Novel major archaeabacterial group from marine plankton

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Marine bacteria often dominate the plankton biomass and are responsible for much of the cycling of organic matter, but bacterial diversity is poorly understood because conventional identification methods (requiring culturing) miss about 99% of the organisms. Recent advances permit characterization of microbial communities by analysis of 16S ribosomal RNA gene sequences directly from biomass without the need to culture the organisms; such studies from surface ocean samples have found only eubacteria and not archaeabacteria (or Archaea), which are profoundly different. Here we report 16S rRNA sequences obtained from Pacific Ocean bacterioplankton samples collected from depths of 100 m and 500 m. Among these we found sequences only distantly related to those of any of the previously characterized by 16S rRNA sequences, with similarities to the nearest such relatives (extreme thermophiles) approximately the same as those between animals and plants. We suggest that these sequences are from a previously undescribed archaeabacterial group that may have diverged from the ancestors of characterized organisms very early in evolution.

Our samples were collected from the western side of the California Current, roughly 350 miles west of San Diego. The area was oligotrophic at the time of sampling, as indicated by the low surface chlorophyll concentrations and a chlorophyll maximum layer at about 100 m depth, near the base of the euphotic zone and substantially below the surface mixed layer (Fig. 1). The samples for genetic analysis came from this biologically active layer (100 m depth) as well as from 500 m, the deeper sample was substantially below the euphotic zone and possessed a significantly lower total bacterial abundance and temperature (Fig. 1). Comparison of 16S rRNA gene sequences from these samples to those from similarly characterized organisms reveals that five of the clones from the 500-m sample and two from a 100-m sample form a cluster within the archaeabacteria, yet this cluster is only distantly related (about 70% sequence identity with 16S rRNA) to any previously described archaeabacterial group (Fig. 2). The sequences most similar to these clones come from extreme thermophiles, such as Pyrodinium. Measurable hybridization (under stringent conditions) of a probe made from one of the clones (NH49-8) to nucleic acid from the archaeabacterium Sulfolobus acidocaldarius, but not that from the eubacterium Escherichia coli or eukaryotic calf thymus, was also consistent with the inferred archaeabacterial origin of these clones.

A majority (5 out of 7) of our clones from 500 m were members of this group, as were a small minority (2 clones out of 10) from only 1 of 3 different 100-m samples collected a few days apart (>30 clones from 100 m were examined); information on other clones will be reported elsewhere. Because the distribution of clones in final polymerase chain reaction (PCR) products may not match the distribution of all 16S rRNA genes in the original unamplified DNA (owing to differences in amplification efficiency), we cannot yet say how prevalent these organisms are in sea water. This may best be determined by hybridization of specific oligonucleotide probes to RNA isolated from the natural communities (not possible with these samples owing to the limited material remaining). But community DNA from the 500-m sample hybridized very strongly to DNA from 1,000 m (ref. 13), suggesting that the dominant organisms from 500 m may dominate a large depth range. It is therefore possible that this new group may be extremely abundant, and may be a significant component of deep-sea metabolism.

At this point in our studies, we know nothing about these organisms other than their 16S rRNA sequences. Although the closest characterized apparent relatives are extreme thermophiles, the temperature of the water from which our samples came was 5–15 °C, and remote from any known hydrothermal sources. Also, the G+C content, which correlates with thermal stability, of our longest clones (NH49-8 and -9) was only ~51%, compared with 63% for Sulfolobus over the same region. Therefore, we think the sequences probably do not come from thermophiles. Phylogenetic statistical evaluation of the sequence data suggests we cannot at this time confidently place these clone sequences within the extreme thermophile branch of the archaeabacteria. A bootstrap analysis, which involves creation of many phylogenetic trees from random resampling within the sequence, and with the eubacterium Deinococcus radiodurans as the outgroup, indicated that although the closest relative to our clones appears to be Pyrodinium occultum, the branch that leads to our clones does not consistently diverge with the extreme thermophiles; in 40% of 70 trees generated by the bootstrap analysis, the branch leading to our clones diverged within the

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FIG. 1. Depth profiles of parameters at sampling location (within 2 km of 31°30' N 124° W). Error bars indicate range of values of the 7-day sampling period (21–27 April 1989) for the biological parameters, points without error bars were not replicated. Temperature profile was measured on 22 April 1989. Bacterial abundance was measured by epifluorescence microscopy with acridine orange stain, particulate chlorophyll a collected on 0.45-µm-pore-size filters was analysed by fluorometry and temperature measured by an expendable bathythermograph.
extreme thermophile lineage, in 36% the divergence occurred within the methanogen/halophile lineage, and in 24% the branch diverged closer to the eubacteria than the extreme thermophile split from the methanogen/halophile lineage. Therefore we cannot rule out that these organisms may be methanogens (common in anaerobic environments, so perhaps released from anaerobic guts of metazoan; the water is not anaerobic in this region), but the sequences are clearly distinct from all known groups of methanogens.11,12 (Fig. 2). Learning more about the organism will probably require enrichment or isolation of such organisms. The sequences described here include several regions suitable for use as specific probes; therefore, many enrichment conditions can be tried and these can be screened with such probes to see which conditions, if any, enhance growth of this group. Given that other archaeaebacteria usually thrive under conditions in which there is little competition from eubacteria or eukaryotes (for example, high temperatures, low pH, high salt), it would be interesting to learn what niche these organisms fill in the sea. There may also be interest phylogenetically because they appear to have diverged early in the evolution of life and have their closest characterized relatives within a broad group that until now was thought to consist only of extreme thermophiles.11

Although it is unsatisfactory to have only a gene sequence, we now know there is something interesting to look for, and we have a probe to assist in the search. The approach we used to discover new microbial groups supplements traditional microbial techniques13,14,15,16, with this novel archaeaebacterial group appearing in the first few midwater plankton samples investigated.

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