

## Microbial Domains in the Ocean: A Lesson from the Archaea

BY EDWARD F. DELONG

### NEW VIEWS OF SEA MICROBES

Microbial life thrives in virtually every habitat imaginable in the ocean, from the scalding temperatures found at hydrothermal vents, to frigid environments in and under polar sea ice, to high-pressure habitats in the ocean's deepest trenches. Our understanding of microbial life in many of these ocean habitats, especially in plankton, has advanced remarkably over the past 30 years or so. The recognition of the ubiquity and distribution of photoautotrophic cyanobacteria such as *Synechococcus* and *Prochlorococcus* (Johnson and Sieburth, 1979; Waterbury et al., 1979; Chisholm et al., 1988), the isolation of pressure-requiring piezophilic bacteria (Yayanos et al., 1979), the discovery of *Pelagibacter* (Giovannoni et al., 1990), and recognition of the high abundance of marine phage (Bergh et al., 1989) represent just a few recent milestones in microbial oceanography. Even at a level as fundamental as the distribution of life's three major domains

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From the standpoint of oceanography, why should we care about microbial

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diversity or microbial taxonomic distributions and abundance? As a fledgling Assistant Scientist at the Woods Hole Oceanographic Institution in the late 1980s, I remember my chagrin while pondering critical reviewer comments on my (failed) grant proposals that aimed to survey marine microbial diversity, which read something like this: “DeLong is out on a fishing expedition, without any hypothesis.” At the time, some of us wondered: If you don't really know what lives in the ocean, might not a little fishing be a good idea? As it turned out, the early oceanic microbial

“fishing expeditions” pioneered by Norm Pace and Stephen Giovannoni, and later others, in the early 1990s, proved to be reasonably productive enterprises. A strong motivation for these microbial

surveys was the general recognition that microbial activities drive most of the major biogeochemical cycles in the sea. Furthermore, it was suspected that many dominant planktonic microbial groups might be undetected because of their recalcitrance to cultivation. Given the “great plate count anomaly” (e.g., the observation that cultivable planktonic microbes accounted for only a small percentage of total direct epifluorescence microscopic counts [Staley and Konopka, 1985]), it seemed quite possible that dominant planktonic microbial groups, some responsible for critical

biogeochemical cycling processes, likely remained unknown. This presumption turned out to be more or less correct.

A fundamental advance that accelerated relatively unbiased microbial census taking was the invention by Norm Pace and collaborators of cultivation-independent, molecular-phylogenetic survey approaches (Olsen et al., 1986). This strategy uses common molecular sequences found in every cell (e.g., ribosomal RNA sequence) that can serve as a sort barcode to identify and track microbes by “reading” DNA sequences extracted directly from the environment, without the need for cultivation. Such cultivation-independent surveys taught us that large amounts of microbial diversity found in natural habitats had totally slipped beneath the radar of cultivation-based approaches. Indeed, some of the most abundant microbial groups on our planet have been discovered using such molecular-based surveys, and had not been evident from culture-based studies. Characterizing these microorganisms is critical for gaining a deeper and truer understanding of native microbial inhabitants and their fundamental environmental activities (see below). Together, both cultivation-based and cultivation-independent approaches, which now extend to environmental genomic sequencing surveys, are yielding significant contributions to our understanding of microbial taxa and activities in the deep blue sea.

The impact of cultivation-independent surveys of microbes in the environment has been well reviewed (Rappé and Giovannoni, 2003). The following story is one tale of how this approach altered our understanding

of marine microbial life and led to the discovery of a new microbial taxonomic group in the sea, the planktonic Crenarchaea. The pattern of initial discovery using these techniques, and subsequent in-depth biological and ecological characterization, is now a recur-

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ring theme in marine microbial ecology. While this story represents only one example, it illustrates how characterization of dominant microbial inhabitants in the sea can lead to new insights into global biogeochemical processes. As well, the important interplay and synergy between cultivation-dependent and cultivation-independent approaches for characterizing marine microbes in the wild is quite evident (see also the article by Giovannoni et al., this issue).

