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New Microbial Biodiversity in Marine Sediments

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Abstract

Microbes in marine sediments represent a large portion of the biosphere, and resolving their ecology is crucial for understanding global ocean processes. Single-gene diversity surveys have revealed several uncultured lineages that are widespread in ocean sediments and whose ecological roles are unknown, and advancements in the computational analysis of increasingly large genomic data sets have made it possible to reconstruct individual genomes from complex microbial communities. Using these metagenomic approaches to characterize sediments is transforming our view of microbial communities on the ocean floor and the biodiversity of the planet. In recent years, marine sediments have been a prominent source of new lineages in the tree of life. The incorporation of these lineages into existing phylogenies has revealed that many belong to distinct phyla, including archaeal phyla that are advancing our understanding of the origins of cellular complexity and eukaryotes. Detailed comparisons of the metabolic potentials of these new lineages have made it clear that uncultured bacteria and archaea are capable of mediating key previously undescribed steps in carbon and nutrient cycling.

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INTRODUCTION

Marine sediments make up more than two-thirds of the Earth's surface. Here, on the ocean floor, is where particulate organic matter from the water column settles. As a result, sediments contain the largest pool of organic carbon on the planet. Subseafloor microbes have been estimated to constitute up to five-sixths of the planet's total biomass and one-third of its living biomass (Whitman et al. 1998). These microbial communities process both organic and inorganic carbon and contribute to the cycling of nutrients such as sulfur, nitrogen, and iron (Parkes et al. 2014). Despite the global importance of these organisms, marine sediments are among the least understood environments. This is due partly to the difficulty of sampling, especially in the deep sea, as well as the complexity of inhabiting communities. Most of our understanding of sediment microbiology is derived primarily from the culture-based approaches, which have tended to be biased toward readily cultivated lineages (Fry et al. 2008, Teske & Sørensen 2008).

In the past 15 years, surveys of gene diversity (e.g., of 16S rRNA genes) in marine sediments have identified several uncultured archaeal taxa. Some of these lineages were detected throughout the oceans and are very abundant, such as the Miscellaneous Crenarchaeotic Group (MCG) (Kubo et al. 2012), now known as the phylum Bathyarchaeota. rRNA sequences falling within the MCG clade have been detected in a wide array of soils, lakes, deep subsurface environments, and hot springs (Barns et al. 1996, Inagaki et al. 2003, Takai et al. 2001, Vetriani et al. 1999). Another prominent archaeal group, Marine Benthic Group B (MBG-B), which was first recovered from the North Atlantic abyssal plain sediments (Inagaki et al. 2003, Vetriani et al. 1999), has also been shown to be widely distributed in sediments. The phylogenetic positions of MBG-B and two other commonly seen and deeply branched archaeal lineages, the Ancient Archaeal Group (AAG) and the Marine Hydrothermal Vent Group (MHVG), have varied considerably in different studies (Teske & Sørensen 2008). AAG and MHVG were initially discovered at hydrothermal vent sites (Takai & Horikoshi 1999), while related lineages have been seen in cold sediments (Inagaki et al. 2003, Sørensen & Teske 2006). Other frequent sediment taxa contain distinct lineages within the Euryarchaeota, including MBG-D and the South African Gold Mine Euryarchaeal Group (SAGMEG). MBG-D and SAGMEG were first described in samples from the Atlantic continental slope and the deep terrestrial subsurface, respectively (Takai et al. 2001, Vetriani et al. 1999), and both have since been shown to be broadly distributed in the oceans (Teske & Sørensen 2008). Marine Group I (MGI) and sister group MBG-A appear to be less common in many environments yet have been detected in a variety of locations, from shallow to deep-sea sediments (Inagaki et al. 2006, Parkes et al. 2014).

The dominant sediment bacterial types have not received as much attention and tend to fall within well-known taxa, including the Proteobacteria, Bacteroidetes, Chlamydiae, Firmicutes, Chloroflexi, Gemmatimonadetes, and Planctomycetes, as well as several common candidate phyla, including OP1, OP3, OP8, OP10, OP11, WS1, JS1, and WS3 (Durbin & Teske 2011). This may reflect a general researcher bias to resolve the diversity of archaea; often bacterial sequences are not given the same phylogenetic characterization that archaeal sequences are. However, when sediment bacterial sequences have been analyzed phylogenetically, novel lineages have been identified. For example, two novel phyla referred to as NT-B2 and NT-B6 were obtained from the Nankai Trough (Reed et al. 2002) and were later detected broadly in the oceans (Durbin & Teske 2011; Fry et al. 2008; Inagaki et al. 2006; Parkes et al. 2005, 2014; Webster et al. 2006).

