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In situ microbial ecology of hydrothermal vent sediments

David B. Hedrick [a], Ralph D. Pledger [b], David C. White [c] and John A. Baross [b]

[a] *Institute for Applied Microbiology, Knoxville, TN,* [b] *Department of Oceanography, University of Washington, Seattle, WA, and* [c] *Department of Microbiology, University of Tennessee, Knoxville, TN and Environmental Science Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

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1. SUMMARY

Lipid analysis of a preliminary sampling of the Endeavor Ridge hydrothermal vent site was performed in order to estimate the extent and nature of the diversity of the microbial community. The vent microbial community was found to be highly variable in density and composition. Evidence was found for a dense microbial community of archaebacteria and possibly Thiobacilli in the interior of the flange of a black smoker, a red *Beggiatoa*-type colony in a sediment sample, and large amounts of polyenoic fatty acids of the type previously found in barophilic eubacteria. Lipid analysis provided a 'snapshot' of the in situ biomass, community structure, and metabolic status of the microbial community, a valuable addition to the techniques available to the microbial ecologist.

Correspondence to: D.B. Hedrick, Institute for Applied Microbiology, 10515 Research Drive, Knoxville, TN 37932-2567 USA.

2. INTRODUCTION

The ecosystems associated with hydrothermal vents are based upon chemosynthetic primary production rather than photosynthesis [1]. Physiological studies of eubacterial and archaebacterial isolates from this environment have been useful in determining potential bacterial activities in hydrothermal vent systems [2,3]. Isolates, however, provide limited insight into ecology since only a small percentage of bacteria can be isolated [4]. A few in situ radiotracer experiments have been performed [5] in hydrothermal vent environments, as well as microscopic [6] and lipid biomarker analyses [6,7]. One approach to the in situ ecology of invertebrates of hydrothermal vents has been the placement of recruitment panels and the enumeration of the attaching species [8].

Polar lipid fatty acids (PLFA) have been validated as a measure of eubacterial and eukaryotic biomass in complex ecosystems, such as estuaries [9] and deep-sea sediments [10], without the need to isolate or cultivate the organisms. Comparison with the lipid profiles of authentic isolates facili-

tates data interpretation in terms of the community structure. More recently, supercritical fluid chromatographic analysis of ether lipids [11] has been used to estimate the archaebacterial biomass and community structure of environments such as methanogenic bioreactors [12] and hydrothermal vent sediments [7]. More studies are needed to establish the validity of this approach. Cluster analysis of environmental lipid biomarker profiles allows an estimation of the degree of relatedness of different samples [12].

In this study, PLFA and archaebacterial ether lipids were used to study the in situ microbial ecology of different environments associated with the deep-sea hydrothermal vents at Endeavor Ridge. Cluster analyses of the lipid profiles were performed to compare microbial communities in different samples analyzed. Although the number of samples was limited, the results provide the first quantitative data on the extreme spatial heterogeneity of the microbial communities of the hydrothermal vent ecosystem.

3. MATERIALS AND METHODS

Samples of hydrothermal vent sulfide deposits were collected from a depth of 2200 m during the 1988 expedition to the Endeavor Segment of the Juan de Fuca Ridge off the west coast of the United States (47° 57′ N, 129° 06′ W). Samples were archived and frozen on ship (after about 4 h at 16°C), and lyophilized upon return to the laboratory. Fig. 1 is a schematic of the hydrothermal vent environment, illustrating the variety of samples obtained. Black smokers such as the one illustrated may reach 15–20 m in height. Table 1 describes the physical characteristics and environments of the samples analyzed. The mud and red bacterial mat samples were scoop samples collected by the submersible ALVIN. The temperature of the oxic overlaying seawater was 2°C. Chimney scrapings were obtained from the sides of black smokers by ALVIN. The 'flange' was a horizontal extension of the black-smoker sulfide mineral deposits with a pool of trapped hy-