#### OCEANIC ARCHAEA?

The Archaea are a curious phylogenetic domain (formerly kingdom) comprised of an odd assortment of cultured microbes that fall into three major groupings: extreme halophiles, methanogens, and extreme thermophiles and thermoacidophiles (Woese, 1987). Why such an odd assortment of salt-loving, or anaerobic, or heat-loving microbes should form such a coherent phylogenetic grouping is still not that well understood. The dogma until 1992 was that archaea inhabit mainly “extreme” environments, inhospitable

to most other life forms. The existence of novel archaeal types was first hinted at during cultivation-independent ribosomal RNA surveys in open-ocean and coastal marine waters. Initial work used the polymerase chain reaction (PCR) to amplify ribosomal RNA genes

from mixed microbial populations. In 1992, Jed Fuhrman of the University of Southern California first reported the existence of a new type of archaeal ribosomal RNA sequence from deep-water planktonic microbes in the Pacific Ocean (Fuhrman et al., 1992). At about the same time, I independently discovered and reported on the distribution and abundance of two different coastal archaeal groups, one related to the deep-water archaea (planktonic Crenarchaea) and another, new group (planktonic Euryarchaea) that were phylogenetic neighbors to halophiles and methanogens (DeLong, 1992). I was also able to demonstrate quantitatively that marine archaea contribute significantly to marine microbial plankton biomass.

The surprise then was that *any* archaea could be found in cold, aerobic habitats of coastal and open-ocean waters—and, to top it off, they were abundant. No cultivated, characterized archaea were known to grow at the combined salinity, temperature, and oxygen concentration found in temperate oceanic waters, shallow or deep.

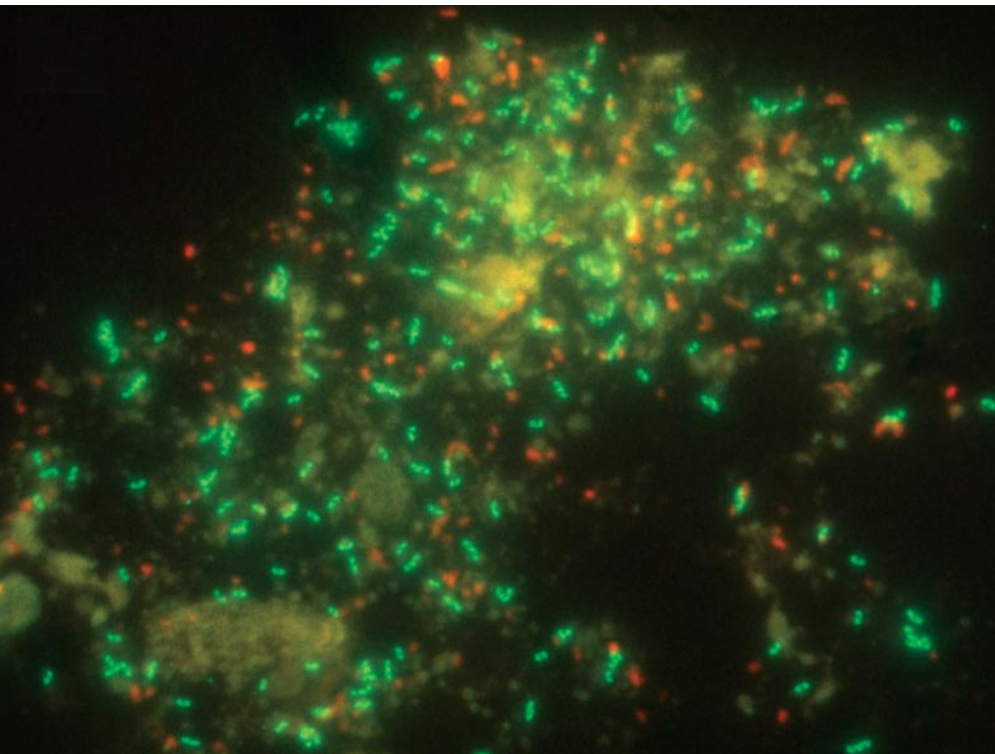


Figure 1. Epifluorescence micrograph of *Cenarchaeum symbiosum*, a symbiotic marine crenarchaeon (green cells) closely related to planktonic archaea. The green fluorescence is derived from fluorescein-labeled, rRNA-targeted probes used for in situ nucleic acid hybridization to identify archaeal cells.

Following on the heels of these first oceanic sightings, archaeal groups began cropping up in many unexpected habitats. The next steps were to quantify the distribution and abundance of these unusual microbes and to begin to understand their biological properties and ecological significance.

