Diversity of deep-sea hydrothermal vent *Archaea* from Loihi Seamount, Hawaii

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Received 1 May 1997; received in revised form 6 September 1997; accepted 12 September 1997

Abstract

Through an examination of SSU rDNA (genes coding for SSU rRNA), the molecular phylogeny of the domain *Archaea* (e.g. one of the three major lineages of life) was analyzed from a microbial mat at an active, deep-sea hydrothermal vent ecosystem located at Pele's Vents on the summit of Loihi Seamount, Hawaii. These SSU rDNAs were amplified from extracted microbial mat genomic DNA by PCR, cloned into a plasmid vector, and sequenced. The derived archaeal sequences were then used to infer the evolutionary relationships between these microbial mat community members and their closest known relatives. Of the four clones initially chosen for sequence analysis, a cluster of three phylogenetically similar PVA (Pele's Vents *Archaea*) clones all contained in the archaean group I lineage of the marine *Crenarchaeota* were detected. A single PVA clone was contained in the archaean group II lineage of the marine *Euryarchaeota*. All four of the PVA clones are novel and constitute the discovery of new archaean taxa. From further rarefaction results of 75 archaean SSU rDNA clones, we estimate the organismal diversity of this domain from the microbial mats located at Pele's Vents to be significantly greater than that of the bacterial domain from this same ecosystem. Analyses of archaean diversity at both the organismal (i.e. rarefaction) and phylogenetic level suggest that hydrothermal vents, such as Pele's Vents, are intimately linked with marine archaeoplankton (a recently discovered component of marine picoplankton) detected from oceans around the world. © 1998 Elsevier Science Ltd. All rights reserved.

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Pll: S0967-0645(97)00081-7
1. Introduction

Although issues of deep-sea benthic macrofaunal diversity have long been of interest (e.g. Hessler and Sanders, 1967; Sanders and Hessler, 1969; Dayton and Hessler, 1972) and continue to be so (e.g. Snellgrove et al., 1996; Paterson et al., 1998; Smith et al., 1998), comparatively little is known of microbial diversity in the deep sea. In part, the lack of knowledge is methods-related; only recently has it been possible to assess both the genetic and organismal diversity of microorganisms collected in environmental samples. Furthermore, definitions of microbial diversity are more problematic than those for macrofauna; most notably, the Darwinian concept of “species” does not apply well (if at all) to prokaryotes. Even when a combined organismal and genetic diversity is delineated (e.g. Moyer et al., 1994, 1995), what is the metric that unequivocally separates related forms (Moyer et al., 1996)? Given the urgency to catalog the world’s microbial diversity (Staley et al., 1997), this question looms large not only in deep-sea research, but for microbial ecology in all the Earth’s environments.

The modern paradigm in microbial ecological studies is to use a phylogenetic approach to establish evolutionary relationships among organisms and use this as a framework for making inferences about community structure and physiological adaptation. This paradigm is possible due to the detailed theory of evolutionary relationships among the domains Bacteria, Archaea and Eucarya that has emerged from comparisons of SSU rRNA “signature” sequences (Olsen et al., 1994; Woese, 1994; Pace, 1997). While several types of cell components are informative, ribosomal RNAs offer a quality and quantity of information, making them one of the best macromolecular descriptors of micro-organisms (Ward et al., 1992). Each SSU rDNA (genes coding for small subunit ribosomal RNA) contains both highly conserved regions found among all living organisms, as well as diagnostic variable regions unique to a particular population or a closely related group. SSU rDNAs are widely used as informative biomolecules because: (i) they are essential components of the protein synthesis machinery and therefore are ubiquitously distributed and functionally conserved in all organisms, (ii) they lack the interspecies horizontal gene transfer found with many prokaryotic genes, (iii) they are readily isolated and identified, and (iv) they contain variable regions interspersed among highly conserved regions of primary and secondary structure, permitting phylogenetic comparisons to be inferred over a broad range of evolutionary distances. These features make SSU rDNAs particularly useful for studies of microbial ecology, because the overall diversity of micro-organisms remains largely unexplored (Torsvik et al., 1990). Indeed, it is as a result of these phylogenetic studies that we are beginning to recognize the true diversity of the microbial world (Amann et al., 1995).

