Old Dominion University
ODU Digital Commons

OEAS Faculty Publications | Ocean, Earth & Atmospheric Sciences

4-1995

Phylogenetic Diversity of the Bacterial Community from a Microbial Mat at an Active, Hydrothermal Vent System, Loihi Seamount, Hawaii

Craig L. Moyer

Fred C. Dobbs
Old Dominion University, fdobbs@odu.edu

David M. Karl

Follow this and additional works at: https://digitalcommons.odu.edu/oeas_fac_pubs

 Part of the Biotechnology Commons, and the Environmental Microbiology and Microbial Ecology Commons

Repository Citation

Moyer, Craig L.; Dobbs, Fred C.; and Karl, David M., "Phylogenetic Diversity of the Bacterial Community from a Microbial Mat at an Active, Hydrothermal Vent System, Loihi Seamount, Hawaii" (1995). *OEAS Faculty Publications*. 11.
https://digitalcommons.odu.edu/oeas_fac_pubs/11

Original Publication Citation

Moyer, C.L., Dobbs, F.C., & Karl, D.M. (1995). Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Applied and Environmental Microbiology, 61*(4), 1555-1562.

This Article is brought to you for free and open access by the Ocean, Earth & Atmospheric Sciences at ODU Digital Commons. It has been accepted for inclusion in OEAS Faculty Publications by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1995, p. 1555–1562 Vol. 61, No. 4
0099-2240/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Phylogenetic Diversity of the Bacterial Community from a Microbial Mat at an Active, Hydrothermal Vent System, Loihi Seamount, Hawaii†

CRAIG L. MOYER,* FRED C. DOBBS,‡ AND DAVID M. KARL

Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, Hawaii 96822

Received 26 September 1994/Accepted 9 January 1995

The phylogenetic diversity of small-subunit rRNA genes associated with the domain *Bacteria* was examined (by using previously defined operational taxonomic units [C. L. Moyer, F. C. Dobbs, and D. M. Karl, Appl. Environ. Microbiol. 60:871–879, 1994]; those for Pele's Vents *Bacteria* are hereafter abbreviated PV*B* OTUs) with samples from a microbial mat at an active, deep-sea hydrothermal vent system. A cluster of phylogenetically related PV*B* OTUs (OTUs 2, 3, 6, and 8) was closely affiliated with *Thiovulum* sp. contained within the ε subclass of the class *Proteobacteria* and accounted for 60.5% of the small-subunit rRNA bacterial clone library from Pele's Vents. A second, smaller cluster of PV*B* OTUs (OTUs 1 and 11) was closely affiliated with *Xanthomonas* sp., contained within the γ subclass of the *Proteobacteria* and accounted for a total of 27.1% of the bacterial clone library. The remaining five PV*B* OTUs each accounted for 2.1% of the clones recovered and were affiliated with the following phylogenetic groups: PV*B* OTU 5 was a member of the Alteromonas group; PV*B* OTU 12 was a member of the Colwellia assemblage; PV*B* OTU 4 was loosely determined to be a member of the Thiothrix group, with the endosymbiotic bacteria from *Bathymodiolus thermophilus* and *Calyptogena magnifica* as the nearest relatives; PV*B* OTU 10B was a member of the Myxobacterium group; and PV*B* OTU 9A was a member of the Paraphyletic assemblage, with the Octopus Spring microbial mat type K clone as the closest known relative. PV*B* OTU 7 was determined to be a PCR-generated chimeric structure combined from two described phylotypes detected in this study, thereby decreasing the previously estimated number of major PV*B* OTUs from 12 to 11.

Hydrothermal vent microbial communities are potentially diverse because of a plethora of habitats sustained by both chemical and physical extreme gradients. The most widely accepted (or at least hypothesized) mode of metabolism thought to dominate hydrothermal vent microbial communities is chemolithoautotrophy, principally through the oxidation of reduced sulfur and iron compounds (14). However, even though numerous metabolic pathways are possible at hydrothermal vent systems, quantitative assessments of these independent metabolic pathways to total community metabolism have not yet been possible (17, 18). A recent study of the phylogenetic affiliations of sulfur- and iron-oxidizing bacteria through the use of small-subunit (SSU) rRNA demonstrated the ubiquity of these metabolic pathways throughout the entire domain *Bacteria* (sensu Woese [36, 55]), which suggests an early evolutionary development with respect to life on Earth (25). These data were also consistent with the original phylogenetic studies using 5S rRNA (26, 47), which demonstrated that aerobic sulfur bacteria are phylogenetically and taxonomically very heterogeneous.

The majority of deep-sea bacteria either grow slowly or are dormant as a result of the limitation of bioavailable carbon and energy (33). Ultimately, these primarily heterotrophic bacteria derive their meager existence from recalcitrant organic matter that becomes available only after advection or sedimentation from the euphotic zone. In contrast, the first study to examine the metabolic potential of free-living bacteria from hydrothermal vents revealed the presence of chemolithoautotrophic bacteria utilizing readily available, geothermally reduced sulfur compounds at the Galapagos vents (21). Later, three distinct physiological groups of putative sulfur-oxidizing bacteria were isolated and described as various strains of obligately chemolithoautotrophic *Thiomicrospira* spp. or as obligately heterotrophic thiobacillus-like and pseudomonad-like microorganisms (42). Microbial mats resembling those formed by the sulfur-oxidizing bacteria *Beggiatoa* and *Thiothrix* spp. were also observed in samples from these vents (42). These and other sulfur- and iron-oxidizing bacteria can grow in habitats containing oxic-anoxic interfaces. The isolation of an obligately anaerobic *Spirochaeta* sp. from a marine hydrothermal vent (13) demonstrates the potential for anoxia in such habitats. In addition, Karl et al. (19, 20) conducted physiological studies and microscopic examinations of microbial mats from the Pele's Vents hydrothermal vent system; they found thermophilic bacterial populations and evidence of iron and sulfur oxidation and observed the microbial mats to be dominated by iron-depositing sheathed bacteria.

The use of molecular biological techniques, especially those that take advantage of the SSU rRNA molecule, has eliminated our dependence upon isolations of pure cultures as a means of studying the diversity and structure of natural microbial communities (37, 49). However, this type of molecular biological strategy also has potential pitfalls and limitations, which investigators must consider and attempt to control. For

* Corresponding author. Mailing address: Department of Oceanography, University of Hawaii at Manoa, 1000 Pope Rd., Honolulu, HI 96822. Phone: (808) 956-6050. Fax: (808) 956-9516. Electronic mail address: cmoyer@soest.hawaii.edu.

† Sea Grant publication UNIHI-SEAGRANT-JC-95-16 and contribution 3811 from the University of Hawaii School of Ocean and Earth Science and Technology.