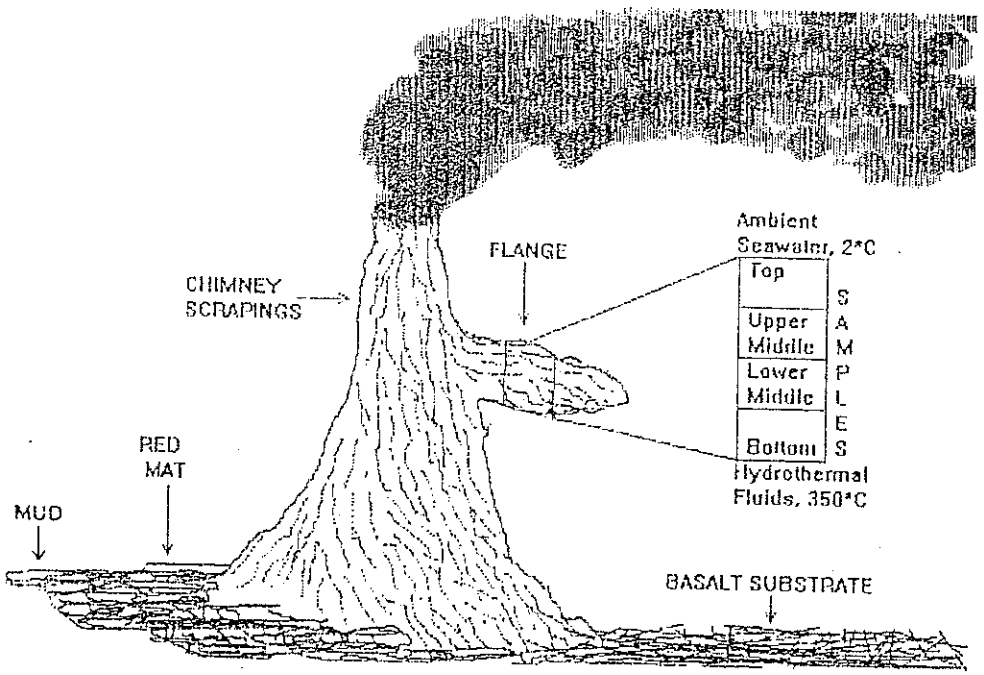

Fig. 1. Schematic diagram of the hydrothermal vent environment, showing the relationship of individual samples. Information on the samples analyzed is presented in Table 1.

drothermal vent fluid below [13]. The flange was recovered by dredge from the support ship. The orientation of the flange in situ was determined by comparison of its morphology and mineralogy with observations of similar flanges from the ALVIN (ref. 15; Delaney, J.R., University of Washington, Seattle, WA, personal communication). Temperature measurements made from the ALVIN on a similar flange established that the overlaying oxic seawater was at the ambient temperature for the deep sea (~ 2°C), and that the pool of anaerobic hydrothermal vent fluid trapped underneath was at up to 350°C [13].

The lyophilized samples were extracted with the one-phase chloroform-methanol-water method of Bligh and Dyer [16,17], separating the total lipid from the lipid-extracted residue (Res). The total lipid extract was fractionated into neutral lipid, glycolipid (GL), and polar lipid (PL) fractions by silicic acid column chromatography [9]. PL fractions were treated with a mild alkaline methanolysis [18] which trans-esterified the eubacterial and eukaryotic ester-linked fatty acids to their PLFA methyl esters. A second silicic acid column chromatography step separated the fatty acid methyl esters as neutral lipids from the unchanged PL ethers. The GL and Res fractions, and the PL ethers were then treated with a strong

Table 1

Description of samples analyzed from the Endeavor Ridge expedition August 1988

Sample code	Sample description	Location (coordinates)	Temperature	Environment	Microscopy observations
6042	Mud	Near smoker 8L (6156/2198)	2°C (seawater above sediment)	Rich organic mud exposed to seawater	NA
6044	Red mat	2 m from smoker 8L (6143/2198)			20–50 μm 'Beggiatoa' filaments, coccoid cells
6067	Chimney scraping	Smoker 86 (6212/5042)	> 2°C (effluent 281°C)	Smoker exterior exposed to seawater	Amorphous mineral, unusual thin objects (cells?)
6068	Chimney scraping	Smoker 98 (6165/4914)	> 2°C (effluent 240–279°C)		Like sample 6067, except fewer thin objects
	Flange samples seawater above		2°C		
6195	Top	North to South dredge through the Endeavour vent field	Unknown	Exposed to seawater	NA
6196	Near top		Unknown	Interior	
6197	Near bottom		Unknown	Interior	
6198	Bottom		Unknown	Exposed to vent fluids	
	Hydrothermal fluid below		> 300°C		

