Archaebacterial ether lipid diversity analyzed by supercritical fluid chromatography: integration with a bacterial lipid protocol

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Abstract A strategy has been developed for archaebacterial lipid analysis which provides three times the information to describe archaebacterial isolates and is compatible with simultaneous eubacterial/eukaryotic lipid analysis of environmental samples. Eubacterial and micro-eukaryotic biomass, community structure, and nutritional status have been routinely defined in environmental samples by lipid analysis. Lipid profiles are also useful in eubacterial identification and taxonomy. Polar lipid or whole cell ester-linked fatty acids are generally analyzed by gas chromatography–mass spectroscopy. Archaebacteria are characterized by their ether-linked membrane lipids. There is, however, less diversity in the side chains of archaebacterial membrane lipids as compared the eubacterial ester-linked membrane lipids. The information content of the archaebacterial lipid profile was increased by separately analyzing the polar lipid, glycolipid, and lipid-extracted residue fractions. Identification and quantification were performed by supercritical fluid chromatography. Results are presented for three species of methanogens and four thermoacidophile isolates, and compared with a literature review. —**Hedrick, D. B., J. B. Guckert, and D. C. White.** Archaebacterial ether lipid diversity analyzed by supercritical fluid chromatography: integration with a bacterial lipid protocol. *J. Lipid Res.* 1991. **32:** 659–666.

Supplementary key words methanogens • thermoacidophiles • halophiles • taxonomy • ecology

The archaebacteria are a recently defined and highly diverse kingdom which, together with the eubacteria, make up the prokaryotes (1). They include the anaerobic methanogens, extreme halophile aerobe/anaerobes, and aerobic and anaerobic thermoacidophiles. One of the characteristics that distinguishes the archaebacteria from the eubacteria and eukaryotes, and unites their disparate phenotypes, is ether-linked isoprenoid membrane lipids (2).

The capillary gas chromatography and gas chromatography–mass spectroscopy (GC–MS) of polar lipid fatty acids have been extensively used in eubacterial taxonomy and identification (3), and in ecological studies as measures of eubacterial biomass, community structure, and metabolic status (4). The polar lipid ethers of the archaebacteria show much less structural diversity than eubacterial polar lipid fatty acids, being dominated by the diether (DE) and tetraether (TE). Although there is some variation in isoprenoid chain length (5) and cyclization (6), there is inadequate diversity for speciation or for determining ecological treatment effects. A second problem is that ether lipids are very difficult to analyze by gas chromatography, and liquid chromatographic detectors have insufficient sensitivity for many applications. In this work, we propose specific solutions to these problems by analysis of three lipid fractions by supercritical fluid chromatography.

Using the traditional Bligh-Dyer extraction (7), we found surprisingly little ether lipid in the polar lipid fraction of *Methanobacterium formicicum* and *Methanococcus maripaludis*. Nishihara and Koga (8) reported a modification of the Bligh-Dyer extraction which increased the yield by a factor of 5.7 for *Methanobacterium thermoautotrophicum*. This modification includes a trichloroacetic acid addition to the extraction system. Acidic extractions, however, are known to result in hydrolysis of eubacterial and eukaryotic ester-linked fatty acids, and degradation of the acid-labile eubacterial cyclopropyl fatty acids (9).

A different approach to improving archaebacterial ether lipid extraction efficiency while still preserving other types of lipid is an acidic treatment of the lipid-extracted cell residue. Pauly and Van Vleet (10) reported that 20%

Abbreviations: DE, diether; GC–MS, gas chromatography–mass spectroscopy; GL, glycolipid; PL, polar lipid; Res, lipid-extracted residue; SDE, synthetic diether, 1,2-di-O-hexadecylglycerol; SFC, supercritical fluid chromatography; TE, tetraether.

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of the ether lipids in a fresh-water swamp sediment were not extracted by the exhaustive procedure used, but only released after acid hydrolysis of the lipid-extracted residue. Mayberry-Carson et al. (11) reported a lipopolysaccharide-like compound in *Thermoplasma acedophilum* that contained TE. Eubacterial lipopolysaccharide fatty acids are routinely recovered by an acid methanolysis of the lipid-extracted residue (12). In addition to improving recovery, this protocol increases the information content of lipid analysis by providing an additional class of lipids, the lipid-extracted residue (Res) lipids. The information available was increased further by separating the lipid extract into neutral lipid (NL), glycolipid (GL), and polar lipid (PL) fractions.

