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Unsaturated diether phospholipids in the Antarctic methanogen *Methanococcoides burtonii*

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1. SUMMARY

The Antarctic methanogen *Methanococcoides burtonii* contained only diether phospholipids. These membrane components were analysed by gas chromatography and gas chromatography mass spectrometry. Of particular interest was the occurrence of unsaturated diether lipids in *M. burtonii*; unsaturated ether lipids accounted for 57% of the diether phospholipids. To our knowledge, unsaturated ether lipids have not been previously reported in a methanogen. The presence of the unsaturated ether lipids in *M. burtonii* is probably the result of temperature adaptation by the bacterium. It may be possible to use these components as a chemical signature for methanogens in Antarctic and Southern Ocean environments.

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2. INTRODUCTION

Methanogens are widely distributed in nature. They are terminal mineralizers of organic material in sulphate depleted environments, and, by definition, produce methane, a fuel and greenhouse gas.

We have been investigating the microbial ecology of Ace Lake in the Vestfold Hills of Antarctica from which a number of new bacterial types have been characterized [1–3], including a new psychotrophic methanogen, *Methanococcoides burtonii* [4]. Methane is present in the hypolimnion of Ace Lake at concentrations approaching saturation [5], although rates of methanogenesis are extremely slow when compared with rates measured in temperate and tropical environments [6].

The salinity of Ace Lake is very close to that of sea water [7], and the microbial processes occurring within the lake may therefore serve as potential models for microbial processes in the Southern Ocean. Methanogenesis occurs in oxic waters

of the worlds' oceans in anaerobic microenvironments associated with particulate organic carbon [8]. Members of the genus *Methanococcoides* have been implicated in this process [9]. A chemical signature for members of this genus, particularly for Antarctic species, would be a useful tool for the detection and quantification of the biomass of methanogens in the Southern Ocean.

In the present study, the phospholipids of *M. burtonii* were analysed. As well as the normal diphytanyl diether, the organism contained several additional novel diether lipids.

3. MATERIALS AND METHODS

Methanococcoides burtonii was isolated from Ace Lake, characterized [4], and deposited in the DSM (German National Culture Collection), Braunschweig, FRG, as strain DSM 6242. Cells were grown in 1-l batches in modified methanogen growth medium [4] containing 3 g l^{-1} trimethylamine · HCl under a nitrogen gas phase at 20°C for 21 days. Cells were harvested by centrifugation (10 000 × *g*) for 20 min, washed in artificial basal salts solution [4], centrifuged and lyophilized.

Extraction, fractionation and preparation of diphytanyl diethers and bi-diphytanyl tetraethers were performed using well-documented procedures [10–12]. Brief outlines are provided below.

Samples were extracted quantitatively by the modified one-phase $CHCl_3$-MeOH Bligh and Dyer method [10,11]. After phase separation, the lipids were recovered in the lower $CHCl_3$ layer (solvents were removed in vacuo) and stored sealed under nitrogen at −20°C. Total lipids were fractionated on a 1 g column of Unisil by an elution sequence of 10 ml chloroform (neutral lipid), 20 ml acetone (glycolipid) and 10 ml methanol (phospholipid). Glycerol di- and tetraethers were formed by acidic hydrolysis of phospholipids [12]. Ether lipids were converted to their corresponding O-trimethyl silyl ethers by treatment with bistrimethylsilyltrifluoroacetamide (50 μl, 60°C, 60 min).

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890 GC equipped with a 3 m × 0.25 mm i.d. BPX5 fused-silica capillary column (Scientific Glass Engineering Pty Ltd.), an OCI3 on column injector and a flame ionization detector. Samples were injected at 90°C and, after 2 min, the GC oven was temperature-programmed to 190°C at 30°C/min and then to 380°C at 10°C/min. The final temperature was maintained for 15 min, after which the GC oven returned to 90°C at 10°C/min. Hydrogen was used as the carrier gas; the column head pressure was 3 psi. The detector was maintained at 385°C. Peak areas were quantified using chromatography software (DAPA Scientific software, Kalamunda, WA, Australia) operated using an IBM compatible personal computer. Identification of individual components was based on comparison of retention time data with that obtained for authentic and laboratory standards, including di- and tetraether lipids derived from *Methanobacterium thermoautotrophicum*. GC-MS analyses of diphytanylglyceryl ethers were performed on a HP 5890 GC and 5970 Mass Selective Detector fitted with a direct capillary inlet and a split/splitless injector. Data were acquired and processed on an HP 59970C Workstation operated in scan aquisition mode. Operating conditions are described in detail elsewhere [13]. A nonpolar column (50 m × 0.22 mm i.d., HP1 crosslinked methyl silicone) was used for these analyses.

4. RESULTS AND DISCUSSION

A representative gas chromatogram of the ether lipids from *M. burtonii* is shown in Fig. 1 and the ether lipid composition of *M. burtonii* is presented in Table 1. The phospholipid-derived ether lipids from *M. burtonii* consisted of only diether lipids. Under the GC conditions used in this study, tetraether lipids appeared between 25–30 min; no peaks were present in this region (Fig. 1). Tetraethers have not been detected in the type species of the genus *Methanococcoides*, *Methanococcoides methylutens* [14]. In addition to the expected diphytanyl diether lipid which was the major component (peak 1), two other major (peaks 2 and 4) and two minor components (peak 3 and 5) were also present.

