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Lipid biomarkers and carbon-isotopes of modern travertine deposits (Yellowstone National Park, USA): Implications for biogeochemical dynamics in hot-spring systems

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Abstract—Lipid biomarkers and ^{13}C fractionation patterns were used to understand the dynamics of carbon cycling during microbial metabolisms in different environments of travertine precipitation (called facies) at Spring AT-1 on Angel Terrace in the Mammoth Hot Springs complex of Yellowstone National Park, USA. Microbial mats that encrust travertine deposits were collected for analyses of lipid biomarkers and carbon isotopes along the continuous drainage outflow system of Spring AT-1. The spring water exhibits a continuous temperature drop from 71°C in the vent at top to 24°C in the distal slope at bottom. Phospholipid fatty acids (PLFA) and glycolipid fatty acids (GLFA) exhibit distinctly different compositions in each of the facies, which are consistent with partitioning of the bacterial 16S rRNA gene sequences in the Spring AT-1 travertine facies (Fouke et al., 2003).

The $\delta^{13}C$ composition of total biomass within the microbial mats decreases from −16.1‰ in the vent to −23.5‰ in the distal slope. However, lower values occur in the pond (−26.0‰) and the proximal slope (−28.0‰) between the vent and the distal slope. Isotopic compositions of PLFA and GLFA have variations similar to those of total biomass. The average $\delta^{13}C$ values of PLFA are −12.4 ± 5.2‰ (n = 10 individual fatty acids, same below) in the vent, −33.0 ± 3.1‰ (n = 11) in the pond, −33.7 ± 3.8‰ (n = 16) in the proximal slope, and −22.4 ± 3.4‰ (n = 10) in the distal slope; the average $\delta^{13}C$ values of GLFA are −19.6 ± 3.0‰ (n = 3) in the vent, −30.4 ± 4.7‰ (n = 8) in the pond, −36.9 ± 2.8‰ (n = 12) in the proximal slope, and −27.9 ± 3.1‰ (n = 13) in the distal slope. In particular, fatty acids in the vent are enriched in ^{13}C relative to the total biomass, which is consistent with the notion that the biosynthetic pathways of the extant microbial community in the vent may be dominated by *Aquificales* using the reversed tricarboxylic acid cycle. Fractionations between fatty acids and total biomass in the pond, the proximal slope and the distal slope suggest the involvement of other biosynthetic pathways for CO_2 fixation by extant microbial populations. The results indicate that lipid biomarkers provide valuable information on the changing diversity and activity of microbial communities in different depositional environments. Carbon-isotope fractionations, on the other hand, can provide insight into the operating biosynthetic pathways associated with different organisms in the changing environment. This integrated approach may serve as a powerful tool for identifying functional metabolism within a community and identify shifts in microbial community structure in modern hot-spring systems. *Copyright © 2004 Elsevier Ltd*

Keywords: Phospholipid fatty acids, glycolipid fatty acids, lipid biomarkers, stable carbon isotopes, *Aquificales*, cyanobacteria, green sulfur bacteria, green non-sulfur bacteria, Angel Terrace, Mammoth Hot Springs, Yellowstone National Park

INTRODUCTION

Lipid biomarkers provide quantitative information about the structure of extant microbial communities without the need for culturing and isolation (White, 1988). Lipids are also one of the most useful biochemical measures of in situ interactions between microbial species and their environments because lipid compositions can indicate temperature-, redox-, stress-, or nutritional conditions (Ray et al., 1971; Fork et al., 1979; Ringelberg et al., 1989; Jahnke, 1992). Furthermore, bacteria commonly have ester-linked lipids and some bacterial species contain additional non-phytanyl ether lipids (Langworthy et al., 1983; De Rosa et al., 1988; Huber et al., 1992). Archaea, on the other hand, have phytanyl ether lipids (Langworthy et al., 1983). Thus, analysis of lipid structures permits identification of these two microbial domains in unknown environmental samples.

Different microorganisms have different biosynthetic pathways. In particular, autotrophic microorganisms can use several pathways for CO_2 fixation (Ratledge and Wilkinson, 1988). A variety of phototrophs, which include cyanobacteria, use the Calvin cycle, which requires the key enzymes of ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) and ribulokinase. Methanogens and several groups of known chemolithoautotrophic bacteria use the acetyl-CoA pathway for CO_2 fixation (Fuchs, 1989; Vorholt et al., 1995). Other bacteria, such as *Hydrogenobacter* and *Aquifex* are known to use the reversed tricarboxylic acid (rTCA) cycle for CO_2 fixation (Fuchs, 1989; Beh et al., 1993). Another pathway is the 3-hydroxylpropionate (HP) cycle, which fixes HCO_3^- rather than CO_2. This pathway has been observed in green non-sulfur bacteria (Strauss and Fuchs, 1993) and Archaea (Menendez et al., 1999). It is suggested that the latter three pathways require

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less ATP for biosynthesis than the Calvin cycle (Karl, 1995); thus they may have significant ecological and evolutionary implications for life that survives under extreme conditions.

Different biosynthetic pathways may result in distinct isotope-fractionation patterns. Microorganisms using the Calvin cycle normally produce fractionations about −26‰ between biomass and CO_2 (O'Leary, 1988; Sirevåg et al., 1977; Madigan et al., 1989; Sakata et al., 1997; Popp et al., 1998); those using the acetyl-CoA pathway can produce fractionations as low as −36‰ (Preuß et al., 1989). On the other hand, the rTCA cycle and the 3-HP pathway can produce fractionations greater than (more positive) −17‰ between biomass and CO_2 (Quandt et al., 1977; van der Meer et al., 2001; Zhang et al., 2002; Londry et al., 2004).

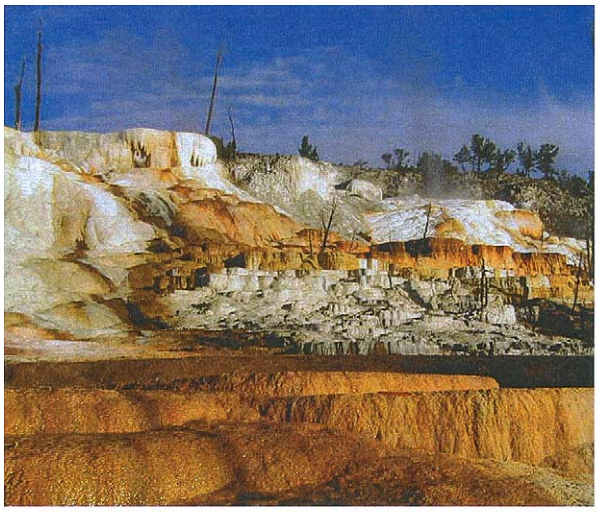
Distinct fractionations have also been observed between lipid biomarkers and biomass for CO_2 fixation pathways used by different organisms. For example, fractionations associated with the Calvin cycle range from −7.6 to −9.9‰ between fatty acids and biomass for a cyanobacterium (Sakata et al., 1997). Fractionations associated with the 3-HP pathway range from 0.2 to −1.9‰ for a green non-sulfur bacterium (van der Meer et al., 2001). Fractionations associated with rTCA cycle in *Aquificales* and green sulfur bacteria range from 2.0–16.0‰ with fatty acids being enriched in ^{13}C relative to biomass (van der Meer et al., 1998; Jahnke et al., 2001; Zhang et al., 2002); however, a fractionation value of −11.8‰ has recently been reported for a sulfate-reducing bacterium (*Desulfobacter hydrogenophilus*) using rTCA for CO_2 fixation (Londry et al., 2004). These results illuminate the potential for using lipid biomarkers and molecular stable isotopes to understand diverse CO_2 fixation pathways and their effects on carbon flow associated with or mediated by microorganisms.

