgers traditions in soil microbiology were initiated by J. Lipman, Waksman's predecessor (R. Bartha, personal communication; Waksman, 1952). Waksman produced numerous treatises that summarized the history, status, and frontiers of soil microbiology, often in collaboration with R. Starkey. Among the prominent works published by Waksman are "Soil microbiology in 1924: an attempt at an analysis and a synthesis" (Waksman, 1925), *Principles of Soil Microbiology* (Waksman, 1927), "Soil microbiology as a field of science" (Waksman, 1945), and *Soil Microbiology* (Waksman, 1952). A steady flow of Rutgers-based contributions to environmental microbiology continue to be published (e.g., Young and Cerniglia, 1995; Haggblom and Bossert, 2003).

In the 1920s and 1930s, E. B. Fred and collaborators, I. L. Baldwin and E. McCoy, comprised a unique cluster of investigators whose interests focused on the *Rhizobium*–legume symbiosis. Several decades later at the University of Wisconsin, T. D. Brock and his students made important contributions to microbial ecology, thermophily, and general microbiology. Another graduate of the University of Wisconsin, H. L. Ehrlich earned a Ph.D. in 1951 and, after moving to Rensselaer Polytechnic Institute, carried out studies on the bacteriology of manganese nodules, among other topics. Author of four comprehensive editions of *Geomicrobiology*, H. L. Ehrlich is, for many, the founder of this discipline.

Another University of Wisconsin graduate, M. Alexander, moved to Cornell University in 1955. For four decades prior to Alexander's arrival, soil microbiological research was conducted at Cornell by J. K. Wilson and F. Broadbent. From 1955 to the present, Alexander's contributions to soil microbiology have examined a broad diversity of phenomena, which include various transformations of nitrogen, predator–prey relations, microbial metabolism of pesticides and environmental pollutants, and advancements in environmental toxicology. Many environmental microbiologists have received training with M. Alexander and become prominent investigators, including J. M. Tiedje.

Other schools and individuals in Britain, Italy, France, Belgium and other parts of Europe, Japan, Russia and other parts of Asia, Africa, Australia, the United States and other parts of the Americas certainly have contributed in significant ways to advancements in environmental microbiology. An insightful review of the history of soil microbiology, with special emphasis on eastern European and Russian developments was written by Macura (1974).

The many historical milestones in the development of environmental microbiology (most of which are shared with broader fields of biology and microbiology) have been reviewed by Atlas and Bartha (1998), Brock (1961), Lechevalier and Solotorovsky (1965), Macura (1974), Madigan and Martinko (2006), van Niel (1967), Waksman (1925, 1927, 1952), and others. Some of the highlights are listed in Table 1.3.

Table 1.3
Selected landmark events in the history of environmental microbiology

- The first visualization of microscopic life by van Leeuwenhoek in 1684
- The role of microorganisms as causative agents of fermentations discovered by Pasteur in 1857
- The use of gelatin plates for enumeration of soil microorganisms by Koch in 1881
- Nitrogen fixation by nodules on the roots of legumes discovered by Hellriegel and Wilfarth in 1885
- The use of elective enrichment methods, by Beijerinck and Winogradsky, in the isolation of single organisms able to carry out ammonification, nitrification, and both symbiotic and nonsymbiotic nitrogen fixation
- Recognition of the diverse populations in soil (e.g., bacteria, fungi, algae, protozoa, nematodes, insect larvae)
- Documentation of anaerobic cellulose decomposition by Omelianskii in 1902
- The study of sulfur-utilizing phototrophic bacteria by van Niel and others
- The specificity of legume-nodulating bacteria (Fred et al., 1932)
- The discovery and development of antibiotics
- Direct microscopic methods of examining environmental microorganisms via staining and contact-slide procedures
- The development of radiotracer techniques
- A diversity of advancements in analytical chemistry for detecting and quantifying biochemically and environmentally relevant compounds
- Developments in molecular phylogeny (Woese, 1987, 1992; Pace, 1997)
- The application of molecular methods to environmental microbiology (Olsen et al., 1986; Pace et al., 1986; Amann et al., 1991, 1995; Ward et al., 1993; White, 1994; van Elsas et al., 1997; Madigan and Martinko, 2006)