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Signature Lipids and Stable Carbon Isotope Analyses of Octopus Spring Hyperthermophilic Communities Compared with Those of *Aquificales* Representatives

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The molecular and isotopic compositions of lipid biomarkers of cultured *Aquificales* genera have been used to study the community and trophic structure of the hyperthermophilic pink streamers and vent biofilm from Octopus Spring. *Thermocrinis ruber*, *Thermocrinis* sp. strain HI 11/12, *Hydrogenobacter thermophilus* TK-6, *Aquifex pyrophilus*, and *Aquifex aeolicus* all contained glycerol-ether phospholipids as well as acyl glycerides. The *n*-$C_{20:1}$ and *cy*-C_{21} fatty acids dominated all of the *Aquificales*, while the alkyl glycerol ethers were mainly $C_{18:0}$. These *Aquificales* biomarkers were major constituents of the lipid extracts of two Octopus Spring samples, a biofilm associated with the siliceous vent walls, and the well-known pink streamer community (PSC). Both the biofilm and the PSC contained mono- and dialkyl glycerol ethers in which C_{18} and C_{20} alkyl groups were prevalent. Phospholipid fatty acids included both the *Aquificales* *n*-$C_{20:1}$ and *cy*-C_{21}, plus a series of *iso*-branched fatty acids (*i*-$C_{15:0}$ to *i*-$C_{21:0}$), indicating an additional bacterial component. Biomass and lipids from the PSC were depleted in ^{13}C relative to source water CO_2 by 10.9 and 17.2‰, respectively. The $C_{20–21}$ fatty acids of the PSC were less depleted than the *iso*-branched fatty acids, 18.4 and 22.6‰, respectively. The biomass of *T. ruber* grown on CO_2 was depleted in ^{13}C by only 3.3‰ relative to C source. In contrast, biomass was depleted by 19.7‰ when formate was the C source. Independent of carbon source, *T. ruber* lipids were heavier than biomass (+1.3‰). The depletion in the $C_{20–21}$ fatty acids from the PSC indicates that *Thermocrinis* biomass must be similarly depleted and too light to be explained by growth on CO_2. Accordingly, *Thermocrinis* in the PSC is likely to have utilized formate, presumably generated in the spring source region.

Based on phylogenetic analysis of small-subunit rRNA sequences, hyperthermophilic organisms proliferate in the deepest branches of the Bacterial and Archaeal domains. The branch lengths of these hyperthermophilic lineages tend to be short, which further suggests that such organisms are the closest known extant descendants of the last common ancestor and retain many ancestral phenotypic properties (49). The recent discovery of filamentous microfossils preserved in a 3,235-million-year-old submarine volcanogenic deposit lends considerable weight to the theory that hydrothermal vent organisms have had a very long history on Earth (41). Hyperthermophilic microbes are also attracting astrobiological and biogeochemical interest because of their potential role in the formation of many kinds of mineral deposits and the generation of rock textures and mineral assemblages that may be diagnostic for extant or extinct life beyond Earth (5).

A well-known example of a hyperthermophilic chemolithotrophic ecosystem is the pink filamentous streamers found at Octopus Spring in Yellowstone National Park (YNP), United States that were described by Brock in 1965 (3, 4). Similar streamer communities were first reported by Setchell in 1903 (45) and have subsequently been identified in neutral to alkaline springs of geothermal areas in Iceland, Japan, and Kamchatka, Russia (21, 48, 56) and, more recently, as distinct black streamers at Calcite Springs, YNP (42).

Molecular analysis of the small-subunit 16S rRNA sequences of the filamentous pink streamer community (PSC) indicates dominance of the domain Bacteria, in particular two deeply diverging phylotypes affiliated with the *Aquificales* and *Thermotogales* (43). From the PCS, the first pink streamer isolate, *Thermocrinis ruber*, was recently brought into culture (18). *T. ruber* forms a separate lineage within the order *Aquificales* and shares many features with the two previously isolated genera, *Aquifex* and *Hydrogenobacter* (22, 28, 29).

The use of lipid biomarkers for revealing microbial community structure is well established. Branched-chain fatty acids are not uncommon in thermophilic organisms (31) and have been identified in pink streamer samples from Octopus Spring previously (1). Other more distinctive lipids are now recognized as valuable biomarkers in some thermophiles. *Thermomicrobium roseum* contains internally methyl-branched C_{18} fatty acid and long-chain 1,2-diols as major components (38). The core lipids of members of the order *Thermotogales* are composed of unusual dicarboxylic fatty acids and a recently discovered ether lipid, 15,16-dimethyl-30-glyceroloxytriacontanoic acid (20). Other novel mono- and dialkyl glycerol ether

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TABLE 1. Growth conditions for *Aquificales* cultures

Organism	Medium	Gas phase (300 kPa, by vol)	pH	Reference
Aquifex aeolicus VF5	SME + 0.2% thiosulfate + 0.1% $NaHCO_3$	H_2-CO_2-O_2 = 79:20:1 (gassing)	6.5	21
Aquifex pyrophilus Ko15a	SME + 0.2% thiosulfate + 0.1% $NaHCO_3$	N_2-CO_2-O_2 = 79:20:1 (gassing)	6.5	22
Hydrogenobacter thermophilus TK-6	TK-6 + 0.1% thiosulfate	H_2-CO_2-O_2 = 79:20:1	7	23
Thermocrinis ruber OC 1/4				
Expt 1	OS + 0.1% thiosulfate	H_2-CO_2-O_2 = 79:20:1 (gassing)	6.5	18
Expt 2	OS + 0.1% $NaHCO_3$ + 0.1% thiosulfate	N_2-O_2 = 97:3	6.5	23
Expt 3	OS + 0.1% $NaHCO_3$	N_2-O_2-H_2 = 94:3:3	6.5	18
Expt 4	OS + 0.1% Na formate	N_2-O_2 = 99:1	6.5	18
Thermocrinis sp. strain HI 11/12	OS + 0.1% thiosulfate + 0.1% $NaHCO_3$	N_2-O_2-H_2 = 96:1:3	6.5	18

(GME and GDE, respectively) lipids have been described in *Thermodesulfobacterium commune* (30) and *Aquifex pyrophilus* (22).

In addition to carrying distinctive chemical structures, lipid biomarkers also encode the stable isotopic signature that provides information about the physiologies of the source organisms (10, 26). However, interpretation of these isotopic signatures requires specific knowledge about C isotopic discriminations associated with the biochemical pathways involved in carbon fixation and lipid synthesis. Only a limited amount of information is available on the bulk isotopic fractionation factors for cultured *Aquificale*s (17), and to our knowledge, nothing has been reported on the isotopic composition of lipids.

In this study, we initially set out to examine the microbial composition of the Octopus Spring PSC and nearby vent biofilms through a comprehensive lipid analysis. The resultant data revealed a more complex situation than was apparent from genomic analysis alone and also indicated a need for appropriate supporting data from pure-culture studies. A comparison of the lipid profiles of several genera within the *Aquificales* as well as measurements of the carbon isotopic fractionation associated with autotrophic and heterotrophic growth of *T. ruber* formed a framework for improved understanding of the population structure of the Octopus Spring PSC and associated vent microbiota.