‡ Present address: Department of Oceanography, Old Dominion University, Norfolk, VA 23529.

example, the in vitro generation of chimeric SSU ribosomal DNA (rDNA) structures during PCR amplification procedures has recently been documented with mixtures of pure culture isolates (29) and environmentally derived, uncultivated bacteria (23, 34). In addition to analysis of SSU rRNA secondary structures, an automated procedure for the detection of chimeric SSU rRNA sequences through similarity analysis (CHECK_CHIMERA) is now available through the Ribosomal Database Project (RDP) (28), and the utility of this program is thoroughly discussed by Kopczynski et al. (23). With the combined use of these quality control procedures, the identities of possible chimeric sequences can be ascertained and the exact location of a chimeric splicing site can potentially be detected.

In the present study, we examined the phylogenetic diversity of the bacterial community from Pele's Vents, an active deep-sea hydrothermal vent system located on the summit of Loihi Seamount, Hawaii. Our focus was on the operational taxonomic units (OTUs) previously characterized by a restriction fragment length polymorphism (RFLP) distribution analysis of bacterial SSU rRNA genes. In the initial SSU rRNA molecular biological study of the microbial mats at Pele's Vents, we determined that the bacterial community was dominated by two OTUs and that several others were present at reduced levels of abundance (34). In addition, we reported that the SSU rDNA clone library generated to represent the bacterial community at Pele's Vents had been sampled sufficiently to detect a majority of the phylogenetic diversity present in the native habitat (34). In this paper, we report the sequence analyses of the SSU rRNA genes from each of the 11 major bacterial OTUs to (i) determine the genetic relatedness of the OTUs to one another and (ii) determine each OTU's ancestry (phylogeny) by comparison with a database of known SSU rRNA sequences (28).

MATERIALS AND METHODS

Sample collection, generation of bacterial clone library, and RFLP distribution analysis. The collection of hydrothermal vent microbial mat samples, the construction of the PCR-amplified bacterial clone library, and the screening of SSU rDNA clones through RFLP distribution analysis and rDNA fingerprinting are described elsewhere (34).

rDNA sequencing. Representative bacterial SSU rDNA clones were sequenced with an automated DNA sequencer (model 373A; Applied Biosystems, Foster City, Calif.). Sequencing was performed according to the manufacturer's specifications by using plasmid templates with fluorescently labeled dideoxy terminators and PCR (*Taq* polymerase) cycle sequencing. Oligonucleotides used as primers at various positions internal to the bacterial SSU rRNA gene were the same as those described by Lane (24) and were synthesized and purified as previously described (34).

Phylogenetic analysis. Sequences were manually aligned to a database of previously determined SSU rRNA sequences obtained from the RDP (28). Sequence alignments were based on primary and secondary structural considerations and were constructed by using the GDE multiple sequence editor distributed through the RDP (28). Sequences also were manually aligned into complete secondary structures and were submitted to the CHECK_CHIMERA program to detect for the presence of possible chimeric artifacts (23, 28). Phylogenetic analyses were restricted to the comparison of highly to moderately conserved nucleotide positions that were unambiguously alignable in all sequences, corresponding to residues 101 to 183, 220 to 451, 482 to 840, 846 to 1005, 1037 to 1133, and 1141 to 1445 (*Escherichia coli* numbering system). Initial phylogenetic screening was conducted by using the DeSoete algorithm (5), which fits distance matrix data to an optimal additive tree. Corrected pairwise distances were computed from percent similarities by the method of Jukes and Cantor (16), as modified by G. J. Olsen to accommodate the actual base ratios (51). Final phylogenetic placement was conducted through maximum likelihood analyses with the fastDNAml program (version 1.0.6c) written by G. J. Olsen and distributed by the RDP (28). This software was derived from J. Felsenstein's DNAml program (version 3.3; part of the PHYLIP package) and uses the generalized two-parameter model of evolution (22, 48). Final phylogenetic trees were constructed by using jumbled orders for the addition of taxa and allowed for the global swapping of branches. Using these parameters, we repeated the search for an optimal tree until the best log likelihood score was reached in at least three independent searches. Bootstrapping methods were used with each data set so that node reproducibilities for tree topologies could be estimated (9). Each data set was bootstrapped 500 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.

Nucleotide sequence accession numbers. The SSU rRNA sequences representing the OTUs for Pele's Vents *Bacteria* (PV*B* OTUs) used in the present analysis have been submitted to GenBank and assigned accession numbers U15100 through U15107 and U15111 through U15118.

RESULTS

A total of 48 clones were screened by using an RFLP distribution analysis, and 12 PV*B* OTUs were initially detected, each containing an ~1.5-kb SSU rDNA insert (34). Three representative clones were sequenced in toto from OTUs 1, 2, and 3, which contained 12, 23, and 3 clones, respectively. The only other OTU that contained multiple clones was OTU 6, in which two clones were detected and sequenced in toto. The remaining eight OTUs were represented by a single clone, and each was sequenced in toto. Upon examination of sequence data through the comparison of SSU rRNA secondary structure models and the use of the CHECK_CHIMERA program, we determined that three of the eight OTUs represented by a single clone were PCR-generated chimeric structures. Two of the chimeric clones (representing OTUs 9 and 10) were still made up predominantly of novel, phylogenetically contiguous segments of DNA sequence (phylotypes). These clones were defined as OTU 9A, which conservatively was represented by a 1,007-bp segment contiguous from the 5′ end of the SSU rRNA gene, and OTU 10B, which conservatively was represented by a 790-bp segment contiguous from the 3′ end of the SSU rRNA gene. The sequences of each of the remaining segments from the clones representing OTUs 9 and 10 were identical to sequences from similar positions in OTUs 2 and 1, respectively. The third chimeric OTU detected was OTU 7, which was determined to be constructed of segments found in OTUs 2 and 9A (the 5′ end to position ~295 was OTU 9A, and position ~295 to the 3′ end was OTU 2). This analysis lowered our overall estimate of detected OTUs (phylotypes) from 12 to 11, thus eliminating OTU 7 and the sequence segments from OTUs 9B and 10A from further phylogenetic analysis.

The phylogenetic affiliations of all PV*B* OTUs are summarized in Table 1, together with the relative abundance of each OTU. By far the most frequently encountered group of OTUs was the OTU 2 cluster, which comprised OTUs 2, 3, and 8 and the more distantly related OTU 6. The OTU 2 cluster was closely affiliated with the Thiovulum group, which is contained within the ε subclass of the class *Proteobacteria* (ε-*Proteobacteria*) (Fig. 1; Table 1), and accounted overall for 60.5% of the bacterial SSU rRNA clones recovered from Pele's Vents. The close association between the OTU 2 cluster and *Thiovulum* sp. was also confirmed through SSU rRNA secondary structure analysis (data not shown), by which it was determined that both taxa have the characteristic short-short helical pattern of the SSU rRNA cruciform structure described by Lane et al. (25). OTUs 2, 3, and 8 are closely related members of the OTU 2 cluster, which all together incurred a total of 18 variable positions. Of these, two sites exhibited compensatory base changes, where complementary bases changed on both sides of a stem structure, thereby preserving the secondary structure of the SSU rRNA molecule. Perhaps in relation to the large abundance of clones from the OTU 2 cluster, some of the variability in OTUs 2, 3, and 8 may be accounted for by microheterogeneity between different *rrn* operons within the same bacterial population. This explanation is certainly not the case with OTU 6, as it was by far the most phylogenetically distant related member of the OTU 2 cluster (Fig. 1) and had numer-

