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The evolution of microbial life: paradigm changes in microbiology*

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Resum. Els bacteris no són ni estructuralment ni funcionalment tan senzills com en un principi pensàvem. Els bacteris viuen i moren en comunitats complexes que podrien semblar organismes multicel·lulars. L'alliberació de feromones provoca en els bacteris d'una població canvis en l'expressió dels gens, fenomen denominat «percepció de quòrum». Els bacteris volen allò que altres organismes volen: créixer, menjar, reproduir-se; si les condicions on es troben els resulten favorables, s'hi quedaran; si les condicions són millors en altres llocs, marxaran; si detecten un perill, fugiran; si el món al seu voltant canvia, ells han de canviar: Tot això, constitueix la base de la vida: accés als nutrients, consum dels nutrients per a la reproducció, dispersió, fugir dels depredadors i diferenciació. Avui, sabem que els microorganismes porten a terme una funció essencial en el manteniment de la vida sobre la Terra. Nosaltres, com els altres "macrobis", depenem de les activitats de l'«invisible» món microbià. La minúscula mida dels seus membres amaga la seva enorme importància.

Paraules clau: estructura bacteriana · mort cel·lular programada · biofilms · diversitat i activitat microbianes

Abstract. Bacteria are not as structurally or functionally simple as we believed. Bacteria live and die in complex communities that in many ways resemble multicellular organisms. The release of pheromones induces bacteria in a population to respond in concert by changing patterns of gene expression, a phenomenon called "quorum sensing". Bacteria want what all other organisms want: to grow, to eat, to reproduce themselves; if their surrounding conditions are good, they will stay; if things are better somewhere else, they will move; if threatened, they will escape; and if the world around them changes, they must change. These are the basics of life: access to nutrients, consumption of nutrients for reproduction, dispersion, escape from predators, and differentiation. Today, it is common knowledge that the majority of microorganisms play essential roles in maintaining life on Earth. We, and our fellow "macrobes", are ultimately reliant on the manifold activities of the "invisible" microbial world. The miniscule size of its members belies their tremendous importance.

Keywords: bacterial structure · programmed cell death · biofilms · microbial diversity and activity

The three stages of microbiology

Progress made in understanding the natural world is usually preceded by technological innovations that allow for new measurements and observations, as well as for the design of experiments previously considered to be impossible. Nonetheless, technology, with its unquestionable utility, is a mere tool, one that depends on intellectual preparation to achieve knowledge and interpret reality. In the words of Louis Pasteur: "Chance favors the prepared mind." Indeed, scientific development is the continuous interaction between ideas and facts.

For a very long time, our knowledge about the microbial world was extremely limited and tainted by fear, since microbes are the agents responsible for infectious disease, food spoilage, the deterioration of different materials, etc. More recently, however, we have come to realize that humans are—and always have been—completely dependent on microbial life. Life itself not only began with prokaryote microorganisms, but its continuity on Earth depends on them.

The significance of this change in paradigm—the acknowledgement of the microbial origin of a disease long held to be non-infectious in origin—many years after the discoveries made by Koch and Pasteur and their collaborators or direct disciples, can best be appreciated within the context of the history of microbiology and thus of scientists' notions of the immense and "invisible" world of microbes.

We can distinguish three epistemological stages, or Ages, in the discovery of microbes: (i) the Microscopic Age; (ii) the Pathogenic Age, and (iii) the Ecological Age [1,2].