MATERIALS AND METHODS

Sample collection and preparation. Biomass consisting of the PSC was collected using forceps from an 87°C, pH 8.3 site in the main outflow just below the source pool vent of Octopus Spring in May 1997. The filaments were placed in glass tubes, sealed with Teflon-lined caps, frozen on dry ice within 3 h, and maintained so in transit to the National Aeronautics and Space Administration (NASA) Ames Research Center. A vent wall geyserite sample, approximately 25 cm^2, was removed in 1996 from the shallower main pool that contains the main effluent of Octopus Spring (≈92°C, pH 8.0). The geyserite sample was also kept frozen until it was prepared for analysis.

Working in a glove box, the topmost 1 to 2 mm of the frozen geyserite biofilm was carefully removed by scraping with a sterilized spatula. The remaining material was then crushed in a sapphire mortar and pestle that had been cleaned with methanol and transferred to sterilized glass vials. PSC and vent geyserite samples were lyophilized and then ground to a powder in a glass mortar previously cleaned with sequential solvent washes of dichloromethane, methanol, and acetone. All glassware and metal implements used in our procedures were baked at 450°C for a minimum of 4 h. Only Teflon stoppers and/or Teflon-lined screw caps were used in analyses.

Strains and culture conditions. *Thermocrinis ruber* OC 1/4 (DSM 12173), *Aquifex pyrophilus* Kol5a (DSM 6858), *Hydrogenobacter thermophilus* TK-6 (IAM 12695), *Aquifex aeolicus* VF5 (21), and *Thermocrinis* sp. strain HI 11/12 (18) were obtained from the culture collection of the Lehrstuhl für Mikrobiologie, Universität Regensburg, Regensburg, Germany. Cell masses of the *Aquificales* strains were grown at 85°C (70°C for *H. thermophilus* and 80°C for *T. ruber* with formate) with stirring (up to 400 rpm) in a 300-liter enamel-protected fermentor (Bioengineering, Wald, Switzerland) as described in Table 1. For growth of *T. ruber* in experiment 1 (isotope study), the cell titer was monitored and the culture was gassed with increasing flow rates (2, 5, 7.5, and 10 liter min^{-1}) to maintain the growth rate.

Phylogenetic analyses. For the analyses, an alignment of about 11,000 homologous full primary sequences available in public databases (ARB project [32, 33]) was used. The *Aquificales* 16S rRNA gene sequences were fitted in the 16S rRNA tree by using the automated tools of the ARB software package (33). Distance matrix (Jukes and Cantor correction), maximum parsimony, and maximum likelihood (fastDNAml) methods were applied as implemented in the ARB software package (34).

Lipid extraction, separation, and analysis. Lipids were extracted from lyophilized ground sinter or *Aquificales* biomass using a single-phase modification of the Bligh and Dyer procedure, and water-soluble contaminants were removed as previously reported (24). Elemental sulfur was removed by passing the total lipid extract over activated copper powder. The total lipid extract (TLE) was dried under nitrogen and then maintained in a vacuum desiccator over Drierite until it reached a constant weight.

A portion of the PSC total lipid was used for an oxidation-reduction procedure to convert bacteriohopanepolyol to its hopanol derivative (44) and analyzed as previously reported (24).

Fatty acid methyl esters (FAME) and glycerol ethers (GME and GDE) were prepared by two procedures. In procedure I, FAME were prepared by subjecting a portion of the TLE to mild alkaline methanolysis (36) with heating at 37°C for 1 h. FAME were separated from the remaining polar ether lipids (GME and GDE) by thin-layer chromatography (TLC) using a methylene chloride mobile phase as previously reported (24). The FAME (R_f = 0.80) were recovered by eluting the silica gel with methylene chloride, and the ether-linked components were recovered from the origin of the TLC plate by Bligh and Dyer extraction of the silica gel zone. The polar ether components were hydrolyzed in 1 ml of chloroform-methanol-concentrated HCl (1:10:1) by heating to 100°C for 2 h (36), and the glycerol ethers were separated by TLC using hexane-diethyl ether-acetic acid (70:30:1) into GME (R_f = 0.04), GDE (R_f = 0.40), and diphytanylglycerol ether (R_f = 0.49) using reference compounds 1-*O*-hexadecyl-glycerol and 1,2-di-*O*-hexadecyl-glycerol (Sigma, St. Louis, Mo.) and diphytanylglycerol ether isolated from a *Halobacterium* sp. (52).

Procedure II was used in an attempt to analyze small samples such as the Octopus Spring vent geyserite. In this approach, the TLE was directly hydrolyzed with acid as described above, followed by trimethylsilyl (TMS) derivatization of the resulting free glycerol ethers, and gas chromatography-mass spectrometry (GC-MS) of the treated TLE. Some TLE samples were also analyzed for free fatty acids and glycerides by preparation of TMS derivatives. Abundance calculations were based on comparison of peak areas to internal standards, methyl tricosanoate (C_{23}) for FAME and cholestanol for glycerol ethers. Weight percent of FAME (Table 2) was calculated based on flame ionization detector (FID) response of individual fatty acids (C_{14} to C_{22}) relative to C_{23}; for GME and GDE, values are based on the areas of the total ion chromatographs and should be considered semiquantitative.

Some of the PSC and *T. ruber* (experiment 1) TLE were also preparatively separated into a polar lipid (phospholipids) and a neutral lipid (glycolipids and glycerides) fraction by precipitation in cold acetone (27). The components of the polar and neutral fractions were then separated by thin layer chromatog-

TABLE 2. Comparison of ester-linked fatty acid and glycerol ether composition of Octopus Spring PSC and *Aquificales* cultures[a]

