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Microbial diversity form and function in prokaryotes: Association to species diversity and phylogenetics diversity

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Abstract
Prokaryotes were originally defined as single celled entities by their cellular structure, such as the lack of a nucleus, division by fission, special structure of the cell wall. Prokaryotes are found nearly everywhere in the modern world and their presence defines the biosphere. They are also phylogenetic ally the most diverse; as two out of the currently recognized three major divisions domains of living organisms Bacteria, Archaea and Eucarya consist of prokaryotes. Prokaryotes are subdivided into 30 phyla in the domain Bacteria and five phyla in the domain Archaea. Most described prokaryotic species belong to only four of the 30 bacterial phyla, whereas the majority of phyla are hardly represented by living isolates. The absence of living isolates to calibrate overall bacterial diversity resulted in pragmatic challenges to taxonomically characterize the bacterial diversity known only from culture independent methods. Thus, searching as an alternative that could eventually lead to a more realistic understanding of prokaryotic biodiversity, provide biotechnology with new tools and maybe even contribute to develop a model of prokaryotic evolution. Many bacteria are known to regulate their cooperative activities and physiological processes through a mechanism called quorum sensing (QS), which bacterial cells communicate with each other by releasing, sensing and responding to small diffusible signal molecules. The ability of bacteria to communicate and behave as a group for social interactions. That provided benefits to bacteria in host colonization, formation of biofilms, defense against competitors and adaptation to changing environments.

Keywords: Microbial diversity form, function in prokaryotes, diversity, phylogenetics diversity

Introduction
Our knowledge about bacteria in natural environments is limited, and studying microbial diversity in nature is not an easy task (Fakruddin and Shahnewaj, 2013) [8]. Microbial diversity can be seen in many forms, including cell size and cell morphology, physiology, motility, pathogenicity, developmental biology, adaptation to environmental extremes, phylogeny and mechanism of cell division (Madigan *et al.*, 2012; Aryal *et al.*, 2015) [26, 2]. Microbial diversity can be defined by the number of species or different groups (e.g. operational taxonomic units) of microbes living in a certain environment, as well as the evenness of the species abundance distribution.

In natural environments microbial communities are typically complex and the diversity is difficult to assess and compare. In order to quantify the diversity, a variety of diversity indices and richness estimates have been developed and applied (Magurran, 2004; Koskinen, 2013) [15, 33]. These estimators present the diversity data as a single number that takes various aspects, depending of the indices used, of diversity into consideration. The diversity within individual samples or locations can be assessed using alpha diversity measurements whereas comparing the community membership and structure between samples or habitats is accomplished by applying beta diversity calculators (Koskinen, 2013) [33].

Microbial diversity and prokaryotes
Generally, prokaryotes' numerical abundance and importance in biogeochemical transformations, the absence of detailed knowledge of prokaryotic diversity is a major omission in our knowledge of life on Earth' thus appears fully justified (Oren, 2004) [16]. Biodiversity has been defined as the range of significantly different types of organisms and their relative abundance in an assemblage or community. Diversity can be defined as the number of prokaryotic species and their relative abundance in a community, or as the amount and distribution of information in a community (Torsvik *et al.*, 2002) [21].

In natural ecosystems, microorganisms exist in high numbers despite the fact that there are several thousands of microbial species that have not yet been described (Fakruddin and Shahnewaj, 2013) [8]. Microorganisms are present everywhere on Earth that will support life. These include habitats we are all familiar with soil, water, animal and plants as well as virtually any structures made by humans. Some microbial habitats are ones in which humans could not survive, being too hot or too cold, too acidic or too caustic or too salty. Although such environments would pose challenges to any life forms, they are often teeming with microorganisms. Organisms inhabiting such extreme environments are called extremophiles, a remarkable group of microorganisms that collectively define the physiochemical limits to life (Prescott *et al*., 2002) [18].

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 (Jemes *et al*., 2002) [27]. 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 (Greek for ''before karyon'' or ''before nucleus'') are simple, single cell organisms that lack a membrane bound nucleus. Eukaryotes (Greek for ''true nucleus'') divide by mitosis and possess a membrane bound nucleus, an intricate cytoskeleton, mitochondria and in the case of algae and plants cells, also chloroplasts (Whitman *et al*., 2013) [25].

