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Bacterial community structure in sediments from Guaymas basin, Gulf of California, as determined by analysis of phospholipid ester-linked fatty acids

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Abstract. The Guaymas basin is a tectonically active basin located in the Gulf of California along the fault system and that extends from the East Pacific Rise to the San Andreas fault. Samples of rocks and sediments located near active hydrothermal mounds were collected during a scientific cruise (1992) with the French research vessel *Nadir* and the submarine *Nautile*. The present study was carried out to determine the distribution of bacterial biomass and community structure in this particular environment, which are also discussed in relation to the environmental parameters. Lipid analysis of samples recovered from a hydrothermal vent site of the basin was performed in order to estimate the extent and nature of the diversity of the microbial community. Examination of the ester-linked phospholipid fatty acids (PLFA) was conducted through the preparation of pentafluorobenzyl (PFB) esters and subsequent GC analysis with an electron capture detector (ECD). The results indicated that the eubacterial biomass was concentrated in the first centimeters of the oil-rich sediment and decreased drastically with depth. Eubacterial density as high as 10^9 cells/g dry weight sediment was determined in samples recovered from white or yellow–brownish bacterial mats. Conversely, a density as low as 10^4 cells/g dry weight was found on rocks. PLFA profiles were characterized by a large proportion of saturates. Saturated PLFA may be adjusted in response to environmental conditions. In situ temperatures ranged from 3°C to 150°C in sediments and pieces of rocks collected from smoking vents. Lipid signatures indicated that sulfur-oxidizing bacteria were present primarily in bacterial mats along with methanotrophs and sulfate-reducing bacteria. Astonishingly, preliminary analyses showed little indication of significant archaebacterial biomass.

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The Guaymas basin (Gulf of California) is a tectonically active basin unusual among other hydrothermal sites in that the ocean floor at the Guaymas basin consists of a deep layer of 300–400 m of unconsolidated sediments through which seawater circulates (Simoneit and Lonsdale 1982; Von Damm et al. 1985; Simoneit 1990). The hydrothermal fluid coming out of the sediments is either emitted from smokers or can reach the sea floor by slow percolation through these sediments, causing temperature gradients from 2°C at the surface to 180°C at a sediment depth of 80 cm. Furthermore, this organic-rich sediment contains large amounts of petroleum-like hydrocarbons as a consequence of pyrolysis of organic diatomaceous residues (Simoneit et al. 1979, 1988, 1992; Welhan and Lupton 1987; Bazylinski et al. 1988; Wehlan et al. 1988). Some hydrothermal petroleum fractions can provide suitable carbon sources for a great variety of microorganisms (Colby et al. 1977; Haber et al. 1983; Simoneit 1985; Bazylinski et al. 1989; Marchand et al. 1994).

Microbial communities can be described by quantifying those extractable cellular compounds that define the viable bacterial biomass and the community structure. Microbial biomarkers are chemical components of microorganisms that can be analyzed directly from the environment and be interpreted both quantitatively and qualitatively in terms of in situ biomass. Membrane lipids and their associated fatty acids are particularly useful eubacterial biomarkers as they are essential components of every living cell and have great diversity coupled with high biological specificity. Phospholipid ester-linked fatty acids (PLFA) have proved to be of great value in describing bacterial community structure in sediments, in understanding bacterial phylogenic and taxonomic classifications (Lechevalier 1977; White et al. 1979a; Guckert et al. 1985), or in microbially influenced corrosion studies (Dowling et al. 1988; Guezennec 1991). The use of PLFA from the polar lipid fraction greatly increases the selectivity of the analysis, as many contaminants or endogen-

eous storage lipids present in the neutral or the glycolipid fractions of the extractable lipids are not analyzed. Phospholipids are not found in storage lipids and have relatively rapid turnover in sediments. So the assay of these lipids gives a measure of the "viable" cellular biomass (White et al. 1979b). PLFA are usually analyzed as fatty acid methyl esters (FAME) by gas chromatography equipped with a flame ionization detector (FID) (Bobbie and White 1980). The electron capture detection (ECD) provides an useful alternative to the FID for trace analysis of microbial constituents when the sensitivity of the latter is insufficient (Odham et al. 1985; Daneshwar and Brooks 1988; Tunlid et al. 1989).

The extreme conditions in hydrothermal vent systems raise interesting questions about both survival and growth of microorganisms adapted to these particular physical and chemical parameters. It is anticipated that biotechnologically important microorganisms will be isolated from the deep-sea vents as sources of new thermostable enzymes, new biologically active molecules, and polysaccharide- and polyhydroxyalkanoate- (PHA) producing bacteria.

This study was carried out on samples recovered during a cooperative Mexican–French cruise operated in the Guaymas basin, Gulf of California, in 1992. The aim of this study was to describe the bacterial community structure in sediments, rocks, active smokers, and deposits collected in this active hydrothermal area. The distribution of bacterial biomass in the sediments and rocks is also discussed in terms of environmental parameters.

Materials and methods

Samples from sediments and rocks were collected during the oceanographic cruise performed by IFREMER with the research vessel *Le Nadir* and the submarine *Nautile* in the Guaymas basin (Fig. 1). Rocks and sediment cores were collected from different locations and kept at −80°C. Upon arrival at the lab, the samples were lyophilized and kept at −20°C until further analyses. The sediment samples were predominantly black with a strong odor of petroleum or hydrogen sulfide.

Lipid extraction

Lyophilized sediment samples were extracted by using a modified Bligh-Dyer method (Bligh and Dyer 1959; White et al. 1979b). The extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids by silicic acid column chromatography using appropriate volumes of chloroform, acetone, and methanol, respectively. The methanol fraction containing polar ether and ester lipids was dried under vaccum and stored under N_2 until further analysis. An aliquot of the polar phase was subjected to alkaline methanolysis and the unsaponifiable lipid removed by partition with hexane and water. Prior to methanolysis, nonanoic acid (C9:0) and tetracosanoic (C24:0) fatty acids were added as internal standarts. The partition was repeated twice to ensure a complete removal of residual hydrocarbon compounds. Free fatty acids were extracted with methylene chloride after acidification with HCl (Tunlid et al. 1989). The same overall procedure was carried out for the blank.

Preparation of PFB esters

The free fatty acids were dissolved in 30 µl of acetonitrile and derivatized with 100 µl of PFB-bromide (α-bromo-2,3,4,5,6-pentafluorotoluene, 3.5% in acetonitrile) and 100 µl of triethylamine (TEA) (Greving et al. 1978; Strife and Murphy 1984). After 15 min at room temperature, the PFB derivatives were extracted with isooctane and purified on a short disposable silicic column.