TABLE 1. Phylogenetic affiliations and percent recoveries of bacterial SSU rRNA genes from a microbial mat at Pele's Vents

Phylogenetic affiliation[a]	Sequenced clone no.	GenBank no.	RFLP group[b]	Clones recovered (%)[c]
ε-*Proteobacteria* (Thiovulum group)	PV*B* 7	U15100	PV*B* OTU 2 (23)	47.9
	PV*B* 15	U15101		
	PV*B* 63	U15102		
	PV*B* 10	U15103	PV*B* OTU 3 (3)	6.3
	PV*B* 12	U15104		
	PV*B* 55	U15105		
	PV*B* 28	U15106	PV*B* OTU 6 (2)	4.2
	PV*B* 73	U15106		
	PV*B* 32	U15107	PV*B* OTU 8 (1)	2.1
γ-*Proteobacteria*				
Xanthomonas group	PV*B* 3	U15111	PV*B* OTU 1 (12)	25.0
	PV*B* 5	U15111		
	PV*B* 25	U15112		
	PV*B* 47	U15113	PV*B* OTU 11 (1)	2.1
Alteromonas group	PV*B* 18	U15114	PV*B* OTU 5 (1)	2.1
Colwellia assemblage	PV*B* 54	U15115	PV*B* OTU 12 (1)	2.1
Thiothrix group	PV*B* 13	U15116	PV*B* OTU 4 (1)	2.1
δ-*Proteobacteria* (Myxobacterium group)	PV*B* 46	U15117	PV*B* OTU 10B (1)	2.1
Paraphyletic assemblage	PV*B* 36	U15118	PV*B* OTU 9A (1)	2.1
Chimera[d]	PV*B* 30		PV*B* OTU 7 (1)	2.1

[a] As described by the RDP, version 4.0.
[b] As determined by Moyer et al. (34); in that study OTUs were defined through RFLP distribution analysis. Numbers in parentheses indicate the total number of recovered clones contained within each PV*B* OTU. The designation of PV*B* OTUs 9A and 10B refers to contiguous phylotypes as described in Materials and Methods.
[c] Calculated by dividing the number of group-specific bacterial clones by the total number of clones analyzed ($n = 48$).
[d] Chimeric SSU rRNA clone combined from sequences belonging to PV*B* OTUs 9A and 2.

ous variable positions (relative to OTUs 2, 3, and 8), including several compensatory base changes, while still maintaining the same overall secondary structure characteristic of the Thiovulum group (data not shown). In addition, the occurrence of OTU 6 was detected twice, and in both instances the sequence data for each clone were identical, as indicated by the common GenBank accession numbers (Table 1).

The second most abundantly represented phylotype was the PV*B* OTU 1 cluster, which comprised OTUs 1 and 11 and accounted for 27.1% of the bacterial clone library recovered from Pele's Vents. The OTU 1 cluster was affiliated with the Xanthomonas group and, in addition, was closely related to several *Thiobacillus* spp., some of which are contained in the Chromatium assemblage and all of which are contained phylogenetically near the root of the γ-*Proteobacteria* (Fig. 2; Table 1). Sequences from the clones representing OTUs 1 and 11 varied by a single *Rsa*I restriction site and had a total of seven variable positions. We assume that this level of variation approximates the lower limit of resolution for the RFLP distribution analysis used to determine the OTUs (34). However, the possibility of microheterogeneity between different *rrn* operons within the same bacterial population cannot entirely be ruled out. Again, identical sequences were detected for two of three of the clones constituting PV*B* OTU 1, as indicated by common GenBank accession numbers (Table 1).

Five other phylogenetically distinct PV*B* OTUs, each represented by a single clone, were also detected, and these cumulatively accounted for 10.5% of the SSU rRNA clone library (Table 1). Four of these were contained within the *Proteobacteria*, with the first being OTU 5, a member of the heterogeneous Alteromonas group and having *Alteromonas haloplanktis* as the closest described relative (Fig. 2). The second was OTU 12, a member of the Colwellia assemblage, which had the environmentally isolated marine aggregate clone 53 (3) and *Colwellia psychroerythrus* as the closest described relatives (Fig. 2). The final OTU affiliated with the γ-*Proteobacteria* was OTU 4, which was most closely related to the chemolithoautotrophic bacterial endosymbionts from *Bathymodiolus thermophilus* and *Calyptogena magnifica* and thereby was determined to be a member of the Thiothrix group. However, OTU 4 was also closely related to *Thiobacillus ferrooxidans* M-1, and so the possibility of placement in the Chromatium assemblage cannot entirely be ruled out (Fig. 3). OTU 10B was affiliated with the Myxobacterium group of the δ-*Proteobacteria*, having *Chondromyces* spp. and *Polyangium* spp. as the closest relatives (Fig. 4). This result was further confirmed by a comparison of several

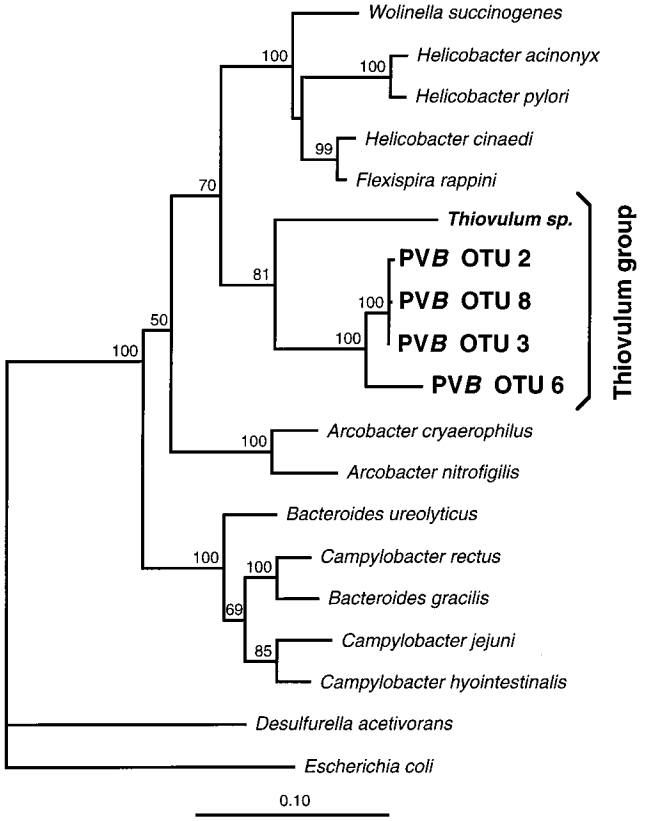

FIG. 1. Phylogenetic tree demonstrating relationships of the PV*B* OTU 2 cluster with ε-*Proteobacteria* as determined by maximum likelihood analysis of SSU rDNA sequences. Numbers at nodes represent bootstrap values (percent) for that node (based on 500 bootstrap resamplings). Outgroups are represented by *E. coli* and *Desulfurella acetivorans* (40) SSU rDNA sequence data. Sequences not determined in this study were provided by the RDP (28), except as noted. The scale bar represents 0.10 fixed mutations per nucleotide position. Bootstrap values are shown for frequencies at or above a threshold of 50%.