Within natural microbial populations, a large amount of genetic information is "waiting" to be discovered. It has been recorded that culturable bacteria represent a minor fraction of the total bacterial population present. However, it is important to continue the work both on the culturable as well as the non-culturable bacteria from different environments. Diversity studies are also important for comparison between samples. Another important reason for studying microbial diversity is the lack of adequate knowledge about the extant and extinct microbes. The capability of an ecosystem to resist extreme perturbations or stress conditions, can partly be dependent of the diversity within the system. Diversity studies are important in order to: increase the knowledge of the diversity of genetic resources, understand the distribution of organisms, increase the knowledge of the functional role of diversity, identify differences in diversity associated with management disturbing, understand the regulation of biodiversity, understand the consequences of biodiversity (Giovannoni *et al*., 1990; Fakruddin and Shahnewaj, 2013) [10, 8].

Prokaryotes were originally defined in a seminal paper as single celled entities by their cellular structure, e.g. the lack of a nucleus, the division by fission and not by mitosis, and the special structure of the cell wall. This is an important difference from animals or plants, since bacterial cells are independent entities that carry out their life processes typically independently of other cells. In 1990, Carl Woese suggested splitting prokaryotes into two domains of life, the 'Bacteria' and the 'Archaea', with the consequence of grouping all other organisms into the third domain of life, the Eucarya (Sikorski, 2015) [34].

Evolution of Prokaryotes

Geochemical and fossil evidence indicates that life on earth is at least 3.5 billion years old (Whitman, 2009) [24]. While the form of ancient microfossils resembles that of modern prokaryotes, there is little additional evidence in the fossil record for their molecular nature. However, by 2.5 billion years ago, there is evidence for abundant prokaryotic life, including widespread microfossils and stromatolites or fossilized microbial mats and major signatures of biological processes in the geochemical record, such as depletion of inorganic carbonates for ^{12}C and deposits of complex organic carbon enriched in ^{12}C. By this time, the oxygenation of the earth was also well under way and it is likely that oxygenic photosynthesis was fully evolved within the domain *Bacteria* (Eigenbrode and Freeman, 2006; Whitman, 2009) [7, 24].

The oldest direct evidence for life on Earth is well preserved microfossils of prokaryotes found in 3.9 billion year old rocks in Western Australia. Based on the fossil record, single cell eukaryotes first appeared ca. 1.8 billion years ago (Whitman *et al.*, 2013) [25]. According to these data, prokaryotes were the only cellular form of life for 2.1 billion years. During this time, prokaryotes evolved most of the biochemistry present in all forms of life, including DNA replication, the genetic code, protein synthesis via transcription and translation, photosynthesis, anaerobic and aerobic metabolism. Furthermore, based on differences in their ribosomal RNA gene sequences, molecular microbiologists have concluded that during this time, prokaryotes split into two groups, or domains, titled Bacteria and Archaea (Pace *et al*., 2012; Whitman *et al.*, 2013) [17, 25].

Molecular clocks based upon both rRNA and protein coding genes suggest that the domains *Archaea* and *Bacteria* had both diverged near 2.5 billion years ago. Moreover, within the domain *Bacteria*, many of the deep groups or phyla had already formed, including the modern lineages of *Cyanobacteria*, *Proteobacteria*, and *Firmicutes*. Within the domain *Archaea*, the *Crenarchaeota* and *Euryarchaeota* had already diverged, as well as many of the major lineages of methanogens within the phylum *Euryarchaeota* (Sheridan., 2003) [19]. The presence of many diverse and highly specialized lineages, many of which share large numbers of complex biochemical pathways and molecular processes suggests that the biochemical complexity of the prokaryotes was fully evolved and that prokaryotes very similar to modern organisms were abundant on earth 2.5 billion years ago. In contrast, the first fossils of clearly eukaryotic organisms appeared about 1.8 billion years ago. Analyses of the molecular diversity within the modern eukaryotes suggest that this group began to diversify about 1.1 to 2.0 billion years ago. Thus, it is likely that the eukaryotes only evolved after the prokaryotes had obtained their modern complexity (Douzery *et al*., 2004; Hedges., 2004) [6, 12].