Glycerol ether analysis

A second aliquot of the polar phase was subjected to strong acid hydrolysis and the resulting core ether lipids digested with 55% HI solution for 18 h at 100°C. The resulting alkyl iodides were extracted with hexane and successively washed with $Na_2S_2O_3$ and Na_2CO_3 solutions (Kates 1964; Langworthy et al. 1983; Pauly and Van Vleet 1986). After extraction with hexane, the iodide derivatives were analyzed using GC equipped with a electron capture detector. Authentic glycerol diethers and diglycerol tetraethers were purchased from Sigma or isolated from cells of *Sulfolobus* sp.

Gas chromatography and gas-chromatography–mass spectrometry analyses

GC analyses were performed on a Carlo Erba (Rodano, Italy) HRGG 5300 gas chromatograph equipped with a fused silica column coated with a nonpolar phase (60 m × 0.2 mm ID; film thickness 0.25 µm) and an electron capture detector. Hydrogen was used as carrier gas. The electron capture detector was a ^{63}Ni foil (10 mCi) and the temperature program was as follows: 100°C for 1 min, 20°C min to 140°C, then 2°C min to 300°C.

GC-MS analyses were performed on a Carlo-Erba (Rodano, Italy) model 4165 gas chromatograph coupled to a quadrupole Nermag (Delsi) R10-10C mass spectrometer with an INCOS (Finnigan Mat, San Jose, CA, USA) data system. The sample was introduced through a Ross injector and the fatty acid separation was achieved on a DB-5 capillary column (30 m × 0.32 mm ID; film thickness 0.25 µm; J & W, Folsom, CA, USA) with helium as carrier gas (1 bar). The GC analysis was temperature programmed from 150°C to 320°C at a rate of 5°C min. Negative ion chemical ionization was performed using ammonia as reactant gas and conducted using both mass scanning from 100 to 700 amu (0.65 s/scan) and selected ion monitoring mode (SIM). Standard fatty acids were purchased from Sigma along with fatty acids of pure culture of *Vibrio natriegens* and *Desulfovibrio desulfuricans* and derivatized as PFB esters for GC-MS analysis. The position and the geometry of the double bond of monounsaturates were identified using the DMDS derivatives by the procedure previously described (Nichols et al. 1986).

Nomenclature

Samples. Samples were identified using the following system. GY indicates the origin of the samples (Guaymas) followed by a number related to the dive and to the location near the vents. The next two numbers are associated with a more precise location of the sediment cores, rocks or smokers. The letter following these numbers is related to the nature of the samples (T = sediment, R = rocks). Finally, the last number corresponds to the subbottom depth of sample in the sediment (1: 0–5 cm; 2: 5–10 cm; 3: 10–15 cm; 4: 15–20 cm; 5: 20–25 cm and 6: 25–30 cm). Thus GY06T6(1) corresponds to a sample of sediment collected during the dive 6 from 0–5 cm including the surface sediment.

Fatty acid nomenclature. A shorthand nomenclature is used which is in the form of numbers separated by a colon. The number before the colon indicates the carbon chain length and the figure after corresponds to the number of double bonds. The position of the double bond is defined by the symbol ω followed by the number of carbons from the methyl end. The prefixes i and a refer to iso and anteiso, respectively. The geometry of the double bonds is indicated by *cis* and *trans*.

Results

Sediments recovered during this cruise were characterized by high levels of hydrocarbons (up to 12,000 ppm) and hydrogen sulfide ranging from 0 to 300 ppb. Carbon and sulfur represented up to 20% of the sediments in some samples (Table 1). H_2S and SO_2 were also present in the water column and primarily in the emitted fluids at concentrations ranging from 0 to 300 ppb. Temperatures ranged from 3°C to 11°C on the sediment surfaces and increased

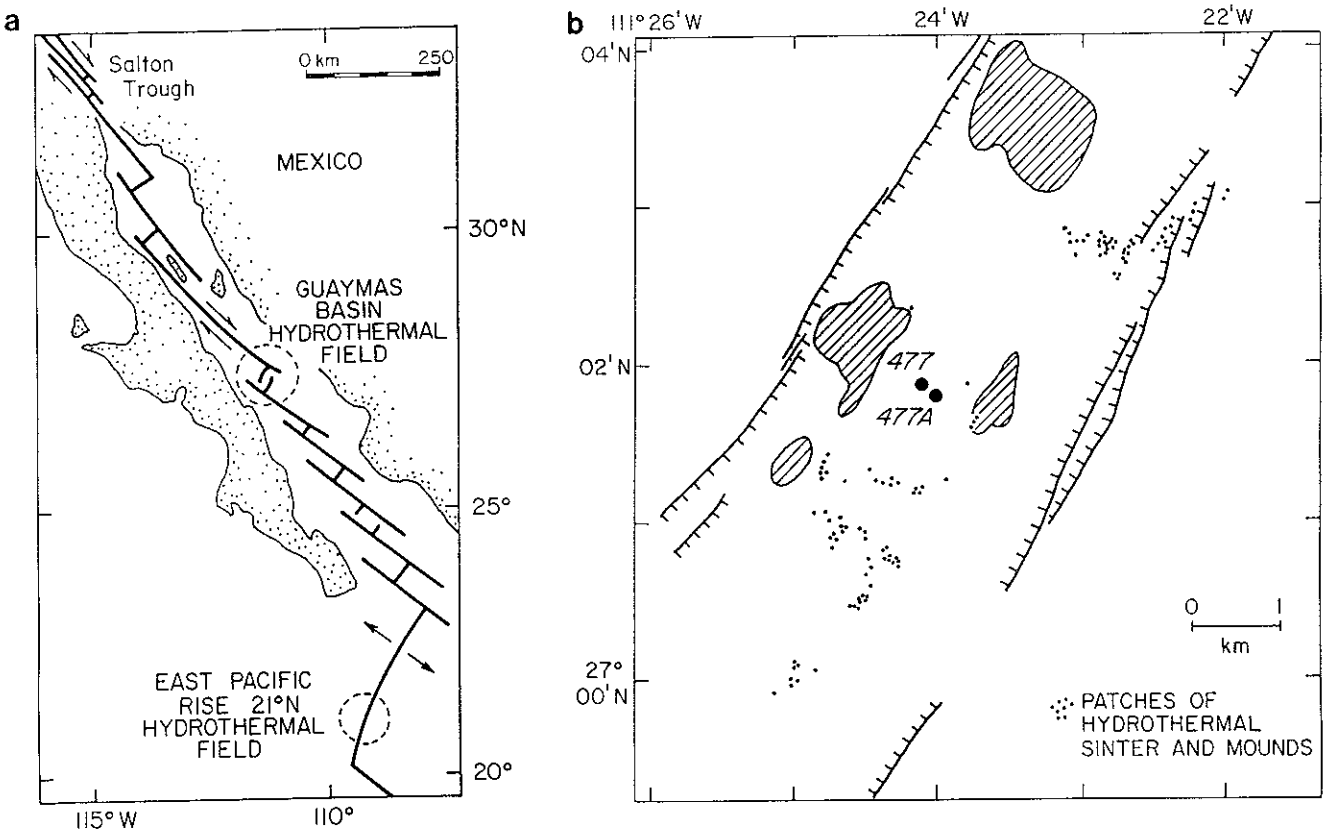

Fig. 1. Location of the Guaymas hydrothermal site in the Gulf of California (with permission of Dr B.R.T. Simoneit).

with depth of sediments, ranging from 67°C to 150°C from 20 to 30 cm.

