JOURNAL OF OLEO SCIENCE
Copyright ©2004 by Japan Oil Chemists' Society
J. Oleo Sci., Vol. **53**, No. 1, 41-44 (2004)

JOS

NOTE

The Core Lipid Composition of the 17 Strains of Hyperthermophilic Archaea, *Thermococcales*

Akihiko SUGAI[1*], Ikuko UDA[1], Yuko H. ITOH[2] and Toshihiro ITOH[1]

[1] *Division of Chemistry, Faculty of General Studies, 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)*

Edited by T. Itoh, Kitasato Univ., and accepted August 19, 2003 (received for review August 6, 2003)

Abstract: The core lipid compositions of the strains of the genus *Pyrococcus* (3 strains), *Thermococcus* (13 strains) and *Palaeococcus* (1 strain) belonging to the Order *Thermococcales*, Euryarchaeota were examined. In the 85℃ culture, the main core lipid of every strain was caldarchaeol (dibiphytanyldiglycerol tetraether). No cyclopentane rings were detected in the C_{40} isoprenoid chains of caldarchaeol from any of the tested strains. Archaeol (diphytanylglycerol diether) was also detected in these strains, and the contents were 5.9% to 42.1% for the total core lipids. The H-type caldarchaeol in which two isoprenoid chains were linked to each other by a covalent bond around the center of both isoprenoid chains was detected in 4 strains, with no relationship to the classification of the genera. The profile of the core lipids in these strains showed that the core lipid composition of *Thermococcales* is close to that of the thermophilic methanogen in Euryarchaeota.

Key words: Archaea, lipid, *Thermococcales*, caldarchaeol, archaeol

1 Introduction

Although the phylogenetic taxonomy of the microorganisms is performed by two types of methods, the gene dependent and those of the phenotype dependent, the relation between a phylogenetic classification and membrane lipids is particularly important in Archaea. The specific membrane lipid of Archaea is capable of clearly distinguishing that of bacteria and eukaryota. That is, the membrane lipid of Archaea has a peculiar core lipid in which the isoprenoid chains are conjugated with ether bonds at the *sn*-2 and *sn*-3 sites of glycerol (1,2).

In recent years, the isolation and identification of many hyperthermophilic Archaea obtained from the sea around the world have been carried out, and are being watched with keen interest. In hyperthermophlic Archaea, *Thermococcales* consist of three genera, the *Pyrococcus*, *Thermococcus*, and *Palaeococcus* (3). Most of these strains could propagate at 90℃ or more, and all of them were anaerobic microorganisms with the optimum pH around neutral.

De Rosa *et al*. reported that the main core lipid of *Thermococcus celer* is the diether type archaeol (4). Furthermore, it was reported by Lanzotti *et al*. that the main core lipid of *Pyrococcus wosei* is archaeol (5). However, when we analyzed the lipid of *Pyrococcus horikoshii*, we detected caldarchaeol as the main core lipid and archaeol as the minor component at the cultivation temperature of 82-98℃ (6). Although the lipid

*Correspondence to: Akihiko SUGAI, Division of Chemistry, Faculty of General Studies, Kitasato University, 1-15-1 Kitasato, Sagamihara-shi, Kanagawa-ken, 228-8555, JAPAN
E-mail: sugai@clas.kitasato-u.ac.jp

analysis was important in Archaea, a detailed lipid analysis of the strain belonging to *Thermococcales* has not yet been reported.

In this report, a comparison was carried out on the core lipid of *Pyrococcus* (3 strains), *Thermococcus* (13 strains), and *Palaeococcus* (1 strain), in order to examine the relationship between the phylogenetic classification based on the 16S rRNA genes and the distribution of the lipids which are regarded as one of the phenotypes.

2 Experimental

2·1 Sources of Organisms and Culture Conditions

Pyrococcus horikoshii (JCM 9974, growth temperature 80-102℃), *P. furiosus* (JCM 8422, 70-103℃), *P. woesei* (JCM 8421, 97-105℃), *Thermococcus celer* (JCM 8558, 75-97℃), *T. stetteri* (JCM 8559, 60-85℃), *T. litoralis* (JCM 8560, 65-95℃), *T. guaymasensis* (JCM 10136, 56-90℃), *T. aggregans* (JCM 10137, 60-94℃), *T. fumicolans* (JCM 10128, 73-103℃), *T. gorgonarius* (JCM 10552, 68-95℃), *T. pacificus* (JCM 10553, 70-95℃), *T. peptonophilus* (JCM 9653, 60-100℃), *T. profundus* (JCM 9378, 50-90℃), *T. zilligii* (JCM 10554, 55-85℃), *T. waiotapuensis* (JCM 10985, 60-90℃), *T. aegaeus* (JCM 10828, 90℃), and *Palaeococcus ferrophilus* (JCM 10417, 60-88℃) were purchased from the Japan Collection of Microorganisms (JCM).