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The Microscopic Age. Late in the 17th century, circumstances and a common interest in microscopes led two very different men, Robert Hooke (1635–1703), an English "professional" scientist and Member of the Royal Society of London, and Antony van Leeuwenhoek (1632–1723), a Dutch draper, an outsider in the scientific circles, to the discovery of the microbial universe. Robert Hooke's *Micrographia or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses with Observations and Inquiries thereupon* (1665), is the first published description of the microscopic world and the founding record of all biological sciences. Leeuwenhoek, who lacked any kind of scientific training but was gifted with a great amount of patience, ability, and curiosity, observed the first protists (mostly ciliates) in pond scum in 1674. He referred to them in Dutch as *beesjes* (small beasts) or *cleijne Schepsels* (minute organisms), and in Latin as *animaculi* (animalcules), the term used in his translated letters published by the Royal Society. Years later, in 1683, he observed the bacteria present on the surface of his own teeth. In another book *Microscopium* (1678), Hooke corroborated Leeuwenhoek's observations, thus increasing the Dutch enthusiast's reputation and the acceptance of his work. Leeuwenhoek built by himself (and only for himself) his simple microscopes, examined diverse materials from many sources, and afterwards wrote letters describing his observations (in Dutch, the only language he knew) to different correspondents. It was only later that these letters were collected and published in book form. The Salvador Collection in the Botanical Institute of Barcelona possesses one of the world's bibliographic marvels: a copy of the five volume collection *Arcana Naturae Detecta* (Discoveries of the secrets of nature), printed during Leeuwenhoek's life (*Arcana Naturae Detecta*, Leiden: Jon Arnold Langerak) and comprising 165 letters out of the 375 that Leeuwenhoek wrote to the Royal Society between 1718 and 1722 (Fig. 1). The development of the microscope allowed the observation of "minute bugs," a previously unknown world that now existed wherever one looked: in water, soil, animals, plants, and even the human body. However, the implications of this knowledge were unappreciated and were to remain so for the next 200 years.

The Pathogenic Age. The importance of microorganisms as the cause of infectious disease was not recognized by the scientific community, much less by the population in general, until well into the 19th century. Instead, diseases were thought to be a consequence of supernatural forces (poisonous gases or "miasmas," a divine punishment) or of the disequilibrium between the human body's four humors: blood, phlegm, yellow bile (cholera), and black bile (melancholy). Technological advances of the second half of the 19th century (such as autoclaves, filters, incubators, etc.) and the development of the basic techniques for the isolation and culture of bacteria, had allowed the founders of modern microbiology, Pasteur and Koch, to prove that microorganisms were the cause of infectious diseases, the contaminating agents in water and food, and that specific microorganisms caused specific diseases. Among the many well-known infectious diseases, two in particular stand out for their rapid and evident effects: syphilis and the plague.

Fig. 1. Frontispice of *Arcana Naturae Detecta*, a book by Antony van Leeuwenhoek published by the Royal Society (London in 1722). (Photos by R. Guerrero of the book from the Salvador Collection, Botanical Garden, Montjuïc, Barcelona.)

Syphilis first became known early in the 16th century. In 1520, the Italian physician Girolamo Fracastoro (1478–1553) wrote the epic poem *Syphilis, sive morbus Gallicus* ("Syphilis or the French disease"), about a shepherd named Syphilis, in order to express his ideas about the origin of this disease, which he thought was propagated through invisible beings ("spores") and intimate contact. A few years afterwards, he described contagious disease as occurring directly or indirectly, in the latter case due to contact of the hands or clothes with these spores, or even "at a distance," with spores that were dispersed in the air (*De contagione*, 1546). Frascatoro thus anticipated by over 300 years the consolidation of Louis Pasteur's (1822–1895) and Robert Koch's (1843–1910) microbial theory of disease during the 1880s, which was to revolutionize the practice of medicine.

The plague, by contrast, has been present throughout the history of humanity. In Europe, it caused several very serious epidemics— some occurring well into the 19th century. The most "famous" one, the Black Plague, spread from the seaport of Genoa to the rest of Europe from 1347 onwards, and resulted in the death of a third of the population during the second half of the 14th century. Eventually, a series of relatively effective hygienic rules were established to prevent the plague from spreading even further; these included burning clothes, closing houses, establishing periods of isolation or quarantine, etc. In Barcelona (as elsewhere in Europe), the plague served as a "justification" for the fierce attacks on the Jewish community in 1348, when the Jews were accused, as has often been the case during calamities, of being responsible for the epidemic. The *aljamas* or Jewish quarters of Cervera, Tàrrega, Lleida, and Girona were also attacked.