Biomarkers have been widely utilized as indicators of past biologic activities on Earth (Mackenzie et al., 1982; Simoneit, 1986; Ward et al., 1989; Brassell, 1992; Summons et al., 1996; Freeman, 2001; Hayes, 2001; Simoneit, 2002). The study of lipid biomarkers in hot-spring systems has particular implications for the evolution of life because the earliest common ancestor of life on earth might have evolved from a thermophilic organism (Woese, 1987; Barns and Nierzwicki-Bauer, 1997).

The present study focused on Spring AT-1 on Angel Terrace in the Mammoth Hot Springs complex of Yellowstone National Park, USA. The depositional facies and associated water chemistry, mineralogy and phylogenetic diversity have been characterized (Fouke et al., 2000, 2003). Our goal in this study was to delineate the carbon cycling pathways in each of the five different travertine depositional facies that occur along the outflow of Spring AT-1, and to use Spring AT-1 as a model for understanding microbial-biogeochemical dynamics in modern and perhaps ancient hot-spring travertine deposits.

Site Description and Previous Studies of Chemistry and Microbiology

Angel Terrace is located in the New Highland Terrace area within the Mammoth Hot Spring complex (Allen and Day, 1935; Bargar, 1978; Fouke et al., 2000). The hydrologic system at Angel Terrace is dynamic with multiple vents appearing, sealing, and reopening at a frequency of months to tens of years (Friedman, 1970; Bargar, 1978; Sorey and Colvard, 1997).



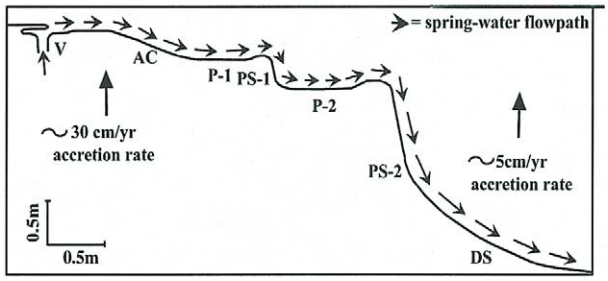


Figure 1. (Upper) Photograph of Angel Terrace Spring AT-1 (taken on July 7, 2001). (Lower) A cross section indicating facies distribution (Table 2) and flow directions (from Fouke et al., 2000).

Fouke et al. (2000) characterize Angel Terrace deposits into multiple facies based on water temperature, chemistry, mineralogy, physical structure, and travertine accumulation rates. These facies include the vent (71–73°C), the apron and channel (69–74°C), the pond (30–71°C), the proximal slope (28–54°C), and the distal slope (28–30°C) (Fig. 1). As the spring water cools, CO_2 degasses and the water chemistry changes from pH ~6 in the vent to pH 8 or higher in the distal slope (Fouke et al., 2000). As a result, total dissolved inorganic carbon (DIC) decreases from ~190 mg/L in the vent to ~70 mg/L in the distal slope. On the other hand, the carbon-isotope composition of DIC increases from ~−1‰ in the vent to ~5‰ in the distal slope (Fouke et al., 2000).

Changes in the microbial communities inhabiting the outflow drainage systems of Angel Terrace are first described by Farmer and Des Marais (1994) and substantiated by Fouke et al. (2003). In particular, Fouke et al. (2003) observe that the phylogenetic diversity of bacteria is strongly partitioned among five different travertine depositional facies in the surface drainage system of the Angel Terrace (Table 1). For example, the vent is dominated by bacterial sequences affiliated with *Aquificales*; whereas, other facies are dominated by sequences affiliated with bacteria including Bacteroides, Cytophagales, Flexibacter, Firmicutes, OP11, OP8, Planctomycetales, Proteobacteria, Thermus-Deinococcus, and Verrucomicrobia (Table 1). The relative proportions of gene sequences for photo-

Table 1. Percentage of gene sequences for chemolithoautotrophs (*Aquificales*), photoautotrophs (green-sulfur bacteria, green non-sulfur bacteria, and cyanobacteria), and other bacterial species recovered from surface environments (facies) at Angel Terrace travertine in Yellowstone (Fouke et al., 2003).

	Facies				
Division	Vent	Apron and channel	Pond	Proximal slope	Distal slope
Aquificales	91	25	7	15	5
Green nonsulfur bacteria	3	4	1	16	ND
Green sulfur bacteria	<1	4	1	9	3
Cyanobacteria	1	ND	9	2	18
Other bacteria[a]	3	67	72	42	74

ND = not detected.

[a] Other bacteria include BCF (Bacteroides, Cytophagales, and Flexibacter), Firmicutes, OP11, OP8, Planctomycetales, Proteobacteria, Thermus-Deinococcus, and Verrucomicrobia in different proportions in each facies, which are either heterotrophs or undetermined.

autotrophs such as green sulfur bacteria, green non-sulfur bacteria, and cyanobacteria vary from facies to facies (Table 1).

Fouke et al. (2003) also observe that there is little down stream transport of bacterial cells at the Angel Terrace Spring AT-1. This indicates that the distribution of microorganisms may be directly controlled by the physical and chemical conditions of each depositional facies. Results of this study support this hypothesis and indicate that Spring AT-1 would be an excellent natural laboratory for studying biogeochemical dynamics in the hot spring environments. Specifically, lipid biomarkers and their molecular isotope compositions would allow us to delineate pathways for CO_2 fixation within a mat community and the shifts in microbial community structure from facies to facies.

MATERIAL AND METHODS

3.1. Sample Collection

Microbial mats or fresh travertine accumulations (carbonate rocks in the vent and the apron and channel) were collected at Angel Terrace Spring AT-1 during daylight-hours in July 2001. The samples were collected from the same spring reported by Fouke et al. (2003) but not from the exact locations. This is because the flow of the spring water is dynamic and changes pathways frequently, which results in the formation of the same facies in different locations and times. The temperature in the same facies, however, is comparable between locations, which makes this study relevant to that of Fouke et al. (2003).

In each facies, the mat material was physically peeled off and collected into 50-mL sterile plastic tubes using forceps. Non-calcified mats grew very thin in the vent and in the apron and channel because of high rates of travertine precipitation. As a result, a piece of the fresh carbonate rock was cut using a knife and transferred into the plastic tube using forceps. Two samples (A and B) were collected from each of the five selected facies (Table 2). Water temperature and pH were measured at the time of collection using a combination probe. Samples were stored on ice in the field (<10 h) and frozen immediately on dry ice at the end of the day. Samples were transported to the home institution on dry ice and lyophilized before lipid extraction and carbon-isotope determinations.

3.2. Lipid Extraction and Fatty Acid Identification

Freeze-dried materials were extracted following White et al. (1979) and (Zhang et al., 2002, 2003). The procedure employed a single-phase organic solvent system comprised of chloroform, methanol, and aqueous 50-mM phosphate buffer (pH 7.4) in the ratio of 1:2:0.8 (v:v:v). In this study, samples were powdered and a portion of the material (0.34–1 g) was used for lipid extraction. After extraction overnight, chloroform and nano-pure water were added to the extractant in equal volumes, which resulted in a two-phase system. The lower phase contained the lipids, which were collected and fractionated on a silicic acid column into neutral lipids, polar lipids (phospholipids), and glycolipids (Guckert et al., 1985). The phospholipids and glycolipids were treated using a mild alkaline methanolysis to produce fatty acid methyl esters (FAMEs), namely the phospholipids fatty acids (PLFA) and glycolipid fatty acids (GLFA).

