

### 3. PHOSPHOLIPID FATTY ACID-DERIVED MICROBIAL BIOMASS AND COMMUNITY DYNAMICS IN HOT, HYDROTHERMALLY INFLUENCED SEDIMENTS FROM MIDDLE VALLEY, JUAN DE FUCA RIDGE<sup>1</sup>

Melanie Summit,<sup>2</sup> Aaron Peacock,<sup>3</sup> David Ringelberg,<sup>3</sup>  
David C. White,<sup>3</sup> and John A. Baross<sup>2</sup>

#### ABSTRACT

Phospholipid fatty acids were measured in samples of 60°–130°C sediment taken from three holes at Site 1036 (Ocean Drilling Program Leg 169) to determine microbial community structure and possible community replacement at high temperatures. Five of six samples had similar concentrations of phospholipid fatty acids (2–6 pmol/g dry weight of sediment), and biomass estimates from these measurements compare favorably with direct microscopic counts, lending support to previous microscopic measures of deep sedimentary biomass. Very long-chain phospholipid fatty acids (21 to 30 carbons) were detected in the sediment and were up to half the total phospholipid fatty acid measured; they appear to increase in abundance with temperature, but their significance is not known. Community composition from lipid analysis showed that samples contained standard eubacterial membrane lipids but no detectable archaeal lipids, though archaea would be expected to dominate the samples at high temperatures. Cluster analysis of Middle Valley phospholipid fatty acid compositions shows that lipids in Middle Valley sediment samples are similar to each other at all temperatures, with the exception of very long-chain fatty acids. The data neither support nor deny a shift to a high-temperature microbial com-

<sup>1</sup>Summit, M., Peacock, A., Ringelberg, D., White, D.C., and Baross, J.A., 2000. Phospholipid fatty acid-derived microbial biomass and community dynamics in hot, hydrothermally influenced sediments from Middle Valley, Juan De Fuca Ridge. *In* Zierenberg, R.A., Fouquet, Y., Miller, D.J., Normark, W.R. (Eds.), *Proc. ODP, Sci. Results*, 169 1–19 [Online]. Available from World Wide Web: <[http://www-odp.tamu.edu/publications/169\\_SR/VOLUME/CHAPTERS/SR169\\_03.PDF](http://www-odp.tamu.edu/publications/169_SR/VOLUME/CHAPTERS/SR169_03.PDF)>. [Cited YYYY-MM-DD]

<sup>2</sup>Oceanography Box 357940, University of Washington, Seattle WA 98195, USA

[summit@ocean.washington.edu](mailto:summit@ocean.washington.edu)

<sup>3</sup>Center for Environmental Biotechnology, University of Tennessee, 10515 Research Drive, Suite 300, Knoxville TN 37932-2575, USA

Date of initial receipt: 3 March 1999  
Date of acceptance: 13 September 1999  
Date of publication: 15 May 2000

munity in hot cores, so at the present time we cannot draw conclusions about whether the microbes observed in these hot sediments are active.

## INTRODUCTION

Prokaryotic microorganisms are now known to exist in many hot environments. Some of these hot microbial habitats are associated with volcanic sources of heat, such as terrestrial (Brock, 1985) and shallow marine hot springs (Hafenbradl et al., 1996) along with deep-sea hydrothermal vents (Baross and Deming, 1995), and some are hot because of the deep nature of the environment, such as deep terrestrial geological formations (Boone et al., 1995) and oil wells (Grassia et al., 1996). The proposed upper temperature limit for life varies from 120° to 150°C or higher (Stetter et al., 1990; Daniel, 1992; Seegerer et al., 1993), and the extent of the deep biosphere is often drawn along that nebulous temperature contour under the assumption that life will exist in all locations within its physical limits (e.g., Whitman et al., 1998).

In contrast to terrestrial hot springs where microbes are obviously thriving, in many hot subsurface environments it is difficult to determine the level of microbial activity (Kieft and Phelps, 1997). For instance, whereas microorganisms have certainly been identified in deep, hot marine sediments (Cragg and Parkes, 1994), it is not possible to determine from the direct microscopic counts whether the microbes are active, dormant, or no longer viable, as fluorescent stains can still bind to intact dead cells (Kepner and Pratt, 1994). It is possible that, though the upper temperature limit of life is currently 120°C or above, the practical limit of activity for a buried microbial community is significantly lower.

If buried microbial communities remain active at high temperatures, the community composition must change with temperature. Buried microbial communities are subjected to ever-increasing temperatures, but the temperature range of any given microorganism is finite. In general, microbes have a temperature range of 30°–50°C, and thus each spans only a subset of the temperatures possible for life; this range appears to be dictated by the underlying biochemistry of the organism (Brock et al., 1994). High-temperature microbial communities studied to date are therefore composed of very different organisms than their low-temperature counterparts; this generalization should hold true in deep environments as well. As most sedimentary geological formations are emplaced at 2°–30°C and can heat up to over 150°C, if the community of microbes seen at a temperature of 80°C (well within the limits of life as we know it) is to be active, it cannot consist of the same microbes that were most active soon after the time of deposition. Microbial succession must take place, replacing a low-temperature microbial community with one that is adapted to high temperatures.

This process of burial and heating is occurring at Middle Valley, Juan de Fuca Ridge, but on a more accessible spatial scale than in most geological environments. Middle Valley is a sedimented ridge off the coast of British Columbia, Canada; young, hot oceanic crust is covered by 200–1000 m of clays and turbiditic silts (Shipboard Scientific Party, 1998). Seawater that has been hydrothermally heated and altered penetrates up through the sediments, discharging locally with temperatures around 270°C (Butterfield et al., 1994). Near these venting locations thermal gradients within the sediments are extremely steep (3°–12°C/m) (Shipboard Scientific Party, 1998), much greater than average geo-

thermal gradients in deep-sea sediments or continental systems that are closer to 0.01°–0.1°C/m (Garland, 1971). Previous sampling at Middle Valley showed evidence of microbial communities within these hot sediments throughout the known temperature range of life and beyond (Cragg and Parkes, 1994), but the species composition of these communities is unknown.

We sampled sediment cores taken on Ocean Drilling Program Leg 169 from Site 1036, the Dead Dog vent field in Middle Valley, to confirm the report of microorganisms in very hot sediments and to determine the composition of the microbial communities inhabiting these hot sediments. Samples were taken from warm (50°–70°C) and hot (90°–130°C) sections of core, and phospholipid analyses were performed as well as standard Acridine orange direct counts to estimate microbial biomass, community composition, and temperature-driven microbial succession.

## METHODS

### Sediment Sampling and Preservation

Whole-round cores (10 cm) were taken from hydraulic piston cores at depths picked on the basis of estimated temperatures. No acetone was used to seal the end caps of the cut cores. Whole-round cores were immediately processed inside an ethanol-sterilized glove bag filled with argon. As the surface of the cut core was potentially contaminated, a fresh face was exposed by removing the top 1 cm with a sterile spatula. The 1 cm of core closest to the core liner was similarly avoided when sampling. Subsamples were preserved for Acridine orange direct counts and lipid analyses. Samples for Acridine orange direct counts were taken with sterile 5- or 10-cm<sup>3</sup> truncated syringes inserted 3–4 cm into the sediment. Samples of 1–2 cm<sup>3</sup> were sectioned with an ethanol-sterilized spatula or razor blade into a clean scintillation vial containing 5 mL of sterile, filtered 4% formalin in artificial seawater. The vials were shaken or vortexed to disperse the sediment and stored at 4°C. Samples for lipid analysis were taken similarly, but with a 60-cm<sup>3</sup> truncated syringe; 30–40 cm<sup>3</sup> were sectioned into a sterile 50-cm<sup>3</sup> Falcon centrifuge tube and immediately stored at –20°C. Aliquots of sediment were also removed for culture of high-temperature, anaerobic microorganisms and DNA extraction.