Chromatography of PFB esters

Figures 2 and 3 represent the chromatograms of fatty acid pentafluorobenzyl esters extracted from sediments and rocks, respectively. The profiles are similar to that of the usual methyl ester derivatives with retention times being 1.6 times longer than the corresponding methyl esters.

NICI mass spectrometry of PFB esters

The mass spectra of C16:1ω7 and C16:0 PFB esters are shown in Fig. 4. The fragmentation produces the negative carboxylate ion from the loss of the PFB radical as base peak with little additional fragmentation.

Biomass

The biomass, expressed as total PLFA, ranged from 15 μg/g (dry weight) to 2 μg/g (dry weight) on the surfaces of the sediment cores and decreased with depth of the sediments (Fig. 5). In some samples, a significant biomass was present in the 5- to 10-cm sediment layer. In most cases very low biomasses were observed below 15 cm. Samples recovered from rocks contained low concentrations of PLFA, as low as 50 ng/g (dry weight).

Fatty acid profiles

The percent distribution of PLFA observed in the samples is shown in Tables 2 and 3. Identified PLFA included saturated, branched-chain, and monounsaturated fatty acids. No polyunsaturated or cyclopropane fatty acids were detected in the sediments. Fatty acid profiles indicated large percentages of saturated fatty acids with C12:0, C14:0, C16:0, and C18:0 predominating. Saturates accounted for 60–95% of the total fatty acids both in sediments and rocks. Longer chain fatty acids with more than 20 carbon atoms were present in low concentrations and primarily in deep sediments and rocks. Monounsaturated fatty acids were present in concentrations ranging from 4% to 31% with the C16:1ω7c and C18:1ω7c predominating. Unsaturations in ω8, ω6, and ω5 were confirmed for both the 16 carbon and 18 carbon fatty acids with concentrations ranging to 0 from 8%. The *cis* geometry was the only configuration detected in the monounsaturated fatty acids.

Branched-chain fatty acids were present as iso and anteiso C15:0 and C17:0 with the higher percentages observed in sediments recovered from the site GY12T2(2). iC14:0 and iC16:0 were also detected in some samples. Monounsaturated branched-chain fatty acids, as iC17:1ω7 and iC15:1ω7 were found in low concentrations in all sediments and rocks with the exception of surface and deep sediments from site GY12.

Concentrations of total PLFA were used to calculate approximate cell numbers of nonarchaebacterial microbes

Table 1. Description of samples analyzed from Guaymas basin (Marchand et al., 1994).

Sample	Sampling Site	Indications (T°C)	Carbon (%)	Sulfur (%)	Hydrocarbons $\mu g.g^{-1}$.dry.wt
GY06T6 (1)	Bacterial mat	Surface: 3–11°C	.49	2.3	Nd
		0–30 cm:	Nd	Nd	Nd
GY06T7	Boundary of the mat	Surface: 3–10°C	Nd	Nd	Nd
GY06T8	Bacterial mat	Surface: 3–10°C			
(1)	0–5 cm		3.61	1.4	2100
(2)	5–10 cm		2.09	2.0	3600
(3)	10–15 cm		5.92	2.1	12700
(4)	15–20 cm		2.29	2.3	2700
(5)	20–25 cm		1.66	2.7	1600
GY12T1	Bacterial mat	Surface: 6.7°C			
		30 cm: 59.7°C			
(1)			1.96	10.5	3200
(2)			1.39	4.4	2200
GY12T2	Bacterial mat	Surface: 3.5°C			Nq
		20 cm: 107°C			Nq
		30 cm: 95°C			
GY12T3	Bacterial mat	Idem	1.59	8.7	2850
GY14T1	Deposits with emanating fluids.	10 cm: 139°C			
		30 cm: 150°C			
GY09R1	Black smoker				
GY10R1	Black smoker				
GY12R1	Black smoker (Top)		2.64	25.8	5070
GY14R1	Smoker (Black area)	86°C	3.93	20.3	6600
GY14R2	Smoker (Yellow area)	Nd	Nd	Nd	Nd

Nd: Not determined. Nq: Not quantified.