The horrendous effects of an epidemic in the 15th century led the Valencian poet and physician Lluís Alcanyís (ca. 1440–1506) to write, in the introduction to his book *Regiment preservatiu e curatiu de la pestilència* (València: Nicolau Spindeler, 1490): "Looking at human nature in countless dangers and mortal cases, all of the causes of death I have not seen a sadder, crueler or more acute one than this epidemic, which silently descends through our main limbs, something proven

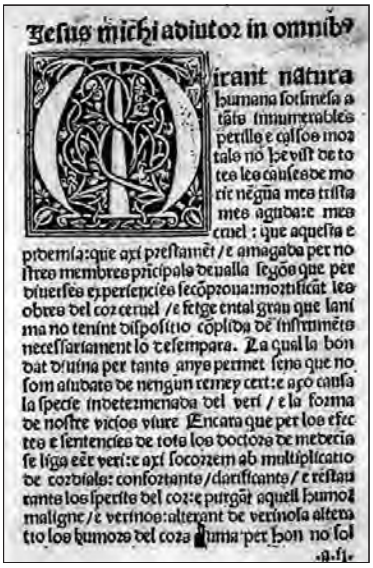
Jesus michi adiutor in omnib9
Mirant natura humana sosmesa a tants innumerables perills e cassos mortals no he vist de totes les causes de mort neguna mes trista mes aguda: e mes cruel: que aquesta epidemia: que axi prestamēt / e amagada per nostres membres principals devalla segons que per diverses experiencies se cōprova: mortificāt les obres del cor cervel / e fetge en tal grau que la anima no tenint disposicio cōplida de instrumēts necessariament lo desempara. La qual la bondat divina per tants anys permet seus que no som aiudats de nengun remey cert: e aço causa la specie indeterminada del veri / e la forma de nostre vicios viure. Encara que per los efectes e sentencies de tots los doctors de medecina se liga eēr veri: e axi socorrem ab multiplicatio de cordials: confortants / clarificants / e restaurants los sperits del cor: e purgāt aquell humor maligne / e verinos: alterant de verinosa alteratio los humors del cor a huma per hon no sol
a.ii.

Fig. 2. First page of the book *Regiment preservatiu e curatiu de la pestilència,* by Lluís Alcanyís (València, Nicolau Spindeler, ca. 1490).

over several experiences, mortifying the heart, brain and liver to the point where the soul, not having these instruments at its disposal, abandons the body" (Fig. 2). Although his influential role on the Valencian society of his time —or just because of that— Alcanyís was finally burned at the stake in November 25th, 1506, accused by the Inquisition of practicing Jewish rituals.

It was not until 1894 that Alexander Yersin, a bacteriologist at the Pasteur Institute, identified a bacterium, nowadays named *Yersinia pestis*, as the etiological agent responsible for the plague.

The Ecological Age. The role of microorganisms in nature and the relationships between microorganisms, other living beings, and the environment are encompassed by the field of microbial ecology, which was initiated by the pioneering work of Martinus Beijerink (1851–1931) and Sergei Winogradsky (1856–1952). However, it was not until 1966, when the first textbook devoted to the subject (*Principles of Microbial Ecology*, by Thomas D. Brock) was published, that microbial ecology received broad recognition, and it was not until late in the 20th century, with the development of molecular biological and genomic techniques, that research in the field exploded. Microbial ecology has also resulted in the recognition of the importance of microorganisms and their activities, which are essential to the planet's global functioning and contribute to the biosphere's sustainable development. Thus, microorganisms are responsible for closing the cycles of matter; they form the basis of trophic webs and control the composition of the gases in the atmosphere [3,4]. The observation of a wide range of natural habitats has established that bacteria essentially never function as isolated individuals; instead, the vast majority of bacteria in natural and pathogenic systems live in multicellular aggregates commonly referred to as biofilms, that is, bacterial surface-associated communities attached to solid substrata. The bacterial communities in a biofilm grow into and are embedded in a polymer matrix produced by the bacteria. This proclivity to form biofilms, and thus to multicellularity, makes bacterial cells similar to many other types of living cells: capable of unicellular existence and yet generally thriving within multicellular communities [5,6,7].

Bacteria as complex organisms

The current paradigm for biological classification of life is based on the prokaryote/eukaryote model, e.g., the existence of two kinds of cells: prokaryotic, those without nuclei (specifically, without nuclear membranes), and eukaryotic, those with a classical membrane-bounded nucleus [4].

