

Oceans of Archaea

Abundant oceanic *Crenarchaeota* appear to derive from thermophilic ancestors that invaded low-temperature marine environments

Edward F. DeLong

Earth's microbiota is remarkably pervasive, thriving at extremely high temperature, low and high pH, high salinity, and low water availability. One lineage of microbial life in particular, the *Archaea*, is especially adept at exploiting environmental extremes. Despite their success in these challenging habitats, the *Archaea* may now also be viewed as a cosmopolitan lot. These microbes exist in a wide variety of terrestrial, freshwater, and marine habitats, sometimes in very high abundance. The oceanic Marine Group I *Crenarchaeota*, for example, rival total bacterial biomass in waters below 100 m. These widespread *Archaea* appear to derive from thermophilic ancestors that invaded diverse low-temperature environments. Novel cultivation strategies, in situ biochemical and geochemical methods, and environmental genomics promise to further our understanding of these and other ubiquitous and abundant *Archaea*.

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Brief Overview of Archaea

In the late 1970s, Carl Woese at the University of Illinois, Urbana-Champaign, and his collaborators, through their ribosomal RNA analyses, first recognized *Archaea* as one of three major monophyletic lineages on Earth. Their analyses of several microbial groups, including extreme halophiles and methanogens, revealed a unique prokaryotic lineage that diverges from most familiar bacterial groups at the deepest phylogenetic levels. In recognizing the *Archaea* as a separate evolutionary unit, Woese reclassified organisms into three domains: *Eucarya* (all eu-

karyotes), *Archaea*, and *Bacteria*. Although alternative taxonomic schemes have been recently proposed, whole-genome and other analyses tend to support Woese's three-domain concept.

Well-known and cultivated archaea generally fall into several major phenotypic groupings: these include extreme halophiles, methanogens, and extreme thermophiles and thermoacidophiles. Early on, extremely halophilic archaea (haloarchaea) were first noticed as bright-red colonies growing on salted fish or hides. For many years, halophilic isolates from salterns, salt deposits, and landlocked seas provided excellent model systems for studying adaptations to high salinity. It was only much later, however, that it was realized that these salt-loving "bacteria" are actually members of the domain *Archaea*.

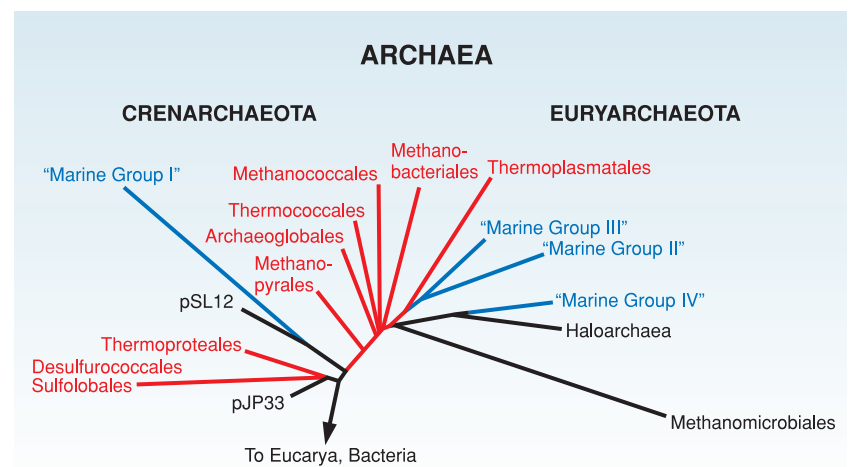
Another major archaeal group, the strictly anaerobic methanogenic archaea, produce most of the methane in our atmosphere. Methanogens are one of the most cosmopolitan of cultivated archaeal groups, occupying diverse habitats that include anoxic sediments and rice paddies, the rumen of cattle, the guts of termites, hydrothermal vents, and deep subterranean habitats. A third phenotypic group of cultured *Archaea* are the heat- and acid-loving thermophiles and thermoacidophiles. These unusual *Archaea* dwell at hydrothermal vents, hot springs, deep subterranean environments, oil reservoirs, and even on burning coal refuse piles.

