Bacteria and Archaea: Molecular techniques reveal astonishing diversity

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Abstract. *The last decade has produced a significant advance in our appreciation of the diversity of prokaryotic organisms (commonly given the generic term "bacteria"). The need for this improvement was clear as the current list of approximately 5,000 accredited species has long been known to be a major underestimate of living prokaryotic species. The primary reasons for this poor census were 1) the inability to cultivate the vast majority of prokaryotic species in the laboratory and 2) a classification system that inherently required laboratory culture. Fortunately, the impact of DNA-based methods has remarkably improved our knowledge by providing both a new alternative classification system (i.e. phylogenetic classification), and critically, new experimental strategies to identify non-culturable species. The resulting data have not only highlighted the true breath of prokaryotic diversity but have also changed some of our previous views of biological evolution. Phylogenetic analysis of gene sequences retrieved from both cultured and uncultured bacteria has shown that all cellular life can be ordered into three taxa (termed Domains) - Bacteria, Archaea, and Eucarya. Intriguingly, this has resulted in a major taxonomic promotion for the Archaea, which were previously thought to be a series of unusual bacterial species. In addition, the use of DNA-based methods to identify and catalogue non-culturable species has radically improved our knowledge of the diversity found within living prokaryotes. This paper describes our current view of prokaryotic diversity describing the impact over the last decade of DNA-based methods. It is a popular adaptation of a previously published paper (2001).*

INTRODUCING THE HIDDEN WORLD OF THE PROKARYOTES

One of the most significant developments in microbiology has been the discovery of many new bacterial species that are so unique that taxonomists have accorded them the rank of new phyla and even kingdoms. The collective scientific name for these organisms is "Prokaryote," meaning a cell characterized by the lack of a distinct membrane-bound nucleus. (In contrast, cells whose chromosomes are contained within a membrane-bound nucleus are termed eukaryotes.) Far more commonly prokaryotes are given the generic term "bacteria." They are found throughout the entire planetary ecosystem including niches where eukaryotic species are rare or absent (e.g. the ocean depths, the planet's subsurface, thermal and polar environments, and oxygen-free environments). This wide ecological range reflects their vast metabolic capabilities that allow different prokaryotic species to inhabit different environments.

Prokaryotes also occur in great abundance. A recent analysis suggested that the total number of living prokaryotic cells is 4 - 6 x 10^{30} composed of 1.2 x 10^{29} cells in the ocean, 2.6 x 10^{29} cells in soil, and 0.25 - 2.4 x 10^{30} cells within the Earth's subsurface (Whitman et al 1998). An alternative way to appreciate these figures is that even while accounting for the idea that a prokaryote cell is typically about 10,000-fold smaller in volume than a eukaryotic cell, the total amount of prokaryote biomass is still approximately 10,000 times greater than the amount of human biomass currently living on Earth. Because of these large numbers, their metabolic capabilities, and their ubiquity, prokaryotes play an essential function in the planet's biochemical processes including decomposition in soil, the provision of atmospheric components, nitrogen fixation, and photosynthesis.

Despite this significance, we have as yet only a very poor description of living prokaryotic species, and perhaps for obvious reasons, surveys of biodiversity often overlook bacteria. There are severe technical limitations among the traditional census-gathering methods of microscopy and bacteriology. Most species are indistinguishable under the microscope, and it has long been observed that only a fraction of the bacteria observed under the microscope can be successfully cultivated in the laboratory. Compounding this, those prokaryotic species that readily adapt to

ABOUT THE AUTHORS. *James O. McInerney* and *Richard Powell* are university lecturers whose research groups are interested in bioinformatics and molecular microbial ecology respectively. For several years, the two groups have combined their interests in a research study of uncultured marine bacteria. Under their supervision, *Marice Malarkey* and *Martina E. Wernecke* have recently successfully completed their postgraduate studies using molecular techniques to analyze uncultured Archaea in Irish coastal waters.

This article is a popularized adaptation of the authors' paper "The impact of taxonomic gene libraries on our knowledge of bacterial diversity: the example of the marine Archaea," published in Rushton B.S., P. Hackney, and C.R. Tyrie (eds), 2001, Biological collections and biodiversity, Linnean Society Occasional Publications 3. (See review of this title in Book Review section, page 45.) Special thanks to R. Powell for his work in popularizing the original.

Table 1. Approximate number of species, described and estimated, for the major groups of organisms (adapted from Watson et al 1995). The relevant figures for the prokaryotes are highlighted.