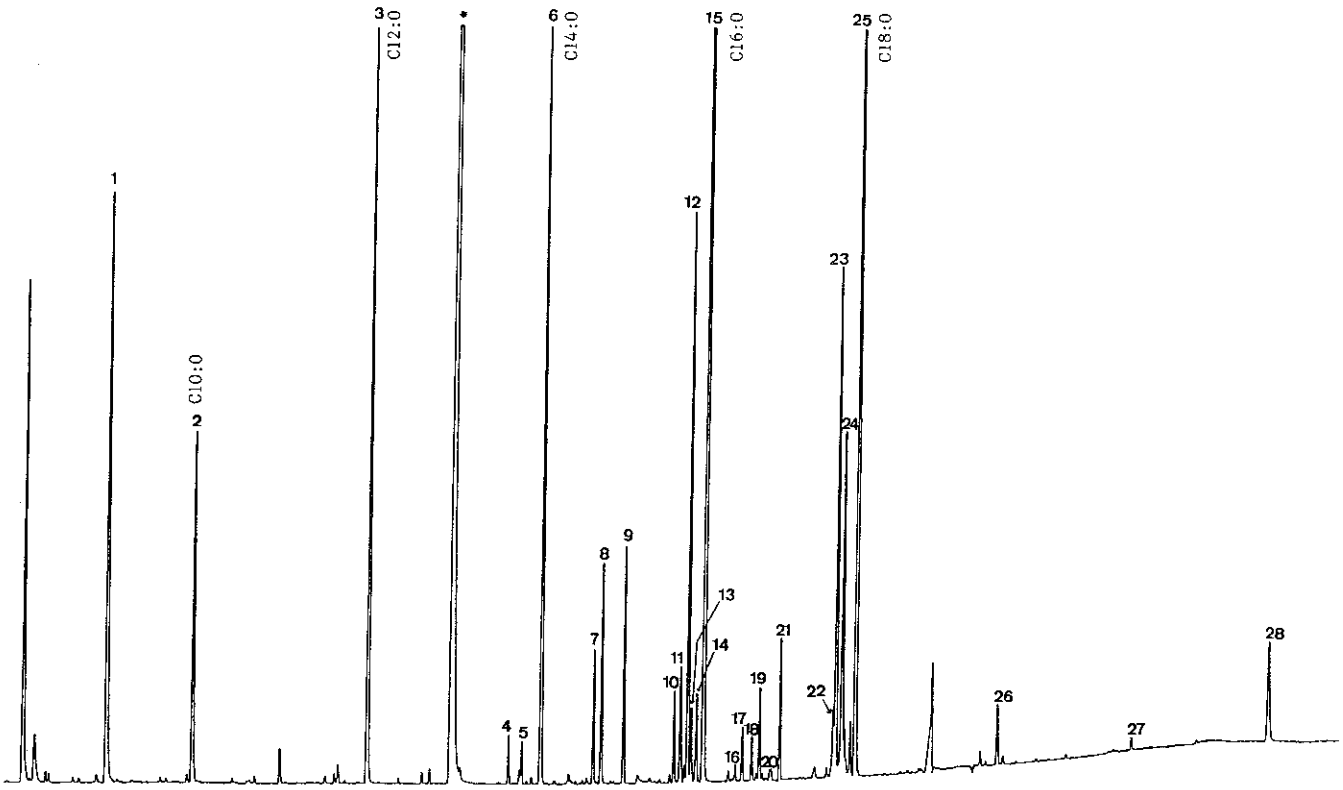

Fig. 2. Gas chromatogram of PFB esters of fatty acids recovered from hydrothermal sediment. 1: C9:0(IS); 2: C10:0; 3: C12:0; *: by-product; 4: C14:1ω7; 5: iC14:0; 6: C14:0; 7: iC15:0; 8: a C15:0; 9: C15:0; 10: iC16:0; 11: C16:1ω8; 12: C16:1ω7; 13: C16:1ω6; 14: C16:1ω5; 15: C16:0; 16: iC17:1ω7; 17: aC17:1ω7; 18: iC17:0; 19: a C17:0; 20: cyclo C17:0; 21: C17:0; 22: C18:1ω8; 23: C18:1ω7c; 24: C18:1ω5; 25: C18:0; 26: C20:0; 27: C22:0; 28: C24:0 (IS).

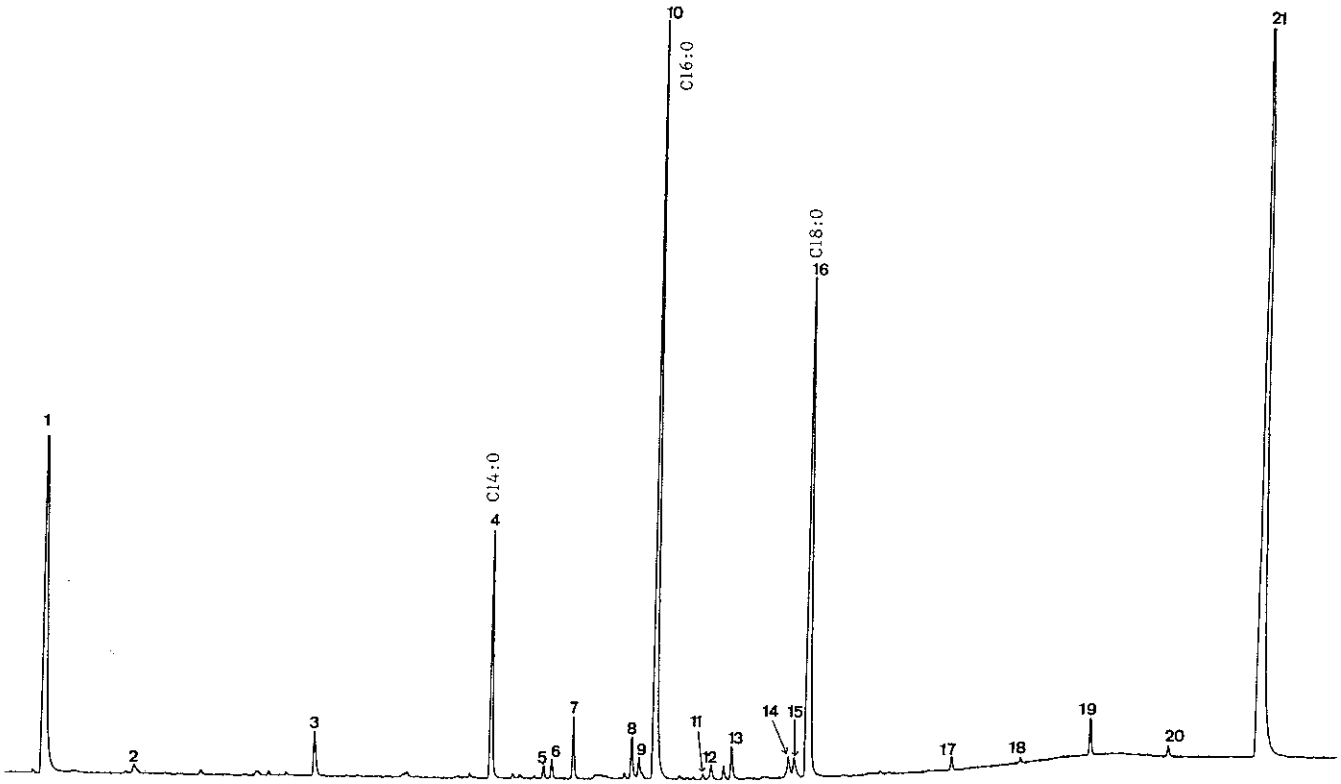

Fig. 3. Gas chromatogram of PFB esters of fatty acids recovered from a rock collected in the hydrothermal Guaymas basin. 1: C9:0(IS); 2: C10:0; 3: C12:0; 4: C14:0; 5: iC15:0; 6: a C15:0; 7: C15:0; 8: C16:1ω7c; 9: C16:1ω5; 10: C16:0; 11: iC17:0; 12: a C17:0; 13: C17.0; 14: C18:1ω7c; 15: C18.1ω5; 16: C18:0; 17: C20:0; 18: C21:0; 19: C22:0; 20: C23:0; 21: C24:0 (IS).