Supercritical fluid chromatography (SFC) provides a simple, sensitive, and reliable method for determination of archaebacterial ether lipid profiles and other large molecular weight or thermally labile compounds. These cannot be analyzed by GC due to the temperature limits of the chromatograph or the column, or the thermal liability of the compounds. The archaebacterial DE, with a molecular weight of 652, can be determined by modifying a gas chromatograph for that purpose (10). The TE, with a molecular weight of 1300, cannot be analyzed by gas chromatography. TE has been analyzed by hydrogen iodide cleavage of the ether bond and GC analysis of the liberated hydrocarbon (13), but the procedure has many steps and low recovery, and therefore is not appropriate for routine or quantitative analysis. While these compounds may be separated by liquid chromatography, sensitive detectors are not available.

In this report we present a lipid analytical scheme appropriate for pure cultures of archaebacteria or environmental samples containing archaebacteria and eubacteria. The utility of SFC analysis of archaebacterial membrane ether lipids in three fractions of cellular material (GL, PL, Res) is proposed to complement the information obtained by GC analysis of eubacterial PL fatty acids. This protocol triples the lipid variables available for the chemotaxonomic description of archaebacterial species, or for the ecological description of natural communities.

MATERIALS AND METHODS

Supercritical fluid chromatograph construction

The mobile phase was SFC-grade CO_2 (Scott Specialty Gasses, Plumbsteadville, PA) delivered in a dip-tube cylinder and pressurized with a helium headspace. The mobile phase was delivered by an ISCO LC 2600 syringe pump (Lincoln, NE). The pump was controlled by a Commodore 64 BASIC language program available from the authors. The pump and computer were connected with an Omnitronix Deluxe RS232 serial interface. A Varian 3700 gas chromatograph (Varian Instrument Group, Palo Alto, CA) was used for the gas plumbing, valves, oven, and flame ionization detector. A Rheodyne 7520 injection valve (Rheodyne, Cotati, CA) with a 0.2-μl injection loop was used to introduce sample in place of the standard Varian injector plumbing. The analytical column was a 10 m $\times$ 100 μm I.D. SB Phenyl-5 (Lee Scientific, Salt Lake City, UT) with 0.5 μm coating. The flow restrictor used was the integral Guthrie restrictor incorporated into the column (14). An injection splitter was constructed out of 20 cm of 1/16 inch O.D. stainless steel tubing, a Swaglok T union drilled out to 1/16 inch I.D., and 5 cm of 5 μm I.D. uncoated fused silica capillary tubing (15). Peak integration was done with the Nelson Analytical 3000 Chromatography System (Nelson Analytical, Paramus, NJ).

The operation of the flame ionization detector of the Varian GC required no modification. The position of the end of the capillary restrictor within the detector and the flow rates for air, hydrogen, and nitrogen were varied to determine the optimal combination. The standard settings for capillary gas chromatography (Varian 3700 Gas Chromatograph Instruction Manual, Varian Inc., Palo Alto, CA) were unchanged for supercritical fluid chromatography—makeup gas and hydrogen at 30 ml/min, air at 300 ml/min, and the end of the capillary restrictor inserted 11.5 cm into the detector.

All SFC analyses were performed with an oven temperature of 120°C and the detector at 350°C. A typical pressure program began at 1500 psi, increased at 50 psi per minute for 40 min to 3500 psi, then at 100 psi per min for 20 min to end at 5500 psi. A synthetic diether (SDE, 1,2-di-O-hexadecyl-glycerol, Sigma Chemical Co.) was used as a retention and concentration standard.

Capillary gas chromatography

Capillary gas chromatography on a Schimadzu GC-9A was used to identify and quantify the polar lipid fatty acids (16). The column was a nonpolar HP-1, 50 m, 0.2 mm ID, 0.11 μm film thickness. The temperature program was 80°C for 1 min, 10°C per min to 150°C, 3°C per min to 240°C, 5°C per min to 280°C, and held at 280°C for 5 min. Peak identities were confirmed with a Hewlett-Packard 5995A GC–MS with the same column and operating conditions.