Compound	Distribution (%, wt/wt)								
	PSC	OC 1/4				HI 11/12	Ko15a	VF5	TK-6
		Expt 1	Expt 2	Expt 3	Expt 4				
Fatty acids[b]									
14:0	<0.1	<0.1	0.1	0.1	<0.1	<0.1	0.3	0.1	<0.1
i-15:0	0.1	nd	nd	nd	nd	nd	nd	nd	nd
15:0	0.1	nd	nd	nd	<0.1	<0.1	0.1	<0.1	<0.1
i-16:0	0.9	nd	nd	nd	nd	nd	nd	nd	nd
16:1*c*Δ7	0.1	0.2	0.3	0.3	0.8	0.3	0.5	0.4	0.2
16:1*c*Δ9	<0.1	nd	nd	nd	nd	<0.1	0.1	0.1	<0.1
16:0	1.5	0.3	0.9	1.0	0.8	0.5	2.3	1.0	0.4
i-17:0	18.9	nd	nd	nd	nd	nd	nd	nd	nd
ai-17:0	0.7	nd	nd	nd	nd	nd	nd	nd	nd
17:0	0.8	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	nd	<0.1
cy-17	0.1	nd	nd	nd	nd	nd	nd	nd	nd
i-18:0	4.4	nd	nd	nd	nd	nd	nd	nd	nd
18:1*c*Δ9	0.6	2.3	4.7	2.3	9.0	2.4	4.0	3.4	3.5
18:1*c*Δ11	<0.1	2.4	1.3	0.2	5.1	1.1	1.8	2.2	2.5
18:1*t*Δ9	<0.1	0.4	0.3	0.1	tr	0.6	0.7	nd	0.2
18:0	12.9	12.1	25.5	31.0	12.6	14.1	27.3	14.0	16.5
i-19:0	16.6	nd	nd	nd	nd	nd	nd	nd	nd
ai-19:0	0.6	nd	<0.1	<0.1	nd	<0.1	0.1	nd	nd
cy-19 (2 isomers)	1.8	2.2	0.7	3.8	1.4	2.9	2.6	1.8	0.6
19:0	2.4	<0.1	<0.1	0.1	<0.1	0.1	0.2	nd	<0.1
i-20:0	0.7	nd	nd	nd	nd	nd	nd	nd	nd
20:1*c*Δ11	3.6	23.1	43.0	8.9	50.9	10.7	18.4	17.5	49.4
20:1*c*Δ13	0.3	14.3	1.7	1.1	3.1	8.2	1.3	3.7	11.3
20:1*t*Δ11	0.3	9.4	nd	1.1	nd	5.4	5.4	11.1	nd
20:1*t*Δ13	nd	3.8	nd	nd	nd	3.5	nd	nd	nd
20:0	6.9	3.9	5.3	7.0	1.9	4.6	5.6	3.4	3.2
i-21:0	0.7	nd	nd	nd	nd	nd	nd	nd	nd
cy-21 (2 isomers)	23.4	24.7	15.7	42.3	14.2	42.4	29.8	40.3	11.6
21:0	0.8	<0.1	<0.1	<0.1	nd	0.1	0.1	0.1	nd
22:1*c*Δ13	0.5	0.7	0.3	0.4	0.2	0.8	0.4	0.3	0.3
22:0	0.2	0.3	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1
FA concn	5.5	92.2	123.7	92.6	89.2	103.5	13.8	35.3	38.8
Glycerol ethers[c]									
GME-18:1	nd	1.8	nd	1.9	10.7	Tr	3.9	2.2	2.7
GME-18:0	62.4	75.2	90.6	86.7	38.5	78.5	57.3	73.0	82.7
GME-19:0	11.5	nd	nd	0.9*	0.6	Tr	2.5*	0.2*	nd
GME-20:1	nd	13.2	7.0	2.9	37.5	17.6	14.4	13.9	11.1
GME-20:0	24.1	4.1	2.5	5.1	4.1	2.0	13.8	10.3	3.5
GME-21:1	nd	3.8	nd	2.6	5.7	1.9	8.1	0.5	nd
GME concn	3.0	5.9	1.7	4.5	1.6	0.9	3.1	19.3	1.2
GDE-17:0, 17:0	0.7	nd	nd	nd	nd	nd	8.9	nd	nd
GDE-17:0, 18:0	0.8	nd	nd	nd	nd	nd	2.1	nd	nd
GDE-18:0, 18:0	33.0	nd	Tr	Tr	73.2*	nd	34.5*	32.2	nd
GDE-18:0, 19:0	7.7	nd	nd	nd	nd	nd	10.0	2.5	nd
GDE-18:0, 20:0	16.2	nd	nd	nd	26.8*	nd	13.5	9.1	nd
GDE-18:0, 21:1	33.1	nd	nd	nd	nd	nd	23.4	44.9	nd
GDE-20:0, 20:0	3.1	nd	nd	nd	nd	nd	7.7	11.2	nd
GDE-19:0, 21:1	3.5	nd	nd	nd	nd	nd	nd	nd	nd
GDE concn	1.2	nd	trace	trace	0.1	nd	1.1	0.6	nd

[a] For culture identification and growth condition, see Table 1. nd, not detected.

[b] FAME were prepared by procedure I. Acyl-alkyl chain nomenclature designates carbon number as saturated (:0) or monounsatured (:1) with double bond of *cis* (*c*) or *trans* (*t*) configuration and position relative to carboxyl end (Δ) or cyclopropyl ring (*cy*), with *iso*- (*i*) or *anteiso*- (*ai*) methyl branching. FA concn, fatty acids in micromoles per gram (dry weight).

[c] GME and GDE results for PSC and *Thermocrinis* (1) from procedure II, remaining samples from procedure I. * presence of multiple GDE isomers with saturated and/or unsaturated alkyl chains (:1 or *cy*). GME and GDE concentrations given in micromoles per gram (dry weight).

raphy on Silica gel G plates (Merck) using acetone-benzene-water (91:30:8) (37) or, in some cases, chloroform-methanol-water (65:25:4) (27). Preliminary characterization of TLC zones was made based on migration of standard diacyl compounds (phosphatidylcholine, phosphatidylethanolamine, and di- and monogalactosyl diglycerides) and staining with specific detection reagents, phosphomolybdic acid, ninhydrin, and α-naphthol (27). TLC zones for lipid analysis were detected by UV fluorescence with rhodamine 6G and recovered by the Bligh and Dyer elution. FAME and glycerol ethers were prepared from each fraction as described above. The double bond positions of the monounsaturated FAME were determined by preparing the dimethyl disulfide adducts (57). TMS derivatives of the glycerides were prepared using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (1:1 in pyridine).

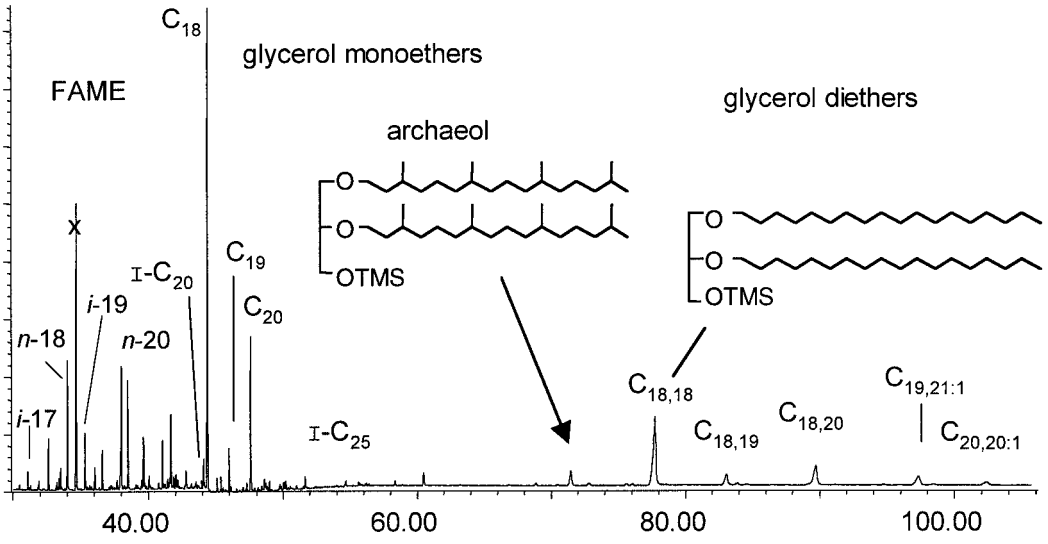

FIG. 1. Total ion chromatogram of biofilm-associated siliceous sinter from within Octopus Spring vent pool (92°C) showing predominance of C_{18} mono- and dialkyl glycerol ethers. Structures are shown for 1,2-di-*O*-phytanylglycerol and $C_{18,18}$-diakylglycerol ether. Symbols designate FAME (*n*- or *i*-18), isoprenoid lipid (I-), monoalkyl glycerol (C_{18}), and dialkyl glycerol ($C_{18,18}$) with accompanying carbon chain lengths.

Alkyl moieties were released from the glycerol ether compounds by reaction with BBr_3 as reported previously (50).