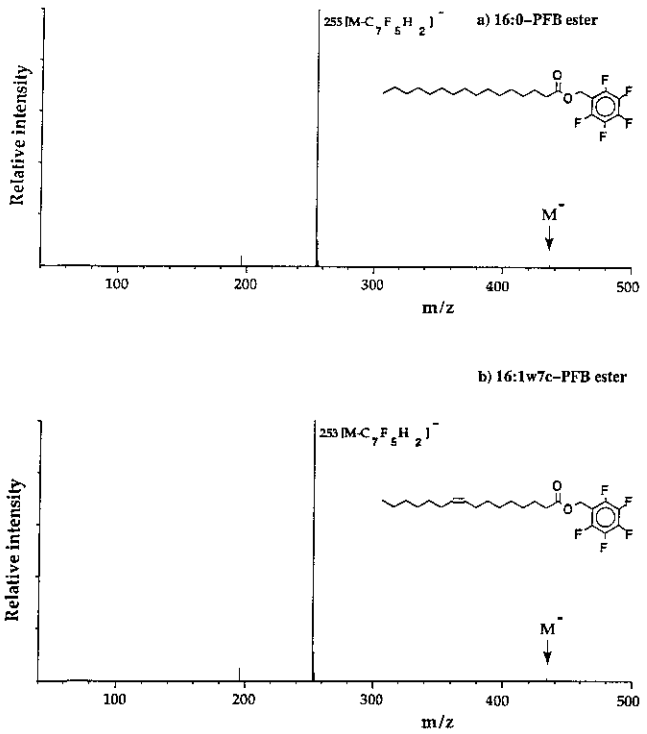

Fig. 4. Mass fragmentograms using NICI of selected PBF esters from an hydrothermal sediment: **(a)** C16:0 PFB ester; **(b)** C16:1ω7c PFB ester.

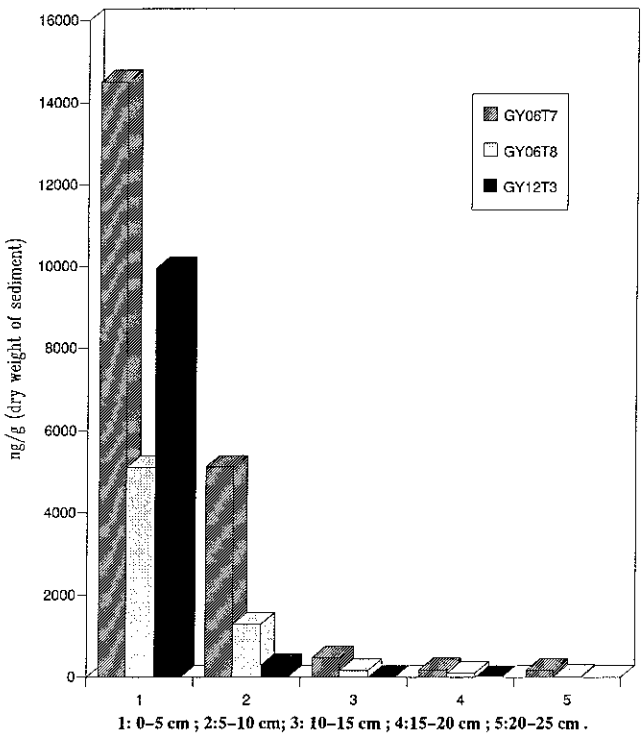

Fig. 5. Biomass, expressed as total PLFA per gram of dry weight, versus depth of the sediments.

Table 2. Fatty acid distribution (% total fatty acids) in hydrothermal sediments.[a]

Fatty acid	06T6(1)	06T6(2)	06T6(3)	06T6(4)	06T7(1)	06T7(2)	06T7(3)	06T7(4)	06T7(5)	06T7(6)	06T8(1)	06T8(2)	06T8(3)	06T8(4)
C10:0					0.54									
C12:0	16.72	19.01	19.52	0.56	1.85	5.08	1.36				10.95	15.94	1.06	1.23
C13:0	0.37	0.80	0.38				7.58	5.64	4.63		1.30	5.35		
iC14:0		0.1		0.08	0.65									
C14:0	10.83	13.3	9.6	5.81	9.97	13.87	19.54	15.44	15.83	25.59	4.74	12.79	5.79	3.87
iC15:1ω7		0.67			0.39								0.25	1.05
aC15:1ω7		0.47			0.34								0.81	1.12
iC15:0	0.47	0.44	0.38	0.2	7.08	2.68	3.34	2.97	2.26		0.82	0.54	0.54	0.40
aC15:0	1.01	1.91	1.07	0.35	11.77	5.1	4.96	3.80	3.41	1.56	1.96	1.62	0.58	0.37
C15:0	3.87	4.11	3.58	0.96	2.28	4.74	4.02	8.55	10.05	9.55	2.31	4.66	1.14	1
iC16:0		0.21		0.22			0.27							
C16:1ω7c	7.4	3.57	3.90	0.48	1.24	3.19	2.84	1.75		0.85	3.85	3.80	0.42	0.94
C16:1ω5	0.57	0.53	0.46		0.41	0.2		0.50	0.48		0.57		0.42	
C16:0	26.73	26.74	33.42	29.82	29.47	21.7	23.05	28.44	26.18	28.73	31.24	32.24	48.54	39.33
iC17:1ω7c	0.24	0.58	0.21											
aC17:1ω7c		0.28			0.41									
iC17:0	0.32	0.11	0.09	0.12	1.25	1.30	1.50	0.52	1.52	1.01	0.50	0.35	0.22	0.10
aC17:0	0.64	0.31	0.21	0.34	1.79	7.8	3.38	1.23	2.67		1.26	0.71	0.45	0.49
Cyclo C17:0		Tr										Tr		
C17:0	1.63	2.11	0.99	1.18	7.47	5.64	5.15	4.61	5.25	5.85	1.59	2.14	1.04	0.75
C18:1ω9														
C18:1ω7c	14.29	8.52	6.78	1.12	3.72	7.28	4.48	4.23	1.81	1.52	13.84	7.58	0.55	1.12
C18:1ω5		0.20	0.78	0.92		2.10	1.50	2.18	0.52		1.10		0.19	0.62
C18:0	14.91	14.82	17.08	37.16	19.37	19.32	15.86	17.17	16.92	18.95	24.07	12.28	36.04	47.61
C20:0		0.32	1.55	10.66					3.2	2.32			0.45	
C21:0								1.32	1.25	0.72				
C22:0		0.89		10.02			1.17	1.65	0.41	2			0.30	
C23:0									2.54	1.35			1.21	
Amount (ng/g dry wt)	2329	2538	525	25	14519	5121	475	186	176	218	5107	1290	164	104
Saturates	75.06	82.1	86.12	96.17	70.95	70.35	77.73	82.82	86.26	95.06	76.20	85.40	95.57	93.79
Branched	2.44	3.08	1.75	1.31	22.54	16.88	13.45	8.52	9.86	2.57	4.54	3.22	1.79	1.36
Unsaturates	22.5	14.82	12.13	2.52	6.51	12.77	8.82	8.66	3.88	2.37	19.26	11.38	2.64	4.85

[a] Each value is the mean of three determinations.

in the sediments. Using approximations described earlier (White et al. 1979a; Balkwill et al. 1988), i.e., 1.7×10^{-17} mol PLFA/bacterial cell, the calculated eubacterial biomass in the sediments and rocks was found to range from 10^9 cells/g (dry weight) in the surface sediments to 10^4 cells/g (dry weight) in the rocks.