An Agilent 6890 series gas-chromatograph (GC) interfaced to an Agilent 5973 mass selective detector was used for the identification of FAMEs. The GC used a 60-m non-polar column (0.25-mm I.D., 0.25-μm film thickness) and the injector and detector were maintained at 230°C and 300°C, respectively. The column-temperature was programmed from 60°C for 1 min, ramping at 20°C/min to 150°C and holding for 4 min. This was followed by ramping at 7°C/min to 230°C and holding for 2 min, and finally ramping at 10°C/min to 300°C and holding for 3 min. Mass-spectra were determined by electron impact at 70 eV. Methyl Heneicosanoate was used as the internal standard. PLFA and GLFA were expressed as equivalent peaks against the internal standard. Double-bond positions of monounsaturated FAME were determined by GC-mass-spectrometry (MS) analysis of the dimethyl

Table 2. Water chemistry and mineralogy of Angel Terrace Spring AT-1.

	Facies				
Parameter	Vent	Apron & Channel	Pond-2	Proximal Slope-2	Distal Slope
Temp. (°C)[a]	71.4 ± 0.5	66.8 ± 0.3	50.9 ± 0.6	46.8 ± 0.5	24.3 ± 0.0
pH[a]	6.3 ± 0.1	6.7 ± 0.1	7.5 ± 0.0	8.1 ± 0.0	8.4 ± 0.0
DIC (mg/L)[b]	192	111	135	104	75
$\delta^{13}C_{DIC}$ (‰)[b]	−0.9	1.0	0.4	1.6	4.6
$\delta^{13}C_{CO2\text{-}d}$ (‰)[c]	−5.4	−3.9	−5.9	−5.1	−4.5
$\delta^{13}C_{TB}$ (‰)	−16.1 ± 4.2	ND[d]	−26.3	−28.0 ± 0.6	−23.5 ± 0.0
Flow rate[b]	High	High	Low	Moderate	Low
Mineralogy[b]	Aragonite	Aragonite	Aragonite/Calcite	Aragonite/Calcite	Calcite
Mat color	Tan-gray travertine	Pink-white	White/green	Dark green/orange-brown	Orange

[a] Mean ± 1 standard deviation for two or three replicate measurements.

[b] Data are from Fouke et al. (2000). DIC = dissolved inorganic carbon.

[c] Isotopic compositions of dissolved CO_2 ($\delta^{13}C_{CO2\text{-}d}$) was calculated from $\delta^{13}C_{HCO3}$ ($\cong \delta^{13}C_{DIC}$) using the equations of $\delta^{13}C_{CO2\text{-}d} = \varepsilon_{(CO2\text{-}HCO3)} \times (1000 + \delta^{13}C_{HCO3})/1000 + \delta^{13}C_{HCO3}$ and $\varepsilon_{(CO2\text{-}HCO3)} = 24.12 - 9866/T$, where ε is the fractionation between dissolved CO_2 and HCO_3^- and T is absolute temperature (Mook et al., 1974).

[d] Not determined.

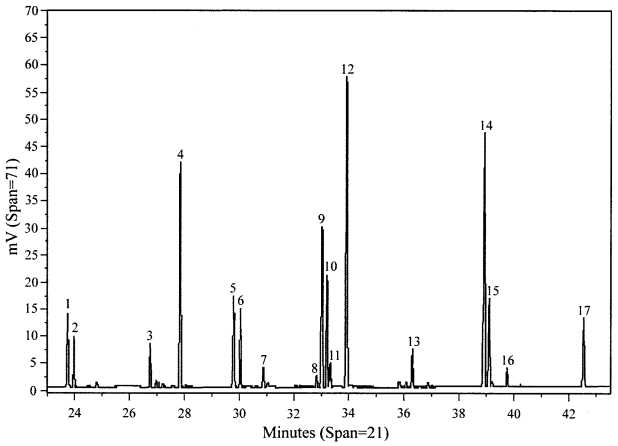


Figure 2. Gas chromatograph of phospholipid fatty acids from a vent sample collected from the Angel Terrace hot-spring complex in Yellowstone National Park (see Fig. 2 for description). Peak identifications are: **1**, i15:0; **2**, a15:0; **3**, i16:0; **4**, 16:0; **5**, i17:0; **6**, a17:0; **7**, 17:0; **8**, i18:0; **9**, 18:1w9c; **10**, 18:1ω7c; **11**, 18:1ω7t; **12**, 18:0; **13**, cy19:0; **14**, 20:1ω9c; **15**, 20:1ω9t; **16**, 20:0; **17**, 21:0 (internal standard).

disulfide adducts (Nichols et al., 1986). *Cis* and *trans* isomers of compounds were identified by known standards. Figure 2 shows an example of fatty-acid profiles for the vent facies.

3.3. Stable Carbon Isotopes

Carbon-isotope compositions of the FAMEs for PLFA and GLFA were determined following Zhang et al. (2002) using a HP 6890 gas-chromatograph connected to a Finnigan MAT Delta$^+$-XL mass-spectrometer. Measurements were corrected for the methyl moiety according to Abrajano et al. (1994) using the following equation:

$$\delta^{13}C_{FA} = [(Cn + 1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/Cn$$

where $\delta^{13}C_{FA}$ is the $\delta^{13}C$ of the fatty acid, Cn is the number of carbons in the fatty acid, $\delta^{13}C_{FAME}$ is the $\delta^{13}C$ of the methylated fatty acid, and $\delta^{13}C_{MeOH}$ is the $\delta^{13}C$ of the methanol used for the methylation reaction.

Carbon-isotope compositions of total biomass were determined using bulk samples after dissolution of the associated carbonate in 1% EDTA. The residual material was then rinsed with distilled water and dried in an oven at 50°C overnight. The $^{13}C/^{12}C$ ratio of total biomass was then determined on a Delta Plus isotope ratio mass spectrometer interfaced with an Elemental Analyzer.

RESULTS

4.1. General Chemistry

Water temperature decreased from 71.4°C in the vent to 24.3°C in the distal slope at the time of sampling. The pH increased from 6.3 in the vent to 8.4 in the distal slope (Table 2). These measurements are in the same range as previous recordings (Fouke et al., 2000). Water samples were not preserved properly and not analyzed for concentration and stable carbon-isotopes of DIC. Previously however, the water had been repeatedly collected and analyzed, and the results gave relatively consistent DIC values for each facies (Fouke et al., 2000). We use these values as well as the reported $\delta^{13}C$ of DIC as references for this study (Table 2). The isotopic composition of dissolved CO_2 is estimated to be in a narrow range of −3.9‰ to −5.9‰ across these facies (Table 2). The $\delta^{13}C$ values of total biomass, however, show a much wider but systematic variation along the temperature gradient, decreasing from −16.1‰ in the vent to −28.0‰ in the proximal slope followed by a slight increase to −23.5‰ in the distal slope (Table 2).

