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Comparative Lipid Composition of Aerobically and Anaerobically Grown *Desulfurolobus ambivalens*, an Autotrophic Thermophilic Archaeobacterium

By A. TRINCONE,[1] V. LANZOTTI,[1]† B. NICOLAUS,[1] W. ZILLIG,[2] M. DE ROSA[1,3] AND A. GAMBACORTA[1]*

[1] *Istituto per la Chimica di Molecole di Interesse Biologico del Consiglio Nazionale delle Ricerche, Via Toiano 6, 80072-Arco Felice, Napoli, Italy*

[2] *Max-Planck Institut für Biochemie, Martinsried, FRG*

[3] *Istituto di Biochimica delle Macromolecole, I Facolta' di Medicina e Chirurgia dell'Universita', Via Costantinopoli 16, 80138-Napoli, Italy*

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Lipids from the autotrophic thermophilic archaeobacterium *Desulfurolobus ambivalens* grown under aerobic and anaerobic conditions were analysed and compared with those of *Sulfolobus solfataricus*, a related micro-organism. The ether lipids of aerobically and anaerobically grown *D. ambivalens*, as well as those of *S. solfataricus*, had the same general features except for the degree of cyclization of the C_{40} isopranic chains. The quinone content of *D. ambivalens* was strongly affected by growth conditions. Aerobically grown cells contained caldariellaquinone, 6-(3,7,11,15,19,23-hexamethyltetracosyl)-5-methylthiobenzo[*b*]thiophen-4,7-quinone (83% of the quinone pool), sulfolobusquinone, 6-(3,7,11,15,19,23-hexamethyltetracosyl)-5-methylbenzo[*b*]thiophen-4,7-quinone (16%) and the tricyclic quinone benzo[1,2-b; 4,5-b']dithiophen-4,8-quinone (trace amounts). In anaerobically grown *D. ambivalens* sulfolobusquinone was the only quinone present.

INTRODUCTION

Growth conditions of extreme pH and/or temperature and the information they provide on the evolution of life make the thermophiles the most interesting representatives of the archaeobacteria. All species grow anaerobically, and most require sulphur as an energy source. However, a few species can grow aerobically and not all of those that use sulphur require it as an energy source (Woese, 1987). Such thermophilic archaeobacteria have been assigned to the orders *Sulfolobales* and *Thermoproteales* (Stetter, 1986; Woese, 1987).

The genus *Sulfolobus* contains isolates that are respiratory heterotrophs capable of growth on a variety of carbon sources, or are facultative chemolithoautotrophs capable of obtaining their energy by the oxidation of sulphur to sulphuric acid or sulphide to molecular sulphur (Stetter, 1986).

Zillig *et al.* (1986) isolated a novel thermophilic sulphur-dependent archaeobacterium which can grow either aerobically as do *Sulfolobus* species by the oxidation of sulphur to sulphuric acid or anaerobically, like species belonging to the order *Thermoproteales*, by the reduction of sulphur to hydrogen sulphide. On the basis of its unique ability to grow facultatively via sulphur reduction, its immunochemical crossreactions and its G + C content, the authors suggested the separation of the new isolate from the genus *Sulfolobus* and named it *Desulfurolobus ambivalens* (Zillig *et al.*, 1986).

† Present address: Facolta' di Agraria, Universita' del Molise, Campobasso, Italy.

Abbreviations: GDGT(s), glycerol-dibisphytanyl-glycerol tetraether(s); GDNT(s), glycerol-dibisphytanyl-nonitol tetraether(s); GLI, glycolipid I; GLII, glycolipid II; SGLII, sulphoglycolipid II; PLI, phospholipid I; PGLI, phosphoglycolipid I; PGLII, phosphoglycolipid II; Glc*p*, glucopyranosyl; Gal*p*, galactopyranosyl.