Discussion

Sediment samples from Guaymas basin are characterized by a high content of sulfur compounds as hydrogen sulfide, SO_2, or sometimes sulfur-rich hydrocarbons. Temperature ranges from 3°C to 11°C near the surfaces of the sediments to 150°C at 30 cm depth. The temperature profile was not homogeneous from site to site, and the microbial ecology of the environments sampled was dominated by access to the oxidants (oxygen and sulfate) in the ambient seawater. Reductants, such as hydrothermal fluids and crude petroleum, were abundant.

The study of deep-sea environments by the lipid signature approach is appropriate, as more conventional methods of bacterial analysis require that the bacteria be grown on selected media (Baird and White 1985). In addition, culturing of microorganisms recovered from hydrothermal vents is difficult due to the temperature and pressure extremes under which these microorganisms naturally exist.

Although the methyl esters of fatty acids represent the most common derivatives used in GC-MS analyses, other derivatives such as halogenated fatty acids offer considerable advantages. The ECD provides a useful alternative to the FID for trace analysis of microbial constituents with increased sensitivity. The presence of large amounts of contaminants, such as petroleum-like and sulfur compounds, in the sediments from the Guaymas basin may interfere with the ECD response, and cleanup procedures along with fixed conditions are required. In addition, mass spectrometry, when operated in the selected ion mode (SIM), may increase the sensitivity.

The most effective electronegative derivatives of fatty acids and related compounds are the pentafluorobenzyl (PFB) and pentafluorobenzoyl (PFBO) esters. Utilization of negative ion for quantification of fatty acids as PFB ester derivatives allows for a detection limit of fatty acids in the range of picograms per milliliter or less (Strife and Murphy 1984; Sonesson et al. 1987; Daneshwar and Brooks 1988).

Biomass estimation

Bacterial cellular biomass expressed as total PLFA is significantly higher for the surface sediments and decreased with depth in the sediment. Conversion factors used in this study were applied on the basis of bacteria of the size of *E. coli*. Many organisms can regulate their fatty acid and lipid composition in response to environmental conditions in order to maintain the effective functioning of the biological membrane (Lechevalier 1977; Russel and Kukunaga 1990). However, this biomass was of the same order of magnitude as

Table 2. *Continued*

Fatty acid	06T8(5)	12T1(1)	12T1(2)	12T2(1)	12T2(2)	12T3(1)	12T3(2)	12T3(3)	12T3(4)	14T1(1)	14T1(2)	14T1(3)	14T1(4)	12T4(1)
C10:0											1.73		1.64	1.64
C12:0	1.99	2.92	2.30	7.87	5.63	7.18	0.34	4.14	3.62	1.95	4.53	2.05	4.33	3.66
iC14:0				0.19	0.12		0.20	0.18			0.42	0.13	0.42	0.47
C14:1ω7		1.16	0.36		0.91									
C14:0	7.69	9.89	10.85	9.26	10.73	4.85	10.91	11.88	11.47	11.22	11.63	9.18	10.98	11.22
iC15:1ω7		1.55	0.15	0.23	0.05	0.28	1.53	2.10	0.51		0.21	0.13	0.26	
aC15:1ω7	1.09	4.33	0.25	1.04	0.12	0.77	5.62	6.18	0.79		0.21	0.26	0.26	
iC15:0	1.01	4.30	2.11	3.53	4.59	1.44	0.68	0.60	0.71	0.80	1.18	0.48	1.11	0.84
aC15:0	1.09	7.48	3.75	6.18	7.01	2.41	1.07	0.95	1.18	1.19	3.34	0.81	3.48	1.21
C15:0	1.56	3.33	2.26	2.72	2.80	2.70	2.60	2.16	3.90	1.61	3	1.87	3.01	2.16
iC16:0				1.10	0.80	1.84	1.59	0.61						
C16:1ω8						1.70	0.80							
C16:1ω7c		1.63	3.30	4.92	3.73	11.02	4.48	0.91	3.66	0.60	1.39	0.70	1.48	0.89
C16:1ω5		6.03	2.55	2.30	1.61	3.40				0.60		0.20		
C16:1ω6						2.03	0.60							
C16:0	42.09	18.35	31.87	23.12	28.76	19.44	27.28	31.35	40.45	42.31	35.63	36.47	36.77	37.97
iC17:1ω7c		0.18	0.17	0.16	0.10	0.11	0.28	0.20			0.34	0.17	0.47	0.33
aC17:1ω7c		0.36	0.28	0.37	0.35	0.61	0.75	0.10			0.21	0.08	0.32	0.28
iC17:0	0.55	1.28	1.3	2.55	4.32	0.73	0.54	1.40	0.50	0.33	0.46	0.29	0.42	0.37
aC17:0	0.82	3.61	2.79	5.21	5.18	1.24	1.09	0.60	1.13	0.98	1.35	0.82	1.21	0.98
CycloC17:0							Tr							
C17:0	1.49	2.11	1.85	1.80	2.21	2.10	1.27	1.40	1.25	1.68	2.37	1.21	1.85	1.74
C18:1ω8		4.80	2.81	2.20		8.33	4.72							
C18:1ω7c		2.23	1.89	4.33	3.84	5.45	9.23	4.75	2.46	2.61	3.04	1.88	3.70	5.77
C18:1ω5		3.34	2.18	3.10		1.20	1.00							
C18:1ω6		1.00	1.00	1.14	1.36	3.20	1.10	1.25		0.74		0.79		
C18:0	36.28	17.12	25.98	11.39	12.91	17.05	18.51	23.56	23.90	32.17	29.06	36.08	28.29	30.47
C20.0	0.87			1.38	0.24	0.81	0.51	0.41	0.77	0.21		0.67		
C21:0				1.07	2.10		0.44	0.18	0.43	0.71		2.16		
C22:0	2.59			1.38	1.25	0.11	1.45	1.51	2.52	0.32		2.03		
C23:0	0.58			1.46	1.28		1.71	3.58	0.85			1.55		
Amount (ng/g dry wt)	19	15383	277	12783	3329	9953	299	30.7	7.50	3.5	2.40	43	1.90	2.10
Saturates	95.44	56.72	76.11	61.45	67.91	54.24	64.92	80.17	89.16	92.18	87.85	93.27	86.87	88.86
Branched	3.47	19.65	10.31	18.76	20.02	7.66	5.17	4.34	3.52	3.30	6.75	2.52	6.64	3.87
Unsaturates	1.09	22.53	13.58	19.79	12.07	38.10	29.91	15.49	7.32	4.52	5.40	4.21	6.49	7.27

those found in deep-sea sediments collected in the North Atlantic at a depth of 4820 m (Baird et al. 1985). Some surface sediments covered with white mats had a greater biomasses than that measured in other deep-sea sediments (Deming and Colwell 1982; Harvey et al. 1984). These mats are common to the deep-sea hydrothermal environments and are suggested to be of bacterial origin (Nelson et al. 1989). Bacterial biomass as high as 10^9 cells/g (dry weight) have been found within these mats (Wirsen et al. 1987; Jacq et al. 1989; Jannasch et al. 1989). Lower biomasses found in rocks and deep sediments are associated with higher temperatures up to 100°C.