Archaea, once thought to be restricted to unusual or extreme aquatic and terrestrial habitats, are now recognized as common members of most naturally occurring microbial communities. This change in our understanding of microbial diversity was facilitated by the application of molecular biological techniques that focus on the examination of SSU rDNAs. The first reports were derived from coastal subsurface marine habitats from temperate regions of the Pacific and Atlantic Oceans. Two novel
lineages were detected, marine archaeal groups I and II (DeLong, 1992; Fuhrman et al., 1992), representing deeply rooted lineages contained within the *Crenarchaeota* and *Euryarchaeota* kingdoms. Marine archaeal groups, both I and II, are exclusive to the classical lineages of culturable *Crenarchaeota* and *Euryarchaeota*. A tremendous *Crenarchaeota* phylogenetic diversity was reported from hot spring habitats at Yellowstone National Park (Barns et al., 1994), with a putative third archaeal kingdom identified and provisionally named "Korarchaeota" (Barns et al., 1996). In addition, *Crenarchaeota* have been reported from several other "ubiquitous" habitats, including microbe-animal associations with marine invertebrates (McInerney et al., 1995; Preston et al., 1996), freshwater lake sediments (MacGregor et al., 1997; Schleper et al., 1997), agricultural (Ueda et al., 1995; Bintrim et al., 1997) and forest (Jurgens et al., 1997) soils, and from ancient Siberian permafrost (Petrova et al., 1997). Incredibly, as much as 30% of the marine picoplankton (planktonic organisms with an average diameter of 0.2–2.0 μm) from both polar and temperate coastal waters fall into the domain *Archaea* (i.e. archaeoplankton), and the majority of these are associated with the marine archaeal group I lineage (DeLong et al., 1994; Massana et al., 1997). Despite this cosmopolitan distribution in nature, little is known about marine archaeal physiological properties or ecology. This study reports the discovery of archaeal community members in microbial mats from the deep-sea hydrothermal vent ecosystem located at Pele's Vents on the summit of the mid-plate hotspot volcano, Loihi Seamount, Hawaii.

All hyperthermophilic isolates from deep-sea hydrothermal vents belong to the domain *Archaea*, with the exception of isolates from the *Thermotogales* and their closest relatives, which are deeply-rooted in the domain *Bacteria*. Isolates from archaeal enrichment cultures have been achieved primarily with samples taken from the hydrothermal vent habitats located at the East Pacific Rise (21° and 11°N), the Juan de Fuca Ridge, and Guaymas Basin (Baross and Deming, 1995; Karl, 1995). So far, all archaeal isolates have demonstrated the "classic" hyperthermophile phenotype (e.g. thriving at temperatures between 80 and 110°C and unable to grow below 60°C). In addition, Huber et al. (1990) used DNA/DNA hybridization experiments with enrichment cultures generated from hydrothermal-plume samples taken during an eruptive event at Macdonald Seamount (another mid-plate hotspot volcano in the South Pacific) to demonstrate the presence of the hyperthermophilic archaeal genera, *Pyrodictium*, *Pyrococcus*, *Archaeoglobus*, and *Thermococcus*. In all of these enrichment-culture studies, representatives from the presumably mesophilic or psychrophilic marine archaeal groups I or II have yet to be detected or isolated. Likewise, no hyperthermophile has yet been identified within either of the marine archaeal group I or II lineages.

Because of the well-recognized resistance to cultivation of the majority of microorganisms found in natural habitats, molecular biological techniques have provided an alternative approach to assess the diversity (i.e. both organismal and phylogenetic) of naturally occurring microbial communities. By taking advantage of the informational content of the SSU rDNA molecule, a sequence-based phylogenetic approach has been used to detect the presence of previously unknown micro-organisms (Pace et al., 1986; Ward et al., 1992). This approach has been especially important for the marine archaeal group I and II lineages, as no culturable analogs have yet been
identified from any marine habitat (planktonic or hydrothermal). Comparative phylogenetic analyses of recovered sequences are used to determine the evolutionary relationships between members of the archaeal community and cultivated microbial taxa, which allows for an estimate of genetic diversity. In addition, these results allow inferences to be made for otherwise unknown micro-organisms, based on the properties of their closest known affiliated relatives. Rarefaction analysis of operational taxonomic units (OTUs), based on restriction fragment length polymorphisms (RFLPs) from an archaeal SSU rDNA clone library, allows the determination of diversity at an organismal level, as these data are assumed to represent the relative occurrence of discrete archaeal taxa.

