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High proportions of tetraether phospholipids in *Methanobacterium thermoautotrophicum* strain Hveragerdi measured by high performance liquid chromatography

(Archaebacteria; diether : tetraether ratio; HPLC/FT-IR; methanogens; signature lipids)

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1. SUMMARY

The diether and tetraether lipids were isolated from the phospholipids of *Methanobacterium thermoautotrophicum* strain Hveragerdi. These membrane components were assayed by high performance liquid chromatography. The ratio of diether to tetraether lipids was 1 : 14 on a weight basis and represents the highest proportion of tetraether yet reported in a methanogenic bacterium. This data and the application of this method has relevance in microbial ecology and organic geochemistry where these chemical signatures may be used to assess the contributions of methane-forming bacteria to biological processes in natural environments.

2. INTRODUCTION

Methanogenic bacteria are widely distributed in nature. They participate in terminal carbon metabolism and are responsible for one of the major end products from the anaerobic decomposition of organic matter [1–3]. As significant members of the microbial community in anoxic sediments, methane-forming bacteria have relevance from an ecological as well as organic geochemical standpoint [2,4–6]. In order to accurately and routinely evaluate the population of these organisms in the environment, a measure of the viable biomass in terms of phospholipid chemical markers [7] can be performed.

As archaebacteria, methanogens characteristically possess isoprenoid-branched ether-linked membrane lipids, diphytanyl glycerol diethers (DE) and bidiphytanyldiglycerol tetraethers (TE) [8,9]. The ratio of the diether to tetraether lipids has been determined by TLC for several strains of methanogens and ranges from 1 : 0 (DE : TE) in *Methanosarcina barkeri* to 1 : 6.4 (DE : TE) in *Methanothermus fervidus* [10,11]. The high proportion of tetraether found in *Mt. fervidus* is unusual for methanogens. In a recent study of swamp sediments, methanogen signature ether lipids were correlated to methane fluxes in the sediments [12]. Tetraethers were more abundant than diethers in the sediment, averaging 82.5% of the methanogen ether lipids.

In the present study, the phospholipids of *Mb.*

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thermoautotrophicum strain Hveragerdi were analyzed and the relative proportions of diether and tetraether lipids were determined.

3. MATERIAL AND METHODS

Mb. thermoautotrophicum strain Hveragerdi was isolated from an Icelandic hot spring [13]. It is a chemolithotrophic thermophile which grows optimally at 58°C. Exponential growth and methane formation also occurs as low as 33° [13]. Lyophilized cells grown according to procedures described elsewhere [13], were provided by J.P. Kaiser and K. Hanselmann of the Department of Microbiology at the University of Zurich, Switzerland.

Techniques for lipid extraction, silicic acid column chromatography fractionation of lipids, hydrolysis of the phospholipid-containing ether lipids have been described [14]. The ether lipids were separated by high performance liquid chromatographic (HPLC) analysis with an isocratic solvent system of hexane : *n*-propanol (99 : 1) on an amino-bonded silica column with a refractive index detector [15]. Phospholipid-derived lipid phosphate was measured calorimetrically as described by White et al. [7].

Diether standard, 1,2-di-*O*-hexadecyl-rac-glycerol, was purchased from Sigma (St. Louis, MO). Authentic tetraether standard was a gift of G. Pauly and E.S. Van Vleet of the University of South Florida, Tampa, FL. Identification of ether lipids was confirmed with Fourier transform-infrared (FT-IR) spectrometry by diffuse reflectance of separated components as described previously [15].

4. RESULTS AND DISCUSSION

Peak areas of dilutions of ether lipids correlated well to levels of both diether ($r = 0.93$) and tetraether ($r = 0.99$) standards, giving a linear response in the concentration range used (0.05–1.00 mM). An equal response factor for the diether and tetraether was determined. Chromatograms of the ether lipids from *Mb. thermoautotrophicum* and