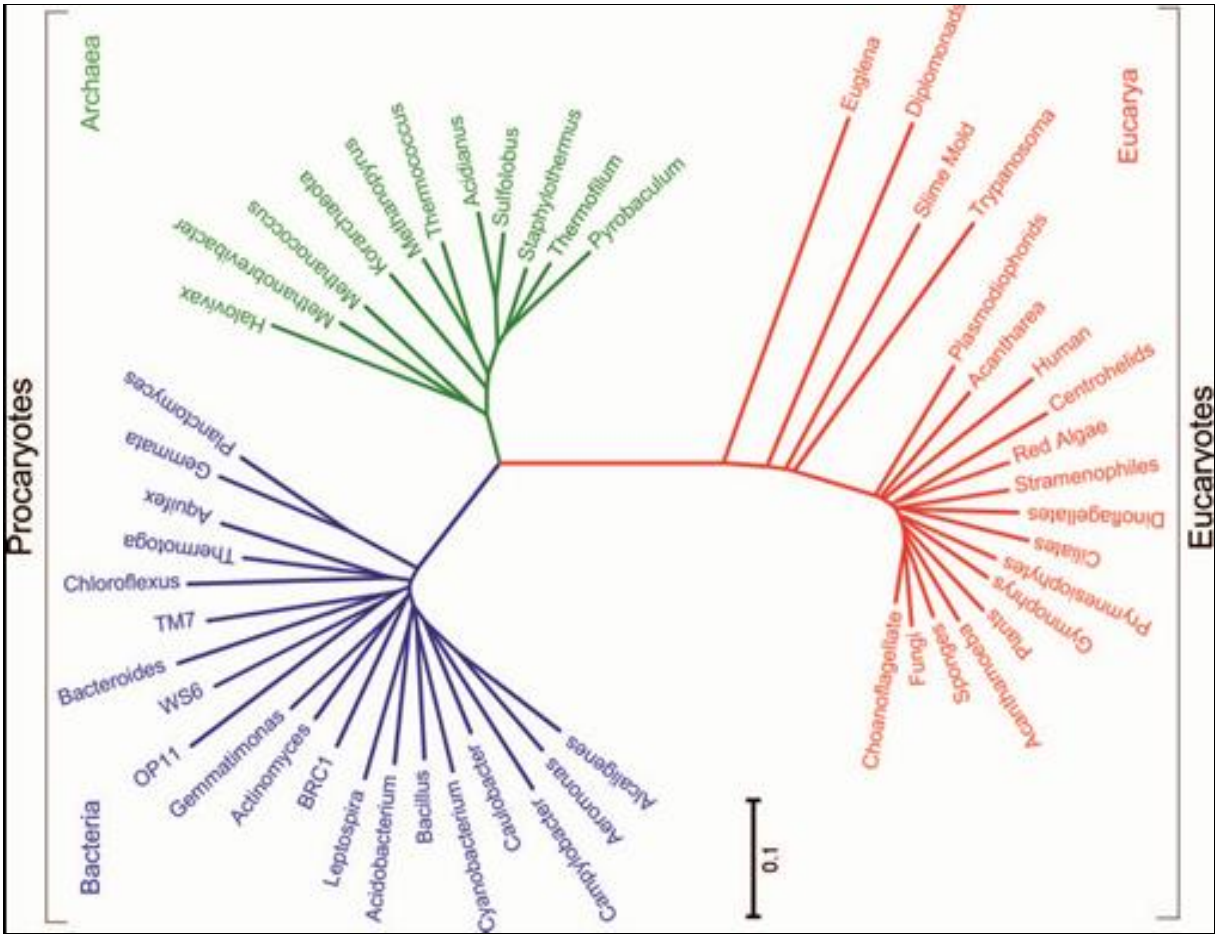

Fig 1: Phylogenetic tree of the 16S rRNA genes in the three domains *Archaea*, *Bacteria* and *Eucarya.* The prokaryotes comprise the archaeal and bacterial domains. The eukaryotes contain solely the domain *Eucarya* (Whitman, 2009) [24].