The mud, red mat, and chimney scrapings were collected by the ALVIN submersible, the flange by dredge from the support ship. NA = not applicable.

acid methanolysis [19] to free the archaebacterial ether-lipid hydrocarbon cores as the ether-alcohols.

The PLFA methyl esters were analyzed by gas chromatography and the structures verified by gas chromatography-mass spectroscopy [18]. Nonadecanoate methyl ester was used as the internal standard for fatty acid quantitation. Fatty acid nomenclature is as follows: number of carbon atoms, colon (:), number of double bonds, omega (ω), number of carbon atoms of the nearest unsaturation from the methyl end of the molecule. The prefix 'i' indicates iso-branching, 'a' anteiso-branching, 'cy' cyclopropyl modification, and '10me' the addition of a methyl group 10 carbons from the carboxyl terminus. The suffix 'c' indicates the *cis* geometric isomer of the unsaturation and 't' the *trans* isomer. For example, 16:1ω7c is a 16-carbon fatty acid with an unsaturation 7 carbons from the methyl end in the *cis* configuration.

The ether lipids of the GL, PL, and Res fractions were determined by supercritical fluid chromatography [11]. 1,2-di-*O*-hexadecyl-glycerol was the external standard for SFC. An internal standard was not needed in SFC due to the high precision of the LC-type injector used. The structures of diether (DE), tetraether (TE), and cyclopentyl-modified TE were verified by co-elution with authentic standards isolated from *Methanococcus maripaludis*, *Methanobacterium formicicum*, and *Thermoplasma acidophilum*, respectively, by supercritical fluid chromatography [11], and by 2 thin-layer chromatography methods [20,21]. DE1 was an unidentified structure appearing immediately after the DE peak on the chromatogram, probably the $C_{20}C_{25}$ diether as found in *Methanosarcina barkeri* [22], *Methanobacterium thermoautotrophicum* [23], and in alkalophilic halobacteria [24]. The TE with cyclopentyl modifications were named TE1 to TE3, representing TE with from 1 to 3 cyclopentane modifications per biphytanyl moiety. The name used for an ether lipid was formed by adding the name of the fraction it was found in (GL, PL, Res) to the name of the structure (DE, DE1, TE, TE1 to TE3). Hence GLDE represents diether found in the glycolipid fraction.

Cluster analyses were performed on the lipid data using the cluster analysis option of the Systat statistical package. Individual lipids were expressed as μg/g dry weight [11] and normalized in the standard fashion, (value-average)/standard deviation [25]. The 1-Pearson's r correlation coefficient and Euclidean distance measures, and the centroid, average, and complete linkage methods were then applied.

4. RESULTS

The measure of eubacterial biomass (PLFA, Table 2) was much higher in the mud, red mat, and scrapings than in the flange samples. The red mat sample had the highest eubacterial biomass, 1056 μg PLFA/g dry weight. No archaebacterial ether lipids were detected in the red mat or at the top of the flange. The amount of PLFA decreased from top to bottom through the flange (samples from $\sim$ 2°C to $\sim$ 350°C), while the total ether lipids reached a maximum just below the top, then decreased.

The PLFA profiles are presented as the weight% of total PLFA and summarized as the weight% of monounsaturated, polyunsaturated, and cyclopropyl fatty acids. The ether lipids are presented as weight% of total ethers (Table 2). The chimney scrapings were very similar in PLFA profiles, within the range expected of replicate environmental samples. The 2 PLFA named as 'cy17:0' were isomers of each other differing in the position of the cyclopropyl moiety on the carbon chain.