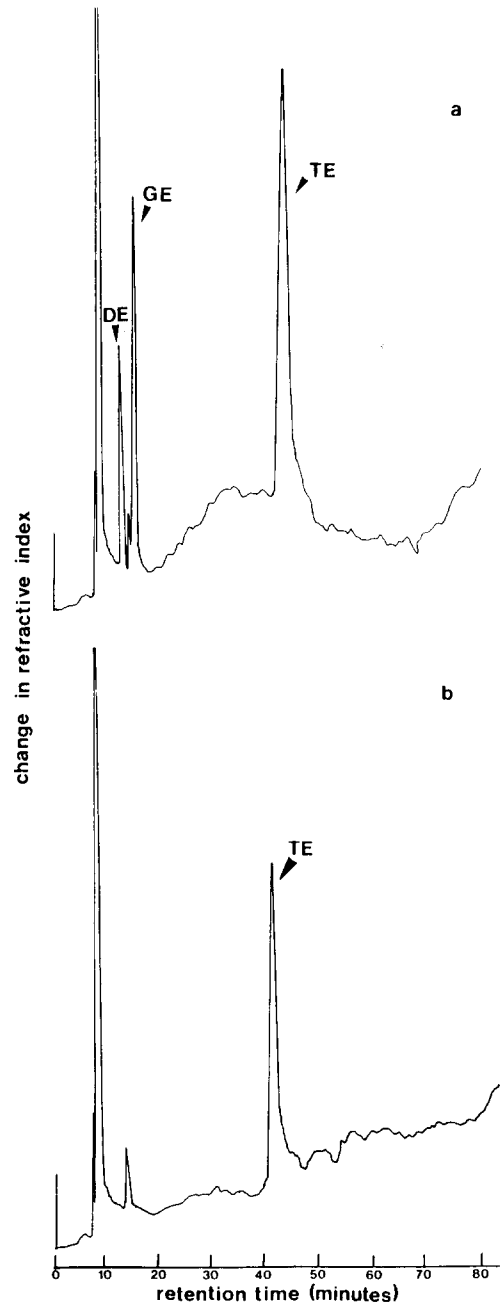

Fig. 1. HPLC traces illustrating separation of archaebacterial ether lipids in (a) *Mb. thermoautotrophicum* strain Hveragerdi (diether: 2.4 nmoles; tetraether: 17.7 nmol) and diether standard (6 nmol); and (b) tetraether standard (10 nmol). Chromatographic conditions were: solvent, hexane : *n*-propanol 99 : 1; flow, 0.5 ml/min; chart speed, 10 cm/h; RI detector at R = 1/4. Abbreviations used are diether (DE), tetraether (TE) and diether standard (GE).

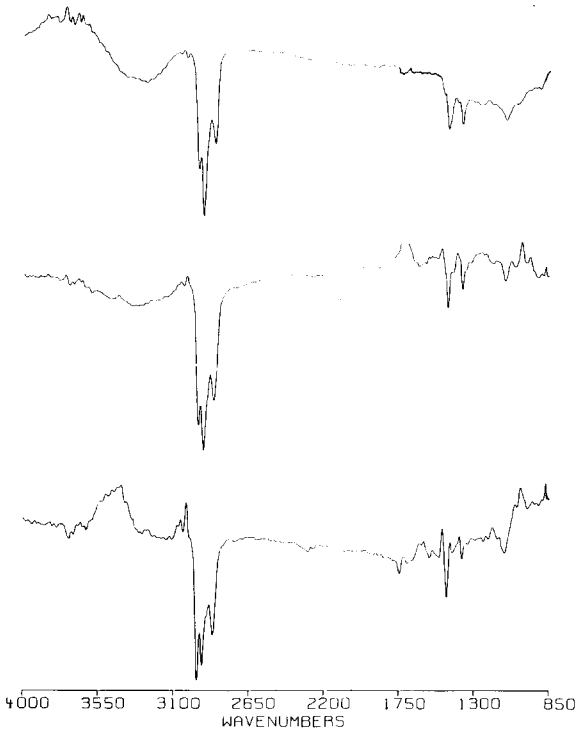

Fig. 2. FT-IR spectra (4000–850 cm^{-1}, transmittance mode) of diether (upper spectrum) and tetraether (middle spectrum) from *M. thermoautotrophicum* and authentic tetraether (lower spectrum).

tetraether standard are shown in Fig. 1a and b, respectively.

The identity of components designated diether and tetraether lipids based on HPLC retention time data, when compared to lipid standards, were confirmed by FT-IR spectroscopy (Fig. 2). HPLC fractions containing these components showed bands corresponding to alkyl (1377, 1463, 2856, 2926 and 2952 cm^{-1}), primary carbinol (1051 cm^{-1}), ether (1115 cm^{-1}) and hydroxyl (3450 cm^{-1}) functional groups (Fig. 2). Ester absorbances at 1750–1710 cm^{-1} were not present in all spectra, indicating the absence of carbonyl-containing phospholipid contaminants.

In *Mb. thermoautotrophicum* strain Hveragerdi, the diether and tetraether lipids were present in a ratio of 1 : 14, on a weight basis (Fig. 1a) with a total recovery of 2.6 μmol (0.3 μmol DE, 2.3 μmol TE, 3.1 mg total) ether lipid/g dry wt. of lyophilized cells. This data was supported by the phospholipid phosphate measurement (2.5 μmol/g dry wt.). The 1 : 14 ratio of phospholipid-derived diethers to tetraethers is the highest proportion of tetraethers yet reported in the phospholids of methanogenic archaebacteria [10]. In analyses of swamp sediments by Pauly and Van Vleet [12], tetraethers were in high abundance relative to diethers. Tetraether also predominated in marine sediments analyzed by Chappe et al. [16]. The data presented here lend support to these studies and suggests that other, as yet unidentified, archaebacterial methanogens may account for the high abundance of tetraether.

In the future, the relative proportions of diethers and tetraethers will be determined for a wide range of monocultures produced under manipulated growth parameters. Differences in the DE : TE ratio in these cultures may aid in describing better the methanogenic community structure in the environment. Together these studies will allow archaebacterial markers to be used more accurately in defining the methanogenic contribution to biological as well as sedimentary processes occurring in natural systems.

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