2. Materials and methods

2.1. Sample collection and generation of archaeal clone library

Hydrothermal-vent microbial mat samples were collected at Pele's Vents on dives #240-247 (3-19 September 1993) with the DSRV Pisces V (Fig. 1). Temperatures ranging from 15 to 30°C were recorded during 18 September-18 October 1993, from two sites located at Pele's Vents (Moyer, 1995). Sampling techniques, construction of the PCR-amplified archaeal clone library and the screening of SSU rDNA clones were described by Moyer et al. (1994, 1995). In addition, a "slurp-gun" sampler was used in conjunction with a rotating rosette bucket sampler, allowing for the collection of multiple samples during a single dive. PCR primers for the initial amplification of archaeal SSU rDNAs templates were analogous to those used by DeLong (1992); priming sites were identical. The 21F primer was synthesized as follows: [5'-TTC YGG TTG ATC CYG CCR GA], where Y = pyrimidine analog "P" and R = purine analog "K" (Glen Research, Sterling, VA). The 958R primer was synthesized as follows: [5'-YCC GGC GTT GAN TCC AAT T], where Y = pyrimidine analog "P" and N = an equal mixture of analogs "P" and "K" at a single position. In addition, the 5' ends of both primers were constructed with phoshalink amidite (Applied Biosystems, Foster City, CA), which aids in cloning efficiency via synthetic phosphorylation.

2.2. SSU rDNA TRE-RFLP analysis

Archaeal SSU rDNA clones (n = 75) were successfully used as templates for PCR (using the 21F and 958R primers). Amplification products from each positive clone were further screened by treatment with two sets of tandem tetrameric restriction enzymes (TREs); therefore a total of four TREs were used. The TRE pairs used were HhaI plus RsaI and MspI plus AluI (4 h at 37°C). The use of four TREs has been determined to detect the majority of bacterial OTUs, including those that are closely related (Moyer et al., 1996). TRE digestion products or RFLPs from each archaeal clone were examined by comparison following gel electrophoresis with 3.5% Metaphor agarose (FMC Bioproducts, Rockland, ME) for 4 h at 5 V/cm in a cold room ( ~ 5°C). Archaeal clones with similar patterns were grouped with UPGMA cluster analysis
and the number of representative SSU rDNA clones per OTU was quantified using the software program GelCompar version 4.0 (Applied Maths BVBA, Kortrijk, Belgium).

2.3. Rarefaction analysis

To estimate archaeal diversity at the organismal level (as described by representative SSU rDNA clones), the estimated number of OTUs (i.e. phylotypes) was plotted as a function of the overall number of clones. This technique, known as rarefaction, estimates OTU richness from a deterministic transform of OTU abundance data. Rarefaction allows for comparisons of diversity using unequal sample sizes and estimates the number of phylotypes ($E_s$) in a random sample of $n$ clones sampled without replacement from a finite parent collection of $N$ clones, where $n_i$ is the number of clones of the $i$th phylotype (Tipper, 1979).

Rarefaction is described by the following equation:

$$E_s = \sum_{i=1}^{s} \left\{ 1 - \binom{N-n_i}{n} \binom{N}{n}^{-1} \right\}$$

Estimates for the number of phylotypes ($E_s$) and corresponding standard deviations were calculated using the algorithm developed by Simberloff (1978), and ported to Matlab (Matlab version 5.0, Mathworks, Natick, MA). The Matlab rarefaction program used herein is available upon request via email (cmoyer@hydro.bioll.wwu.edu).

2.4. SSU rDNA sequencing

Representative archaeal SSU rDNA clones were sequenced using an automated DNA sequencer Model 373 A (Applied Biosystems). Sequencing was performed in both directions according to the manufacturer's specifications, using plasmid templates with fluorescently labeled dideoxy-terminators and PCR (Taq polymerase) cycle sequencing. Oligonucleotides used as primers at the various positions internal to the archaeal SSU rDNA were as described by Lane (1991), with the exception of the universal priming site located between positions 515 and 533 (E. coli reference numbers), which required redesigning to accommodate novel marine group I archaeal sequences. The corresponding primers were designed to accommodate and maintain this universally conserved site as follows: 533 → 515R [5'-GTG YCA GCM GCC GCG GTA A], where Y = pyrimidine analog “P” and M = A/C and 515 → 533F [5'-TTA CCG CGG CKG CTG RCA C], where K = T/G and R = purine analog “K”. All oligonucleotides used in this study were synthesized and purified by thin-layer chromatography (Moyer et al., 1994).

