Environmental Microbiology

Principles and Applications

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Preface

Environmental Microbiology as a subject is relatively young compared to other traditional subjects. It deals with processes in the environment that are directly mediated by microorganisms. However, it is also a rapidly growing field that cuts across various traditional disciplines such as Biochemistry, Ecology, Engineering, Geography, and Microbiology. It is difficult to pinpoint the origin of Environmental Microbiology as a subject but its foundation lies in the initial interest to understand and provide safe drinking water coupled with proper sanitation at the onset of industrialization and associated urbanization. However, during the past three decades the scope of Environmental Microbiology has tremendously expanded to include a variety of other issues, such as the fate of organic pollutants, transformation of metals, and aerobiology.

This book was written for an audience that has a basic understanding of microbiology. Oftentimes, microbiologists tend to overzealously focus on bacteria, inadvertently ignoring other microbes (i.e., algae, fungi, protozoa, and viruses). This discrepancy is redressed herein. Scholars of environmental microbiology come from a variety of disciplines including Microbiology majors, Social Scientists, Engineering, Law (Environmental Law), Agriculturalists, Geography (GIS), Chemists (Environmental Chemists), Toxicologists and so forth. Considering such a diverse audience, not everyone will be content with the depth accorded to all aspects of the topic. However, the reader will find the extensive references rich resources for more in-depth data. The material presented here recognizes the basic foundations and importance of conventional microbiological techniques (which focused greatly on culture-based studies), linking them with information from more recent nonconventional techniques. Various principles are also applied which attest to the undisputable reality that microbes in pure culture may function somewhat differently than in complex multispecies environmental matrices.

This book is unique in that the subject is approached from a **history of microbes** and their place in shaping the environment, rather than a *history of microbiology.* This approach properly introduces the reader to the several different microorganisms and then unveils the role of each (negative or positive) in the environment. That environmental degradation is more prevalent in developing countries is a commonly recognized fact. Quite a number of books address important environmental microbiology issues, such as water treatment, but sad to say orient their presentation exclusively to high-investment treatment systems. This book reaches beyond such economically burdensome schemes by covering the basic concepts of water treatment and modes of application in a variety of backgrounds and economic settings. Basic microbiological concepts such as physiology, genetics, and metabolism are discussed with reference to ecological concepts and biochemical cycling. A chapter on environmental biotechnology is also included.

While writing this book professional advice, editorial opinions, support and encouragement were received from several individuals, including Drs. O. Roger Anderson, Brian K. Kinkle, Mark LeChevallier, Eugene L. Madsen, Lynn Margulis, Sharon Parker, Boakai Robertson, Dorion Sagan, Angela Sessitsch, and Guenther Stotzky, to name but a few. The editorial skills of the

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Microbial Evolution and Diversity

1.1 INTRODUCTION

The importance of the environment in our day-to-day activities only became obvious to the general public in the early part of the 1960s when Rachel Carson's book *Silent Spring* was published. Carson (1962) opened her book by describing an imaginary town in the heart of America where all life had at one time lived in harmony with its surroundings. Over a short period of time, mysterious maladies swept the community and all its forms of life. Everywhere in this town was a shadow of death attributed to humans and their activities. Even though Carson clearly indicates in her description of these maladies that this town did not actually exist, the picture she painted brought home humanity's detrimental activities against the environment. Soon after her book was published, the public outcry in the United States prompted Congress to establish the Environmental Protection Agency (US EPA). Various developed countries also created either full ministries or departments within specific ministries to oversee matters pertaining to the environment. In the United Nations, the United Nations Environmental Program (UNEP) was established to help member countries deal with environmental management problems. Today, most countries have, at least in principle, shown a commitment to slow down or even reverse the human-driven devastating effects against the environment.