Properties of prokaryotes

It is well known that prokaryotic cells outnumber the eukaryotic cells on Earth by several order of magnitudes, e.g. each of us contains 10 to 100 times (10^{14-12}) more bacterial than own cells (10^{13}) (Whitman *et al.*, 1998) [28]. There are even more important and unique properties of prokaryotic microbes. Prokaryotes provide the foundation of our biosphere. Biogeochemical cycles would be incomplete without the help of these microbes. The global biosphere is largely shaped by their geochemical activities. They impact and are impacted by virtually all geochemical processes that occur at the Earth's surface. In particular, complete nitrogen and sulfur cycles as well as metal reductions and oxidation would be impossible without the activity of prokaryotic microbes. Some of them obtain energy for growth by transferring electrons to a wide range of harmful metals, such as uranium, chromium, arsenic and plutonium (Whitman *et al.*, 1998; Schleifer, 2004) [28-29].

Prokaryotic microbes show an unusual high physiological and biochemical versatility. Moreover, certain metabolic pathways, such as special fermentations, nitrogen fixation, methane formation and an oxygenic photosynthesis, are only found among prokaryotic microbes. The metabolic, physiological and genetic diversity of prokaryotic microorganisms is far greater than that found in higher organisms. Microbial, in particular bacterial endosymbionts are fundamental to the survival of higher organisms. Without bacterial endosymbionts most animals would not survive. Symbionts carry out essential biochemical reactions for their eukaryotic hosts, e.g. the biosynthesis of essential amino acids, vitamins or the degradation of certain macromolecules. Non-cultured prokaryotes represent a huge genetical and biotechnological potential and therefore an enormous source of new products and processes (Schleifer, 2004) [29].

Distribution of Prokaryotes

Prokaryotes are found nearly everywhere in the modern world and their presence defines the biosphere. They have been detected at altitudes of 77 km in the atmosphere and depths of 2 km in the subsurface. Soil, water, sea ice, leaves and roots of trees, guts of invertebrate and vertebrate animals and subsurface aquifers are all fully colonized by highly specialized populations of Prokaryotes. The number of individual cells is probably on the order of 5 X1030 and their biomass is comparable to that of plants (Whitman *et al.*, 1998) [28].

Aharon, (2004) [1] attempted to make an inventory of prokaryotes on Earth, based on the latest estimates of their numbers in different ecosystems (table 1). The 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. The Bacteria and Archaea present in the gastrointestinal tracts of animals contribute relatively little: the number of prokaryotes found in the bovine rumen (2.9 x 10^{24} in 1.3 x 10^{9} animals) is 4-6 orders of magnitude less than the numbers found in soil, the subsurface and seawater. The prokaryotes present in the colon of 5.6 x 10^{9} humans contribute 3.9 x 10^{23} cells.

Table 1: Number and biomass of prokaryotes in the world (Aharon, 2004).

Environment	Number of prokaryotic cells $\times 10^{28}$	Carbon in prokaryote biomass ($\times 10^{15}$ g)
Aquatic habitats	12	2.2
Oceanic subsurface soil	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

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 (Jemes *et al.*, 2002) [27]. However, approximately 6000 species of prokaryotes and 100,000 species of protists have been formally described. In the case of the diversity of microorganisms, even the right order of magnitude is unknown and the issue is highly controversial (Aryal *et al.*, 2015) [2].

Diversity of Prokaryotes

The prokaryotes are by far the most abundant organisms inhabiting Earth planet. They are also phylogenetic ally the most diverse; as two out of the currently recognized three major divisions (domains) of living organisms (Bacteria, Archaea and Eucarya) consist of prokaryotes. They thus represent a large proportion of life's genetic diversity. Moreover, the prokaryotes are metabolically far more diverse than the eukaryotic organisms and they are responsible for many of the key processes in the biogeochemical cycling on Earth. Despite their significance and large number, 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 (Madigan *et al.* 2003; Aharon, 2004) [14, 1].