2.5. Phylogenetic analysis

Sequences were manually aligned to a database of SSU rRNA sequences obtained from the ribosomal database project (RDP; Maidak et al., 1997). Sequence alignments
were based on primary and secondary structure considerations, and were constructed using the GDE multiple sequence editor distributed through the RDP. Sequences were also manually aligned into complete secondary structures as well as submitted to the CHECK_CHIMERA program to detect for the presence of possible chimeric artifacts (Kopczynski et al., 1994; Robison-Cox et al., 1995). Phylogenetic analyses were restricted to the comparison of highly to moderately conserved nucleotide positions that were unambiguously alignable in all sequences. Initial phylogenetic screening was conducted using the DeSoete algorithm, which fits distance matrix data to an optimal additive tree (DeSoete, 1983). Final phylogenetic placement was conducted through maximum likelihood analysis with the fastDNAML program (version 1.0.6c) distributed by the RDP (Maidak et al., 1997). This software was derived from J. Felsenstein's DNAml program and uses the generalized two-parameter model of evolution (Kishino and Hasegawa, 1989; Thorne et al., 1991). Final phylogenetic trees were constructed using jumbled orders for the addition of taxa and allowing for the global swapping of branches. Using these parameters, the search for an optimal tree was repeated until the best log likelihood score was reached in at least three independent searches. Bootstrapping methods were used so that node reproducibility for the overall tree topology could be estimated (Felsenstein, 1985). The data set was bootstrapped 100 times with the jumbled addition of taxa, and the search for an optimal tree was repeated until the best log likelihood score was reached in at least two independent searches.

2.6. Nucleotide sequence accession numbers

The SSU rDNA sequences representing the OTUs for Pele's Vents Archaea (PVA OTUs) used in the present analysis have GenBank accession numbers U46677–U46680.

3. Results and discussion

The results presented herein are part of an ongoing project studying the microbial community structure and diversity of hydrothermal vent habitats at Loihi Seamount, Hawaii (Fig. 1; Moyer et al., 1994, 1995). The double–double TRE digest has been successfully used as a tool to estimate the organismal diversity using rarefaction; however, only a few compatible data sets are available for comparison (Tiedje et al., 1997). These results are the first example of the rarefaction analysis of archaeal OTUs generated through comparable double–double TRE digests of SSU rDNA (across > 900 bp). These data demonstrate that the relative organismal diversity of Pele's Vents Archaea nearly reached the level of lake bacterioplankton, indicating that the diversity of these two groups are more similar than that of the archaeal and bacterial components of the microbial community from: Pele's Vents (Fig. 2). RFLP pattern differences found using four TREs discriminate among bacterial taxa with > 2% difference in SSU rDNA sequence (Moyer et al., 1996). This is a conservative estimate for archaeal OTU discrimination, as many less representative SSU rDNA sequences are currently available for comparison. Rarefaction analysis of bacterial
OTUs similarly generated (across \(>1400\) bp) indicated diversity of Pele’s Vents Bacteria is relatively low, whereas it is intermediate for lake bacterioplankton, and relatively high for soil communities (Tiedje et al., 1997). Considerable phenotypic differences are still present in the different micro-organisms that share common OTU
Fig. 2. Rarefaction curves as indicators of microbial diversity (at the organismal level) from four different habitats. For Bacteria, soil communities are most diverse, hypolimnion bacterioplankton is intermediate, and hydrothermal vent mats are least diverse. For Archaea, hydrothermal vent mat diversity is virtually identical to that of Wintergreen Lake bacterioplankton. The comparative data for the hydrothermal vent microbial mat Bacteria were taken from Moyer et al. (1994), and the hypolimnion bacterioplankton and Hawaiian volcanic and Siberian tundra soil communities were from studies described by Tiedje et al. (1997). Estimated number of OTUs and associated standard deviations (error bars) were calculated according to Simberloff (1978).

patterns (Moyer, unpublished result). Hence, the level of microbial diversity revealed by this method and evaluated in Fig. 2 should also be regarded as a conservative estimate.

The high level of organismal diversity in soil compared to other habitats is suggested by the higher diversity of the OTUs in Hawaiian volcanic and Siberian tundra soils, versus the diversity of OTUs from the anaerobic, phototrophic hypolimnion of a eutrophic lake (Wintergreen Lake, MI) and from the microbial mat from a deep-sea hydrothermal vent (Pele's vents). The greater diversity of microniches and variety of bioavailable energy resources may explain the extreme degree of diversity found in soil. Furthermore, elevated temperatures, geochemical extremes, and the presence of chemosynthetic-based food chains (found at hydrothermal vents in general) may act to restrict the diversity of Pele's Vents Bacteria, i.e. the overall number of bacterial populations capable of existing in this hydrothermal vent community. Rarefaction indicates a significantly greater level of organismal diversity within the archaeal domain with respect to the bacterial domain within the microbial mats at Pele's Vents, most likely affected by the ecological distribution and physiology of the archaeal populations.
Through a cursory examination of archaeal specific SSU rDNA clones generated from samples taken at Pele's Vents, four clones were selected after successful primary restriction with BamHI and PstI (Moyer et al., 1994). These four clones were then sequenced in toto, for a total of ~915 bp each. Following examination of the entire cloned SSU rDNA sequence through the comparison of secondary structure models and using the CHECK CHIMERA program, it was determined that each of these clones was a phylogenetically contiguous DNA sequence (i.e. phylotype) and free of potential chimeric artifacts.