LIFE GROUPS	DESCRIBED SPECIES	NUMBER OF ESTIMATED SPECIES		ACCURACY
		HIGH	LOW	
Viruses	4,000	1,000,000	50,000	Very Poor
Prokaryotes	4,000	3,000,000	50,000	Very Poor
Fungi	72,000	2,700,000	200,000	Moderate
Protozoa	40,000	200,000	60,000	Very Poor
Algae	40,000	1,000,000	150,000	Very Poor
Plants	270,000	500,000	300,000	Good
Nematodes	25,000	1,000,000	100,000	Poor
Crustaceans	40,000	200,000	75,000	Moderate
Arachnids	75,000	1,000,000	300,000	Moderate
Insects	950,000	100,000,000	2,000,000	Moderate
Molluscs	70,000	200,000	100,000	Moderate
Vertebrates	45,000	55,000	50,000	Good
others	115,000	800,000	200,000	Moderate
TOTALS	1,750,000	111,655,000	3,635,000	Very Poor

growth under laboratory conditions may not be representative, or even major components of, the prokaryotic community of which they are natural members. The result is that prokaryotic diversity remains almost unexplored. A comparison of the numbers of identified species from other life groups (e.g. fungi, algae, plants, and animals) quickly highlights the fact that the current description of 5,163 validly named species of bacteria (Garrity & Holt 2001) constitutes an almost insignificant number in terms of the inventory of all species currently residing on Earth (Table 1). Indeed, a recent estimate of the number of living prokaryotic species was between 10^5 - 10^7 (Hammond 1995).

This large numerical discrepancy is primarily because microbiologists have relied on the traditional ecological tools of microscopy and bacterial culture. Problematically, when the results from both approaches are compared, the number of bacteria observed from the microscopic analysis usually exceeds the number of bacteria cultivated in the laboratory by at least two orders of magnitude (Jannasch and Jones 1959; Kogure et al 1979). Current classification (i.e. phenetic classification) of bacterial species also compounds the difficulty as, crucially, it requires pure cultures of bacterial strains for examination and is therefore limited by the bias inherent in laboratory cultivation. Also, it is not designed to provide information on the evolutionary relatedness of different bacterial species. This is unfortunate as prokaryotes provide neither a useful fossil record of past species nor rich anatomical detail in living species for comparative studies. However, the few ancient bacteria-like fossils that do exist show the presence of bacterial cells or bacterial community activity in some of the Earth's oldest rocks dated to over 3.5 billion years ago (Schopf 1993). By comparison, the oldest microfossils of multi-cellular red algae date to 1.25 billion years ago (Butterfield et al 1990) while the oldest metazoan fossils date to the Ediacaran era of approximately 600 million years ago (Schopf 1999).

The clear deduction from the limited fossil record is that cell-based life arose comparatively quickly after the planet's formation 4.5 billion years ago, and for two-thirds of the time since then, it was limited to prokaryotic-like life. Indeed, it was the impact and evolution of prokaryotic life that provided a suitable environment for the subsequent evolution of animal and plant species. For example, in geochemical terms, the formation of an oxygenated atmosphere suitable for the evolution of many eukaryotic species was primarily due to bacterial photosynthesis. Or, in biological terms, think of the endosymbiotic events whereby bacteria provided the chloroplast and mitochondrial organelles found in many current eukaryotic cells (Taylor 1974; Margulis 1993).

Therefore, although microbiologists were well aware of its potential significance, the technical limitations meant that an exploration of prokaryotic diversity was impossible to perform in any systematic manner. Thankfully, the advent of DNA-based methods and the insightful ideas of a few researchers have recently provided a radical solution to this problem.

A THREE DOMAIN RATHER THAN FIVE KINGDOM CLASSIFICATION

In 1987, Carl Woese summarized ten years of work and proposed a phylogenetic classification system for prokaryotic species based on the nucleotide sequences of small subunit ribosomal RNA (SSU rRNA) molecules. He reported that SSU rRNA gene sequences could be used for comparative analysis between different species to provide a tree of relatedness based on common ancestry or genealogy. Significantly, as both prokaryotic and eukaryotic cells contain SSU rRNA genes, phylogenetic analysis could also be used to compare both prokaryotic and eukaryotic species.