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 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 (fungi, algae, plants and animals) quickly highlights the fact that the current description of 5,163 validly named species of bacteria. Constitutes an almost insignificant number in terms of the inventory of all species currently residing on Earth. Indeed, a recent estimate of the number of living prokaryotic species was between 10^5-10^7 (James *et al.*, 2002) [13].

Several attempts to estimate the number of species living on Earth have been made, to date less than 5000 prokaryotic species have been described. This relative low number is caused by the problems encountered for the isolation of microorganisms in pure cultures and their characterization. Problems such as hitherto unculturability, lack of proper research funding and in some cases the underestimation of the isolation efforts are responsible for such numbers. However, the isolation of an organism in pure culture is to date an indispensable requisite for the recognition of prokaryotic species. On the other hand, it is of general knowledge among microbiologists that there is a large potential of prokaryote diversity made up of hitherto uncultured microorganisms. Molecular techniques, most notably those based on 16S rRNA, which are directed towards analyzing community composition of environmental samples indicate that the hitherto classified prokaryotic species account for a very small portion of the real prokaryote diversity. Thus, if we only consider the recognized prokaryotic species for diversity calculations, their total number would never be regarded as a significant proportion of the total Earth's biodiversity.

Diversity estimates for natural bacterial communities have traditionally depended on cultivable species, but results from the use of molecular techniques to measure diversity suggest that reliance on culture has led to a longstanding underestimate of bacterial diversity. DNA and RNA analyses imply prokaryotic diversity far greater than was predicted and are beginning to hint at the role of bacterial and viral diversity in global ecological cycles. For instance, most investigations of prokaryotic diversity relate to surface environments, but recent research suggests that the biota extend deep into Earth's crust and that the majority of prokaryotic organisms might occur in the oceanic and terrestrial subsurface (Torsvik *et al.*, 2002) [21].

Recently, it has been possible to investigate prokaryotic diversity quantitatively. For instance, surveys of prokaryotic 16S rRNA genes in environmental samples have detected greater than 50 bacterial "phyla," of which only half have cultivated representatives. Prokaryotic phyla represent the deepest classification within the domain *Bacteria* or *Archaea*. Molecular clocks and correlations with the biogeochemical record indicate that these phyla probably formed greater than 2.5 billion years ago (Sheridan 2003) [19]. The antiquity of these lineages is consistent with their enormous diversity. Importantly, prokaryotic phyla are much more diverse than eukaryotic phyla, which formed much later. For instance, the mammals, reptiles, and amphibians probably formed within the last 450 million years (Douzery *et al.*, 2004; Hedges 2004) [6, 12]. If they were classified by the same criteria used for many prokaryotes, they would be placed in separate genera within the same family.

Given the diversity of ancient groups, it is not surprising that the number of modern groups is enormous. A prokaryotic species is much deeper than common in eukaryotic biology and includes strains with >70% DNA-DNA hybridization and a change in the melting temperature of the DNA hybrids of <5 °C. By this same criterion, most of the primates would be considered a single species (Whitman, 2009) [24]. At present, there are no certain

estimates of the total number of prokaryotic species on earth. Within soil, which contains a relatively diverse population, various methods have detected 10^3 to 10^4 different molecular species or operational taxonomic units (OTUs) per sample (Torsvik 2002) [21]. Theoretical estimates suggest that soil could contain well over 10^6 OTUs. Similar observations have been made in the deep sea. In the most extensive study to date, partial sequencing of 900,000 16S rRNA prokaryotic genes from two deep-sea sites encountered 36, 087 unique sequences representing 20,468 OTUs. The OTUs detected in these experiments are defined at 97% sequence similarity of the 16S rRNA and are deeper taxonomic groups than a conventional prokaryotic species as defined above. Importantly, only a small fraction of the total number of species known to exist have ever been characterized.