Microbes, a term we must emphasize, that includes bacteria, protozoa, fungi, algae, and for lack of a better collective term, viruses, play a very significant role in influencing environmental dynamics. Microbes preceded photosynthesis. The subject of environmental microbiology includes microbial ecology which is basically the study of the distribution, activities, and interactions of microorganisms with their habitats (i.e., soil, sediments, freshwater, groundwater, etc.). Such studies normally entail the isolation, identification, and measurement of the activities of organisms in both pure and mixed culture, assessment of their interactions with other living cells, and determination of their response to abiotic environments.

An attempt to understand environmental microbiology requires an examination of microorganisms in the establishment (origin) of life on Earth and their contributions to life through evolution. Based on radiodating measurements, the solar system is estimated to be about 4.6 billion years (Ba) old. It originated when a very hot star (supernova) exploded and generated a new star (the sun) and other components in the galaxy including the Earth. During this early time, the intensity of sunlight was approximately 30% less than it is now (Philander, 1998). We are sure that the Earth's origin was connected with the explosion of a supernova because to date, it is radioactive and made of elements such as iron, silicon, and oxygen. These elements cannot be made in the normal processes of stellar evolution and it takes energy to make them. This radioactivity in turn provides an accurate clock which has been used to establish the time since this explosion occurred and the inception of Earth.

1.2 THE ORIGIN OF LIFE

The earliest forms of life on Earth have been estimated to be 3.5-4.0 Ba old, dating from the time when the planet cooled to a point at which liquid water was present. Several scientific theories to explain the origin of life on Earth have been advanced. Most widely subscribed to is the possibility that life started from a primordial soup. It is likely that a rich mixture of gases accumulated on primitive Earth, setting the stage for biological evolution. Ultraviolet light or lightning could have struck the primordial soup of the Archean ocean, causing the fusion of carbon and hydrogen atoms that helped to produce the first life (Margulis and Sagan, 1986). Before the advent of an oxygen atmosphere on Earth, the planet was likely a predominantly reducing environment, the abundant gases being carbon dioxide, methane, ammonia, and nitrogen. Traces of carbon monoxide, hydrogen, and HCN, the latter formed as a result of ammonia reacting with methane, were also present. The different forms of energy that were present even at that early period, including geothermal, ultraviolet (UV) radiation, and radioactivity, could have facilitated the abiotic formation of organic matter, leading to macromolecules that aggregated to form membrane-like interfaces in the surrounding liquid. The absence of an ozone layer enabled fluxes of UV light to reach the Earth's surface. The primordial soup-UV light theory is supported by the fact that some biochemically important molecules such as amino acids, sugars, fatty acids, purines, and pyramidines can be synthesized abiotically when some gases are irradiated with UV or electron discharge. Miller and Urey (1959) bombarded a mixture of water vapor, ammonia, methane, and hydrogen with a lightning-like discharge for a week and synthesized alanine and glycine, as well as a number of other organic substances. The conditions they provided during this synthesis were somewhat similar to those that existed on prebiotic Earth. Before their experiments, such organic molecules were thought to be produced only by living cells. Since that discovery, more components of complex cellular molecules including ATP, adenine, cytosine, guanine, thiamine and uracil have been synthesized in the laboratory by subjecting various mixtures of gases and mineral solutions to different energy sources such as UV, heat, electric discharge, and electric sparks, reenforcing the possibility of life originating from a primordial soup (Chang et al., 1983; Dickerson, 1978; Robertson and Miller, 1995). When oxygen is included in these synthesis experiments, the synthesis fails since O_2 rapidly oxidizes the organic products before they accumulate. In the absence of oxygen and microbial decomposition, these initial life-forming processes may have enabled organic products to accumulate over millions of years, forming the basis of cellular organisms. During this chemical evolution, carbon was abundant and clay minerals, with their surface charge and repeating crystalline structure, could have provided surfaces for polymerization of the more complex organic molecules such as RNA and proteins.

