Effect of Growth Temperature on the Lipid Composition of two Strains of *Thermus* sp.

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Growth-temperature-dependent alterations in the total extractable lipid and polar lipid components of two strains of *Thermus* sp. isolated from a Portuguese hot spring were studied between 50 °C and 78 °C. The total extractable lipid varied between 8.0 and 10.6% of the cell dry weight; there were no alterations in the phosphorus and carotenoid content of the lipid extract, but the carbohydrate content increased as the growth temperature was raised. Three glycolipids and the four phospholipids were separated by thin-layer chromatography. The relative concentration of the major glycolipid (GL₁) of strain SPS 17 (yellow-pigmented) increased with the growth temperature, whereas the relative concentration of a minor glycolipid (GL₂) decreased. There were no temperature-dependent alterations in the relative concentrations of GL₁ and GL₂ from strain SPS 11 (colourless). The proportion of the major phospholipid (PL₂) decreased in both strains as the growth temperature increased, whereas that of a minor phospholipid (PL₁) increased. The major fatty acyl chains of both strains were 13-methyltetradecanoate (iso-C₁₅) and 15-methylhexadecanoate (iso-C₁₇). The GL₁ in strains SPS 11 and SPS 17 had a glucose:glucosamine:glycerol:long fatty acyl chain ratio of 3:1:1:3.

INTRODUCTION

Many thermophilic eubacteria, assigned to the genus *Thermus*, have been isolated from natural and man-made thermal environments since Brock & Freeze (1969) described the type species, *T. aquaticus* strain YT-1 (Pask-Hughes & Williams, 1977; Hudson *et al.*, 1986). These strains are normally yellow pigmented but some are colourless; they have an optimum growth temperature of 70–75 °C. Another species, *T. ruber*, with a lower growth temperature (60 °C) was described by Loginova *et al.* (1984).

The polar lipid fraction of five yellow-pigmented and colourless strains that have been studied contains a major glycolipid and a major phospholipid (glycophospholipid). The major glycolipid is a diacyl-diglycosyl-(*N*-acyl)glycosaminylglucosylglycerol which, depending on the strain, contains glucose, or glucose and galactose, and glucosamine or galactosamine (Oshima & Yamakawa, 1974; Pask-Hughes & Shaw, 1982). Branched fatty acyl chains (C₁₅ and C₁₇) comprise the major aliphatic moieties of the polar lipids of these strains (Ray *et al.*, 1971*a*; Oshima & Yamakawa, 1974; Oshima, 1978; Pask-Hughes & Shaw, 1982). The effect of the growth temperature on the fatty acyl composition of strains YT-1 and HB-8 has been studied in detail, but little attention has been paid to growth-temperature-dependent alterations in other lipid components.

This paper describes the effect of the growth temperature on the total and polar lipid content of two strains of *Thermus* sp. isolated from a Portuguese thermal spring and provides a preliminary analysis of the major glycolipids in both strains.

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METHODS

Organisms and growth conditions. *Thermus* sp. strains SPS 11 and SPS 17, isolated from the São Pedro do Sul Spa in central Portugal (unpublished results), were maintained at -80°C in *Thermus* medium (Brock, 1978) containing 15% (v/v) glycerol. Liquid cultures were grown in 1 litre Erlenmeyer flasks containing 200 ml *Thermus* medium at temperatures between 50°C and 78°C in a water-bath shaker (New Brunswick, RW-650) at 120 r.p.m. For larger cell masses, the strains were grown in a 25 l fermenter (New Brunswick, MF-1285) at 72°C until late exponential growth phase.

Extraction and separation of lipids. Freshly harvested cells were extracted twice by the method of Bligh & Dyer (1959) and the extract was washed once with 0.5 M-KCl to induce phase partition and remove non-lipid impurities (Ray *et al.*, 1971 *b*). The polar lipids were separated from the neutral lipid fraction containing the vast majority of the carotenoids on preparative silica gel G plates (2 mm thickness, Merck) developed with acetone. The neutral lipids and the carotenoids migrated with the solvent front, whereas the polar lipids remained at the origin. This fraction was eluted from the silica with chloroform/methanol/water (45:45:10, by vol.).