A simple observation of a bacterium under the microscope is not very revealing. Most bacteria appear as plain rods or small spheres, without any characteristic traits. Despite this apparent morphological simplicity, bacteria present an enormous metabolic diversity that allows them to occupy the most diverse ecological niches imaginable, and to be at the base of every trophic web in the biosphere. Since prokaryotic cells lack the distinctive organelles found in eukaryotes, for many years it was thought that their cytoplasm was merely a sack containing soluble enzymes and the different components of the molecular machinery. We now know that the prokaryotic cytoplasm is incredibly complex with respect to its structure and its ability to synthesize highly specialized molecules [8,9]. Like their eukaryotic counterparts, bacteria employ a full complement of cytoskeletal proteins, localize proteins and DNA to specific subcellular addresses at specific times, and use intercellular signaling to coordinate multicellular events (Fig. 3).

Furthermore, while for over a century bacteria were studied as cell populations that acted independently of each other, it has now become clear that bacterial cells and populations communicate and interact with each other by several mechanisms. These include the production of an enormous quantity of chemical compounds that enable the cells to respond to different environmental stimuli. For example, the "quorum sensing"

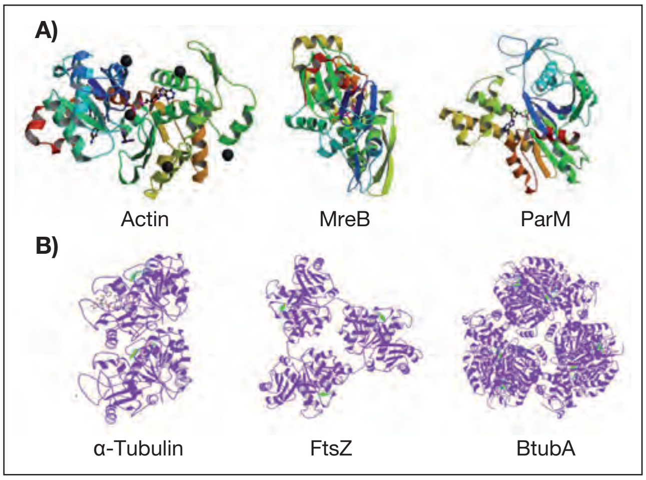

Fig. 3. Structural comparision of prokaryotic and eukaryotic counterpart cytoskeletal proteins. Protein structures were downloaded from the Protein Data Bank (PDB). (**A**) Uncomplexed actin (PDB no. 1J6Z), *Thermotoga maritima* MreB (PDB no. 1JCG), ParM of plasmid R1 *Escherichia coli* (PDB no. 1MWM). (**B**) *Bos taurus* α-tubulin (PDB no. 1JFF), *Methanococcus jannaschii* FtsZ (PDB no. 1FSZ), *Prosthecobacter dejongeii* BtubA (PDB 2BTO). (From *International Microbiology* 10(3), p. 164, with permission [4]).

response not only leads to changes in gene expression when the population reaches a certain size [10], but is also involved in interspecies communication and competition [11]. Quorum sensing is widespread in the bacterial world. Cell–cell communication can occur within and between bacterial species, and between bacteria and their eukaryotic hosts, which suggests that the chemical lexicon is complex. Behaviors controlled by quorum sensing are usually ones that are productive only when carried out simultaneously by many cells. For example, quorum sensing controls secretion of virulence factors, formation of biofilms, conjugation, sporulation and bioluminescence. Quorum-sensing systems can be divided into three primary classes based on the type of autoinducer signal and the apparatus used for its detection. First, gram-negative bacteria typically have enzymes that synthesize acylated homoserine lactone (AHL) autoinducers. The second class of quorum-sensing system is found in gram-positive bacteria, which use modified oligopeptides as autoinducers. The signals are synthesized as precursor peptides, which are subsequently processed and secreted. A third class of quorum-sensing system is a hybrid between the gram-negative and gram-positive systems.

Death of the immortals: programmed cell death

A prokaryotic cell in division form two identical cells if the environment conditions are permissive. Prokaryotes can grow or divide without "aging". Although there are variations of the general model, the typical cell division of bacteria is produced by "binary fission", that gives two equivalent cells. The bacterium *Caulobacter* divides into two cells that are unequal in size and shape, a new smaller flagellated daughter-cell and the stalked-mother cell that no lose her own identity once completed the process of division. Other bacteria divide by budding (resembling yeasts). In the yeasts, each bud leaves a "scar" on the surface of the cell mother, that impedes the formation of another bud in the same place; when all the surface is cover by those "scars of the labor", the yeast is incapable to be divided again, and finally the cell die. With the binary fission growth, the cells, in principle, they do not die. Obviously, as every form of life, bacteria can die by hunger (absence of nutrients), heat (high temperature), high concentration of salt, drying or dehydration, etc.