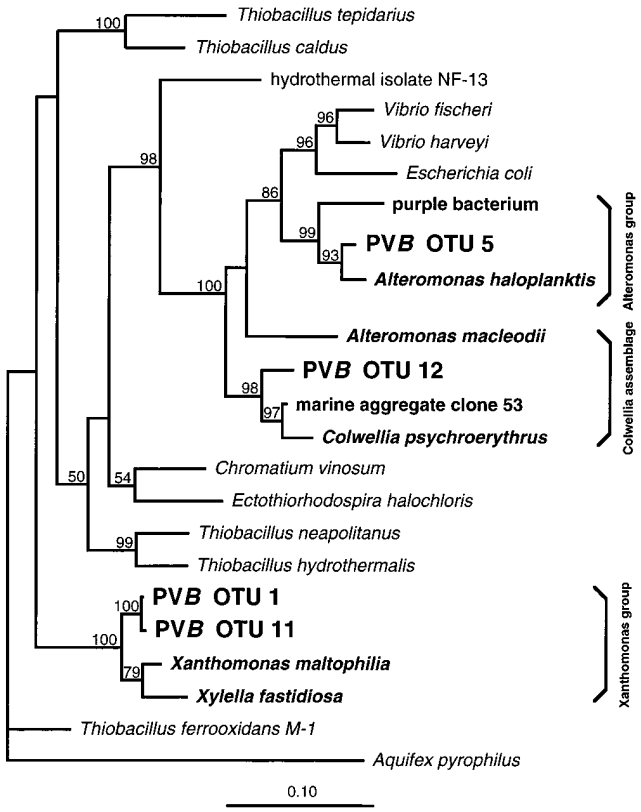

FIG. 2. Phylogenetic tree demonstrating relationships of the PV*B* OTU 1 cluster, PV*B* OTU 5, and PV*B* OTU 12 with γ-*Proteobacteria* as determined by maximum likelihood analysis of SSU rDNA sequences. Numbers at nodes represent bootstrap values (percent) for that node (based on 500 bootstrap resamplings). An outgroup is represented by *Aquifex pyrophilus* SSU rDNA sequence data. Sequences not determined in this study were provided by the RDP (28). The scale bar represents 0.10 fixed mutations per nucleotide position. Bootstrap values are shown for frequencies at or above a threshold of 50%.

distinguishing shared derived characters (synapomorphies) at the secondary structural level, using the myxobacterial sequence signatures determined by Shimkets and Woese (45). The final phylotype examined was OTU 9A, which was placed in the Paraphyletic assemblage (Fig. 5). The Paraphyletic assemblage is deeply rooted in the domain *Bacteria* (36, 55). The closest relative to OTU 9A was another environmental clone isolate, Octopus Spring microbial mat type K (52).

DISCUSSION

At least four generic hydrothermal vent habitats with associated microbially based communities 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 strata, (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 (17, 18). 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. Each of these factors must be considered when one is designing an appropriate sampling scheme that will avoid sampling bias, especially in view of the constraints imposed when a submersible is used for sample collection.

Phylogenetic analyses of SSU rDNA clones provide a method for assessing the structure and diversity of a microbial community without introducing the bias that is inherent with pure culture isolation techniques. However, as with any sampling-detection procedure, the potential for introducing some bias and selectivity still remains. When molecular biological strategies of this type are used, there are potential pitfalls and limitations in addition to the previously described problem of PCR-generated chimeric SSU rDNA structures. Another potential limitation can occur when the efficient and unbiased extraction of nucleic acids from natural microbial communities is attempted. The presence of large concentrations of exopolymeric substances and mineral deposits in hydrothermal vent sample materials may reduce the extraction efficiency or bias the yields of total nucleic acids from natural bacterial populations (18). This problem can potentially be circumvented through the use of both French pressure and enzymatic cellular lysis, microscopic examination of the sample slurry, and purification steps to maximize the recovery of total nucleic acids (34). The differential PCR-mediated amplification and blunt-end cloning of SSU rRNA genes are additional sources of potential selection bias. The use of multiple PCRs and ligation reactions in the generation of a clone library may act to diminish these effects, although the complete elimination of these

potential biases is not yet possible and thus they continue to be drawbacks of these cloning methods (34).

The wide diversity among the bacterial phylotypes recovered in this study suggests that there was no unique bias during our extraction, amplification, and cloning procedures. Chimeric structures accounted for only 6.25% ($n = 3$) of our total number of clones from the bacterial SSU rDNA clone library, and we were able to detect the points of recombination within each of the chimeric SSU rDNA clones. Two of the chimeric clones comprised primarily novel phylotypes (OTUs 9A and 10B), and only a single clone (OTU 7) was recombined completely from other described phylotypes from this study. Additionally, no clones detected more than once through the RFLP distribution analysis were determined to contain chimeric structures. As described above, molecular biological techniques can potentially involve biases that are not yet fully understood, but these biases are much less encumbering than those imposed by the requirements of selective enrichments and pure culture isolation. These and other data (3, 10, 12, 44, 50) demonstrate the effectiveness of evaluating the structure and diversity of a microbial community from environmentally derived mixed populations through the use of SSU rRNA-based molecular biological techniques.

This study assessed the bacterial community structure and diversity through the phylogenetic analysis of bacterial OTUs found at Pele's Vents (Table 1). Assuming that our SSU rDNA clone library approximated the distribution of populations from the bacterial mat community, then the mats at Pele's Vents are dominated (60.5%) by the populations of bacteria represented in the PV*B* OTU 2 cluster. The OTU 2 cluster is most closely related to *Thiovulum* sp. (Fig. 1), a mesophilic obligate chemolithoautotrophic member of the ε-*Proteobacteria*, and may therefore represent a source of autotrophic productivity for the microbial community at Pele's Vents. *Thiovulum* sp. has been studied extensively in the laboratory through the use of enrichment cultures (27, 54). The most characteristic features of *Thiovulum* sp. are that it grows in veils and webs (held together by a polysaccharide matrix) and that it is found in sharply localized white masses in habitats that encompass the interface between sulfide and oxygen (27). Moreover, the suggestion that the OTU 2 cluster represents bacterial populations like *Thiovulum* sp. is supported not only phylogenetically but also by the observation of large clumps of white "streamers" of bacteria surrounding the orifices of Pele's Vents. The veils or webs formed by *Thiovulum* sp. form a stabilized microenvironment by creating an unstirred boundary layer, which minimizes the mixing of sulfide and oxygen and thus also minimizes the chemical oxidation of sulfide (15). The venting waters at Pele's Vents contain extremely high concentrations of total dissolved CO_2 (ca. 300 mM), which are more than 100 times greater than the concentration at the Galapagos Rift Vents (8). Enriched cultures of *Thiovulum* sp. demonstrated significant sulfide-stimulated assimilation of dissolved CO_2 in addition to not assimilating Casamino Acids, acetate, glutamate, or mannitol, further suggesting a chemolithoautotrophic mode of metabolism (54). The present study is the first report of *Thiovulum*-like bacterial populations from a deep-sea hydrothermal vent habitat. Since *Thiovulum* sp. has yet to be grown in pure culture, we do not expect to isolate a representative from Pele's Vents; however, future studies using enrichment culture and in situ oligonucleotide hybridization techniques may provide additional physiological and ecological information.

