Ricardo Guerrero[1]*
Mercedes Berlanga[2]

[1]Department of Microbiology, Faculty of Biology, University of Barcelona, Spain
[2]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Barcelona, Spain

Life's unity and flexibility: the ecological link

Summary. The small size, ubiquity, metabolic versatility and flexibility, and genetic plasticity (horizontal transfer) of microbes allow them to tolerate and quickly adapt to unfavorable and/or changing environmental conditions. Prokaryotes are endowed with sophisticated cellular envelopes that contain molecules not found elsewhere in the biological world. Although prokaryotic cells lack the organelles that characterize their eukaryotic counterparts, their interiors are surprisingly complex. Prokaryotes sense their environment and respond as individual cells to specific environmental challenges; but prokaryotes also act cooperatively, displaying communal activities. In many microbial ecosystems, the functionally active unit is not a single species or population (clonal descendence of the same bacterium) but a consortium of two or more types of cells living in close symbiotic association. Only recently have we become aware that microbes are the basis for the functioning of the biosphere. Thus, we are at a unique time in the history of science, in which the interaction of technological advances and the exponential growth in our knowledge of the present microbial diversity will lead to significant advances not only in microbiology but also in biology and other sciences in general. [**Int Microbiol** 2006; 9(3):225-235]

*Corresponding author:
R. Guerrero
Department of Microbiology
University of Barcelona
Av. Diagonal 645
08028 Barcelona. Spain
Tel. +34-934482373. Fax +34-933341079
E-mail: rguerrero@iec.cat

The Microbe's Contribution to Biology

Scientific development is a continuous interaction between facts and ideas. Advances in knowledge are frequently preceded by a technological innovation that permits a new measurement or observation, or the ability to carry out experimental approaches previously thought to be impossible. Nonetheless, techniques are only tools; to achieve real progress in scientific knowledge considerable intellectual preparation is necessary ("In the field of observation, chance favors only the prepared mind", as Louis Pasteur once said). Fifty years ago, Albert J. Kluyver (1888–1956) and his former student, Cornelis B. van Niel (1897–1985), published *The Microbe's Contribution to Biology* (Harvard University Press, 1956), a small book based on the John M. Prather Lectures that the authors had delivered at Harvard University

in April 1954. The purpose of these lectures was to answer the question: in what respects would biologists' outlook be different if they had continued to ignore—consciously or unconsciously—all forms of life invisible to the naked eye. The immediate answer to such a question would be that it would inevitably lead to a serious undervaluation of life's potentiality. When Pasteur and Koch discovered the essential role played by microbes in human life, microbiology became a science with practical implications for the well-being of humankind, but with scarce importance for the core of biological science. *Microbe's* revealed the contribution of microorganisms to general biological knowledge. In addition, the book offered a synthesis of the advances that had furthered an understanding of prokaryotic genetics and metabolism, as well as a discussion of the biochemical uniformity and diversity of living beings. The results were a new perspective regarding the importance of the microbial

world to the maintenance of life and a paradigm shift in microbiology research and the teaching of this science worldwide. Kluyver, considered to be the father of comparative biochemistry, and his disciple van Niel postulated the metabolic unity of life and proposed the use of microorganisms to elucidate the biochemical pathways and energy transformations occurring in all living beings. The book is organized in six chapters. The first four chapters focus on microbial metabolism, energy production, and regulation. Chapters 5 and 6 describe inheritance, mutations, and the exchange of DNA as sources of genetic variation among prokaryotes and, finally, of the origin of life [17,20,31] (Table 1).

Up to the late 19th century, scientists thought that life had only two mechanisms to fulfill its energetic and nutritional needs; one based on the intake of simple mineral nutrients and fueled with radiant energy (light), and the other based on the intake of complex organic nutrients that are burned with oxygen ("aerobiosis"). However, neither the conversion of radiant into chemical energy by plants, nor the more or less standardized oxygen-consuming respiration of animals could explain the metabolism of all living organisms. Instead, a panoply of chemical conversions of an almost bewildering diversity appears to satisfy the energetic needs of many cells. The demonstration by Pasteur that many microorganisms avoid free oxygen and that oxygen may act on them as a deadly poison produced a serious challenge to the established concepts of the time.

Depending on the type of substrate used, the products formed by microorganisms vary greatly. Moreover, a single substrate may be decomposed to numerous by-products

Table 1. *The Microbe's Contribution to Biology.*

Chapter	Main contributions described in the chapter
1. Microbial metabolism and the energetic basis of life	• Microbes open a new vision on the almost infinite capacity of life, for adapting itself to the immense variety of external conditions realized on Earth. • These tiny unicellular organisms cannot be considered as mere curiosities for life's terrestrial activities. • Many microorganisms are ubiquitous on Earth. • Various microbial types, surprisingly, have enormous diversity regarding their nutritional requirements.
2. Microbial metabolism; further evidence for life's unity	• The general equation of metabolism is: $$AH_2 + B \rightarrow A + BH_2$$ • This conversion will yield compounds with an "energy-rich" phosphate bond. • The continuous flow of hydrogen atoms appears to be essential for the maintenance of life. • Life has diverse ways of solving the problem for obtaining energy.
3. Phototrophic bacteria; key to the understanding of green-plant photosynthesis	• The absorbed radiant energy in photosynthesis permits the conversion of CO_2 into organic compounds. • O_2 produced during oxygenic photosynthesis must come entirely from H_2O. • Only one special pigment is involved in photosynthesis (chlorophyll *a,* in green plants; bacteriochloropyll *b* in purple bacteria, and bacteriochloropyll *e* in green sulfur bacteria. • Red sulfur bacteria are photosynthetic as well as chemosynthetic.
4. Life's flexibility; microbial adaptation	• The study of the microbe has made another important contribution to biology by casting a clear light on the flexibility of the living cell with regard to its metabolism. Microbial species are not characterized by specific and fixed metabolic patterns. • Changing the enzymatic equipment allows a cell to better fit its new environment. Environmental factors may induce (induced enzyme synthesis) the appearance of a new characteristic in a genetically constant cell.
5. Trial and error in living organisms; microbial mutations	• Studies carried out in the 1940s showed that mutations in bacteria are spontaneous, that bacteria can exchange genetic material, and that this material is DNA. These studies provided evidence for the unity of genetics in all living organisms. • A variation in properties may also result from sudden and random changes in genotype, with subsequent selection by environment of those modified microorganisms that can successfully compete with the original type. This illustrates the operation of the "trial-and-error" principle in the world of microbes.
6. Evolution as viewed by the microbiologist	• The same general concepts are also applicable to plants and animals. • The apparent "simplicity" of microbes suggests that they occupy a position somewhere near the base of the evolutionary scale, but the problem of the manner in which microorganisms came into being is still unresolved. • Life is a special property of matter at a certain stage of complexity.

