**NOTE**

**Variation in Molecular Species of Core Lipids from the Order *Thermoplasmales* Strains Depends on the Growth Temperature**

Ikuko Uda¹*, Akihiko Sugai¹, Yuko H. Itoh² and Toshihiro Itoh¹

¹ Division of Chemistry, Center for Natural Sciences, College of Liberal Arts and Sciences, Kitasato University
(1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, JAPAN)
² Department of Bioinformatics, Faculty of Engineering, Soka University
(1-236 Tangi-cho, Hachioji, Tokyo 192-8577, JAPAN)

**Abstract:** The core lipids from five strains of the order *Thermoplasmales* grown at various temperatures have been investigated. The major core lipid of the cell membrane in these strains was caldarchaeol (2, 2′, 3, 3′-tetra-O-di(biphytanyl)-sn-diglycerol). Five types of molecular species of the C₄₀ isoprenoid chains having different numbers of cyclopentane rings were detected in caldarchaeol. In the strains of the genus *Picrophilus*, a novel molecular species of the C₄₀ isoprenoid chain, being a C₄₀-bicyclic isomer, was detected. An increasing degree of cyclization depending on the rising growth temperatures was observed in all the strains examined.

**Key words:** Archaea, ether lipid, *Thermoplasmales*, caldarchaeol

---

**1 Introduction**

The order *Thermoplasmales* includes the wall-less strains in the Archaea. The genus *Thermoplasma* (two species), the genus *Picrophilus* (two species), and the genus *Ferroplasma* (one species) are present. The strains of *Thermoplasma* include the thermophilic acidiophile (*T. acidophilum* grows between 45 and 62 °C, optimum 59 °C, pH 1~4, optimum 1~2 (1); *T. volcanium* does between 33 and 67 °C, optimum 60 °C, pH 1~4, optimum 2 (2)) and the cells are surrounded by a single triple-layer membrane, 5~10 nm thick. The family *Picrophilaceae* is the thermophilic hyperacidophile growing between 47 and 65 °C and between pH 0 and 3.5. This family is separated from the family *Thermoplasmaceae* based on their base sequence of the 16S rRNA gene, DNA-dependent RNA polymerase, and the presence of an S-layer (3). In contrast to these families, the family *Ferroplasmaceae*, also a wall-less Eur-yarchaeon, grows in the quite different temperature range between 15 and 45 °C, optimum 35 °C, pH 1.3~2.2, optimum 1.7 (4).

Among these wall-less representatives, only the structure of the membrane lipids of *T. acidophilum* has been well investigated. The structure of the main membrane lipids of *T. acidophilum* is caldarchaeol (2, 2′, 3, 3′-tetra-O-di(biphytanyl)-sn-diglycerol) (5, 6), one of the ether core lipids of the archaeal strains. Five types of molecular species of the C₄₀ isoprenoid chain, classified by the number of cyclopentane rings, have been detected in the caldarchaeol of the extreme thermophilic Crenarchaeon such as *Sulfolobus solfataricus*. These are acyclic-, monocyclic-, bicyclic-, tricyclic-, and tetracyclic-C₄₀ isoprenoid chains (7). It has been observed that a decrease in the number of double bond in the membrane depends on lowering the environmental temperature for controlling the membrane fluidity in Eucarya and Bacteria. In *S. solfataricus*, it has been
reported that the average number of cyclized isoprenoid chains in the lipids increased by raising the growth temperature (8). The change in the average number of that in *T. acidophilum* showed the same tendency as *S. solfatarius* which depends on the cultivation temperature (9).

In this report, we describe the novel structure of a core lipid and the structural changes related to the cultivation temperatures of the cells using five strains belonging to the order *Thermoplasmales*.

## 2 Experimental

### 2.1 Organisms and Culture Conditions

The *Thermoplasma acidophilum* strain (ATCC 27658) was obtained from the American Type Culture Collection. *Thermoplasma volcanium* (JCM 9571), *Picrophilus oshimae* (JCM 10054), *Picrophilus torridus* (JCM 10055), and *Ferroplasma acidiphilum* (JCM 10970) were purchased from the Japan Collection of Microorganisms (JCM). The strains of *Thermoplasma* spp. and *Picrophilus* spp. were grown in modified JCM designated medium: \((\text{NH}_4)_2\text{SO}_4, 0.2 \text{ g}; \text{KH}_2\text{PO}_4 3.0 \text{ g}; \text{MgSO}_4\cdot7\text{H}_2\text{O} 0.5\text{ g}; \text{CaCl}_2\cdot2\text{H}_2\text{O} 0.25 \text{ g}; \text{Bacto yeast extract (Difco)} 0.6 \text{ g}; \text{glucose} 10.0 \text{ g/L})\). The pH of the medium for the *Thermoplasma* strains and the *Picrophilus* strains was adjusted to 1.5 and 1.3, respectively, using 18 mol/L H\(_2\)SO\(_4\). *Ferroplasma acidiphilum* was grown in the JCM medium #283 at pH 1.6. Incubation was aerobically carried out at different temperatures in the range of each growth temperature using 1.5 L-medium in flasks with low stirring and/or standing cultures of 10 mL-medium in loosely capped glass tubes. The cells were harvested during the late exponential phase by centrifugation, washed with distilled water and then lyophilized.