One of the striking characteristics of these uncultured lineages is that they are globally distributed (Teske & Sørensen 2008). They are also predominant to great depths in the subseafloor (Parkes et al. 2005). The large proportion of uncultured lineages, estimated to make up more than 75% of sediment genera (Lloyd et al. 2018), has led to the realization that we have a limited

understanding of marine sediment microbiology. Given the importance of these communities to the oceans, there is a need to better resolve the diversity and ecological roles of these uncultured taxa. 16S rRNA gene-based analyses have offered limited information to advance our understanding of their ecological roles. Moreover, despite the large number of uncultured taxa being described from these diversity analyses, polymerase chain reaction (PCR)-based approaches only detect taxa that the PCR primers target (Baker et al. 2006). There are many biogeochemical processes that have not been attributed to microbes, and thus we have little understanding of the biodiversity and biochemical mechanisms that mediate these processes—for example, how recalcitrant detrital carbon is processed in the deep sea is largely unknown (Arnosti 2011). Moreover, cryptic processes are likely occurring that have not been detected geochemically, similar to those described for sulfur cycling in oxygen minimum zones (Canfield et al. 2010). Thus, it stands to reason that there is untapped biodiversity and physiologies in sediments, several new examples of which are covered in this review.

In the past 15 years, there have been several rapid advancements in DNA sequencing technologies (e.g., Illumina) and computational approaches to reconstruct genomes from nature (Baker et al. 2020). This review covers how this approach is transforming our understanding of the biodiversity and ecological potential of uncultured sedimentary lineages. These new genomes are also revolutionizing our understanding of the tree of life and early evolution on the planet.

THE GENOMIC RECONSTRUCTION AND CHARACTERIZATION OF KEY SEDIMENTARY LINEAGES

The reconstruction of individual genomes from sediments has provided the first glimpses of the metabolic capabilities of several uncultured lineages, including the candidate phyla described above. Also, several distinct lineages that were overlooked by PCR-based diversity surveys have been described, including new phyla (e.g., Thorarchaeota, Helarchaeota, and Brockarchaeota). A look at the variety of archaeal phyla that are broadly distributed in the marine sediments reminds us how diverse these communities are (**Figure 1**). This also illustrates how most of the prominent taxa in sediments are currently uncultured. The genomes of many of these lineages were first described in marine sediments (Thorarchaeota, Lokiarchaeota, Heimdallarchaeota, Brockarchaeota, Bathyarchaeota, and some new bacterial phyla) and subsequently studied in other environments (Liu et al. 2018). In most cases, all we have at this point are a variety of genomes. However, these genomes are providing insights into the physiological capabilities and evolutionary past of these phyla.

Expansion of the Bathyarchaeota Phylum and Its Potential Ecological Roles

MCG is likely one of the most diverse and broadly distributed sedimentary archaeal groups (Zhou et al. 2018). Several subgroups have been described (Lazar et al. 2015), and when phylogenomic analyses showed that this group was a unique phylum, it was named Bathyarchaeota (Meng et al. 2014). The first draft genome of this group (estimated to be ~41% complete) was obtained using single-cell genomics (Lloyd et al. 2013). Comparative analyses of these genomes indicated that they are capable of protein degradation, which is obviously a prominent niche to fill in marine sediments. A few years later, several bathyarchaeotal metagenome-assembled genomes (MAGs) were obtained from estuary and deep-sea sediments (He et al. 2016, Lazar et al. 2016), and metabolic inference from these genomes indicated that they may be capable of homoacetogenesis.

A large number of MAGs belonging to the Bathyarchaeota have now been recovered from different environments, and their gene content indicates an ability to degrade a wide range of

Phylogenomics: the generation of phylogenetic trees from the concatenation of multiple proteins

Metagenome-assembled genome (MAG): a genome reconstructed from microbial communities