### Acridine Orange Direct Counts

A 1-cm<sup>3</sup> sample of sediment was diluted in 10 mL of filter-sterilized (0.2 µm) 4% formalin in artificial seawater (FFSW). This slurry was vortexed vigorously, 100 µL was removed with a wide-bore pipet tip, diluted in 10 mL of FFSW with 0.02% Triton X-100 (Sigma), and sonicated for 10 min at 1 mA. Samples were mixed thoroughly before removing an aliquot for filtration; the volume was adjusted so that 50%–75% of the slide was covered by sediment (generally 0.2–0.4 mL). This aliquot was diluted in 5–8 mL of FFSW to aid in dispersion and filtered onto 0.2-µm black-stained polycarbonate filters (Poretics).

A filter-sterile solution of 0.1% Acridine orange (Sigma) in artificial seawater was overlain on the filter for 10 min, and the filter was rinsed with 10 mL of FFSW. Some filters were counterstained with the fluorochrome DAPI (4',6-diamidino-2-phenylindole) (Sigma); a filter-sterile

solution of 20 µg/mL DAPI in deionized water was overlain on the Acridine orange-stained, rinsed filters for 1 min, and the filters were given a final rinse with 1 mL of FFSW. Filters were removed from the filtration unit under vacuum and allowed to air dry before mounting in paraffin oil (Fluka).

Samples were examined under blue excitation on a Zeiss epifluorescence microscope using a 100× Neofluor lens with the optivar set at 1.25. Particles that fluoresced bright green (or red orange, in some cases), were 0.2–1 µm in size, round or rod shaped, and had well-defined edges were classed as bacteria for this study. Random grids were counted until 200 bacteria were found or 500 grids had been examined. Two slides were counted from each sample and, following the recommendation of Cragg and Parkes (1994), a third was prepared and counted if the calculated number of bacteria per cubic centimeter for the first two filters varied by more than half an order of magnitude. Blanks were made and examined with every batch of slides.

### Lipid Analyses

Frozen sediment samples were extracted with the single-phase chloroform-methanol-buffer system of Bligh and Dyer (1954), as modified by White et al. (1979a). Next, 37 mL of chloroform, 75 mL of methanol, and 30 mL of phosphate buffer (50 mM, pH 7.4) were added to the sediment, mixed, and allowed to extract for 6 hr. The single-phase extractant was separated from the solid material by centrifugation at 2000 rpm for 20 min and decanting into a separatory funnel. A further 37 mL of chloroform was used to wash the pelleted solids, which were then re-centrifuged, and the chloroform added to the extract. An additional 37.5 mL of water was added to the extract to force the separation of the aqueous from the organic phase. After separation for ~12 hr, the organic phase was drained into a round-bottomed flask and the solvent removed with a rotary evaporator.

The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography (Guckert et al., 1985). Large Pasteur pipets (1 cm diameter) partially blocked with a plug of glass wool were prepared, and 0.5 g of silicic acid was added as a slurry in chloroform. The columns were pre-eluted with 2 mL of chloroform, and the sample was transferred to the column with three 100-µL washes of chloroform. Neutral lipids were eluted with 5 mL of chloroform, glycolipids with 5 mL of acetone, and polar lipids with 5 mL of methanol. The solvent was removed from the polar lipids under a stream of dry nitrogen at 37°C.

The polar lipids were transesterified to the fatty acid methyl esters by a mild alkaline methanolysis (Guckert et al., 1985). The polar lipid extract was dissolved in 1 mL of chloroform/methanol (1:1), 1 mL of methanolic KOH was added, and the mixture was heated at 37°C for 1 hr. Fatty acid methyl esters were recovered from the organic fraction of the sample after adding 2 mL of hexane and 2 mL of water to break phase.

A strong acid methanolysis was performed on the glycolipid and polar lipid residue (Hedrick et al., 1992). The lipid was dissolved in 1 mL of methanol:chloroform:concentrated hydrochloric acid (10:1:1) and heated for 1 hr at 100°C. Next, 2 mL each of lipid-free water and hexane:chloroform (4:1) were added to the cooled methanolizate. The mixture was thoroughly mixed and centrifuged, and the upper organic was layer transferred.

The methanolyzate was extracted twice more with 2 mL hexane:chloroform (4:1), and the solvent was removed from the pooled organic extracts in a stream of dry N<sub>2</sub> at 37°C. The samples were then derivatized to the tetramethyl silyl derivative using O-bis(trimethylsilyl)trifluoroacetamide (Pierce) before analyzing on the gas chromatograph-mass spectrometer.

The fatty acid methyl esters were analyzed by capillary gas chromatography with flame ionization detection on a Hewlett-Packard 5890 Series 2 chromatograph with a 50-m nonpolar column (0.2-mm ID; 0.11- $\mu$ m film thickness). The injector and detector were maintained at 270° and 290°C, respectively. The column temperature was programmed from 60°C for 2 min, ramped at 10°C/min to 150°C, and then ramped to 312°C at 3°C/min. Preliminary peak identification was by comparison of retention times with known standards. Detailed identification of peaks was by gas chromatography-mass spectroscopy (GC-MS) of selected samples using a Hewlett-Packard 5890 series 2 gas chromatograph interfaced to a Hewlett-Packard 5971 mass selective detector using the same column and temperature program previously described. Mass spectra were determined by electron impact at 70 eV. Methyl nonodecanoate was used as the internal standard, and the phospholipid fatty acid (PLFA) was expressed as equivalent peak response to the internal standard.

GC-MS analysis of the ether-lipid cores was performed with high-temperature gas chromatography as described by Nichols et al. (1993). Samples were analyzed by a Hewlett Packard 6890 gas chromatograph (GC) interfaced with a 5973 mass selective detector. The GC was equipped with a 3 m  $\times$  0.25 mm ID, 5% phenylsiloxane fused silica column and an on-column injector. The samples were injected at 90°C, and after 2 min the GC oven temperature was programmed to 190°C at 30°C/min and then to 380°C at 10°C/min. The final temperature was held for 15 min. Helium was used as the carrier gas, and column head pressure was 3 psi.

### Lipid Nomenclature

Fatty acids were named according to the convention X:Y $\omega$ Z, where "X" is the number of carbon atoms in the chain, "Y" is the number of unsaturations, and "Z" is the number of carbon atoms from the methyl end of the molecule to the first unsaturation encountered. Prefixes are as follows: "i" = iso-branched (methyl branch on the second carbon from the methyl end), "a" = anteiso-branched (methyl branch on the third carbon from the methyl end), "10Me" = methyl branch on the tenth carbon from the carboxylate end, and "cy" = cyclopropyl. The suffixes "c" and "t" stand for the *cis* and *trans* geometric isomers of the unsaturation, respectively. A subtle ambiguity in this naming convention is that the number of carbons given for iso, anteiso, and cyclopropyl fatty acids includes all of the carbons in the fatty acid, but 10Me16:0 indicates a methyl group attached (17 carbons in the fatty acid).

## RESULTS

### Summary of Sedimentary Environment

Middle Valley sediments are composed of hemipelagic silty clays with occasional turbidite sequences (Shipboard Scientific Party, 1998). The sediments recovered at Site 1036 show the influence of the nearby venting of 270°C hydrothermal fluid (Butterfield et al., 1994) in their high temperatures (Shipboard Scientific Party, 1998). However, in the intervals sampled for this study (Table T1), all three holes were experiencing hydrothermal recharge. Recharge was demonstrated by direct measurements of negative pore pressures in the upper layers of sediment and is also seen in the chemistry of the pore fluids (Shipboard Scientific Party, 1998). Pore fluids above 20, 40, and 50 mbsf at Holes 1036A, 1036B, and 1036C, respectively, are consistent with seawater that has been conductively heated and subsequently reacted with the surrounding sediment (Shipboard Scientific Party, 1998). Therefore, these sediment samples have markedly different chemistry than typical hydrothermal samples in the same temperature range (60°–130°C), which are thought to achieve their intermediate temperatures through mixing of seawater and hot, reduced hydrothermal fluids.