The hydrothermal vent samples were clustered based upon the weight of membrane lipids, the PLFA and the GL, PL, and Res ethers, per gram dry weight of sample (Fig. 2). The greatest difference was found between the seawater exposed samples (top of flange, mud, red mat, and chimney scrapings) and the hydrothermal fluid-exposed and interior samples (the bottom and the 2 middle of the flange samples). Within the seawater exposed samples, there was a distinction found between those with and without hydrothermal vent fluids percolating through, and within the lower 3 flange samples there was a distinction

Table 2

Detailed lipid profiles of the Endeavor Ridge samples

Sample:	Mud	Red mat	Chimney scrapings		Flange			
Description:					Top			Bottom
Sample Code:	6042	6044	6067	6068	6195	6196	6197	6198
Polar lipid fatty acids (weight %)								
14:0	0.7	0.4	1.2	1.4	0.9	6.1	–	0.8
il5:0	0.4	0.2	0.5	0.5	0.6	1.5	0.2	2.2
al5:0	0.5	0.2	0.6	0.8	1.0	4.6	0.4	2.3
15:0	0.4	1.3	0.1	0.2	0.2	1.0	0.4	1.0
16:1w7c	23.0	11.9	17.1	15.5	39.5	0.5	2.8	6.0
16:1w7t	2.9	3.5	1.4	1.2	0.7	–	1.9	1.0
16:1w5c	2.5	1.3	0.9	1.0	1.9	1.0	7.5	0.6
16:0	8.8	19.0	16.9	16.5	14.5	10.2	13.6	27.7
10me16:0	0.3	0.2	0.2	0.4	–	3.0	0.2	3.4
il7:0	0.3	0.2	0.6	0.9	0.1	0.7	0.2	4.2
al7:0	0.7	0.3	0.4	0.8	0.3	0.8	1.2	3.2
cyl7:0	0.5	0.3	0.6	0.7	0.4	47.2	2.1	10.8
cyl7:0	–	–	–	–	–	17.6	9.6	0.8
17:0	0.5	0.7	0.5	0.7	0.1	0.6	8.1	1.1
18:2	1.1	0.3	0.9	0.6	–	–	–	–
18:2w6	1.1	0.3	3.4	3.2	2.2	–	0.3	0.7
18:3w3	0.6	0.3	0.4	0.3	1.7	0.3	0.8	0.7
18:1w9c	2.5	1.0	2.8	3.1	2.0	–	0.9	3.9
18:1w7c	16.2	15.5	22.3	19.1	21.8	0.4	7.7	11.9
18:1w7t	1.1	0.7	1.8	2.4	0.3	–	0.7	0.9
18:1w5c	0.9	0.4	0.5	0.5	0.1	0.5	15.4	0.3
18:0	2.1	1.0	6.0	5.1	0.8	1.8	17.2	9.8
cy19:0	0.1	0.3	0.2	0.3	–	1.6	8.8	4.0
20:4w6	3.3	–	3.3	3.6	1.2	–	–	–
20:5w3	18.4	32.7	4.7	5.9	6.8	–	–	–
20:3w6	0.3	0.1	1.9	1.9	0.4	–	–	–
20:4w3	0.6	1.4	1.2	1.0	0.2	0.5	–	–
20:2w3	0.4	0.1	0.8	0.7	0.2	–	–	1.1
20:1w13	2.2	0.5	5.0	5.8	–	–	–	–
20:1w7	2.9	1.5	1.3	1.7	–	–	–	–
20:1w9c	–	–	–	–	1.4	–	–	1.5
22:6w3	0.8	0.3	–	–	–	–	–	–
22:5w3	3.6	4.2	1.8	2.7	0.6	–	–	–
22:1w9c	0.2	0.1	0.6	1.3	–	–	–	–
Fatty acid functional groups (weight %)								
Monouns.	54.4	36.5	53.8	51.6	67.6	2.5	36.9	26.3
Polyuns.	30.1	39.6	18.4	20.1	13.4	0.8	1.1	2.5
Cycloprop.	0.7	0.6	0.8	1.0	0.4	66.4	20.5	15.6
Archaebacterial ether lipids (weight %)								
GLDE	–	–	33.5	36.2	–	11.0	14.5	56.1
GLDE1	–	–	–	–	–	–	–	31.8
GLTE	1.0	–	27.8	21.9	–	19.0	28.3	4.7
GLTE1	–	–	17.0	13.2	–	35.7	25.1	7.4
GLTE2	–	–	–	–	–	2.8	11.6	–
GLTE3	–	–	–	–	–	21.1	–	–
PLDE	53.7	–	3.8	4.0	–	2.4	–	–
PLDE1	–	–	0.2	0.2	–	1.2	–	–
PLTE	–	–	5.0	1.3	–	2.3	–	–
PLTE1	–	–	2.9	0.4	–	3.1	–	–
PLTE2	–	–	–	–	–	1.5	–	–
ResDE	45.4	–	9.7	22.7	–	–	20.5	–