Because the atmosphere on primitive Earth had predominantly reducing conditions with abundant hydrogen and carbon dioxide, the early life forms must have been microbial. Initially anaerobic, they evolved photosynthesis and production of oxygen as a waste product, eventually leading to its accumulation in the atmosphere. People who study evolution call this primitive type of cell which eventually evolved into the present day prokaryotic cells, eugenotes or cenancester. These could in turn have evolved from even more primitive cell-like organisms called progenotes. The progenotes and eugenotes existed in anaerobic environments containing abiotically formed organic matter. As will be discussed in the next chapter, the ability of prokaryotes to spread their genes rapidly must have transformed the planet from a sterile, hostile environment into one abundantly endowed with a variety of species.

Ferrous iron and hydrogen sulfide are thought to have been abundant on primitive earth and the reaction between these two compounds could have been the potential source of energy to the progenitors. The energy generated from these reactions may in turn have formed ATP.

$$FeCO_3 + 2H_2S \rightarrow FeS_2 + H_2 + H_2O + CO_2 \quad \Delta G^{o\prime} = -61.7 \text{ kJ/reaction} \quad \ldots(1)$$

$$FeS + H_2S \rightarrow FeS_2 + H_2 \quad \Delta G^{o\prime} = -61.7 \text{ kJ/reaction} \quad \ldots(2)$$

Both reactions also yield H_2 which could have been used to reduce elemental sulfur (S^o) to hydrogen sulfide (H_2S), replenishing the supply of H_2S in the environment. Both reactions are fairly simple and require few enzymes, notably hydrogenase and an ATPase to trap the energy released from the reaction. As expected, the early life forms on Earth were biochemically simple, possessing a few enzymes; the more complex forms appeared through mutation and selection. Most hyperthermophilic Archea, which are closest to the earliest organisms on Earth, are also able to reduce S^o and Fe(III) with H_2 and form H_2S, an observation that offers some validity to the contention that both ferrous iron and H_2S could have initially supplied energy for the earliest progenitors. Thus, iron and sulfate reduction may have been the first forms of microbial respiration.

Isotopic studies of ancient Isua rocks from Greenland and Fig Tree fossils (South Africa) and stromatolites formed in Western Australia (Warrawoona Formations), discussed below, strongly suggest that early life processes were entirely microbial.

1.3 MICROBIAL DIVERSITY AND ABUNDANCE

It is important to appreciate how diverse microbes are and the scope of processes with which they are associated. Textbooks devoted solely to the diversity of microorganisms have been published (Goodfellow and O'Donnell, 1993; Priest et al., 1994; Colwell et al., 1996). Bacteria are the most abundant, possibly as a result of their minute size and rapid rate of multiplication. In temperate regions, vis-à-vis the tropics, the diversity of microorganisms and their processes have been more extensively studied. However, the tropics harbor the greatest diversity of organisms compared to other geographic regions on the planet (Croll, 1966; Ehrlich, 1986). Why this is so remains uncertain but the following explanations have been postulated:

(1) productivity (e.g. photosynthesis) is much higher in the tropics than elsewhere;
(2) the tropics are more environmentally stable, making it easier for numerous small populations to exist and less likely to be subjected to accidental extinction; and/or
(3) the tropics are more climatically stable for longer periods of time, allowing species to coevolve and branch more freely.

