Available online at www.sciencedirect.com
ScienceDirect
Systematic and Applied Microbiology 31 (2008) 169–182
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

A dendrogram of archaea based on lipid component parts composition and its relationship to rRNA phylogeny

Yosuke Koga[a,*,1], Masahiro Nakano[b]

[a]*Department of Chemistry, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan*
[b]*Department of Health Information Science, School of Health Sciences, University of Occupational and Environmental Health, Yahatanishi-ku, Kitakyushu 807-8555, Japan*

Received 30 October 2007

Abstract

The results of two objective and quantitative, computer-assisted analyses of the lipid component parts distribution pattern among various archaeal organisms belonging to Euryarchaeota are reported. One was a cluster analysis and the other a selection of unique combinations of lipid component parts found exclusively in a given taxon. The cluster analysis revealed that the distribution of lipid component parts was correlated with phylogeny based on small subunit rRNA sequences, although there was some discrepancy with rRNA phylogeny. A hypothesis that may explain the reason for the correlation and the discrepancy is proposed. In our scenario, we assumed that random and independent mutations on the rRNA and lipid biosynthesis genes may result largely in coincided evolution. The fact that RNA and lipid are semantide and episemantic molecules, respectively, is the fundamental difference between the phylogeny of RNA and lipid. Moreover, different selective pressures on RNA and lipids exert different effects on their evolution. Unique lipid component parts were detected for eight out of nine orders, 14 families, and 22 genera of the Euryarchaeota analyzed. A unique lipid component parts combination pattern characterized the taxon. The results confirm and extend a previously reported conclusion based on a more statistical basis.

Keywords: Archaea; Ether lipid; Phospholipid; Glycolipid; Cluster analysis; Chemotaxonomic marker

Introduction

More than 100 types of phospholipids and glycolipids (polar lipids) have been identified from various archaea. Polar lipid composition varies depending on the family or genus to which the archaeal organism belongs, and is, therefore, one of the useful chemotaxonomic markers for archaea, especially Euryarchaeota [10]. For chemotaxonomic purposes, we developed a simplified analysis of lipid component parts (LCP) as a method partly based on chemical structural information [7]. Polar lipids of archaea are composed of glycerol or a glycerophosphate backbone with ether-linked isoprenoid hydrocarbon chains (core lipid) and polar groups (phosphodiester-linked alcohols or sugar groups) [9,11] (Fig. 1A). These are designated as LCP. One species of

*Corresponding author. Present address: 1-1 Hinosato, Munakata City, Fukuoka 811-3425. Tel./fax: +81 940 37 2657.
E-mail addresses: yokoga@ams.odn.ne.jp, kogay@med.uoeh-u.ac.jp (Y. Koga).
[1]Tel.: +81 93 691 7215; fax: +81 93 693 9921.

0723-2020/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2008.02.005

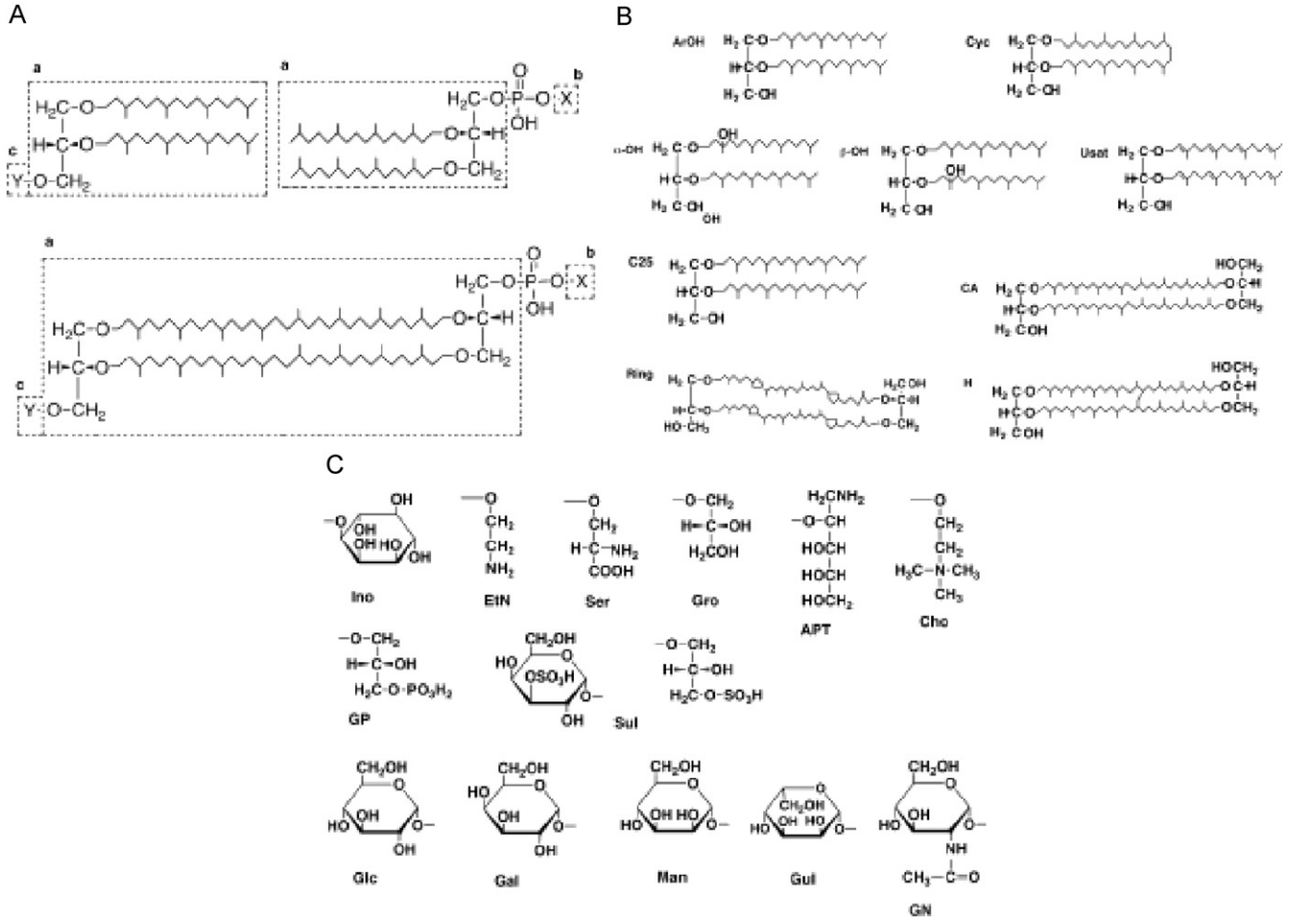

Fig. 1. LCP of archaea. (A): a, core lipids; b, phosphodiester-linked polar head groups; c, glycolipid sugars. B: variety of core lipids. ArOH, archaeol; Cyc, cyclic archaeol; α-OH, α-hydroxyarchaeol; β-OH, β-hydroxyarchaeol; Usat, unsaturated archaeol; C25, archaeol with one or two C25 isoprenoid chains; CA, caldarchaeol; Ring, caldarchaeol with one to eight cyclopentane rings on the isoprenoid chains; H, caldarchaeol with a covalent bond between two isoprenoid chains. C: variety of polar groups. Ino, *myo*-inositol; EtN, ethanolamine; Ser, serine, Gro, glycerol; APT, aminopentanetetrol either with two or three methyl groups on the amino group or without a methyl group. Cho, choline; GP, glycerophosphate as a polar head group; Sul, a sulfate group bound with a sugar or glycerol.