Table 2 continued on next page

Table 2 (continued)

Sample:	Mud	Red mat	Chimney scrapings		Flange			
Description:					Top			Bottom
Sample Code:	6042	6044	6067	6068	6195	6196	6197	6198
Lipid content (μg/g dry weight)								
PLFA	247	1056	45	98	4.5	0.37	0.04	0.01
Ether lipids	17	–	4.2	7.2	–	6.3	0.12	0.17

Lipid nomenclature is described in MATERIALS AND METHODS.

made between those interior to the flange and the 1 exposed to hydrothermal vent fluids.

5. DISCUSSION

Due to limited sample availability from this environment only one pair of replicates was available, the chimney scrapings, precluding tests of statistically significant differences. Cluster analysis was used to relate the hydrothermal vent samples in a hierarchy of increasing differences, based upon their PLFA and ether lipid profiles. The 1-Pearson's r correlation coefficient metric used in the cluster analyses (the mathematical algorithm for calculating differences between samples, represented by lengths in the dendrogram, Fig. 2) was chosen since it clusters samples together based upon similarity in the pattern of lipid biomarkers (covariance). Hence, it gave exactly the same clustering pattern for the data whether μg/g dry weight or normalized data were entered. Normalization was required for the Euclidean metric because it arranges the samples in order of decreasing amount of lipid if μg/g dry weight data were entered.

Validation

There are 3 indications that the clusters obtained in Fig. 2 reflect the true relationships of the samples. The first is that the clustering of the hydrothermal vent samples organizes them in agreement with what we know about their environments. The 5 seawater-exposed samples (flange top, red mat, mud, and the chimney scrapings) were separated from the 3 interior and hydrothermal vent fluid exposed horizons of the flange. The red bacterial mat with its high eubac-