The individual polar lipids were separated by thin-layer chromatography (TLC) on silica gel G plates (0.25 mm thickness, Merck) with solvent systems consisting of chloroform/methanol/ammonia (25%)/water (70:30:4:1, by vol.) (solvent A) or chloroform/methanol/water (65:25:4, by vol.) (solvent B). The glycolipids and phospholipids were eluted from the silica developed with solvent A as described above, after spraying the plates with water to locate the lipid-containing areas. Further purification of the major glycolipids was done by TLC with solvent B. The purity of the glycolipids was confirmed by two-dimensional TLC using chloroform/methanol/ammonia (25%)/water (65:35:0.3:4, by vol.) as a solvent system in the first dimension and chloroform/methanol/acetone/acetic acid/water (50:20:10:10:5, by vol.) in the second dimension.

Analytical methods. The dry weight of the cells was determined before extraction by passing a sample of the suspension through a pre-weighed dry Sartorius type SM membrane filter (0.45 μm , pore size) and drying at 80°C to constant weight. The weight of the total lipid extract was determined gravimetrically on very small tared dry aluminium foil pans. The carotenoids were estimated by the absorption peak at 455 nm.

The polar lipids were visualized on the TLC plates using iodine vapour; phosphorus, carbohydrate and free amino groups were detected using the Dittmer-Lester reagent, α -naphthol and ninhydrin respectively (Christie, 1982). The phosphorus content of the total lipid extract, polar lipid fraction and individual polar lipids was determined using the Bartlett (1959) modification of the Fiske and Subbarow method after hydrolysis of samples at 180°C in 70% HClO_4 (Bottcher *et al.*, 1961). The total carbohydrate of the same lipid fractions was determined using the phenol/sulphuric acid method (Dubois *et al.*, 1956).

Samples of the major glycolipids were hydrolysed under N_2 in 2 M-HCl at 100°C for 3 h in sealed ampoules. The hydrolysates were extracted three times with light petroleum (b.p. $40-60^{\circ}\text{C}$) to recover fatty acids. The aqueous phase was neutralized with NaOH, and the free sugars and glycerol separated by descending paper chromatography on Schleicher and Schuell no. 2043 paper using a solvent system of ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by vol.). The components were visualized with alkaline silver nitrate (MacLean & Scott, 1976) and with ninhydrin.

The total carbohydrate in the aqueous phase was determined using the phenol/sulphuric acid method, D-glucose with glucose oxidase (Sigma), total polyol using the method of Bok & Demain (1977), glycerol with glycerol kinase (Sigma) and glucosamine using the Elson and Morgan procedure (Dittmer & Wells, 1969). Sugars and glycerol hydrolysed under the same conditions were used as standards.

Fatty acid methyl esters were prepared with 14% (v/v) boron trifluoride-methanol (Dittmer & Wells, 1969) using phosphatidylcholine as a standard. Gas liquid chromatography (GLC) was done using a Varian Aerograph gas chromatograph (model 2700) with a flame ionization detector. An open tubular capillary column SP-2340 (25% methyl, 75% cyanopropyl silicone, 0.2 μm phase thickness, 60 m $\times$ 0.75 mm i.d.) was eluted with N_2 at 4 ml min^{-1} , and operated at 160°C . Some separations were confirmed with a 4 m $\times$ 3.2 mm i.d., column packed with 10% SP-2340 (25% methyl, 75% cyanopropyl silicone) on Chromosorb WAW 100/120 mesh eluted with N_2 at 6.7 ml min^{-1} and operated at 145°C . Fatty acid methyl esters were identified by comparison of their retention times with those of known standards.

Infrared spectrophotometry was done on samples applied in KBr discs using a Perkin-Elmer (model 1430) spectrophotometer.

RESULTS

Effect of growth temperature on the total lipid extract

Strain SPS 17 (yellow) was grown between 50°C and 78°C , whereas strain SPS 11 (colourless) could only be grown up to 75°C . The total lipid content of both strains varied between about 8.0 and 10.6% of the cell dry weight (Table 1). There appeared to be only a very slight increase in the total lipid extract as the growth temperature was raised. The phosphorus content of the lipid

Table 1. *Effect of growth temperature and growth phase on the total lipid composition of Thermus SPS 11 and SPS 17*

Values without error terms are from a single determination; other values are the means $\pm$ SD of the number of experiments indicated in parentheses.