Cultivated members within one kingdom of the domain *Archaea*, the *Crenarchaeota* (Fig. 1), consist entirely of thermophilic species. Remarkably, a recent report by Derek Lovely at the University of Massachusetts, Amherst, suggests

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FIGURE 1



Schematic tree showing the relationships of the four major groups of planktonic marine *Archaea* relative to cultivated groups.

that some *Crenarchaeota* can grow at temperatures exceeding 120°C (see p. 484). In addition to high-temperature growth, some archaeal thermophiles like *Picrophilus oshimae* also thrive at extremely low pH, growing best at hydrogen ion concentrations approaching 1 M.

Archaeal Ubiquity?

Despite expanding information on cultivated *Archaea*, only a small fraction of naturally occurring archaeal diversity is currently represented in culture collections. Cultivation-independent survey approaches pioneered by Norman Pace of the University of Colorado, Boulder, have greatly expanded our current appreciation of natural microbial diversity. Such surveys indicate that a significant amount of microbial diversity found in natural habitats has so far eluded cultivation in the laboratory. Characterizing these elusive and understudied but ubiquitous microorganisms remains a critical goal for gaining a deeper and truer appreciation of microbial evolution, physiology, and ecology.

Although *Archaea* were thought to preferentially occupy environments that are inhospitable to *Eucarya* and *Bacteria*, their ecological and physiological diversity turns out to be far greater than previously supposed. Some early hints came from PCR-based, ribosomal RNA-gene

surveys of mixed microbial populations from open ocean and coastal marine waters.

From such studies, Jed Fuhrman of University of Southern California in Los Angeles presented evidence in 1992 for one new type of archaeal ribosomal RNA sequence, recovered from planktonic microbes at depths of 500 m in the Pacific Ocean. At about the same time, I reported the discovery of two archaeal groups in coastal seawater, one identical to that found by Fuhrman (the Group I planktonic *Crenarchaeota*, Fig. 1), and an additional group (Group II planktonic *Euryarchaeota*, Fig. 1) distantly related to halophiles and methanogens (Fig. 1). We found that these *Archaea* contribute significantly to the total extractable rRNA in marine surface waters.

The surprise at that time was that any *Archaea* could be found in cold, aerobic habitats of coastal and open ocean waters. No cultivated, characterized *Archaea* were then known to grow at the salinities, temperatures, and oxygen concentrations found in temperate oceanic waters, shallow or deep. Soon, researchers were reporting finding rRNAs belonging to divergent archaeal groups in all sorts of habitats, including soils, freshwater and marine sediments, and anaerobic digestors. Many of the new, nonmarine archaeal types in soils and freshwater sediments were related to the marine Group I *Crenarchaeota*. Recently, a survey of the Great Lakes by Randall Hicks and colleagues at the University of Minnesota, Duluth, has also revealed the prevalence of similar types of *Crenarchaeota*. It has been surprising to realize that *Archaea*, initially thought to be relegated to extreme environments, actually thrive all over our planet, including the vast world oceans, the Great Lakes, and the very soil beneath our feet.

Among the four major groups of planktonic marine *Archaea*, the most abundant appear to be the first groups recognized, Group I *Crenarchaeota* and Group II *Euryarchaeota* (Fig. 1). Two other planktonic archaeal groups have also been detected, but appear to be lower in abundance, according to recent surveys. The Group III planktonic *Archaea*, found in waters below the photic zone, are peripherally related to the

Breaking the Ice, Finding New Habitats for *Archaea*

Marine biologist Edward DeLong received an unforgettable lesson about eight years ago during a field trip to Antarctica, when the ship he was on became lodged in ice. What ordinarily should have been a four-day journey from South America to Antarctica stretched into two weeks with no rescue in sight. "When you're down in Antarctica, nature calls the shots and you learn to be adaptable," says DeLong, a senior scientist with the Monterey Bay Aquarium Research Institute in Moss Landing, Calif.