The four PVA OTUs have as nearest relatives (based on phylogenetic affiliation) only other recently discovered environmental clones from the following locations: Palmer Peninsula, Antarctica; Santa Barbara Channel, CA; Woods Hole, MA; and the Oregon Coast (Fig. 3). A cluster of three phylogenetically similar PVA OTUs was detected (i.e. PVA OTU 2 cluster), which was contained in the marine crenarchaeal group I lineage. A single PVA OTU was contained in the marine euryarchaeal group II lineage. Although the four PVA OTUs were contained within two discrete phylogenetic lineages, they each represent the detection of a novel archaeal taxon. It is assumed that these archaeal OTUs represent an additional component contained within the microbial community located at Pele's Vents. Currently, none of the Pele's Vents Archaea lineages has any known culturable analogs as phylogenetically affiliated relatives.

The genetic diversity of archaeal SSU rDNA clones recovered from the microbial mats at Pele's Vents is limited to two distinct lineages, in striking contrast to the broad phylogenetic diversity found in bacterial SSU rDNA library originating from these samples (Moyer et al., 1995). This overall pattern is similar to that observed in pelagic marine habitats from the world’s oceans, where currently only two lineages of marine archaea (groups I and II) have been recovered (DeLong, 1992; DeLong et al., 1994; Massana et al., 1997), compared to the numerous lineages of pelagic marine bacteria (Giovannoni et al., 1990; Schmidt et al., 1991; Fuhrman et al., 1993). Classically isolated Archaea (i.e. culturable isolates) have been divided into two kingdoms by SSU rRNA phylogenetic analyses (Woese et al., 1990; Olsen et al., 1994; Woese, 1994): the Crenarchaeota, which predominately contains the phenotype of the sulfur-metabolizing extreme thermophiles (noted exceptions are Pyrolobus aerophilum and Pyrolobus fumarius), and the Euryarchaeota, which contains four phenotypes of extreme halophiles, methanogens, thermophilic sulfate reducers, and additional sulfur-metabolizing extreme thermophiles. Each marine archaeal lineage is distinct from any of the previously cultured archaeal groups, although both the Crenarchaeota and Euryarchaeota kingdoms contain one of these marine archaeal lineages. All methanogens and most extreme thermophiles are strict anaerobes, which is in direct contrast to most of the oxygenated marine environment. It may be possible that members of these novel archaeal lineages grow anaerobically in marine habitats such as hydrothermal vents, submarine hydrocarbon seeps, or within marine sediments (specialized niches devoid of oxygen). These potential anaerobic habitats, are replete with bioavailable energy sources. However, these novel archaeal lineages may also be facultative anaerobes or even aerobes (with respect to their physiology), due to their high abundance in oxygenated seawater. It is unlikely that either of these
Fig. 3. Phylogenetic tree demonstrating relationships of the PVA OTU 2 cluster within the marine crenarchaeal group I and PVA OTU 1 within the marine euryarchaeal group II as determined by maximum likelihood analysis of SSU rDNA sequences. Numbers at nodes represent bootstrap values (percent) for that node (based on 100 bootstrap resamplings). Outgroups are represented by *Aquifex pyrophilus*, PVB OTU 9A, and *Thermotoga maritima*. Sequences not determined in this study were provided by the Ribosomal Database Project (Maidak et al., 1997). The scale bar represents 0.10 fixed mutations per nucleotide position. Bootstrap values are shown for frequencies at or above a threshold of 50%.
marine archaeal lineages are hyperthermophiles, because of their relatively low SSU rDNA G + C ratios (DeLong, 1992) and because of the absence of high temperature (> 100°C) effluent waters at the Pele's Vents habitat.