1.4 GEOLOGICAL EVIDENCE OF EARLY MICROBIAL LIFE ON EARTH

The history of life is not a continuum of development but a record punctuated by brief, sometimes geologically instantaneous episodes of mass extinction and subsequent diversifications. Most of the evidence for the existence of early life has, therefore, been based on circumstantial evidence from fossil records. Such records indicate the build-up of oxygen in the atmosphere which, besides improving species diversity, also provided for better aeration that in turn ironically enhanced the rate of decomposition, thus minimizing the preservation of fossil records. Although somewhat better fossils are left by plants and animals compared to microorganisms, plant and animal life is more recent on Earth. The oldest animal and plant macrofossils are about 0.7 Ba old compared to microbial life which has existed for more than 3.5 Ba, a period that only began 1.0 Ba after the Earth was formed and 3.0 Ba before the appearance of plant and animal life. Paleontologists have long struggled to measure evolutionary change during the 2.0 Ba-period before the Cambrian age, known as the Proterozoic, but their analysis has been stymied by the lack of hard-shelled fossilized cells. There are fossils of such groups as the blue-green algae but these are rare and do not provide a complete track through time. Borrowing the words of that famous evolutionary biologist, Charles Darwin, fossil records are oftentimes imperfect and can be equated to a book in which just a few pages are preserved, on which a few lines, a few words, and a few letters still exist. Despite all this, some evidence to document the much earlier existence of microbial life on Earth has been accumulated and is discussed below.

1.4.1 The Isua Formation

The oldest rocks found so far on Earth, come from the Isua formation in western Greenland. This 3.75 Ba old sedimentary rock formation offers a record of the cooling and stabilization of the Earth's crust. These strata are too altered by heat and pressure (metamorphosed) to preserve the morphological remains of living creatures. However, the Isua rocks provide a geochemical signature of $^{12}C/^{13}C$ isotope ratios and show the enhanced ^{12}C that arises as a product of organic activity, thereby suggesting the existence of a prolific microbial life on the early Earth (Schidlowski, 1988). The proportion of the ^{12}C and ^{13}C isotopes in carbon of rocks made in the absence of life is recognizably different from the proportion of carbon from rocks that were once living matter. This evidence conveys the possibility that autotrophic C-fixation is an old process that dates as early as 3.5-3.8 Ba ago. Most carbon is in the stable, light form (^{12}C), with less than 1% in the heavy ^{13}C and an even smaller amount in the unstable radioactive ^{14}C. The chemistry of living matter segregates the isotopes such that the lighter ^{12}C isotope is preferentially used compared to the heavier ^{13}C isotope, a phenomenon called isotope discrimination. Both the organic and inorganic sedimentary C retain the isotopic composition of their progenitor organisms and carbonate rocks respectively. A steady flux of both inorganic C (mostly carbonate) and organic C enter newly formed sediments. Such fluxes have led to the accumulation of organic C. This discrimination raises the ratio of $^{12}C/^{13}C$ above the values that would be measured if all the sedimentary C had an organic source.

The sedimentary rocks in the Isua formation are of further evolutionary interest because their formation suggests that liquid water was present at the time of their formation (i.e., 3.75 Ba ago), a sign that at this early period, the conditions for some form of life existed. Prior to this period, however, the temperatures on Earth were greater than 100°C and thus free water could only have existed as vapor until the Earth subsequently cooled. Microfossils that are 3.75 Ba old which closely resemble cyanobacteria have been found embedded in rocks (Schopf, 1978).

1.4.2 Stromatolites and Fig Tree formations

Further evidence of early microbial life on Earth is based on stromatolites and Fig Tree formations in Swaziland and South Africa respectively. In 1977, Dr. Barghoorn hacked off pieces of flintlike rocks from the side of Mt. Barberton which had been formed 3.5 Ba ago when a silica-rich lava repeatedly poured on top of it, forming a chert of organic sediments that appear to have been deposited. Samples from this chert were sliced into thin sections which were placed under the microscope, revealing "microbial fossils". In other samples from a nearby Kromberg formation were found filamentous cyanobacteria-like fossils, evidence that by this early period, photosynthesis already thrived. This evidence is further collaborated by Stanley M. Awramik who, in 1979, discovered well-preserved multicellular filamentous microstructures in the 3.4 Ba-old rocks of the Warrawoona formation in Australia.