The lipids of archaeobacteria are characterized by unusual structural features that can be considered as specific taxonomic markers (Langworthy, 1985; De Rosa & Gambacorta, 1988). The complex lipids of archaeobacteria are mainly based on two classes of isopranoid ether core lipids that are characterized as diethers and tetraethers. The diethers are essentially based on glycerol, with a 2,3-*sn* configuration, linked to phytanyl, sesterterpanyl or head-to-head bisphytanyl chain(s). The second class contains macrocyclic tetraethers based on two head-to-head bisphytanyl ω–ω' diols, linked via an ether linkage to two 2,3-*sn*-glycerol moieties (glycerol-dibisphytanyl-glycerol tetraether, GDGT) or to one 2,3-*sn*-glycerol and one branched-chain nonitol moiety (glycerol-dibisphytanyl-nonitol tetraether, GDNT). The aliphatic C_{40} chains may have as additional features up to four cyclopentane rings, the extent of cyclization of which varies according to species, and to growth conditions such as temperature, heterotrophy, autotrophy, etc. (De Rosa & Gambacorta, 1988).

GDNT lipids are found only in members of the *Sulfolobales* and seem to be a specific taxonomic marker. The *Sulfolobales* display the widest lipid spectrum found in archaeobacteria at the level both of polar end structure and of isoprenic chain variety (De Rosa & Gambacorta, 1988). On the basis of their quinone distribution thermophilic archaeobacteria can be divided into two groups: those with naphthoquinone isoprenoids and those with benzothiophen-quinones (Collins & Jones, 1981; Thurl *et al.*, 1986; De Rosa & Gambacorta, 1988). Species of the order *Sulfolobales*, whose quinones are all based on a benzothiophene nucleus, are unique to the second group. Thus, data on the distribution of the quinones, although still fragmentary, and data on the complex and core lipid patterns found in archaeobacteria, indicate that a correlation between classifications based on other criteria and that based on lipid distribution is possible.

This paper reports on the detailed analysis of lipid and quinone patterns in *D. ambivalens* grown either aerobically or anaerobically. These data are compared with those on lipids from *Sulfolobus solfataricus* to provide further information on the phylogenetic position of *Desulfurolobus* within the order *Sulfolobales*.

METHODS

Micro-organisms and culture conditions. *Desulfurolobus ambivalens* was isolated from solfataric sources in Iceland and at the Vulcano Island beach in Italy and from the Pisciarelli hot springs in Italy. Cells were grown in the standard medium described by Zillig *et al.* (1986). Anaerobic batch cultures were grown at 80 °C, pH 2·5, in the presence of 1 g sulphur l^{-1} with continuous gassing with 80% CO_2/20% H_2 (v/v). Aerobic batch cultures were grown under the same culture conditions as above in an atmosphere of air supplemented with 5% (v/v) CO_2.

Extraction and isolation of lipids. Dried cells (about 6 g) were extracted (Soxhlet) first for 15 h with $CHCl_3/CH_3OH$ (1:1, v/v) and then for 15 h with CH_3OH/H_2O (1:1, v/v). The extracts were pooled and evaporated to give the total lipid residue of which 20% was directly hydrolysed to analyse the core lipid pattern. The remaining 80% of the lipid extract was treated with light petroleum (b.p. 40–70 °C) and the soluble material chromatographed on a silica gel column (Merck Kieselgel 70–230 mesh, 20 g) (20 cm × 10 mm, i.d.). The column was eluted with 1 l of a step gradient of 0–15% (v/v) diethyl ether in light petroleum in 5% increments (250 ml for each gradient step). The quinone fraction was eluted with 5% diethyl ether and further resolved by preparative HPLC.