depending on the nature of the microbial species involved. An analysis of this great diversity reveals a fundamental unity: "hydrogenation" (richness in hydrogen, or a "less positive" valence) as the basis of assimilative energy, and "transhydrogenation" (hydrogen transference, or the movement of electrons) as the essence of dissimilation. As *The Microbe's Contribution to Biology* proposes (p. 19), the general equation of metabolisms is

$$AH_2 + B \rightarrow A + BH_2$$
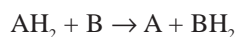
AH_2 represents any substrate amenable to dehydrogenation, i.e., the "electron donor". The hydrogen (or electron) acceptor B in the standard reaction may be an organic compound, as in fermentation, or free oxygen, as in aerobic respiration, or inorganic compounds (anaerobic respiration) containing N, S or C. These substrates are converted into their most reduced stage, thereby providing a continuous flow of hydrogen atoms (or electrons) that is essential for the maintenance of life. Hydrogen conversion, in turn, yields compounds (ATP) containing "energy-rich" phosphate bonds. The formation of such ATP has proved to be indispensable for the synthesis of many cell constituents.

The discovery of the phenomenon of induced enzyme synthesis (the "operon theory") represented another valuable gift of the microbe to biology. Each microbial species is not characterized by a specific and fixed metabolic pattern. Instead, by altering its enzymatic equipment the cell is better able to adapt to its new environment. By this process, new characteristics can be induced in a genetically constant microbial cell in response to a changing environment.

Studies started in the 1940s but which were developed in the 1950s and early 1960s showed that mutations in bacteria are spontaneous, that prokaryotes can exchange genetic material, and that this material is DNA. These studies provided evidence for the unity of genetics and established microbiology as a discipline at the core of biology.

Diversity and flexibility: the energetic basis of life

Microbes provide the best evidence of the almost infinite capacity of life and its ability to adapt itself to the immense variety of external conditions found on Earth. In the microbial world, diversity is expressed in terms of metabolism rather than structure, and prokaryotes have optimized their biochemistry for the uptake and utilization of a wide variety of nutrients [28]. The manner in which many microorganisms obtain energy differs greatly from the mechanisms of energy production in animals and plants; nevertheless, an understanding of energy production in microbes provided strong evidence for the unity of biochemistry, that is, that in all living cells chemical energy is generated by the transfer of electrons and protons to a variety of acceptors. The electron donors are determined by the electron acceptors because of thermodynamic principles, which tell us that an energy-yielding reaction must always involve electron flow from a more negative to a more positive redox potential. Organisms that use electron acceptors of relatively low negative potential are restricted to electron donors that are even more negative, e.g., hydrogen and certain organic compounds in the case of methanogens, acetogens, sulfur/sulfate reducers, or iron reducers. In contrast, organisms that use more positive electron acceptors can oxidize a greater variety of substances, e.g., aerobic lithotrophs; thus, certain nitrate reducers (anaerobic respiration) can oxidize sulfide, ferrous iron, methane, nitrite, and ammonia.

At life's origin, certain biosynthetic processes probably occurred spontaneously, either because of primordial physico-chemical conditions on the surface of the early Earth, or because of direct coupling to energy-yielding reactions (Fig. 1). Subsequent evolution depended on a mechanism to conserve energy from the environment by a distinct catabolic mechanism [27]. All living systems produce two forms of usable energy: (i) energy-rich chemical bonds of ATP (and similar molecules, including other nucleoside triphosphate), in which several metabolic reactions are coupled to ATP hydrolysis, and (ii) transmembrane ion gradients such as those used in flagellar movement and in the uptake of certain nutrients. ATP can be made by substrate-level phosphorylation (the key steps in fermentation pathways) or by electrochemical gradients generated in the electron-transport chain during respiration or photosynthesis. Probably all major types of catabolism arose early, but sequentially. It has been proposed that carbon dioxide respiration with hydrogen (methanogenesis and acetogenesis) was the first type of catabolism [19]. Several forms of respiration may also have appeared early in metabolic evolution, but they would have been limited by the availability of substrates and/or electron acceptors. Photosynthesis could have played a key role in the evolution of complex mechanisms and the production of oxidized products. Fermentation evolved independently from other forms of catabolism and could have occurred at any stage.