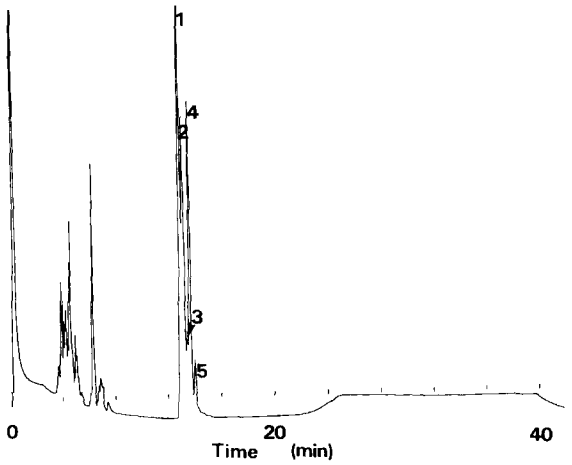

Fig. 1. Capillary gas chromatogram showing phospholipid-derived ether lipid distribution of *M. burtonii*. Peak 1 off scale. Components eluting between 4 and 8 min include phytadienes and monophytanyl glyceryl diols. Peak numbers refer to Table 1.

Identification of peaks 2 and 4 as monounsaturated ether lipids (rather than containing a pentacyclic ring within the diether side-chain structure, although the latter compounds have not been reported to date) was based on comparison of GC retention time data and GC-MS data (for OTMSi ethers) with data obtained for the halophilic archaeon *Halobacterium lacusprofundi* ([15,16] John Volkman, personal communication). Major ions, m/z (relative abundance), were: peak 2, 55 (30), 57 (67), 69 (41), 71 (41), 81 (100), 95 (28), 123 (26), 131 (29), 165 (13), 278 (19), 445 (16); peak 4, 55 (41), 57 (100), 69 (52), 71 (64), 83 (80), 95 (46), 123 (37), 130 (45), 165 (18), 278 (31), 445 (40). Common ions resulting from fragmentation of the branched or straight chain alkane and alkene sidechains are m/z 57, 71, 85 and m/z 55, 69, 83 respectively. The latter are enhanced in peaks 2 and 4 relative to the saturated diphytanyl diether lipid. Ion m/z 445 results from cleavage at the ether link of the phytenyl sidechain accompanied by double hydrogen rearrangement. A similar cleavage together with hydrogen rearrangement produces phytadiene (m/z 278). In comparison, the mass spectra of the saturated diphytanyl diether lipid showed a lower abundance of ions m/z 55, 69, 83; additional diagnostic ions are also observed at m/z 309, 369, 412 and 426.

Table 1

Phospholipid-derived ether lipid composition of *Methanococcoides burtonii*

Peak number	Compound	Number double bonds	Percentage composition
1	diphytanyl glycerol ether (DPGE)	0	43.3
2	monounsaturated DPGE	1	26.2
3		ND	4.0
4	monounsaturated DPGE	1	23.6
5		ND	2.9

Peak numbers refer to Fig. 1.
ND: not determined due to insufficient material.

In *H. lacusprofundi*, the position and geometry of unsaturation of the major monounsaturated ether lipid (equivalent to peak 4 in *M. burtonii*) was identical to that of *trans* phytol from NMR data.

Further confirmation that the monounsaturated ether lipid (peak 4) is the same as that of *H. lacusprofundi* is the presence of phytadienes and monophytanyl glyceryl diols; 1,2-diol (sn-3-O-phytanyl glycerol) and 1,3-diol (sn-2-O-phytanyl glycerol) eluting early in the GC trace (Fig. 1). These components were also present in extracts from *H. lacusprofundi* and are thought to be artifacts derived from the acid conditions employed for conversion of the phospholipid ether lipids to the free diether lipids (Volkman and Neill, personal communication). The second major monounsaturated ether lipid (peak 2) was not detected in *H. lacusprofundi*; we consider the two monounsaturated diethers may differ by only the position in which the chain containing the double bond is located (position 2 or 3 on the glycerol).

To our knowledge, the high relative abundance of unsaturated ether lipids present in the phospholipids of the Antarctic methanogen, *M. burtonii*, has not been previously reported in a methanogen. Unsaturated ether lipids have only been previously reported in the halophilic archaeon *H. lacusprofundi*, also isolated from an Antarctic lake [15]. The presence of unsaturated ether lipids in both *M. burtonii* and *H. lacuspro-*

fundi is probably the result of adaptation by the bacteria to low temperature, as has been observed for the phospholipid ester linked fatty acids from a wide variety of eubacteria [17,18]. It may be possible to use these components as signature lipids for methanogens in ecological investigations of Antarctic lakes and polar waters where considerable interest exists in the role of methanogens in biogeochemical and microbial processes.

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