Species Diversity

Microbial diversity refers unequivocally to biological diversity at three levels: within species, species number and community diversity. The term species diversity consists of two components; the first component is the total number of species present, which can be referred to as species richness. In other words, it refers to the quantitative variation among species. The second component is the distribution of individuals among these species, which is referred to as evenness or equability (J). One problem is that evenness often is unknown in bacterial systems because individual cells very seldom are identified to the species level. An attractive possibility for the measurement of biodiversity is to use divergence in molecular characters, especially the percentage of either nucleic acid homology or base sequence difference (Fakruddin and Shahnewaj 2013) [8].

The various modern concepts of species all attribute certain dynamic properties to species: that each species should be a cohesive group, whose diversity is limited by an evolutionary force; that different species is irreversibly separate; that species is ecologically distinct; and that species are each founded only once. Efforts to define prokaryotic species according to these properties have differed most profoundly in the forces of cohesion deemed to be most important for prokaryotic species. In the ecotype concept of species, a prokaryotic species (or ecotype) is a clade whose members are ecologically similar to one another, so that genetic diversity within the ecotype is limited by a cohesive force, either periodic selection or genetic drift, or both. The prokaryotes appear to dwarf the eukaryotes in the number of species as well. Estimates of total eukaryotic diversity fall within the range of 10–50 million species. Although only about 9000 species of prokaryotes have been described. Indirect molecular approaches based on annealing of DNA extracted from the environment (without cultivation) suggest the existence of a billion or more prokaryotic species worldwide and ten million species within a given habitat (Cohan and Koeppe, 2008) [3].

The “phylophenetic” definition circumscribes the species as a “monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity in many independent characteristics and is diagnosable by a discriminative phenotypic property”. Second, a species can be defined as an assemblage of strains sharing 70% or more DNA homology. Third, in an ecological definition the species and niche concept are linked and thus a species consists of the organisms occupying the same niche (Torsvik *et al.*, 2002) [21]. The description of species of prokaryotes is based on living cultures and one isolate is designated as the nomenclatural type. The basis of the taxonomic hierarchy is the species. However, the concept of a prokaryote species still lacks a theoretical basis and all existing definitions are pragmatic ones, such as, for example: ‘A species consists of an assemblage of individuals (or, in micro-organisms, of clonal populations) that share a high degree of phenotypic similarity, coupled with an appreciable dissimilarity from other assemblages of the same general kind’ or ‘a collection of strains showing a high degree of overall similarity, compared to other, related groups of strains’ (Oren, 2004) [16] called for the establishment of a ‘natural’ species concept for the prokaryotes.

Phylogenetic Diversity

Phylogenetic diversity (PD) represents the summed branch lengths of the evolutionary tree connecting species within a set, frequently defined by geographical proximity. While a completely sampled phylogeny depicts the evolutionary divergences between extant taxa, allowing us to reconstruct ancestral states and diversification rates. The sub tree includes only species within a given sample; hence not all diversification events are represented, but the evolutionary distances separating included taxa are preserved (Davies and Buckley, 2011) [4].

Molecular techniques, most notably those based on 16S rRNA and DNA-DNA hybridization when studying microbial diversity and phylogeny. Moreover, attempts to elucidate the phylogeny of prokaryotes based on the ssu-rRNA have been quite successful. However, saturation is a problem due to the restricted length of the molecule and functional restrictions limiting the number of mutable sites. This issue can be addressed to some degree by using additional phylogenetic markers, such as 23S rRNA, the β - subunit of F1 F0 ATPase. Another well-known problem associated with this type of approach is that the evolutionary history of any single gene may differ from the phylogenetic history of the whole organism from which the corresponding molecule was isolated. Indeed, the sequence requirement grows exponentially with time as one attempts to resolve deeper and deeper divergences. Therefore, it seems important to make use of as much genetic information as available for the reconstruction of the prokaryotic phylogeny. Now, a growing number of complete prokaryotic genomes is available and the question arises how to derive phylogenies based on the whole genomic information of organisms rather than based on a small number of genes (Henz *et al*., 2005) [30].