Gas chromatographic analyses. FAME were analyzed by using a Perkin-Elmer Sigma 3B gas chromatograph equipped with an FID and 30-m megabore columns (J & W Scientific), either a DB-5ms programmed to increase at 4°C/min from 160 to 280°C, or a DB-27 programmed to increase at 4°C/min from 120 to 220°C. Compound identification was based on retention times on the nonpolar and the polar columns and on mass spectral analysis (see below).

GC-MS analyses of fatty acids as FAME or TMS esters and glycerol ethers as TMS derivatives, were performed using an HP 6890 gas chromatograph equipped with a J&W DB-5 (60 m by 0.32 mm, 0.25-μm film) capillary column and an HP 5973 mass-selective detector operated at 60°C for 10 min, then programmed at 10°C/min to 320°C, and held for 60 min (Fig. 1). The bond positions of the monounsatured FAME were determined by analyzing their dimethyl disulfide adducts as previously described (25).

Isotopic measurements. The dissolved inorganic carbon (DIC) was measured by taking three 40-ml water samples from the outflow site using a syringe and immediately filtering through Whatman GF/F filters into preevacuated 130-ml serum bottles sealed with silicone stoppers and containing a few drops of saturated $HgCl_2$ to inhibit bacterial growth. The bottles were kept chilled until analysis. At Ames, samples were withdrawn and acidified, and the CO_2 gas was collected on a vacuum line for isotopic analysis on a Nuclide 6-60RMS mass spectrometer modified for small samples (9, 14). Analysis of the DIC composition of the *Thermocrinis* culture medium was similar except that samples were collected by filling three glass tubes with the gassed culture medium prior to inoculation and immediately stoppering them with crimp seals before shipment by air to Ames from Germany. Biomass and total lipid were determined at Ames using a Carlo Erba CHN EA1108 elemental analyzer interfaced to a Finnigan Delta Plus XL isotope ratio mass spectrometer (EA-IRMS). Compound specific isotope analyses were done at the Australian Geological Survey Organisation (AGSO) as previously described using a Finnigan MAT 252 mass spectrometer equipped with a CuO/Pt microvolume combustion furnace and a Varian 1400 gas chromatograph with DB-5 column (25). Reported δ values for FAME and GME were the averages of two runs which agreed within ±1.2‰ and have been corrected for the presence of carbon added during derivatization (−43‰ in the case of TMS-C and −50‰ in the case of methanol carbon used to form FAME).

RESULTS

Comparison of PSC and vent biofilm lipids. The total organic carbon (TOC) recovered from the vent geyserite surface was only 0.24%, compared with 7.2% for PSC and 36.7% for *T. ruber* biomass (experiment 1). Procedure II, direct acid hydrolysis, was used to minimize loss of material during preparation of the vent sample (Fig. 1) and to allow comparison with PSC and *T. ruber* samples. Although this method does degrade the cyclopropyl FAME present in these extracts (6), it provides valuable comparative information.

The fatty acids of vent communities and PSC were predominantly C_{17} to C_{22} chain lengths, distinguished by *iso*-homologues of the C_{16} to C_{21} acids plus a diminished (see above) amount of *cy*-C_{21}. The TMS derivatives of the free fatty acids present in the vent TLE confirmed that high amounts of *cy*-C_{21} (25% of total) were characteristic of the Octopus Spring vent biofilm community. The fatty acid composition of vent and PSC extracts, while qualitatively similar, differed quantitatively. Streamers had more abundant branched-chain fatty acid (41% in PSC versus 13% in Octopus Spring vent) with a somewhat shorter chain distribution: the ratio of *i*-$C_{17:0}$ to *i*-$C_{19:0}$ was 1:2.1 in PSC but 1:3.7 in the Octopus Spring vent. The *anteiso* analogues *ai*-$C_{17:0}$ and *ai*-$C_{19:0}$ were present in PSC (1.3%) but almost absent in the Octopus Spring vent. The Octopus Spring vent TLE also contained small amounts of even-carbon-numbered chain fatty acids, *n*-22:0 to *n*-30:0, and a mid-chain branched octadecanoic acid (1%), possibly 10- or 12-methyl-$C_{18:0}$. Alkyl glycerol ethers were abundant in both vent and PSC, however, the vent GDE represented a higher proportion (43%) of total ether lipids than in PSC (29%). Significant amounts of archaeal biomarkers, 1,2-di-*O*-phytanylglycerol (archaeol) and both C_{20}- and C_{25}-isoprenoid glycerol monoethers, were present in the vent biofilm and PSC (≈5% of total ether lipids). BBr_3 cleavage of the ether alkyl chains showed that, apart from the isoprenoid moieties, only straight-chain compounds were present, with carbon numbers consistent with the GC-MS characterization of the intact glycerol ethers. C_{18} chains dominated both GME and GDE (Fig. 1).

PSC and *Aquificales* polar lipids. The acid hydrolysate of the *T. ruber* TLE (procedure II) was composed primarily of saturated and monounsaturated C_{20} fatty acids and a C_{18}-GME

TABLE 3. Stable carbon isotopic composition ($\delta^{13}C$) and distribution of fatty acids in major lipid fractions isolated from PSC[a]

Fatty acids[b]	PL- 2		PL-4		NL-2	
	$\delta^{13}C$ (‰)	Distribution % (wt/wt)	$\delta^{13}C$ (‰)	Distribution % (wt/wt)	$\delta^{13}C$ (‰)	Distribution % (wt/wt)
i-17	−27.6	22.7	−27.2	2.8	−27.4	28.6
i-18	−27.9	6.1	nm	0.2	−27.2	5.5
18:1	nm	0.3	nm	1.2	nm	0.8
18:0	−24.7	12.3	−23.2	19.1	−24.9	10.4
i-19	−27.2	18.9	−27.2	2.6	−26.6	26.2
cy-19	nm	1.5	−23.2	2.6	nm	1.0
19:0	−23.7	2.1	−22.5	4.1	nm	1.1
20:1	−21.6	3.1	−20.4	7.6	nm	2.1
20:0	−23.6	5.8	−23.3	8.9	−23.7	6.6
cy-21	−24.5	20.9	−23.8	42.3	−24.2	8.4
Avg[c]	−25.1		−23.4		−26.2	

[a] PSC site at main vent outflow of Octopus Spring was 87°C, pH 8.3, with a total DIC of 5.4 mM and $\delta^{13}C$ of −1.5‰ (CO_2 = −4.7‰). No information is available for potential dissolved organic carbon sources. PL-2, PL-4, and NL-2 represented 45.5, 14.6, and 29.1% of total FAME, respectively. nm, insufficient material for measurement.

[b] Compound-specific isotope analysis of individual FAME. Values represent averages of two runs which agreed to within at least ±1.2%. The $\delta^{13}C$ FAME are reported for baseline resolved peaks or, in the case of unsaturated positional isomers, the integrated area of the unresolved peaks.

[c] $\delta^{13}C$ calculated by mass balance for each fraction based on relative abundance and $^{13}C/^{12}C$ ratio for individual fatty acids, where $\delta^{13}C$ FAME = [(ratio FAME − ratio Peedee Belemnite)/ratio Peedee Belemnite] × 1,000.

(Table 2). No *iso*-branched fatty acids or GDE were detected. Additional analyses of the lipid using an alkaline methanolysis procedure (I) confirmed the high proportion of C_{20} fatty acids with $C_{20:1}$, which together comprised over 49% of the total for *T. ruber* OC1/4 (Table 2, experiment 1). Large amounts of *cy*-C_{21} were also recovered in *T. ruber* and PSC by this procedure, and all subsequent FAME analyses were so carried out (Tables 2 and 3).