archaea typically contains several to 20 or more types of polar lipids. The simplified analysis of the lipid component parts is such that, without separating the individual lipids, LCP are liberated from the total lipids by appropriate chemical degradation methods and identified by appropriate chromatography [7,10]. The results of the analysis are qualitatively recorded only for the ''presence'' or ''absence'' of the individual component parts. Since an ether polar lipid is composed of one core lipid with a phosphodiester-linked polar head group and/or a few monosaccharide units, an intact lipid structure can be generally inferred from a given set of LCP. For example, the presence of archaeol and serine in an archaeon suggests the occurrence of archaetidylserine in the organism. Another example is that the presence of glucosyl caldarchaetidylinositol is supposed from the presence of LCP such as caldarchaeol, inositol, and glucose. However, in this case, the number of glucose units, the linkage position between sugars, and the anomeric configuration of glucose cannot be specified.

Our previous paper described the LCP composition in methanoarchaea as being characteristic of a family- or genus-level taxon, which is classified basically by the 16S rRNA sequence similarity [10]. This means that lipid composition is correlated with rRNA phylogeny. In order to confirm the relationship between lipid composition and rRNA phylogeny, a more quantitative, computer-assisted cluster analysis (Ward method) of the LCP distribution was undertaken for various archaeal organisms belonging to Euryarchaeota, using previously reported LCP. It was suggested from the cluster analysis that the distribution of the LCP correlated with the rRNA-based phylogenetic type,

although with some significant exceptions. The relationship of membrane lipid evolution to rRNA phylogeny is discussed and a hypothesis that may explain both the similarity and the discrepancy is presented. In addition to the cluster analysis, the LCP combination exclusively found in a taxon (a unique LCP or hallmark LCP) was selected with the aid of a computer program, and this unique hallmark combination characterized the taxon. The present results confirmed and extended the conclusion in a previous paper [10] based on a more statistical approach. The archaeal lipid nomenclature proposed by Nishihara et al. [16] was used throughout the text.

Materials and methods

Archaea analyzed

All of the archaea analyzed belonged to Euryarchaeota, which is classified into nine orders, 14 families, 34 genera, and 46 species (including two different strains of *Methanosarcina mazeii*). Among them, 38 strains of 25 genera were methanoarchaea. Since it is not known which isomer of hydroxyarchaeol (α- or β-isomer, see below) is contained in *Methanopyrus kandleri* and *Thermoplasma acidophilum*, the species with each isomer were presumed to be separate strains (Table 1). Therefore, 49 strains in all were used for the present analyses.

LCP composition

The distribution of LCP in Euryarchaeota may be obtained from our previous papers [7,10,12,24] and other published literature [3,5,6,14,15,20–23,25]. Archaeol was not used as a variable to calculate the distance between two organisms because it was found in all of the archaeal organisms used in the analysis. In addition to archaeol, at least one of the following core lipids was found in one organism used in this analysis: caldarchaeol (CA), α-hydroxyarchaeol (α- OH), β-hydroxyarchaeol (β-OH), archaeol with one or two C25 isoprenoid chain(s) (C25), cyclic archaeol (Cyc), archaeol with unsaturated isoprenoid chains (Usat), caldarchaeol with cyclopentane rings on the isoprenoid chains (Ring), and caldarchaeol with an H-shaped C80 isoprenoid chain (H; Fig. 1B). *myo*-Inositol (Ino), ethanolamine (EtN), L-serine (Ser), glycerol (Gro), a phosphorylated glycerol group (irrespective of being methylated or not on the phosphate group, and including cyclic phosphate on the glycerol moiety, GP), aminopentanetetrols with or without methyl groups on their amino group (APT), and choline (Cho) were phosphodiester-linked polar head groups. D-glucose (Glc), D-galactose (Gal), D-mannose (Man), *N*-acetyl-D-glucosamine (GN), and L-gulose (Gul) constituted a glycolipid sugar group. A sulfate or sulfonate group on a glycolipid sugar (sulfated sugar) or on the unalkylated glycerol of archaetidylglycerol (archaetidylglycerosulfate; Sul) was also recorded. The structures of these LCP are shown in Fig. 1C. The presence and absence of LCP were coded as "1" and "−1", respectively. Missing data were coded as "0" (Table 1). The LCP composition may vary depending on the culture conditions. Consequently, although the effects of growth conditions on lipid composition have not been specifically established, standardized conditions were used, as far as possible, for the fastest (best) growth of each archaeon. For details of chemical degradation methods and identification methods, refer to our previous papers [7,9–11].

Cluster analysis

Cluster analysis of the distribution of 21 kinds of LCP (other than archaeol) in 49 strains of 46 species of Euryarchaeota was performed by the hierarchical grouping method of Ward [26] and the computer software at URL: http://aoki2.si.gunma-u.ac.jp/BlackBox/BlackBox.html, and http://www.stat.psu.edu/~jglenn/stat505/18_cluster/09_cluster_wards.html was used for the calculations. Squared distances between the organisms or clusters were used to construct a dendrogram. Two other methods for cluster analysis, the nearest neighbor method and the group average method (http://case.f7.ems.okayama-u.ac.jp/statedu/hbw2-book/node115.html), were also used to confirm the results obtained by the Ward method.

Selection of taxon-specific hallmark LCP combinations

The unique hallmark LCP combination of an order-, family-, or genus-level taxon (an LCP combination found exclusively in a given taxon and not detected in other taxa) was selected by use of a computer program developed by the present authors (posted at URL: http://nqube.health.uoeh-u.ac.jp/uniqueLCP/). When one LCP or combination of two, three, or four LCP was surveyed, one to several thousand combinations were produced. From this enormous output, the combinations comprised of the least number of LCP were chosen. If there were two or more LCP combinations produced, one of their options was adopted as a hallmark LCP combination. For example, +Ino+Ser-Gro is shown as a unique LCP combination of the family *Methanobacteriaceae* (Table 3); however, three more combinations of +Ser-Gro-GN, −Gro-Gal-GN, and -β-OH+Ser-GN were found. The latter three are not shown in Table 3. In this text, an LCP combination is expressed for the sake of brevity, such as, for example, +Ino+Ser-Gro, which means the presence of