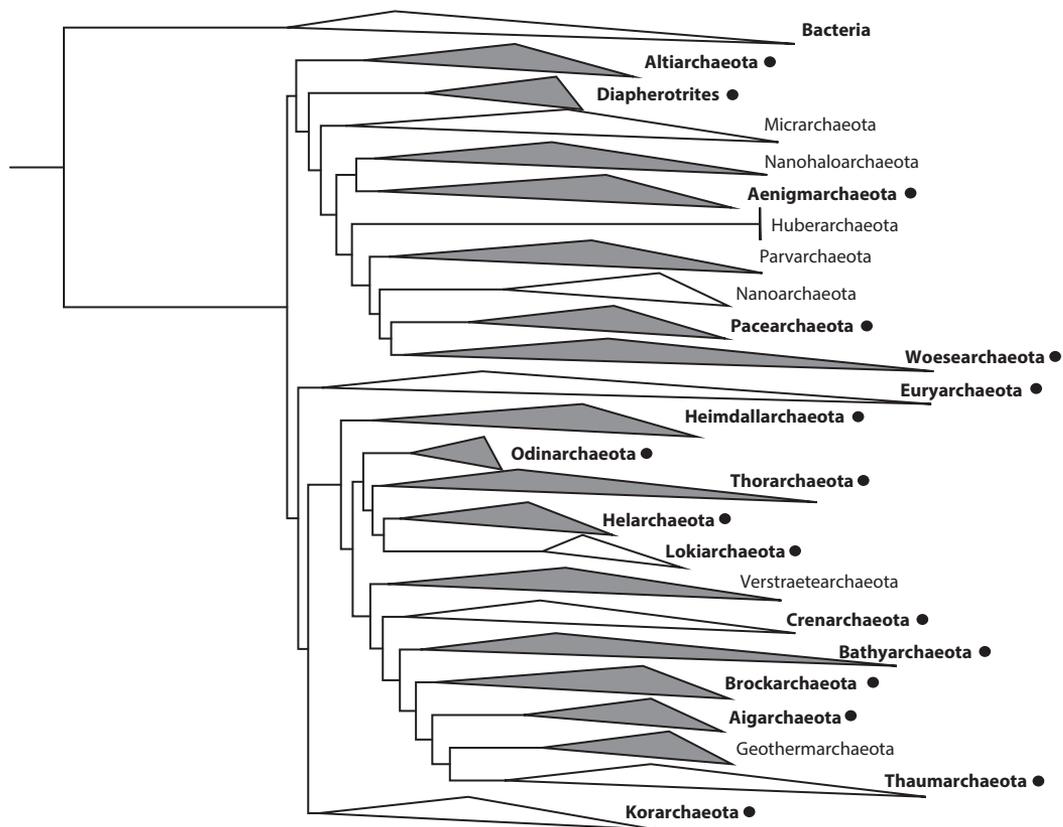


Figure 1

Phylogenomic analyses of 37 marker proteins identified using PhyloSift (Darling et al. 2014), based on 4,962 archaeal genomes. Phyla in bold with black dots have been shown to be prominent members of marine sediments; those with a shaded wedge have not been cultured. As this figure clearly shows, most of the key phyla in marine sediments have not been cultivated.

organics, including carbohydrates, fatty acids, and aromatic compounds (Dombrowski et al. 2018, Zhou et al. 2018) (**Figure 2**). Interestingly, a small subset of the bathyarchaeotal MAGs contain methyl-coenzyme M (*mcr*) genes (Evans et al. 2015), which was an important discovery because it was the first example of *mcr* genes identified outside of the Euryarchaeota. Phylogenetic analyses of the Mcr proteins revealed them to be highly divergent from those present in Euryarchaeota. At the time, Mcr was only known to be involved in methane production and methane oxidation, but enrichment of a distinct Euryarchaeota species, *Syntrophoarchaeum* sp., revealed that these Mcr-like proteins (which are related to those in Bathyarchaeota) are involved in the anaerobic oxidation of butane and propane (Laso-Pérez et al. 2016). Nevertheless, it has not been determined which alkane (butane or some other alkane) Bathyarchaeota are capable of oxidizing. Recently, isotopic tracer experiments have indicated that members of this phylum (subgroup 8) are able to fix inorganic carbon, which is assimilated into cellular material via anabolic metabolism, when grown with lignin as an energy source, further expanding the range of organic carbon substrates they are able to utilize (Yu et al. 2018). Even so, subsets of this phylum have the potential to oxidize hydrocarbons, nitrogen, and sulfur (Zhou et al. 2018), and the commonality that is emerging is that they are key players in the degradation of organic matter in sediments.

