Distribution of Complex and Core Lipids within New Hyperthermophilic Members of the Archaea Domain

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Summary

Core and complex lipids of several new hyperthermophilic archaeal isolates were analyzed. The organisms belong to the Sulfolobales, Archaeoglobus, Pyrobaculum, and Methanococcus. A detailed structural investigation of complex lipids of Pyrobaculum species is reported. The different lipid structures are of help for a rapid and simple phylogenetic classification of the new isolates. They are in agreement with the classification based on other features.

Key words: Archaea – Hyperthermophilic – Lipids – Isopranyl ether

Introduction

The archaeal domain of life had been discovered and its phylogenetic relationship had been investigated by comparison of 16S rRNA catalogues and total sequences (Woese, et al., 1978; Woese et al., 1990). It comprises many phylogenetically and physiologically diverse groups with distinct features. Within the Archaea, two phylogenetic domains are evident: (1) the Euryarchaeota containing the methanogens, extreme halophiles, the cell-wall deficient organotrophic Thermoplasma, the sulfate reducing Archaeoglobus, and the organonothrophic Thermococcales, and (2) the Crenarchaeota comprising the sulfur-metabolizing genera Pyrodictium, Desulfurococcus, Staphylothermus, Hyperthermus, Thermoproteus, Pyrobaculum, Thermofilum, Sulfolobus, Acidianus, Desulfurolobus, and Metallosphaera (Stetter et al., 1990). Hyperthermophiles with optimal growth temperatures between 80 and 110°C are so far members of the genera Methanococcus (only M. jannaschii and M. igneus), Methanothermus, Methanopyrus, Archaeoglobus, Thermococcus, Pyrococcus, and of all the genera of the subdomain Crenarchaeota. Archaeoglobus represents a biochemical missing link between the methanogens and the sulfur-metabolizing archaea (Achenbach-Richter et al., 1987). Acidianus and its close relative Desulfurolobus represent a missing biochemical link between the sulfur-oxidizing and sulfur-reducing archaea (Segerer et al., 1985; Segerer et al., 1986; Zillig et al., 1986; Huber et al., 1987).

All archaeal membrane lipids isolated to date are based on isopranyl glycerol ethers in contrast to the fatty acid glycerol esters usual in bacteria. Many of their unusual structural features can be considered to be specific taxonomic markers of this group of microorganisms. The complex lipids of archaea are based on two classes of isoprenoid ether core lipids: diethers and tetraethers. The former is structurally derived from glycerol in which the oxygens in 2- and 3-sn positions are linked via ether link to C20 or C25 isopranyl alcohol. The 2,3-di-O-phytanyl-sn-glycerol a (Fig. 1) could be considered a universal core lipid in archaea. This glycerol diether represents 100% of ether core lipids in the majority of neutrophilic halophiles and in some coccoid forms belonging to methanogens and thermophiles (De Rosa et al., 1991).

A macrocyclic glycerol diether b (Fig. 1), characterized by the presence of a 36 membered ring originated from the condensation of a glycerol with a C40 to C50 isopranyl diol, is found until now in Methanococcus jannaschii and this lipid characterizes this species (Comita et al., 1984).

Other structural varieties are found among archaeal
diethers such as substitution of glycerol with tetritol in a unique lipid found in Methanosarcina barkeri and different alkyl chains such as sesterterpanyl (C25) or phytanyl (C20) with an OH group in the position 3 found in aliphilic halophiles and in Methanothrix concilii GP6, respectively, (Ferrante et al., 1988; De Rosa et al., 1989).

The tetraethers found in the archaea are classified in two series: in the first series, a tetraether molecule has a 72-member ring formed by two 2,3-sn-glycerol moieties bridged through ether linkages by two isoprenoid C40 diols formally derived from head to head linkage of two O-phytanyl residues. The presence of differently cyclized C40 diols originates a series of nine compounds named GDGTs (glycerol dialkyl glycerol tetraethers). The second series has a structural organization similar to that of GDGTs, but a more complex polyol with nine carbon atoms replaces one of the glycerols. By analogy this series is named GDNTs (glycerol dialkyl nonitol tetraethers). The uncyclized GDGT a (Fig. 2) and 2,3-di-O-phytanyl-sn-glycerol in different proportions are the basic components of complex lipids of methanogenic archaea belonging to the orders Methanobacteria and Methanomicrobiales. Only in M. barkeri and in M. sociabilis tetraethers with cyclopentane rings are detected (Trincone et al., 1988). Tetraethers are the main components of lipids of Crenarchaeota, moreover the presence of the lipids based on GDNTs characterizes the microorganisms belonging to the Sulfolobales order.