This sequence-based phylogenetic system represented a new model for evaluating the relatedness of any species, in terms of shared ancestry and evolution. Figure 1 shows a copy of the first presentation of the universal phylogenetic tree (Woese et al 1990). For the first time, the placement of the prokaryotes was firmly positioned on a universal tree of life. The resulting picture radically changed previous perceptions and convention by contradicting the five-kingdom classification of cellular life, i.e. Prokaryotes, Protists, Fungi, Plants, and Animals (Whittaker 1959). The phylogenetic tree of life supported the proposal that the five-kingdom system be replaced with a three-domain system wherein the differences between each domain are of a more profound nature than the differences that separate each kingdom. With a revolutionary impact on microbiology, the prokaryotes were split into two domains, the *Bacteria* (previously termed eubacteria) and the *Archaea* (previously termed archaebacteria). The third domain, the *Eucarya*, contained the other four eukaryotic kingdoms of protists, fungi, animals, and plants. The innovative nature of the phylogenetic tree was clear: Now both prokaryotic and eukaryotic species could be analyzed together to give (a) a picture of the comparative genetic diversity of all cellular life, and (b) a true view of the range of diversity accounted for by the prokaryotes.

The inferences that can be drawn from the universal phylogenetic tree are new and exciting for microbiology. As the geological evidence suggests the presence of both thriving cyanobacteria-like and sulphate-reducing *Bacteria* 3.5 billion year ago (Schopf 1993; Shen et al 2001), the origin of the last common ancestor and the division of prokaryotes into the *Bacteria* and *Archaea-Eucarya* lineages must have occurred before this time. This places the origin of life surprisingly early in the planet's development at a time when it was inhospitable by today's standards. Interestingly, these conditions correlate with inferences that can be deduced from the phylogenetic tree. The current members of the deepest branches of the domains *Bacteria* and *Archaea* live at high temperatures and in oxygen-free environments.

The work of Woese and his colleagues had provided microbiologists with a new framework to examine the role and impact of prokaryotes in the context of the evolution and diversity of life on Earth. However, the challenge remained to solve, or at least manage, the problem posed by the fact that the vast majority of living bacterial species will not culture under laboratory conditions.

DNA-BASED STUDIES OF PROKARYOTIC ECOLOGY

Concurrently with the development of the phylogenetic classification, Norman Pace and his colleagues were developing a new approach for the study of bacterial ecology. Their aim was to study natural bacterial communities by directly retrieving informative molecules, i.e. DNA sequences, as opposed to