4.2. Lipid Profiles in Different Facies

4.2.1. Phospholipid fatty acids (PLFA)

PLFA in the vent facies are dominated by 20:1ω9c and 20:1ω9t, which collectively make up about one third (32.1%, average of samples A and B; same reporting below) of the total fatty acids (Table 3). The next most abundant fatty acid is 18:0 (22.2%) followed by 18:1 with three isomers, 18:1ω9c (8.2%), 18:1ω7c (7.6%), and 18:1ω7t (1.7%). Collectively, these isomers make up ~17.4% of total PLFA (Table 3). The universal fatty acid, 16:0, accounts for ~10.0% of total PLFA. Less abundant fatty acids include 20:0, cy19:0, and *iso*- or *anteiso*-15, −16 and −17 carbon chains, which are <5% each (Table 3).

The pond-2 and the proximal slope-2 (Fig. 1) have similar lipid profiles with 16:0 accounting for about half of total PLFA in both facies (Table 3). This is followed by 18:1ω9c and 18:1ω7c, which collectively account for ~15.5% of total PLFA in both facies. The most abundant fatty acid (20:1ω9) in the vent nearly disappears in these facies and a15:0, i17:0, a17:0, and cy19:0 also decrease to below 1.0% (Table 3). On the other hand, 16:1ω7c and 18:2ω6 appear in relatively high abundance (5.0–6.0%) with cy17:0 being a minor compound (1.5%) in the pond-2. In addition, the proximal slope-2 shows a sharp increase in 14:0, which accounts for ~2.7% of total PLFA (Table 3).

In the distal slope, 16:0 decreases to 30.0% of total PLFA but still remains the most abundant. The compound 18:0 continues to decrease to a value of 4.4%. On the other hand, 18:1ω9c remains relatively unchanged (~14.0%) compared to its abundances in the pond-2 or the proximal slope-2, but 18:1ω7c increases to a greater proportion of ~11.0%. Collectively, 18:1ω9c and 18:1ω7c account for ~25.0% of total PLFA in the distal slope and are the most abundant in all four facies. Also increased in the distal slope are i15:0 (~7.0%), a15:0 (~1.3%), 16:1ω7c (~6.8%), i17:0 (~1.3%), 18:3ω3 (~5.4%), and 20:0–23:0 (1.0–2.2%) (Table 3). These changes may reflect shifts in microbial community structure from facies to facies.

4.2.2. Glycolipid fatty acids (GLFA)

In all facies GLFA have major fatty acids that are also found in PLFA (Table 4). However, the relative proportions of major fatty acids in glycolipids may be different from those in the phospolipids. For example, GLFA in the vent have a significantly greater proportion of 18:1ω9c (44.2%, sample B) and lower proportions of 20:1ω9c (4.8%, sample B) and 20:1ω9t (3.1%, sample B) than PLFA in the same facies (Table 4). In the pond-2 and the proximal slope-2, major GLFA such as 16:1ω7c, 16:0, 18:2ω6, 18:1ω9c and 18:0 have similar proportions as the PLFA (Table 4). In the distal slope, GLFA have greater proportions of 16:1ω7c (average 9.5% between samples A and B, same below) and 16:0 (~60.0%) but lower proportions of i15:0 (1.2%), 18:2ω6 (~3.4%), 18:1ω9c (7.1%), and 18:1ω7c (5.6%) than the PLFA (Table 4).

Table 3. Distribution of phospholipid fatty acids in microbial mats from different facies of Angel Terrace Spring AT-1.

Fatty acids (mol%)	Facies							
	Vent		Pond-2		Proximal slope-2		Distal slope	
	A	B	A	B	A	B	A	B
14:0	0.0	0.0	0.3	0.2	3.0	2.4	0.6	0.9
i15:0	2.3	1.5	1.8	1.4	1.8	2.0	6.5	7.5
a15:0	1.6	1.3	0.0	0.0	0.0	0.0	1.2	1.3
15:0	0.0	0.0	0.3	0.2	0.3	0.3	0.3	0.4
i16:0	1.5	1.2	2.8	2.3	2.2	2.8	0.6	0.6
16:1ω7c	0.0	0.0	6.2	5.2	3.7	4.6	6.8	6.7
16:1ω7t	0.0	0.0	0.7	0.6	0.0	0.0	0.3	0.4
16:1ω5c	0.0	0.0	0.1	0.0	0.2	0.4	1.0	1.1
cy16:0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
16:0	9.4	10.0	54.7	58.7	58.2	50.7	29.6	30.3
i17:1ω7c	0.0	0.0	0.1	0.0	0.3	0.5	0.7	0.8
br17:1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
i17:0	3.9	4.6	0.5	0.4	0.3	0.4	1.4	1.2
a17:0	3.5	4.5	0.5	0.4	0.3	0.4	0.5	0.7
17:1ω6c	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0
cy17:0	0.0	0.0	1.7	1.3	0.0	0.1	0.0	0.0
17:0	1.2	1.2	0.6	0.5	0.5	0.5	0.6	0.5
18:3ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
i18:0	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0
18:2ω6	0.0	0.0	5.5	4.3	5.8	7.9	6.1	6.8
18:3ω3	0.0	0.0	0.0	0.3	0.9	1.3	4.8	5.9
18:1ω9c	9.3	7.0	13.7	13.7	12.9	15.6	15.7	12.3
18:1ω7c	7.2	8.0	1.6	1.5	1.2	1.4	11.0	10.5
18:1ω7t	1.3	2.0	0.4	0.0	0.0	0.0	0.0	0.0
18:1ω5c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
18:0	19.6	24.7	7.8	8.4	7.9	7.5	4.2	4.4
10Me18:0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
i19:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
cy19:0	2.5	1.4	0.0	0.5	0.0	0.0	0.0	0.0
19:0	0.0	0.0	0.7	0.0	0.1	0.1	0.7	0.2
20:2ω6	0.0	0.0	0.0	0.0	0.2	0.5	0.8	1.4
20:1ω9c	25.5	18.6	0.0	0.0	0.1	0.2	0.0	0.5
20:1ω9t	8.6	11.5	0.0	0.0	0.0	0.0	0.3	0.4
20:0	1.9	2.0	0.0	0.1	0.1	0.2	1.1	1.0
22:0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	1.9
23:0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	1.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

4.3. Stable Isotope Compositions of PLFA and GLFA

Isotopic compositions of individual PLFA and GLFA were obtained only for those having concentrations high enough to be detected on the isotope-ratio-mass-spectrometer via the GC (Table 5, Table 6). Each sample was analyzed at least twice for the isotopic compositions of individual fatty acids. The average of the replicate runs is reported, which has a standard deviation less than 1.0‰ in most samples (Table 5, Table 6). Large standard deviations are associated with PLFA 18:0 (±2.2–4.6‰) in the distal slope (Table 5).

4.3.1. Isotopic compositions of PLFA

PLFA in the vent have $\delta^{13}C$ values ranging from −18.7 ± 5.6‰ (average of samples A and B, n = 2; same reporting below unless otherwise noted) for 16:0 to −6.3 ± 1.6‰ (n = 2) for Σ18:1ω9c/7c. Collectively, the PLFA in the vent have an average value of −12.4 ± 5.2‰ (n = 10, Fig. 3).

PLFA in the pond-2 and in the proximal slope-2 have $\delta^{13}C$ values with similar variations. For example, i15:0 is most enriched in ^{13}C and has $\delta^{13}C$ values of −26.7‰ in the pond-2 and −26.8 ± 0.3‰ (n = 2) in the proximal slope-2 (Table 5). The second most ^{13}C-enriched fatty acid is i16:0, which has $\delta^{13}C$ values of −27.1‰ and −28.6 ± 0.2‰ (n = 2) in these two facies, respectively. The fatty acid 16:1ω7c is mostly depleted in ^{13}C and has $\delta^{13}C$ values of −35.4 ± 0.4‰ (n = 2) in the pond-2 and −37.8 ± 0.3‰ (n = 2) in the proximal slope-2 (Table 5). Collectively, PLFA have average values of −33.0 ± 3.1‰ (n = 11) in the pond-2 and −33.7 ± 3.8‰ (n = 16) in the proximal slope-2 (Fig. 3). These average values are ~21‰ more negative than that in the vent (Fig. 3), suggesting a dramatic change in the mechanisms of carbon-isotope fractionation in these facies.