At another site in Swaziland, the Earth's oldest non-metamorphosed sediments, actual fossilized cells that date between 3.5-3.6 Ba have been reported (Knoll and Baghoorn, 1977). Stromatolites are fossilized versions of sediment trapped and bound by bacteria and cyanobacteria. Because the Earth was still anoxic during the time of the early formations, these ancient stromatolites were probably made of anoxygenic phototrophs. The anoxygenic phototrophic bacteria in the upper and subsequent layers make the mat opaque. The lower layer contains chemoorganotrophic bacteria (such as sulfur-reducing bacteria). Some of these microfossils have smooth organic walls and, occasionally, internal organic contents. More specifically, some microfossils were preservations in a process of binary fission. It is hypothesized that generation after generation of bacteria in the topmost layers died from exposure to radiation, their remains shielding the lower layers which accumulate sand and sediments to form a sort of living rug. Microbial mats occur in various environments such as hot springs and shallow marine basins where photosynthesis in the uppermost layer of the mat is balanced by decomposition from below (Awramik et al., 1983). Overall, however, such evidence from microbial mats and microscopic sections through chet rocks like those at Fig Tree is rare.

The time of origin of eukaryotes has been estimated by several approaches which include maximum sizes of organic-walled microfossils (1.75-2.0 Ba ago), carbonaceous megafossils (>1.8 Ba ago), modified sterol molecules extracted from Proterozoic rocks (>1.7 Ba ago) and molecular chronometry (1.8$\pm$0.4 Ba ago). This period saw a concentrated episode of diversification (the "Cambrian explosion"), an appearance of multicellular animals with hard parts in the fossil record. Han and Runnegar (1992) suggested that the very earliest eukaryotes such as *Grypania* probably lacked mitochondria and were therefore incapable of aerobic respiration until oxygen levels reached at least 1% of the present atmospheric levels. The Burgess fauna, which existed soon after the Cambrian explosion (approximately 530 Ma ago), are the only known major soft-bodied fauna from this primordial time. Considering the fact that the Earth is 4.5 M old (Gould, 1989; Margulis and Sagan, 1986), this Cambrian explosion and subsequent existence of familiar macroscopic life occupies only about 10% of earthly time. A key question by evolution scholars is why plants, animals, and fungi appeared so late and why these complex creatures have no direct simpler precursors in the fossil record of Precambrian times.

1.5 ONSET OF PHOTOSYNTHESIS AND RESULTANT DIVERSIFICATION

Early photosynthesis is thought to have been anoxygenic as found in present-day Chlorobiaceae, Rhodospirillaceae, and Chromatiaceae organisms that lack photosystem II and are not able to utilize the hydrogen from water to reduce carbon dioxide. The photosynthesizing organisms possibly used sulfur-reducing compounds such as H_2S and FeS (See Section 1.2), the process probably operating at temperatures close to the boiling point of water. Oxygen was probably not a product from these earlier photosynthetic processes.

About 2 Ba ago, the oxygen levels on Earth started increasing as a by-product of photosynthesis by the photosynthetic ancestors of the present day cyanobacteria (Fig. 1.1). The evolution of photosynthesis is the most important metabolic invention in the history of life on Earth. At its inception, the photosynthesizers used the abundant CO_2 and converted it to organic matter and oxygen or its equivalent just