Culture media, which are designated by JCM, were used. All of the microorganisms were anaerobically cultivated for 2 ~ 3 days at pH 7.0 and 85℃.

2·2 Preparation and Analyses of Lipids

Lipid extraction from the cells was carried out according to the method using alkaline solvents with ammonia as previously described (6).

The core lipids were obtained from the crude total lipids by acid methanolysis, i.e., adding 5% HCl-methanol at 100℃ for 2 h. The polar lipids of *Thermococcales* were completely cleaved into the polar head group and the core lipid, and the core lipids were obtained from the organic phase after partition between chloroform and water. Negative fast atom bombardment mass spectrometries (FAB-MS) of the core lipids were performed using a JMS AX-505H (Japan Electron Optics Laboratory, Tokyo, Japan) with a matrix of glycerol. The preparation of the hydrocarbons from the core lipid was performed by the following methods. The core lipids were treated with 57% HI at 100℃ for 2 h. The alkyl iodides were extracted with hexane, then reduced to hydrocarbons with zinc powder and acetic acid for 2 h at 100℃ after removal of the solvent.

GLC analyses were performed using a GC-18A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a CP-SimDist Ultimetal column (Chrompack, I.D. 0.53 mm × 10 m, df=0.17 μm) by increasing the temperature from 110℃ (5 min) to 380℃ at the rate of 15℃ / min.

2·3 Thin-layer Chromatography

The TLC (thin-layer chromatography) of the core lipids was carried out on precoated silica gel 60 plates (Merck. Art. 5641) which were activated at 120℃ for 2 h before use. The TLC of the core lipids was developed using hexane / ethyl acetate (7:3, v/v) solvent system. After spraying the TLC plates with 50% H_2SO_4, it was charred at 160℃ for 10 min to detect the core lipids. The purification of the core lipids was performed by preparative TLC.

3 Results and Discussion

The optimum growth temperature of the 17 strains were distributed between 75℃ and 103℃. Cultivations in this experiment were performed at 85℃ in which all 17 strains can grow. The cells of the early stationary phase were used for these experiments. The core lipids of each strain were compared using TLC (data not shown). The caldarchaeol of the tetraether type was detected as the major core lipid from all the strains. Also, the archaeol was detected from all strains. Moreover, the H-type caldarchaeol (6), which has already been detected in *P. horikoshii*, was also detected in *T. celer*, *T. guaymasensis*, and *T. waiotapuensis*. The spots of the archaeol (*Rf*=0.64), the caldarchaeol (*Rf*=0.38) and the H-form caldarchaeol (*Rf*=0.26) on the TLC plate were clearly separated, when the solvent system of hexane / ethyl acetate (7:3, v/v) was used. In the caldarchaeol of the strain belonging to the order *Sulfolobales* of acidothermophilic Crenarchaeota, another subdomain of Archaea, they were divided into some bands on the plate as a molecular species according to the number of cyclopentane rings in the isoprenoid chains when the same solvent system was used (7). However,

in the case of *Thermococcales*, at the culture conditions of 85℃, the caldarchaeol of all strains was detected as a single spot on the TLC.

When the FAB-MS measurement of these caldarchaeol spots on the TLC were carried out, the molecular ionic peak of $(M-1)^-$ was revealed at *m/z* 1300, and it was proved that these caldarchaeols consisted of isoprenoid chains without a cyclopentane. Isoprenoid chains constituting the core lipids of the 17 kinds of strains were analyzed by GLC. These results are shown in **Table 1**. As seen in the table, these isoprenoid chains corresponded to the hydrocarbon chains, that is, C_{20} to archaeol, C_{40} to caldarchaeol, and C_{80} to the H-type caldarchaeol. Similar to the results of the TLC analysis, the C_{40} isoprenoid chains derived from the tetraether type caldarchaeol was detected as a major hydrocarbon component in the strain belonging to *Thermococcales*. Since C_{80} is also a tetraether type, if this is included, the tetraether type lipids constituted 58% or more of the total in almost all the strains. In the case of *Thermococcales*, C_{20} is contained just over or below 20%, and differed from the strain of *Sulfolobales* in which the content of archaeol is as low as 5 ~ 8% (7). It appeared that the cyclopentane ring was not in the C_{40} isoprenoid chain at the cultivation temperature of 85℃, and significantly differed from the strain of *Sulfolobales*. In *Sulfolobales*, it has been postulated that the archaeol type lipids contained as a very small component were the precursor of the tetraether type lipid, but in the case of the strain of *Thermococcales* containing many archaeols (7), the possibility can be considered that the archaeol-type lipid exists as a constituent of the membrane.

Table 1 Hydrocarbon Compositions (%) of *Thermococcales*.