DeLong and his colleagues were en route to the Antarctica Peninsula in August 1995, the austral winter, to study *Archaea*, then newly discovered to inhabit cold-ocean waters. They were traveling by icebreaker, but that didn't prevent the ship from getting trapped in the ice. Ice formed

around the boat, and nothing budged. Fortunately, the situation wasn't dire, but it was unlikely that another icebreaker would be available to dislodge them. So they waited, wondering whether they would have to remain there until the summer thaw.

When a shift in the winds led the ice to break up, they were free but also low on fuel. They had to turn back, refuel, and start again. "It wasn't scary, just an interesting experience in sociology, to see how you and your colleagues adapt to being stuck on a boat for two weeks with nothing to do," DeLong says. "Actually, it was fun."

DeLong has been having fun for his entire career. It began during his childhood with a love of the sea. DeLong, now 45, grew up in Sonoma, Calif., where he learned to skin dive for aba-

lone, scuba dive, and paddle a kayak. "I fell in love with the ocean as a kid. I really do love the sea," he says.

"I always tell people that it's amazing I get paid to do what I do."

After finishing high school, DeLong moved to Alaska for a year "to find myself," then returned to California to start college. He studied biology at Santa Rosa Junior College, Calif., in 1980, before obtaining a B.S. degree in bacteriology from the University of California, Davis (UCD) in 1982, and a Ph.D. in marine biology from the Scripps Institute of Oceanography, San Diego, Calif., in 1986. In 1989, he received an



Continued

order *Thermoplasmatales*. Members of the pelagic Group IV *Archaea*, discovered by Francisco Rodriguez-Valera and collaborators of the Universidad Miguel Hernandez in Spain, appear to be related to haloarchaea, and apparently inhabit deep ocean waters. Currently, hundreds of ribosomal RNA sequences derived from marine planktonic *Archaea* exist in the public databases. These data indicate that substantial genetic variability (e.g., microheterogeneity) exists among very closely related sequence types, reflecting unexplored evolutionary, genetic, and ecological complexities.

Planktonic Archaeal Abundance, Distribution, and Variability

Archaeal Groups I and II appear to be the most abundant in the ocean and so far have been the easiest to study in situ. Early reports using radiolabeled oligonucleotide probes to quantify

archaeal rRNA in marine plankton yielded some unexpected surprises. Perhaps most startling was the high relative abundance of *Archaea* in winter surface waters off Antarctica, where planktonic *Crenarchaeota* sometimes comprise as much as 20% of total microbial rRNA. Those coastal waters are -1.8°C , a far cry from the 80°C or higher growth temperature optima of cultivated *Crenarchaeota*. Similar surveys in temperate waters off the coast of California show that the planktonic *Crenarchaeota* tend to be most abundant in depths below about 100 m or so.

Modified fluorescence in situ hybridization assays have led to better estimates of archaeal cell concentrations throughout the oceanic water column. Off the California coast, we showed that planktonic *Crenarchaeota* represent more than 20% of the total picoplankton cells in water from 80 m to 3,000 m. Our later studies with David M. Karl and colleagues of the University



independent study award from Woods Hole Oceanographic Institute, Woods Hole, Mass., where he met his wife, Kathleen Ledyard, a biogeochemist. They have a six-year-old son.

Earlier, DeLong thought he would end up becoming a medical technologist, specializing in microbiology. But at UCD, he met Paul Baumann, a professor and microbiologist who is an expert in marine bacterial taxonomy and biochemistry. "Paul got me into this whole research world of marine microbiology, and I gradually figured out you could make a career doing this sort of thing," DeLong says.

After finishing his thesis work with Art Yayanos at Scripps, DeLong did his postdoctoral work with Norman Pace, then at Indiana University, Bloomington, who introduced him to the techniques of using DNA sequences to identify microbes. These ap-

proaches allow scientists to survey entire microbial communities without the requirement of cultivation in the lab.

"There was a real big impact when these techniques became available to study naturally occurring microbial communities en masse, rather than studying microbial cultures one by one," DeLong says. "Science jumps forward a lot of times in lockstep with new technologies, and this opens up new world views for us."