#### MARINE ARCHAEOAL ABUNDANCE, DISTRIBUTION, AND VARIABILITY

Once it was clear that archaea were reasonably abundant players in microbial plankton, scientists initiated studies that employed radiolabeled, archaea-specific oligonucleotide probes to quantify total, extractable archaeal rRNA in marine plankton (Figure 1). Surprisingly,

planktonic Crenarchaea were found to contribute as much as 20% to the total microbial rRNA in late-winter Antarctic coastal waters at  $-1.8^{\circ}\text{C}$  (DeLong et al., 1994). Surveys in temperate waters off the coast of California also showed that the planktonic Crenarchaea tended to be most abundant in waters below the euphotic zone (Massana et al., 1997). This trend has generally held in a global sense in both coastal and open-ocean settings.

Ribosomal RNA-targeted fluorescence probes (DeLong et al., 1989) have been used to provide estimates of archaeal cell numbers in the water column. Off the California coast, for example, planktonic Crenarchaea represent  $> 20\%$

of the total picoplankton cell counts from water depths of 80–3000 m. At the Hawaii Ocean Time-series (HOT) station ALOHA, Dave Karl and collaborators showed that Crenarchaea comprised as much as 30% of the total microbial counts in deep waters below the euphotic zone (Karner et al., 2001). In aggregate, these and other data suggest that pelagic Crenarchaea comprise a significant proportion of overall planktonic microbial biomass throughout the world's ocean.

#### BIOLOGY AND ECOLOGY OF PLANKTONIC MARINE CRENARCHAEA

Creative application of biochemical, geochemical, and genomic techniques have provided considerable data on the planktonic Crenarchaea. These studies, combined with isolation in pure culture of a marine crenarchaeon (see below), now provide some specific clues regarding the biogeochemical and ecological importance of this abundant marine microbial group.

Lipid analyses of cold marine sediments by Jaap Damste's group at the Netherlands Institute for Sea Research (NIOZ) revealed a first for this environment—high levels of tetraether lipids, that were previously found only in thermophilic Crenarchaea (Hoefs et al., 1997). Stuart Wakeham at the Skidaway Institute of Marine Science and my group at the University of California, Santa Barbara, then showed that marine plankton samples with high numbers of planktonic Crenarchaea also contained high levels of the same tetraether lipids (DeLong et al., 1998). In addition, Preston et al. (1996) showed that *Cenarchaeum symbiosum*, a crenarchaeal

symbiont of marine sponges, contained the very same tetraether lipids. These could be used to infer the lipid's detailed chemical structure. Collectively, these data establish that the sources of abundant marine tetraether lipids in the plankton are indeed derived from planktonic Crenarchaea (Figure 2).

A real surprise came when Ann Pearson, during her graduate work with Tim Eglinton at Woods Hole Oceanographic Institution, provided radioisotopic data suggesting pelagic Crenarchaea may be chemoautotrophic (Pearson et al., 2001). She purified large amounts of marine archaeal lipids from deep-sea surficial sediments derived from the deep-water planktonic Crenarchaea. Natural  $^{14}\text{C}$  isotope analyses of archaeal lipids suggested that deep-water archaea were not consuming much organic matter derived from surface primary productivity. Rather, deep-water Crenarchaea appeared to be using dissolved inorganic carbon as their main carbon source. Pearson's analyses are now supported by a variety of independent studies. For example, when Cornelia Wuchter, a graduate student at NIOZ, added  $^{13}\text{C}$ -labeled bicarbonate to a sample from the North Sea, then incubated the seawater in the dark, the heavy-isotope label from  $\text{CO}_2$  was nearly exclusively incorporated into crenarchaeal lipids (Wuchter et al., 2003).

The observation of  $\text{CO}_2$ -fixing Crenarchaea of course immediately led to the question: exactly what are these ubiquitous  $\text{CO}_2$ -fixing marine Crenarchaea using as their energy source? Early clues came from the discovery of an ammonia monooxygenase gene in Crenarchaea that encodes a key