Fatty acid profiles

Fatty acid analysis can provide an insight into the bacterial community structure. Chemotypes based on fatty acid patterns have already been proposed by several authors (Gillan and Hogg 1984; Findlay et al. 1990). Gram-positive bacteria as well as anaerobic gram-negative bacteria contain predominantly branched saturated fatty acids, while aerobic gram-negative bacteria are characterized by larger amounts of monounsaturated fatty acids.

Fatty acid profiles in sediments are characterized by large amounts of saturated fatty acids. C16:0 and C18:0 predominate while C10:0, C12:0, and C14:0 are also present in some samples in significant concentrations. Saturates as C16:0 are ubiquitous to all microorganisms, and other straight-chain fatty acids, particularly longer chains, are known to be present in both prokaryotes and eukaryotes.

Higher concentrations in monounsaturates were found at the surface of the sediments [GY06T6,T8(1), (2); GY12T1(1,2), T3(1)] associated with white bacterial mats. C16:1ω7 and C18:1ω7 fatty acids predominated among the monounsaturates. It has been reported that the monoenoic acids C16:1ω7 and C18:1ω7 can also be contributed by microalgae (Findlay et al., 1983). However, the absence of polyunsaturated fatty acids in these sediments suggest these fatty acids are of bacterial origin. Sulfur-oxidizing bacteria have been reported in the white bacterial mats on the sea floor near hydrothermally active areas. Membrane lipids of thio-oxidizing bacteria are usually characterized by large amounts of monounsaturated fatty acids with either C16:1ω7 or C18:1ω7 predominating (Larkin 1980; Jacq et al. 1989; Durand 1992). Filamentous bacteria, identified as members of the genus *Beggiatoa* by gliding motility and internal globules of elemental sulfur, have been identified in massive aggregations at the deep-sea hydrothermal vents of Guaymas basin (Nelson et al. 1989). These massive mats, observed during numerous dives, were several centimeters thick and occurred in patches interspaced by areas of black

Table 3. Fatty acid distribution (%) in rocks and hydrothermal deposits.[a]

Fatty acid	09R1	09R2	10R1	12R1	14R1	14R2	16R1
C12:0	2.62	2.58	2.34	1.48	3.11	2.78	2.32
C13:0							
iC14:0	0.26		0.15	0.59	0.25	0.51	0.16
C14:1ω7							
C14:0	11.54	11.94	8.20	7.21	9.33	11.86	12.95
iC15:1ω7	0.27	0.23	0.14				0.25
aC15:1ω7	0.31	0.36	0.21				0.19
iC15:0	1.07	0.52	0.89	3.31	0.38	0.63	0.82
aC15:0	1.31	0.12	0.76	5.20	1.80	1.18	1.06
C15:1ω6							
C15:0	4.59	2.74	1.84	1.07	1.97	2.77	3.25
iC16:0				0.50			
C16:1ω7c	2.42	2.63	1.73	3.17	2.11	1.82	2.17
C16:1ω6	1.41	1.54	1.05	0.57	1.61	0.47	1.14
C16:0	42.74	38.91	32.58	35.77	36.80	38.82	40.04
iC17:1ω7c	0.40	0.21	0.07		0.36	0.21	0.25
aC17:1ω7c	0.25	0.3	0.14		0.16	0.20	0.18
iC17:0	0.25	0.22	0.28	1.26	0.28	1.30	0.33
aC17:0	0.57	0.50	0.86	3.70	0.50	3.94	0.82
Cyclo C17:0							
C17:0	1.47	1.15	1.15	0.95	1.32	2.02	1.72
C18:1ω9							
C18:1ω7c	1.89	1.56	1.54	2.95	8.14	1.59	1.93
C18:1ω6	1.69	0.97	1.17	3.25	2.22	0.80	0.66
C18:0	24.94	33.52	40.39	29.02	24.74	27.90	28.80
C20:0			0.99		1.1	0.37	0.71
C21:0			0.56		0.7	0.25	0.25
C22:0			1.90		1.05	0.58	
C23:0			1.06		2.07		
Amount							
(ng/g dry wt)	48	1	4.4	1.80	26	50	1.9
Saturates	87.9	90.84	91.01	75.50	82.19	87.35	90.04
Branched	3.46	1.36	2.94	14.56	3.21	7.56	3.19
Unsaturates	8.64	7.80	6.05	9.94	14.60	5.09	6.77

[a] Each value is the mean of three determinations.

sediments. The occurrence of these microorganisms is supported by the constant H_2S supply by diffusion or percolation through the chimney wall, the particular temperature resulting from the mixing of reduced warm hydrothermal fluid and the oxygenated cold seawater, and the organic matter that is supplied by invertebrates (Jannasch 1985). The development of sulfide-oxidizing bacteria such as *Beggiatoa* species in a habitat depends strongly on the kind of anoxic–oxic transition zone. These filamentous gliding bacteria are typical gradient organisms that actively position themselves at favorable sulfide and oxygen concentrations (Jogersen 1982; Nelson and Jannasch 1983).

The presence of monounsaturates with the ω6 and ω8 double-bond positions in some of the samples recovered from hydrothermal sediments was interesting. However, these fatty acids were only observed in sites GY12 T1, T2, and T3 and were totally absent from the other sites (Table 2). Most samples were collected within bacterial mats associated with hydrocarbon contents up to 3200 μg/g dry wt and temperatures ranging from 3°C to 7°C at the surfaces of the sediments. Unfortunately, no quantitative data on hydrocarbon contents were available for GY12T2 even though the strong odor of samples suggested large amounts of those compounds in the sediments. Type I methanotrophs have been characterized by 16-carbon monounsaturates with the ω6 and ω8 unsaturations, while some type II methanotrophs are characterized by the ω6 and ω8 unsaturated 18-carbon fatty acids (Makula 1978; Martz et al. 1983; Urakami and Komagata 1984). Morever the C18:1ω8 fatty acid, present at significant concentrations in sites GY12T1(1) and GY12T3(1,2), is highly specific to *Methylosinus* and *Methylocystis* spp. (Bowman et al. 1993). The type II methane oxidizers seems to predominate over the type Is in the Guaymas sediments. Methylotrophs are widespread in soil and water, where they oxidize methane and methanol in aerobic conditions. Recently, eubacterial mesophiles that oxidized alkanes up to C_{20} anaerobically have been isolated (Aeckersberg et al. 1991). The Guaymas basin sediments are characterized by a large variety of C_1–C_8 hydrocarbons and concentrations of methane up to 7500 ng/g sediment were analyzed along with significant levels of other C_2–C_8 alkanes (Simoneit et al. 1979, 1988). In this oil-rich environment, the presence of these microorganisms is quite obvious, even if the overall methanotrophic biomass appears to be not very important.