Today, instead of the traditional rank based biological classification, phylogenetic systematics, which aims at postulating phylogenetic trees rather than focusing on what taxa to delimit, has been used commonly (Shifman *et al*., 2014) [31]. Comparative analysis of the hundreds of sequenced bacterial and dozens of archaeal genomes leads to several generalizations on the principles of genome organization and evolution. Comparative genomics also shows that horizontal gene transfer (HGT) is a dominant force of prokaryotic evolution, along with the loss of genetic material resulting in genome contraction. A crucial component of the prokaryotic world is the mobilome, the enormous collection of viruses, plasmids and other selfish

elements, which are in constant exchange with more stable chromosomes and serve as HGT vehicles. Thus, the prokaryotic genome space is a tightly connected, although compartmentalized, network, a novel notion that undermines the 'Tree of Life' model of evolution and requires a new conceptual framework and tools for the study of prokaryotic evolution (Koonin and Wolf, 2008) [32].

Quorum sensing

For a long time, it was considered that the most basic forms of life, single cell prokaryotic bacteria lacking a nucleus, are not able to develop a basic form of social behavior as a result of chemical communication among members of a population. Cooperative behavior by using autoinducer molecules was discovered first in bacteria that are living in symbiosis with a marine squid. The basic of this molecular communication, which is called "quorum sensing" (QS) and the signaling molecules involved were demonstrated via a very elementary experiment: by adding a so-called conditioned supernatant of a densely grown bacterial culture to a fresh, low cell density culture, the properties of the high density culture were conferred (Waters and Bassler, 2005) [23].

Many environmental and interactive important traits of bacteria, such as antibiotic, siderophore or exoenzyme (like cellulose, pectinase) production, virulence factors of pathogens, as well as symbiotic interactions, are regulated in a population density dependent manner by using small signaling molecules. This phenomenon, called quorum sensing (QS), is widespread among bacteria. Many different bacterial species are communicating or "speaking" through diffusible small molecules. The production often is sophisticatedly regulated via an auto inducing mechanism. A good example is the production of N-acyl homo serine lactones (AHL), which occur in many variations of molecular structure in a wide variety of Gram-negative bacteria. In Gram-positive bacteria, other compounds, such as peptides, regulate cellular activity and behavior by sensing the cell density. The degradation of the signaling molecule called quorum quenching is probably another important integral part in the complex quorum sensing circuit (Hartmann and Schikora, 2012) [11].

The term quorum sensing (QS) is used to describe the communication between bacterial cells, whereby diffusible molecules produced by individuals control a coordinated population response. QS has not only been described between cells of the same species (Intraspecies), but also between species (interspecies) and between bacteria and higher organisms (inter-kingdom). The fact that QS-based communication appears to be widespread among microbes is strange, considering that explaining both cooperation and communication are two of the greatest problems in evolutionary biology. From an evolutionary perspective, intraspecies signaling can be explained using models such as kin selection, but when communication is described between species, it is more difficult to explain. It is probable that in many cases this involves QS molecules being used as 'cues' by other species as a guide to future action or as manipulating molecules whereby one species will 'coerce' a response from another. In these cases, the usage of QS molecules cannot be described as signaling. This review seeks to integrate the evolutionary literature on animal signaling with the microbiological literature on QS, and asks whether QS within bacteria is true signaling or whether these molecules are also used as cues or for the coercion of other cells (Diggle *et al*., 2007) [5]

Nitrogen Fixation and prokaryotes

Prokaryotes capable of fixing N share the ability to produce an enzyme called nitrogenase that ultimately catalyzes the reaction that splits the triple bonded N2 gas into two separate ammonia (NH3) molecules. Nitrogen fixing prokaryotes are quite diverse and exist in a variety of environments both as free living microbes and as organisms that have evolved an associative relationship with various plants. The nitrogenase enzyme is sensitive to oxygen and is irreversibly inactivated in the presence of free oxygen. Consequently, N fixing prokaryotes have developed a range of strategies to protect nitrogenase from exposure to oxygen. For example, under N limited conditions *Anabaena*, a filamentous cyanobacteria, transform vegetative cells within a filament into specialized cells called heterocysts that are designed specifically to protect the nitrogenase enzyme (Walley, 2013) [22].