The second most highly abundant group (27.1%) was represented by the PV*B* OTU 1 cluster, which is a member of the Xanthomonas group (as defined by the RDP) but is also closely related to several *Thiobacillus* spp., some of which are contained in the Chromatium assemblage (Fig. 2); all of these taxa are contained in the γ-*Proteobacteria*. The closely related *Thiobacillus* spp., all of which are mesophilic obligate chemolithoautotrophs, include *Thiobacillus hydrothermalis*, isolated from a deep-sea hydrothermal vent in the Fiji Basin (7). Xanthomonads are known for their production of characteristic yellow pigments (xanthomonadins) and their production of copious amounts of complex extracellular polysaccharides (38). We consider this phylotype (the PV*B* OTU 1 cluster) to represent a free-living addition to the pseudomonad rRNA similarity group V, as described by Palleroni et al. (39). We suggest that this phylotype may contribute to the observed yellow color of the mats at Pele's Vents, as well as to the complex extracellular polysaccharides found therein. However, it must be realized that production of yellow pigments and production of complex extracellular polysaccharides are characteristics which are phylogenetically widespread and which are plastic even among individual bacterial strains.

Two additional, phylogenetically distinct PV*B* OTUs, each represented by a single clone, were contained in the taxonomically defined, highly heterogenous genus *Alteromonas*, which falls within the γ-*Proteobacteria* and is composed primarily of heterotrophic marine bacteria. The first phylotype detected was OTU 5, a member of the Alteromonas group (as defined by the RDP), which had *A. haloplanktis* and an as-yet-undescribed purple bacterium as the closest relatives (Fig. 2). The entire Alteromonas group taxonomically centers around *A. haloplanktis* and includes most of the recognized *Alteromonas* species (11). The second phylotype detected was OTU 12, a member of the Colwellia assemblage (as defined by the RDP), which had the environmentally isolated marine aggregate clone 53 (3) and *C. psychroerythrus* as the closest described relatives (Fig. 2). This group taxonomically centers around *Alteromonas macleodii* and is clearly separate from the other *Alteromonas* species. Most *Alteromonas* species have been isolated from seawater, including many from the open ocean around the Hawaiian archipelago (1). We did not, however, detect any phylotypes from the cyanobacteria, prochlorophytes, or α-*Proteobacteria* (e.g., the SAR 11 cluster), which are known to occur in great abundance within the surface mixed layer of the Atlantic and Pacific Oceans (3, 10, 12, 44). We conclude from these observations that OTUs 5 and 12 are genuine members of the bacterial mat community contained within the hydrothermal ecosystem at Pele's Vents. In addition, we hypothesize that hydrothermal vent habitats, in general, may be an important source of carbon and energy for many of these types of heterotrophic marine bacteria, which are otherwise in the starvation-survival state when found throughout the water column (31, 32). An alternative hypothesis (the two are not mutually exclusive) is that these particular bacteria may reside in the microbial mats as a result of the cold seawater circulation through this hydrothermal vent system. This hypothesis is supported by the ecological distribution of planktonically isolated psychrophiles and barophiles (30), the closest relatives of OTUs 5 and 12, which are contained almost exclusively in the genera *Colwellia* and *Alteromonas* (4, 11).

The final phylotype affiliated with the γ-*Proteobacteria* was PV*B* OTU 4 (Fig. 3). This phylotype was most closely related to the chemolithoautotrophic bacterial endosymbionts from *B. thermophilus* and *C. magnifica* and thereby was determined to be a member of the Thiothrix group (as defined by the RDP). However, we could not rule out the possibility that OTU 4 belongs to the Chromatium assemblage (as defined by the RDP), because of the similarity to *T. ferrooxidans* M-1. Gill endosymbionts from lucinid clams and the trophosome endo-

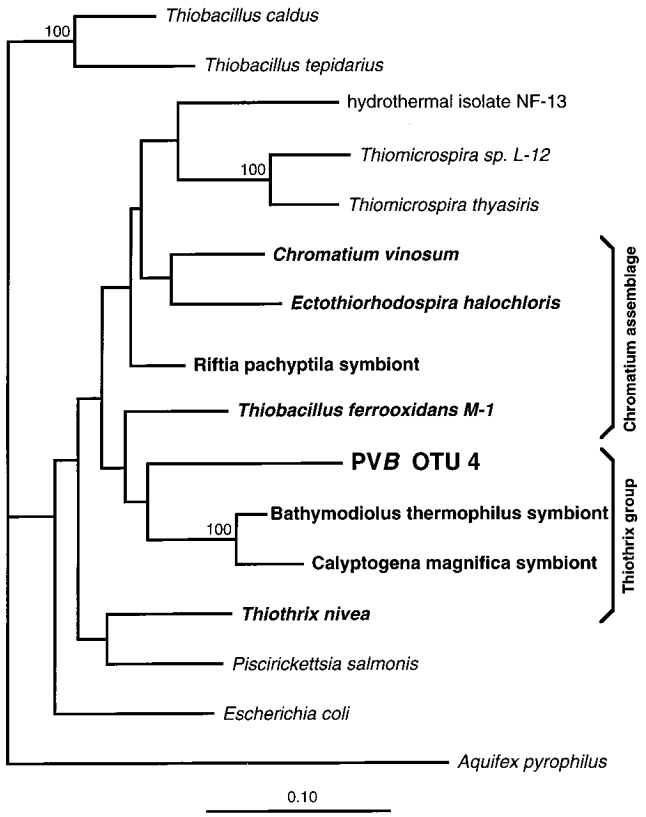

FIG. 3. Phylogenetic tree demonstrating relationships of PV*B* OTU 4 with γ-*Proteobacteria* as determined by maximum likelihood analysis of SSU rDNA sequences. Numbers at nodes represent bootstrap values (percent) for that node (based on 500 bootstrap resamplings). An outgroup is represented by *Aquifex pyrophilus* SSU rDNA sequence data. Sequences not determined in this study were provided by the RDP (28). The scale bar represents 0.10 fixed mutations per nucleotide position. Bootstrap values are shown for frequencies at or above a threshold of 50%.