Culture samples

Cultures of *Methanobacterium formicicum* and *Methanococcus maripaludis* were gifts from Dr. William Whitman, University of Georgia, Athens, GA. They were grown at 30°C in the media described by Balch et al. (17) and by Whitman et al. (18), respectively. *Methanosarcina barkeri* DSM 800 was a gift from Dr. Harry Peck, University of Georgia, Athens, GA, and grown according to Balch et al. (17). The thermoacidophilic archaebacteria were isolated from the Endeavor Ridge submarine vent system, ES1

(19) and ES2 (20) by Dr. John Baross, and AL1 and AL2 by Dr. Jody Deming (21), both at the University of Washington at Seattle. *Bacillus subtilis* and *Escherichia coli* were obtained from Sigma Chemical Co.

Lipid preparation

Lyophilized cultures (ca. 25 mg dry weight) were extracted by the method of Bligh and Dyer (7) as modified (22) or by the Koga extraction (8). In all modified Bligh-Dyer extractions, 30 ml of chloroform, 60 ml of methanol, and 27 ml of phosphate buffer (pH 7.2, 50 mM) were combined with lyophilized cells in a separatory funnel to form the single-phase extraction solvent. After at least 2 h extraction, 30 ml each of chloroform and water were added and the organic and aqueous phases were allowed to separate overnight. The lower organic phase was filtered through a Whatman 2v folded filter paper (Whatman, Inc., Clifton, NJ), and reduced to dryness at 37°C by rotary evaporation. The lipid-extracted residue fraction (Res) was recovered from the aqueous phase by rotary evaporation at 50°C to remove methanol, and then lyophilization (12).

The Koga extraction (8) was the same as above except that 10% trichloroacetic acid replaced the phosphate buffer, and the chloroform extract was washed with 1.9 times its volume of methanol–water 1:0.8.

The total lipid extract was fractionated into neutral lipid (NL), glycolipid (GL), and polar lipid (PL) using silicic acid column chromatography (23). One gram of Unisil (100–200 mesh, Clarkson Chemical Co., Williamsport, PA) was slurried with 5 ml of chloroform and packed wet into a 1-cm glass column. The total lipid was dissolved in a minimum volume of chloroform and applied to the column. The NL were eluted with 10 ml chloroform, the GL with 10 ml acetone, and the PL with 10 ml methanol.

The polar lipid fatty acids were released as fatty acid methyl esters by a mild alkaline transesterification (24) and the lipids were extracted with chloroform against water.

The ether lipids were released by strong acid methanolysis. The GL, PL, or Res fraction to be treated was dissolved or suspended in 1 ml of methanol–chloroform–conc. hydrochloric acid 10:1:1 in a screw-cap test tube with a Teflon-lined cap. The tubes were heated at 100°C for 1 h. To the cooled methanolyzate, 2 ml water was added and it was extracted three times with 2 ml hexane–chloroform 4:1. The pooled extracts were dried under a stream of dry nitrogen at 37°C.

RESULTS

Supercritical fluid chromatography performance

The sensitivity of the SFC was calculated from a 0.2-μl injection of 20 μg/ml synthetic diether standard. Given the convention that the minimum detectable peak is 2 times as high as the width of the noise, the minimum detectable quantity corresponds to approximately 0.6 ng of diether. A minimum of 20 μl of solvent is required to make the 0.2-μl injection, so the practical limit of detection is 60 ng per sample.

The reproducibility of quantification was estimated from 22 injections of the standard synthetic diether over 11 days of analyzing routine samples. The first injection of each day was eliminated from calculations due to low peak area, as is often observed for aging chromatography columns. The relative standard deviations for injections of 20, 200, and 2000 μg/ml were 9.3%, 6.5%, and 6.0% of the mean, respectively.

Comparison of the Bligh-Dyer and Koga extractions

The PL ethers of *Methanococcus maripaludis* and *Methanobacterium formicicum*, and the PL fatty acids of *Bacillus subtilis* and *Eshcerichia coli* were extracted by both the Bligh-Dyer and Koga methods. **Table 1** presents these data as micromoles of lipid per gram dry weight cell material for each extraction method, and the ratio of lipid recovered by the Koga relative to the Bligh-Dyer extraction. The ratios for PL DE were 0.80 and 0.87, and 2.00 for PL TE. The ratios for PL fatty acids were between 0.60 and 0.66 for the saturated, iso-branched, anteiso-branched, and total fatty acids. The cyclopropyl fatty acids were almost completely eliminated by the Koga extraction; 0.06 of the amount found in the Bligh-Dyer extraction was found.