The complex lipids of archaea are identified as phospho-, phosphoglyco-, amino-, aminophospho-, and glycolipids by the use of specific staining tests and by spectroscopic and chemical procedures. Aminolipids and aminophospholipids were found to be widely distributed in methanogens in which they prevail in contrast with the other two phenotypes (De Rosa et al., 1991). Usually in thermophilic archaea the polar lipids are based on tetraethers. Glycolipids are present in lesser amount than acidic lipids. Phospholipids and phosphoglycolipids contain usually inositol as head group. Moreover only in the thermophiles belonging to the genera Thermococcus, Pyrococcus and in AN1 isolate are found complex lipids based on 2,3-di-o-phytanyl-sn-glycerol.

In this paper we describe the core and the complex lipids of several new hyperthermophilic archaeal isolates belonging to the Sulfolobales, Archaeoglobus, Pyrobcaculum and Methanococcus.

Materials and Methods

Cultivation of strains. Strains of hyperthermophilic Archaea were grown in 300 l batch cultures using an enamel-protected fermentor (HTE, Bioengineering, Wald, Switzerland). Metallosphaera sedula, TH2 and Sp3a the same as the isolates VE2, VE6 and HV5 were cultivated aerobically on S° (1%) and yeast extract (0.02%) in modified Allen medium (Allen, 1959; Huber et al., 1989). Sulfolobus metallicus strains Kra22 and Kra23 were grown autotrophically in modified Allen medium in the presence of S° (1%). Pyrobcaculum organotrophum and P. islandicum were grown anaerobically on modified Allen medium as described (Huber et al., 1987). Archaeoglobus fulgidus VC16 and Archaeoglobus profundus AV18 and Kol3 were grown on modified Wolfe's medium 3 (Balch et al., 1979; Stetter et al., 1987; Stetter, 1988; Burggraf et al., 1990a). Methanococcus igneus Kol5 was grown chemolithoautotrophically on modified Wolfe's medium 3 with H2/CO2 as gas phase (Burggraf et al., 1990b).
Thermoproteus tenax was generously given by Prof. W. Zillig, Max Planck Institute.

**Extraction of lipids.** Lyophilized cells were extracted continuously (Soxhlet), first for 15 h with CHCl₃/MeOH (1:1, v/v) and then with MeOH/H₂O (1:1, v/v). The combined extracts were dried under vacuum and dissolved in CHCl₃/MeOH (1:1, v/v). The soluble material represented total lipid extract. Quinone extraction was performed by treating total lipid extract with light petroleum b.p. 40–70°C, the soluble fraction represented the quinone extract.

**Fractionation of the lipid.** The total lipid extract of Pyrococcus furiosus and P. islandicum was generously given by Prof. W. Zillig, Merck. Extraction of lipids was performed by treating total lipid extract with light petroleum b.p. 40–70°C, the soluble fraction represented the quinone extract.

**Chemical procedures.** Total lipid extracts or purified complex lipids were hydrolyzed with methanolic HCl (1N at reflux for 3 h). The hydrolysis mixture was dried in vacuum and then dissolved in CHCl₃/H₂O (1:1, v/v) yielding a biphasic system; the CHCl₃-soluble and the H₂O-soluble material were analyzed chromatographically.