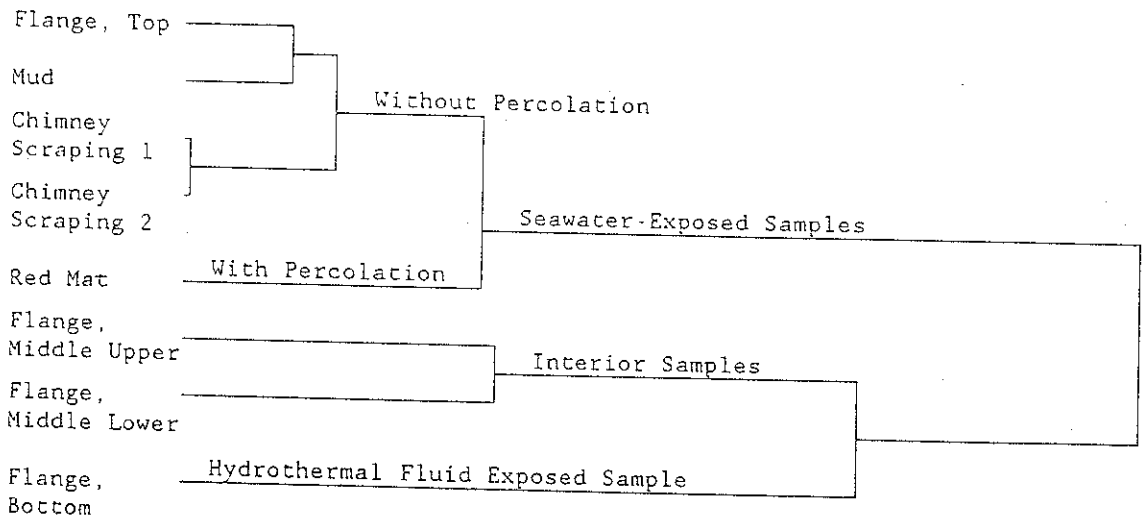

Fig. 2. Clustering of the hydrothermal vent samples according to the similarity of their lipid profiles. Horizontal distance represents degree of dissimilarity.

terial biomass of filamentous bacteria similar to *Beggiatoa* was separated from the other seawater-exposed samples. The second is that the only pair of replicate samples, the chimney scrapings, clustered much more closely than any other 2 samples. The third indication of the significance of the relations shown in the dendrogram (Fig. 2) is that the same clusters were obtained using the Pearson's r or Euclidean metrics, and the average, centroid, or complete linkage methods in all combinations, indicating that the clustering obtained was robust to changes in the clustering method.

Spacial heterogeneity

The hydrothermal vent system showed wide variation in microbial biomass per gram dry weight sample and in the composition of the communities making up that biomass. The sum of eubacterial and eukaryotic biomass as measured by PLFA varied by a factor of 4 between the mud and red mat samples, and by over 2 orders of magnitude within the transect of the flange (Table 2). The archaebacterial ether lipids showed the same high variation, but in a different pattern. They were undetectable in the sample with the highest PLFA, the red bacterial mat (detection limit $\ll$ 3.5 μg/g dry weight), and in the horizon of the flange with the highest PLFA, the top of the flange (detection limit $\ll 0.04$ μg/g dry weight). The horizon of the flange below the top contained 6.3 μg/g dry weight ether lipids. This represents a very high microbial density since the only room for organisms within the flange was the interstitial spaces between mineral grains.

The composition of the microbial communities sampled was also highly variable as revealed by the lipid profiles. For example, the weight% of the PLFA as polyunsaturates ranged from 0.8% to 39.6% and as monounsaturated PLFA varied from 2.5% to 67.5% over the 8 samples (Table 2).

Eubacterial polyunsaturated fatty acids

The seawater-exposed samples contained high proportions of polyunsaturated PLFA, ranging from 13.4 to 39.6 weight% of the PLFA, relative to the 0.8 to 2.5 weight% in the 3 lower horizons of the flange. Polyunsaturated fatty acids (PUFA) have been used to indicate the presence of eukaryotic or cyanobacterial input to environmental samples [26]. However, DeLong and Yayanos established that 9 barophilic heterotrophs (out of 11 isolates from 1000 to 11000 m deep) synthesized 20:5 or 22:6, incorporated them into membrane lipids, and that the degree of unsaturation increased with pressure [27]. The fatty acid 20:5 had also been reported in a marine *Flexibacter* [28]. In another study [29], 112 out of 7391 marine isolates tested produced 20:5. All of the 20:5-producing strains had been isolated from the guts of marine fish as opposed to invertebrate gut or sediment isolates. More research is required to determine which bacteria are capable of polyunsaturating fatty acids, and the role of PUFA in barophily. The proportions of the biomass contributed by eukaryotes and by barophilic eubacteria such as DeLong and Yayanos isolated can not be determined from this data since the prevalence of PUFA-containing eubacteria has not been determined. However, it is noted that the percent of PUFA found by DeLong and Yayanos [28] in barophilic eubacteria ranged from 7.2% to 36.7% compared with the 13.4% to 39.6% found in these seawater-exposed samples (Table 2), and that the most prevalent PUFA in the seawater-exposed samples was a 20:5 as found in deep-sea eubacteria, indicating a possible significant contribution by PUFA-containing eubacteria to the microbial biomass in the seawater-exposed samples.