In the late 1970s, microbiologist Carl Woese had begun to compare microorganisms by using purely genetic information—and learned that *Archaea* are not ordinary bacteria, as originally thought. Later, scientists learned that these and related microorganisms do not live only in extreme environments, such as boiling water, acid-laden streams, or super-salty brine. "We didn't even know that they lived in cold-

ocean water, but it turns out they are major ocean inhabitants," he says. "If you would have asked any microbiologist 10 years ago whether *Archaea* were abundant in the seawater, they would have justifiably said 'no—it's not an extreme environment.' In actual fact, oceanic *Archaea* represent one of the major groups of *Archaea* on Earth. What we are up to now is trying to learn more about their biology and ecology," he adds.

The goal of that nearly ill-fated, ice-breaking field trip in 1995 was to gather samples of cold-sea water, hoping to find *Archaea*. When they arrived on the continent, they crossed the sea ice on skis, drilling holes to extract seawater. "We found, as predicted, that the frigid Antarctic waters were chock-full of *Archaea*," he says.

Marlene Cimons

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of Hawaii, Manoa, show a similar trend in the North Pacific Ocean Gyre, with pelagic *Crenarchaeota* comprising more than 30% of the total microbial cells from 200 m down to 5,000 m (Fig. 2).

Estimates from these cell counts indicate that planktonic *Crenarchaeota* account for a large fraction of the total microbial biomass throughout the world's oceans—consisting of about 1×10^{28} cells, or about 20% or so of all the bacterial and archaeal cells found in the sea. Recent estimates based on the abundance of archaeal lipids in seawater are consistent with these cell counts. In some locations, archaeal abundance appears to vary with the seasons. For instance, off the Antarctic Peninsula, the pelagic *Crenarchaeota* (Group I) appear more abundant in surface winter waters and subsurface summer waters. In temperate seas, Marine Group II *Euryarchaeota*, unlike Marine Group I *Crenarchaeota*,

seem to be more abundant at the surface. This group also shows evidence of seasonal fluctuations, reaching highest abundance in summer in the North Sea. Marine Group II *Archaea* spring "blooms" have been observed off the coast of California that are restricted to the photic zone.

Another Surprise—an Archaeal Symbiont of Sponges, *Cenarchaeum symbiosum*

While a graduate student at the University of California, Santa Barbara, Christina Preston made an unexpected discovery. She found that a single species of cold-loving crenarchaeote lives within the tissues of the coastal marine sponge *Axinella mexicana* (Fig. 3). Dubbed *Cenarchaeum symbiosum*, this symbiotic crenarchaeote can thrive at low temperatures—in this case, about 10°C. This sponge-archaeal association is specific, with *C. symbiosum* detectable only within *Axinella mexicana*, and not in surround-

ing seawater or other sponges. This symbiotic archaeon grows well at temperatures of 10°C, more than 60°C below the growth temperature optimum of its closest cultivated relatives in the *Crenarchaeota* (Fig. 1). Since *C. symbiosum* can be successfully maintained in laboratory aquaria within the tissues of its host, it provides a useful source of cold-dwelling crenarchaeotal biomass. Because *C. symbiosum* is the only archaeon present, archaeal cell preparations from the sponge are useful for biochemical studies—for example, to define the lipid composition of cold-dwelling *Crenarchaeota*. This archaeon should also be useful to help further define the physiological, biochemical, and genomic characteristics of cold-dwelling *Crenarchaeota*. Similar species from other habitats, including Mediterranean sponges of the genus *Axinella*, now also have been reported to specifically harbor archaea closely related to *C. symbiosum*.

Physiological Ecology of Oceanic Archaea

Relatively little is known about the physiology of oceanic *Archaea* species, due in part to the unavailability of pure cultures. Creative applications of biochemical, geochemical, and genomic techniques, however, are now providing valuable insights into planktonic *Crenarchaeota*, including information about specific enzymes, membrane lipid composition, and potential carbon sources.