### Direct Counts of Microorganisms

The number of microbes was determined from formalin-fixed samples for all whole-round cores where complementary lipid analyses were performed. Objects counted as cells under Acridine orange appeared to be small (0.2–0.4 µm), green-fluorescing coccoids; most but not all of these also stained with DAPI. Counts were extremely low in all samples (Table T1), and the detection limit for direct counts in this study was  $1.9 \times 10^5$  cells/cm<sup>3</sup>, which amounts to one Acridine orange-stained bacterially shaped object per 1500 microscope fields. Some samples had bacterial densities below this detection limit, but even in samples above the detection limit, fewer than 20 cells were observed per prepared slide. The 95% confidence intervals for these counts are estimated at  $\pm 0.3$  log units.

### Lipid Analyses

PLFAs were detected at low levels in all samples (Table T1), although Sample 169-1036C-4H-5, 140–150 cm, was near the detection limit of 0.5 pmol/g. Eubacterial abundances corresponding to these PLFA contents were calculated using a conversion factor of  $2.5 \times 10^4$  cells/pmol PLFA (Balkwill et al., 1988) and an average factor of 1.2 g (dry weight) of sediment per cm<sup>3</sup> (Shipboard Scientific Party, 1998). All PLFAs (including very long-chain saturated fatty acids) were included in the biomass estimate. No ether-linked lipids were detected; the detection limit for ether-linked lipids is also 0.5 pmol/g.

Detected PLFAs are shown in Table T2 as mole percentages. Sample 169-1036C-4H-5, 140–150 cm, is excluded from this summary because the total PLFA in this sample was so near the detection limit that only the very most abundant PLFAs were observed. The normal saturates 16:0 and 18:0 were the most abundant PLFAs in these sediments, accounting for 44%–65% of all detected PLFA. Monounsaturates 16:1 $\omega$ 7, 18:1 $\omega$ 7, and 18:1 $\omega$ 9 formed the second most predominant group (21%–

---

T1. Characteristics of sediment samples taken at Site 1036, p. 18.

---

---

T2. Mole percentages of phospholipid fatty acids in sediment samples, p. 19.

---

34%) and branched-chain saturates (i15:0, a15:0, i16:0, i17:0, and a17:0) were 11%–23% of the total PLFA. Cyclopropyl fatty acids cy17:0 and cy19:0 were 3%–9% of the total PLFA. No polyunsaturated, branched-chain unsaturated, or trans-monounsaturated PLFAs were detected in these samples. Very long-chain saturated PLFAs (VLCFAs, greater than 20 carbons) were also measured in quantities comparable to those that contained 20 carbons or fewer (Table T1).

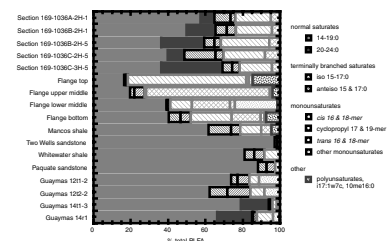
Samples were divided on the basis of temperature into warm (Samples 169-1036B-2H-1, 140–150 cm, and 169-1036C-2H-5, 130–140 cm), hot (Samples 169-1036B-2H-5, 140–150 cm, and 169-1036C-3H-5, 140–150 cm), and very hot samples (Samples 169-1036A-2H-1, 140–150 cm, and 169-1036C-4H-5, 140–150 cm). PLFA-derived biomass was not significantly different between warm and hot groups (t-test,  $p > 0.5$ ), although VLCFA abundance did vary significantly between those two groups (t-test,  $p < 0.02$ ). To test the hypothesis that temperature was the main factor controlling community composition, arcsine-transformed PLFA mole percentages (including VLCFAs) were subjected to *K*-means cluster analysis using SYSTAT (Wilkinson et al., 1992). The *K*-means analysis splits samples into a user-specified number of groups and swaps samples among the groups until between-group variation has been maximized (Hartigan, 1975, as modified by Wilkinson et al., 1992). *K*-means analysis on this set of samples failed to divide the samples into groups; instead single samples split off the main group as the number of groups was increased.

PLFA profiles (mole percentages) from Middle Valley sediments were compared with other PLFA analyses of samples from deep or hot environments (Fig. F1). Cluster analysis was used to gauge the similarity and relationship between samples; for this comparison, VLCFAs greater than 24 carbons in length were excluded from the analysis as they are not routinely measured in all samples. Euclidean distances and Pearson correlation coefficients (Pearson's *R*), joined with average value cluster linkage in SYSTAT (Wilkinson et al., 1992), gave similar branching topologies (Fig. F2). Hot samples (Samples 169-1036B-2H-5, 140–150 cm, and 169-1036C-3H-5, 140–150 cm) clustered apart from the rest of the Middle Valley sediments, and this clustering was mainly driven by differences in the amounts of VLCFA. If VLCFAs were excluded from the analysis, the partitioning between Middle Valley samples became much less robust (Fig. F2B). Middle Valley sediment samples form a cluster distinct from hot sediments from Guaymas Basin (Guezennec et al., 1996) and deep shales and sandstones from New Mexico (Ringelberg et al., 1997). One very hot sample of active sulfide (Hedrick et al., 1992) from the Endeavour Segment, Northeast Pacific, was similar to Middle Valley sediments, although other sulfide samples were less similar.

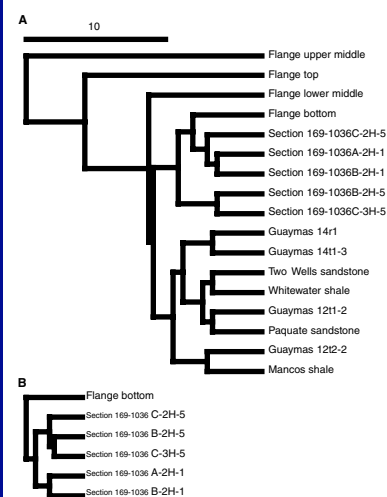
## Discussion

Middle Valley sediments do not provide an especially enticing microbial habitat. These sediments are not rich in microbial energy sources, unlike many hydrothermally influenced areas (Baross and Deming, 1995; Jannasch, 1995). Potential electron donors in the sediments include organic carbon (0.2 wt%), methane at about 10 ppm, and ammonium (Table 1; Shipboard Scientific Party, 1998). Oxygen and nitrate, favored electron acceptors, were not measured; although, by comparison with other sediments, at 10 mbsf oxygen is certainly depleted. Sulfate is present at all depths in all samples at concentrations 50%–100% of that in seawater (Table T1). These sediments, although hydrothermal

F1. Mole percentages of phospholipid fatty acids, p. 16.



F2. Dendrogram of samples in Figure F1 clustered using Euclidean distance and average distance linkage, p. 17.



in proximity, are unlike other hydrothermal locales previously sampled for microbiology and, in many respects, are much more comparable to terrestrial deep environments.

A few basic predictions can be made about the composition of the microbial community based on the physical and chemical environmental parameters. These sediment samples are 60°–130°C, and, therefore, any active community should be composed of high-temperature microorganisms. Sulfate is present in all samples and, because sulfate reduction is a dominant anaerobic lifestyle in marine sediments (Jørgensen, 1982), sulfate-reducing microbes should be prevalent. Any heterotrophic organisms that are active are probably oligotrophic, adapted to growing under conditions of low nutrients.

### Biomass Estimates

Bacterial densities were extremely low but measurable (in most cases) in sediments from all three holes at Site 1036 (Cragg et al., [Chap. 2](#), this volume). All intervals sampled in this study were near the detection limit of Acridine orange direct counts (this study:  $1.9 \times 10^5$  cells/cm<sup>3</sup>) and some were near the detection limit of PLFA (0.5 pmol/g sediment). Biomass estimates from PLFA analyses are usually presumed to represent viable cells, as phospholipids have been demonstrated to have extremely short half-lives in near-surface sediments (White et al., 1979b), likely because of phospholipase activity (Harvey et al., 1986). However, it is possible that an environment with very low microbial activity might preserve PLFA in an atypical fashion, so these biomass estimates must represent an upper boundary. Free fatty acids have been synthesized abiotically under hydrothermal conditions in the laboratory (McCollom et al., 1999), but these should not interfere with the biomass estimates because the extraction procedure used in this study specifically separates free fatty acids from PLFA.