### 2.2 Preparation and Analysis of Polar Lipids

The total cellular lipids were extracted from the lyophilized cells using an ultrasonicated basic solvent system of chloroform / methanol / 1 mol/L aqueous ammonia (1:2:0.5, by vol). Neutral lipids and acidic lipids were fractionated from the total lipids as described in a previous report (10). Thin layer chromatography (TLC) of the total lipids and fractionated lipids was performed as previously described (10). Each main polar lipid in the acidic lipid fractions of *P. oshimae* and *P. torridus* were purified by preparative TLC using boronic acid-impregnated high performance (HPTLC) plates of silica gel (Merck, Darmstadt, Germany). Both solvent systems, i.e., chloroform / methanol / 0.2% CaCl\(_2\) (55:45:7, by vol) and chloroform / methanol / 1 mol/L aqueous ammonia (65:35:5, by vol), were used for developing the preparative TLC. Analyses of the purified main polar lipids from the *Picrophilus* strains were performed according to a previous report (11).

### 2.3 Analysis of Core Lipids

Lyophilized cells or intact lipids were methanolyzed using 5% HCl-methanol to obtain the core lipids and polar head groups. The core lipids were extracted by a chloroform / methanol mixture from the methanolyzate. TLC analysis of the core lipids was performed using a HPTLC plate of silica gel and double solvent systems, chloroform / methanol (4:1, v/v) and hexane / diethyl ether / acetic acid (60:40:2, by vol). The core lipids were detected by spraying with 18 mol/L H\(_2\)SO\(_4\) followed by heating at 150°C, then calculated using the TLC scanning (CS-930, Shimadzu, Kyoto, Japan). The hydrocarbon chains from the core lipids were obtained by HI degradation as previously described (10) and subsequently analyzed by gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS). The GLC analyses were performed using a CP-SimDist Ultimetal column (Chrompack, i.d. 0.53 mm × 15 m, df = 0.17 μm), attached to a GC-18 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame-ionization detection system. The hydrocarbon chains were analyzed with a temperature gradient from 110°C (5 min) to 300°C at the rate of 15°C/min.

### 2.4 GC-MS, FAB-MS

GC-MS (electron ionization, EI) was carried out using a JMS-AX505H gas chromatograph-mass spectrometer (JEOL, Tokyo, Japan) with a capillary column (DB-1, i.d. 0.53 mm × 15 m). Fast atom bombardment-mass spectrometry (FAB-MS) was carried out in the negative mode with an m-nitrobenzylalcohol (m-NBA) matrix using a JMS-DX300 (JEOL, Tokyo, Japan).

## 3 Results and Discussion

TLC profiles of the total lipids obtained from the five
strains of order *Thermoplasmales* are shown in Fig. 1. The main polar lipid (MPL) of *T. acidophilum*, and *T. volcanium* was identified as β-L-gulopyranosyl caldarchaetidylglycerol (Rt = 0.67) by Swain *et al.* (12). On the other hand, the structure of the main polar lipid of *F. acidiphilum* was identified as β-D-glucopyranosyl caldarchaetidylglycerol (MPL′, Rt = 0.65) by Batrakov *et al.* (13) and that of *P. oshimae* and *P. torridus* had the same Rt value (0.65) as MPL′. The main polar lipid of *Thermoplasma* spp. and *F. acidiphilum* were about 50% of the total lipids and that of *Picrophilus* spp. being about 80%. MPL′ is a homologue of MPL which differs in the sugar moiety as mentioned above. These homologues are chromatographically isolated by the solvent system: chloroform / methanol / 1mol/L aqueous ammonia (65/35/5, by vol), and the boronic acid impregnated silica gel plate (11). Both of them were detected in the total lipid extract of *T. acidophilum*; the gulosyl-homologue is major and the glucosyl-homologue is minor. In this paper, we describe the chemical structure of the main polar lipid (MPL) of *P. oshimae* and *P. torridus* based to our previous paper (11).