The number of completely sequenced genomes has been rapidly increasing since the first complete cellular genome was obtained, that of the bacterium *Haemophilus influenzae*, in 1995. The exploitation of this information for understanding the organizing principle of genetic and metabolic networks is one of the important areas of research in the post-genome era. The phylogenetic distribution of key enzymes, such as those involved in aerobic and anaerobic respiratory
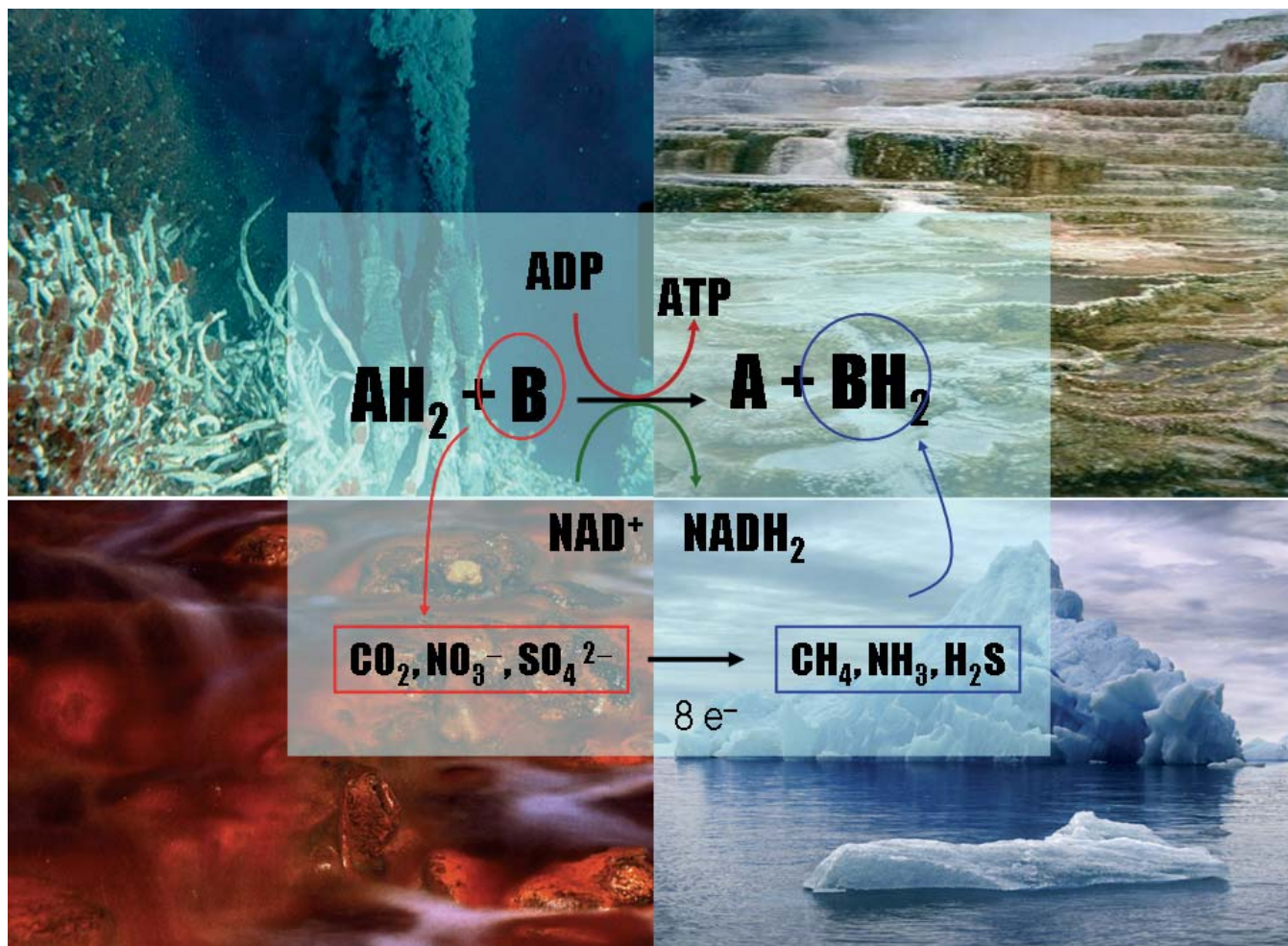
Fig. 1. The general equation of metabolism. In all living cells, chemical energy is generated by the transfer of electrons and protons to a variety of acceptors (see text for more information). The great metabolic diversity in microbes (especially prokaryotes), allow them to inhabit all possible locations in which liquid water is found. The background includes those areas representing extreme environments: life in deep sea vents, in hot springs (i.e., Yellowstone, USA), in acid (i.e., Río Tinto, Spain), and in ice (i.e., Antarctica).

pathways and in photosynthesis, is very irregular in prokaryotes. A dogma of bioenergetic evolution has long been that photosynthesis was one of the earliest ways of generating cellular energy, whereas aerobic metabolism appeared late in evolution, and in several independent lineages, after the release of oxygen by cyanobacteria. This reasoning was mainly based on the interpretation of certain geological and ancient fossil data. On the contrary, the phylogenetic analysis of cytochrome oxidase sequences indicated that aerobic organisms belonging to very diverse bacterial and archaeal groups used a homologous cytochrome oxidase and, therefore, that aerobic metabolism must have had a single and ancient origin. The availability of oxygen for aerobic respiration would not have been a problem, since there is evidence that a small amount of free oxygen was present in the early Earth [6]. Phylogenetic analysis at the genome level of meta-

bolic potential shows a close relationship between eukaryotes and archaea. These studies imply that, although horizontal gene transfer is important for an organism to improve its metabolic capacity and to obtain new metabolic functions, microorganisms are constrained by certain pre-existing metabolic organizing principle(s) that arose during evolution [22].