T. ruber biomass from a variety of growth conditions, as shown in Table 1, was analyzed. In experiment 1, *T. ruber* was grown using thiosulfate with a constant gassing of H_2-CO_2-O_2 to maintain high substrate levels for measurement of the carbon isotopic discrimination associated with CO_2 fixation (results below). These conditions resulted in accumulation of relatively large amounts of intracellular sulfur (≈15% of dry weight). S^0 accumulation was not apparent in *T. ruber* grown with thiosulfate but without H_2 (experiment 2) or in PSC extracts. The lipid composition of additional *Aquificales* cultures (Table 1) was also analyzed to assess the potential use of *Thermocrinis*-like lipids as group biomarkers to characterize the pink streamer community (Table 2). All *Aquificales* cultures contained GME, and no *iso*-branched fatty acids were detected (Table 2). GDE were only present in the lipids of the PSC and the two marine *Aquifex* cultures, *A. aeolicus* VF5 and *A. pyrophilus* Kol 5a. BBr_3 cleavage of the *Aquificales* glycerol ethers showed only straight-chain alkyls with chain length distributions similar to those characterized for the intact molecules.

Using the methods of Rohmer et al. (44), no hopanoids were detected in the PSC total lipid extract, either as the polar bacteriohopanepolyol or as the free lipids, diplopterol or diploptene.

A preliminary attempt was made to separate the complex lipids of the PSC extract to aid in biomarker identification and as a preparative step to carbon isotope analysis. The diversity of polar head groups and the occurrence of both diacyl and dialkyl moieties made separation by one-dimensional TLC difficult. TLC separation of the polar lipid (PL) fraction using the chloroform-methanol-water system recovered 92% of PL-FAME in two zones, PL-2 (R_f = 0.35 to 0.25) and PL-4 (R_f = 0.60 to 0.56) that roughly comigrated with diacylphosphatidylcholine (R_f = 0.33) and diacylphosphatidylethanolamine (R_f = 0.66) (Table 3). All PL zones showed the presence of multiple components, and PL-4 was dominated by an aminolipid similar to that observed from *A. pyrophilus* (27).

FAME present in the neutral lipid (NL) fraction were separated using the acetone-benzene-water system. Both a glycolipid zone that migrated closely with a digalactosyl diglyceride standard (R_f = 0.43) and a rapidly migrating component (R_f = 0.95), a probable free glyceride designated NL-2, accounted for 12 and 82% of recovered NL-FAME, respectively (Table 3). The GDE present in the PL fraction were recovered primarily in PL-4 and those in the NL fraction in NL-2. Together these two zones accounted for 60 and 25% of total GDE, respectively. While the recoveries of FAME and GDE were in good agreement with analyses made using the TLE, the recovery of GME was poor (see below).

Lipid fractions as described above were also prepared from the total lipid of *T. ruber* (experiment 1). In this case, most of the FAME were recovered from two fractions equivalent to PL-2 and PL-4 with 24 and 70%, respectively. No GDE were detected in any of the isolated fractions, and although most of the GME was also recovered in PL-2 and PL-4, the recovery was not significant (0.2 μmol/g [dry weight]) relative to the amount measured by the direct acid hydrolysis procedure (Table 2). This discrepancy appears to be associated with the use of TLC to separate the polar compounds and not procedure I, as relatively large amounts of GME were recovered during analysis of the additional *Aquificales* cultures using procedure I.

Carbon isotopic composition. The Octopus Spring water had a relatively high DIC content, 5.3 mM, with a $\delta^{13}C$ value of −1.5‰ ± 0.3‰ (n = 3). At the temperature (87°C) and pH (8.3) of the pink streamer site, a dissolved CO_2 concentration ($[CO_2]$) of 71 μM and $\delta^{13}C$ of −4.7‰ can be calculated based on a pK_a for carbonic acid at 90°C of 6.42 and the equation of Mook et al. (35) in which the fractionation between dissolved CO_2 (d) and dissolved bicarbonate (b) is given by $E_{d/b}$ = 24.12‰ − 9866/T, where T is the absolute temperature. The streamer biomass and TLE from this site are depleted in ^{13}C relative to $[CO_2]$ by 10.9 and 17.2‰, respectively (Table 4).

In laboratory cultures (Table 1, experiment 1), a sample of medium removed prior to inoculation measured 14.1 mM DIC with a $\delta^{13}C$ of −25.5‰ ± 0.9‰ (n = 3). At the temperature and pH of the fluid, the $[CO_2]$ was 6.2 mM, with a $\delta^{13}C$ of −27.4‰. The carbon available from the continuously flowing gas mixture far exceeded that assimilated by the culture. The *T. ruber* biomass and lipids were depleted in ^{13}C relative to the $[CO_2]$ by 3.3 and 2.1‰, respectively. As shown in Table 4, this depletion is much smaller than that observed for the PSC. Moreover, the patterns of depletion differ, with lipids depleted relative to biomass in the PSC and enriched in *T. ruber*.

In a second experiment where *T. ruber* was grown with 0.1% formate (Table 1, experiment 4), biomass was much more

TABLE 4. C isotopic composition of isolated components from PSC and *T. ruber* grown as a lithoautotroph with thiosulfate-H_2-O_2-CO_2 and as a chemoorganotroph with formate and O_2[a]

Sample	Carbon source	$\delta^{13}C$ (‰)	Component	$\delta^{13}C$[a] (‰)	$\Delta\delta^{13}C_{component\text{-}source}$ (‰)
PSC	CO_2	−4.7	Biomass	−15.6 ± 0.1*	−10.9
			Extracted residue	−15.2 ± 0.2	−10.5
			Total lipid	−22.6 ± 0.1	−17.2
T. ruber	CO_2	−27.4	Biomass	−30.7 ± 0.1*	−3.3
			Extracted residue	NM	NM
			Total lipid	−29.5 ± 0.1	−2.1
	Formate	−23.3 ± 0.3	Biomass	−42.9 ± 0.9	−19.7
			Extracted residue	−44.3 ± 0.1	−21.0
			Total lipid	−41.9 ± 0.1	−17.9

[a] CO_2 values based on DIC analysis run in triplicate with $\delta^{13}C_{DIC}$ of 1.5‰ ± 0.3‰ for PSC and 25.5‰ ± 0.9‰ for *T. ruber*. All remaining values from EA-IRMS analysis are the averages of two runs. *, analysis after treatment of biomass with 1 N HCl to remove potential carbonate minerals showed no appreciable change in C isotope composition. NM, not measured or not done.

strongly depleted in ^{13}C relative to carbon source (19.7‰). The yield from 250 liters of medium was 5 g (dry weight) (39.2% carbon), which accounted for 0.36% of available carbon. As with growth on CO_2, lipids were slightly enriched relative to biomass.