**Thin layer chromatography (TLC).** Thin layer chromatography (TLC) was performed on 0.25 mm layers of silica gel F₂₅⁴, (Merck) activated at 100 °C for 2 h. Solvents included CHCl₃/EtOAc (60:40, v/v) for GDGTs, and CHCl₃/EtOAc (60:20:20, v/v) for myo-inositol, 2,3-di-O-phytanyl-sw-glycerol; n-hexane-EtOAc (75:25, v/v) for glycerol dialkyl glycerol tetraethers; CHCl₃/MeOH (9:1, v/v) for glycerol dialkyl nonitol tetraethers; CHCl₃/MeOH (9:1, v/v) for GDNTs. All HPLC analyses were made on a Waters Associates apparatus and eluants from column were detected by integrator peak areas in HPLC.

**Results**

Core lipids obtained after acid hydrolysis of total lipid extracts were identified as follows: 2,3-di-O-phytanyl-sn-glycerol (HPLC, EI-MS of acetylated derivative and [α]D value), GDGTs and GDNTs (TLC, 13C NMR and [α]D value).

**Complex lipids of the isolates belonging to the Sulfolobales order, Archaeoglobus and Methanothermobacter genera have been analyzed by comparison with complex lipids of known species. Complex lipids of P. organotrophum and P. islandicum have been analyzed by chemical and spectroscopic methods.**

**Identification of the quinones in the microorganisms belonging to the Sulfolobales order has been made by EI-MS analysis while their relative percentages have been calculated by integration peak areas in HPLC.**

**Sulfolobales order**

Seven new isolates of thermophilic archaea were analyzed for their lipid composition. They were named: VE2, Kra22, VE6, SP3a, Kra23, TH2, and HV5. The quinone composition of the isolates and of other microorganisms belonging to the Sulfolobales order is shown in Table 1. All the isolates analyzed possess caldariellaquinone, a unique benzothiophenquinone present as major component of the quinone fraction of Sulfolobus solfataricus. Only in

<table>
<thead>
<tr>
<th>Species</th>
<th>CQ</th>
<th>SQ</th>
<th>SSQ</th>
</tr>
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<tbody>
<tr>
<td>TH2</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP3a</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kra22</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kra23</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VE6</td>
<td>95</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>VE2</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HV5</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. solfataricus</td>
<td>94</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>D. ambivalens (a)</td>
<td>83</td>
<td>16</td>
<td>trace</td>
</tr>
<tr>
<td>D. ambivalens (an)</td>
<td></td>
<td></td>
<td>99</td>
</tr>
</tbody>
</table>

CQ = Caldariellaquinone (see text)

SQ = Sulfolobusquinone (see text)

SSQ = Tricyclicquinone (De Rosa and Gambacorta, 1988)

a = grown under aerobiosis
an = grown under anaerobiosis
VE6 sulfolobusquinone is present in the same proportion as in *S. solfataricus*.

Total lipids from these thermophilic isolates account for about 7–8% of dried cells. All the isolates show a pattern of complex lipids very close to that reported for *S. solfataricus* (Fig. 2). However the relative proportions of glycolipids GL-I and GL-II (Fig. 2) are different as judged by TLC. Hydrolysis of complex lipids of the isolates gives rise to a mixture of GDGTs and GDNTs and to trace amounts of 2,3-di-O-phytanyl-sn-glycerol. The analysis of cyclic composition of GDGTs performed by TLC as previously reported (Trincone et al., 1988) shows all isolates possess cyclized tetraethers as reported in Table 2.

**Archaeoglobus genus**

To date *Archaeoglobus* is represented by two species *Archaeoglobus fulgidus* VC16 DSM 4304 and *Archaeoglobus profundus* AV18 DSM 5681.

The two species of *Archaeoglobus* genus and an isolate Kol3 are analyzed for their lipid composition. Core lipids of both species of *Archaeoglobus* are based on 2,3-di-O-phytanyl-sn-glycerol and tetraether a (Fig. 2). The former is present only in trace amounts in the isolate Kol3. However, *A. fulgidus* and *A. profundus* are different in the composition of complex lipids as judged by staining tests at TLC level. In *A. fulgidus*, two phosphoglycolipids at Rf 0.10 and 0.25, one phospholipid at Rf 0.30 and one glycolipid at Rf 0.60 are present as major complex lipids. In AV18 the major complex lipids are two phosphoglycolipids at Rf 0.10 and 0.13 and four glycolipids at Rf 0.40, 0.45, 0.60 and 0.65. The pattern of complex lipids in the isolate Kol3 appears to be the same as for AV18. In all isolates of the *Archaeoglobus* genus examined so far aminolipids are absent.