Programmed cell death (PCD), or apoptosis, is crucial for proper embryogenesis, immune system maintenance and the removal of damaged cells, and is a fundamental eukaryotic process that is highly evolutionarily conserved. Apoptosis is distinct from the more random process of necrotic cell death, which apoptosis eliminates individual cells without inducing an inflammatory response. Apoptosis also exists in several unicellular eukaryotes, including several species of the flagellates *Trypanosoma, Leishmania,* the ciliate *Tetrahymena thermophilia* and the dinoflagellate *Peridinium gatunense.* The cell death phenotype of these unicellular organisms shares many features with apoptosis in multicellular organisms, including DNA fragmentation, cytoplasmic blebbing and vacuolization and regulation by extracellular signals or environmental stress [12]. PCD plays an important role in a number of developmental processes in bacteria, such as lyses of the mother cell in sporulation, lysis of vegetative cells in myxobacterial fruiting body formation, and DNA transformation liberated from cells of streptococci undergoing spontaneous autolysis. Suicide could limit the spread of a viral infection. In the case of serious damage by toxic factors, cells will donate their nutrients to their neighbours instead of draining resources from their kin in a futile attempt to repair themselves. The altruistic self-sacrifice of the majority of the population, after exposure to a damaging agent such as antibiotics or to adverse environmental or nutrient-limiting conditions, would ultimately enhance the survival of the few that remain [13]. In bacteria, one of the most studied PCD systems is mediated by a genetic unit that consists of a pair of genes. The second gene encodes a stable toxin, and the first gene encodes a labile antitoxin that interferes with the lethal action of the toxin. Such genetic toxin-antitoxin (TA) systems (e.g., *mazEF* system) have been found in *E. coli* and of some other bacteria, including several pathogens.

The influence of infectious agents on apoptosis has been known for nearly two decades. Programmed cell death has been observed as a response to infection by a wide range of animal and plant pathogens. Pathogen-induced the host cell-death may serve to eliminate key immune cells or evade host defences that can act to limit the infection. Alternatively, suppression of the death pathway may facilitate the proliferation of intracellular pathogens. The pathogen therefore benefits from apoptosis in two complementary ways—inhibition of inflammation and enhanced propagation of the infection. Pathogenic bacteria have been implicated in modulating host-cell apoptosis. Induction of apoptosis has been linked to the production of bacterial toxins that target host-cell membranes (e.g., those produced by *Helicobacter*, *Staphylococcus* and *Listeria* spp.), inhibit host-cell protein synthesis (for example, *Corynebacterium* and *Shigella* spp.) or secrete effector proteins directly into the host-cell cytoplasm via a type III secretion system (for example *Shigella*, *Salmonella* and *Yersinia* spp.). Inhibition of apoptosis has rarely been reported for bacterial pathogens, although the obligate intracellular pathogen *Rickettsia rickettsii* might inhibit apoptosis. The rickettsiae probably interfere with apoptosis to encourage a long-term relationship with the invaded host cell and to prevent this protected environment from collapsing [14].

Bacteria as an unexpected agent of disease: the case of *Helicobacter*

Bacteria colonize every external surface of the human body. In the digestive tract, in addition to their role in the nutrient absorption and uptake, they prevent the proliferation of pathogenic microorganisms, thus safeguarding human health. The difference between microorganisms that are beneficial and essential and those which are definitely harmful is not always clear, as it depends on a delicate and dynamic equilibrium between the host and its resident microorganisms, and upon the environmental conditions. This equilibrium is what marks the

Table 1. Chronological parallelism between Robert Koch and J. Robin Warren and Barry J. Marshall

Koch (Germany)		Warren & Marshall (Australia)	
1882	Koch axenically cultures *Mycobacterium tuberculosis*	1982	Warren & Marshall axenically culture *Helicobacter pylori*
1884	Koch proposes his (and Loeffler's) four Postulates	1984	Marshall proves Koch's Postulates with *Helicobacter pylori* (and himself)
1905	Koch wins the Nobel Prize in Physiology or Medicine	2005	Warren & Marshall win the Nobel Prize in Physiology or Medicine

difference between health and disease, between life and death.