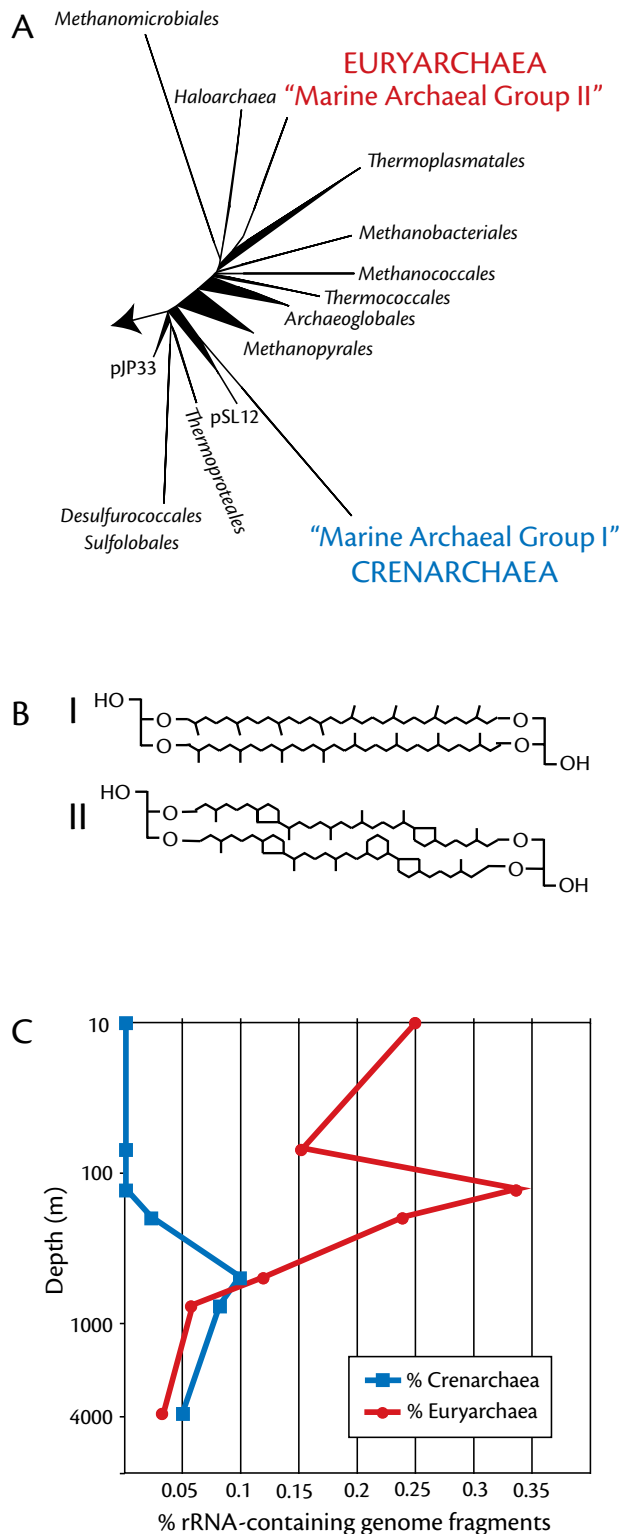


Figure 2. Phylogeny, lipids, and vertical distribution of planktonic marine Crenarchaea. (A) A phylogenetic tree showing the general affiliations of two different planktonic archaeal groups. (B) General structures of abundant tetraether lipids found in Crenarchaea. (C) Distribution of planktonic Crenarchaea and Euryarchaea in the North Pacific Subtropical Gyre, as detected in large DNA-insert genomic libraries (data from DeLong et al., 2006).

enzyme used by nitrifiers in the first step of ammonia oxidation (Treusch et al., 2005). This new archaeal ammonia monooxygenase gene was subsequently detected in many marine environments as well (Wuchter et al., 2006; Mincer et al., 2007). Genomic analyses subsequently revealed many other genes associated with nitrification and CO<sub>2</sub> fixation in marine Crenarchaea (Hallam et al., 2006a, 2006b). A definitive demonstration that marine Crenarchaea are indeed nitrifiers was achieved by Dave Stahl's group at the University of Washington, in collaboration with John Waterbury at the Woods Hole Oceanographic Institution (Konneke et al., 2005). In cultures designed to isolate nitrifying bacteria (bacteria that oxidize ammonia obtain energy and utilize CO<sub>2</sub> as their carbon source), Stahl's group unexpectedly found ammonia-oxidizing cultures that did not appear to be typical bacterial nitrifiers—in fact, these nitrifying microbes did not even belong to the

isolates grew exclusively on ammonia for energy, producing nitrite as the end product. The archaeal nitrifiers were also shown to use CO<sub>2</sub> as their carbon source, and they did not appear capable of growing on organic matter. These data, combined with earlier information on crenarchaeal distributions and abundance, indicate that planktonic Crenarchaea are indeed critical players in the ocean's nitrogen cycle, and they exert a large influence on oceanic nitrification in the sea (Francis et al., 2007).