Table 1. Lipid component parts composition in 49 strains of Euryarchaeota

Euryarchaeal organism	Lipid component parts																				
	CA	α-OH	β-OH	C25	Cyc	Usat	Ring	H	Ino	EtN	Ser	Gro	APT	Cho	GP	Glc	Gal	Man	GN	Gul	Sul
Methanobacterium formicicum DSM1535	1	−1	−1	−1	−1	−1	−1	−1	1	1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanothermobacter thermautotrophicus DSM1053	1	−1	−1	−1	−1	−1	−1	−1	1	1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanothermobacter marburgensis Marburg	1	−1	−1	−1	−1	−1	−1	−1	1	1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanothermobacter wolfeii DSM2970	1	−1	−1	−1	−1	−1	−1	−1	1	1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanobrevibacter arboriphilus A2	1	−1	−1	−1	−1	−1	−1	−1	1	−1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanobrevibacter smithii DSM861	1	−1	−1	−1	−1	−1	−1	−1	1	−1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanosphaera stadtmanae DSM3091	1	1	−1	−1	−1	−1	−1	−1	1	−1	1	−1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanothermus fervidus DSM2088	1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanococcus vannielii DSM1224	−1	1	1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanococcus voltae DSM1537	−1	1	1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanococcus maripaludis DSM2067	−1	1	1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanococcus aeolicus	−1	1	1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanothermococcus thermolithotrophicus DSM2095	1	1	1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanocaldococcus jannaschii DSM2661	1	−1	−1	−1	1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanotorris igneus Kol5	−1	1	1	−1	1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	1	−1	−1	1	−1	−1
Methanomicrobium mobile DSM1539	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanolacinia paynteri DSM2545	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanogenium cariaci DSM1497	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanoplanus limicola OCM101	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanoculleus bourgensis DSM3045	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanocorpusculum parvum DSM3823	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanospirillum hungatei DSM1101	1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	1	−1	−1	−1	−1
Methanosarcina barkeri DSM800	−1	−1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanosarcina mazeii DSM2053	−1	−1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	1	1	−1	−1	−1
Methanosarcina mazeii TMA	−1	−1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	−1	1	−1	−1	−1
Methanosarcina thermophila DSM1825	−1	−1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	1	1	1	−1	−1
"Methanosarcina alcaliphila" NY-728	−1	−1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	−1	−1	−1	−1	−1	−1
Methanolobus tindarius DSM2278	−1	−1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanococcoides methylutens DSM2657	−1	1	1	−1	−1	−1	−1	−1	1	1	−1	1	−1	−1	−1	1	−1	−1	−1	−1	−1

Methanococcoides Strain NaT1	−1	−1	1	−1	−1	−1	−1	−1	1	1	−1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanococcoides burtonii DSM6242	−1	−1	1	−1	−1	1	−1	−1	1	1	−1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanohalophilus mahii DSM5219	−1	−1	1	−1	−1	−1	−1	−1	1	1	−1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanosalsum zhilinae DSM4017	1	1	1	−1	−1	−1	−1	−1	1	1	1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanohalobium evestigatum OCM161	1	−1	−1	−1	1	−1	−1	−1	1	1	−1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
"Methanosalinarium flagellum" NY-218	−1	−1	1	−1	−1	−1	−1	−1	1	1	−1	1	−1	−1	−1	1	−1	−1	−1	−1	−1
Methanosaeta concilii GP6	−1	1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	−1	1	1	1	−1	−1	−1
Methanosaeta thermophila Pt	1	−1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	−1	−1	−1	−1	1	−1	−1	11	−1
Methanopyrus kandleri DSM6324α	1	1	−1	−1	−1	1	1	−1	1	1	1	1	−1	1	−1	1	1	1	1	−1	−1
Methanopyrus kandleri DSM6324β	1	−1	1	−1	−1	1	1	−1	1	1	1	1	−1	1	−1	1	1	1	1	−1	−1
Halobacterium salinarum NRC34002	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	1	1	1	1	−1	−1	1
Haloarcula vallismortis ATCC29715	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	1	1	−1	1	−1	−1	1
Haloferax volcanii NCMB2012	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	1	1	−1	1	−1	−1	1
Halococcus saccharolyticus P-423	−1	−1	−1	1	−1	1	−1	−1	−1	−1	−1	1	−1	−1	1	1	1	1	−1	−1	1
Natronomonas pharaonis	−1	−1	−1	1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	1	−1	−1	−1	−1	−1	−1
Natronococcus occultus	−1	−1	−1	1	−1	−1	−1	−1	−1	−1	−1	1	−1	−1	1	−1	−1	−1	−1	−1	−1
Thermoplasma acidophilum HO-62α	1	1	−1	−1	−1	−1	1	−1	1	−1	−1	1	−1	−1	−1	1	−1	1	−1	1	−1
Thermoplasma acidophilum HO-62β	1	−1	1	−1	−1	−1	1	−1	1	−1	−1	1	−1	−1	−1	1	−1	1	−1	1	−1
Thermococcus celer JCM8558	1	−1	−1	−1	−1	−1	−1	1	1	−1	−1	1	−1	−1	−1	1	0	0	0	−1	−1
Archaeoglobus fulgidus DSM4304	1	0	0	−1	0	−1	−1	−1	1	1	−1	−1	−1	−1	−1	1	1	1	−1	−1	−1

For the abbreviated name of LCP, see "Material and Methods". "1" and "−1" represent the presence and absence of the LCP, respectively. Missing data are coded as "0".