Ether lipid profiles

The lipid profiles over the GL, PL, and Res fractions for three species of methanogens and four recent isolates of thermoacidophilic archaebacteria are presented as weight percents in **Table 2**. The data for the methanogens are the average of four determinations, and a single determination for the thermoacidophiles. The majority of ether lipid in *Methanobacterium formicicum* (74.9 ± 9.9%) was TE in the Res fraction. Almost all of the ether lipid found in *Methanococcus maripaludis* (96.9 ± 0.8%) was GL DE. The ether lipid of *Methanosarcina barkeri* was DE, divided between the GL (34.7 ± 1.9%) and the PL (65.3 ± 1.8%) fractions.

Of the thermoacidophilic isolates, AL1 and ES1 were very similar except for the lack of GL ethers in AL1 and less PL ethers in ES1. Isolate AL2 had most of its ethers as either PL DE (30.0%) or Res TE (43.0%). ES4 was unique in having a large amount of PL TE (57.0%).

Archaebacterial ether lipid contents

A literature search on the ether lipid contents of different fractions and a variety of species is presented in **Table 3**, and compared with this work (5, 25–37). Referred articles were included for which grams of ether lipid per gram of dry weight cells could be calculated from the

TABLE 1. Polar lipid fatty acids (FA) and ethers recovered by the Bligh-Dyer and Koga extractions

Sample	Bligh-Dyer	Koga	Ratio Koga/Bligh-Dyer
		μmol/g	
Methanococcus maripaludis			
Diether	0.44 ± 0.07	0.35 ± 0.02	0.80
Methanobacterium formicicum			
Diether	0.15 ± 0.06	0.13 ± 0.01	0.87
Tetraether	0.08 ± 0.04	0.16 ± 0.02	2.00
Total ether lipids	0.23 ± 0.07	0.29 ± 0.03	1.26
Bacillus subtilis			
Saturated FA	2.7 ± 0.3	1.8 ± 1.1	0.65
Unsaturated FA	2.5 ± 0.3	1.8 ± 0.9	0.72
Iso-branched FA	8.0 ± 1.3	5.0 ± 3.4	0.63
Anteiso-branched FA	3.4 ± 0.5	2.1 ± 1.3	0.62
Total fatty acids	16.6 ± 2.6	10.7 ± 6.9	0.64
Escherichia coli			
Saturated FA	34.2 ± 1.0	22.4 ± 1.9	0.66
Unsaturated FA	2.1 ± 0.1	2.0 ± 0.1	0.95
Cyclopropyl FA	31.7 ± 0.6	2.0 ± 0.7	0.06
Unknown FA	0.1 ± 0.0	14.4 ± 0.7	144
Total fatty acids	68.1 ± 1.5	40.8 ± 2.6	0.60

Values expressed as mean ± SD, triplicate analyses.

data presented. Most researchers reported either the total lipid or the PL ethers. No report was found of the ethers occurring in the GL fraction by silicic acid column chromatography, and only one report of the ethers found in the lipid-extracted Res. The amount of lipid recovered varied from 1.5 to 53.7 mg ether per gram dry weight in different species. In a study of the effects of temperature and salinity on *Sarcina marina* [since renamed *Halococcus morrhuae* (38)], the amount of ethers in the TL fraction varied by a factor of 5.

The amounts of ether lipid recovered in this work, totaled over all three fractions, varied from 21.41 mg ether/g (dry weight) for isolate AL1 down to 0.18 mg ether/g (dry weight) for ES4. The low value found for ES4 was an artifact due to the large amount of solid sulfur inseparable from the cell mass.

DISCUSSION

Supercritical fluid chromatograph performance

The SFC method, patterned after the system of DeLuca et al. (6), showed a sensitivity (0.6 ng of DE) similar to that reported by these workers (1 ng of DE). Lipid chromatography with refractive index detection has been reported to have a sensitivity of 390 ng DE (39). For many applications, the sensitivity of the refractive index system would not be sufficient. Liquid chromatography with fluorescence detection of derivatized DE had a sen-

TABLE 2. Archaebacterial lipid profiles, Bligh-Dyer extraction, and supercritical fluid chromatography analysis

	Glycolipid		Polar Lipid		Residue	
Sample	DE	TE	DE	TE	DE	TE
Methanogens			*wt %, total ether lipid*			
Methanobacterium formicicum	10.6 (1.9)[a]	0.2 (0.1)	4.4 (1.9)	9.3 (4.9)	0.5 (0.1)	74.9 (9.9)
Methanococcus maripaludis	96.9 (0.8)		2.8 (0.7)		0.2 (0.2)	
Methanosarcina barkeri	34.7 (1.9)		65.3 (1.8)			
Thermoacidophiles						
AL1			6.3	1.8	0.8	91.2
AL2			30.0	19.5	6.7	43.0
ES1	0.6	0.1	2.0	0.9	1.3	94.9
ES4	0.7	0.7	22.0	57.0	6.3	13.3

The methanogens were grown as described in the text. ES1 was grown at 80°C, ES4 at 90°C.
[a]In parentheses, standard deviation (n = 4).