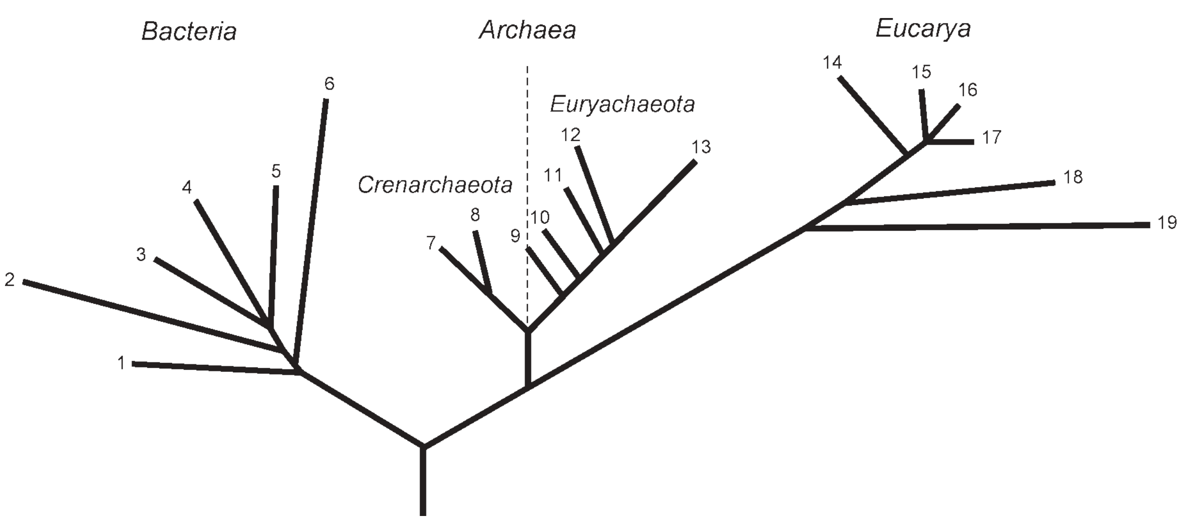


Figure 1.
Universal phylogenetic tree based on comparative SSU rRNA gene sequence analysis (Woese et al 1990). The position of the root was determined by comparing paralogous gene sequences that diverged before the three primary lineages emerged from their common ancestral condition. The numbers of the branch tips correspond to the following groups of organisms.
***Bacteria*:**
1, the Thermotogales; 2, the flavobacteria and relatives; 3, the cyanobacteria; 4, the purple bacteria; 5, the Gram-positive bacteria; and 6, the green non-sulphur bacteria.
***Archaea*:**
the kingdom Crenarchaeota encompassing 7, the genus *Pyrodictium*; 8, the genus *Thermoproteus*; and the kingdom Euryarchaeota encompassing 9, the Thermococcales; 10, the Methanococcales; 11, the Methanobacteriales; 12, the Methanomicrobiales; and 13, the extreme halophiles.
***Eucarya*:**
14, the animals; 15, the ciliates; 16, the green plants; 17, the fungi; 18, the flagellates; and 19, the microsporidia.

bacterial cells for cultivation (Pace et al 1985; 1986). As their informative molecule of choice was the SSU rRNA gene, any such gene sequences retrieved directly from a natural community could be used to produce a phylogenetic description of the natural bacterial community including both culturable and non-culturable members.

Initially, this molecular approach to bacterial ecology was relatively slow to gain a place among the tools used by microbial ecologists. Technically, it was laborious and required the construction of gene libraries from the total genomic DNA isolated from natural communities. Subsequently, large numbers of clones from these libraries had to be screened in order to identify individual clones containing SSU rRNA genes from the various community members. However, the development of PCR amplification technology (Saiki et al 1988) removed this technical obstruction. The initial reports using this PCR-based approach showed that the majority of SSU rRNA genes sequences recovered from natural communities were derived from novel bacterial species (Ward et al 1990; Giovannoni et al 1990). Since then, the large number of similar reports analyzing diverse bacterial communities have repeatedly demonstrated and confirmed the existence of novel bacteria and the limitations of traditional growth-dependent methods. With as many environmentally derived SSU rDNA sequences described as those derived from cultured bacterial species, the phylogenetic tree for the *Bacteria* has flowered in detail since its initial description. The current tree contains at least 36 phyla, 13 of which do not as yet have a cultured representative (Hugenholtz et al 1998).

Prokaryotes are ubiquitous! Numerous species are found in extreme environments, such as this hot spring in Yellowstone National Park, U.S.A. (a thermophilic environment). However, excitingly, in the last decade, many other prokaroytes have been identified in "ordinary" environments such as soybean and rice field soils, forest soils, coastal salt marshes, lake waters and sediments, and the deep planet subsurface. (Photo taken in 1996 by German Jurgens, Department of Applied Chemistry and Microbiology, University of Helsinki, Finland).

THE SURPRISING *ARCHAEA*

The description of the *Archaea* in the first universal pylogenetic tree was novel and surprising. Known beforehand as archaebacteria (Balch et al 1997; Woese and Fox 1977), they represented a small group of highly atypical bacterial species that inhabited unusual or extreme environmental niches (e.g. thermophilic springs, hydrothermal vents, high-saline waters, anoxygenic muds). Even today, there are only 217 accredited, cultured archaebacterial species (Garrity and Holt 2001). In biochemical terms, these cultured species constitute three groups: methanogens, extreme halophiles, and extreme thermophilic sulphur metabolizers. Phylogenetic analysis of approximately 50 archaeal SSU rRNA genes derived from these cultured species showed that rather than simply being composed of obscure bacterial species, the archaebacteria constituted a taxonomic rank of the highest order, i.e. the domain *Archaea*. Within this domain, the *Archaea* split into two major lineages (termed kingdoms): the Euryarchaeota containing the methanogens, extreme halophiles, and sulphur reducers, and the Crenarchaeota containing the extreme thermophiles (Woese 1987; Woese et al 1990).

The *Archaea* held other surprises for microbiologists. The unusual habitats of cultured archaeal species led to the presumption that these living *Archaea* represented ancient or unchanged bacterial forms limited today to niches that reflect early Earth conditions and are devoid of, or limited in, competition from other *Bacteria* and *Eucarya* species. These misconceptions were overturned by the unexpected identification of the presence of *Archaea* SSU rRNA genes in cold oxygenated seawaters (DeLong 1992; Fuhrman et al 1992). These uncultured marine *Archaea* probably play a major role in the bacterioplankton community as other DNA-based analysis suggests that they are responsible for between 2% and 30% of the total bacterial activity in these ocean waters. Furthermore, a recent study calculated that these marine *Archaea* may constitute approximately 1.3 x 10^{28} cells throughout the global oceans, a number that is close to half of the estimated 3.1 x 10^{28} bacterial cells present in the same waters (Karner et al 2001). In fact, as ocean waters constitute one of the largest planetary niches, the marine *Archaea* may be one of the most dominant prokaryotic groups on Earth (Mestel 1994).