The carbon isotopic compositions of several individual lipid biomarkers were also determined (Table 5). In the PSC fractions, fatty acids clustered into two isotopically distinct groups. Among the peaks with sufficient material for isotopic analysis, the *iso*-branched fatty acids (*i*-$C_{17:0}$, *i*-$C_{18:0}$, and *i*-$C_{19:0}$) in PL-2, PL-4, and NL-2 were more depleted in ^{13}C than the longer-chain C_{20} and *cy*-C_{21} fatty acids. Depletions relative to CO_2 averaged 22.6‰ ± 0.4‰ ($n = 8$) for *iso*-branched and 18.4‰ ± 1.4‰ ($n = 8$) for the C_{20} and C_{21} acids. Bulk fractions varied in parallel. PL-2 and NL-2 contained greater proportions of *iso* acids relative to long-chain acids and were depleted in ^{13}C relative to PL-4, in which longer-chain acids were more abundant. Alkyl chains from glycerol ethers analyzed after BBr_3 cleavage yielded δ values of −23.8, −21.8, and −23.8‰ for C_{18}, C_{19}, and C_{20}, respectively.

DISCUSSION

Lipid composition and makeup of PSC and vent communities. A previous study of the pink filamentous streamers at Octopus Spring identified three phylotypes, EM3, EM17, and EM19, from amplification of the mixed-population DNA (43). A phylogenetic tree was constructed to take advantage of a current, more extensive sequence database (Fig. 2) and confirms that the EM17 gene sequence clusters among the *Aquificales* and is closely related (99% sequence identity) to the pink streamer isolate *T. ruber* (18), whereas the EM3 sequence is related to the *Thermotogales*. EM19, however, constitutes a separate, more deeply diverging lineage, well outside the *Aquificales* and *Thermotogales*. In Reysenbach's study (43), the EM17 sequence represented the majority of clones examined (26 of 35) and a fluorescently labeled oligonucleotide probe complementary to EM17 hybridized in situ to the pink filaments. No hybridization was noted for EM3 or EM19 probes even though all morphotypes in the PSC did bind to universal and bacterial probes (43).

TABLE 5. Stable carbon isotopic composition ($\delta^{13}C$) and distribution of fatty acids and glycerol ethers of *T. ruber* grown with CO_2 or formate[a]

Compound	CO_2 (−27.4‰)			Formate (−23.3‰)		
	$\delta^{13}C$[b] (‰)	$\Delta\delta^{13}C$ (‰)	Distribution % (wt/wt)	$\delta^{13}C$ (‰)	$\Delta\delta^{13}C$ (‰)	Distribution % (wt/wt)
Fatty acids						
18:1	−29.6	−2.2	5.1	−41.1	−17.8	14.1
18:0	−29.4	−2.0	12.1	−41.2	−17.9	12.6
cy-19	−23.9	+3.5	2.2	−44.0	−20.7	1.4
20:1	−30.6	−3.2	50.6	−41.7	−18.4	54.0
20:0	−30.3	−2.9	3.9	−40.8	−17.5	1.9
cy-21	−25.2	+2.2	24.7	−42.7	−19.4	14.2
22:1	−29.9	−2.5	0.7	nm	nm	0.2
Avg		−1.2			−18.4	
GME						
GME-18:1	nm	nm	1.8	−41.8	−18.5	10.7
GME-18:0	−27.7	−0.3	75.2	−42.5	−19.2	38.5
GME-20:1	nm	nm	13.2	−42.9	−19.6	37.5
Avg					−19.3	

[a] *T. ruber* cultures were grown either at 85°C and pH 6.5, constantly gassed with 20% CO_2 as the carbon source ($\delta^{13}C = -27.45‰$) at 80°C and pH 6.5 with 14.5 mM sodium formate ($\delta^{13}C = -23.3‰$) (calculation shows that only 0.36% of available formate carbon was recovered as biomass). For the CO_2-grown culture, the measured DIC was 14.3 mM with a $\delta^{13}C$ of −25.5‰ ($CO_2 = -27.4‰$).

[b] See Table 3, footnote *c*, for explanation. nm, insufficient material for measurement.

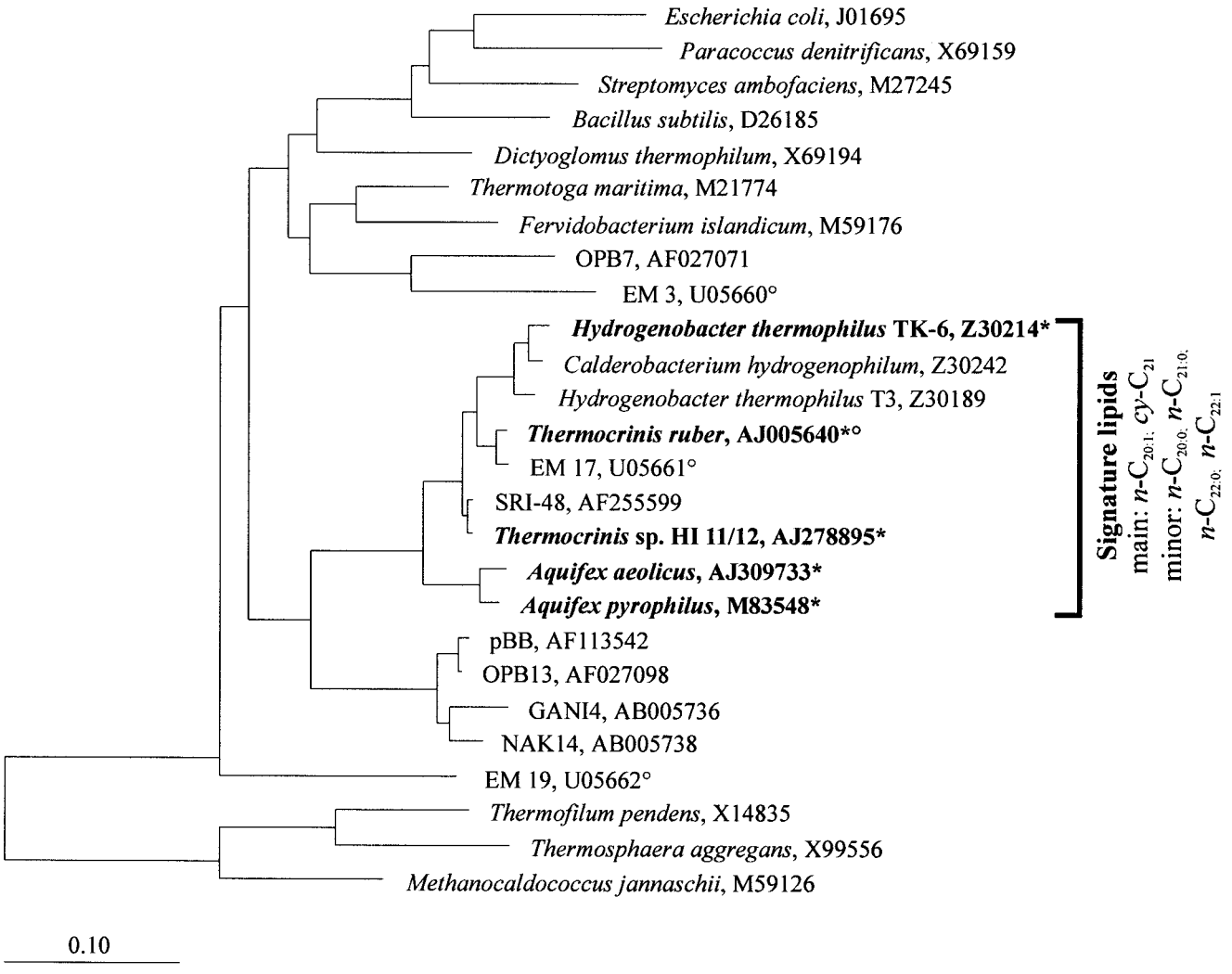

FIG. 2. 16S rRNA gene-based phylogenetic tree of the *Aquificales* based on the results of a maximum-likelihood analysis showing C_{20-22} signature lipids for this group. Reference sequences were chosen to represent the broadest diversity of *Bacteria*. Only sequence positions that have identical residues in 50% or more of all available bacterial 16S rRNA sequences were included for tree reconstruction. Accession numbers for the sequences are indicated. The scale bar represents 0.10 fixed mutations per nucleotide position. Of the sequences previously identified in the PSC (°), only EM17 is closely related to *Aquificales* from this study (*).