The complex lipids that were insoluble in light petroleum were chromatographed on a silica gel (Merck Kieselgel 70–230 mesh, 60 g) column (40 cm × 10 mm, i.d.). $CHCl_3/CH_3OH$ (93:7, v/v) was used to elute the less polar compounds; $CHCl_3/CH_3OH$ (8:2, v/v) eluted GLI and GLII; $CHCl_3/CH_3OH$ (6:4, v/v) eluted PLI; $CHCl_3/CH_3OH/H_2O$ (65:25:1, by vol.) gave SGLII; $CHCl_3/CH_3OH/H_2O$ (65:25:4, by vol.) gave a mixture of PGLI and PGLII.

The mixture of GLI and GLII was further resolved by preparative TLC, washed with 0·1 M-boric acid and activated by heating at 100 °C for 5 h. After elution with $CHCl_3/CH_3OH/H_2O$ (65:25:4, by vol.), the glycolipids, located by I_2 vapour, were scraped off and eluted with $CHCl_3/CH_3OH/H_2O$ (65:25:4, by vol.). The mixture of PGLI and PGLII was subjected to alkaline hydrolysis as reported previously (De Rosa *et al.*, 1980). The resulting mixture of two glycolipids, GLI and GLII, were further resolved as described above.

GLI, GLII, PLI and SGLII, pure as shown by TLC analysis, were weighed to evaluate their relative percentages. The relative percentage of PGLI and PGLII was evaluated as glycolipid derivatives after alkaline hydrolysis.

Identification of lipids was achieved by spectroscopic (^1H and ^{13}C NMR), chromatographic and degradative methods as reported previously (De Rosa *et al.*, 1980, 1983) using, for comparison, authentic lipids obtained from *S. solfataricus*.

Hydrolysis of lipids and purification of isopranoid ether components. Lipids were hydrolysed by heating with 2 M-HCl at 110 °C in a stoppered reaction tube for 16 h. After cooling, the reaction mixture was dried under vacuum and partitioned by addition of equal volumes of $CHCl_3$ and H_2O. The chloroform-soluble fraction was chromatographed on a silica gel column (Merck Kieselgel 70–230 mesh, 20 g) (20 cm × 10 mm, i.d.). $CHCl_3$/diethyl ether (98:2, v/v) eluted 2,3-di-*O*-phytanyl-*sn*-glycerol; $CHCl_3$/diethyl ether (9:1, v/v) eluted GDGTs; and $CHCl_3/CH_3OH$ (95:5, v/v) eluted GDNTs. Purified compounds were weighed to estimate their relative percentages. The GDGTs and GDNTs, the latter as fully acetylated derivatives, were resolved into single differently cyclized components by HPLC. The relative proportion of each lipid was calculated by integration of peak areas on HPLC.

Isopranoid ether acetylation. GDNTs were acetylated with acetic anhydride/pyridine (9:1, v/v) by refluxing for 6 h. The reaction mixture was then dried under vacuum and analysed by HPLC.

Chromatographic procedures. TLC was done on 0·25 mm layers (analytical) and on 2 mm layers (preparative) of silica gel F 254 (Merck) activated by heating at 100 °C for 2 h. Solvents used were light petroleum/diethyl ether (95:5, v/v) for the quinones; $CHCl_3$/diethyl ether (95:5, v/v) for 2,3-di-*O*-phytanyl-*sn*-glycerol; $CHCl_3$/diethyl ether (9:1, v/v) for GDGTs and fully acetylated GDNTs; $CHCl_3/CH_3OH$ (9:1, v/v) for GDNTs; and $CHCl_3/CH_3OH/H_2O$ (65:25:4, by vol.) for complex lipids. All compounds were detected either by exposure to I_2 vapour or by spraying with 0·1% $Ce(SO_4)_2$ in 1 M-H_2SO_4. The Dittmer and Lester (Dittmer & Lester, 1964) reagent was used for phospholipids and α-naphthol/H_2SO_4 for glycolipids.

HPLC analyses were done on a Waters apparatus equipped with a differential refractometer, using a Microporasil column (30 cm × 3·9 mm, i.d.), flow rate 1·5 ml min^{-1}. Solvents used were *n*-hexane/ethyl acetate (99:1, v/v) for quinones, *n*-hexane/ethyl acetate (85:15, v/v) for GDGTs and *n*-hexane/ethyl acetate (7:3, v/v) for GDNT acetates.