Component	Strain	Growth temp. . . .		50 °C		60 °C		70 °C		75 °C		78 °C	
		Growth phase . . .											
		Exp.	Sta.	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.
Total lipid	SPS 11	8.0 $\pm$ 1.1 (4)	9.0	8.7	9.7	10.5	9.1	10.5 $\pm$ 0.7 (2)	9.9 $\pm$ 3.1 (2)	—	—	—	—
(% dry wt)	SPS 17	8.4 $\pm$ 1.5 (2)	9.5	10.5	10.2	9.6	9.3	9.0 $\pm$ 1.2 (6)	9.6 $\pm$ 1.8 (2)	10.4 $\pm$ 1.3 (2)	9.0	—	—
Phosphorus	SPS 11	2.6 $\pm$ 0.2 (5)	2.5	2.6	2.7 $\pm$ 0.1 (2)	2.5	2.6	2.7 $\pm$ 0.2 (3)	2.5 $\pm$ 0.1 (2)	—	—	—	—
[10 ² $\times$ μ mol (mg dry wt) ⁻¹]	SPS 17	3.0 $\pm$ 0.3 (4)	2.6 $\pm$ 0.1 (2)	2.9	3.2 $\pm$ 0.1 (2)	2.9	3.1	2.8 $\pm$ 0.4 (6)	2.9 $\pm$ 0.1 (2)	2.9 $\pm$ 0.3 (2)	2.7	—	—
Sugar	SPS 11	7.0 $\pm$ 0.9 (5)	5.7	8.7	6.7 $\pm$ 0.7 (2)	8.4	8.5	9.9 $\pm$ 1.7 (3)	9.2 $\pm$ 0.9 (2)	—	—	—	—
[10 ² $\times$ μ mol (mg dry wt) ⁻¹]	SPS 17	6.5 $\pm$ 0.9 (4)	6.3 $\pm$ 0.8 (2)	8.7	7.2 $\pm$ 0.3 (2)	9.4	8.3	8.9 $\pm$ 1.0 (6)	9.7 $\pm$ 0.4 (2)	10.0 $\pm$ 0.5 (2)	8.3	—	—
Carotenoids	SPS 11	0.1	0.1	0.2	0.1	0.1	0.1	0.2 $\pm$ 0.1 (2)	0.1	—	—	—	—
[10 ² $\times$ A ₄₅₅ (mg dry wt) ⁻¹]	SPS 17	1.2	0.7 $\pm$ 0.2 (2)	1.4	1.2	1.7	1.6	1.6 $\pm$ 0.3 (3)	1.7 $\pm$ 0.1 (2)	0.8 $\pm$ 0.1 (2)	1.1	—	—
Ratio (μ mol lipid sugar)/ (μ mol lipid phosphorus)	SPS 11	2.7	2.3	3.3	2.5	3.4	3.3	3.7	3.7	—	—	—	—
	SPS 17	2.2	2.4	3.0	2.3	3.2	2.7	3.2	3.3	3.4	3.0	—	—

Table 2. R_F values for the polar lipids of strains SPS 11 and SPS 17 in two solvent systems and their reactions with α -naphthol (carbohydrate), Dittmer-Lester (phosphorus) and ninhydrin (free amino groups) reagents

Lipid	R_F values*		Reaction with:		
	A	B	α -Naphthol	Dittmer-Lester	Ninhydrin
GL ₁	0.06	0.24	+	—	—
GL ₂	0.19	0.42	+	—	—
GL ₃	0.51	ND	+	—	—
PL ₁	0.25	0.50	—	+	+
PL ₂	0.35	0.35	—	+	—
PL ₃	0.60	ND	—	+	—
PL ₄ †	0.56	ND	—	+	—

ND, Not detected.

* A, Chloroform/methanol/ammonia (25%)/water (70:30:4:1, by vol.). B, Chloroform/methanol/water (65:25:4, by vol.).

† PL₄ was detected only in SPS 17 at 78 °C.

extract from both *Thermus* strains did not vary with the growth temperature or phase of growth. On the other hand, there was an increase in the carbohydrate content of the total lipid extract of both strains; this increase was more pronounced in SPS 17, which grew at 78 °C. The rise in the carbohydrate content was reflected in an overall increase in the carbohydrate: phosphorus ratio.

The content of carotenoids, as measured by the absorption peak at 455 nm, remained relatively stable throughout the temperature range studied, although there appeared to be a slight increase in the carotenoid content of strain SPS 17 between 60 °C and 75 °C. No alteration in the absorption spectrum of the carotenoids, between 350 and 600 nm, could be detected within the temperature range.