Mud and red mat

The red bacterial mat had the highest eubacterial biomass (as measured by polar lipid fatty acids), the highest proportion of PUFAs, most of its PUFAs as 20:5, and no detectable ether lipids. Based upon the estimate of 10^{-4} mol of fatty acids per gram dry weight of a 'typical' eubacterium [17], the red mat is approximately 4% bacteria by dry weight. The high biomass of the red mat relative to the others indicate that there were hydrothermal fluids percolating through to support the biomass.

Filamentous bacteria of a white mat from a shallow-water hydrothermal vent [6] were found to closely resemble bacteria such as *Begiatoa,*

Thiothrix, or *Thioploca* by light and electron microscopy. Cells 20–50 μm in diameter resembling *Begiatoa* [30] were also common in the red mat. The most abundant PLFA in both samples were 16:1ω7c, 16:0, 18:1ω7c, and 20:5. In the shallow water sample 20:5 was the least abundant of the 4, while in the deep-sea hydrothermal vent sample it was the most abundant, in agreement with the experiments of DeLong and Yayanos [27] on the effects of pressure on 20:5 production. The significance of the color of the red mat is unknown.

Middle of flange

The lower 3 flange samples had from 15.6 to 66.4 weight% of their PLFA with cyclopropyl moieties, much higher than the from 0.4 to 1.0 weight% found in the seawater-exposed samples. The proportion of cyclopropyl PLFA (66.4%, Table 2) in the near top horizon of the flange is much higher than other environmental samples from, for example, the deep sea [10], estuaries [31], or methanogenic bioreactors [12]. Many eubacteria synthesize cyclopropyl fatty acids in stationary growth phase [32], or under toxic stress such as acid pH or solvents [33]. A personal database of 550 published eubacterial fatty acid profiles collected from the literature representing all 10 of the eubacterial phyla according to Woese [34] was examined for species with unusually high cyclopropyl fatty acid contents. Only 6 species with greater than 40 mol% cyclopropyl fatty acids were found: *Thiobacillus intermedius, T. acidophilum, T. novellus,* and *T. thiooxidans* [35], and *Serratia marcescens*, and *Escherichia coli* [36]. In a recently published book [37] containing 656 eubacterial fatty acid profiles, only 11 profiles had more than 40% of their fatty acids containing cyclopropyl moieties: *Pediococcus cerevisiae, Pe. halophilus, Lactobacillus buchneri, L. yamanashiensis, Pseudomonas halosaccharolytica, Alcaligenes eutrophus, Brucella melitensis, B. ovis, Megasphaera elsdenii, Thiobacillus concretivorus,* and *T. thiooxidans*. The Thiobacilli are the only autotrophs in this list of possible dominant members of this microbial community [38,39]. They fix energy by the oxidation of sulfide and sulfur with oxygen [40]. This could be explained by a horizon of the flange where reduced sulfur compounds from hydrothermal vent fluids and oxygen from seawater inter-diffuse, supporting a very dense community of metabolically stressed Thiobacilli. It is interesting that the highest archaebacterial biomass in the flange profile occurs in the same sample which contained the PLFA indicative of Thiobacilli. Whether these 2 groups have some co-metabolic relationship or simply live in adjacent horizons of the flange can not be resolved by this sampling.

Conclusions

The potential of lipid analysis to provide measures of in situ microbial biomass and community structure has been demonstrated. In the absence of sufficient replication for statistical tests of significant differences, cluster analysis provided information on the relative similarity of the samples analyzed, which directly related to the in situ conditions. More work is needed on the validation of archaebacterial ether lipids as measures of biomass and on sampling hydrothermal vents more rapidly in order to not change the microbial lipids by stressing the organisms. Given adequate sampling and replication of hydrothermal vent environments and lipid analysis of appropriate isolates for comparison, quantitative determination of the location of viable biomass and potential activities within a hydrothermal vent site will be possible.

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