The direct counts and PLFA estimates of biomass agree to within half an order of magnitude, which is relatively close considering the uncertainty in the direct counts. Agreement between direct counts and PLFA biomass estimates is variable in other studies pertaining to the subsurface: in some studies they agree within an order of magnitude (Balkwill et al., 1988; Fredrickson et al., 1995), whereas in others there is an order of magnitude offset (Kieft et al., 1994), or even two (Haldeman et al., 1995). Cell densities calculated from PLFA are vulnerable to the choice of conversion factor (PLFA per cell). The conversion factor chosen,  $2.5 \times 10^4$  cells per pmol PLFA, was calculated for subsurface cells with similar diameters (Balkwill et al., 1988). Other studies have used conversions as large as  $5 \times 10^5$  cells per pmol PLFA (Fredrickson et al., 1995).

The concordance between PLFA biomass and direct counts in this study supports previous measures of microbial biomass in deep sediments using Acridine orange direct counts (Cragg et al., 1990, 1992; Cragg, 1994; Cragg and Parkes, 1994). Although bacteria observed in direct microscopic counts are not necessarily active, PLFA in samples imply intact lipid membranes and at least indicate some form of microbial survival. The biomass found in Middle Valley sediments is comparable to biomass found in other hot, energetically depauperate environments. Piceance Basin in Colorado shows similar PLFA biomass (10 pmol/g) at 45°C, though this biomass decreases with depth and temperature (Colwell et al., 1997). Other very high-temperature environments where PLFA biomass has been measured include samples of active sulfide structures (10–370 ng/g, temperatures estimated at 50°–350°C)



fide structures (10–370 ng/g, temperatures estimated at 50°–350°C) (Hedrick et al., 1992) and near-surface sediments in zones of diffuse hydrothermal upflow (200 pmol/g, temperatures estimated at 60°C) (Guezennec et al., 1996); these samples have the benefit of hydrothermal energy sources and could be expected to have larger biomass than the Middle Valley samples.

### Community Composition

Lipid analysis can give insight into the composition of the microbial community. Whereas most organisms cannot be individually identified from a lipid profile, some classes of microbes have distinctive lipids (e.g., the Kingdom Archaea), and the presence or absence of these biomarkers is an indicator of the presence or absence of these classes. Other types of distinctive lipids are associated with the state of the cell and can be used as stress or growth indicators.

Surprisingly, archaeal diether and tetraether lipids were below the detection limit in all samples. The strong acid methanolysis performed on the whole sediment samples is designed to remove ether lipid residues from a solid matrix (Hedrick et al., 1992), and it is unlikely that there were ether lipids in the samples that went undetected. As the detection limit for the PLFA analysis and the ether lipids are similar, this implies that the archaeal biomass was less than 10% of the bacterial biomass in all samples. Most known organisms that grow in the temperature range 80°–110°C fall in the Kingdom Archaea (Brock et al., 1994), and archaeal lipids made up the bulk of the total microbial lipids in samples of hot sulfide (Hedrick et al., 1992). However, in these Middle Valley sediment samples, archaea were not a significant fraction of the microbial community. Preliminary analyses of hot, hydrothermally influenced surface sediments from Guaymas basin also showed no detectable archaeal biomass (Guezennec et al., 1996), but it is possible that study missed ether lipids present in the sediment because they did not use the robust strong acid methanolysis ether lipid extraction protocol.

Specific PLFAs are characteristic of different classes of Eubacteria. Monounsaturated PLFAs like the 16:1 $\omega$ 7, 18:1 $\omega$ 7, and 18:1 $\omega$ 9 found in this study are indicative of gram-negative bacteria, whereas the terminally branched saturates i15:0, a15:0, i16:0, i17:0, and a17:0 are usually found in gram-positive bacteria or anaerobic microbes (White et al., 1996). The normal saturates 16:0 and 18:0 are abundant in all microorganisms.

Cyclopropyl PLFAs are generally found in gram-negative bacteria, and their abundance tends to vary with growth phase. As gram-negative bacteria enter the stationary phase of growth, they increasingly convert the monounsaturates 16:1 $\omega$ 7 and 18:1 $\omega$ 7 into the cyclopropyl fatty acids cy17:0 and cy19:0 (Guckert et al., 1985; Kieft et al., 1994). Cyclopropyl fatty acids are important components of the phospholipids in these sediment samples, and the cy17:0/16:1 $\omega$ 7 and cy19:0/18:1 $\omega$ 7 ratios range from 0 to 0.45 and 0.27 to 0.71, respectively. These ratios are midway between ratios seen in actively doubling cultures (0.05 or less) and cultures in stationary phase (2.5 or more) (Guckert et al., 1985); this may indicate that the sedimentary microbes are still actively growing to some extent.

In these sediment samples, a number of specific PLFAs are notable by their absence. No polyunsaturated PLFAs were detected, which are found in microeukaryotes (White et al., 1996) and some barophilic bac-

teria (DeLong and Yayanos, 1986). No *trans*-monounsaturated PLFAs were detected; these tend to be produced from the corresponding *cis* isomer during periods of stress (Kieft et al., 1994; Findlay et al., 1990a), including starvation stress. However, a subsurface microbe (*Arthrobacter* sp.) did not accumulate *trans*-PLFA in starvation experiments (Kieft et al., 1994; Kieft et al., 1997), so the ratio of *trans*- to *cis*-PLFA may not be a robust measure of stress in all environments.

No branched monounsaturate or mid-chain branched saturate PLFAs were detected. *Desulfovibrio* and *Desulfobacter*, two of the most widely distributed and easily cultured sulfate-reducing genera, have distinctive terminally branched monounsaturates (i17:1 $\omega$ 7) and mid-chain branched saturates (10Me16:0), respectively (Kohring et al., 1994). *Desulfotomaculum* spp. also show the branched monounsaturate i17:1 $\omega$ 7 (Kohring et al., 1994; Liu et al., 1997). Typically, these PLFAs make up 10%–20% of the total in cultured representatives of these genera (Kohring et al., 1994; Liu et al., 1997); therefore, as these PLFAs were not detectable in the sediments sampled, these genera cannot be dominant members of the microbial community. Whereas the absence of these biomarkers does not mean that all sulfate-reducing bacteria are absent from these sediments, these three genera are believed to be abundant in deep environments. Thermophilic *Desulfotomaculum* spp. have been isolated from deep, hot boreholes in the Taylorsville Triassic basin (Liu et al., 1997). Both *Desulfovibrio* and *Desulfobacter* have been identified in oil wells (Magot et al., 1992; Brink et al., 1994). All cultures of sulfate-reducing bacteria isolated from deep marine sediments (Bale et al., 1997; Barnes et al., 1998) are *Desulfovibrio* spp. by 16S rRNA sequence analysis. Hot sediments may have different suites of sulfate-reducing microbes, though; sulfate reduction was measured in hot sediments from Guaymas Basin to temperatures of 110°C (Jørgensen et al., 1992), and pushcores taken at Guaymas do not show any i17:1 $\omega$ 7 or 10Me16:0 (Fig. F1). Sulfate reduction at these temperatures might be expected to be moderated by *Archaeoglobus* or other sulfate-reducing archaea (Baross and Deming, 1995).

VLCFAs were detected in these sediment samples (21:0 through 30:0). Long-chain saturates of 20–23 carbons were found in lipids from sediments collected at the Guaymas basin hydrothermal area (Guezennec et al., 1996) and have been noted in cultured isolates of oligotrophic soil bacteria (Rezanka et al., 1991). VLCFAs are not necessarily routinely analyzed with other phospholipids (as the gas chromatography run may be terminated before they elute), so the unreported VLCFAs in other studies of PLFAs cannot always be taken as evidence of absence. These lipids are more abundant in the two 100°C samples than in the two 60°C samples, but they are a large fraction of the total lipids (16%–49%) in all five samples. The significance or function of these very long-chain saturated PLFAs is not known, but they could be a signature of oligotrophic lifestyles or perhaps involved in thermal protection of membranes, as increased chain length raises the melting point of lipids.