RESULTS AND DISCUSSION

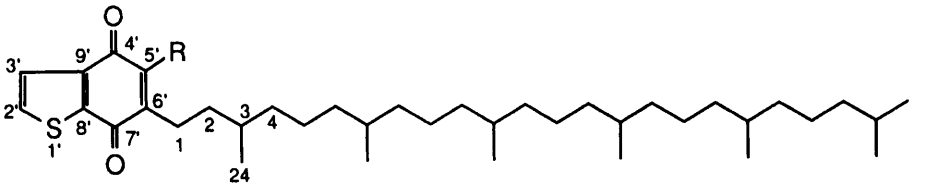
Total lipids from *D. ambivalens* grown aerobically and anaerobically accounted for about 8% of dried cells in both cases, a value very close to that of *S. solfataricus* (De Rosa *et al.*, 1980).

The total quinone content as well as the type of quinone present in *D. ambivalens* seemed to be affected by growth conditions (Table 1). While the quinone fraction represented 0·16% of cell dry wt in aerobically grown cultures, in the absence of oxygen the quinone content fell to only 0·05%. A similar trend has been reported for facultative anaerobic micro-organisms in which the quinone content may depend to some extent on the extent of anaerobiosis and increases with increased oxygen tension (Brodie, 1965).

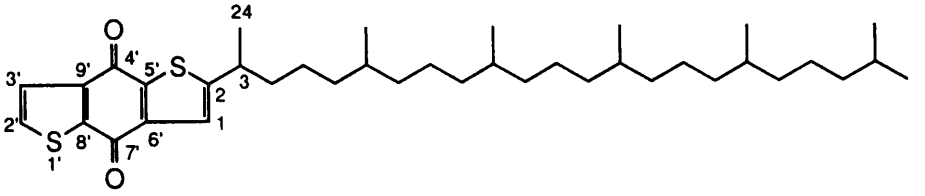
In aerobically grown cells caldariellaquinone (Fig. 1*a*) represented 83% of the quinone pool and sulfolobusquinone (Fig. 1*b*) 16%, while tricyclic quinone (Fig. 1*c*) was present in only trace amounts. In contrast, in anaerobically grown cells sulfolobusquinone was the only quinone present. Conflicting data have been provided by Thurl *et al.* (1986), showing that in anaerobically grown *D. ambivalens* equal amounts of both caldariellaquinone and sulfolobusquinone are present.

The complex lipid patterns of *D. ambivalens* (grown aerobically and anaerobically) closely resembled those of *S. solfataricus*, the same complex lipids being present in both genera. Moreover, the relative proportions of the different complex lipids were quite similar, with the exception of PGLI (Table 2).

The hydrolysis of total lipid extract of *D. ambivalens* gave rise to a mixture of GDGTs and GDNTs (Fig. 2, Table 3) and to trace amounts of 2,3-di-*O*-phytanyl-*sn*-glycerol. The relative ratios of GDGTs and GDNTs in *D. ambivalens* grown under different conditions and in *S. solfataricus* were comparable. In contrast, the cyclic composition of GDGT and GDNT fractions showed significant differences. In particular, *D. ambivalens* tetraethers were less cyclized and showed less structural variety when compared to those from *S. solfataricus* grown under standard conditions (De Rosa *et al.*, 1980, 1989). In *D. ambivalens* the GDGT and GDNT composition was strongly affected by the presence or absence of oxygen during growth. In



(*a*) R = SCH_3; caldariellaquinone
(*b*) R = CH_3; sulfolobusquinone



(*c*) Tricyclic quinone

Fig. 1. Chemical structures of benzothiophenquinones occurring in the order *Sulfolobales*.