Cold marine sediments contain high levels of tetraether lipids (Fig. 4), derived from the membrane lipids of planktonic *Archaea*, according to Jaap S. Sinninghe-Damsté of the Royal Netherlands Institute for Sea Research (NIOZ), Texel, the Netherlands. Freshly collected marine plankton samples containing abundant planktonic *Crenarchaeota* also contain high levels of similar tetraether lipid sediments. Further, *Cenarchaeum symbiosum*, a member of the Marine Group I archaeal

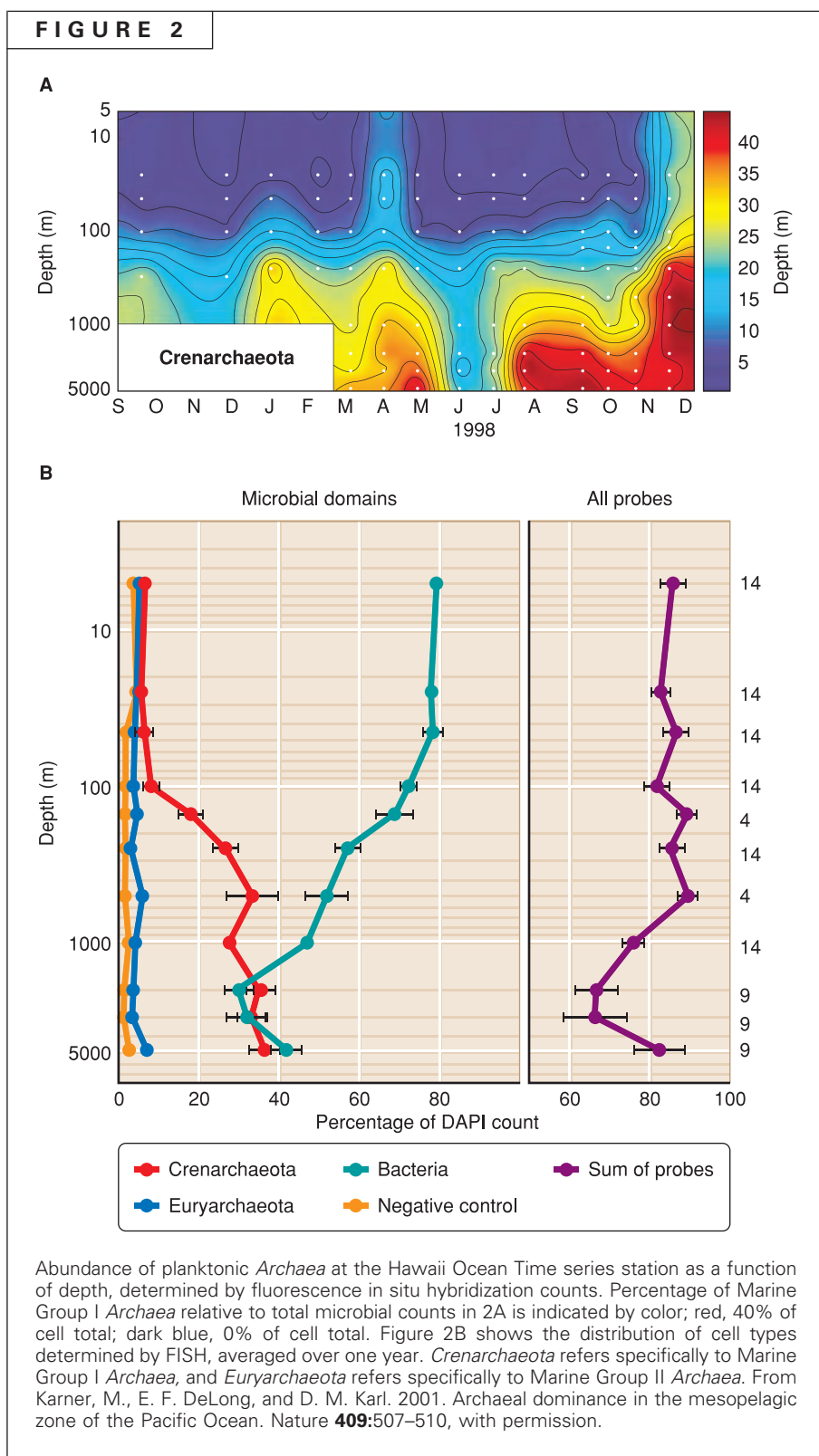
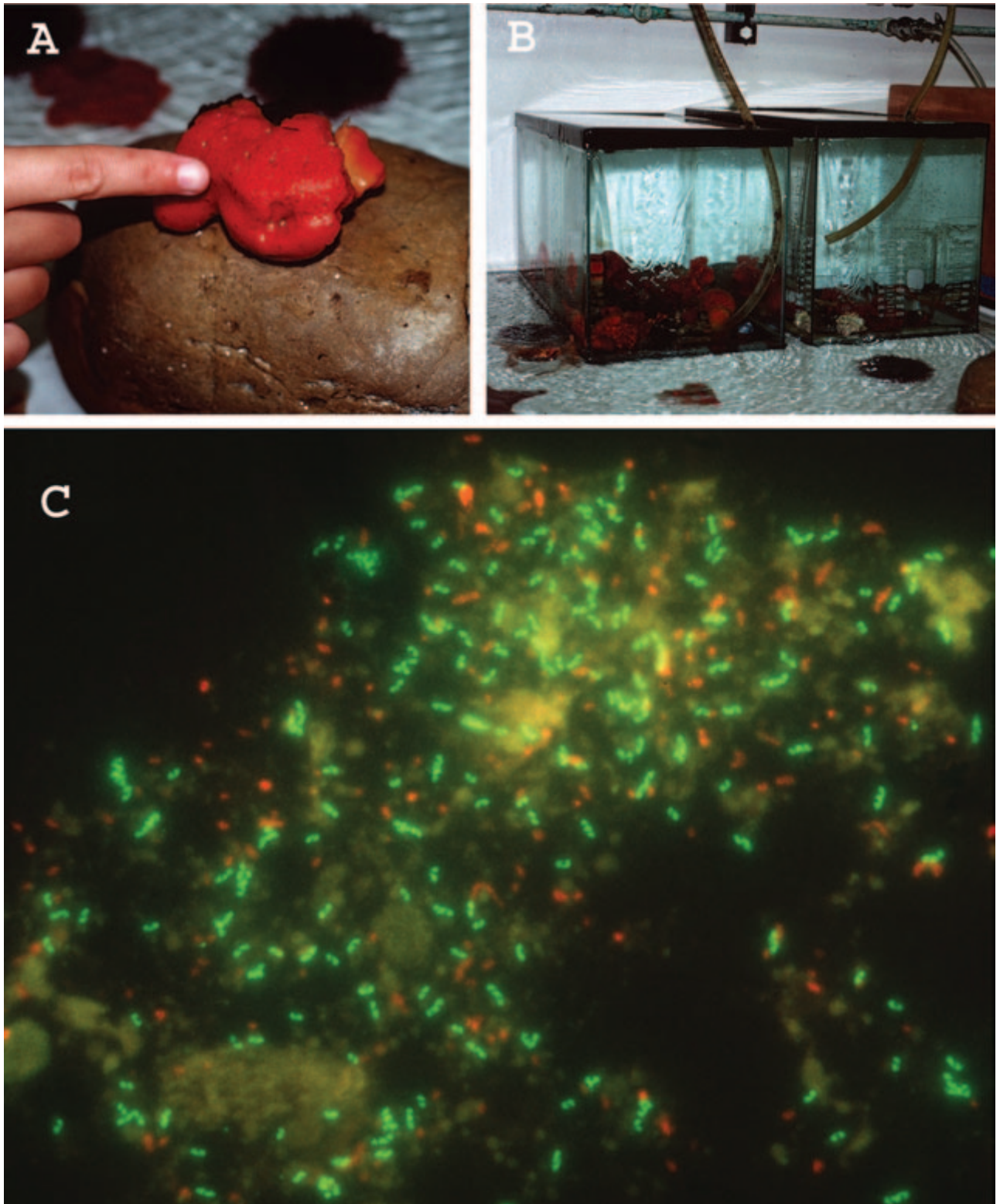