**Pyrobaculum genus**

*Pyrobaculum islandicum* (DSM 4184), a facultative autotrophic archaean, which is the type species of a new genus of hyperthermophilic archaea, and *Pyrobaculum organotrophum* (DSM 4185) that is obligately heterotrophic, are analyzed in detail for their lipid composition.

Core lipids of *Pyrobaculum* species are restricted to tetraether of GDGT type and trace amounts of 2,3-di-O-phytanyl-sn-glycerol. Analysis of cyclization of GDGTs, performed by TLC (Trincone et al., 1988) shows the presence of tetrathers a–e (Fig. 2) in different proportions in both organisms. The more cyclized products, d and e are found to be the major components in *Pyrobaculum organotrophum*, while less cyclized tetrathers a–c (Fig. 2) are more abundant in *Pyrobaculum islandicum*.

In Table 3 is reported the composition of complex lipids from these two organisms. Both possess glyco- and phos-
phoglycolipids being the sole difference the presence of a phosphopholipid at Rf 0.20 in *P. organotrophum* instead of the glycolipid at the same Rf found in *P. islandicum*. Aminophospholipids are absent in both organisms.

In Table 4 is reported the downfield part of the $^{13}$C NMR spectra of four major complex lipids that account for 85% of total lipids of both organisms.

<table>
<thead>
<tr>
<th>Glycerol units</th>
<th>GL-IV</th>
<th>GPL-II</th>
<th>GL-II</th>
<th>GPL-I</th>
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<tr>
<td>Glycosylated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>70.9</td>
<td>as in</td>
<td>as in</td>
<td>as in</td>
</tr>
<tr>
<td>C-2</td>
<td>78.0</td>
<td>GL-IV</td>
<td>GL-IV</td>
<td>GPL-II</td>
</tr>
<tr>
<td>C-3</td>
<td>70.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free or --P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>62.0</td>
<td>65.3</td>
<td>as in</td>
<td>as in</td>
</tr>
<tr>
<td>C-2</td>
<td>78.0</td>
<td>77.8</td>
<td>GL-IV</td>
<td>GPL-II</td>
</tr>
<tr>
<td>C-3</td>
<td>70.2</td>
<td>70.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-myo-inositol</td>
<td>P-O-C</td>
<td>72.5</td>
<td>73.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.3</td>
<td>71.3</td>
<td>GPL-II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70.2</td>
<td>69.9</td>
<td></td>
</tr>
<tr>
<td>Anomeric signals</td>
<td>C-1</td>
<td>103.5</td>
<td>103.6</td>
<td>103.7; 104.0; 103.7; 104.0; 104.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>104.2</td>
</tr>
</tbody>
</table>

| Anomeric signals | C-1 | 103.5  | 103.6 | 103.7; 104.0; 103.7; 104.0; 104.1 |
|                 |      |        |       | 104.2  |

Acid hydrolysis of GL-IV yielded a mixture of GDGTs (compounds a-e Fig. 2) and glucose. The glucose purified from the hydrolysate by HPLC had a specific rotation $[\alpha]_{D20}^\circ = + 52^\circ$ establishing its absolute D-configuration.

The $^{13}$C NMR signal at 103.5 ppm as well as $^1$H NMR signal at 4.35 ppm ($J_{H1-H2} = 7.8$ Hz) indicate the presence of a $\beta$-glucosidic linkage. The signal at 62.0 ppm in the $^{13}$C NMR spectrum, besides to the C-6 signal of the glucose moiety (61.8 ppm), is due to the free CH$_2$OH of the glycerol in the molecule. Glucosyl carbon signal assignments were made by DEPT experiments and by comparison with values reported for methyl $\beta$-D-glucopyranoside (Kalinosky et al, 1990) and for related archaeal glycolipids of similar structure (Ferrante et al., 1990).

Comparison of TLC mobilities and of $^{13}$C NMR spectrum of GL-IV compound with the glycolipid GL-1 from *Thermoproteus tenax*, identified as $\beta$-D-glucopyranosyl GDGT (Thurl and Schäfer, 1987) fully confirms its structure as indicated in Fig. 3.