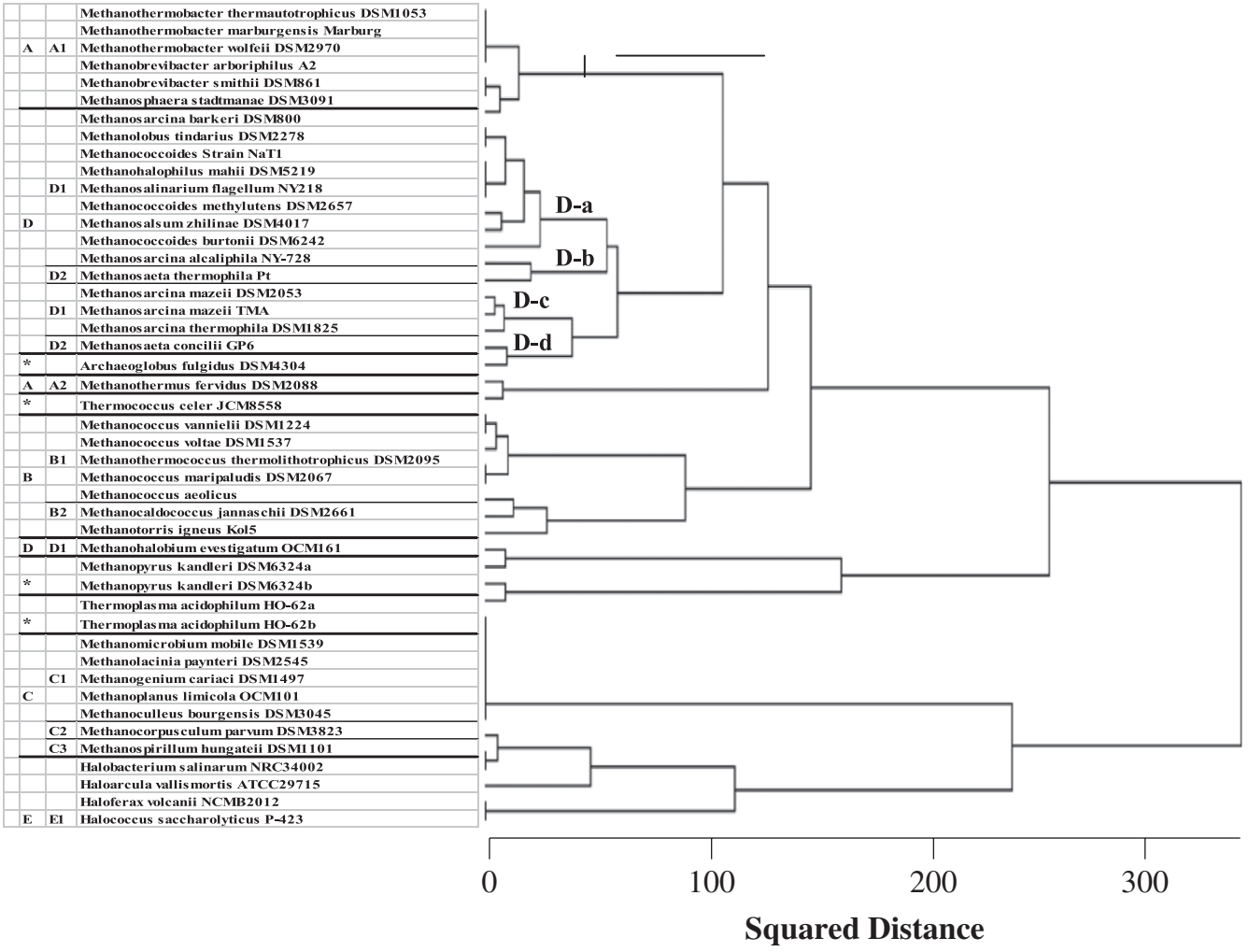

Fig. 2. A dendrogram constructed by the Ward method for the cluster analysis of LCP from Euryarchaeota. A, *Methanobacteriales*; A1, *Methanobacteriaceae*; A2, *Methanothermaceae*; B, *Methanococcales*; B1, *Methanococcaceae*; B2, *Methanocaldococcaceae*; C, *Methanomicrobiales*; C1, *Methanomicrobiaceae*; C2, *Methanocorpusculaceae*; C3, *Methanospirillaceae*; D, *Methanosarcinales*; D1, *Methanosarcinaceae*; D2, *Methanosaetaceae*; E, *Halobacteriales*; E1, *Halobacteriaceae*; *, other Euryarchaeota. D1-a to D1-d, subclusters of *Methanosarcinaceae*.

inositol (Ino) and serine (Ser), and the absence of glycerol (Gro).

Results

Clustering of euryarchaeota

The species of each order of methanoarchaea (*Methanobacteriales* (A), *Methanococcales* (B), *Methanomicrobiales* (C), and *Methanosarcinales* (D)) and the extreme halophile *Halobacteriales* (E) almost clustered in the dendrogram of the cluster analysis of LCP composition (Fig. 2). The tree topology of the LCP dendrogram at the order level was compared with the topology of the rRNA phylogenetic tree [18] (Fig. 3). In the LCP dendrogram, the branching order of *Methanobacteriales*, *Methanosarcinales*, *Methanococcales*, and *Methanopyrales* was fundamentally the same as that in the RNA tree provided that *Methanomicrobiales* and *Halobacteriales* were driven out of the main cluster of the RNA tree. However, the relative position of *Methanomicrobiales* and *Halobacteriales* in the LCP tree revealed a significant difference from that in the rRNA tree. In the LCP tree, these two orders stood outside the main clusters of the above four methanogenic orders, while they were the closest relatives of the order *Methanosarcinales* in the RNA tree.

Since only one specis from each of the orders *Methanopyrales*, *Thermococcales*, *Thermoplasmatales*, and *Archaeoglobales* was analyzed, it is not certain whether each order made a cluster.

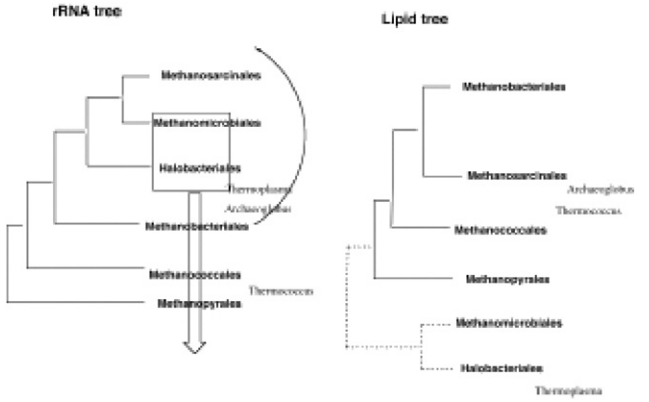

Fig. 3. Schematic representation of an rRNA phylogenetic tree and an LCP dendrogram for methanoarchaeal orders. The topology of the rRNA tree for methanogens and extreme halophiles is almost similar to that of the LCP dendrogram except for *Methanomicrobiales* and *Halobacteriales*, supposing they are taken out of the main cluster for the methanogens.

Methanobacteriales and Methanococcales

The three genera *Methanobacterium*/*Methanothermobacter* (regarded as one genus because of the same LCP composition pattern; see below), *Methanobrevibacter*, and *Methanosphaera* in the family *Methanobacteriaceae* of the order *Methanobacteriales* were divided into respective subclusters by the LCP cluster analysis, while the family *Methanothermaceae* was placed outside the main clusters of *Methanobacteriales* and *Methanosarcinales*. Among the three genera of the family *Methanobacteriaceae*, *Methanobacterium* and *Methanothermobacter* had the same LCP composition, whereas the other one, *Methanobrevibacter* revealed the minimum difference. Thus, clustering at the genus- or family-level was observed in this order.

In the *Methanococcales* cluster, methanococci were classified, after close examination with the dendrogram, into four groups, while the whole order was united in a cluster. The four species of the mesophilic genus *Methanococcus* were divided into two groups. The moderately thermophilic *Methanothermococcus* was more related to one of the mesophilic methanococci (*M. vannielii* and *M. voltaea*) groups. The hyperthermophilic *Methanocaldococcus* and *Methanotorris* were clearly divided into subclusters. In these orders of methanoarchaea (*Methanobacteriales* and *Methanococcales*), each specis could not be distinguished in a single genus, whereas, within the order, the LCP distribution did discriminate each genus (except for *Methanobacterium* and *Methanothermobacter*) or family. As previously discussed [10], this means that LCP are specific to the genus or family of methanoarchaea in most orders (see below) and LCP composition clearly relates to rRNA phylogeny in these orders.