FIGURE 3



The marine sponge *Axinella mexicana* and its archaeal symbiont, *Cenarchaeum symbiosum*. 3A., *Axinella mexicana* a bright red demosponge found off the California coast. 3B., *A. mexicana* maintained in laboratory aquaria. 3C., FISH experiment showing *C. symbiosum* population present in the sponge tissues (in green). Many of the *C. symbiosum* cells are visibly dividing.

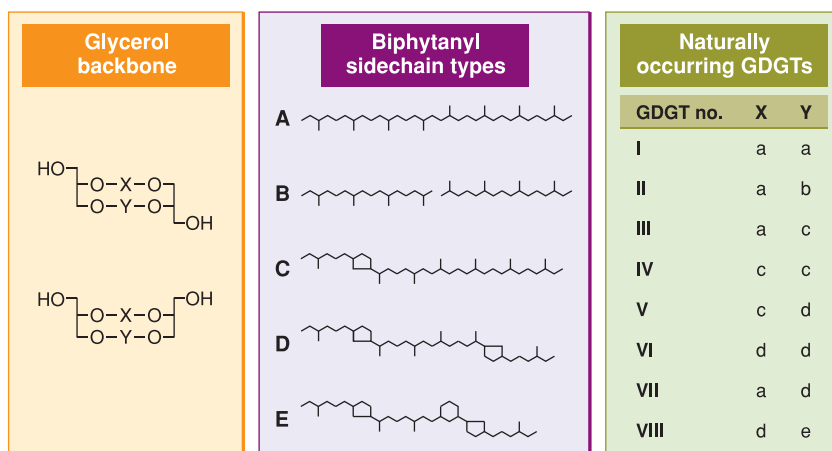
clade, contains the exact same tetraether lipids as are found in plankton. Taken together, these data establish that Marine Group I *Archaea* are a main source of the tetraether lipids, although additional archaeal sources (e.g., Group II) for these lipids in plankton cannot be ruled out.

Stefan Schouten and colleagues of NIOZ found structural differences between the lipids of planktonic archaea and those of archaeal thermophiles such as *Sulfolobus solfataricus*. Glycerol dibiphytanyl glycerol tetraethers (GDGTs) are commonly found archaeal lipid structures in marine sediments and plankton, and they include a novel ether lipid, called crenarchaeol, containing a six-membered cyclohexyl ring (Fig. 4, structure e). Studies with *C. symbiosum* have confirmed that these novel lipids are definitely found in members of the Marine Group I *Crenarchaeota*. This uniquely characteristic cyclohexyl ring structure likely has adaptive significance for the low-temperature *Archaea*. The Netherlands group proposes that this change from the standard, five-membered ring (found in thermophilic archaeal lipids) adds a kink in the tetraether chains, rendering the membrane structures they form more fluid at lower temperatures.

If planktonic archaea are globally abundant, what niche do they occupy? The Netherlands group analyzed stable isotopes in marine sediment-derived archaeal lipids in attempting to determine the carbon sources that support archaeal growth. Their initial findings suggested that planktonic archaea use either algal carbohydrates, proteins, or dissolved bicarbonate and thus do not indicate whether these archaea are autotrophs or heterotrophs. Other studies using radiolabeled amino acids, microautoradiography, and fluorescent in situ hybridization (FISH) have provided some evidence indicating that planktonic archaea might incorporate exogenous amino acids.

In her thesis work Ann Pearson, working with Tim Eglinton and John Hayes at the Woods Hole Oceanographic Institution, Woods Hole, Mass., purified large amounts of marine archaeal lipids from the deep sea, presumably derived from planktonic *Crenarchaeota*. Using

FIGURE 4



Archaeal lipid structures found in marine plankton and sediments. GDGT, glycerol dibiphytanyl glycerol tetraether. The most common forms found in marine plankton, incorporating sidechains a, or d and e, are GDGTs I and VIII. Modified after S. Schouten, E. C. Hopmans, R. D. Pancost, and J. S. S. Damste'. 2001. Widespread occurrence of structurally diverse tetraether membrane lipids: Evidence for the ubiquitous presence of low-temperature relatives of hyperthermophiles. Proc. Natl. Acad. Sci. USA **97**:14421–14426, Copyright 2001, National Academy of Sciences, U.S.A.