### Community Comparisons and Dynamics

Whereas some classes of organisms have distinctive lipids and can be identified directly from a lipid profile, most organisms will only contribute to the overall pattern. This profile of lipids, or lipid fingerprint, will be different for different microbial communities, and comparison of lipid fingerprints over time or between locations to determine differ-

ences in microbial community structure is the most powerful way to use environmental PLFA data. PLFA profiles have been used to compare microbial community structures in a range of sediments and rocks: boreal peatlands (Sundh et al., 1997), wetland sediments (Boon et al., 1996), field manipulations of marine sediments (Findlay et al., 1990b), contaminated sediments of marine bays (Rajendran et al., 1994), deep sandstones and shales (Ringelberg et al., 1997), hot surficial sediments near deep-sea hydrothermal vents (Guezennec et al., 1996), and metal sulfide precipitates from deep-sea hydrothermal vents (Hedrick et al., 1992).

The microbial communities in the sampled sediments from Middle Valley are relatively similar to one another, regardless of temperature differences. *K*-means analysis showed that communities did not readily partition into distinct groups. Cluster analysis grouped the two hot samples (Samples 169-1036B-2H-5, 140–150 cm, and 169-1036C-3H-5, 140–150 cm) together, though this difference was mainly driven by the distribution of VLCFAs. Removal of VLCFAs from the data set increases the self-similarity of this group (Fig. F2B). VLCFA showed an increase from warm (60°C) to hot (100°C) samples, though the VLCFA in the hottest sample (130°C) was much lower than either the warm or the hot samples.

Comparison of Middle Valley PLFA community profiles with PLFA profiles from other deep or hot environments (Figs. F1, F2) shows that these deep marine sediments are more similar to each other than they are to other sampled environments, with the exception of an extremely hot sample (100°–350°C) of sulfide (Hedrick et al., 1992). Pushcore samples taken in hot hydrothermally influenced sediments at Guaymas Basin (Guezennec et al., 1996) are more similar to deep cores of sandstone and shale from New Mexico (Ringelberg et al., 1997) than they are to the Middle Valley sediments. Guaymas sediments are influenced by the upflow of hydrothermal fluids and thus are expected to differ from Middle Valley sediments, which are similarly hot but experiencing downflow of seawater. Unexpectedly, Guaymas sediments intermingled with deep sandstones and shales in the cluster analysis.

If buried microbial communities at Middle Valley remain active at high temperatures, the low-temperature community active at the time of deposition must be replaced by a high-temperature community. Thermophilic replacements may have been dormant in the sediment (as was shown in Pacific abyssal sediments by Dobbs and Selph, 1997) or potentially advected into the hot region. Middle Valley sediments are mainly silty clays and, because downflow through the sediments is 10–100 cm/yr (P. Schultheiss, pers. comm., 1997), micrometer-sized particles such as bacteria are likely to have difficulty penetrating down through sediments of comparable grain size. There is no evidence for lateral advection at any of the depths sampled (Shipboard Scientific Party, 1998). It is likely that the microbes making up the communities in Middle Valley sediments were present at the time of burial, and any succession events were composed of microbes (active or inactive) initially deposited at the sediment/water interface.

These microbial communities do not show large or coordinated PLFA shifts with temperature, with the exception of the increase in VLCFA with temperature. This relatively consistent PLFA fingerprint implies that the overall structure of the microbial community has not changed or else microorganisms have been cryptically replaced with other microbes that have the same fatty acids. To determine which of these possibilities might have occurred, DNA extraction was attempted on

preserved splits of the sediment samples, but no measurable or amplifiable DNA could be recovered. If a community shift occurred as temperature increased, thermophiles with similar lipid compositions have replaced mesophilic organisms. It is difficult to reconcile this scenario with the lack of detectable archaea in hot samples; based on our knowledge of high-temperature microorganisms, archaea should have dominated the biomass of samples in the thermal range 80°–110°C. A community shift to archaea was seen in the active sulfide structure samples analyzed by Hedrick et al. (1992), where, as the temperature increased, the ratio of ether-linked (archaeal) to ester-linked (bacterial) lipids increased from 0.1 to more than 20. Thus, it is likely that the organisms present at higher temperatures in Middle Valley sediments may be remnant communities from lower temperatures and therefore unlikely to be active. However, the caveat remains that our knowledge of prokaryotic diversity is meager (Pace, 1997), and it is possible that hot Middle Valley sediments contain previously unknown high-temperature, active bacterial communities that are similar in lipid composition to moderate temperature Middle Valley sediment communities.

## CONCLUSIONS

Hydrothermally influenced sediments from Middle Valley contain microorganisms, detectable microscopically and through phospholipid fatty acid analysis. Their abundances are estimated at about  $10^5$  cells/cm<sup>3</sup> and are similar to other estimates of biomass in deep, hot environments. Agreement between direct counts and lipid analysis lends support to other studies of direct counts in deep marine sediments, and, in the future, lipid analyses could be used advantageously in such deep sediments, especially in conjunction with nucleic acid studies. The microbial community structure as seen from lipid biomarkers shows that the organisms that might otherwise be predicted as most abundant in these environments (high-temperature archaea, *Desulfovibrio* spp.) are undetectable. Communities in the five sediment samples are relatively similar to each other, except for changes in very long-chain fatty acids that increase with temperature. Otherwise the sediments do not show large or coordinated changes with temperature. Microbial communities in Middle Valley sediments may not have actively adapted to the changing environment downcore. Alternatively, as much remains to be discovered about microorganisms, active communities in these sediments at high temperature may represent new communities of high-temperature bacteria expressing very long-chain fatty acids.

## ACKNOWLEDGMENTS

We wish to thank the Ocean Drilling Program for sediment samples and especially the core technicians of Leg 169 for providing us minimally disturbed samples as soon as possible after core recovery. Thanks to Barry Cragg for help through the challenges of direct counts in low-abundance samples and to Jody Deming and Steve Summit for providing helpful suggestions on the manuscript. This work was funded by a JOI/USSSP grant to MS and JAB.