What prokaryotes cannot do

For a long time, the enrichment or detection of organisms capable of anaerobic growth on methane and ammonium compounds was unsuccessful. This led to the idea that ammonium and methane were inert under anoxic conditions, but there is no chemical basis for this conclusion. During the 1970s, several independent studies showed that in marine

sediments methane concentrations decreased from the sediments toward the water column. At the same time, sulfate concentrations decreased from the water column into the sediments, indicating that sulfate might be the electron acceptor for anaerobic methane oxidation. Recent studies using molecular techniques showed that the syntrophic consortium of an archaeon and a sulfate-reducing bacterium is involved in anaerobic methane oxidation, although, it has been suggested that several archaea can oxidize CH_4 without the need for a syntrophic partner bacterium [38].

Presently, the microbial oxidation of methane is thus believed to proceed only with oxygen or sulfate. But, anaerobic oxidation of methane coupled to the reduction of nitrate (denitrification) is also possible [29]. A microbial consortium, enriched from anoxic sediments, was shown to oxidize methane to carbon dioxide coupled to denitrification in the complete absence of oxygen. This consortium consisted of two microorganisms, a bacterium representing a new division (or phylum) of *Bacteria* and an archaeon distantly related to marine methanotrophic *Archaea*.

Anaerobic ammonium oxidation ("anammox" as it has been named) contributes significantly to biological nitrogen cycling in the world's oceans—up to 50% of marine N_2 production. Anammox is mediated by a monophyletic group of bacteria that branches deeply in the Planctomycetales. The group comprises two freshwater species, *Candidatus "Brocadia anammoxidans"* (first discovered anammox bacteria) and *Candidatus* "Kuenenia stuttgartiensis", one marine species discovered in the Black Sea, *Candidatus* "Scalindua sorokinii", and a new anammox specie enriched in a laboratory scale bioreactor "*Candidatus Anammoxoglobus propionicus*" [14,15]. These genera share the same metabolism and have a similar ultrastructure (characterized by the presence of an "anammoxosome"). Planctomycetes produce sterols, contain ether lipids (in the anammox case), and have a proteinaceous cell wall comparable to that of *Archaea*, most likely without peptidoglycan. In addition, they have a differentiated cytoplasm with membrane-bound intracytoplasmic compartments that are unique for each species [11].

The capability for anammox evolved only once. Still, the evolutionary distances among anammox genera are large; the sequence identity of the 16S rDNA genes of *Candidatus* "Scalindua" and *Candidatus* "Brocadia" is, on average, only 85%. For comparison, among the proteobacteria, two species that also have 85% sequence similarity on the 16S level have completely different phenotypes, i.e., *Nitrosomonas europeae* and *Neisseria gonnorhoeae* [34].

The almost complete sequence of the 4.2-megabase genome of *Kuenenia stuttgartiensis* will shed light on the biochemical pathway of anaerobic ammonium oxidation. In the *K. stuttgartiensis* genome, more than 200 genes related to anammox catabolism and respiration have been identified! So far, a comparable level of redundancy has only been observed for versatile heterotrophic bacteria, such as *Geobacter sulfurreducens* and *Shewanella oneidensis*, while the aerobic ammonia oxidizer *Nitrosomonas europaea* has only 50 such genes. Anammox bacteria use the energy from their catabolism for autotrophic growth. Based on the isotopic composition of cell carbon ($\delta^{13}C$ –24 to –50 per thousand relative to bicarbonate), it was proposed that anammox bacteria make use of the acetyl-coenzyme A (CoA) pathway for carbon fixation [35].

Lifestyle and metabolic flexibility

Adaptations evolved by microorganisms of the central pathways allow them to grow under a wide range of ecological circumstances. The Krebs cycle, or tricarboxylic acid (TCA) cycle, is the core of most contemporary intermediary metabolism, yielding reduced NADH and $FADH_2$ for bioenergetic purposes. In 1966, the discovery was made that the genus *Chlorobium* uses the reductive tricarboxylic acid (rTCA) cycle to "fix" carbon. This pathway has also been found in *Aquifex*, *Hydronenobacter*, and *Desulfobacter*. The organism *Desulfofacter hydrogenophilus* can function as an oxidative heterotroph, requiring organic substrates, or as a reductive autotroph. In the rTCA cycle, carbon obtained from carbon dioxide is incorporated into the cell's organic compounds. The cycle therefore serves as an engine of synthesis instead of one of energy transformation, as in most aerobes. Energy must be supplied from outside the cycle by environmental oxidation-reduction reactions.

It has been proposed [33] that in the map of intermediary metabolism, the rTCA cycle is at the universal anabolic core. This is not merely a result of common ancestry but rather a solution imposed on life already within the energetically structured environment of the early Earth. The argument is based on three empirical observations: (i) The 11 carboxylic acids of the TCA cycle serve as the unique anabolic core for all of life; (ii) the sequence of reactions in the cycle is run oxidatively in modern photoautotrophs and oxidizing heterotrophs but reductively in several chemolithoautotrophs; and (iii) the reducing chemistry likely to have characterized at least some environments on the early Earth makes the reductive cycle a candidate primordial form.

The reductive cycle requires two key enzymes not present in the oxidative cycle: citrate lyase and 2-oxoglutarate synthase. Organisms having both enzymes should be able to drive the entire TCA reductively; with only one of the two, they might be able to drive part of the reductive cycle. By

enabling organisms to adapt to a changing environment, the ability to drive the cycle both ways could ensure survival and propagation, not only for free-living organisms, but especially for those that have adapted to intracellular residence in a suitable host. An example of this is found in *Mycobacterium tuberculosis,* which can alter its mode of metabolism to survive in granulomes, which are progressively nutrient-starved, microaerophilic, and oxygen-depleted. NADH, NADPH, and FADH$_2$ are generated under oxic conditions via glycolytic, TCA, and β-oxidation pathways. Under oxygen-limiting microaerophilic and anoxic conditions, however, redox imbalance can result in toxic accumulation of the reduced compounds. Since granulomes are enriched in CO_2, the reductive TCA cycle may allow these reducing equivalents to be used efficiently to fix carbon in the anabolic mode. Since *M. tuberculosis* has both of the key enzymes of the rTCA cycle, a fully functional cycle operating in the reverse direction would enable the bacterium to fix carbon in the relatively reductive environment of the granulome while maintaining replication at a low level that facilitates persistence anaerobically [40]. This may be a common survival strategy among bacteria that cause persistent infections, including *Salmonella enterica* serovar Typhi and *Helicobacter pylori* [26].