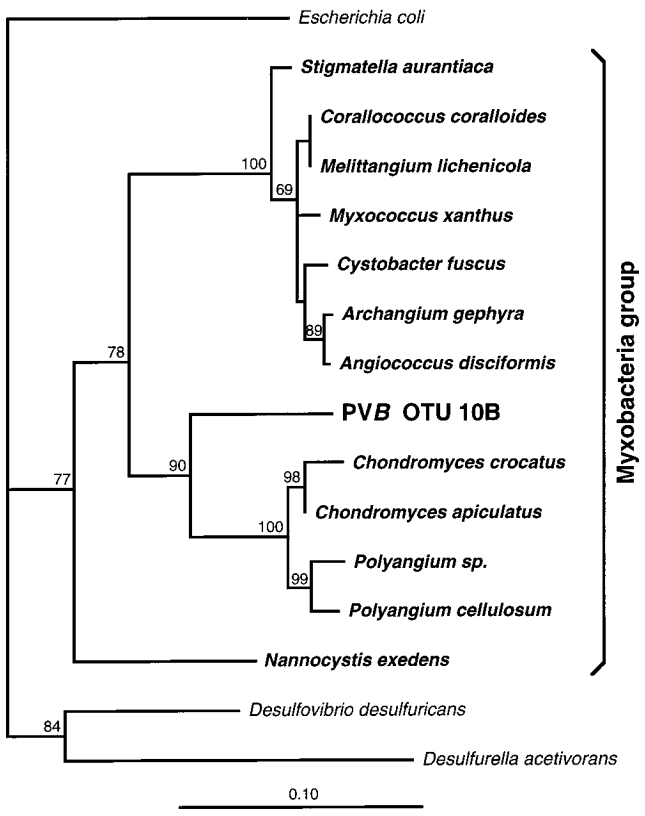

FIG. 4. Phylogenetic tree demonstrating relationships of PV*B* OTU 10B with other myxobacteria as determined by maximum likelihood analysis of SSU rDNA sequences. Numbers at nodes represent bootstrap values (percent) for that node (based on 500 bootstrap resamplings). Outgroups are represented by *E. coli*, *Desulfovibrio desulfuricans*, and *Desulfurella acetivorans* (40) SSU rDNA sequence data. Sequences not determined in this study were provided by the RDP (28), except as noted. The scale bar represents 0.10 fixed mutations per nucleotide position. Bootstrap values are shown for frequencies at or above a threshold of 50%.

symbiont from *Riftia pachyptila* have a lineage phylogenetically distinct from the one associated with the gill endosymbionts from the hydrothermal vent bivalves *B. thermophilus* and *C. magnifica* (6). These two endosymbiont lineages are contained in the Chromatium assemblage and the Thiothrix group, respectively. Thus, the phylogenetic uncertainty about OTU 4 indicates that this phylotype either belongs to one of these lineages or may be an ancestral phylotype of both lineages. Pele's Vents do not have the luxuriant macrofauna found at other hydrothermal vent systems; however, recently a novel species of bresiliid shrimp (53) and a novel pogonophoran worm (48a) have been detected in association with this hydrothermal vent system. At this time, it is not known whether the OTU 4 phylotype is an endo- or exosymbiotic partner with either of these species of macrofauna.

The PV*B* OTU 10B phylotype was closely affiliated with the myxobacteria, which are contained in the δ-*Proteobacteria*, and had *Chondromyces* spp. and *Polyangium* spp. as the closest relatives (Fig. 4). We found this result unusual since no myxobacteria are known to grow at the salt concentrations found in seawater (41), even though several species of myxobacteria have been isolated from seashore sediments (2, 43). The principal habitats for myxobacteria are associated with soil and are ubiquitously distributed worldwide. Almost all myxobacteria are "micropredators" (46) and are attracted to habitats with rich microbial communities (41). If the OTU 10B phylotype is indeed a typical myxobacterium-like organism, then the relatively concentrated biomass within the microbial mats at Pele's Vents may provide a niche for such a micropredator, thereby constituting the next step in trophic level structure.

The PV*B* OTU 9A phylotype was included in the Paraphyletic assemblage, which is itself deeply rooted in the domain *Bacteria* (Fig. 5). The closest relative to OTU 9A was another environmental clone isolate, Octopus Spring microbial mat type K (52). We found this result most intriguing because this Octopus Spring clone (type K), which occurred only once in a bacterial community consisting of 15 detected phylotypes, came from a predominantly cyanobacterium-like-organism (i.e., photoautotrophic)-based community (49) and not a *Thiovulum*-like-organism (i.e., chemoautotrophic)-based one. The inability to relate these phylotypes to others from the domain *Bacteria* suggests that these microbial mat communities are inhabited by additional as-yet-undiscovered phylotypes (i.e., without culturable analogs). We agree with Ward et al. (49), who speculated that these phylotypes (on the basis of their overall low abundance) may represent community members that occupy a secondary trophic level and that diversity within the community may increase as does the variety of substrates made available by primary producers to microorganisms occupying higher trophic levels. However, our present inability to relate these phylotypes to better-defined phyloge-

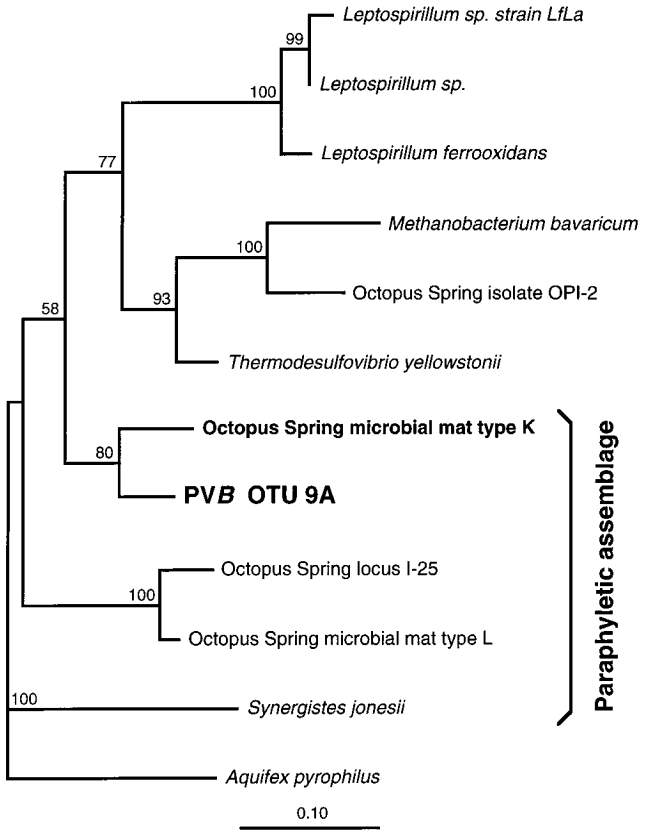

FIG. 5. Phylogenetic tree demonstrating relationships of PV*B* OTU 9A with the Paraphyletic assemblage and the Leptospirillum group as determined by maximum likelihood analysis of SSU rDNA sequences. Numbers at nodes represent bootstrap values (percent) for that node (based on 500 bootstrap resamplings). An outgroup is represented by *Aquifex pyrophilus* SSU rDNA sequence data. Sequences not determined in this study were provided by the RDP (28). The scale bar represents 0.10 fixed mutations per nucleotide position. Bootstrap values are shown for frequencies at or above a threshold of 50%.

netic or physiological groups makes it difficult to determine the ecological significance of these results.