At least four generic microbially based communities associated with hydrothermal vent habitats are known to exist; these include (i) free-living bacterial populations associated with the discharged vent fluids and presumably growing and reproducing within the sub-seabed conduits, (ii) free-living microbial mats growing on surface strata that are exposed to flowing vent waters, (iii) endo- and exosymbiotic associations of microorganisms and vent fauna, and (iv) microorganisms within the deep-sea hydrothermal vent plumes (Karl, 1987, 1995). In addition, cold seawater surrounds and permeates the entire hydrothermal vent ecosystem and provides physical, chemical, and biological inputs, thereby affecting all of the habitats contained therein. During studies of the Pele's Vents Bacteria (Moyer et al., 1994, 1995), it was hypothesized that hydrothermal vent habitats may be an important source of bioavailable carbon and energy for many of these free-living types of marine bacteria, which are otherwise in the starvation-survival state (i.e. viable but nonculturable) in the water column. An alternative hypothesis, which is not mutually exclusive, is that these free-living bacteria may survive in the microbial mats as a result of the cold seawater circulation through this hydrothermal vent system (i.e. recruited to the microbial mats due to the hydrothermal flow regime). This hypothesis is supported by the broad ecological distribution of planktonic psychrophiles and barophiles (Morita, 1975), representative of the closest relatives of PVB OTUs 5 and 12, which are bacterial OTUs also found in the microbial mats at Pele's Vents (Moyer et al., 1995).

Results of both organismal and phylogenetic diversity analyses suggest hydrothermal ecosystems such as Pele's Vents may act as a potential source of marine archaeoplankton. Alternatively, these hydrothermal ecosystems may act as a sink, with deep seawater percolating through microbial mats, depositing and concentrating archaeoplankton. Although evidence for Pele's Vents acting as a source or sink for these archaeal lineages remains equivocal, archaeal OTUs detected at this location strongly support a link between marine hydrothermal and pelagic habitats. Moreover, this study emphasizes the necessity for culturable isolates so that the true physiology of these phylogenetically distinct archaeal lineages can be ascertained.

Another important question regarding the archaeoplankton is their respective evolutionary origins. Based on the recently developed theory of evolutionary relationships among the domains Bacteria, Archaea and Eucarya, it has been hypothesized that the earliest prokaryotes were thermophilic lithotrophs (e.g. Pace, 1997). The question remains as to whether the common ancestor of the marine crenarchaeal group I lineage and extant hyperthermophilic Crenarchaeota was hot or cold. The results of this phylogenetic analysis (Fig. 3) suggest that the marine crenarchaeal group I lineage evolved from a thermophilic ancestor, thereby indicating the invasion of colder marine habitats from hotter ones. This also may be the case for common ancestor of the marine euryarchaeal group II lineage and extant thermophilic Euryarchaeota, as this lineage consistently exhibits Thermoplasmataceae as the closest cultivated relative. The conclusion of a "hot" ancestor is not as robust as with the marine crenarchaeal group I lineage because of the physiological diversity present in
the *Euryarchaeota* (e.g. the relative proximity of the halophile lineage). However, the potential exists for polyphyletic origins (e.g. multiple occurrences) regarding the evolution of thermophilic "hot" ancestors invading colder habitats within the archaeoplankton. Warm, as opposed to hot, hydrothermal habitats such as Pele's Vents may have acted as stepping stones during this process.

During September–October 1993, temperatures ranged from 15 to 30°C at Pele's Vents (Moyer, 1995). During July and August 1996, the largest swarm of seismic activity ever observed at any Hawaiian volcano occurred at Loihi Seamount (Duennebier et al., 1997). In response to this event, microbial mat samples were collected at the newly formed Pele's Pit Vents where temperatures up to 200°C were recorded. Studies are currently underway to examine the effect of habitat disturbance on the bacterial and archaenal domains of the microbial mat community as well as the relative impact to both bacterial and archaenal diversity.

**Acknowledgements**

The authors gratefully acknowledge the assistance of the officers and crew of the R/V *Kila*, the DSRV *Piaces V* operations team, and the staff of the Hawaiian Undersea Research Laboratory. This research was funded by the NOAA-University of Hawaii Sea Grant College Program and the NOAA-National Undersea Research Program, U.S. Department of Commerce (to F.C.D. and D.M.K.). We also thank the guest editor, Craig R. Smith, and two anonymous reviewers for their constructive criticisms of an earlier draft of the manuscript. Maximum likelihood analysis was made possible with computers provided by Western Washington University (to C.L.M). This project was also funded by NSF grant BIR-9120006 (to J.M.T.). SOEST contribution #4580.

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