The surprising identification of non-cultured *Archaea* inhabiting a relatively non-extreme environment has primed further DNA-based searches for *Archaea*. To date, the presence of uncultured *Archaea* have been reported in a variety of general terrestrial and aquatic environments including soybean and rice field soils, forest soils, coastal salt marshes, lake waters and sediments, and the deep planet subsurface. This data shows that our previous picture of archaeal ecology was limited from its dependence on the analysis of cultured species. Clearly, the *Archaea* are ubiquitous, occur in great abundance, and inhabit both unusual niches as well as a full range of large and non-extreme environments containing robust competition from other *Bacteria* and *Eucarya* species.

A CURRENT VIEW OF THE PHYLOGENY OF THE *ARCHAEA*

As initially described, and based on the SSU rRNA gene sequences of approximately 50 cultured species, the *Archaea* constituted two kingdoms, Crenarchaeota and Euryarchaeota (Woese 1987). As with the domain *Bacteria*, the addition of the uncultured archaeal SSU rRNA gene sequences is now changing this view of

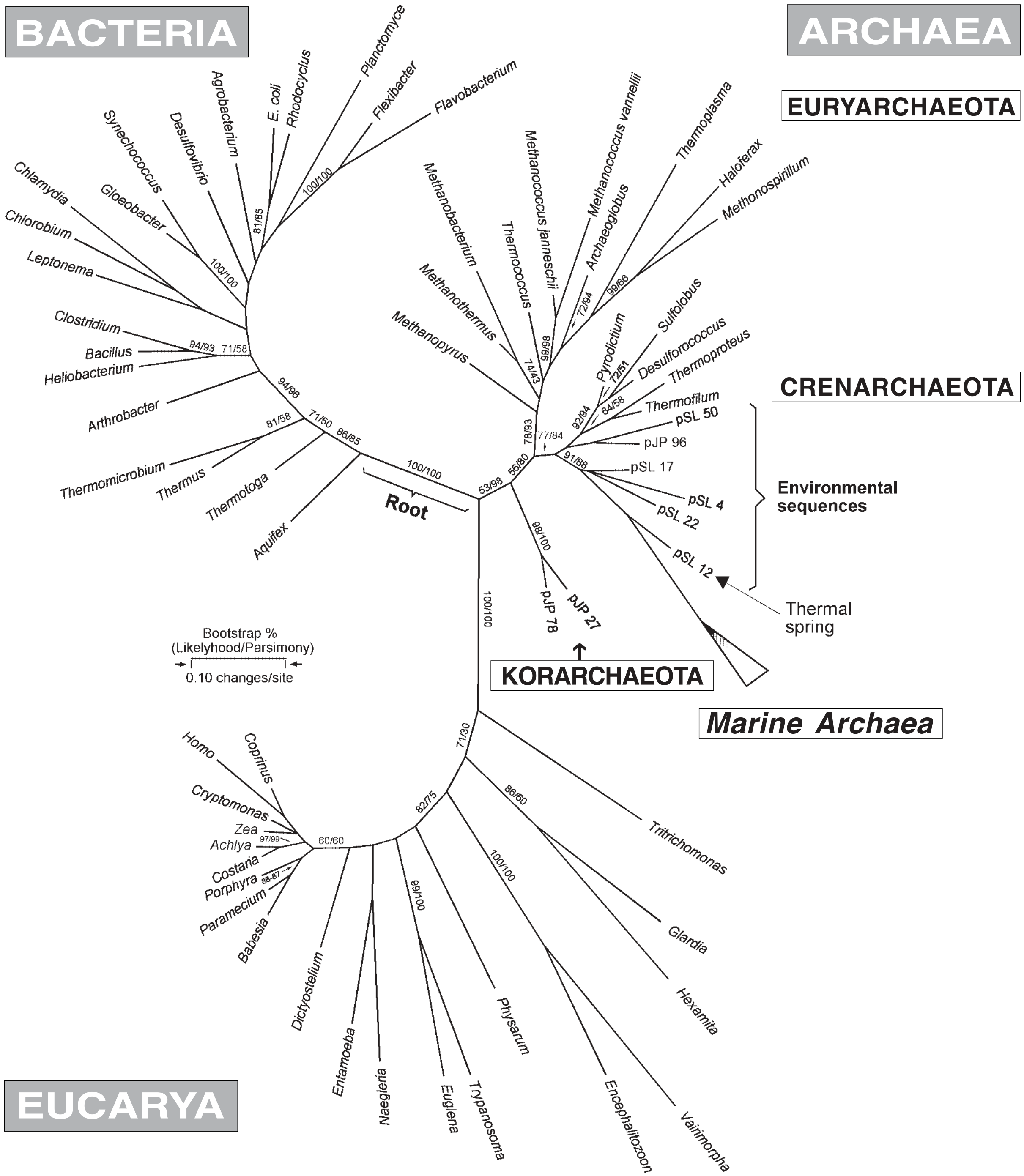
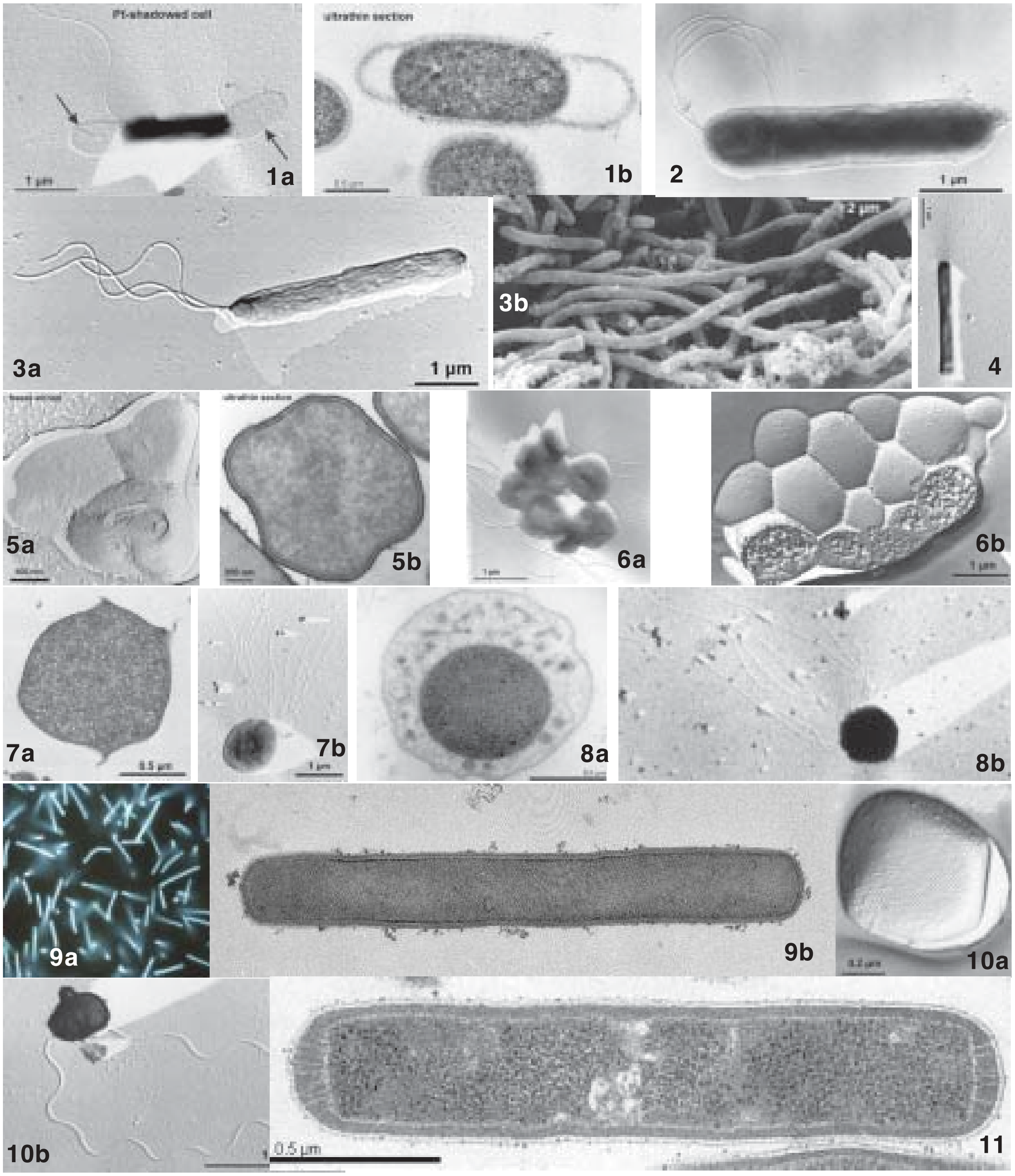