This study has focused on the phylogenetic analyses of SSU rRNA sequence data from previously defined OTUs (34) to estimate the genetic diversity contained within the bacterial community from Pele's Vents. This approach is free of the bias introduced by classical microbial cultivation techniques but may still contain some selective biases from the types of molecular biological techniques used here. We determined that the community is composed primarily of a cluster of *Thiovulum*-like phylotypes and secondarily of a cluster of xanthomonad-like phylotypes, assuming the nearly equivalent recoveries of the bacterial community members. With this assumption in mind, it is interesting that both of the dominant PV*B* OTU clusters (1 and 2) contain a single dominant phylotype. The remaining phylotypes represent a wide variety of SSU rRNA genetic diversity spanning the domain *Bacteria*. The phylogenetic analysis of recovered SSU rDNA clones from the domain *Archaea* are reported elsewhere (35). Further examination of the bacterial community from Pele's Vents by using in situ hybridization techniques will enable the confirmation and localization of specific phylotypes and begin to delineate their spatial and temporal variability. Pele's Vents is the first hydrothermal vent microbial community to be dissected by using this type of molecular biological approach, making it a model against which to compare other microbially based hydrothermal vent communities.

ACKNOWLEDGMENTS

We gratefully acknowledge the helpful assistance, technical expertise, and thoughtful discussions of Neil Reimer, Martin Bento, and Lisa Sampson of the University of Hawaii Biotechnology-Molecular Biology Instrument and Training Facility. We thank the captain and crew of the R/V *Kila*, the DSRV *Pisces V* operations team, and the staff of the Hawaiian Undersea Research Laboratory. We also gratefully acknowledge Edward DeLong for his assistance with the phylogenetic sequence data analysis. We also thank the two anonymous reviewers for their constructive criticisms of an earlier draft of the manuscript.

This project was funded by a grant from the National Oceanic and Atmospheric Administration, project R/OM-8, which is sponsored by the University of Hawaii Sea Grant College Program (SOEST), under Institutional Grant NA89AA-D-SG063 from the National Oceanic and Atmospheric Administration Office of Sea Grants; by the National Oceanic and Atmospheric Administration-National Undersea Research Program, Department of Commerce; and by a Research and Training Revolving Fund Award from the University of Hawaii Research Council.

REFERENCES

1. **Baumann, L., P. Baumann, M. Mandel, and R. D. Allen.** 1972. Taxonomy of aerobic marine eubacteria. J. Bacteriol. **110:**402–429.
2. **Brockman, E. R.** 1967. Fruiting myxobacteria from the South Carolina coast. J. Bacteriol. **94:**1253–1254.
3. **DeLong, E. F., D. G. Franks, and A. L. Alldredge.** 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. **38:**924–934.
4. **Deming, J. W., L. K. Somers, W. L. Straube, D. G. Swartz, and M. T. MacDonell.** 1988. Isolation of an obligately barophilic bacterium and description of a new genus, *Colwellia* gen. nov. Syst. Appl. Microbiol. **10:**152–160.
5. **DeSoete, G.** 1983. A least squares algorithm for fitting additive trees to proximity data. Psychometrika **48:**621–626.
6. **Distel, D. L., D. J. Lane, G. J. Olsen, S. J. Giovannoni, B. Pace, N. R. Pace, D. A. Stahl, and H. Felbeck.** 1988. Sulfur-oxidizing bacterial endosymbionts: analysis of phylogeny and specificity by 16S rRNA sequences. J. Bacteriol. **170:**2506–2510.
7. **Durand, P., A. L. Reysenbach, D. Prieur, and N. Pace.** 1993. Isolation and characterization of *Thiobacillus hydrothermalis* sp. nov., a mesophilic obligately chemolithotrophic bacterium isolated from a deep-sea hydrothermal vent in Fiji Basin. Arch. Microbiol. **159:**39–44.
8. **Edmond, J. M., A. C. Campbell, M. R. Palmer, K. K. Falkner, and T. S. Bowers.** 1987. Chemistry of low temperature vent fluids from Loihi and Larson's Seamounts. EOS, Trans. Am. Geophys. Union **68:**1553–1554.
9. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution **39:**783–791.
10. **Fuhrman, J. A., K. McCallum, and A. A. Davis.** 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific oceans. Appl. Environ. Microbiol. **59:**1294–1302.
11. **Gauthier, M. J., and V. A. Breittmayer.** 1991. The genera *Alteromonas* and *Marinomonas*, p. 3046–3070. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York.
12. **Giovannoni, S. J., T. B. Britschgi, C. L. Moyer, and K. G. Field.** 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature (London) **345:**60–63.
13. **Harwood, C. S., H. W. Jannasch, and E. Canale-Parola.** 1982. Anaerobic spirochete from a deep-sea hydrothermal vent. Appl. Environ. Microbiol. **44:**234–237.
14. **Jannasch, H. W., and M. J. Mottl.** 1985. Geomicrobiology of deep-sea hydrothermal vents. Science **229:**717–725.
15. **Jørgensen, B. B., and N. P. Revsbech.** 1983. Colorless sulfur bacteria, *Beggiatoa* spp. and *Thiovulum* spp., in O_2 and H_2S microgradients. Appl. Environ. Microbiol. **45:**1261–1270.
16. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism. Academic Press, New York.
17. **Karl, D. M.** 1987. Bacterial production at deep-sea hydrothermal vents and cold seeps: evidence for chemosynthetic primary production. Symp. Soc. Gen. Microbiol. **41:**319–360.
18. **Karl, D. M.** Ecology of free-living, hydrothermal vent microbial communities. *In* D. M. Karl (ed.), Microbiology of deep-sea hydrothermal vents, in press. CRC Press, Boca Raton, Fla.
19. **Karl, D. M., A. M. Brittain, and B. D. Tilbrook.** 1989. Hydrothermal and microbial processes at Loihi Seamount, a mid-plate hot-spot volcano. Deep-Sea Res. **36:**1655–1673.