In the distal slope, isotopic compositions of individual PLFA become enriched in ^{13}C but not to the level of enrichment observed in the vent. The $\delta^{13}C$ ranges from −18.4 ± 0.1‰ (n = 2) for i15:0 to −27.0 ± 0.2‰ (n = 2) for 16:0. One exception is the isomers 18:1ω9c and 18:1ω7c, which have a

Table 4. Distribution of glycol-lipid fatty acids in microbial mats from different facies of Angel Terrace Spring AT-1.

Fatty acids (mol%)	Facies							
	Vent		Pond-2		Proximal slope-2		Distal slope	
	A[a]	B	A	B	A	B	A	B
14:0	0.0	0.0	0.8	0.0	7.1	6.7	1.3	1.6
i15:0	0.0	0.0	0.4	0.0	0.3	0.3	1.0	1.3
a15:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
15:0	0.0	0.0	0.2	0.0	0.1	0.1	0.0	0.2
i16:0	0.0	0.0	0.5	0.0	0.4	0.4	0.0	0.2
16:1	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.5
16:1ω7c	0.0	0.0	8.9	5.9	6.4	6.0	10.2	8.8
16:1ω7t	0.0	0.0	0.3	0.0	0.1	0.1	0.0	0.4
16:0	0.0	11.9	62.0	72.1	52.4	49.7	61.3	58.6
i17:0	0.0	0.7	0.2	0.0	0.1	0.1	0.5	0.6
a17:0	0.0	1.3	0.0	0.0	0.0	0.1	0.0	0.1
Unknown	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
17:0	0.0	1.1	0.3	0.0	0.2	0.2	0.4	0.5
18:2ω6	0.0	1.4	6.8	5.1	13.6	14.2	3.0	3.8
18:3ω3	0.0	0.0	0.4	0.0	1.4	1.8	3.5	4.0
18:1ω9c	0.0	44.2	12.4	12.3	12.7	12.5	9.3	4.9
18:1ω7c	0.0	9.7	1.0	0.0	0.9	1.1	5.8	5.4
18:1ω7t	0.0	1.7	0.0	0.0	0.1	0.0	0.0	0.1
18:1ω5c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
18:0	0.0	18.5	5.8	4.6	2.7	3.1	3.6	3.5
10Me18:0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
19:2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2
br19:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
br19:1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
br19:0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.1
20:2ω6	0.0	0.0	0.0	0.0	0.2	0.2	0.0	1.5
20:3ω3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
20:1ω9c	0.0	4.8	0.0	0.0	0.1	0.0	0.0	0.0
20:1ω9t	0.0	3.1	0.0	0.0	0.1	0.1	0.0	0.3
20:0	0.0	1.5	0.0	0.0	0.1	0.1	0.0	0.4
br20:0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.2
br21:0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
22:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Unknown	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
22:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
Unknown	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
23:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
24:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Total	0.0	100.0	100.0	100.0	99.6	100.0	100.0	99.4

[a] Sample was lost.

composite $\delta^{13}C$ of −40.0‰ (Table 5). This value is suspiciously low compared with $\delta^{13}C$ values of other PLFA in the same facies. Because we didn't have a replicate measurement in sample A to confirm this value, it is excluded for further calculation. As a result, the overall PLFA have an average value of −22.4 ± 3.4‰ (n = 10) in the distal slope (Fig. 3). This is approximately 11‰ enriched in ^{13}C relative to PLFA in the pond-2 and the proximal slope-2 and ~10‰ depleted in ^{13}C relative to PLFA in the vent (Fig. 3).

4.3.2. *Isotopic compositions of GLFA*

Fewer fatty acids in the glycolipids were available for isotopic analyses (Table 6). Nevertheless, the data show that most of the GLFA are depleted in ^{13}C relative to the same fatty acids in PLFA. In the vent, for example, the $\delta^{13}C$ values of individual GLFA are depleted by 7.6‰ (18:0) to 11.5‰ (Σ18:1ω9/7c) relative to the same fatty acids in PLFA (Table 5). Most of the GLFA in the proximal slope and the distal slope are also depleted in ^{13}C (0.1–8.2‰) relative to the PLFA. Exceptions are 18:2ω6 and 18:0 in sample B of the proximal slope-2, for which the GLFA is ~1–3.3% enriched in ^{13}C relative to PLFA (Table 5, Table 6). In the pond-2, however, most of the GLFA are enriched in ^{13}C (2.8–6.0‰) relative to PLFA with the exception of 16:0 (Table 5, Table 6).

The average $\delta^{13}C$ values of GLFA vary from facies to facies in a manner similar to that of PLFA. For example, the average $\delta^{13}C$ of GLFA is −19.6 ± 3.4‰ (n = 3) in the vent, −30.4 ± 4.7‰ (n = 8) in the pond-2, −36.9 ± 2.8‰ (n = 12) in the proximal slope-2, and −27.9 ± 3.1‰ (n = 13) in the distal slope (Fig. 3). The isotopic variations of PLFA and GLFA are also consistent with that of total biomass among these facies (Fig. 3).

Table 5. Isotopic compositions of phospholipid fatty acids of microbial mats from different facies of Angel Terrace Spring AT-1.

	$\delta^{13}C$ (‰)[a]							
	Vent		Pond-2		Proximal slope-2		Distal slope	
Fatty acid	A	B	A	B	A	B	A	B
14:0					−35.2 (0.4)	−35.4 (0.1)		
i15:0				−26.7 (0.2)	−27.0 (0.2)	−26.6 (0.1)	−18.5 (0.2)	−18.3 (0.1)
i16:0				−27.1 (0.4)	−28.8 (0.2)	−28.5 (0.8)		
16:1ω7c			−35.1 (1.0)	−35.7 (0.1)	−37.7 (0.2)	−38.0 (0.1)	−19.6 (0.0)	−19.6 (0.5)
16:0	−22.7 (0.4)	−14.8 (0.3)	−34.7 (0.1)	−35.1 (0.0)	−36.3 (0.1)	−36.0 (0.1)	−26.8 (0.0)	−27.1 (0.1)
Σi17:0,a17:0[b]	−16.7	−14.8						
18:2ω6				−34.3 (0.3)	−34.6 (0.3)	−34.9	−22.8 (0.2)	−23.2
Σ18:1ω9/7c[b]	−7.4	−5.2	−33.2	−33.8	−36.7	−35.0		−40.0
18:0	−13.8 (0.5)	−11.9 (1.1)	−33.1 (0.5)	−34.0 (0.1)	−34.7 (0.4)	−33.7	−26.0 (4.6)	−21.8 (2.2)
Σ20:1ω9t/c[b]	−8.2	−8.7						

[a] Average ± 1 standard deviation (in parentheses) of two or three replicate runs. The $\delta^{13}C$ values of individual fatty acids were corrected for methylation (see text).
[b] Baseline resolution was not possible; a composite $\delta^{13}C$ value was obtained for coeluting fatty acids by integrating individual $\delta^{13}C$ values using weight percentage of mass 44 [V] of each peak.