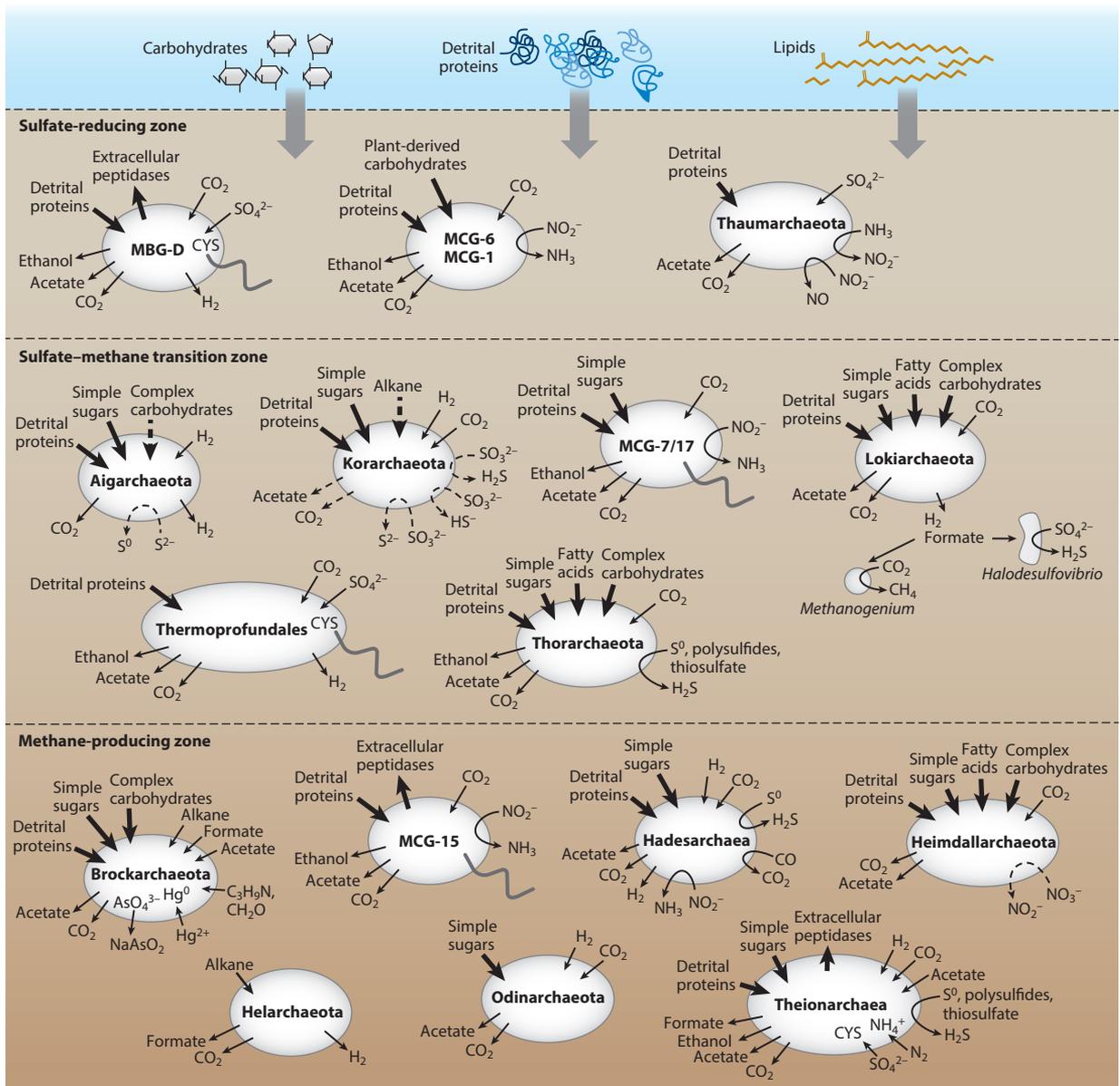


Figure 2

Overview of the inferred ecological niches of broadly distributed uncultured archaeal taxa in marine sediments. Most of these processes have been proposed based only on the presence of metabolic pathways in genomic data. Abbreviations: MBG, Marine Benthic Group; MCG, Miscellaneous Crenarchaeotic Group.

New Branches in the Korarchaeota

The first Korarchaeota 16S rRNA gene sequences were described from the Obsidian Pool hot spring (Yellowstone National Park, USA), and phylogenetic analyses indicated that the Korarchaeota branch independently from the two other archaeal phyla known at the time,

Crenarchaeota and Euryarchaeota (Barns et al. 1996). Their basal branching in the archaeal phylogeny suggested they were descendants of an ancient ancestor. Their high abundance in this hot spring made it possible to obtain an enrichment culture, from which the first complete genome was obtained of “*Candidatus* Korarchaeum cryptofilum” (Elkins et al. 2008). This hot spring korarchaeote has a fairly limited metabolic capacity and relies on peptide fermentation for survival. Due to the limited distribution of the Korarchaeota (they have been detected only in hot environments), very few other genomes were obtained until recently, when 10 MAGs were obtained from deep-sea hydrothermal sediments from the Guaymas Basin (Gulf of California) (Dombrowski et al. 2018). The reconstruction of these additional korarchaeotal genomes has been valuable for more robust phylogenomic analyses of the archaea (Baker et al. 2020). These deep-sea genomes also indicate that the Korarchaeota have limited physiological capabilities; however, they contain sulfide-quinone reductase genes, suggesting that they are capable of sulfide oxidation (**Figure 2**).

More recently, two metagenomic populations, “*Ca.* Korarchaeum cryptofilum” OPF8 and “*Ca.* Methanodesulfokores washburnensis,” were characterized from hot springs (McKay et al. 2019). These korarchaeotes each contain *mcr* and dissimilatory sulfite reductase (*dsr*) genes, suggesting they are capable of coupling sulfur reduction with anaerobic methane oxidation. However, these genotypes have not been seen in marine sediments.

Despite being the third archaeal phylum to be named, the Korarchaeota are among the least studied lineages. Given their deep position in the archaeal tree, their roles in archaeal evolution will likely be the subject of further evolutionary studies.

Asgard Archaea Have Shaken the Tree of Life

Undoubtedly, some of the most appealing lineages to be described from marine sediments in recent years are those belonging to the Asgard archaea. The first genomes from this group were recovered from sediments near the Loki’s Castle hydrothermal vent and were thus named Lokiarchaeota (Spang et al. 2015). This phylum comprises archaea that had been previously described by rRNA phylogenies as MBG-D and the Deep Sea Archaeal Group (DSAG). The reconstruction of these first Lokiarchaeota genomes enabled a more robust phylogenetic characterization using an array of marker proteins, which revealed that the Lokiarchaeota are monophyletic with eukaryotes. This finding and the presence of several proteins previously identified only in eukaryotes indicated that the Lokiarchaeota are descendants of the last eukaryotic common ancestor.

Some of the first large-scale sequencing targeting coastal and marine sediments resulted in the reconstruction of additional Asgard genomes, and three additional phyla—Odinarchaeota, Thorarchaeota, and Heimdallarchaeota (which comprise AAG and MHVG 16S rRNA gene clades)—have been described (Zaremba-Niedzwiedzka et al. 2017). This broader sampling of genomes provided more robust support for eukaryotes to branch from within the Asgard archaea and indicated that the Heimdallarchaeota are the closest representative phylum. The addition of these genomes to the tree of life provided further support for the relationship with eukaryotes. More recently, another phylum has been proposed, Helarchaeota, which was recovered from the Guaymas Basin (Seitz et al. 2019).