	Hydrocarbons (%)		
	C_{20}	C_{40}	C_{80}
Pyrococcus horikoshii	5.9	60.1	34.0
P. furiosus	31.2	68.8	-
P. woesei	26.5	73.5	-
Thermococcus celer	38.8	45.7	15.5
T. stetteri	9.6	90.4	-
T. litoralis	26.8	73.2	-
T. guaymasensis	12.7	75.3	12.0
T. aggregans	42.1	57.9	-
T. fumicolans	27.3	72.7	-
T. aegaeus	27.2	72.8	-
T. gorgonarius	16.8	83.2	-
T. profundus	16.0	84.0	-
T. peptonophilus	14.3	85.7	-
T. zilligii	33.2	66.8	-
T. waiotapuensis	15.2	35.9	48.9
T. pacificus	25.4	74.6	-
Palaeococcus ferrophilus	12.2	87.8	-

In *T. celer*, which was cultivated at 85℃, the content of archaeol was about 40% and caldarchaeol was the major core lipid. On the other hand, De Rosa reported that the main core lipid of *T. celer* is archaeol (4). This difference was also observed in *P. woesei*. Since many archaeols are contained in *T. celer*, it is probable that a quantitative relation with caldarchaeol will be reversed with growth temperature, etc. In this respect, it is necessary to change the cultivation conditions and examine the quantitative relation in detail.

The distribution of the hydrocarbon chain of the strains of *Thermococcales* as shown in **Table 1** suggested that *Thermococcales* is more closely related to a methanobacterium than an acidothermophile of Archaea (8). In this comparison of the core lipid, although a correlation was not found between the classification of a genus and lipid composition, a possible correlation in terms of *Thermococcales* was seen. That is, in the 85℃ culture, the core lipid mainly consists of archaeol and caldarchaeol, and the major core lipid is caldarchaeol. Moreover, no cyclopentane rings existed in the C_{40} hydrocarbon chains of caldarchaeol. The H-type caldarchaeol was initially detected only from the thermophilic methanobacterium of Archaea (8), but they are also widely distributed in *Thermococcales* though they were not in all strains. Consequently, by combining the features of these core lipids and cultivation conditions, there is a significant possibility that an identification can be made simply as to whether the hyperthermophilic microorganisms separated from the natural hot springs belong to *Thermococcales*.

References

1. M. De ROSA, A. GAMBACORTA and A. GLIOZZI, Structure, Biosynthesis, and Physicochemical Properties of Archaebacterial Lipids, *Microbiol. Rev.*, Vol. **50**, 70-80 (1986).
2. A. GAMBACORTA, A. GLIOZZI and M. De ROSA, Archaeal Lipids and Their Biotechnological Applications, *World J. Microbiol. Biotech.*, Vol. **11**, 115-131 (1995).

3. K. TAKAI, A. SUGAI, T. ITOH and K. HORIKOSHI, *Palaeococcus ferrophilus* gen. nov., sp. nov., a Barophilic, Hyperthermophilic Archaeon from a Deep-Sea Hydrothermal Vent Chimney, *Int. J. Syst. Evol. Microbiol.*, Vol. **50**, 489-500 (2000).
4. M. De ROSA, A. GAMBACORTA, A. TRINCONE, A. BASSO, W. ZILLIG and I. HOLZ, Lipids of *Thermococcus celer*, a Sulfur-Reducing Archaebacterium: Structure and Biosynthesis, *System. Appl. Microbiol.*, Vol. **9**, 1-5 (1987).
5. V. LANZOTTI, A. TRINCONE, B. NICOLAUS, W. ZILLIG, M. De ROSA and A. GAMBACORTA, Complex Lipids of *Pyrococcus* and AN1, Thermophilic Members of Archaebacteria Belonging to *Thermococcales*, *Biochim. Biophys. Acta*, Vol. **1004**, 44-48 (1989).
6. A. SUGAI, Y. MASUCHI, I. UDA, T. ITOH and Y.H. ITOH, Core Lipids of Hyperthermophilic Archaeon, *Pyrococcus horikoshii* OT3, *J. Jpn Oil Chem. Soc.*, Vol. **49**, 695-700 (2000).
7. A. SUGAI, I. UDA, K. KON, S. ANDO, Y.H. ITOH and T. ITOH, Structural Identification of Minor Phosphoinositol Lipids in *Sulfolobus acidcaldarius* N-8, *J. Jpn Oil Chem. Soc.*, Vol. **45**, 327-333 (1996).
8. H. MORII, T. EGUCHI, M. NISHIHARA, K. KAKINUMA, H. KOIG and Y. KOGA, A Novel Ether Core Lipid with H-Shaped C_{80}-isoprenoid Hydrocarbon Chain from the Hyperthermophilic Methanogen *Methanothermus fervidus*, *Biochim. Biophys. Acta*, Vol. **1390**, 339-345 (1998).