GL-II (Rf 0.30) is a triglycolipid as indicated by its $^{13}$C NMR spectrum in which three anomeric carbons at 103.7, 103.9, 104.1 ppm are present, by integration of protons in $^1$H NMR spectrum and by mild hydrolysis that gave parent GL-III and GL-IV (Fig. 3). Glucose was the sole compound identified in the water phase after acid hydrolysis of this lipid and its absolute D-configuration was determined as above. The downfield glycosylation shift of the C-2 of the glucose of about six ppm (79.7 ppm) indicates that this carbon atom is attached to another glucose molecule as confirmed by methylation analysis. These data are consistent with the $(1 \rightarrow 2)$-$\beta$-D-glucopyranosyl sequence in the carbohydrate moiety. The Rf of this lipid is similar to that of GL-4 from *T. tenax* which has however the $(1 \rightarrow 6)$-$\beta$-D-glucopyranosyl sequence in its sugar head-group (Thurl and Schäfer, 1987).

GPL-II (Rf. 0.16) responds to phosphate reagent. In the $^{13}$C NMR spectrum of this compound the downfield shift (65.3 ppm) of the signal at 62.0 ppm, indicates the location of a phosphate group on the CH$_2$OH of the glycerol; the remaining signals accord with the presence in the molecule of a P-myo-inositol and $\beta$-D-glucose (De Rosa et al., 1987; Ferrante et al., 1988).

Acid hydrolysis of GPL-II yielded glucose, P-myoinositol and a mixture of GDGTs (compounds a-e Fig. 2). Selective alkaline hydrolysis of GPL-II, releasing only

$$\begin{align*}
R_1' &-O-CH_2 \\
H \cdots C \cdots O-(C_{40}H_{78}-80)O-O-CH_2 \\
H_2C-\cdots O-(C_{40}H_{78}-80)O-O-C-H \\
CH_2O-R_2
\end{align*}$$

![Fig. 3. R$_1$](image-url)

<table>
<thead>
<tr>
<th>R$_1$</th>
<th>R$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLIV OH</td>
<td>$\beta$-D-glcp</td>
</tr>
<tr>
<td>GLIII OH</td>
<td>$\beta$-D-glcp(1→2) $\beta$-D-glcp</td>
</tr>
<tr>
<td>GLII OH</td>
<td>$\beta$-D-glcp(1→2) $\beta$-D-glcp(1→2) $\beta$-D-glcp</td>
</tr>
<tr>
<td>GLPII P-myo-inositol</td>
<td>$\beta$-D-glcp</td>
</tr>
<tr>
<td>GLPI P-myo-inositol</td>
<td>$\beta$-D-glcp(1→2) $\beta$-D-glcp(1→2) $\beta$-D-glcp</td>
</tr>
</tbody>
</table>
phosphoinositol moiety, gave rise to GL-IV parent glycolipid. TLC comparison with lipids from T. tenax shows that GPL-II has the same RF value of PL-3. These data indicate for GPL-II the structure of Fig. 3.

GPL-I (RF 0.05) has the same \( ^{13}\text{C} \) NMR spectrum of GL-II except for the signal at 65.07 ppm assigned to phosphorylated glycerol moiety and for signals of inositol. GPL-I responds to phosphate reagent and alkaline hydrolysis of this lipid gave rise a compound with the same RF value of GL-II. The structure of the latter has been assigned as described for GL-II. The comparison with lipids from T. tenax indicates that GPL-I has the same RF as PL-5 which is built up from one tetraether, three glucose residues, one inositol and one phosphate (Thurl and Schäfer, 1987).

Minor complex lipids

Minor lipids are detected in the pattern of complex lipids of both species of Pyrobaculum. The structures of these minor compounds have been tentatively assigned on the basis of their RF values and their response to staining tests and acid hydrolysis.