The physiologies of the Asgard archaea have broad implications for early eukaryotic evolution and biogeochemistry in sediments. The first description of Thorarchaeota was based on the reconstruction of genomes from estuary sediments, and their gene contents indicated that they are capable of producing acetate from the degradation of proteins (Huang et al. 2019, Seitz et al. 2016). A comprehensive characterization of several Asgard phyla has provided new insights into their metabolic capabilities (Spang et al. 2019). The Odinarchaeota have the smallest genomes among the Asgard archaea and appear to be only capable of fermenting simple carbon compounds.

The Lokiarchaeota and Thorarchaeota have many metabolic commonalities, and both groups are capable of carbon dioxide fixation via the Wood–Ljungdahl pathway (WLP). They can also utilize a variety of organic substrates, including peptides, amino acids, complex carbohydrates, alcohols, hydrocarbons, and fatty acids. The presence of genes encoding nicotinamide-dependent group 3b, ferredoxin-consuming group 3c [NiFe]-hydrogenases, and enzymes for formate production indicates potential mechanisms for syntrophic interactions under conditions with limited electron acceptors.

Given that the Heimdallarchaeota are currently thought to be the Asgard group most closely associated with eukaryotes, their physiologies are of particular interest with respect to understanding the archaeal host in eukaryogenesis. Functional predictions of genes in the Heimdallarchaeota indicate that they are quite metabolically versatile and are able to grow heterotrophically via fermentation. They are distinct from other Asgard archaea in that they lack most of the WLP. Some of the lineages contain genes that encode nitrate reductase, A-type heme-copper oxidase, and respiratory chain complex I, which suggests that they are able to use oxygen and nitrate as electron acceptors via aerobic or anaerobic processes (Spang et al. 2019). Thus far, they are the only Asgard archaea that have been seen in oxic environments, and a MAG has been reconstructed from ocean waters (Tully et al. 2018). This MAG encodes a terminal oxidase with similarity to a protein present in Heimdallarchaeum LC2, suggesting that some Heimdallarchaeota are capable of aerobic respiration. However, this ability is lacking in other Heimdallarchaeota.

A broad comparison of commonalities among the Asgard archaea indicated that they had the potential to use organics (fatty acids, alkanes, and aromatic compounds) for growth (Figure 2). The by-products of this usage vary considerably depending on the organism and environment. The lack of the WLP, which is an electron sink for acetogens, and the gain of membrane-bound hydrogenases, as seen in some Heimdallarchaeota, may be resulting in a selective pressure for a syntrophic partnership (Spang et al. 2019). In this scenario, electrons produced by the Heimdallarchaeota (and perhaps the archaeal host in eukaryogenesis) during the oxidation of organics could be transferred to a partner via H₂, via formate, or directly. Another possibility is that the ancestor of the Asgard archaea and eukaryotes may have coupled the anaerobic oxidation of a short-chain alkane (such as butane) through electron transfer to a bacterial partner. This possibility has been reinforced by the discovery of a newly described lineage, the Helarchaeota, which contain methyl-coenzyme M reductases, the WLP, and beta-oxidation pathways similar to those that have been confirmed to be syntrophic anaerobic butane oxidizers (Seitz et al. 2019). This scenario is not compatible with the close association of the Heimdallarchaeota with eukaryotes in current phylogenies. However, it does suggest that syntrophic interactions are broadly distributed in the Asgard archaea. There is considerable evidence that the first eukaryotic cell was the result of a similar interaction between an archaeon and bacterium (Eme et al. 2017), with the bacterium becoming mitochondria (Martin et al. 2015).

Recently, a member of the Lokiarchaeota has been cultured in a syntrophic partnership with a bacterial sulfate reducer (*Halodesulfovibrio*) and a methanogen (*Methanogenium*) (Imachi et al. 2020). After 12 years of enrichment, a coculture of the Lokiarchaeum and *Methanogenium* was eventually obtained. This isolate, named *Prometheoarchaeum syntrophicum* (strain MK-D1), is anaerobic, degrades amino acids, and produces hydrogen and formate, which are utilized by the bacteria partner. Although this species is less closely related to eukaryotes, the confirmation of syntrophic interactions makes this finding of interest to the origin of eukaryotes. Surprisingly, microscopic examination indicated that *P. syntrophicum* is a relatively small cell, with an average diameter of approximately 550 nm. This small size is unusual since other small cells—for example, the DPANN archaea (named for a cluster of phyla comprising Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, and Nanohaloarchaeota) (Rinke et al. 2013)—also have small

Eukaryogenesis: the biological events that led to the formation of the first eukaryotic cell

genomes (generally ~1 Mb) (Baker et al. 2010, Comolli et al. 2009), yet the *P. syntrophicum* genome is 4.46 Mb. It may be difficult to fit a genome that size in a cell that small, with ribosomes and other biomolecules. Microscopy also revealed that *P. syntrophicum* makes long protrusions, which have been suggested to be involved in facilitating the symbiotic interactions.

While the cultivation of a member of the Asgard archaea is a great advance in many ways, its slow growth rate makes physiological experiments challenging, and as a result, much of our understanding of its metabolism still rests on predictions from its genomic composition. However, this work certainly demonstrates that these organisms can be cultured and will surely lead to more species being grown in laboratories.