One common feature of parasites and symbionts is the reduced size of their genomes—a result of a long evolutionary process of adaptation to the hosts' cells. Phylogenetic trees of metabolic networks based on genomic studies have shown that all organisms with genomes of less than 1400 kb (*Buchnera*, *Chlamydia*, *Rickettsia*, *Treponema*, *Borrelia*, *Ureaplasma*, *Mycoplasma*, etc.) cluster together [22]. Parasites have lost most of their metabolic genes and thus have a relatively simple metabolic network (as few as 77–150 enzymes). Symbionts also have a simple metabolic network; however, they possess several enzymes for the synthesis of certain metabolites that cannot be produced by their hosts. This feature distinguishes them from parasites. For instance, *Buchnera aphidicola* contains genes encoding host-essential amino acids, whereas those for non-essential amino acids are missing, but *Rickettsia*, a predator of amino acids produced by its hosts, possess only a few amino-acid biosynthetic genes. To compensate for the lack of amino acid biosynthetic genes, the *Rickettsia prowazekii* genome contains as many as 15 genes encoding amino-acid transporters [36].

The ecological link

Our Sun began its existence some 5000 million years ago as part of the Milky Way. Together with our galaxy, it moves in an almost circular orbit that takes about 226 million years to complete. The Sun, like other stars in its class, possesses smaller non-luminous bodies that revolve around it, the planets ("wanderers", in Greek). Occupying the third position from the center, Earth clearly distinguishes itself from Venus and Mars by having an atmosphere that is not in equilibrium. In addition, because of large forest fires and luminous cities, it produces its own light. Every year. the Earth spins around its star, and just as a year is not much in the life of a person, neither are 226 million years in the life of the Earth. Biopoiesis may have taken place in our planet many times after there was liquid water, some 3850 million years ago.

What made the maintenance of life on Earth possible was the development of ecosystems, or ecopoiesis, which prevented depletion of the biogenic elements of the planet's surface, something that would have taken place sometime around a maximum of 200 or 300 million years ago. Thus, without ecopoiesis, early life would have been extinguished. Instead, since then, the establishment of trophic chains, in which the metabolic products of some organisms serve as nutrients for others, has allowed the recycling of matter. The activity of these ecosystems determined the subsequent evolution of the planet, which until approximately 1800 million years ago had prokaryotes as its sole inhabitants [9,12]. There is life on Earth but not on the Moon. In the 21st century, perhaps we will discover whether there is, or was, life on Mars, or in other parts of our solar system, such as on Europa (Jupiter's moon) or Titan (Saturn's moon). On Mars, Europa, and Ganymede (Jupiter's) there is some evidence for the past or present existence of liquid water, the availability of energy, and an inference of organic compounds. As for Titan and Enceladus (Saturn's moon) despite their physically extreme conditions, energy sources and a complex chemistry may be possibly suitable for life forms unknown on Earth.

Life is a special property of matter at a certain stage of complexity [17]. Nonetheless, the distribution and the function of microbial populations are hardly influenced by diverse abiotic factors. The four basic elements of the Greek cosmology dictate the four basic requirements for life: water (metabolism), air (respiration), fire (heat), and land (food). The first three exclude the existence of microorganisms in different environments, according to the law of the tolerance of Shelford, which establishes that each organism needs a series of abiotic conditions to survive and to develop. The fourth element, land or food, regulates the biomass of a microorganism according to the law of the minimum of Liebig [1], which states that growth is regulated by the amount of the scarcest nutritional element available. The search for food and cell movement were properties that emerged during the first phases of life, when, independent of

the richness of the "soup", the presence of microorganims led necessarily to oligotrophy and to famine. Obviously, the continuance of a population in an ecosystem depends on both its nutritional needs and its tolerance of abiotic factors. Microbial ecology is the study of how microorganisms interact with their environments at the microscopic scale. The study of biodiversity in a particular ecosystem (a forest, a lake, a sea) would be incomplete without the inclusion of microorganisms, since they are essential contributors to the global functioning of the planet and thus to the sustainable development of the biosphere, because the primary role of microbes in the biosphere is as a catalysts of biogeochemical cycles. The results of microbial metabolic activities can dramatically affect the physical and chemical characteristics of the macroenvironment [23]. Observations of a wide range of natural habitats have established that bacteria do not function as individuals; rather, the vast majority of bacteria in natural and pathogenic ecosystems live in aggregates commonly referred to as biofilms [3,13]. These bacterial surface-associated communities are attached to solid substrata, and they grow into and become embedded in a polymer matrix produced by the bacteria themselves.Such metabolically integrated consortia consisting of multiple species can adopt specific spatial configurations. Indeed, the bacterial consortia of biofilms reach levels of complexity nearing those of multicellular eukaryotes [7].