Methanosarcinales

Seven out of eight individual genera of the order *Methanosarcinales* were also grouped into four subclusters in the LCP dendrogram. These subclusters were the "*Methanosarcina alcaliphila*" and *Methanosaeta thermophila* Pt group (D–b), the *M. mazeii* and *Methanosarcina thermophila* group (D–c), the *Methanosaeta concilii* along with *Archaeoglobus fulgidus* group (D–d), and the largest and main *Methanosarcinales* group, including *Methanosarcina barkeri*, with species of the genera *Methanolobus*, *Methanococcoides*, *Methanohalophilus*, *Methanosalsum*, and *Methanosalinarium* (D–a). In terms of the entire order, the LCP composition was less uniform and the dendrogram was polyphyletic. This exception made it difficult to select specific LCP for the order *Methanosarcinales* (see below). *Methanohalobium evestigatum*, one of the members of the family *Methanosarcinaceae*, was located outside the main *Methanosarcinales* cluster, and was instead related to the *Methanocaldococcaceae* cluster. *M. evestigatum* and *Methanocaldococcus jannaschii* resembled each other with the pattern +CA+Cyc-α−OH. These lipid composition patterns were unlike those of many of the *Methanosarcinaceae* members. Four species of the genus *Methanosarcina* were divided into three groups, *M. barkeri*, *M. mazeii*+*M. thermophila*, and "*M. alcaliphila*" in the *Methanosarcinaceae* cluster. The scattered distribution of *Methanosarcinales* species in the lipid dendrogram was mainly due to the diversity in glycolipid sugars and core lipids (caldarchaeol and hydroxyarchaeol). In other words, some species of this genus possessed glucose, galactose, and mannose as their glycolipid sugars, while other species only had glucose. In general, these sugars were found at quite a low level in these *Methanosarcina* species compared to the level of glycolipid sugar in other methanogens.

Two species of the genus *Methanosaeta*, *M. concilii* and *M. thermophila*, had a different lipid composition, for example, in terms of CA, α−OH, Glc, and Man. While the type species of the genus (*M. concilii*) was grouped into the *Methanosarcinales* cluster (most closely related with *A. fulgidus* (D–d)), *Methanosaeta thermophila* was clustered with *"M. alcaliphila"* (D–b). Thus, two species of *Methanosaeta* were dispersed into different subclusters. In spite of the diversity of LCP composition and polyphyletic genealogy of the LCP dendrogram, most species of the order *Methanosarcinales* were, in general, concentrated in the main cluster.

Methanopyrales

M. kandleri, the only species of the order *Methanopyrales*, was located furthest outside the methanoarchaea rRNA tree [18]. However, in the LCP dendrogram,

the order, along with *Thermoplasmatales*, was distantly related to a large cluster composed of three orders *Methanobacteriales*, *Methanosarcinales*, and *Methanococcales* (A + D + B, Fig. 2), but *Methanopyrus* was less related to the orders *Methanomicrobiales* and *Halobacteriales*. This indicated the unique lipid composition of *M. kandleri*, although it was not as different as the lipid composition of the organisms in the order *Methanomicrobiales*.

Methanomicrobiales and Halobacteriales

Three families and seven genera of the order *Methanomicrobiales* were clearly united into a cluster by LCP clustering and were the most sharply distinguished from the other orders of methanoarchaea (Fig. 2) because all the organisms of *Methanomicrobiales* displayed exactly the same, unique LCP pattern. Although each family or genus of the order could not be discriminated by means of this analysis, they could be distinguished as a whole from other archaeal orders or families.

Four neutrophilic and two alkaliphilic species of the order *Halobacteriales* made a clearly distinct cluster in the dendrogram. Among the six species, *Haloarcula vallismortis* and *Haloferax volcanii*, and *Natronomonas pharaonis* and *Natonococcus occultus* could not be discriminated by the LCP composition, respectively; however, four genera were distinguished by the LCP dendrogram and they were clustered in a monophyletic fashion. The cluster of the order *Halobacteriales* was the most distantly related to the majority of the methanoarchaeal orders but it was the most closely related to the order *Methanomicrobiales*, in accordance with the fact that the order *Methanomicrobiales* was the closest relative of *Halobacteriales* in the RNA tree. Nine LCP differed between *Methanobacterium formicicum* and *Halobacterium salinarum*, and this was one of the largest differences in LCP composition.

Other euryarchaeal species

In the dendrogram, the archaeal organisms of the genera *Thermococcus*, *Archaeoglobus*, and *Thermoplasma*

Table 2. Hallmark LCP combination of each order of Euryarchaeota

Order	CA	Ring	H	Ino	Ser	Gro	APT	Cho	GP	Gal	Man	Gul
Methanobacteriales				+Ino		−Gro				−Gal		
Methanococcales				−Ino	+Ser							
Methanomicrobiales							+APT					
Methanosarcinales[a]												
Methanopyrales								+Cho				
Halobacteriales									+GP			
Thermoplasmatales												+Gul
Thermococcales			+H			+Gro						
Archaeoglobales	+CA	−Ring									+Man	

[a]No unique LCP combination was detected for this order.

Table 3. Hallmark LCP combination of each family of Euryarchaeota

Family	CA	Cyc	Ring	H	Ino	EtN	Ser	Gro	APT	Cho	GP	Gal	Man	GN	Gul
Methanobacteriaceae					+Ino		+Ser	−Gro							
Methanothermaceae				+H										+GN	
Methanococcaceae		−Cyc			−Ino		+Ser								
Methanocaldococcaceae		+Cyc					+Ser								
Methanomicrobiaceae/															
Methanocorpusculaceae/									+APT						
Methanospirillaceae															
Methanosarcinaceae						+EtN		+Gro		−Cho					
Methanosaetaceae		−Cyc						−Gro				+Gal			
Methanopyraceae										+Cho					
Halobacteriaceae											+GP				
Thermoplasmataceae															+Gul
Thermococcaceae				+H				+Gro							
Archaeoglobaceae	+CA		−Ring										+Man		

Table 4. Hallmark LCP combination of each genus of Euryarchaeota

Genus	CA	α-OH	β-OH	C25	Cyc	Ring	H	Ino	EtN	Ser	Gro	APT	Cho	GP	Gal	Man	GN	Gul	Sul
Methanobacterium/ Methanoothermobacter								+Ino	+EtN	+Ser	−Gro								
Methanobrevibacter		−α–OH							−EtN	+Ser									
Methanosphaera		+α–OI	−β–OH													−Man			
Methanothermus							+H										+GN		
Methanococcus	−CA	+α–OH			−Cyc												+GN		
Methanothermococcus	+CA	+α–OH						−Ino											
Methanocaldococcus	+CA				+Cyc					+Ser									
Methanotorris		+α–OH			+Cyc														
Methanosalsum	+CA	+α–OI	+β–OH						+EtN										
Methanohalobium					+Cyc			+Ino											
Methanosaeta					−Cyc						−Gro				+Gal				
Methanopyrus													+Cho						
Halobacterium				−C25										+GP	+Gal				
Haloarcula/Haloferax														+GP	−Gal				+Sul
Halococcus				+C25												+Man			
Natronomonas/ Natronococcus				+C25											−Gal				
Thermoplasma																		+Gul	
Thermococcus							+H				+Gro								
Archaeoglobus	+CA					−Ring										+Man			

For seven genera of Methanomicrobiales, see Table 3 since they have the same LCPs.

clustered most closely to *Methanothermus fervidus*, *M. concilii*, and *M. kandleri*, respectively. The relationship of these orders based on LCP did not coincide with the relationship based on the rRNA sequences, but since only one specis of each order was analyzed, this finding should be confirmed by further analysis with more species. On the other hand, according to Ludwig and Klenk [13], a stable and significant tree topology resolving the relationship between four orders (such as *Methanobacteriales*, etc.) and *Archaeoglobus*, *Thermococcales*, and *Methanopyrales* cannot be deduced from the current database. Therefore, an LCP dendrogram of these archaea may help to make the relationship clearer.