New Lineages in the Euryarchaeota

The first genomes belonging to SAGMEG were obtained from shallow coastal sediments (Baker et al. 2016). Single-cell genomes from a sister group, referred to as Mediterranean Sea Brine Lakes 1 (MSBL1), were identified in brine pools in the Red Sea (Mwirichia et al. 2016). Robust phylogenomic analyses [using 48 archaeal clusters of orthologous genes (arCOGs)] of the SAGMEG genomes indicated that they are a basal group within the Euryarchaeota (Baker et al. 2016). Thus, they were originally proposed to be a new class, named Hadesarchaea; more recently, they were suggested to be a unique phylum and renamed Hadesarchaeota, but this renaming lacked phylogenetic analyses (Chuvochina et al. 2019). Their relatively small genomes are likely adapted to life in nutrient-limited subsurface environments. They contain genes that are similar to those that encode pathways for common subsurface gases (CO and H₂) and appear to be capable of carbon fixation via the Calvin–Benson–Bassham cycle. Those present in Red Sea brines are thought to be capable of fermenting sugars (Mwirichia et al. 2016). Many Hadesarchaea have several pathways similar to those for methane utilization and generation (Baker et al. 2016). However, no genomes containing methyl-coenzyme M reductase genes have been described, indicating that they are not capable of methane cycling. This does suggest, however, that they evolved from a methanogenic ancestor (Evans et al. 2019).

Another ubiquitous archaeal lineage in marine sediments is MBG-D. The first genomic information (partial genomes 32–70% complete) about this group was obtained via single-cell approaches (Lloyd et al. 2013). A few years later, MAGs estimated to be up to 95% complete were obtained from estuary sediments (Lazar et al. 2017). Inference of their metabolisms from these first genomes suggested they are involved in the mineralization of carbon from proteins and acetogenesis. They are likely excreting extracellular peptidases, and given their predominance in sediments, they likely play a large role in global carbon cycling. Recently, a comprehensive phylogenomic characterization indicated that they should be classified as a new order, named Thermoprofundales, within the class Thermoplasmata (Zhou et al. 2019). However, a more robust phylogenomic analyses (based on Bayesian inference of 41 proteins) indicated they are a unique class within the Euryarchaeota, named Izemarchaea (Adam et al. 2017). Transcriptomic activity supports their utilization of acetate and amino acids in mangrove and intertidal mud flats in China. An additional four MAGs were obtained in this study, and further metabolic analyses suggested that these organisms are also capable of carbon fixation via the WLP. There are still relatively few genomes available for the Izemarchaea, and as a result, our knowledge of their physiologies is limited.

Two MAGs belonging to a unique clade within the Euryarchaeota were recovered from the methane-rich layer in estuary sediments (Lazar et al. 2017). Comparison of the 16S rRNA genes in the genome showed that they are associated with a group previously designated as Z7ME43. Phylogeny based on 16 ribosomal proteins indicated that they constitute a new class, proposed to be named Theionarchaea (Lazar et al. 2017). Soon after, they were shown to be associated

with the Thermococci and Methanofastidiosia, and the three classes were grouped into a super-class, Acherontia (Adam et al. 2017). Searches of rRNA databases revealed that Theionarchaea are broadly distributed in marine sediments and the terrestrial subsurface. The genomes reveal that they are metabolically versatile, containing genes encoding the enzymes capable of utilizing intermediate sulfur compounds, including S^0 (sulfhydrogenase) and thiosulfate (thiosulfate reductase) reduction to H_2S . They also contain nitrogen fixation genes, suggesting that they are able to use N_2 as a sole source of nitrogen in nutrient-limiting conditions. More conclusively, they are likely involved in the degradation of detrital proteins, like other Euryarchaeota in sediments. They also encode a complete WLP and thus may also be able to fix CO_2 .

New Bacterial Phyla Described from Sediment Communities

The first large-scale metagenomic sequencing and MAG reconstruction from estuary sediments resulted in individual genomes from several previously described uncultured bacterial phyla, including the first genomes belonging to TA06 and KD3-62 (Baker et al. 2015). In addition, genomes belonging to three new phyla—WOR-1, WOR-2, and WOR-3—were described. Further phylogenetic analyses revealed that WOR-2 actually belongs to Omnitrophica (Hug et al. 2016). WOR-1 and WOR-3 have since been named Saganbacteria (Matheus Carnevali et al. 2019) and Stahlbacteria (Dombrowski et al. 2017), respectively. Saganbacteria are positioned at the base of the cyanobacteria, and their ancestors are thought to have played an important role in the rise of oxygenic photosynthesis (Matheus Carnevali et al. 2019). Like their sister groups, they encode a variety of hydrogenases, suggesting that H_2 metabolism was central to their ancestors. Surprisingly, some Saganbacteria may be able to use O_2 via a novel type of heme copper O_2 reductase, an ability that appears to have been acquired recently. Stahlbacteria are capable of fermenting lipids, protein, and carbohydrates (Dombrowski et al. 2017).