Figure 2. SSU rRNA sequence-based unrooted universal phylogenetic tree showing the placement of the proposed new Archaeal kingdom, Korarchaeota (Barns et al 1996). The numbers indicate percentage bootstrap re-sampling scores. Paralogous gene sequence analysis places the root of the tree on the branch at the base of the *Bacteria*.

archaeal evolution. Phylogenetic analysis of the marine archaeal SSU rDNA sequences indicates that the planktonic *Archaea* constitute two separate evolutionary groups. Group I represents a novel deep-branching lineage that is either loosely associated with the Crenarchaeota (DeLong 1992) or, perhaps, representing a new archaeal kingdom (McInerney et al 1997). Group II marine *Archaea* represent a series of novel lineages within the Euryarchaeota (DeLong 1992). Interestingly, the other environmental archaeal SSU rRNA genes sequences predominantly tend to associate with the Group I marine *Archaea* and occasionally with the Group II marine *Archaea*. A further group of uncultured *Archaea* SSU rRNA gene sequences recovered from a hot spring sediment (Barns et al 1994) showed even greater genetic divergence from the cultured Euryarchaeota and Crenarchaeota than the other environmentally derived archaeal SSU rRNA gene sequences (Figure 2). This lineage has been proposed as a new kingdom-level taxon within the *Archaea* and termed the Korarchaeota (Barns et al 1996).

Therefore, our current view of archaeal evolution, although limited when compared to the *Bacteria*, also shows that the vast majority of the *Archaea* remain uncultured, i.e. there are as yet no cultivated representatives of two of the four *Archaea* kingdoms.

A DIVERSITY OF HIDDEN LIFE! With thanks to Prof. Dr. Karl Stetter and Dr. Reinhard Rachel, Universitaet Regensburg, Lehrstuhl Mikrobiologie, for allowing us to reprint the following selected images from their website. http://www.biologie.uni-regensburg.de/Mikrobio/Stetter/bilder.html



Figs. 1-11. 1a-b, *Thermotoga maritima* represents a genus of extremely thermophilic bacteria growing up to 90°C [R. Huber et al. 1986. *Archives of Microbiology* 144: 324-333]; **2, *Aquifex pyrophilus*** represents a group of marine hyperthermophilic hydrogen-oxidizing bacteria [R.Huber et al. 1992. *Systematic and Applied Microbiology* 15:340-351]; **3a-b, *Thermocrinis ruber***, a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park, U.S. [R.Huber et al. 1998. *Applied and Environmental Microbiology* 64:3576-3583]; **4, *Pyrobaculum aerophilum*** is a nitrate-reducing hyperthermophilic archaeum [P.Völkl et al. 1993. *Applied and Environmental Microbiology* 59: 2918-2926]; **5a-b, *Pyrolobus fumarii*** represents a group of Archaea, extending the upper temperature limit for life to 113°C [E.Blöchl et al. 1997. *Extremophiles* 1:14-21]; **6a-b,** Sulfur-inhibited ***Thermosphaera aggregans*,** a genus of hyperthermophilic Archaea isolated after its prediction from environmentally derived 16S rRNA sequences [R.Huber et al. 1998. *International Journal of Systematic Bacteriology* 48:31-38]; **7a-b, *Thermococcus chitonophagus***, a chitin-degrading, hyperthermophilic archaeum from a deep-sea hydrothermal vent environment [From R.Huber et al. 1995. *Archives of Microbiology* 164:255-264]; **8a-b, *Ignicoccus***, a genus of hyperthermophilic, chemolithotrophic Archaea, represented by two species, *Ignicoccus islandicus* and *Ignicoccus pacificus* [H.Huber et al. 2000. *International Journal of Systematic and Evolutionary Microbiology*. 50:2093-2100]; **9a-b, *Methanopyrus kandleri***, a genus of abyssal, hyperthermophilic, methanogenic archaea growing at 110°C [R.Huber et al. 1989]. *Nature* 342:833-83; M.Kur et al. 1991. *Archives of Microbiology* 156: 239-247]; **10a-b, *Archaeoglobus veneficus*,** a facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers [H.Huber et al. 1997. *Systematic and Applied Microbiology* 20: 374-380]; **11, *Methanothermus fervidus***, an extremely thermophilic methanogen isolated from an Icelandic hot spring [K.O.Stetter et al. 1981. *Zentralbl. Bakterial. Parasitenkde. Infektionskr. Hyg. Abt. 1 Orig. Reiche C* 2:166-178].