TABLE 3. Ether lipids recovered from archaebacteria

Archaebacteria	TL	GL	PL	Res	Reference
		mg ether lipid/g dry wt of cells			
Methanococcales					
Methanococcus maripaludis		14.2	0.4	ND	this work
Methanococcus jannaschii	4.5				25
Methanobacteriales					
Methanobacterium formicicum		1.02	0.89	5.47	this work
Mb. thermoautotrophicum			3.2		26
Mb. thermoautotrophicum str. Hveragerdi			3.1		27
Mb. thermoautotrophicum			4.7		28
Halobacteriales					
Halobacterium cutirubrum	31.3				29
Sarcina marina	4.2				30
Sarcina marina	20.3				30
Alkalophile	12.4				31
Methanomicrobiales					
Methanosarcina barkeri		2.29	4.35	ND	this work
Ms. barkeri str. Jolich			1.5		5
Ms. barkeri	13.4				
Methanospirillum hungatei			31.35		32
Methanothrix concilii GP6			53.67		33
Thermoacidophiles					
Thermoplasma acidophilum	30.0				34
Thermoplasma acidophilum				5.9	35
Sulfolobus acidocaldarius			48.5		36
Desulfurococcus mobilis			13.6		37
Isolate ES1		0.023	0.11	3.5	this work
Isolate ES4		0.0026	0.15	0.037	this work
Isolate AL1		ND	1.71	19.7	this work
Isolate F1		ND	1.14	1.14	this work

ND, none detected.

sitivity of 48 pg DE (46), but did not detect the archaebacterial tetraether. The highest sensitivity (5 pg of phytanol) has been reported with GC–MS of the heptafluorobutyric acid-derivatized alcohol cleaved from the glycerol backbone by HI cleavage of the ether linkage (41). This method, however, is not recommended for routine or quantitative analysis due to the great number of chemical manipulations with the potential for sample loss and/or contamination. While many researchers might hesitate to attempt SFC due to unfamiliarity with the method, it was found in this laboratory that it was no more difficult to maintain and operate than a capillary gas chromatograph, and easier than a liquid chromatograph with refractive index or fluorescence detection.

Comparison of the Bligh-Dyer and Koga extractions

The Koga extraction was compared with the Bligh-Dyer extraction for *Methanococcus maripaludis*, *Methanobacterium formicicum*, *Bacillus subtilis*, and *Escherichia coli* (Table 1). The loss of PL DE from *Mc. maripaludis* and *Mb. formicicum* by the Koga method was possibly due to the need to wash the extract with methanol–water to remove the trichloroacetic acid. Twice as much PL TE was extracted from *Mb. formicicum* by the Koga than by the Bligh-Dyer method. The benefit of increased recovery of TE in the PL was minimal, however, since by the proposed procedure the TE would be recovered in the Res. In *E. coli* and *B. subtilis*, the low amount of saturated, iso-branched, anteiso-branched, and total fatty acids recovered (0.60 to 0.66 relative to the Bligh-Dyer) may be due to hydrolysis of the ester bond in the acidic extraction medium (9). Cyclopropyl fatty acids were decreased by the Koga extraction to 0.06 of that extracted by the Bligh-Dyer. Under acidic conditions, cyclopropyl fatty acids degrade to a variety of products. In the same sample that saw drastic loss of cyclopropyl fatty acids, the amount of unknown fatty acids increased 144 times. In environmental analysis, the Koga extraction would underestimate the eubacterial biomass and degrade cyclopropyl fatty acids. The Koga extraction was not intended for eubacterial lipids (8).

Koga's extraction clearly documented the inadequacy of a simple Bligh-Dyer extraction in the analysis of archaebacterial lipids. For analysis of archaebacterial and eubacterial lipids in environmental samples, or for analysis of pure cultures to be compared with environmental data, an integrated approach is more efficient and powerful. In our laboratory, polar lipid fatty acids and ethers are routinely determined on the same sample (42). Mild alkaline methanolysis releases the fatty acids as their

methyl esters and does not affect the ether lipids. Silicic acid column chromatography (see Materials and Methods) quantitatively separates the fatty acid methyl esters in the chloroform and the polar lipid ethers in the methanol eluate. Then strong acid methanolysis released the ether lipids for SFC analysis.