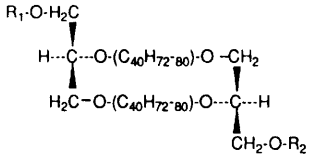
Table 1. *Quinone composition of D. ambivalens and S. solfataricus*

CQ, caldariellaquinone; SQ, sulfolobusquinone; TQ, tricyclic quinone. (For structures see Fig. 1.)

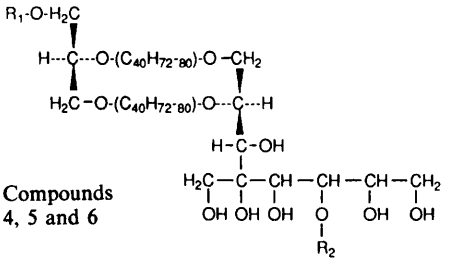
	Quinone fraction (% dry wt)	CQ (% of quinone mixture)	SQ (% of quinone mixture)	TQ (% of quinone mixture)
D. ambivalens (aerobically grown)	0·16	83·0	16·0	tr.
D. ambivalens (anaerobically grown)	0·05	–	100	–
S. solfataricus	0·30	94·0	5·0	tr.

tr., Trace.

Table 2. *Structures of major complex lipids and percentage lipid composition of D. ambivalens and S. solfataricus*



Compounds 1, 2 and 3



Compounds 4, 5 and 6

Compound		R_1	R_2	Lipid composition (%) *D. ambivalens* Aerobically grown	*D. ambivalens* Anaerobically grown	*S. solfataricus*
1	GLI	H	β-D-Glc*p* → β-D-Gal*p*	3·5	7·2	5·8
2	PLI	Phosphoinositol	H	7·0	6·3	7·8
3	PGLI	Phosphoinositol	β-D-Glc*p* → β-D-Gal*p*	18·7	16·5	7·8
4	GLII	H	β-D-Glc*p*	11·5	12·5	13·6
5	SGLII	H	β-D-Glc*p*-sulphate	9·0	8·0	10·4
6	PGLII	Phosphoinositol	β-D-Glc*p*	50·3	49·5	54·5

Table 3. *Composition of the GDGT and GDNT fractions isolated from lipid hydrolysates of D. ambivalens and S. solfataricus*

The GDNT/GDGT molar ratio was 2 for aerobically grown *D. ambivalens* and 3 for anaerobically grown *D. ambivalens* and for *S. solfataricus*.

	GDGT composition (%)		
	D. ambivalens		
No. of cyclopentanes per molecule*	Aerobically grown	Anaerobically grown	*S. solfataricus*
0 + 0 (a)	6·2	–	1·0
0 + 1 (f)	10·1	–	1·5
1 + 1 (b)	16·0	–	3·9
2 + 1 (g)	28·0	7·0	13·2
2 + 2 (c)	35·6	93·0	26·9
3 + 2 (h)	4·1	–	25·6
3 + 3 (d)	–	–	17·0
4 + 3 (i)	–	–	6·3
4 + 4 (e)	–	–	4·6
Mean cyclization value†	2·9	3·9	4·7
	GDNT composition (%)		
	D. ambivalens		
No. of cyclopentanes per molecule*	Aerobically grown	Anaerobically grown	*S. solfataricus*
0 + 0 (a′)	–	–	0·5
0 + 1 (f′)	–	–	1·2
1 + 1 (b′)	–	–	1·8
2 + 1 (g′)	7·0	100	10·2
2 + 2 (c′)	93·0	–	21·8
3 + 2 (h′)	–	–	32·2
3 + 3 (d′)	–	–	19·6
4 + 3 (i′)	–	–	7·6
4 + 4 (e′)	–	–	5·1
Mean cyclization value†	3·9	3·0	4·9

* See Fig. 2.