## REFERENCES

- Bale, S.J., Goodman, K., Rochelle, P.A., Marchesi, J.R., Fry, J.C., Weightman, A.J., and Parkes, R.J., 1997. *Desulfovibrio profundus* sp. nov., a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan Sea. *Int. J. Syst. Bacteriol.*, 47:515–521.
- Balkwill, D.L., Leach, F.R., Wilson, J.T., McNabb, J.F., and White, D.C., 1998. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer systems. *Microb. Ecol.*, 16:73–84.
- Barnes, S.P., Bradbrook, S.D., Cragg, B.A., Marchesi, J.R., Weightman, A.J., Fry, J.C., and Parkes, R.J., 1998. Isolation of sulfate-reducing bacteria from deep sediment layers of the Pacific Ocean. *Geomicrobiol. J.*, 15:67–83.
- Baross, J.A., and Deming, J.W., 1995. Growth at high temperatures: isolation and taxonomy, physiology, and ecology. In Karl, D.M. (Ed.), *Microbiology of Deep-Sea Hydrothermal Vents*: Boca Raton, FL (CRC Press), 169–217.
- Bligh, E.G., and Dyer, W.J., 1954. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37:911–917.
- Boon, P.I., Virtue, P., and Nichols, P.D., 1996. Microbial consortia in wetland sediments: a biomarker analysis of the effects of hydrological regime, vegetation and season on benthic microbes. *Mar. Freshwater Res.*, 47:27–41.
- Boone, D.R., Liu, Y., Zhao, Z.J., Balkwill, D.L., Drake, G.R., Stevens, T.O., and Aldrich, H.C., 1995. *Bacillus infernus* sp. nov., an Fe(III)- and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. *Int. J. Syst. Bacteriol.*, 45:441–448.
- Brink, D.E., Vance, I., and White, D.C., 1994. Detection of *Desulfobacter* in oil field environments by non-radioactive DNA probes. *Appl. Microbiol. Biotechnol.*, 42:469–475.
- Brock, T.D., 1985. Life at high temperatures. *Science*, 29:132–138.
- Brock, T.D., Madigan, M.T., Martinko, J.M., and Parker, J., 1994. *Biology of Microorganisms*: Englewood Cliffs, NJ (Prentice Hall).
- Butterfield, D.A., McDuff, R.E., Franklin, J., and Wheat, C.G., 1994. Geochemistry of hydrothermal vent fluids from Middle Valley, Juan de Fuca Ridge. In Mottl, M.J., Davis, E.E., Fisher, A.T., and Slack, J.F. (Eds.), *Proc. ODP, Sci. Results*, 139: College Station, TX (Ocean Drilling Program), 395–410.
- Colwell, F.S., Onstott, T.C., Delwiche, M.E., Chandler, D., Fredrickson, J.K., Yao, Q.J., McKinley, J.P., Boone, D.R., Griffiths, R., Phelps, T.J., Ringelberg, D., White, D.C., LaFreniere, L., Balkwill, D., Lehman, R.M., Konisky, J., and Long, P.E., 1997. Microorganisms from deep, high temperature sandstones: constraints on microbial colonization. *FEMS Microbiol. Rev.*, 20:425–435.
- Cragg, B.A., 1994. Bacterial profiles in deep sediment layers from the Lau Basin (Site 834). In Hawkins, J., Parson, L., Allan, J., et al., *Proc. ODP, Sci. Results*, 135: College Station, TX (Ocean Drilling Program), 147–150.
- Cragg, B.A., Harvey, S.M., Fry, J.C., Herbert, R.A., and Parkes, R.J., 1992. Bacterial biomass and activity in the deep sediment layers of the Japan Sea, Hole 798B. In Pisciotto, K.A., Ingle, J.C., Jr., von Breymann, M.T., and Barron, J., et al., *Proc. ODP, Sci. Results.*, 127/128 (Pt. 1): College Station, TX (Ocean Drilling Program), 761–776.
- Cragg, B.A., and Parkes, R.J., 1994. Bacterial profiles in hydrothermally active deep sediment layers from Middle Valley (NE Pacific), Sites 857 and 858. In Mottl, M.J., Davis, E.E., Fisher, A.T., and Slack, J.F. (Eds.), *Proc. ODP, Sci. Results*, 139: College Station, TX (Ocean Drilling Program), 509–516.
- Cragg, B.A., Parkes, R.J., Fry, J.C., Herbert, R.A., Wimpenny, J.W.T., and Getliff, J.M., 1990. Bacterial biomass and activity profiles within deep sediment layers. In Suess, E., von Huene, R., et al., *Proc. ODP, Sci. Results*, 112: College Station, TX (Ocean Drilling Program), 607–619.
- Daniel, R.M., 1992. Modern life at high temperatures. *Origins Life Evol. Biosphere*, 22:33–42.

- DeLong, E.F., and Yayanos, A.A., 1986. Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. *Appl. Environ. Microbiol.*, 51:730–737.
- Dobbs, F.C., and Selph, K.A., 1997. Thermophilic bacterial activity in a deep-sea sediment from the Pacific Ocean. *Aquatic Microbiol. Ecol.*, 13:209–212.
- Findlay, R.H., Trexler, M.B., Guckert, J.B., and White, D.C., 1990a. Laboratory study of disturbance in marine sediments: response of a microbial community. *Mar. Ecol.: Prog. Ser.*, 62:121–133.
- , 1990b. Response of a benthic microbial community to biotic disturbance. *Mar. Ecol.: Prog. Ser.*, 62:135–148.
- Fredrickson, J.K., McKinley, J.P., Nierzwicki-Bauer, S.A., White, D.C., Ringelberg, D.B., Rawson, S.A., Li, S.-M., Brockman, F.J., and Bjornstad, B.N., 1995. Microbial community structure and biogeochemistry of Miocene subsurface sediments: implications for long-term survival. *Mol. Ecol.*, 4:619–626.
- Garland, G.D., 1971. *Introduction to Geophysics: Mantle, Core, and Crust*: Philadelphia, PA (Saunders).
- Grassia, G.S., Mclean, K.M., Glenat, P., Bauld, J., and Sheehy, A.J., 1996. A systematic survey for thermophilic fermentative bacteria and archaea in high-temperature petroleum reservoirs. *FEMS Microbiol. Ecol.*, 21:47–58.
- Guckert, J.B., Hood, M.A., and White, D.C., 1985. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. *Appl. Environ. Microbiol.*, 52:794–801.
- Guezennec, J.G., Dussauze, J., Bian, M., Rocchiccioli, F., Ringelberg, D., Hedrick, D.B., and White, D.C., 1996. Bacterial community structure in sediments from Guaymas basin, Gulf of California, as determined by analysis of phospholipid ester-linked fatty acids. *J. Mar. Biotech.*, 4:165–175.
- Hafenbradl, D., Keller, M., Dirmeier, R., Rachel, R., Rossnagel, P., Burggraf, S., Huber, H., and Stetter, K.O., 1996. *Ferroglobus placidus* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe<sup>2+</sup> at neutral pH under anoxic conditions. *Arch. Microbiol.*, 166:308–14.
- Haldeman, D.L., Amy, P.S., Ringelberg, D., White, D.C., Garen, R.E., and Ghiorse, W.C., 1995. Microbial growth and resuscitation alter community structure after perturbation. *FEMS Microbiol. Ecol.*, 17:27–38.
- Hartigan, J.A., 1975. *Clustering Algorithms*: New York, NY (John Wiley and Sons, Inc.)
- Harvey, H.R., Fallon, R.D., and Patton, J.S., 1986. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. *Geochim. Cosmochim. Acta*, 50:795–804.
- Hedrick, D.B., Pledger, R.J., White, D.C., and Baross, J.A., 1992. In situ microbial ecology of hydrothermal vent sediments. *FEMS Microbiol. Ecol.*, 101:1–10.
- Jannasch, H.W., 1995. Microbial interactions with hydrothermal fluids. In Humphris, S.E., Zierenberg, R.A., Mullineaux, L.S., and Thomson, R.E. (Eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*: Washington, D.C. (American Geophysical Union), 273–296.
- Jørgensen, B.B., 1982. Mineralization of organic matter in the seabed—the role of sulphate reduction. *Nature*, 296:643–645.
- Jørgensen, B.B., Isaksen, M.F., and Jannasch, H.W., 1992. Bacterial sulfate reduction above 100°C in deep-sea hydrothermal vent sediments. *Science*, 258:1756–1757.
- Kepner, R.L., Jr., and Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental sample: past and present. *Microbiol. Rev.*, 58:603–615.
- Kieft, T.L., and Phelps, T.J., 1997. Life in the slow lane. In Amy, P.S., and Haldeman, D.L. (Eds.), *The Microbiology of the Terrestrial Subsurface*: Boca Raton, FL (CRC Press), 137–164.
- Kieft, T.L., Ringelberg, D.B., and White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. *Appl. Environ. Microbiol.*, 60:3292–3299.