In 1982, Barry J. Marshall and J. Robin Warren, two Australian physicians, described the presence of a "new species related to the genus *Campylobacter*" (later reclassified as *Helicobacter pylori*) in the human stomach, and related it to the development of gastritis and peptide ulcers [15]. The confirmation that gastroduodenal ulcer is caused by a bacterium, and therefore treatable with antibiotics, radically changed the concept of infectious disease. For clinicians, it meant abandoning the long-held belief that these ulcers were a psychosomatic or hereditary condition whose sole treatment in the most serious cases was surgery. Moreover, *H. pylori* has also been implicated in stomach cancer and in the MALT form of lymphoma.

Histopathologists had already detected the presence of spiral bacteria in the human stomach since 1906. And although similar observations were made repeatedly over the years, they received little attention since the bacteria observed could not be cultured. Furthermore, it was thought that microorganisms were unable to permanently reside in the stomach given the low pH (0–1) of the gastric secretions. Marshall and Warren, however, not only described spiral or curved bacteria in histological sections of the gastric mucous, they also found bacteria of the same shape in ulcerated or malignant gastric tissues. Moreover, they were able to culture the new bacterium, eventually named *Helicobacter pylori* (helical bacterium of the pylorus), from the gastric mucosa. Definite proof came from their studies demonstrating that elimination of this bacterium completely changed the clinical development of the ulcer. These observations were corroborated by Marshall himself, who voluntarily ingested a culture of isolated bacteria and, a few days later, experienced gastritis—a prelude to gastric ulcer. He was cured when treated with antibiotics. (Warren could not join the experiment since he already suffered from a gastroduodenal ulcer.) Afterwards, other patients with gastroduodenal ulcers were successfully treated with antibiotics that targeted *H. pylori*, clearly demonstrating that a bacterium was the cause of the disease. In 1994, *H. pylori* was the first bacterium and the second infectious organism, after hepatitis B virus, to be considered a class I carcinogen, according to the criteria of the World Health Organization.

Despite the reasonable expectation that the medical community would immediately embrace Marshall's and Warren's convincing findings, this was by no means the case. To change a deeply rooted idea, medical or otherwise, is not an easy task. For many years, most clinicians refused to believe that ulcers had an infectious origin. The idea went against the traditional "dogma" that had been accepted for decades, in which the disease was ascribed to stress and excess acid secretion in the stomach. It was not until 15 years later, in 1997, following a massive campaign to educate physicians as well as the public that the role of *H. pylori* in gastroduodenal ulcer was finally accepted, and medical practice and the approach to therapy changed accordingly. In 2005, Marshall and Warren received the Nobel Prize in Physiology or Medicine, which was awarded for the discovery of the role of *Helicobacter pylori* as the causal agent of gastric ulcers (Table 1) [16].

Ecological diversity

Both the number of different types of life forms and the relative importance of these individual elements contribute to ecological diversity. Our planet's microbial diversity is immense. It has been estimated that in microbial mats the diversity at the division level is among the highest in known ecosystems: out of the 55 bacterial divisions identified to date, 44 are present in microbial mats (complex biofilms) [17]. In comparison, soil can contain more than 20 bacterial divisions [18], approximately 12 divisions are represented in the Sargasso Sea [19], and there are eight in the adult human gastrointestinal tract, which, however, is tremendously diverse at the strain and species level, with more than 7000 phylotypes or species (Fig. 4) [20].

Evolutionary information about the functionality of microbes in their particular environment may offer us the key to understanding the functional diversity of microbial communities and ultimately of ecosystem function as a whole. Indeed, one of the central aims of ecology is to identify mechanisms that maintain biodiversity in general. This knowledge that has taken on particular urgency as biodiversity has become increasingly threatened by climate change and other adverse effects imposed by human populations. Structured environments (physicochemical gradients) promote genetic divergence between identical populations (spatial segregation) and select those genotypes that are best adapted to these new, unexploited niches (e.g., the adaptation of *Prochlorococcus*, found in marine habitats, and of the different populations of cyanobacteria and *Sulfolobus*, found in hot-springs, to light). The functional stability of an ecosystem is the outcome not of population diversity per se, but of functional redundancy—the presence of a reservoir of species able to perform the same ecological function. Increasing this functional redundancy ensures a range of responses to the disturbances that inevitably occur over time. If several individuals are lost after a challenge, many other almost-identical