Ecosystems expand over time and along space. Whereas time is an intrinsic component of the ecosystem itself, space is an extrinsic factor that contributes to change but which also limits the number of effective links between components of the ecosystem [13]. The consumption of resources and the formation of metabolic products by spatially separated microbial populations constitute the driving force that leads to gradient formation. Nutrient limitation (source of C) normally results in a decrease or cessation of metabolic activity (synthesis of biomass), but a lack of energetic substrates (electrons donors) forces a population to switch to another type of metabolism or may even cause a change in the composition of the microbial population. The metabolic activities in the living portion of an ecosystem result in a net flow of electrons, and all ecosystems have a gradient of redox potential. Life is a type of system that organizes in response to a gradient. Gradients enabled microbiota to diversify metabolically and they lead to more complete cycling of nutrients and to community-level interactions over a range of temporal and spatial scales [5,32]. Communities can be regarded as assemblages of microbial heterogeneous populations living together at a given place or habitat. The community is the highest biological unit in an ecological hierarchy.

Ecological diversity is considered to be a function of both the number of different types (richness or variety) of life forms and the relative importance of individual elements among them (evenness or equitability) [8]. Species richness can be expressed by simple ratios between total species and total numbers. It measures the number of species in the community, but not how many individuals of a species are present. Equitability, which measures the proportion of individuals distributed among the species, indicates whether there are dominant populations. Population identification is the first step in establishing relationships between the whole (community) and its parts (populations). The study of microbial communities has raised questions about their composition, structure, and stability as well as about the activity and function of the individual inhabitants.

We need appropriate tools for quantifying microbial diversity in natural communities. The enrichment and isolation approach establishes artificial conditions under which only the "fittest" of microorganisms will successfully compete; those conditions must be discovered by the ability, persistence, and luck of the researcher. It should thus come as no surprise that the vast majority of extant microbial life remains uncultivated. The application of molecular techniques in environmental microbiology provides a doorway to the discovery of organisms that are difficult to grow in the laboratory. Sequencing the microbial world will result in the discovery of hitherto unknown microorganisms and hitherto unknown functions of microorganisms. Microbial diversity on our planet is vast. To date, 55 divisions of *Bacteria* and 13 divisions of *Archaea* it have been described, although much diversity remains unexplored. Soil can contain more than 20 bacterial divisions [10], and approximately 12 division are represented in the Sargasso Sea [39]. In the adult human gastrointestinal tract, eight divisions have been identified, but there also appears to be tremendous diversity at the strain and species level, with more than 7000 phylotypes or species recognized [2]. In comparison, the diversity of microbial mats at the division level is among the highest of all environments, with 42 phyla that include 15 novel candidate phyla [21]. A microbial mat is a multilayered community of many bacterial populations—each with a particular structural, physiological, and taxonomic diversity—that form thin horizontal layers. These layers have active growth and can be several millimeters to a few centimeters thick. Lamination results from a light gradient along the vertical axis due to light extinction with depth, and from physicochemical microgradients due to the metabolism of different bacterial populations [13].

One of the central aims of ecology is to identify mechanisms that maintain biodiversity. Coexistence of competing species is usually associated with a marked degree of spatial segregation (separation) between these species [16].

Structured environments may accelerate evolutionary diversification by promoting genetic polymorphisms within populations, thereby increasing the complexity of genetic constraints that allow divergence among replicate populations. Only in the physically structured habitat are population cells exposed to gradients in concentrations of carbon, oxygen, moisture, and metabolites (both useful and toxic) released by other cells. This spatial heterogeneity, which is an important feature of many microbial communities, may contribute to within-population variability by promoting frequency-dependent selection for genotypes that are adapted to unexploited niches [18]. Examples include the light adaptation of marine *Prochlorococcus* and distinct hot-spring *Sulfolobus* and cyanobacteria populations [41]. The functional stability of an ecosystem is the outcome not of population diversity per se, but of functional redundancy, which is ensured by the presence of a reservoir of species able to perform the same ecological function. Increasing this functional redundancy ensures a reservoir of responses to disturbances through time. If several individuals are lost after a challenge, many other almost-identical individuals are available to replace them, thus repairing the system [4].

Perhaps the greatest challenge facing microbiology today is the problem of linking phylogeny and function. Methods based on 16S rRNA analysis provide extensive information about the taxa present in an environment, although they provide little insight into the functional role of each phylogenetic group. Metagenomic analysis provides some functional information through the genomic sequence and what it reveals about the expression of traits, but other methods are required to link specific functions with the group responsible for them. For example, analyses of the expression of both rRNA genes and genes encoding key enzymes in relation to environmental factors can be used to obtain information about the phylogeny and ecology of functional bacterial groups responsible for processes like denitrification, nitrification, and methane oxidation. Evolutionary information about the functionality of microbes in their particular environment may give us the key to understanding the functional diversity of microbial communities and ultimately of ecosystem function as a whole.

The force of the cooperation

Symbiotic associations among prokaryotes were the progenitors of all the complex and varied biological forms that followed and that now exist on Earth, giving place to the new eukaryotic cell, or eukarypoiesis. Eukarypoiesis might have taken place on our planet about 1.8 billion years ago. Protists

(i.e., protozoans, algae, etc.) were the first eukaryotic-celled organisms. Protists, fungi, plants, and animals (all of them composed of eukaryotic cells) emerged within a prokaryotic world and have retained intimate connections with, and dependency upon, prokaryotes. For example, the mitochondria of all plants and animals are derived from α-proteobacteria. Similarly, the photosynthetic organelle, the chloroplast, found in all plants and algae, is descended from a group of photosynthetic bacteria, the cyanobacteria [24].