The core lipids of each MPL of *Picrophilus* spp. were co-chromatographed on the TLC with the caldarchaeol. The sugar moieties of the MPL were identified as glucose at the molar ratio of 1 to 1 versus caldarchaeol. The phosphorus containing polar head groups were identified as glycerophosphate. Both of the negative FAB-MS spectra of the MPL showed a molecular ion peak at 1614 as m/z [M-H]⁻. This ion peak corresponds to the structure of the glucosyl-caldarchaetidylglycerol in a core lipid composed of the C₄₀-acyclic and C₄₀-monocyclic isoprenoid chains. It seems that the glucosyl-caldarchaetidylglycerol is commonly distributed among the order *Thermoplasmales*.

Figure 2 shows the TLC profile of core lipids prepared from the total lipids of the five strains of the order *Thermoplasmales*. The main core lipid of each strain was co-chromatographed with caldarchaeol (Rt = 0.55) on a TLC plate, and the spots presented at the same Rt value as caldarchaeol are more than 95% of the total core lipids. The archaeol (2, 3-di-O-phytanyl-sn-glycerol), the other core lipid commonly distributed in the archaeal strains, existed at about 1% in *Thermoplasma* spp., at about 5% in *Picrophilus* spp., and at a trace in the Ferroplasma strain at the Rt value of 0.39. It is clear that the main core lipid of the order *Thermoplasmales* strains was caldarchaeol independent of their optimum growth temperature or surface structure of the cells.

We have focused on the comparison of the molecular species of the isoprenoid chain constituting caldarchaeol from the five strains at different cultivation temperatures. Figure 3 shows a gas chromatogram of the isoprenoid chains of caldarchaeol from *P. torridus* cultiv-
I. Uda, A. Sugai, Y.H. Itoh et al.

ed at 62°C. It is shown that five peaks corresponded to the C40 isoprenoid chains detected in S. solfataricus (14, 15) and an unknown peak were observed. The peaks for [M]+ obtained from the GC-MS (EI) spectra at m/z 562, m/z 560, m/z 558, m/z 556, and m/z 554 were identified as C40H82 (acyclic), C40H80 (monocyclic), C40H78 (bicyclic), C40H76 (tricyclic), and C40H74 (tetracyclic), respectively. The unknown peak between the peaks of C40-monocyclic and C40-bicyclic on the gas chromatogram of the isoprenoid chains was detected from each Picrophilus spp.

**Figure 4** shows the structure of the C40-bicyclic and proposed structure of the unknown hydrocarbon presumed from the fragmentation of the GC-MS. The GC-MS (EI) spectrum of the unknown peak showed [M]+ as m/z 558 corresponding to C40H78 (bicyclic). The structure of the C40-bicyclic previously reported is a symmetrical structure having a cyclopentane ring at carbon 7. From the GC-MS (EI) spectrum of the C40-bicyclic, the fragment ion peaks corresponding to have cleaved between carbon 6 and carbon 7 were obtained at m/z 99 (C7H15+) and m/z 459 (C33H63+). The fragment ion peak at m/z 99 (C7H15+) including a methyl-branch at carbon 3 was also obtained from the spectra of the C40-acyclic and C40-monocyclic.

On the other hand, the fragment ion peaks of m/z 97 (C7H13+) and m/z 461 (C33H65+) instead of m/z 99 (C7H15+) and m/z 459 (C33H63+) were obtained from the GC-MS (EI) spectrum of the unknown peak. The fragment ion peak m/z 461 (C33H65+) was also obtained from the C40-monocyclic corresponding to the structure which was cleaved between carbon 6 and carbon 7 (including one cyclopentane ring). The existence of the partial structure m/z 461 (C33H65+) in the unknown peak was deduced from the GC-MS analyses of the C40-acyclic and C40-monocyclic. The fragment ion peaks of m/z 393(C35H57+) and m/z 365(C36H53+) were obtained from the C40-acyclic, C40-monocyclic, and the unknown peak. When the cyclopentane rings exist at the position of carbon 3 and carbon 7 in the structure of the C40-tricyclic and C40-tetracyclic, the fragment ion peaks at m/z 97 (C7H13+) and m/z 165 (C12H21+) were observed. These fragment ion peaks were also obtained from the unknown peak. Therefore, it was presumed that the structure of the unknown peak is C40-bicyclic including the cyclopentane rings positioned at carbon 3 and carbon 7.

**Table 1** shows the distribution of molecular species of the C40 isoprenoid chain and the average cyclization number in the core lipids of the whole cells from the five strains of the order Thermoplasmales grown at different temperatures. In case of the Picrophilus strains, the percentage of the C40 bicyclic was calculated from the sum of the peak area of the already-known C40-bicyclic and newly identified C40-bicyclic isomer. In Thermoplasma spp., the C40-tetracyclic is not detected at the growth temperatures lower than their optimum. In all the strains examined here, the decrease in the C40-acyclic and the increase in the C40-tricyclic and C40-tetracyclic were accomplished by increasing the cultivation temperatures. Therefore, the average number of cyclopentane rings in the isoprenoid chains in the strains of the order Thermoplasmales increases with the increase in the growth temperatures. The average degrees of cyclization at higher cultivation temperatures were increased 1.2 ~ 1.5 times that at the lower temperature.