The pink streamers of the Octopus Spring outflow channel were characterized by high levels of *iso*- and cyclopropane ester-linked fatty acids and straight-chain ether-linked alkyl lipids (Table 2). Similar lipids were associated with the biofilm growing on the siliceous sinter walls of the vent pool (Fig. 1). The presence of two fatty acid pools, the C_{15}-C_{19} *iso*-branched fatty acids and the C_{20}-*cy*-C_{21} fatty acids (Tables 2 and 3), together with the absence of *iso*-branched fatty acids in *Aquificales* cultures (Table 2), indicated that the PSC contained more than one distinct bacterial population.

To date, *T. ruber* OC 1/4 is the only cultivated isolate from the PSC (18, 21). Its fatty acid composition is similar to that reported previously for a number of *Hydrogenobacter thermophilus* strains (28) and to those of the additional *Aquificales* cultures analyzed here (Table 2). The fatty acids of these *Aquificales* were dominated by *n*-$C_{18:0}$, *n*-$C_{20:1}$, and *cy*-C_{21} (Table 2). Two sets of monounsaturated isomers, $C_{18:1\Delta9}$ and $C_{18:1\Delta11}$, and their chain elongation products, $C_{20:1\Delta11}$ and $C_{20:1\Delta13}$, were detected. Since cyclopropane fatty acids are formed by the addition of a methylene group from *S*-adenosylmethionine across the double bond of a monounsaturated fatty acid, the two *cy*-C_{21} isomers are probably derivatives of the *n*-$C_{20:1}$ isomers. C_{20} fatty acids are rare in bacteria, and the presence of large amounts of the *n*-$C_{20:1}$ and *cy*-C_{21}, with lesser amounts of *n*-$C_{20:0}$, *n*-$C_{21:0}$, *n*-$C_{22:0}$, and *n*-$C_{22:1}$ in representatives of four distinct subclusters within the *Aquificales* (Fig. 2, Table 2), demonstrates a phylogenetic clustering for these membrane lipids and suggests that these fatty acids can serve as taxonomic marker signatures for this order. Notably, the pink streamers also contained similar C_{20}, C_{21}, and C_{22} fatty acids.

Nonisoprenoid alkyl glycerol ethers are being increasingly recognized as bacterial membrane lipids. In addition to the GME and GDE with the *n*-C_{16-18} alkyl chains previously de-

scribed in *A. pyrophilus* (22), GME and GDE with *iso*- and *anteiso*-branched chains have also been identified as major membrane lipids in two anaerobic thermophiles, *Thermodesulfobacterium commune* (30) and *Ammonifex degensii* (19). An unusual glycerol monoether with a dimethyltriacontanyl chain has been identified in another thermophile, *Thermotoga maritima* (8). Additionally, small amounts of glycerol monoethers with normal or methyl-branched chains have been detected in mesophilic and thermophilic clostridia. These presumably are derived from 1-*O*-alkylglycerols in which an ester-linked fatty acid is initially present at position 2 (31). Environmental analyses have identified small amounts of *n*-C_{18} and *br*-C_{17} 1-*O*-alkyl ethers and a C_{15},C_{15} 1,2-*O*-alkyl diether in hot spring cyanobacterial mats (58, 59), and more recently, relatively abundant *n*- and *br*-$C_{14–18}$ 1-*O*-alkylglycerols have been found in association with anaerobic methane-oxidizing consortia in marine sediments (15).

All of the *Aquificales* cultures in our study synthesize at least some alkyl glycerol ether lipids (Table 2). The *A. pyrophilus* ether lipids most closely approximated the distribution observed for the PSC and vent alkyl ether lipids. To date, however, the only identified *Aquifex* spp. are marine bacteria. *T. ruber* does not appear to be the source of the PSC ether lipids. The relative abundance of GME and GDE and the alkyl chain distribution in the PSC suggest that an additional *Aquificales*-like organism was present in these thermophilic communities or that some environmental condition is responsible for the presence of GDE. Although we attempted to grow *T. ruber* under a variety of conditions in our study, we cannot preclude this latter point. Simulation of natural flow conditions in the laboratory results in growth of *T. ruber* as filaments rather than as the individual cells characteristic of our batch cultures (18). It is interesting to speculate that the physical environment of more natural growth conditions might allow expression of GDE synthesis.

Although limited by the small amount of biomass available, our analyses suggest that the community of organisms present in the biofilm of the Octopus Spring vent differs somewhat from the PSC. Specifically, the organisms producing the *iso*-fatty acids appear to be less abundant than the *Aquificales*. *Iso*-$C_{17:0}$ and *iso*-$C_{19:0}$ are abundant in the PSC, and, while present in the vent biofilm, the *iso*-$C_{19:0}$ is now the only major branched acid and is present in much lower amounts relative to the $C_{20–22}$ fatty acids and the glycerol ethers representing the *Aquificales* community members. The GDE also make up a much higher proportion of the glycerol ether lipids in the vent biofilm than in the PSC, which may reflect an environmental effect (i.e., higher temperature of vent water) or possibly a different *Aquificales* population.

Carbon isotopic patterns. Four CO_2 fixation pathways have been described for bacteria (see reference 13 for a review). Carbon isotopic fractionation varies widely, depending on CO_2 assimilation pathway. Generally, the enzymes of the reductive acetyl-coenzyme A (CoA) pathway express the largest ^{13}C discriminations, with $\delta^{13}C$ values for biomass relative to CO_2 ranging from 20 to 36‰ (11, 39). Organisms using the Calvin-Benson cycle and ribulose-1,5-bisphosphate carboxylase (Rubisco) for CO_2 incorporation display somewhat less discrimination, with $\Delta\delta^{13}C$ from 11 to 26‰ (12, 39, 40, 47). A much broader range of values is found for the metabolically diverse organisms using the hydroxypropionate cycle ($\Delta\delta^{13}C$ values from 2 to 13‰ relative to DIC [16, 53, 55]), and those using the reductive citric acid cycle (3 to 13‰ [39, 40, 47]). A particular hallmark of organisms of this last group, reductive tricarboxylic acid (TCA), is enrichment of ^{13}C in lipids relative to biomass (54).

The enzymes of the reductive citric acid cycle are present in *A. pyrophilus*, *A. aeolicus,* and *H. thermophilus,* whereas Rubisco is absent (2, 7, 46). However, no information has been available about the C isotopic discrimination associated with growth of these obligately autotrophic bacteria. Moreover, the enzymology and carbon fixation pathways of *T. ruber* which can grow either autotrophically or organotrophically (18), have not been investigated. Our results indicate that *T. ruber* cells grown autotrophically at 85°C are depleted in ^{13}C relative to CO_2 by 3.3‰ and that the lipids are enriched in ^{13}C relative to biomass by 1.2‰. Although both of these results are consistent with fixation of inorganic carbon by the reductive citric acid cycle, a novel metabolism cannot be precluded.