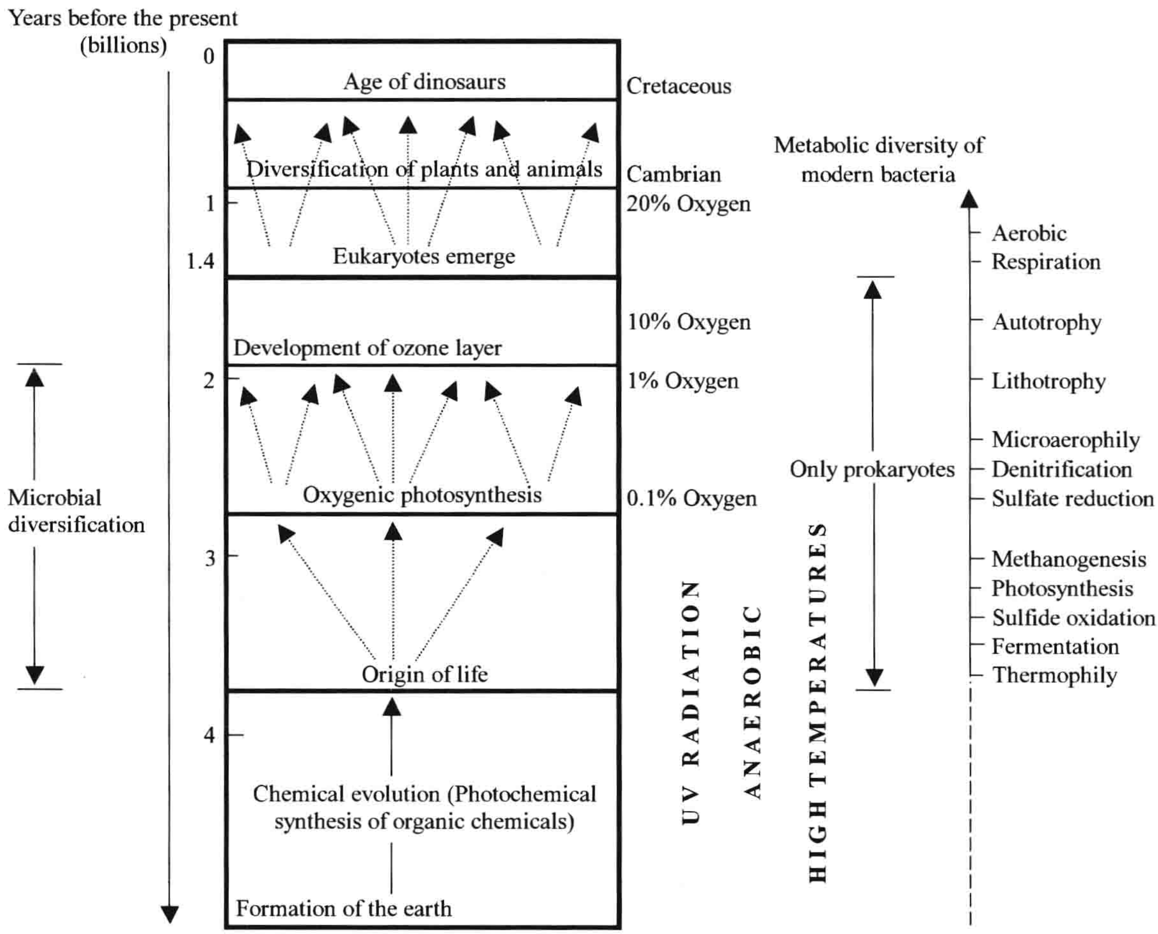

Fig. 1.1 Schematic diagram of the evolution of life on Earth.

like plants routinely do today. The O_2 generated from this process would have been mopped up immediately by the ubiquitous oxidizable matter in the environment, notably the iron (Fe) and sulfurs. Under these conditions, only microorganisms capable of anaerobic existence, notably the methanogens, prevailed. These photosynthetic microorganisms were also using hydrogen from hydrogen gas and hydrogen sulfide, more abundant then than now. Dissociation of H_2S is a less demanding photochemical reaction than dissociating the O-H bond in water.

Based on 16S rRNA chronometry (see below) there is sufficient evidence that methanogens also developed early in the ecosystem. The methanogens lived by decomposing organic matter and converting the carbon which had been generated by photosynthesizers, the CO_2 and methane thus replenishing these gases in the atmosphere. As photosynthesis continued, the abundance of these gases dwindled. In a frantic search for alternative sources of H_2 for photosynthesis, these organisms must have stumbled on water (H_2O) which until that time they had not been able to use for photosynthetic purposes because its H-O bond is stronger than the H-H and the H-S bonds in H_2 and H_2S respectively.