DISCUSSION

5.1. Lipid Profiles of Different Facies

Fouke et al. (2003) have provided a phylogenetic survey of bacterial species in each facies at Angel Terrace Spring AT-1 based on 16S rRNA gene sequences. The results show strong partitioning between dominant microbial species and types of depositional facies (Table 1). In particular, the vent is dominated by *Aquificales* (91% of total sequences). Gene sequences of the green sulfur bacteria (i.e., *Chlorobium*) are most abundant in the proximal slope (9%) and less than 1% in the vent; those of the green non-sulfur bacteria (i.e., *Chloroflexus*) range from 4% in the apron and channel to below detection in the distal slope (Table 1). The cyanobacteria have consistently higher proportions in the relatively low-temperature environments (the pond, the proximal slope and the distal slope) than in the high-temperature environments (the vent and the apron and channel) (Table 1). Except in the vent, the majority of gene sequences belong to other bacteria (Table 1), whose roles in these environments are not well defined. It must be understood, however, that the relative proportion of sequences (clones) representing a given species or division of bacteria is not directly correlated to the actual size of the cellular population of that species or division.

Lipid biomarkers can provide valuable information about microbial structures in geological settings. Pioneering studies of microbial lipids in Yellowstone hot springs were lead by Ward and colleagues, who performed extensive analyses of lipid biomarkers of cyanobacterial mats (Ward et al., 1985; Dobson et al., 1988; Ward et al., 1989; Zeng et al., 1992a,b). Zeng et al. (1992a) observe that the distribution of most of the major mat lipids in the Yellowstone hot springs is consistent with lipid profiles of microorganisms isolated from the mats. Jahnke et al. (2001) focus their study on the lipid biomakers in the Octopus Spring hyperthermophilic community in Yellowstone National Park. Lipid extracts of the natural samples show fatty acid biomarkers dominated by cy21:0, 20:1ω9, i17:0, i19:0, and 18:0. The abundance of 20:1ω9, cy21:0, and 18:0 are characteristic of certain known *Aquificales* species (Jahnke et al., 2001). Other culture studies identify 18:1ω9/7 as additional

Table 6. Isotopic compositions of glycolipid fatty acids of microbial mats from different facies of Angel Terrace Spring AT-1.

	$\delta^{13}C$ (‰)[a]							
	Vent		Pond-2		Proximal slope-2		Distal slope	
Fatty acid	A	B	A	B	A	B	A	B
14:0			−20.4		−37.6 (0.3)	−36.7 (0.2)	−32.9	−29.6
i15:0							−21.2 (0.1)	−23.8 (0.5)
16:1ω7c			−32.1	−29.7	−41.1 (0.1)	−39.8 (0.2)	−27.0 (1.1)	−26.2 (0.0)
16:0		−22.6	−35.5	−35.3	−37.9 (0.1)	−37.4 (0.2)	−29.9 (0.2)	−29.3 (0.4)
18:2ω6					−35.9 (0.3)	−33.9 (0.2)	−31.0	−26.8 (0.3)
Σ18:1ω9/7c[b]		−16.7	−30.3	−31.0	−38.7 (0.0)	−38.1 (0.0)		−30.5
18:0		−19.5	−28.5		−34.8 (0.2)	−30.4 (0.2)	−26.7	−27.4 (0.5)

[a] Average ± 1 standard deviation (in parentheses) of two or three replicate runs. The $\delta^{13}C$ values of individual fatty acids were corrected for methylation (see text).
[b] Baseline resolution was not possible; a composite $\delta^{13}C$ value was obtained for co-eluting fatty acids by integrating individual $\delta^{13}C$ values using weight percentage of mass 44 [V] of each peak.

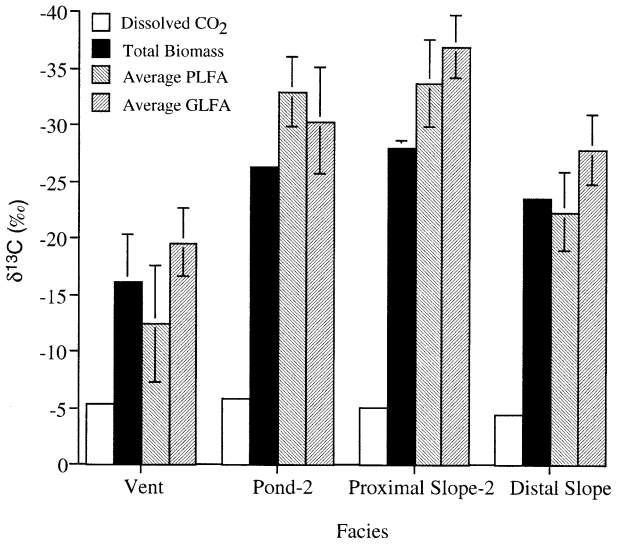


Figure 3. Average isotopic compositions of phospholipids fatty acids (PLFA), glycolipid fatty acids (GLFA), and total biomass in different facies at Angel Terrace Spring AT-1. The isotopic compositions of dissolved CO_2 calculated from total dissolved inorganic carbon (Table 2) are also shown.

major fatty acids instead of cy21:0 (Kawasumi et al., 1984; Nishihara et al., 1990; Stohr et al., 2001a; Zhang et al., 2002).

The above discussion provides a basis for understanding the biogeochemical dynamics of lipids in different facies at Spring AT-1. For example, the dominance of 20:1ω9, 18:0, and 18:1ω9/7 in the vent (Table 3, Table 4) is consistent with the distribution of the 16S rRNA gene sequences of *Aquificales* in the vent (Fouke et al., 2003) and consistent with lipid profiles of known *Aquificales* species (Kawasumi et al., 1984; Huber et al., 1998; Jahnke et al., 2001; Stohr et al., 2001a; Gotz et al., 2002; Zhang et al., 2002). However, *Aquificales* in pure cultures do not produce *iso*- or *anteiso*- fatty acids (Jahnke et al., 2001; Zhang et al., 2002). Thus the presence of *iso*- or *anteiso*-15:0 to −17:0 in the vent are likely derived from other bacterial species. These may include *Thermus aquaticus*, *Bacillus stearothermophilus* and other mat-inhabiting heterotrophs, which are known to produce *iso*- and *aneteiso*- 15:0 to 17:0 compounds as major fatty acids (Yao et al., 1970; Ray et al., 1971; Oshima and Miyagawa, 1974).

The similar lipid profiles between the pond-2 and the proximal slope-2 suggest that the relative proportions of extant microbial populations in these two facies may be similar. The dramatic decrease in 20:1ω9c/t in these facies suggests that the relative abundance of *Aquificales* is much lower than in the vent (Table 3, Table 4). On the other hand, 16:1ω7c and 18:2ω6 in both PLFA and GLFA begin to appear in significant abundance accompanied by increasing 16:0 (Table 3, Table 4), which are consistent with their presence in cyanobacteria or green non-sulfur bacteria (Fork et al., 1979; Ward et al., 1989, Zheng et al., 1992a; Summons et al., 1996).

In the distal slope, the microbial community is also likely dominated by cyanobacteria and green non-sulfur bacteria as demonstrated by high abundances of 16:1ω7c and 18:2ω6, and 18:1ω9c that are similar to those in the pond-2 and the proximal slope-2. The increase in 18:3ω3 in the distal slope may further indicate the presence of cyanobacteria because 18:3 biomarkers have been reported as major fatty acids in cyanobacterium *Phormidium luridum* grown at temperatures similar to the distal slope environment (Summons et al., 1996). In addition, the increase in i15:0 may indicate an increasing population of heterotrophic bacteria growing at low temperatures.