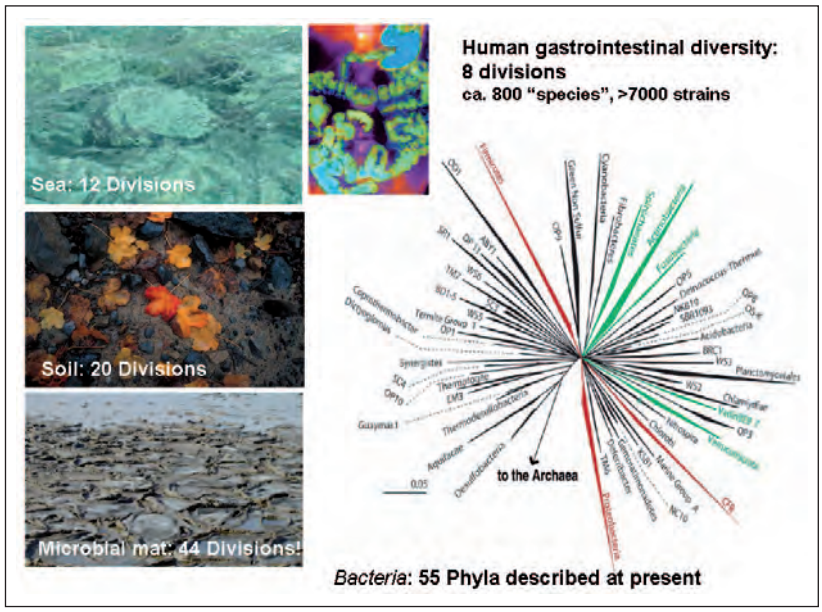

Fig. 4. Bacteria diversity in different habitats and communities, such as sea, soil, microbial mats and human gastrointestinal microbiota.

individuals (with regards to function) are available to replace them, thus repairing the system.

Perhaps the greatest challenge facing microbiology today is the problem of relating phylogeny to function—information crucial in our attempts to understand and appreciate biodiversity. Methods based on 16S rRNA analysis provide extensive data regarding the taxa present in an environment, including those that cannot be cultured, but little insight into the functional role of each phylogenetic group. Metagenomic analysis yields 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 such as denitrification, nitrification, and methane oxidation.

"Sequencing the microbial world" will allow the identification of microorganisms inhabiting a particular environment, the genes necessary for survival in that environment, and how the expression of these genes enables microbial adaptation to other, less common habitats. Furthermore, a sequence-based approach will facilitate predictions regarding which characteristics a new organism must possess based on the activities (genes) of its neighbors. Nonetheless, genomics on its own cannot represent biological reality, and the use of molecular techniques on environmental samples is limited by the fact that the results are qualitative rather than quantitative. In addition to knowing which microorganisms comprise a particular sample, it is also important to be able to determine the relative abundance of different organisms and to convert "inanimate pieces of information" (genes) into knowledge of cellular activity (function). The integration of traditional studies in physiology and genetics with the current genomic techniques of molecular biology has yielded many advances in the study of microbial evolution and has furthered our understanding of how microorganisms control the biosphere. However, ultimately, the only way to understand a genome is to apply the tools of microbial ecology to determine where this genome lives, identify its natural habitat, and understand the complex arrangement of biotic and abiotic interactions of its environment. The environment is the context in which genetic material functions and evolves, and, in the end, it determines the complexity and survival of the genome [21].

Coda

Microorganisms possess remarkable characteristics and are endowed with a functional potential unknown to the rest of the living world. They are small, ubiquitous, and exhibit metabolic variability and flexibility as well as genetic plasticity (horizontal transfer). Together, these properties allow microbes to rapidly adapt to unfavorable and/or changing environmental conditions. In addition, the complex envelopes of prokaryotes contain molecules that are not found in any other part of the biological world. Prokaryotes experience their environment and respond as individual cells to specific environmental challenges; but they also act cooperatively, carrying out activities as a community. In many microbial ecosystems, the functionally active unit is not a single species or population (clonal descendants 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 in science in general.

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