Branched fatty acids have been found in significant concentrations at some stations associated with the surface sediments as well as in deep sediments. Even though iso and anteiso C15:0 and C17:0 are common to many bacteria, including some aerobic bacteria, anaerobic bacteria, and sulfate reducers (Boon et al. 1977; Edlund et al. 1985), a predominance of iso over anteiso appears to be characteristic of the sulfate-reducing bacteria (SRB) (Dowling et al. 1986). With the exception of site GY12T1(1), T3(2), and T3(3),

low concentrations of branched-chain monounsaturates were found in the sediments. With the exception of *Desulfovibrio gigas*, iso and anteiso C15:1ω7 and C17:1ω7 were reported as characteristic fatty acids of *Desulfovibrio* species (Parkes and Taylor 1983). Previous studies performed on sulfate reducers isolated from the deep-sea environment have demonstrated the presence of these markers for *Desulfovibrio* species (Elsgaard et al. 1991, 1995). A low content of iC17:1ω7 fatty acid has been found in some *Desulfovibrio* species associated with high content of the aC15:0 fatty acid. Strains of this group have been isolated from fresh, brackish, and seawater sediments (Vainshtein et al. 1992). In reduced environments, C17:1ω6 and C17:1ω8 fatty acids are characteristic of *Desulfobulbus* species and *Desulfotomaculum* species, respectively. The conjunction of 10Me-C16:0 and cyclo-C17:0 has been demonstrated in *Desulfobacter* species (Dowling et al. 1986). In our analyses, none of these specific lipid biomarkers were present. An oxidation of methane or higher saturated hydrocarbons by sulfate-reducing or other anaerobic bacteria is controversial (Novelli and Zobell 1944), but saturated hydrocarbons can indirectly serve as substrates for anaerobic bacteria under intermittent aerobic/anaerobic conditions. Thus, aerobic hydrocarbon- and petroleum-oxidizing bacteria are known to release monocarboxylic acids that may serve under anaerobic conditions as substrates for sulfate reducers (Atlas and Bartha 1973; Rozanova and Nazina 1982). The presence of hydrogen sulfide in these samples along with fatty acid biomarkers suggested that sulfate-reducing bacteria recovered from the vents contributed significantly to the bacterial input to the hydrothermal sediments.

Archaebacteria do not contain fatty acyl chains in their phospholipids. They are characterized by the presence of phytanyl chains and diphytanyl chains, which have repeating methyl groups and are joined to glycerol by an ether linkage. Ether lipids were analyzed in our studies as iodide derivatives and subsequent GC analysis using an electron capture detector. Analyses of glycerol ether lipid in sediments revealed the presence of small amounts of isoprenoid ethers with C_{20} predominating. Low amounts of C_{20} isoprenoids are in agreement with previous studies performed on the Guaymas basin showing low levels of polar ether lipids, indicative of active archaebacteria (Holzer et al. 1988). Although the number of samples considered in this study was limited, analyses performed on samples recovered from each location indicated low amounts of C_{20} and C_{40} and consequently low archaebacterial biomass.

Fatty acid profiles must also be considered in terms of environmental parameters. Deep-sea hydrothermal vents of the Guaymas basin are characterized by high pressure, high gradients of temperatures, and high concentrations of hydrocarbons (Simoneit 1990). In the cold sediments recovered from this particular site, no monounsaturates or polyunsaturates containing 20 or more carbons have been detected. High percentages of saturates were found in the deeper sediments associated with higher temperatures, while monounsaturates were significantly higher in the surface sediments associated with lower temperatures. Environmental parameters are well known to influence fatty acid biosynthesis in bacteria in order to maintain the fluidity and the physical integrity of the membrane. Eubacteria respond to lower temperature by increasing the levels of unsaturation, i.e., conversion of saturated to monounsaturated fatty acids. High temperatures induce the formation of saturates. Cyclization along with changes in the carbon chain length and configuration of monounsaturates are also considered to be a response to specific environmental conditions (Chapman et al. 1966; Guckert et al. 1986). Bacterial membrane structure is also sensitive to pressure pertubation. The low temperatures and high hydrostatic pressures have lipid-solidifying effects (Mac Elhaney and Souza 1976; Wirsen et al. 1987; Russel and Kukunaga 1990; Kamimura et al. 1993). Previous work on hydrothermal vent bacteria reported increases in the monounsaturated fatty acids as a function of increasing pressure (Delong and Yayanos 1986). Large amounts of C20:5 fatty acid have been determined in a psychrophilic and barophilic deep-sea bacterium (Yayanos et al. 1982) and were related to the maintenance of membrane fluidity. Other authors reported the presence of large amounts of C20:5ω3 in muds and red mats collected from the Endeavor Ridge hydrothermal vent site (Hedrick et al. 1992). In that, sediments from the Guaymas basin must be considered both in terms of specific bacterial contribution and also in terms of bacterial response to extreme environments.

Conclusion

Even though the use of pentafluorobenzyl esters of fatty acids requires more precautions than the corresponding methyl esters, this approach was successfully applied to samples with low bacterial biomass, such as those found in the sediments recovered from the Guyamas basin. Due to the very high sensitivity and selectivity of the ECD, fatty acids as low as 10^{-15} mol can be determined. PLFA analyses indicated bacterial biomasses ranging from 10^9 cells/g (dry weight) for the surface sediments to 10^4 cells/g for deeper sediments and rocks.

Despite the lack of information related to the physical and chemical parameters (redox, pH, S^{2-} and SO_4^{2-}) of the samples collected during this cruise, an attempt to evaluate both the bacterial biomass and community structure has been performed on sediments and rocks from the Guaymas basin. Hydrothermal sediments recovered from the petroleum-rich Guaymas basin are characterized by a bacterial biomass primarily located near the surface, with sulfur-oxidizing bacteria predominating. Along with these bacteria, methanotrophs and sulfate reducers appears to be present in this environment. The occurrence of specific biomarkers supports the presence of these microorganisms in the sediments. The surface sediments are covered with sulfur-oxidizing bacteria common to many hydrothermally active sites. In deeper sediments or associated with the white bacterial mats, methanotrophs were also present in significant concentrations. The occurrence of these microorganisms is related to the large amounts of C_1–C_8 alkanes in the sediments. The sulfate-reducing bacteria were substantiated by the physical conditions of the surface sediments. Reducing conditions, as expressed by the hydrogen sulfide concentration in the water column, favors growth of anaerobic microorganisms and primarily sulfate-reducing bacteria. Archaebacterial biomass, analyzed as iodide ether glycerol derivatives, showed little indication of the presence of these microorganisms in the sediments.

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