Many aspects of the microbial associations of eukaryotic cells are only now being explored. No biological entity has evolved without being molded by the presence of other organisms. Microbial symbioses with animals, plants, and other microorganisms are diverse and common in natural communities. Moreover, they are essential to the livelihood of both the microorganism and its partners; without microbial symbionts, most animals and plants could not survive in natural communities. Obligate mutualists of insects are thought to enable their hosts to survive on restrictive diets, typically consisting of a single food source, by provisioning nutritional supplements such as amino acids and B vitamins. Symbiont loss often results in detrimental fitness costs for the host, such as sterility, growth impairment, and shortened life spans (Fig. 2). The congruence of host and obligate mutualist phylogenies, dating back to ancient times, has been demonstrated for a number of insect systems, including *Buchnera* and aphids, *Wigglesworthia* and tsetse flies, *Blochmannia* and ants, *Carsonella* and psyllids, and *Blattobacterium* and cockroaches. Many of these arthropod-associated mutualists form distinct but related lineages in the γ-proteobacteria, with genome diversification presumably having occurred upon establishment of the respective symbiosis. Analyses of obligates reveal drastically reduced genomes, such as the 450–653 kb of *Buchnera*, the 697 kb of *Wigglesworthia*, the 800 kb of *Blochmannia*, and the 680 kb of *Baumannia*. These reduced genomes are reflective of tight associations with host physiology and ecology, such that pathways for superfluous compounds or compounds deemed beneficial but unnecessary have been eliminated [30].

Wolbachia a endosymbiotic bacteria infect a wide variety of arthropods and filarial nematodes. Phylogenetic classification of *Wolbachia* is within the order Rickettsiaceae. In filarial nematodes, *Wolbachia* endobacteriae are concentrated in intracytoplasmic vacuoles within the hypodermal lateral cords of male and female worms and female reproductive organs, but are also detected by immunohistochemistry in oocytes and microfilariae. Little is known about the interaction between the filarial nematode and the bacterial endosymbiont. One example of a bacterial molecule that may be important in the symbiotic relationship is *Wolbachia* cata-
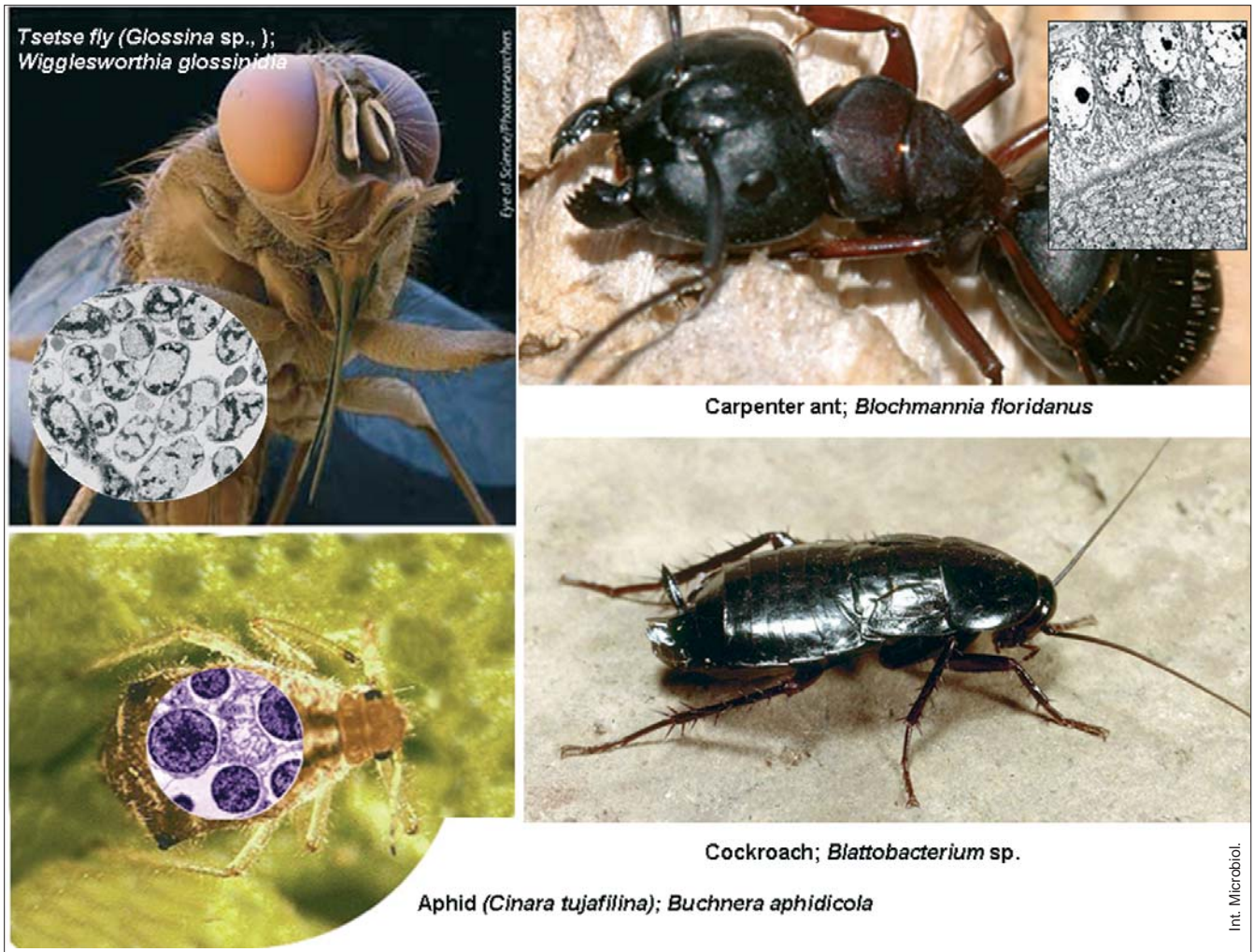
Fig. 2. We are not alone. Organisms live in companionship. Living in relationship to other organisms varies from beneficial to damaging. Negotiations between partners will determine the kind of interaction that prevails. Some examples of insects and their obligate bacteria symbiont are represented. Images of the fly, ant, and cockroach were obtained up from the internet. The aphid photo is courtesy of *Int. Microbiol.* (vol. 5(3), cover).

lase, an enzyme that may protect both bacteria and nematode from oxidative damage. Studies focusing on the mechanism of action of anti-*Wolbachia* drugs (i.e., doxycycline) indicate that *Wolbachia* is required for successful early moulting as well as reproduction. The endosymbiont also appears to play a critical role in the reproductive capacity of the nematodes. Thus, *Wolbachia* seems to play an important role in the pathogenesis of filarial disease [37].