GL-III (Table 3) has the same RF value as for the diglucoside GL-3 from T. tenax (Thurl and Schäfer, 1987). Mild hydrolysis of this product gave GL-IV and, at the end of reaction, GDGTs mixture. On the basis of relationship with parent glycolipid GL-IV and GL-II, its structure could be glucopyranosyl (1 \( \rightarrow \) 2)-glucopyranosyl diglycerol tetraether.

Phospholipid (PL-I, RF 0.20) present only in P. organotrophum has the same RF value as phosphoinositol derivative of GDGT from S. solfatarius and after hydrolysis gave GDGTs mixture. GL-I (RF 0.20) from P. islandicum is a tetraether based glycolipid, giving in fact GDGTs mixture after hydrolysis.

Methodococcus genus

The new isolate Methodococcus igneus Kol5 is a hyperthermophilic methanogen growing up to 92°C. This organism has been isolated from the Kolbeinsey ridge at a depth of 106 m in Iceland (Burggraf et al., 1990b). Core lipids of Kol5 are analyzed in comparison with that of Methodococcus jannaschii. Both organisms possess 2,3-di-O-phthanyln-sn-glycerol (a, Fig. 1), a macrocyclic diether (b, Fig. 1) identified by El-MS analysis of acetylated derivative and [\( \alpha\)]\text{D} value, and the unacylated form of GDGT.

The pattern of complex lipids in both organisms is very complicated as usually found in methanogens. More than 30 spots are present and staining test analysis shows the presence of glyco- and glycophospho-, aminophospho-, and phospholipids.

Discussion

Several unknown isolates have been analyzed for their lipid composition in comparison with representative members of Archaea domain.

Seven metal-mobilizing organisms have been isolated in Pisciarelli, Agnano (Italy) and in Iceland. They grow from 68 to 80°C. One of these isolates, the TH2 strain, on the basis of distinct physiological and molecular properties, has been described as type strain of the new genus Metallosphaera (Metallosphaera sedula TH2, DSM 348), while Sp3a can be considered as a strain of the same species (Huber et al., 1989). The analysis of quinone content of the isolates has been particularly useful in that the organisms belonging to the Sulfolobales order all possess benzo[10]phenquinones instead of naphthoquinone found in halophiles and in other thermophilic organisms (De Rosa and Gambacorta, 1988). All the isolates examined possess the unique caldariellaquinone found so far only in members of the Sulfolobales order. Moreover the analysis of core and complex lipids of these isolates confirms their assignment to this order. In particular the presence of GDNTs is proved to be a very diagnostic tool in ascribing new organisms to the Sulfolobales order.

Archaeoglobus may represent a biochemical missing link between methanogens and sulfur-metabolizing archaea. This genus is represented by the type species Archaeoglobus fulgidus, isolated from a shallow marine hydrothermal system at Vulcano, Italy. Recently the strain AV18, isolated from Guaymas hot vent areas, has been classified as a new species of Archaeoglobus genus and named profundus (DSM 5681). Although core lipids of this new species are the same of those found in the parent species A. fulgidus, complex lipids show the differences confirming the assignment of the isolate AV18 as a new species. Biochemical data about the isolate Kol3, possessing the same complex lipid pattern of AV18, are not available until now.

Three genera belong to Thermoproteales order, Thermoproteus, Pyrobaculum and Thermofilum. A detailed complex-lipid characterization is reported for Pyrobaculum islandicum and P. organotrophum. A close relationship at level of complex lipids is found between T. tenax and Pyrobaculum species. In fact both polar head(s) and core lipid(s) are the same (phosphomyoinositol, ß-D-glucose, GDGTs mixture) being the sole difference the sequence of carbohydrate in the di- and triglycolipids. No data on complex lipids of Thermofilum species are at moment available. Although a close similarity of complex and core lipids in the Thermoproteales order exists, a fine structural definition of complex lipids clearly shows differences among different genera of the same order. Moreover, from a structural point of view, as previously stated for T. tenax and in general as seen for other archaea, also in Pyrobaculum a biosynthetic relationship between glycolipids and phosphoglycolipids could exist.

Within Methodococcus genus the presence of macrocyclic diether (b, Fig. 1) has been reported so far only in the lipids of the species Mc. jannaschii. This is the first report of a new recognized organism, assigned to the genus Methanococcus, possessing this diether.

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