New observations on the biology, ecology, and activity of planktonic Crenarchaea continue to accumulate. These include detailed quantitative analyses modeling in situ carbon sources of planktonic Crenarchaea (Ingalls et al., 2006), microautoradiography studies to track substrate assimilation into different cell types (Herndl et al., 2005), and observations of Crenarchaea in anoxic zones of the Black Sea (Coolen et al., 2007) and the Arabian Sea (Damste et

in the water column (Mincer et al., 2007). Future studies promise to further elucidate the ecophysiological properties of the ubiquitous marine crenarchaeal nitrifiers and to better quantify and constrain nitrification and CO<sub>2</sub> fixation rates, as well as further characterize their ecological interactions.

## CONCLUSION

The discovery and ecological characterization of the planktonic marine archaea represent but one example in a positive and accelerating trend in marine microbial ecology. The general story shows the utility of cultivation-independent, DNA-based survey approaches for identifying and tracking microbes in the environment. The interplay between nucleic-acid-based approaches and geochemical and biomarker studies that leverage stable- and radio-isotopic tracers is also central to the tale. As well, the important synergy between cultivation-dependent and cultivation-independent microbial surveys is also clear. Other similar parables include the discovery, ecological characterization, and isolation of the ubiquitous bacterioplankton *Pelagibacter* (see Giovannoni article, this issue), the recognition of anaerobic ammonia-oxidizing bacteria in marine oxygen-minimum zones (see Ward et al., this issue), and the discovery of anaerobic, methane-oxidizing archaea at methane seeps (Hinrichs et al., 1999). It is clear that combining these and other newly evolving strategies for characterizing microbes in situ and in the lab, including nanoscale DNA sequencing, proteomics, and single-cell stable isotope analyses, will advance our knowledge of microbial life and activity in the global oceans. ☐

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domain Bacteria (Konneke et al., 2005). After some major microbe sleuthing, Stahl's group discovered that the new nitrifiers were, in fact, the same type of Crenarchaea that are so abundant in marine plankton. These crenarchaeal

al., 2002). The potential interactions between Crenarchaea and other bacterioplankton have also recently been suggested by the similar distributions of bacterial nitrite oxidizers belonging to the genus *Nitrospina* and to Crenarchaea