Hallmark LCP unique to a specfic taxon of Euryarchaeota

Unique LCP, specific to a methanogenic taxon, which were intuitively selected by direct observation, have been tentatively described in a previous paper [10]. In order to detect more robustly unique LCP combinations specific to a taxon, unique combinations of one to four LCP were thoroughly searched for with the aid of a computer. The results of the analysis are shown in Tables 2–4.

There were several groups of taxa that possessed the same LCP composition, such as members of the two genera, *Methanobacterium* and *Methanothermobacter*, methanogens of seven genera of three families belonging to the order *Methanomicrobiales*, two pairs of *Methanococcus* species (*M. vannielii* and *M. voltae*, and *M. maripaludis* and *M. aeolicus*), and two pairs of extreme halophiles (*H. vallistmortis* and *H. volcanii*, and *N. pharaonis* and *N. occultus*). Initially, in these cases, a unique LCP combination could not be chosen for the taxa. Therefore, computation was undertaken treating these taxa as one taxon (an imaginary combined taxon).

Hallmark LCP combinations specific to each order

Unique LCP combinations for the eight orders analyzed were selected (Table 2). For example, the combination of +Ino-Gro-Gal specifically characterized the order *Methanobacteriales*. No unique LCP was detected for the order *Methanosarcinales*. This was due to the internal diversity of LCP composition among the species of this order. Only one LCP was selected as a unique characteristic LCP for the orders *Methanomicrobiales* (+APT), *Methanopyrales* (+Cho), *Halobacteriales* (+GP), and *Thermoplasmatales* (+Gul). These patterns were quite unique among all the LCP.

Hallmark LCP combinations for each family

All of the 14 families, including *Methanosarcinaceae*, analyzed thus far (including the three combined families of *Methanomicrobiales*) gave specific LCP combinations (Table 3). In archaea, of which only one family was present in an order, the specific LCP combination for the family was the same as the unique LCP combination of the order (*Methanomicrobiales*, *Methanopyrales*, *Halobacteriales*, *Thermoplasmatales*, *Thermococcales*, and *Archaeoglobales*).

Methanobacteriaceae was characterized by +Ino+Ser-Gro. This LCP combination was almost the same as that described in a previous paper [10], except for caldarchaeol, which was detected by observation. *Methanothermaceae* contains only one genus and the LCP composition was fairly unique because of the presence of the H-shaped caldarchaeol. Therefore, this was characterized by only two LCP (+H+GN).

Methanococcales contains two families, mesophilic and moderately thermophilic *Methanococcaceae*, and hyperthermophilic *Methanocaldococcaceae*. Although they shared −Ino+Ser, the presence or absence of cyclic archaeol clearly distinguished the two families. The +EtN+Gro-Cho pattern characterized the family *Methanosarcinaceae*, unlike the order *Methanosarcinales*, which had no unique LCP combination. *Methanosarcinales* consists of two families, *Methanosarcinaceae* and *Methanosaetaceae*, but since the specific LCP combinations of the two families were quite different (+EtN+Gro-Cho for *Methanosarcinaceae*, and +Gal-Cyc-Gro for *Methanosaetaceae*), the order would not yield a unique LCP combination.

Unique LCP combinations for each genus

Unique LCP combinations for most genera (22 out of 34) were identified (Table 4), while no unique LCP combination was detected for several genera in *Methanosarcinaceae*. The reason why the two genera *Methanosalsum* and *Methanohalobium* had unique LCP combinations was due to the presence of CA and Cyc, unlike other members of the family *Methanosarcinaceae*.

The *Methanobacterium*/*Methanothermobacter* group (which had the same LCP) and *Methanobrevibacter* were discriminated by the presence and absence of EtN, respectively. *Methanobrevibacter* and *Methanosphaera* were different in terms of the presence or absence of α-OH. *M. fervidus* is the organism from which H-shaped caldarchaeol was identified. Therefore, H was the LCP that specified *Methanothermus* along with GN. Since H was also present in *Thermococcus*, GN (*Methanothermus*) and Gro (*Thermococcus*) discriminated the two genera.

The LCP distribution between the two species in the genus *Methanosaeta* was much more diverse than that among the species in the other genera. Four LCP were different (CA, OH, Glc, and Man). These two species were scattered in different clusters by the cluster analysis (see above). It was discussed earlier that the diversity was sufficient for the two species to be placed in separate genera [10] and the present investigation supported this previous suggestion.

Although unique LCP specific to a limited number of species were detected by a computer search, they were not cited in this paper, because several species in one genus generally showed the same LCP distribution.

Discussion

An LCP dendrogram of Euryarchaeota calculated by the Ward method, and unique LCP combinations specific to each taxon were obtained by the aid of computer programs. The results were more sophisticated than those obtained by the direct observations described in a previous paper [10]. In the LCP dendrogram, most orders, families and genera of Euryarchaeota were organized into separate clusters. The tree topology of the LCP dendrogram resembled the topology of the rRNA phylogenetic tree [18] for most methanogenic orders, but major differences were also observed in the branching order of *Methanomicrobiales* and in the internal tree structure of *Methanosarcinales*. The results obtained by the nearest neighbor method and the group average method almost confirmed the results of the Ward analysis, except that *Methanopyrales* stood outside the main clusters the most (data not shown). Although Zuckerkandl and Pauling [27] have discussed the significance of semantides (rRNA in this case) and episemantic molecules (LCP in this case) in molecular phylogeny, the relationship between the evolution of lipid and rRNA has not been specifically discussed, to the best of the authors' knowledge.

rRNA is a more sensitive measure of evolution than lipid. Examples of such can be seen in the comparison of the LCP dendrogram and the rRNA tree. Four species of *Methanobacterium* and *Methanothermobacter*, as well as seven genera of *Methanomicrobiales*, etc. could not be distinguished by LCP composition (the same LCP), whereas every species can be discriminated by rRNA. Although semantides are considered to be the most significant molecules for providing the basis for a molecular phylogeny, a phylogenetic lipid tree, in the same way as a phenotype tree, may constitute a means of determining how the rRNA tree reflects phenotypic genealogy. It is important to estimate how the molecular evolution of rRNA reflects phenotypic evolution, if the rRNA tree is regarded as a representative of phylogenetic trees. This is the main aim in discussing the relationship between trees based on rRNA and lipids. Therefore, we offer a possible explanation for the similarities and differences in the molecular evolution of membrane lipid and rRNA.

Uniformity and diversity of membrane polar lipids and their evolution

The structures of membrane phospholipids have certain common features, which include having one polar head and two hydrophobic tails connected by a three-carbon backbone [8]. This is the minimum requirement for a membrane phospholipid structure. On the other hand, actual phospholipids are diverse in structure within the limited range allowed by their structural uniformity. Various kinds of linkages (ether, ester, and alk-1-enyl ether linkages) are possible between either the stereoisomer of the glycerophosphate backbone (*sn*-glycerol-1-phosphate (G-1-P) or *sn*-glycerol-3-phosphate (G-3-P)) and hydrocarbon chains (fatty acids or isoprenoids) and polar groups (see Fig. 1C). This creates the uniformity and diversity of membrane polar lipids. The diversity of polar lipids is regarded as a result of the evolution of membrane lipids. Lipid evolution is caused by mutations of lipid-synthesizing genes.