† Calculated according to the formula

$$\sum_{n=0}^{n=8} (\%\ \text{tetraethers} \times \text{no. of cyclopentane rings for each molecule, } n) \times 10^{-2}.$$

aerobic conditions, the main tetraethers present were a–c, f–h, c′ and *g*′, whilst under anaerobic condition the tetraethers c, g and g′ were the most representative core lipids (Fig. 2, Table 3). The degree of cyclization of GDGTs and GDNTs obtained by hydrolysis of each pure lipid was similar to that obtained by hydrolysis of total complex lipids.

The results reported in this paper show that the complex lipid composition of *D. ambivalens*, grown both aerobically and anaerobically, is very similar to that described for the genus *Sulfolobus*. Moreover, in *D. ambivalens* GDNTs and benzothiophenquinones, found until now only in the genus *Sulfolobus*, are also present. These similar lipid patterns, the presence of trehalose (Nicolaus *et al.*, 1988) and the use of the same glucose catabolic pathway (data not published) by both *Desulfurolobus* and *Sulfolobus* confirm the close relationship between these two genera.

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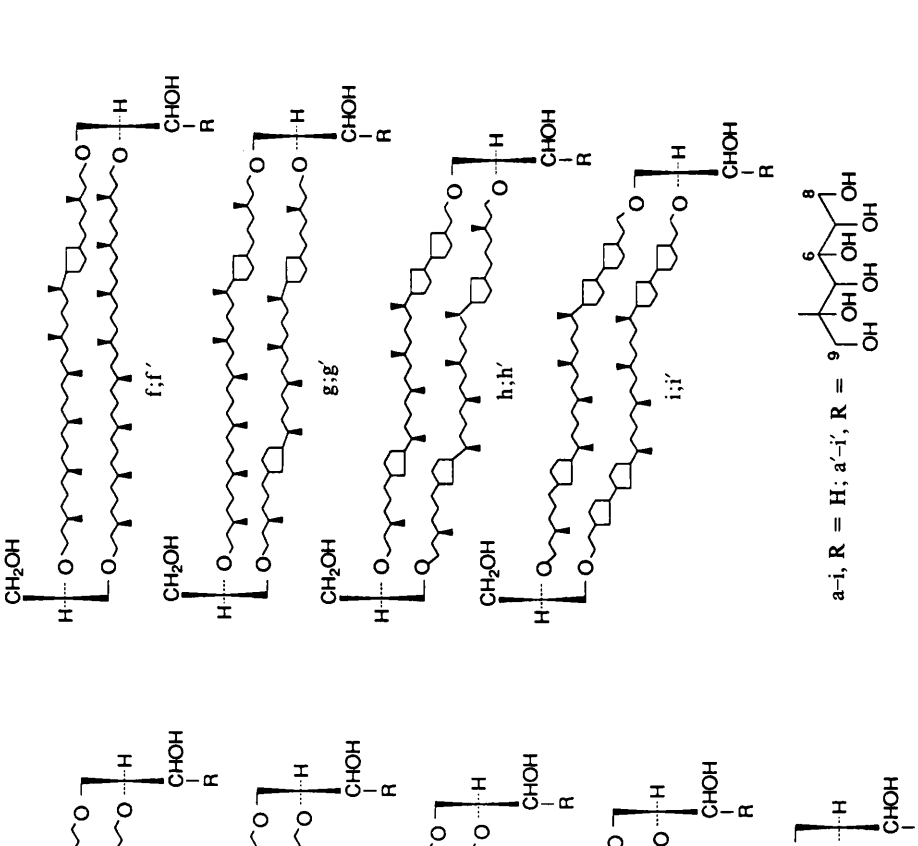

Fig. 2. Chemical structures of isoprenoid tetraethers, basic components of complex lipids in the order *Sulfolobales*, with different degree of cyclization in the molecule from the 0 + 0 cycle up to 4 + 4 cycles for both aliphatic chains. a–i, Glycerol-dialkyl-glycerol tetraethers (GDGTs); a′–i′, glycerol-dialkyl-nonitol tetraethers (GDNTs).

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