naturally occurring ^{14}C in oceanic organic matter and CO_2 as a tracer, their results indicated that deep-water archaea are not using organic matter derived from recent photoautotrophic primary production. Instead, these deep-sea *Crenarchaeota* apparently are incorporating dissolved CO_2 as their main carbon source. Meanwhile, the Netherlands group has recently acquired additional data supporting this hypothesis, via $^{13}\text{CO}_2$ isotope tracer-labeling experiments. This finding, if true, is quite a surprise, implying that oceanic *Archaea* fix CO_2 and may represent an unexpected source of primary productivity in the sea. If correct, what are these *Archaea* using as an energy source to fuel cell growth and CO_2 fixation? Answering this question may provide quite a bit of new information about carbon and energy flux in the deep waters of the world's oceans.

Genomics and Evolutionary Origins of Oceanic *Archaea*

Modern genomic approaches are now proving extremely useful for studying microbes that have resisted cultivation. In the early 1990s, working with Jeff Stein, then at the Agouron Institute, La Jolla, Calif., we isolated large



genomic fragments from the planktonic Marine Group I *Crenarchaeota*. Thus began our explorations in environmental genomics. Later in my lab, Christa Schleper, now at the Technical University of Darmstadt in Germany, developed high-quality genomic libraries from the symbiotic archaeon *Cenarchaeum symbiosum*. Schleper next identified *C. symbiosum* DNA polymerase in those genomic libraries, and expressed, purified, and characterized it biochemically. Although the DNA polymerase amino acid sequence most closely resembles thermostable DNA polymerases from other hyperthermophilic *Crenarchaeota*, the enzyme itself is not thermostable. It is inactivated at about 40°C, consistent with the lower temperature origins and ecological niche of *C. symbiosum*. We later collaborated with a group at Diversa Corporation, San Diego, Calif., to sequence 100 kbp of the *C. symbiosum* genome and learn more about the genome of this archaeon.

More recently, support from the National Science Foundation has allowed us to embark on full-genome sequencing of *C. symbiosum*. With a third of the genome now in hand, new clues about the physiological and genetic architecture of marine *Crenarchaeota* are already beginning to emerge. These new genomic data promise to teach us much about the natural history, genetics, evolution, and physiology of the ubiquitous marine *Crenarchaeota*.

What extraordinary events led to the wide distribution of *Crenarchaeota* in habitats ranging from boiling hot springs of 100°C to frigid polar waters of -1.8°C? There are several clues to the natural history of this fascinating group that come from a variety of disciplines, including molecular evolution, biochemistry, and geochemistry. All of these perspectives tend to suggest that the ancestors of modern-day, cold-adapted marine *Archaea* once lived in anoxic,

high-temperature habitats. It also seems likely, based on phylogenetic analyses, that different lineages within the *Crenarchaeota* adapted to low-temperature habitats on multiple occasions. The journey from hot to cold environments seems to have been a common trek in the course of evolution within different archaeal groups.

There are a number of outstanding puzzles concerning the marine *Archaea* that remain to be solved. For instance, the marine Group I *Crenarchaeota* are eurybathyl, that is, they are distributed throughout the ocean depths from near-surface waters to the bottom of the abyss. Studies of fine-scale genetic and physiological differences in the group are likely to tell us quite a bit more about deep-sea adaptations. Determining the specific carbon and energy sources of Group I *Crenarchaeota* remains another outstanding problem concerning this ubiquitous microbial group. Resolving this mystery will help us better interpret the ecological roles and significance of *Archaea* in oceanic ecosystems.

So, where to next with the marine *Archaea*? Steve Giovannoni at Oregon State University, Corvallis, has recently developed novel cultivation strategies that have successfully recovered elusive and difficult-to-cultivate planktonic bacteria (see *ASM News*, August 2003, p. 370). Similar approaches may eventually lead to the isolation of pure cultures of planktonic *Archaea*. This would aid tremendously in our understanding of the physiology and ecology of these ocean-going microbes. In situ studies using archaeal-specific biomarkers and geochemical tracers also offer much promise for studying indigenous marine *Archaea* on their own turf. Finally, genomic studies will provide us with not only the parts list of marine *Archaea*, but also clues as to the physiological activities of this widespread, abundant, and fascinating component of microbial life on Earth.

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