Overall, the lipid profiles clearly reflect the changing microbial communities from the vent to the pond and the proximal slope and to the distal slope. Temperature appears to be the major controlling factor for the dominant microbial species in these environments. For example, most *Aquificales* can grow at temperatures above 70°C (Kawasumi et al., 1984; Huber et al., 1998, 2002; Reysenbach et al., 2000; Stohr et al., 2001a; Gotz et al., 2002); whereas, cyanobacteria, green sulfur bacteria, green non-sulfur bacteria, and *Hydrogenophilus hirschii* Yel5a grow at temperatures below 60–70°C (Meeks and Castenholz, 1971; Bauld and Brock, 1973; Ward et al., 1998; Miller and Castenholz, 2000; Stohr et al., 2001b). Algae and other eukaryotes are normally found at temperatures below 40–55°C (Castenholz, 1984). On the other hand, fatty acid compositions of individual cells can also vary under different growth temperatures (Ray et al., 1971; Fork et al., 1979; Jahnke, 1992; Könneke and Widdel, 2003). In particular, variations in temperature can induce changes in unsaturation of fatty acids necessary for proper function of cell membranes (Murata et al., 1992; Murata and Wada, 1995; Könneke and Widdel, 2003). Overall, results of this study support similar patterns of temperature distribution of microbial species in hot springs that were determined using molecular DNA techniques (Ward et al., 1998; Miller and Castenholz, 2000; Fouke et al., 2003).

5.2. Carbon Isotopic Compositions of Fatty Acids

The estimated isotopic compositions of dissolved CO_2 (Table 2) show small variation (≤2.0‰) with decreasing DIC from the vent (192 ppm) to the distal slope (75 ppm); whereas, the isotopic compositions of total biomass show a large variation decreasing from −16.1‰ in the vent to −23.5‰ in the distal slope (Table 2). These results suggest that the abundance and isotopic compositions of CO_2 or DIC do not seem to have major influence on the changing isotopic compositions of organic matter in these environments. One exception may be the distal slope, where degassing results in 3.0‰ enrichment in $^{13}C_{DIC}$ relative to DIC in the upper proximal slope. This might have contributed to the 4.5‰ increase in $^{13}C_{Biomass}$ in the distal slope relative to biomass in the proximal slope (Table 2).

Isotopic compositions of PLFA and GLFA are consistent with those of total biomass between different facies (Fig. 3). This suggests that in each facies fatty acids and biomass may reflect the same mechanism of isotope fractionation. Different facies, however, appear to be associated with different mechanisms of isotope fractionation as demonstrated by the plot of $\varepsilon_{FA\text{-}Bio}$ vs. $\varepsilon_{Bio\text{-}CO2}$ (Fig. 4). In this plot, the trend of fractionations for different facies is parallel to that for pure cultures, among which the cyanobacteria represent fractionations for the Calvin cycle (lower end-member), the *Aquificales* represent fractionations for the rTCA cycle (upper end-member), and the green non-sulfur bacteria represent the 3-HP pathway between the two end-members (Fig. 4). Such a comparison indicates that CO_2 fixation in the vent may be dominated by rTCA; whereas,

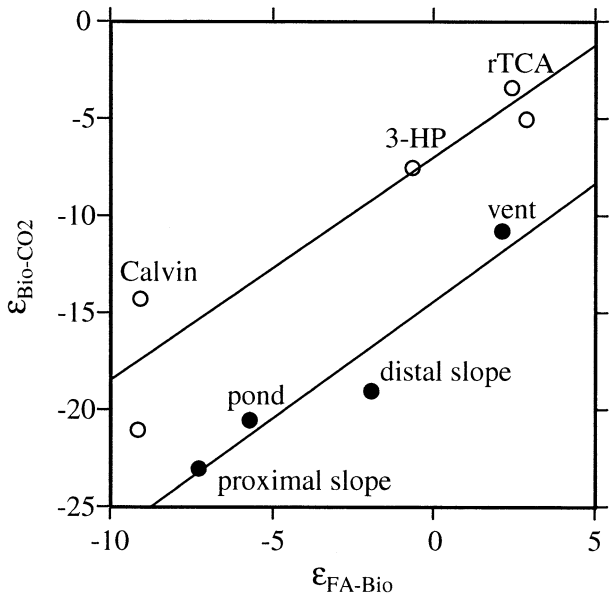


Figure 4. Isotope fractionations between fatty acids (FA) and total biomass (Bio) vs. fractionations between biomass (Bio) and CO_2 for pure cultures (open circles) and for environmental samples from different facies at AT-1 (solid circles). For pure cultures, Calvin represents cyanobacteria (*Synechocystis* UTEX 2470 and *Phomidium luridum*), rTCA represents *Aquificales* (*Persephonella marina* and *Thermocrinis rubber*), and 3-HP represents green non-sulfur bacteria (e.g., *Chloroflexus aurantiacus*) (Table 7).

CO_2 fixation in the pond-2 and the proximal slope-2 may be dominated by the Calvin cycle. CO_2 fixation in the distal slope is consistent with the 3-HP pathway; however, the fractionation can also represent a mixture of the two end-members.

Among the proposed mechanisms of CO_2 fixation, the rTCA cycle can be unambiguously identified in the vent because of the dominance of 20:1ω9 and 18:1ω9/7 biomarkers that are specific for *Aquificales.* This, coincidently, also corresponds to the large proportion of gene sequences of *Aquificales* in the vent (Fouke et al., 2003).

In the pond-2 and the proximal slope-2, it may be difficult to distinguish the relative contributions of cyanobacteria, green non-sulfur bacteria, and/or green sulfur bacteria to the biomass production because these organisms may closely interact with each other. For example, it has been observed that *Chloroflexus* spp. of the green non-sulfur bacteria, are closely associated with cyanobacteria and uses their organic byproducts for photoheterotrophic growth. When this happens, the mixture of these species shows the isotopic composition of the cyanobacteria (Estep, 1984). If that is the case in the pond-2 and the proximal slope-2, we would expect $\delta^{13}C$ of *Chloroflexus* to be similar to that of cyanobacteria, thus underestimating their contribution to the community biomass.

In the distal slope, the fractionation is consistent with the 3-HP pathway, which may be used by the green non-sulfur bacteria such as *Chloroflexus* (Table 7), However, gene sequencing shows a lack of green non-sulfur bacteria in the distal slope (Fouke et al., 2003). Thus it is not clear whether *Chloroflexus* contributes significantly to biomass production in the distal slope. On the other hand, the fractionations can be explained by a mixture of cyanobacteria with more negative fractionations and green sulfur bacteria (i.e., *C. limicola*) with more positive fractionations (Table 7). Both groups are present in the distal slope based on gene sequencing (Fouke et al., 2003).

Large differences in isotopic compositions also occur between individual fatty acids within each facies (Table 5, Table

Table 7. Carbon isotopic fractionations of microbial species associated with known biosynthetic pathways.