Coincidental mutations on the rRNA gene and lipid genes

Both rRNA and lipid-synthesizing enzymes are coded on a genome. rRNA is directly controlled, whereas lipids are indirectly controlled by their genes. Since mutations on an rRNA gene and a lipid-synthesizing gene can be supposed to take place independently and randomly, the mutations of membrane lipids have paralleled (taken on average over a long period of time) that of 16S rRNA, which has led basically to a parallel topology for rRNA and lipid phylogenetic trees.

Mutation of the primary structure of rRNA and changes of phenotypes

16S rRNA is a single molecule and plays the single role of protein synthesis. The construction of the rRNA phylogenetic tree is based on the similarity of the primary structure. The mutations accumulated in a surviving organism are on the sites at which mutations do not affect the ribosome function (non-important sites). A mutation at an active site essential to the function would cause malfunctioning in the ribosome, and such a mutation must be excluded. The rRNA tree is, therefore, a record of the accumulation of mutations at the non-important sites in the primary structure. On

the other hand, the membrane polar lipids of an organism are composed of several kinds of LCP. Therefore, as Zuckerkandl and Pauling [27] described, lipids, as episemantic molecules, are polygenic characters and they only express the information contained in the active centers of enzymes. In contrast to rRNA, mutations at the non-important sites of a lipid gene do not result in any detectable changes in the dendrogram. It is possible that mutations at important sites (e.g., substrate specificity-determining sites) of lipid-enzymes could be allowed if the membrane accepts an altered lipid structure, even though some constraint may be present, because membrane polar lipids are actually diverse in structure, as described in above. This means that mutations at important sites of lipid-enzymes are not necessarily excluded but survive like mutations at non-important sites. This is the main circumstance of the coincidence of both phylogenetic trees, supposing that the mutations were random. Examples of evolutionary changes in the substrate specificity of lipid-enzymes include: G-1-P dehydrogenase derived from glycerol dehydrogenase through a change in substrate specificity [1]; the CDP-alcohol cytidyltransferase family that includes serine-, glycerophosphate-, and *myo*-inositol-phospholipid-synthesizing enzymes, which have been derived from a common ancestral enzyme by multiple mutations of substrate specificity [2]; and, first and second ether bond-forming enzymes of archaeal ether lipids (geranylgeranyl glycerophosphate synthase and digeranylgeranyl glycerophosphate synthase) derived from some kind of prenyl transferases [4,19]. However, this situation is also one of the causes of the difference between the evolution of rRNA and lipid. An altered lipid structure suffers from a different selective pressure (see below).

Different constraint for evolution of rRNA and membrane lipids

rRNA and membrane lipids function in the form of a complex with r-proteins and membrane proteins, respectively. If the lipid structure or RNA is changed, the lipid or RNA should suffer from different constraints due to various factors, such as complexed r-proteins or membrane proteins, a secondary structure by specific base-pairings, or environmental effects. The definite interaction of rRNA with r-proteins is seen in the assembly map of a ribosome subunit [17]. The different constraints would cause separate effects on rRNA and membrane lipids independently.

Not completely independent features

Each LCP is not completely independent. For example, if two LCP are in a precursor–product relationship, the presence of the product LCP is dependent on the presence of the precursor LCP. If the turnover of the precursor LCP is much faster than its synthesis, the precursor LCP may not accumulate at a detectable level in spite of the presence of product LCP. This is seen in the case of (archaetidyl)serine and (archaetidyl)ethanolamine. The absence of a precursor LCP can be caused by one of two factors: (1) the absence of the LCP-forming enzyme, or (2) rapid turnover of the precursor LCP. This is actually observed in the case of *Methanococcoides methylutens*, in which EtN was detected in spite of the apparent absence of Ser. If a precursor LCP is lost, the product LCP would be automatically lost. One change leads to two changes. On the other hand, interactions between sites of an RNA molecule might affect its evolution, since interactions between two LCP, as well as interactions between RNA sites, may produce different evolutionary results.

The effect of APT on the topology of the dendrogram

One trial to investigate the effect of APT (the most unique LCP for *Methanomicrobiales*) was to construct an LCP dendrogram calculated from data excluding APT. In the resultant dendrogram, the cluster of the order *Methanomicrobiales* was placed between *Methanococcales* and *Methanopyrales* (data not shown), and represented a striking difference from the original dendrogram. This is due to the fact that the presence of APT is limited to organisms from this order. Once such an LCP occurred during evolution, the topology of the LCP dendrogram would be greatly deformed as compared to the RNA tree. Since this phenomenon was observed more or less by using the other two cluster analysis methods, it cannot be due to an artificial phenomenon caused by any one particular method.

Conclusion

Generally, although the evolution of rRNA and membrane lipids satisfactorily, though not wholly, coincides, there are other factors (mentioned as above) showing that rRNA and membrane lipids evolved in different ways. The similarities and differences between the two trees are thus largely explained.

There were cases in which two or more similar, but not exactly the same, LCP were used as independent and separate LCP, or cases in which slightly different LCP were reduced to one variable for analyses. An example of the former is hydroxyarchaeol, which is comprised of two isomers, α-hydroxyarchaeol and β-hydroxyarchaeol (see Fig. 1B). Ward analysis in which α-hydroxyarchaeol and β-hydroxyarchaeol are used as two separate

variables for the calculation, or simply as hydroxyarchaeol, resulted in a small but significant difference in the dendrogram. Therefore, we used the two hydroxyarchaeol isomers as independent LCP. On the other hand, the position of a C25 isoprenoid chain bound to the glycerol moiety, the number of cyclopentane rings, and the number of methyl groups on APT were neglected for simplicity of chemical analysis, which is an important factor with a chemotaxonomic purpose.

In *Halobacteriaceae*, intact glycolipid and phospholipid structures have been used for a chemotaxonomic purpose. For example, the members of *Haloarcula* and *Haloferax* could be distinguished by the occurrence of PGS (archaetidylglycerosulfate), TGA-2 (*β*-glucosyl-mannosyl-glucosyl-archaeol) and S-DGA-1 (sulfomannosyl-glucosyl-archaeol) [6], while the LCP compositions of the members of the two genera were the same and could not discriminate the two genera. This clearly shows that the intact lipid structure has a higher resolving power than LCP analysis, although LCP analysis has an advantage over the analysis using an intact lipid in that it is a less time-consuming and less laborious analysis.

The computer-assisted selection of unique LCP combinations was much simpler and exhaustive, and yet it was consistent with the previous conclusion. Therefore, the findings could be used as a chemotaxonomic marker for these archaea, especially among the methanoarchaea. However, when only one species of a genus is analyzed, the reliability of the LCP information might be insufficient for the analysis. The accumulation of additional information in future studies should help construct even more reliable LCP data.