- Kieft, T.L., Wilch, E., O'Connor, K., Ringelberg, D.B., and White, D.C., 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. *Appl. Environ. Microbiol.*, 63:1531–1542.
- Kohring, L.L., Ringelberg, D.B., Devereux, R., Stahl, D.A., Mittelman, M.W., and White, D.C., 1994. Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria. *FEMS Microbiol. Lett.*, 119:303–308.
- Liu, Y.T., Karnauchow, T.M., Jarrell, K.F., Balkwill, D.L., Drake, G.R., Ringelberg, D., Clarno, R., and Boone, D.R., 1997. Description of two new thermophilic *Desulfotomaculum* spp., *Desulfotomaculum putei* sp. nov, from a deep terrestrial subsurface, and *Desulfotomaculum luciae* sp. nov, from a hot spring. *Int. J. Syst. Bacteriol.*, 47:615–621.
- Magot, M., Caumette, P., Desperrier, J.M., Matheron, R., Dauga, C., Grimont, F., and Carreau, L., 1992. *Desulfovibrio longus* sp. nov., a sulfate-reducing bacterium isolated from an oil-producing well. *Int. J. Syst. Bacteriol.*, 42:398–403.
- McCollom, T.M., Ritter, G., and Simoneit, B.R.T., 1999. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. *Origins Life Evol. Biosphere*, 29:153–166.
- Nichols, P.D., Shaw, P.M., Mancuso, C.A., and Franzmann, P.D., 1993. Analysis of archaeal phospholipid-derived di and tetraether lipids by high temperature capillary gas chromatography. *J. Microbiol. Methods*, 18:1–9.
- Pace, N.R., 1997. A molecular view of microbial diversity and the biosphere. *Science*, 276:734–740.
- Rajendran, N., Matsuda, O., Urushigawa, Y., and Simidu, U., 1994. Characterization of microbial community structure in the surface sediment of Osaka Bay, Japan, by phospholipid fatty acid analysis. *Appl. Environ. Microbiol.*, 60:248–257.
- Rezanka, T., Zlatkin, I.V., Viden, I., Slabova, O.I., and Nikitin, D.I., 1991. Capillary gas chromatography-mass spectrometry of unusual and very long-chain fatty acids from soil oligotrophic bacteria. *J. Chromatog.*, 558:215–221.
- Ringelberg, D.B., Sutton, S., and White, D.C., 1997. Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipid fatty acids *FEMS Microbiol. Rev.*, 20:371–377.
- Seegerer, A.H., Burggraf, S., Fiala, G., Huber, G., Pley, U., and Stetter, K.O., 1993. Life in hot springs and hydrothermal vents. *Origins Life Evol. Biosphere*, 23:77–90.
- Shipboard Scientific Party, 1998. Middle Valley: Dead Dog Area (Site 1036). In Fouquet, Y., Zierenberg, R.A., Miller, D.J., et al., *Proc. ODP, Init. Repts.*, 169: College Station, TX (Ocean Drilling Program), 153–203.
- Stetter, K.O., Fiala, G., Huber, G., Huber, R., and Seegerer, A., 1990. Hyperthermophilic microorganisms. *FEMS Microbiol. Rev.*, 75:117–124.
- Sundh, I., Nilsson, M., and Borga, P., 1997. Variation in microbial community structure in two boreal peatlands as determined by analysis of phospholipid fatty acid profiles. *Appl. Environ. Microbiol.*, 63:1476–1482.
- White, D.C., Bobbie, R.J., Nickels, J.S., Fazio, S.D., and Davis, W.M., 1979a. Nonselective biochemical methods for the determination of fungal mass and community structure in estuarine detrital microflora. *Bot. Mar.*, 23:239–250.
- White, D.C., Davis, W.M., Nickels, J.S., King, J.D., and Bobbie, R.J., 1979b. Determination of the sedimentary microbial biomass by extraction of lipid phosphate. *Oecologica*, 40:51–62.
- White, D.C., Stair, J.O., and Ringelberg, D.B., 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. *J. Ind. Microbiol. Biotech.*, 17:185–196.
- Whitman, W.B., Coleman, D.C., and Wiebe, W.J., 1998. Prokaryotes: the unseen majority. *Proc. Nat. Acad. Sci. U.S.A.*, 95:6578–6583.
- Wilkinson, L., Hill, M.-A., and Vang, E., 1992. *SYSTAT: Statistics* (vers. 5.2). Evanston, IL (SYSTAT, Inc.).

**Figure F1.** Mole percentages of phospholipid fatty acids in Middle Valley sediments (this study), active sulfide flange (2°C on top, 350°C at the bottom; Hedrick et al., 1992), Piceance Basin sandstones and shales (40–200 m, Ringelberg et al., 1997), and Guaymas Basin sediments (t) and sulfides (r) (temperatures estimated at 30°–90°C; Guezennec et al., 1996).

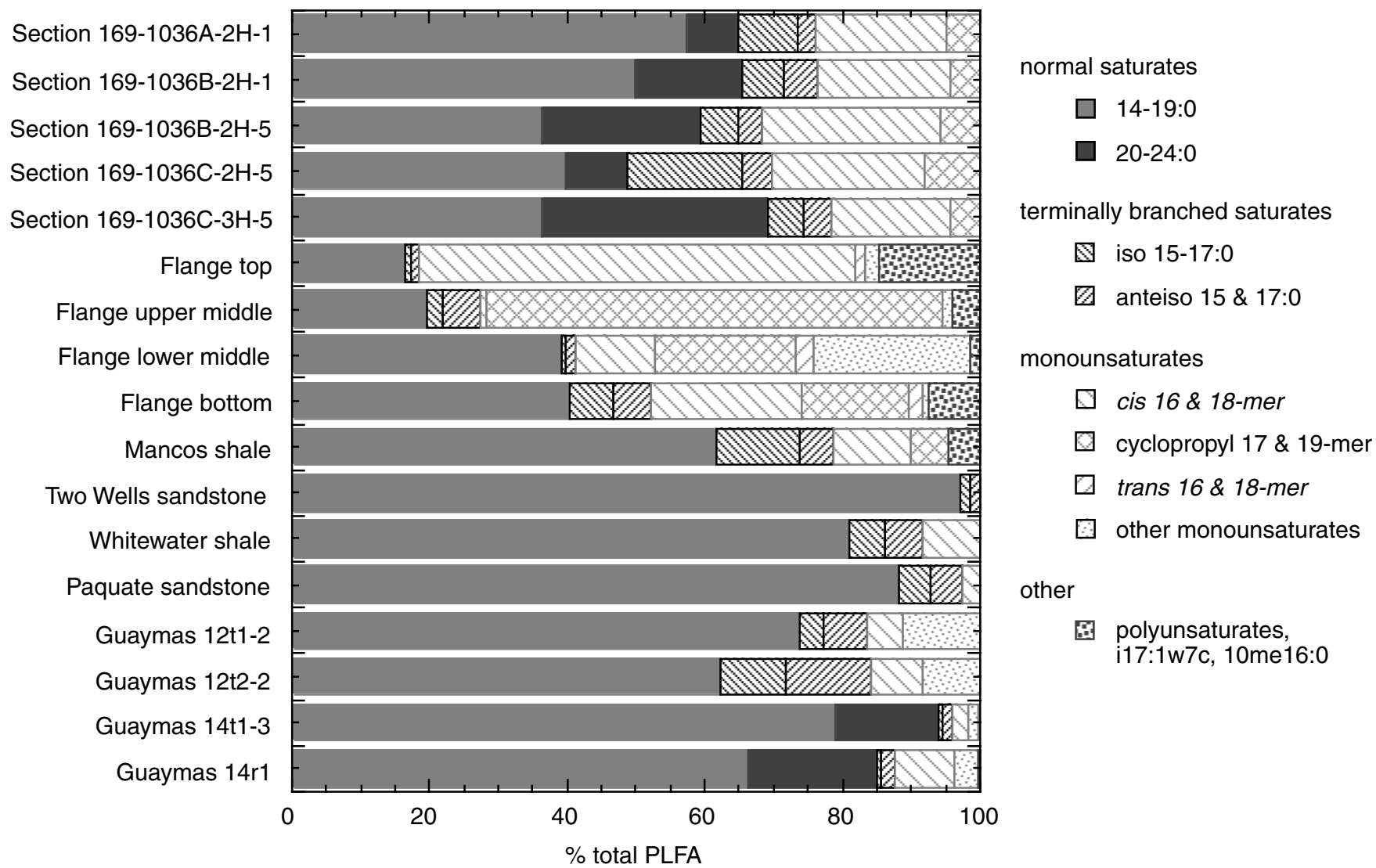




Figure F2. A. Dendrogram of samples in Figure F1, p. 16, clustered using Euclidean distance (scale bar is 10 units) and average distance linkage. Samples from this study form a group; VLCFAs up to 24 carbons are included. B. Excerpt of subcluster containing Middle Valley samples from cluster analysis using a data set excluding all VLCFA (at the same scale as A).