Another broadly distributed bacterial group is the Atribacteria (formerly designated OP9 and JS1). They are most predominant in organic- and hydrocarbon-rich sediments (Lee et al. 2018). MAGs and single-cell-assembled genomes have been recently obtained from oil reservoirs and Antarctic sea sediments (Liu et al. 2019). These genomes and their prevalence in methane-rich sediments seem to suggest that Atribacteria are involved in syntrophic interactions with formate or H_2 exchange with methanogens (Lee et al. 2018). They are capable of fermenting a variety of organic compounds (amino acids and oligosaccharides) and acetate oxidation. MAGs obtained from oil reservoirs are able to mediate anaerobic degradation of hydrocarbons (*n*-alkanes) to produce fatty acids (Liu et al. 2019), which is consistent with the predominance in hydrocarbon-rich sedimentary environments.

Additional sequencing of deep-sea sediments has continued to expand phylum-level bacterial diversity. For example, approximately 500 MAGs were recently recovered from the Guaymas Basin, among which were three likely new phyla, designated GB-BP1, GB-BP2, and GB-BP3 (Dombrowski et al. 2018). Moreover, there were several unique lineages within previously described phyla, which have unique metabolic features. This leads to the question, How many more phyla are out there left to be discovered? Are we close to having genomes from most of the phyla present in sediments? How does the discovery of these new taxa alter our understanding of geochemistry in the oceans? With this in mind, we looked for novel diversity in three deeply sequenced cores from the Guaymas Basin and generated a phylogenetic tree from approximately 3,000 reconstructed bacterial genomes along with other described phyla. This tree revealed that, from this handful of cores, there are conservatively 13 new clades of genomes that do not fit into the phyla that can currently be described (**Figure 3**). In addition, five individual genomes appear to be new phyla as well.