The pink streamer community overall is strongly depleted relative to CO_2 by 10.9‰ (Table 4). Moreover, as shown in Table 3, the individual fatty acids are even more strongly depleted (by 15.7 to 23.2‰). Neither the relatively large depletion in biomass nor the depletion in fatty acids relative to biomass is consistent with dominance of the PSC by *T. ruber* or any other organism utilizing the reductive citric acid cycle for the assimilation of CO_2.

The isotopic composition of the *T. ruber*-like biomass in the PSC can be estimated from the $\delta^{13}C$ value of the relevant biomarkers, namely the C_{20} and C_{21} fatty acids. As shown in Table 4, whether grown on CO_2 or on formate, the lipids in *T. ruber* are enriched in ^{13}C by 1.2 to 1.8‰ relative to biomass. Accordingly, the ≈23‰ value of the C_{20}-C_{21} fatty acids (Table 3) corresponds to a $\delta^{13}C$ biomass of ≈−24.5‰ (Fig. 3). This would be consistent with a CO_2 carbon source with $\delta^{13}C \approx -21$‰ or a formate carbon source with $\delta^{13}C \approx -5$‰ (Fig. 3). The former can be excluded. The isotope composition of the dissolved CO_2 in the pink streamer community is known to be −4.7‰ (Table 4).

It is not known whether formate was present in the waters around the pink streamer community at the time these samples were collected. In subsequent investigations, Shock (E. Shock, personal communication) has found formate downstream but not at the location of the pink streamers and also measured H_2 and CO in the gas phase of the vent source. It is in any case possible that the organisms within the pink streamer communities both produced (from $CO_2 + H_2$ or from $CO + O_2 + H_2O$) and quantitatively consumed HCO_2H. Given the misfit between isotopic composition of DIC and *T. ruber* biomass, this provides the explanation most consistent with the available evidence. *T. ruber* is the only member of the *Aquificales* to grow either autotrophically or as a chemoorganotroph using formate or formamide (18). The nature of this metabolic capacity is unknown (18).

The $\delta^{13}C$ value for the PSC organic carbon overall is −15.6‰ (Table 4). The difference between this value and the −24.5‰ estimated for *T. ruber* biomass requires that the remaining organic carbon be enriched in ^{13}C (Fig. 3). If its abundance is equal to *T. ruber*, by mass balance, it must have

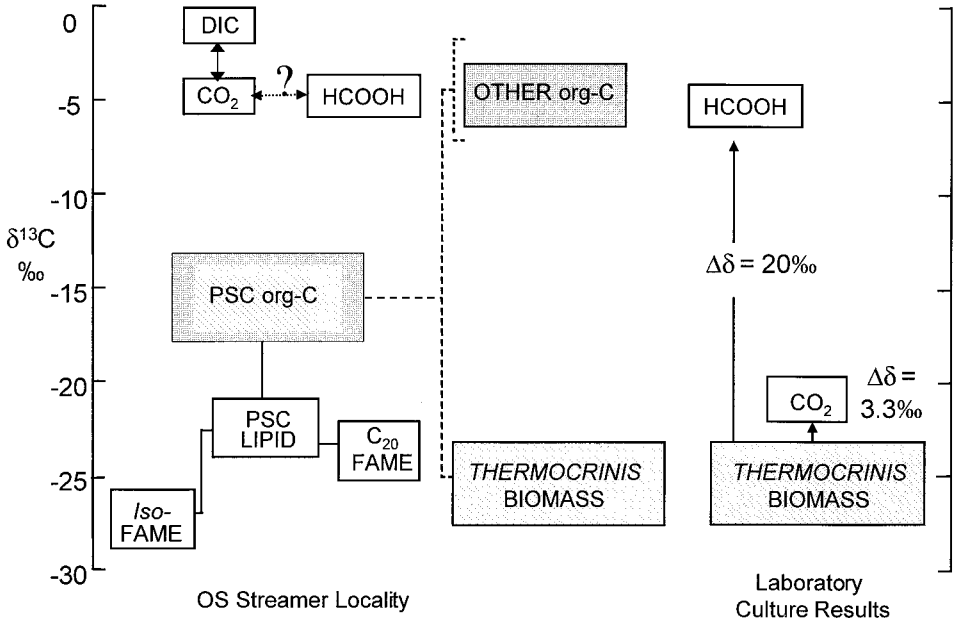

FIG. 3. Proposed carbon isotopic profile for PSC showing measured $\delta^{13}C$ values for isolated PSC components on the left. In the center and to the right, an assumed value of ≈24‰ for *Thermocrinis* biomass is based on the PSC biomarker C_{20} fatty acids and the established relationship between *T. ruber* biomass and fatty acids measured in laboratory cultures. On the right, suggested $\delta^{13}C$ values for formate and CO_2 based on expressed discriminations measured in laboratory culture experiments. Isotopic value for formate in Octopus Spring vent (upper right) is unknown. In this scenario, mass balance between PSC organic C (hatched box) and *Thermocrinis* biomass requires a relatively heavy component (gray box) and leaves unresolved the relationship of *iso*-fatty acids and their associated biomass to the heavy organic carbon pool proposed.

$\delta^{13}C \approx -6‰$ in order to produce an average $\delta^{13}C \approx -15.6‰$.

The nature of this heavy portion of the bulk organic carbon is then problematic. The $\delta^{13}C$ of two additional, independently collected PSC samples (September 1994 and 1999) agreed within ±0.5‰ (data not shown), indicating that the isotopic composition of this community is stable. It does not appear to arise from the *iso*-fatty acids. These have $\delta^{13}C$ values near −27‰. Based on our current knowledge, the biomass to which they are related must have a $\delta^{13}C$ value of −15‰ or lighter (the greatest observed depletion of lipids relative to biomass in microorganisms is ≈12‰ [13]). The total lipid fraction represents a relatively small portion (<10%) of total bulk organic carbon in the PSC and is much too light (−22.6‰), in itself, to account for all of the heavy carbon. Some other nonlipid component, synthesized within the community or brought in from the surrounding environment, must be present to balance the isotopic abundances.

Information is needed about the concentration and isotopic composition of formate in Octopus Spring water, the potential nature of an isotopically heavy component associated with the PSC filaments, the apparent lack of GDE in *T. ruber*, and the physiological mechanisms leading to the novel fractionations associated with its growth on CO_2 and formate in order to fully assess the PSC on physiological, structural, and ecosystem levels.

Hydrogen-oxidizing members of the *Aquificales* are widely distributed in hot springs and thought to play a major role in the biogeochemical processes in these ecosystems (29). The carbon isotopic composition of the biomarker lipids in the PSC points to the expression of a novel metabolic potential by the *Thermocrinis*-like organisms in this hyperthermophilic community, but leaves unanswered the role and/or relationship of the population represented by the *iso*-fatty acids. *Aquificales* are considered the prevalent phylotype in filamentous bacterial communities found in geothermal springs (29, 42, 43, 51), but identification of *Aquificales* signature lipids associated with this vent geyserite suggests a broader ecological role for this group. Electron microscopic images of Octopus Spring geyserite indicate that bacterial communities readily colonize the vent walls and contribute to geyserite morphogenesis (5). Because of their phylogenetic position and their potential microfossil record, this group of organisms is particularly important to a better understanding of Earth's earliest microbial life.

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