Ether lipid profiles of archaebacteria

Polar lipid fatty acid profiles have been extensively used to define the biomass, community structure, and metabolic status of eubacteria in natural environments such as marine sediments (43), polluted aquifers (4), and anaerobic biomass reactors for methane production (44). They have also been very useful in identifying eubacterial species and discovering taxonomic relationships (3). This study shows the potential usefulness of lipid biomarkers for research on archaebacteria.

As shown in Table 2, the archaebacteria differ in which fraction contains most of their ether lipids, and in the proportion of diethers to tetraethers. These differences may be related to the "astonishing variation" in cell wall types found in archaebacteria (45). The patterns of ether lipids clearly differentiate between the three species of methanogens. They were chosen from each of the three orders of methanogens: Methanobacteriales, Methanococcales, and Methanomicrobiales (46). More analyses are required before the range of ether lipid patterns within each order and the differences between them can be defined. Towards this end, the complete analysis of archaebacterial lipid fractions described in this study provides three times the descriptive information than is available by current methods. This approach has potential for the inclusion of lipid patterns in archaebacterial chemotaxonomic evaluations.

The ether lipid profiles for the four recent isolates of sulfur-respiring thermoacidophilic anaerobes from the Endeavor Ridge submarine vent system (47) are also presented in Table 2. Strains AL1 and ES1 gave very similar profiles, suggesting that they should be examined for possible identity of strains. While strains ES4 and AL2 were generally similar, the proportions of individual components were different enough to make identity highly unlikely.

Trends can be detected in the ether lipid profiles of the archaebacteria in this small sample. TE was predominantly found in the Res fraction in four of the five cultures containing TE. The GL fraction of each of the three methanogens was predominantly or entirely DE. None of the thermoacidophiles contained significant amounts of GL ether. The PL faction, however, had both DE and TE in those species containing any TE.

Ether lipid content of archaebacteria

Pauly and Van Vleet (10) reported a conversion factor of 32 mg ether/g (dry weight) of lyophilized methanogen cells on the basis of a literature review. Nichols, Mancuso, and White (26) reported a conversion factor of 3.2 mg ether/g (dry weight) for *Methanobacterium thermoautotrophicum*. The average for the seven isolates in this study was 8.02 ± 7.51 mg ether/g (dry weight). Due to the wide variation in ether contents of pure cultures with differences in species and growth conditions, it is useful to report the ether contents of environmental samples as grams of ether normalized to the sample (per gram dry weight, per cm^2, etc). Conversion to grams of archaebacterial cells or number of archaebacterial cells per unit of sample should only be done on the basis of information about the ether lipid contents of the species in the sample. The ether lipid content should not be expressed in terms of moles of ether lipid since a mole of TE occupies twice as much lipid bilayer surface area as DE (48).

Conclusions

By separately analyzing the glycolipid, polar lipid, and lipid-extracted residue fractions, the information content of the lipid profile is tripled. While the detailed analysis of the structure of individual ether lipids is very important for understanding of the mechanisms of membrane integrity and phylogenic relationships, the gross separation of lipid classes is more practical for routine identification of archaebacterial species and the analysis of environmental samples.

Supercritical fluid chromatography provides a sensitive assay for the archaebacterial ether lipids, 60 ng ether lipid per sample, corresponding to approximately 7 mg of dry cell mass (based upon the isolates analyzed here).

More archaebacterial isolates must be analyzed by this method in order to establish the extent and phylogenic significance of ether lipid diversity. Experiments are underway on the effects of environmental conditions on ether lipid content and diversity, and how this variation contributes to the organism's survival strategy.

This research was supported by grants to Dr. David C. White from the Gas Research Institute, #5086-260-1303, and from the Office of Naval Research, #6083-226-0848. The gifts of viable cultures of methanogens from Dr. William Whitman and Dr. Harry Peck, University of Georgia, Athens, GA are appreciated. The samples and cooperation of Ralph Pledger and Dr. John A. Baross, and Dr. Anna-Louise Reysenbach and Dr. Jody Deming of the School of Oceanography, Washington State University, Seattle, WA are gratefully acknowledged.

Manuscript received 21 September 1990 and in revised form 20 November 1990.

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