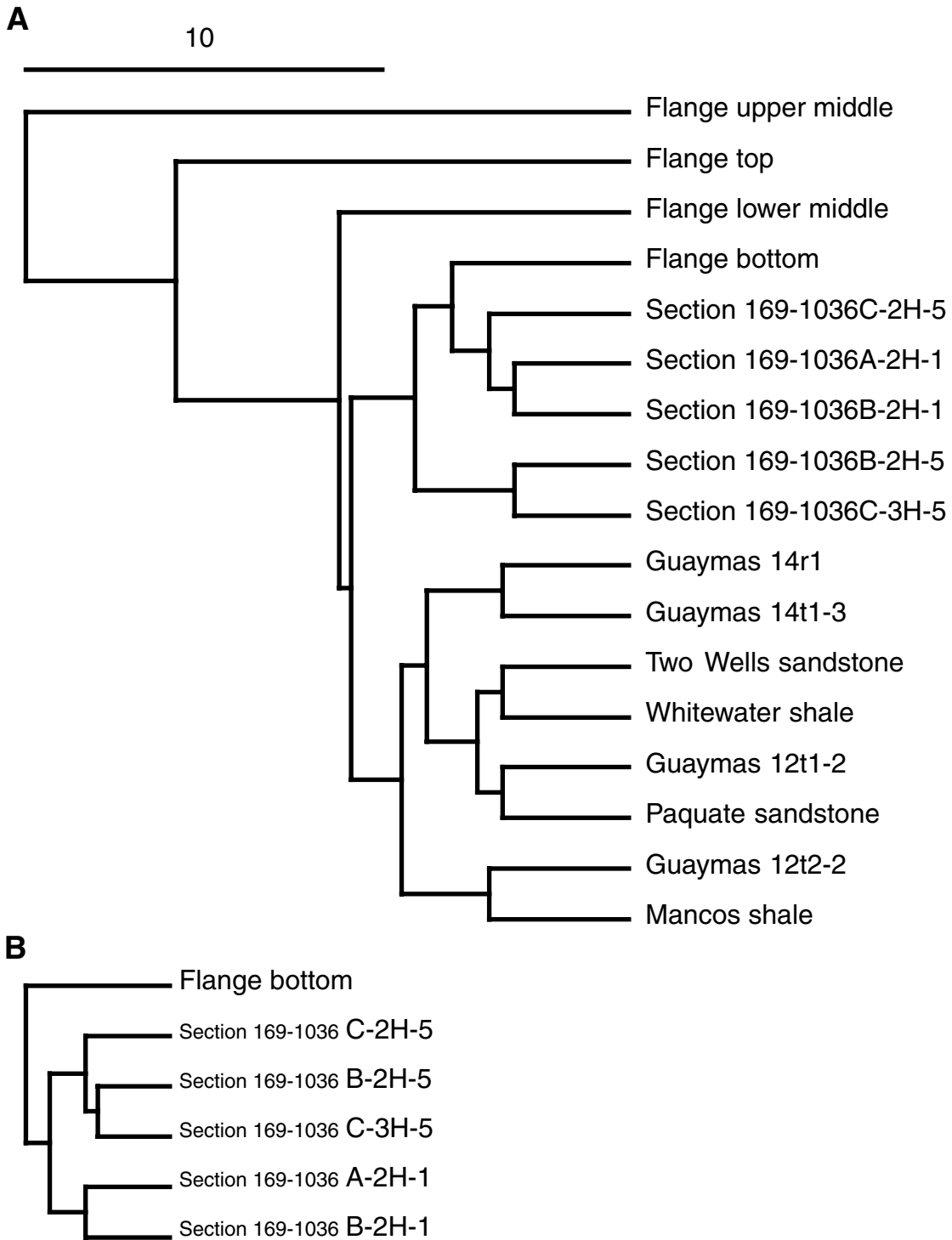


Table T1. Characteristics of sediment samples taken at Site 1036.

Hole, core, section, interval (cm)	Depth (mbsf)	T (°C)	Total PLFAs* (pmol/gdw)	Total VLCFAs (pmol/gdw)	PLFA biomass** (log cells/cm <sup>3</sup> )	Direct counts (log cells/cm <sup>3</sup> )	Cl (mM)	pH	SO <sub>4</sub> (mM)	NH <sub>4</sub> (μM)	Ca (mM)	Mg (mM)
1036A-2H-1, 140-150	10.9	130	1.91	0.36	4.83	5.28	562	7.10	27.3	51	12.6	54.5
1036B-2H-1, 140-150	10.1	60	3.87	1.94	5.24	5.93	590	7.52	29.4	147	23.2	47.4
1036B-2H-5, 140-150	16.1	100	3.90	3.77	5.36	BD	573	7.09	19.3	238	31.8	30.9
1036C-2H-5, 130-140	13.8	64	6.18	1.55	5.37	6.18	561	7.56	27.6	250	15.9	54.2
1036C-3H-5, 140-150	23.3	93	4.34	4.11	5.40	BD	566	7.04	27.9	395	21.1	49.7
1036C-4H-5, 140-150	32.8	118	0.40	0.04	4.12	BD	563	6.70	12.4	560	28.3	25.5

Notes: gdw = gram dry weight; PLFA = phospholipid fatty acids; VLCFA = very long-chain fatty acids; BD = below detection; temperatures for samples at Hole 1036C are interpolated from temperature measurements (Shipboard Scientific Party, 1998), whereas temperatures for samples from Holes 1036A and 1036B are calculated from the estimated thermal gradients of 12°C/m and 6°C/m (Shipboard Scientific Party, 1998), respectively. \* = excluding VLCFAs; \*\* = as calculated in text from PLFAs including VLCFAs; chemistry data are from Shipboard Scientific Party (1998); for reference, seawater values are 557 mM Cl, 28 mM SO<sub>4</sub>, 0 mM NH<sub>4</sub>, 10.6 mM Ca, and 54 mM Mg.

Table T2. Mole percentages of phospholipid fatty acids in sediment samples.

	Section				
	169-1036A-2H-1	169-1036B-2H-1	169-1036B-2H-5	169-1036C-2H-5	169-1036C-3H-5
Normal saturates (found in all bacteria):					
16:0	33.7%	26.1%	15.3%	20.7%	20.2%
18:0	18.6%	13.1%	8.6%	14.3%	7.6%
Total normal	52.3%	39.3%	24.0%	35.0%	27.8%
Branched saturates (typical of gram-positive and anaerobic bacteria):					
i15:0	3.3%	4.6%	2.3%	4.7%	2.0%
a15:0	2.4%	4.0%	2.1%	4.0%	1.7%
i16:0	4.5%	0.0%	1.5%	6.6%	1.3%
i17:0	0.0%	0.0%	0.0%	3.2%	0.8%
a17:0	0.0%	0.0%	0.0%	0.0%	1.3%
Total branched	10.2%	8.6%	5.9%	18.5%	7.0%
Monounsaturates (typical of gram-negative bacteria):					
16:1w7c	5.5%	5.8%	6.3%	6.4%	4.1%
18:1w9c	4.3%	4.6%	3.4%	3.1%	2.5%
18:1w7c	7.5%	4.8%	7.4%	9.8%	6.6%
Total monounsaturates	17.3%	15.2%	17.1%	19.3%	13.2%
Cyclopropyl fatty acids (in gram-negative bacteria, made in stationary phase):					
cy17:0	2.5%	0.0%	0.0%	2.8%	0.8%
cy19:0	2.1%	3.5%	3.8%	4.3%	2.5%
Total cyclopropyl	4.5%	3.5%	3.8%	7.2%	3.3%
Long-chain normal saturates, including very long-chain fatty acids:					
21:0	2.1%	3.0%	2.8%	2.4%	2.7%
22:0	1.2%	3.6%	4.2%	2.2%	7.4%
23:0	1.2%	2.5%	2.9%	1.7%	3.9%
24:0	2.3%	3.3%	5.4%	1.7%	11.0%
25:0	1.2%	4.2%	3.4%	1.5%	3.4%
26:0	3.5%	6.8%	10.6%	3.4%	9.9%
27:0	1.2%	3.6%	3.5%	3.0%	2.0%
28:0	3.0%	6.4%	13.7%	4.2%	5.6%
29:0	0.0%	0.0%	2.7%	0.0%	0.8%
30:0	0.0%	0.0%	0.0%	0.0%	1.8%
Total long saturates	15.7%	33.5%	49.2%	20.0%	48.7%

Note: The most abundant PLFAs in Section 169-1036C-4H-5 were 16:0 (26%), 18:0 (37%), cy17:0 (28%), and a polyenoic 18-carbon PLFA (8%).