20. **Karl, D. M., G. M. McMurtry, A. Malahoff, and M. O. Garcia.** 1988. Loihi Seamount, Hawaii: a mid-plate volcano with a distinctive hydrothermal system. Nature (London) **335:**532–535.
21. **Karl, D. M., C. O. Wirsen, and H. W. Jannasch.** 1980. Deep-sea primary production at the Galapágos hydrothermal vents. Science **207:**1345–1347.
22. **Kishino, H., and M. Hasegawa.** 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. **29:**170–179.
23. **Kopczynski, E. D., M. M. Bateson, and D. M. Ward.** 1994. Recognition of chimeric small-subunit ribosomal DNAs composed of genes from uncultivated microorganisms. Appl. Environ. Microbiol. **60:**746–748.
24. **Lane, D. J.** 1991. 16S/23S rRNA sequencing, p. 115–175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Ltd., London.
25. **Lane, D. J., A. P. Harrison, Jr., D. Stahl, B. Pace, S. J. Giovannoni, G. J. Olsen, and N. R. Pace.** 1992. Evolutionary relationships among sulfur- and iron-oxidizing eubacteria. J. Bacteriol. **174:**269–278.
26. **Lane, D. J., D. A. Stahl, G. J. Olsen, D. J. Heller, and N. R. Pace.** 1985. Phylogenetic analysis of the genera *Thiobacillus* and *Thiomicrospira* by 5S rRNA sequences. J. Bacteriol. **163:**75–81.
27. **La Rivière, J. W. M., and K. Schmidt.** 1991. Morphologically conspicuous sulfur-oxidizing eubacteria, p. 3934–3947. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York.
28. **Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Macke, T. L. Marsh, and C. R. Woese.** 1993. The ribosomal database project. Nucleic Acids Res. **21:**3021–3023.
29. **Liesack, W., H. Weyland, and E. Stackebrandt.** 1991. Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed culture of strict barophilic bacteria. Microb. Ecol. **21:**191–198.
30. **Morita, R. Y.** 1975. Psychrophilic bacteria. Bacteriol. Rev. **39:**144–167.
31. **Morita, R. Y.** 1982. Starvation-survival of heterotrophs in the marine environment. Adv. Microb. Ecol. **6:**171–198.
32. **Morita, R. Y.** 1985. Starvation and miniaturisation of heterotrophs, with special emphasis on maintenance of the starved viable state, p. 111–130. *In* M. Fletcher and G. Floodgate (ed.), Bacteria in the natural environments: the effect of nutrient conditions. Academic Press, New York.
33. **Morita, R. Y.** 1988. Bioavailability of energy and its relationship to growth and starvation survival in nature. Can. J. Microbiol. **34:**436–441.
34. **Moyer, C. L., F. C. Dobbs, and D. M. Karl.** 1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. Appl. Environ. Microbiol. **60:**871–879.
35. **Moyer, C. L., F. C. Dobbs, and D. M. Karl.** The molecular ecology of deep-sea hydrothermal vent *Archaea*. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995, in press. American Society for Microbiology, Washington, D.C.
36. **Olsen, G. J., C. R. Woese, and R. Overbeek.** 1994. The winds of (evolutionary) change: breathing new life into microbiology. J. Bacteriol. **176:**1–6.
37. **Pace, N. R., D. A. Stahl, D. J. Lane, and G. J. Olsen.** 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microb. Ecol. **9:**1–55.
38. **Palleroni, N. J.** 1991. Introduction to the family Pseudomonadaceae, p. 3071–3085. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York.
39. **Palleroni, N. J., R. Kunisawa, R. Contopoulou, and M. Doudoroff.** 1973. Nucleic acid homologies in the genus *Pseudomonas*. Int. J. Syst. Bacteriol. **23:**333–339.
40. **Rainey, F. A., R. Toalster, and E. Stackebrandt.** 1993. *Desulfurella activorans*, a thermophilic, acetate-oxidizing and sulfur-reducing organism, represents a distinct lineage within the *Proteobacteria*. Syst. Appl. Microbiol. **16:**373–379.
41. **Reichenbach, H., and M. Dworkin.** 1991. The myxobacteria, p. 3416–3487. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York.
42. **Ruby, E. G., C. O. Wirsen, and H. W. Jannasch.** 1981. Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos rift hydrothermal vents. Appl. Environ. Microbiol. **42:**317–324.
43. **Rückert, G.** 1975. Zur Verbreitung von Fruchtkörper-bildenden Myxobakterien in europäischen Strand- und Dünenböden. Zentralbl. Bakteriol. Abt. 2 **130:**343–347.
44. **Schmidt, T. M., E. F. DeLong, and N. R. Pace.** 1991. Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. J. Bacteriol. **173:**4371–4378.
45. **Shimkets, L., and C. R. Woese.** 1992. A phylogenetic analysis of the myxobacteria: basis for their classification. Proc. Natl. Acad. Sci. USA **89:**9459–9463.
46. **Singh, B. N.** 1947. Myxobacteria in soils and composts: their distribution, number and lytic action on bacteria. J. Gen. Microbiol. **1:**1–10.
47. **Stahl, D. A., D. J. Lane, G. J. Olsen, D. J. Heller, T. M. Schmidt, and N. R. Pace.** 1987. Phylogenetic analysis of certain sulfide-oxidizing and related morphologically conspicuous bacteria by 5S ribosomal ribonucleic acid sequences. Int. J. Syst. Bacteriol. **37:**116–122.
48. **Thorne, J. L., H. Kishino, and J. Felsenstein.** 1991. An evolutionary model for maximum likelihood alignment of DNA sequences. J. Mol. Evol. **33:**114–124.

48a. **Vrijenhoek, R.** Personal communication.

49. **Ward, D. M., M. M. Bateson, R. Weller, and A. L. Ruff-Roberts.** 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. Adv. Microb. Ecol. **12:**219–286.
50. **Ward, D. M., R. Weller, and M. M. Bateson.** 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature (London) **345:**63–65.
51. **Weisburg, W. G., J. G. Tully, D. L. Rose, J. P. Petzel, H. Oyaizu, D. Yang, L. Mandelco, J. Sechrest, T. G. Lawrence, J. Van Etten, J. Maniloff, and C. R. Woese.** 1989. A phylogenetic analysis of the mycoplasmas: basis for their classification. J. Bacteriol. **171:**6455–6467.
52. **Weller, R., M. M. Bateson, B. K. Heimbuch, E. D. Kopczynski, and D. M. Ward.** 1992. Uncultivated cyanobacteria, *Chloroflexus*-like inhabitants, and spirochete-like inhabitants of a hot spring microbial mat. Appl. Environ. Microbiol. **58:**3964–3969.
53. **Williams, A. B., and F. C. Dobbs.** A new genus and species of caridean shrimp (Bresiliidae) from hydrothermal vents on Loihi Seamount, Hawaii. Proc. Biol. Soc. Wash., in press.
54. **Wirsen, C. O., and H. W. Jannasch.** 1978. Physiological and morphological observations on *Thiovulum* sp. J. Bacteriol. **136:**765–774.
55. **Woese, C. R., O. Kandler, and M. L. Wheelis.** 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. USA **87:**4576–4579.