We are becoming increasingly aware that microorganisms form the basis of the functioning of both the biosphere and the human body. While microbes' infamous reputation as agents of disease has tended to overshadow their vital role in nature, despite the threat represented by pathogens, humans and other forms of life have learned to coexist with microbes. In fact, the interactions of microbial communities with the human body are usually not pathological but symbiotic [25]. Indigenous microbiota maintain human health in multiple ways, such as protecting against exogenous pathogens. They regulate the development of our immune system and mucosa, provide us with nutrients and vitamins, and detoxify harmful dietary constituents [42].

Coda

All living beings on Earth depend on prokaryotic life. Prokaryotes are present in all the places where life can exist, occupying a wide variety of environmental conditions that range from "ideal" growth conditions (ideal, obviously from the macroorganisms' point of view) to extreme environments

(unthinkable for inhabitance by more evolved or recent forms). Without knowledge of microorganisms, biology would be much more limited: We would not know that there is life in conditions of extreme temperature, salinity or pH, photosynthesis would only be aerobic and oxygenic (when, actually, it developed in prokaryotes as anaerobic and anoxygenic), and the most long–lived living beings (such as redwoods) would not be older than a 1,000 (mere "youths" compared to some spores of *Bacillus*, which can be "as old as the hills").

Earth is a great rock in space, but a living rock, with multiple changing forms. In 5 billion years, our Sun—in the same manner as its predecessor star—will become a red giant, expanding and burning all the planets around it. Not even bacteria, who have inhabited our planet since the origin of life and survived some 30 massive extinctions, will escape the catastrophe. Or will they? Some scientists suspect that endospores or some other form of bacterial resistance have escaped the Earth as stowaways in rock fragments shot into outer space due to the earlier, violent impact of asteroids. Since that time, these microbial travelers have been searching for new planets to inhabit and "conquer".

As Louis Pasteur wrote: "I have taken my drop of water from the immensity of creation, and I have taken it full of the elements appropriate to the development of inferior beings. And I wait, I watch, I question it, begging it to recommence for me the beautiful spectacle of the first creation. But it is dumb, dumb since these experiments were begun several years ago; it is dumb because I have kept it from the only thing man cannot produce, from the germs which float in the air, from Life, for Life is a germ and a germ is Life."

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La unidad y flexibilidad de la vida: la conexión ecológica

Resumen. Los microbios son de pequeño tamaño, ubicuos, presentan versatilidad y flexibilidad metabólica, y plasticidad genética (transferencia horizontal) que les permite soportar y adaptarse rápidamente a las condiciones ambientales desfavorables y/o cambiantes. Los procariotas poseen sofisticadas envolturas que contienen moléculas que no se encuentran en ninguna otra parte del mundo biológico. Aunque la célula procariota carece de los orgánulos que caracterizan sus equivalentes eucariotas, su interior es asombrosamente complejo. Los procariotas sienten su ambiente y responden como células individuales a los desafíos ambientales específicos; pero también actúan de forma cooperativa, mostrando actividades comunitarias. En muchos ecosistemas microbianos, la unidad funcionalmente activa no es una sola especie o población (descendencia clónica de la misma bacteria), sino un consorcio de dos o más tipos de células que vive en estrecha asociación simbiótica. La comprensión de los microbios como la base del funcionamiento de la biosfera es algo muy reciente. Nos encontramos en un momento en que la interacción de avances tecnológicos y el conocimiento exponencial de la actual diversidad microbiana permitirán avances significativos no sólo en microbiología, sino en biología y en otras ciencias en general. [**Int Microbiol** 2006; 9(3):225-235]

Palabras clave: contribución de los microbios a la biología · base energética de la vida · cooperación

A unidade e flexibilidade da vida: a conexão ecológica

Resumo. Os micróbios são de pequeno tamanho, ubíquos, apresentam versatilidade e flexibilidade metabólica, e plasticidade genética (transferência horizontal) que lhes permite suportar e adaptar-se rapidamente às condições ambientais desfavoráveis e/ou variáveis. Os procariotas possuem sofisticados revestimentos que contêm moléculas que não se encontram em nenhuma outra parte do mundo biológico. Mesmo que a célula procariota careça das organelas que caracterizam seus equivalentes eucariotas, seu interior é asombrosamente complexo. Os procariotas sentem seu ambiente e respondem como células individuais aos desafios ambientais específicos; mas também atuam de forma cooperativa, mostrando atividades comunitárias. Em muitos ecossistemas microbianos, a unidade funcionalmente ativa não é uma só espécie ou população (descendência clônica da mesma bactéria), mas um consórcio de dois ou mais tipos de células que vive em estreita associação simbiótica. A compreensão dos micróbios como a base do funcionamento da biosfera é algo muito recente. Nós estamos em um momento em que a interação entre os avanços tecnológicos e o conhecimento exponencial da atual diversidade microbiana permitirão avanços significativos em microbiologia e assim como em biologia e outras ciências em geral. [**Int Microbiol** 2006; 9(3):225-235]

Palavras chave: contribuição dos micróbios à biologia · base energética da vida · cooperação