Microorganisms	$\delta^{13}C_{CO2}$ (‰)	$\delta^{13}C_{biomass}$ (‰)	$\delta^{13}C_{fatty\ acids}$ (‰)	$\varepsilon_{FA\text{-}BIO}$[a] (‰)	$\varepsilon_{BIO\text{-}CO2}$[a] (‰)	$\varepsilon_{FA\text{-}CO2}$[a] (‰)	Biosyn. pathway	Ref.[b]
Cyanobacteria								
Synechocystis UTEX 2470	−9.9	−30.7	−39.6	−9.2	−21.0	−30.0	Calvin	1
Synechococcus sp.	−7.8 to −16.9	−26.1 to −33.1			−17.0 ± 1.2		Calvin	2
Phomidium luridum	−7.8 to −39.9	−18.4 to −60.1	−28.7 to −66.4	−9.1 ± 1.5	−14.3 ± 4.4	−23.3 ± 2.9	Calvin	3
Algae								
Tetraedron minimum				−5.0			Calvin	4
Porosira glacialis	−28.3 to −37.9	−34.2 to −58.8			−14.1 ± 5.2		Calvin	2
Aquificales								
Persephonella marina	−40.8	−45.6	−42.9	2.8	−5.0	−2.2	r-TCA	5
Thermocrinis ruber	−27.4	−30.7	−28.4	2.4	−3.4	−1.0	r-TCA	6
Green sulfur bacterium								
Chlorobium limicola		−26.3		12.9			r-TCA	7
Chlorobium thiosulfatophilum	−16.5[c]	−28.7			−12.2		r-TCA	8
Green non-sulfur bacterium								
Chloroflexus aurantiacus	−41.7	−48.9	−49.6	−0.7	−7.6	−8.4	3-HP[d]	9

[a] Fractionation between two substances. $\varepsilon_{A\text{-}B} = ((\delta^{13}C_A + 1000)/(\delta^{13}C_B + 1000) - 1) \times 1000$. FA = total fatty acids; BIO = biomass or total organic carbon. In cases of average ± 1 standard deviation; n = 5–12.

[b] 1. Sakata et al. (1997); 2. Popp et al. (1998); 3. Summons et al. (1996); 4. Schouten et al. (1998); 5. Zhang et al. (2002); 6. Jahnke et al. (2001); 7. van der Meer et al. (1998); 8. Sirevåg et al. (1977); 9. van der Meer et al. (2001).

[c] Calculated from measurement of $^{13}C_{HCO3}$ using a fractionation factor of 7.3‰ between dissolved CO_2 and bicarbonate as used by Sirevåg et al. (1977).

[d] Although the 3-HP pathway uses HCO_3^- rather than CO_2 as a substrate for cell growth, CO_2 is used for calculation in order to compare with other pathways using CO_2 for cell growth.

6). These variations may reflect: 1) different biosynthetic pathways of different microorganisms, 2) different carbon substrates used by the same microorganism, or 3) variation within the same microorganism. Factor one has been discussion above. Factors 2 and 3 cannot be determined using the existing data. However, one example is given by Jahnke et al. (2001) who show that isotopic compositions of lipids of *Thermocrinis ruber* (rTCA cycle) were ~10‰ lighter when grown heterotrophically on formate (−23.3‰) than grown autotrophically on CO_2 (−27.4‰), even though the isotopic compositions of CO_2 and formate are similar. Nevertheless, isotopic compositions of fatty acid biomarkers provide valuable information about changing microbial community structure and biosynthetic pathways from the vent to the pond and the proximal slope to the distal slope at Spring AT-1.

5.3. Geological and Evolutionary Implications

Previous studies of travertine deposits have focused on the inorganic chemistry of the spring water and carbonate mineralogy. While travertine crystal fabrics are strongly influenced by microbes**,** which may provide a substrate for crystal nucleation, the chemical byproducts of microbial respiration and photosynthesis are not well expressed in the travertine crystal geochemistry. This study integrates lipid biomarkers and stable isotopes in the context of microbial communities, water chemistry, and varying facies of carbonate precipitation, which provide substantial insights into the relationships between microbial population dynamics and the changing environment in travertine deposits.

Studies of hot springs in other locations around the world show lipid distributions that are consistent with our observations at Mammoth Hot Springs. For example, hot springs in Iceland and New Zealand have lipid biomarkers consistent with the dominance of cyanobacterial mats (Robinson and Eglinton, 1990; Shiea et al., 1990, 1991). A variety of hot springs and hydrothermal vents harbor *Aquificales*, which all have the characteristic fatty acids of 20:1ω9, 18:0, 18:1ω9/7, or cy21:0 (Kawasumi et al., 1984; Huber et al., 1998; Jahnke et al., 2001; Stohr et al., 2001a; Gotz et al., 2002; Zhang et al., 2002). The latter biomarkers are rare in other bacterial species and may serve as taxonomic biomarkers for *Aquificales* species (Kawasumi et al., 1984; Jahnke et al., 2001). This study has enriched our knowledge about lipid biomarker and isotopic signatures of major microbial populations in geological settings.

Aquificales, Cyanobacteria, green sulfur bacteria, and green non-sulfur bacteria have been frequently observed in hot springs in the Yellowstone National Park (Ward et al., 1984, 1997, 1998; Stahl et al., 1985; Reysenbach et al., 1994; Hugenholtz et al., 1998; Eder and Huber, 2002). These species have also been found in hot springs in other regions of the world (Cohen, 1984; Robinson and Eglinton, 1990; Eder and Huber, 2002; Nakagawa and Fukui, 2002). They are of particular interest for studies of microbial evolution. For example, the *Aquificales* represents one of the deepest lineages of the Bacteria in the phylogenetic tree (Reysenbach et al., 1994). Green sulfur and green non-sulfur bacteria are anaerobic phototrophs and might have evolved before the atmosphere was oxygenated (Oyaizu et al., 1987; Gupta et al., 1999). The cyanobacteria, on the other hand, may provide clues about the early stages of arising oxygen levels on earth (Summons et al., 1996). Recent advances in molecular microbiology and compound-specific isotope signatures will shed new light and provide more precise information about biogeochemical processes mediated by these organisms in modern systems, which help better understand the evolution of life in ancient environments.

SUMMARY AND CONCLUSIONS

Phospholipid fatty acids (PLFA), glycolipid fatty acids (GLFA), and their carbon isotope compositions were determined for microbial mats and rock materials from four different facies that comprise the modern travertine deposits in the Spring AT-1 outflow drainage system of Mammoth Hot Springs in Yellowstone National Park. In the vent (71°C), PLFA and GLFA are dominated by 20:1ω9, 18:0, and 18:1ω9/7, which are consistent with biomarkers observed in *Aquificales*; isotope fractionations between fatty acids and biomass are consistent with rTCA cycle used by *Aquificales* for CO_2 fixation. In the pond (51°C) and the proximal slope (47°C), characteristic biomarkers include 16:1ω7c, 18:1ω9/7, and 18:2ω6, which may represent cyanobacteria, green non-sulfur bacteria, and/or green sulfur bacteria; isotope fractionations are consistent with the Calvin cycle being a potential mechanism for CO_2 fixation. Lipid biomarkers in the distal slope (24°C) also indicate the predominance of cyanobacteria, green non-sulfur bacteria and/or green sulfur bacteria; isotope fractionations, however, are consistent with the 3-HP pathway or a mixture of the Calvin cycle or rTCA cycle.

Results of this study show that lipid biomarkers and their isotopic compositions are only slightly affected by abundance and isotopic compositions of dissolved inorganic carbon in different facies. Instead, lipid biomarkers mostly reflect the changing microbial communities in different environments with temperature being the dominating factor; the isotopic compositions of biomass and fatty-acid biomarkers, on the other hand, provide a valuable insight into the mechanisms of CO_2 fixation by the extant microbial communities in the changing environments.

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