The increasing tendency of the average degree of cyclization with an increase in the growth temperatures was also observed in the strains of thermoacidophilic,
The Core Lipids of Thermoplasmales Strains

Fig. 4 The Structure of C_{40}-bicyclic and Proposed Structure of the Unknown Hydrocarbon Presumed from Fragmentation of GC-MS (EI).

Table 1 Distribution of Cyclopentane Rings in Core Lipids of Whole Cell from the Order Thermoplasmales Strains.

<table>
<thead>
<tr>
<th>Temperature of growth (°C)</th>
<th>% of C_{40} hydrocarbon with</th>
<th>Average degree of cyclization*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acyclic Monocyclic Bicyclic Tricyclic Tetracyclic</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4.9 32.7 62.4 ND** ND</td>
<td>1.58 ± 0.06</td>
</tr>
<tr>
<td>50</td>
<td>2.3 20.7 71.9 5.2 ND ND</td>
<td>1.80 ± 0.09</td>
</tr>
<tr>
<td>60</td>
<td>0.7 7.0 77.6 14.6 &lt; 0.1</td>
<td>2.06 ± 0.04</td>
</tr>
<tr>
<td>45</td>
<td>64.8 27.3 7.9 ND ND</td>
<td>0.43 ± 0.09</td>
</tr>
<tr>
<td>50</td>
<td>34.0 46.9 19.1 ND ND</td>
<td>0.85 ± 0.12</td>
</tr>
<tr>
<td>55</td>
<td>34.4 41.2 21.1 3.3 ND ND</td>
<td>0.93 ± 0.08</td>
</tr>
<tr>
<td>60</td>
<td>14.1 35.4 44.0 6.5 ND</td>
<td>1.43 ± 0.15</td>
</tr>
<tr>
<td>62</td>
<td>12.2 29.1 42.8 14.6 1.4</td>
<td>1.64 ± 0.16</td>
</tr>
<tr>
<td>45</td>
<td>32.0 32.0 28.2 7.2 0.7</td>
<td>1.13 ± 0.29</td>
</tr>
<tr>
<td>55</td>
<td>31.1 32.0 24.4 9.5 2.9</td>
<td>1.21 ± 0.14</td>
</tr>
<tr>
<td>62</td>
<td>18.7 25.8 34.6 15.9 5.0</td>
<td>1.63 ± 0.16</td>
</tr>
<tr>
<td>45</td>
<td>23.3 46.0 26.8 3.8 &lt; 0.1</td>
<td>1.18 ± 0.13</td>
</tr>
<tr>
<td>55</td>
<td>13.1 36.1 36.8 12.0 2.0</td>
<td>1.54 ± 0.12</td>
</tr>
<tr>
<td>62</td>
<td>9.2 28.7 36.6 19.2 6.3</td>
<td>1.85 ± 0.10</td>
</tr>
<tr>
<td>35</td>
<td>ND 31.2 68.8 ND ND</td>
<td>1.69 ± 0.04</td>
</tr>
<tr>
<td>40</td>
<td>ND 12.8 87.2 &lt; 0.1 ND</td>
<td>1.87 ± 0.05</td>
</tr>
</tbody>
</table>

*Average degree of cyclization: (% monocyclic + 2 × % bicyclic + 3 × % tricyclic + 4 × % tetracyclic) × 10^{-2}.

**ND: not detected (< 0.01).
extreme thermoacidophilic, and hyperthermophilic Archaea (8, 9, 16). However, the average degree of cyclization in the strains having a higher optimum growth temperature is not always higher than that having lower ones. For example, in the hyperthermophilic Archaea such as the strains of the order Thermococcales, almost all strains have no cyclopentane rings in the isoprenoid chains (17). To counter the environmental temperatures, there are various modifications of the core lipid structure and polar lipid structure that depend on the strains. Only in the case of the cyclopentane rings presented in the core lipids, a correlation was found between the cyclization number of the isoprenoid rings presented in the core lipids, a correlation was on the strains. Only in the case of the cyclopentane core lipid structure and polar lipid structure that depend on lower ones. For example, in the hyperthermophilic Archaea the fluidity of the membrane versus the environmental temperature, similar to that in the unsaturation of the fatty acylester lipids of Eucarya and Bacteria.

Acknowledgments

This research was financially supported in part by a Kitasato University Research Grant for Young Researchers.

References