## REFERENCES

- Bergh, O., K.Y. Borsheim, G. Bratbak, and M. Haldal. 1989. High abundance of viruses found in aquatic environments. *Nature* 340:467–468.
- Chisholm, S.W., R.J. Olson, E.R. Zettler, R. Goericke, J.B. Waterbury, and N.A. Welschmeyer. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* 334:340–343.
- Coolen, M.J., B. Abbas, J. van Bleijswijk, E.C. Hopmans, M.M. Kuypers, S.G. Wakeham, and J.S. Sinninghe Damste. 2007. Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: A basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. *Environmental Microbiology* 9:1,001–1,016.
- Damste, J.S., W.I. Rijpstra, E.C. Hopmans, F.G. Prahl, S.G. Wakeham, and S. Schouten. 2002. Distribution of membrane lipids of planktonic Crenarchaeota in the Arabian Sea. *Applied and Environmental Microbiology* 68:2,997–3,002.
- DeLong, E.F. 1992. Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences of the United States of America* 89:5,685–5,689.
- DeLong, E.F., L.L. King, R. Massana, H. Cittone, A. Murray, C. Schleper, and S.G. Wakeham. 1998. Dibiphytanyl ether lipids in nonthermophilic crenarchaeotes. *Applied and Environmental Microbiology* 64:1,133–1,138.
- DeLong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N.U. Frigaard, A. Martinez, M.B. Sullivan, R. Edwards, B.R. Brito, and others. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311:496–503.
- DeLong, E.F., G.S. Wickham, and N.R. Pace. 1989. Phylogenetic stains: Ribosomal RNA-based probes for the identification of single cells. *Science* 243:1,360–1,363.
- DeLong, E.F., K.Y. Wu, B.B. Prezelin, and R.V. Jovine. 1994. High abundance of Archaea in Antarctic marine picoplankton. *Nature* 371:695–697.
- Francis, C.A., J.M. Beman, and M.M.M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. *ISME Journal* 1:19–27.
- Fuhrman, J.A., K. McCallum, and A.A. Davis. 1992. Novel major archaeobacterial group from marine plankton. *Nature* 356:148–149.
- Giovannoni, S.J., T.B. Britschgi, C.L. Moyer, and F.G. Field. 1990. Genetic diversity in Sargasso Sea bacterioplankton. *Nature* 345:60–63.
- Hallam, S.J., K.T. Konstantinidis, N. Putnam, C. Schleper, Y. Watanabe, J. Sugahara, C. Preston, J. de la Torre, P.M. Richardson, and E.F. DeLong. 2006a. Genomic analysis of the uncultivated marine crenarchaeote *Cenarchaeum symbiosum*. *Proceedings of the National Academy of Sciences of the United States of America* 103:18,296–18,301.
- Hallam, S.J., T.J. Mincer, C. Schleper, C.M. Preston, K. Roberts, P.M. Richardson, and E.F. DeLong. 2006b. Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *PLoS Biology* 4:e95.
- Herndl, G.J., T. Reinthaler, E. Teira, H. van Aken, C. Veth, A. Pernthaler, and J. Pernthaler. 2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Applied and Environmental Microbiology* 71:2,303–2,309.
- Hinrichs, K.U., J.M. Hayes, S.P. Sylva, P.G. Brewer, and E.F. DeLong. 1999. Methane-consuming archaeobacteria in marine sediments. *Nature* 398:802–805.
- Hoefs, M., S. Schouten, J.W. De Leeuw, L.L. King, S.G. Wakeham, and J. Damste. 1997. Ether lipids of planktonic archaea in the marine water column. *Applied and Environmental Microbiology* 63:3,090–3,095.
- Ingalls, A.E., S.R. Shah, R.L. Hansman, L.I. Aluwihare, G.M. Santos, E.R. Druffel, and A. Pearson. 2006. Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proceedings of the National Academy of Sciences of the United States of America* 103:6,442–6,447.
- Johnson, P.W., and J.M. Sieburth. 1979. Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. *Limnology and Oceanography* 24:928–935.
- Karner, M.B., E.F. DeLong, and D.M. Karl. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* 409: 507–510.
- Konneke, M., A.E. Bernhard, J.R. de la Torre, C.B. Walker, J.B. Waterbury, and D.A. Stahl. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437: 543–546.
- Massana, R., A.E. Murray, C.M. Preston, and E.F. DeLong. 1997. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Applied and Environmental Microbiology* 63:50–56.
- Mincer, T.J., M.J. Church, L.T. Taylor, C. Preston, D.M. Karl, and E.F. DeLong. 2007. Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. *Environmental Microbiology* 9:1,162–1,175.
- Olsen, G.J., D.J. Lane, S.J. Giovannoni, N.R. Pace, and D.A. Stahl. 1986. Microbial ecology and evolution: A ribosomal RNA approach. *Annual Review of Microbiology* 40:337–365.
- Pearson, A., A.P. McNichol, B.C. Benitez-Nelson, J.M. Hayes, and T.I. Eglinton. 2001. Origins of lipid biomarkers in Santa Monica Basin surface sediment: A case study using compound-specific  $\Delta^{14}\text{C}$  analysis. *Geochimica et Cosmochimica Acta* 65:3,123–3,137.
- Preston, C.M., K.Y. Wu, T.F. Molinski, and E.F. DeLong. 1996. A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. *Proceedings of the National Academy of Sciences of the United States of America* 93:6,241–6,246.
- Rappé, M.S., and S.J. Giovannoni. 2003. The uncultured microbial majority. *Annual Review of Microbiology* 57:369–394.
- Staley, J.T., and A. Konopka. 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology* 39:321–346.
- Treusch, A.H., S. Leininger, A. Kletzin, S.C. Schuster, H.P. Klenk, and C. Schleper. 2005. Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environmental Microbiology* 7:1,985–1,995.
- Waterbury, J.B., S.W. Watson, and R.R.L. Guillard. 1979. Widespread occurrence of a unicellular marine, planktonic, cyanobacterium. *Nature* 277:293–294.
- Woese, C.R. 1987. Bacterial evolution. *Microbiological Reviews* 51:221–271.
- Wuchter, C., S. Schouten, H.T. Boschker, and J.S. Sinninghe Damste. 2003. Bicarbonate uptake by marine Crenarchaeota. *FEMS Microbiology Letters* 219:203–207.
- Wuchter, C., B. Abbas, M.J. Coolen, L. Herfort, J. van Bleijswijk, P. Timmers, M. Strous, E. Teira, G.J. Herndl, J.J. Middleburg, and others. 2006. Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 103:12,317–12,322.
- Yayanos, A.A., A.S. Dietz, and R. van Boxtel. 1979. Isolation of a Deep-Sea Barophilic Bacterium and Some of Its Growth Characteristics. *Science* 205:808–810.