Yet another group of N-fixing prokaryotes, collectively called rhizobia, instruct legume roots to form root nodules on their behalf, providing a haven for the rhizobia in which the nitrogenase enzyme is protected, as well as a ready supply of carbohydrates to sustain the rhizobia housed in the nodule (Fig. 2). This relationship between the N-fixing prokaryotes and the legume plants is called a symbiosis, with both partners deriving benefits from the association. Ultimately, the rhizobial partner provides N "fixed" (i.e., captured) from the atmosphere, and in exchange the plant provides C, which is fixed from atmospheric CO_2 during photosynthesis. This type of mutualistic relationship is limited to a relatively narrow group of prokaryotic bacteria, including the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Bradyrhizobium* and *Azorhizobium* (Gage, 2004) [9].

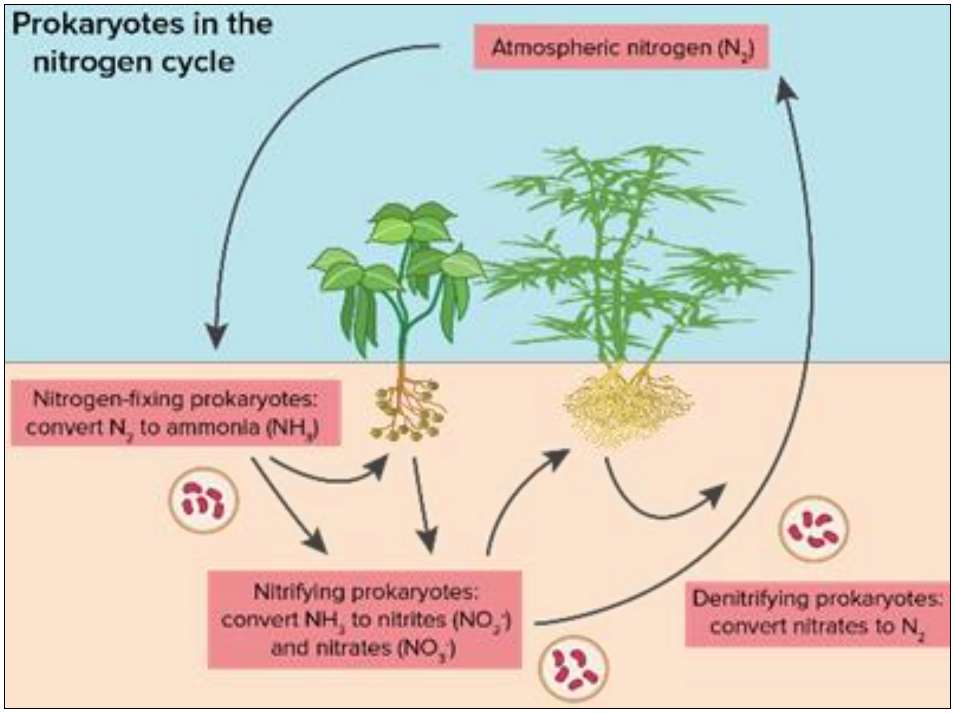

Fig 2: Image modified from "Nitrogen cycle" by Johann (2015) (CC BY-SA 3.0). The modified image is licensed under a CC BY-SA 3.0 license (online accessed 3/30/2017)

Conclusion

Prokaryotes' numerical abundance and importance in biogeochemical transformations, the absence of detailed knowledge of prokaryotic diversity is a major omission in our knowledge of life on Earth' thus appears fully justified. When molecular approaches based on sequencing of small-subunit rRNA (16S in prokaryotes, 18S in eukaryotes) were introduced in the late 1970s, it became clear that the prokaryotes do not form a single, phylogenetic ally coherent group, but consist of two fundamentally different groups, the divisions (domains) Archaea (formerly called Archaebacteria) and Bacteria (formerly named Eubacteria) This splitting-up of the prokaryotes into Archaea and Bacteria is now generally accepted.

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