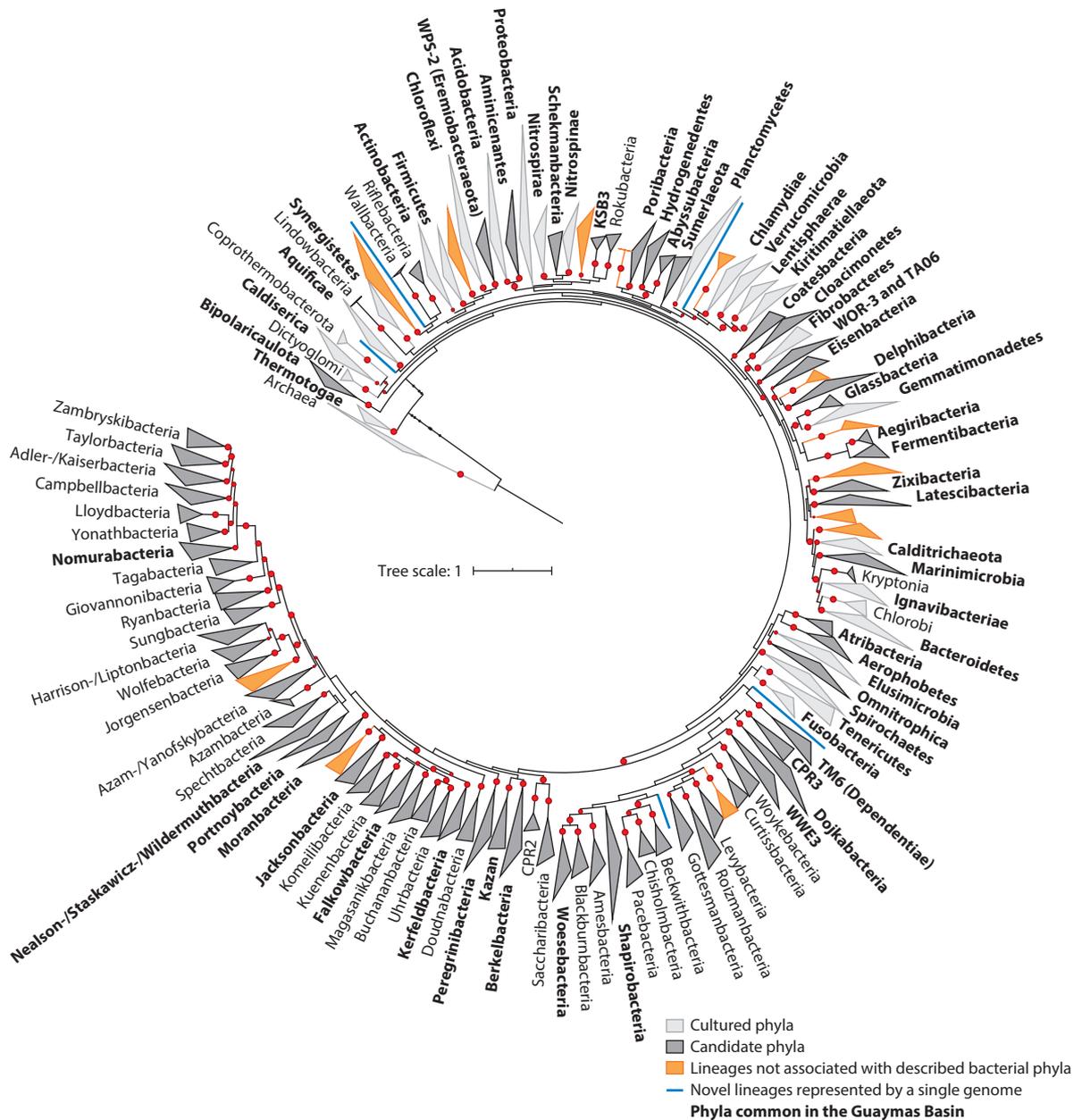


Figure 3

Phylogeny of uncharacterized genomes present in deep-sea sediments from the Guaymas Basin (Gulf of California). The unlabeled clades in orange (13 in total with more than two genomes in them) are lineages that are not associated with described bacterial phyla. The five blue branches are novel lineages represented by a single genome. The phylum names in bold are those that are common in the Guaymas Basin. The clades in light gray are cultured phyla, and those in dark gray are candidate phyla. The phylogeny was generated from 4,084 genomes using RAxML (version 8.2.4) (Minh et al. 2013), with rapid bootstrapping (100 replicates) methods of marker proteins extracted using PhyloSift (Darling et al. 2014).

CONCLUSIONS AND OUTLOOK

Microbial communities in marine sediments play an important role in global elemental cycling. However, the vast majority of taxa present in sediments have not been cultured in a laboratory setting. Primarily, lineages that have been cultured from sediments, such as Gammaproteobacteria and Deltaproteobacteria, are capable of specialized metabolic processes such as sulfate reduction and sulfur oxidation. These processes are relatively energetic, and the microbes that mediate them have relatively rapid growth rates, making them more readily cultured. Many of the ecological niches in sediments yield less energy (such as fermentation), and as a result, the organisms that occupy them have much slower growth rates. Slow-growing microbes require years of culture maintenance to be isolated. This has been demonstrated in several recent cultivations of sediment archaea, such as *Prometheoarchaeum*, which have taken several years (Chen et al. 2019, Imachi et al. 2020). A limited understanding of the substrate needs of uncultured lineages has also impeded cultivation. Furthermore, community interactions are often crucial for the survival of organisms that rely on low-energy physiologies, and these interactions are challenging to mimic in laboratory cultures. Recent successes in cultivation have revealed that interactions in anaerobic processes—for example, the coupling of organic carbon degradation in Asgard archaea with sulfate reducers—suggest that these symbioses may be more common in sediment microbes than previously thought (Imachi et al. 2020). Overall, cultivation is a powerful tool, but it can be costly and time consuming. Moreover, the physiological activity of individual species and small mixtures (enrichments) in a laboratory can be drastically different from what occurs in natural communities. Therefore, we must continue to employ and develop culture-independent approaches to study the vast biodiversity and physiologies of seafloor microbial communities.

Diversity surveys (such as 16S rRNA gene sequencing) have been instrumental in broadening our understanding of uncultured lineages and their distribution. However, the reconstruction of genomes via metagenomics has led to a large number of novel lineages that were overlooked due to primer biases (Baker et al. 2006). Recent data indicate that our current understanding of sediment diversity is limited and that the discovery of phylum-level novelty will continue. Inference of metabolic capabilities from novel genomes has revealed that dominant sedimentary lineages likely play crucial roles in biogeochemical processes, such as the degradation of detrital organic matter among Bathyarchaeota (Lloyd et al. 2013). The growing number of archaea that are capable of oxidizing short-chain alkanes, as described in Helarchaeota, also illustrates this (Seitz et al. 2019). Moreover, many newly described sediment taxa appear to play key roles in denitrification, nitrogen fixation, and intermediate sulfur transformations (**Figure 2**). The metabolic mechanisms of many of these processes have implications for our understanding of the links between microbial ecology and geochemistry. Resolving the microbial partnerships, and those present in individual species, will reveal previously unrecognized couplings between carbon and nutrient cycles. The mechanisms of these microbe-mediated processes are difficult to understand by geochemical analyses alone.

Despite all these advances in our understanding of the biogeochemical roles of previously overlooked sedimentary lineages, several fundamental gaps remain between our knowledge of active biogeochemical processes and our understanding of the metabolic mechanisms and taxa mediating them. For example, it is not known which nonmethanogenic taxa utilize the ample methylate compounds on the seafloor (Trembath-Reichert et al. 2017). We are just beginning to describe the vast biodiversity of microbes and their biogeochemical roles in marine sediments. Genome-based inferences of metabolism provide detailed genetic road maps of biochemical pathways in uncultured lineages; however, these maps are putative, and thus we must also harness *in situ* activity-based techniques to confirm physiological activity in nature. The incorporation of all these new genomes into the tree of life is reshaping our understanding of microbial diversity and evolution.

SUMMARY POINTS

1. The reconstruction of individual genomes from complex marine sediments has revealed vast unexplored diversity across the tree of life.
2. These new lineages have had a large impact on the topology of the tree of life and our understanding of early evolution.
3. Estimates of biodiversity based on traditional molecular approaches and cultivation overlooked several phyla.
4. New archaeal phyla, within what are called the Asgard archaea, share a common ancestor with eukaryotes and have advanced our understanding of the origins of cellular complexity.
5. Many of these newly described lineages are broadly distributed in the oceans and play crucial roles in carbon and nutrient cycling.
6. Description of the metabolisms of these novel taxa is advancing our understanding of their biogeochemical roles, including the coupling of carbon and nutrient cycling, in the oceans.
7. Even though microbes in sediments have expanded the tree of life, there are still many additional undescribed lineages left to be explored.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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