Acknowledgements

We are grateful to Professor H. Toh (Division of Bioinformatics, Medical Institute of Bioregulation, Kyushu University) and Dr. H. Morii (Department of Chemistry, University of Occupational and Environmental Health) for their helpful discussions. We are also indebted to Mr. Carman (Department of Foreign Languages, University of Occupational and Environmental Health) for his kindness in correcting the manuscript.

References

[1] H. Daiyasu, T. Hiroike, Y. Koga, H. Toh, The analysis of membrane stereochemistry with the homology modeling of *sn*-glycerol-1-phosphate dehydrogenase, Protein Eng. 15 (2002) 987–995.

[2] H. Daiyasu, K. Kuma, T. Yokoi, H. Morii, Y. Koga, H. Toh, A study on archaeal enzymes involved in polar lipids synthesis by an approach linking the information about amino acid sequences, genomic contexts and lipid composition, Archaea 1 (2005) 399–410.

[3] M. De Rosa, A. Gambacorta, B. Nicolaus, H.N.M. Ross, W.D. Grant, J.D. Bu'Lock, An asymmetric archaebacterial diether lipid from alkaliphilic bacterial halophiles, J. Gen. Microbiol. 128 (1982) 343–348.

[4] H. Hemmi, K. Shibuya, Y. Takahashi, T. Nakayama, T. Nishino, (*S*)-2,3-di-*O*-geranylgeranylglyceryl phosphate synthase from the thermoacidophilic archaeon *Sulfolobus solfataricus* – molecular cloning and characterization of a membrane-intrinsic prenyltransferase involved in the biosynthesis of archaeal ether-linked membrane lipids, J. Biol. Chem. 279 (2004) 50197–50203.

[5] M. Kates, Glyco-, phosphoglyco- and sulfoglycoglycerolipids of bacteria, in: M. Kates (Ed.), Glycolipids, Phosphoglycolipids, and Sulfoglycolipids, vol. 6, Plenum Press, New York, 1990, pp. 1–122.

[6] M. Kates, Membrane lipids of archaea, in: M. Kates, D.J. Kushner, A.T. Matheson (Eds.), The Biochemistry of Archaea (Archaebacteria), Elsevier Science Publishers B.V., Amsterdam, 1993, pp. 261–295.

[7] Y. Koga, M. Akagawa-Matsushita, M. Ohga, M. Nishihara, Taxonomic significance of the distribution of the component parts of polar ether lipids in methanogens, Syst. Appl. Microbiol. 16 (1993) 342–351.

[8] Y. Koga, H. Morii, Biosynthesis of ether-type polar lipids in archaea and evolutionary considerations, Micobiol. Mol. Biol. Rev. 71 (2007) 97–120.

[9] Y. Koga, H. Morii, Recent advances in structural research on ether lipids from archaea including comparative and physiological aspects, Biosci. Biotechnol. Biochem. 69 (2005) 2019–2034.

[10] Y. Koga, H. Morii, M. Akagawa-Matsushita, M. Ohga, Correlation of polar lipid composition with 16 s rRNA phylogeny in methanogens. Further analysis of lipid component parts,, Biosci. Biotechnol. Biochem. 62 (1998) 230–236.

[11] Y. Koga, M. Nishihara, H. Morii, M. Akagawa-Matsushita, Ether polar lipids of methanogenic bacteria: structures, comparative aspects, and biosynthesis, Microbiol. Rev. 57 (1993) 164–182.

[12] Y. Koga, M. Ohga, M. Nishihara, H. Morii, Distribution of diphytanyl ether analog of phosphatidylserine and an ethanolamine-containing tetraether lipid in methanogenic bacteria, Syst. Appl. Microbiol. 9 (1987) 176–182.

[13] W. Ludwig, H.-P. Klenk, Overview: a phylogenetic backbone and taxonomic framework for prokaryotic systematics, in: D.R. Boone, R.W. Castenholz (Eds.), Bergey's manual of systematic bacteriology – the archaea and the deeply branching and phototrophic bacteria, vol. 1, Springer, New York, 2001, pp. 49–65.

[14] N. Moldoveanu, M. Kates, C.G. Montero, A. Ventosa, Polar lipids of non-alkaliphilic halococci, Biochim. Biophys. Acta 1046 (1990) 127–135.

[15] S. Morth, B.J. Tindall, Variation of polar lipid composition within haloalkaliphilic archaebacteria, Syst. Appl. Microbiol. 6 (1985) 247–250.

[16] M. Nishihara, H. Morii, Y. Koga, Structure determination of a quartet of novel tetraether lipids from

Methanobacterium thermoautotrophicum, J. Biochem. 101 (1987) 1007–1015.
[17] M. Nomura, Assembly of bacterial ribosomes, Science 179 (1973) 864–873.
[18] G.J. Olsen, C.R. Woese, R. Overbeek, The winds of (evolutionary) change – breathing new life into microbiology, J. Bacteriol. 176 (1994) 1.
[19] J. Payandeh, M. Fujihashi, W. Gillon E.F. Pai, The crystal structure of (*S*)-3-*O*-geranylgeranylglyceryl phosphate synthase reveals an ancient fold for an ancient enzyme, J. Biol. Chem. 281 (2006) 6070–6078.
[20] H. Shimada, N. Nemoto, Y. Shida, T. Oshima, A. Yamagishi, Complete polar lipid composition of *Thermoplasma acidophilum* HO-62 determined by high-performance liquid chromatography with evaporative light-scattering detection, J. Bacteriol. 184 (2002) 556–563.
[21] G.D. Sprott, B.J. Agnew, G.B. Patel, Structural features of ether lipids in the archaeobacterial thermophiles *Pyrococcus furiosus*, *Methanopyrus kandleri*, *Methanothermus fervidus*, and *Sulfolobus acidocaldarius*, Can. J. Microbiol. 43 (1997) 467–476.
[22] A. Sugai, I. Uda, Y.H. Itoh, T. Itoh, The core lipid composition of 17 strains of hyperthermophilic archaea, *Thermococcales*, J. Oleo Sci. 53 (2004) 41–44.
[23] M. Swain, J.R. Brisson, G.D. Sprott, F.P. Cooper, G.B. Patel, Identification of β-L-gulose as the sugar moiety of the main polar lipid *Thermoplasma acidophilum*, Biochim. Biophys. Acta 1345 (1997) 56–64.
[24] M. Tarui, N. Tanaka, K. Tomura, M. Ohga, H. Morii, Y. Koga, Lipid component parts analysis of the hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus*, J. UOEH (2007).
[25] M. Torreblanca, F. Rodoriguez-Valera, G. Juez, A. Ventosa, M. Kamekura, M. Kates, Classification of non-alkaliphilic halobacteria based on numerical taxonomy and polar lipid composition, and description of *Haloarcula* gen. nov. and *Haloferax* gen. nov., Syst. Appl. Microbiol. 8 (1986) 89–99.
[26] J.H. Ward, Hierarchical grouping to optimize an objective function, J. Am. Stat. Assoc. 58 (1963) 236–244.
[27] E. Zuckerkandl, L. Pauling, Molecules as documents of evolutionary history, J. Theor. Biol. 8 (1965) 357–366.