TAPPING THE HIDDEN RESOURCE OF UNCULTURED PROKARYOTES

The latest challenge for microbiologists is to develop techniques that will allow much better access to non-culturable prokarytic species rather than simply the retrieval of their SSU rRNA gene sequences. The combined use of both DNA-based methods and traditional fermentation technology has already proved successful for the laboratory cultivating of previously unknown species (Huber et al 1995; Kane et al 1993). The basic experimental strategy is to use the SSU rRNA gene sequence information derived from uncultured species to (a) design and monitor laboratory fermentation protocols that selectively target the unknown species, or (b) modify the actual inoculum so that the desired species has less competition from the more readily adaptable species.

As an alternative strategy, and in this era of genomics, it has become possible to clone and determine the nucleotide sequences of many genes, if not whole genomes, of non-culturable bacteria. This strategy is based on the direct cloning of the genomic DNA from entire natural communities into routine laboratory strains such as *Escherichia coli*. The term "metagenome" has been coined for this strategy (Rondon et al 1999). These *E. coli* clones can then be screened directly for novel biological activity of interest, e.g. new enzyme activity of biotechnological importance. The result is the direct acquisition of novel genes and proteins without ever attempting to cultivate the hidden species.

A series of other DNA-based methods have also been developed that provide useful information on prokaryotic community structure including its diversity without actually having to determine individual SSU rRNA gene sequences. Methods such as denaturing gradient gel electrophoresis (Muyzer et al 1993), amplified ribosomal DNA restriction analysis (Acinas et al 1997), or terminal-restriction fragment length polymorphism (Liu et al 1997) amplify the SSU rRNA gene sequences of all species present in a natural prokaryotic community. This results in a DNA fingerprint that is characteristic of the diversity of the community at the time of sampling. These DNA fingerprints can then be used in comparative analysis to monitor temporal or spatial changes in community composition.

Finally, with the advent of DNA microarray technology, it is now possible to build DNA microarrays, which are glass slides containing several thousand different synthetic DNA molecules that are specific for the various bacterial phyla or groups described on the phylogenetic tree and allow you to examine them simultaneously. DNA or rRNA isolated directly from natural communities can then be used to screen these bacterial "genosensors" producing a rapid, culture-independent view of community composition. Ultimately, these genosensors may have the ability to provide both a quantitative assessment in terms of the number of different groups within a natural community, and also qualitative data with respect to the comparative abundance and activity of the different groups. Guschin et al (1997) demonstrated the utility of this approach in an analysis of nitrifying bacteria that are known for their difficulty to culture due to their long generation times and poor plating efficiencies. Interestingly, DNA microarray technology could plausibly allow the design of the ultimate bacterial genosensor containing a sufficient number of different SSU rRNA-based synthetic DNA molecules to cover every theoretical SSU rRNA gene sequence combination. Such an approach might well unearth other currently hidden prokaryotic phyla.

> *"If I could do it all over again, and relive my vision in the twenty-first century, I would be a microbial ecologist. Ten billion bacteria live in a gram of ordinary soil, a mere pinch held between thumb and forefinger. They represent thousands of species, almost none of which are known to science. Into that world I would go with the aid of modern microscopy and molecular analysis."* E. O. Wilson. 1994. *Naturalist*. Island Press, Washington D.C., U.S.A.

CONCLUSIONS

In the last decade, the use of DNA-based technology has provided a major stimulus for studies of both prokaryotic classification and ecology. It has produced a much clearer and perhaps a more equitable view of the contribution of prokaryotic organisms to life's evolution and current biodiversity. In spectacular fashion, it has uncovered the hidden significance of the *Archaea* and placed them as an equivalent taxonomic rank to the *Bacteria* and the *Eucarya*. It has provided a natural system for classifying the various bacterial groups, many of which we note are more distantly related to each other in evolutionary terms than are plants and animals. It has also produced a rational framework for ecological studies that are independent of the bias associated with laboratory cultivation. The result is the now constant flow of publications describing novel SSU rRNA gene sequences isolated from an ever-increasing range of natural populations and environments.

Microbiologists have never had a better time to discover new species. The current prokaryote world represents a major biological resource that is as yet hardly described, although biotechnology companies have already focused it in their sights for exploitation. Alternatively, as concern is expressed about the condition of biosphere Earth, and the impact of anthropogenic activity, the challenge of ecosystem study remains difficult if one wishes reasonably to include the hidden prokaryotes.

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