Effect of growth temperature on the polar lipids and fatty acyl chains

The separation of the polar lipids from the neutral lipids and carotenoids by TLC with acetone as solvent was rapid and the polar lipid fraction obtained by this method contained, in all cases, over 90% of the initial lipid phosphorus and carbohydrate in the lipid extract.

The TLC patterns of the polar lipids were similar for both *Thermus* strains. Three phospholipids and three glycolipids were detected; a fourth phospholipid (PL₄) was only detected in SPS 17 grown at 78 °C. Phospholipid 1 (PL₁) gave a positive ninhydrin reaction in both strains (Table 2). One minor α -naphthol-positive lipid co-migrated with a polar carotenoid in all solvent systems used.

Glycolipid 1 (GL₁) and phospholipid 2 (PL₂) were the major polar lipids in both strains at all the growth temperatures (Table 3 and 4). A rise in the growth temperature from 50 °C to 78 °C increased the relative concentration of GL₁ in SPS 17 from 87% to 96% of the polar lipid carbohydrate, with a concomitant decrease in GL₂ from 7–10% to 1–2%, depending on the growth phase (Table 3). The very minor component, GL₃, also decreased as the growth temperature increased (Table 3). In contrast, the relative concentrations of GL₁ and GL₂ in strain SPS 11 remained relatively constant over the growth temperature range (Table 4).

The major phospholipid (PL₂) decreased in concentration in both strains as the growth temperature increased, whereas PL₁ increased in concentration, reaching 19.4% of the total phosphorus in SPS 17 at 78 °C (Tables 3 and 4). PL₃ appeared to reach its highest concentration in SPS 17 at 70 °C and 75 °C during the stationary phase (Table 4). PL₄ was detected only at 78 °C in strain SPS 17 (Table 4).

The major acyl chains in both strains were 13-methyltetradecanoate (iso-C₁₅); 12-methyltetradecanoate (anteiso-C₁₅), 14-methylpentadecanoate (iso-C₁₆); 15-methylhexadecanoate (iso-C₁₇) and 14-methylhexadecanoate (anteiso-C₁₇). An unknown fatty acyl chain reached

Table 3. *Effect of growth temperature and phase of growth on the glycolipid and phospholipid compositions of Thermus SPS 17*

Values are given as the percentages of total carbohydrate or phosphorus. Those without error terms are from a single determination; other values are the means $\pm$ SD of the number of experiments indicated in parentheses.

Component	Growth temp. . . . Growth phase . . .	50 °C		60 °C		70 °C		75 °C		78 °C	
		Exp.	Sta.	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.
Glycolipids											
GL ₁		87.4	87.1 ± 5.9 (2)	93.6	90.0 ± 1.3 (2)	95.8	93.4	95.7 ± 0.2 (2)	92.7 ± 3.8 (2)	96.4	97.5
GL ₂		7.1	9.9 ± 4.4 (2)	5.0	8.3 ± 3.8 (2)	2.9	4.9	2.9 ± 0.5 (2)	2.2	2.1	1.4
GL ₃		1.2	1.2 ± 0.8 (2)	0.5	1.3	0.6	0.8	0.6	0.9	0.7	0.4
CA*		4.3	1.8 ± 0.7 (2)	1.0	2.1	0.7	0.8	1.1 ± 0.5 (2)	1.6 ± 1.1 (2)	0.8	0.7
Phospholipids											
PL ₁		1.6	1.7 ± 0.6 (2)	3.1	1.9 ± 0.2 (2)	5.6	5.5	6.7 ± 0.4 (2)	8.9 ± 0.6 (2)	19.4 ± 0.1 (2)	14.3
PL ₂		95.4	96.0 ± 1.8 (2)	92.0	93.4 ± 0.5 (2)	91.5	87.2	90.1 ± 0.7 (2)	84.7 ± 1.0 (2)	75.4 ± 3.2 (2)	79.1
PL ₃		1.6	1.4 ± 0.7 (2)	1.5	3.2 ± 0.4 (2)	0.9	5.3	1.6 ± 0.2 (2)	5.4 ± 0.1 (2)	2.0 ± 0.6 (2)	2.1
PL ₄		—	—	—	—	—	—	—	—	1.2 ± 0.1 (2)	3.0
Origin		1.5	0.9 ± 0.6 (2)	3.4	1.5 ± 0.1 (2)	2.1	2.0	1.6 ± 0.1 (2)	1.2 ± 0.6 (2)	2.1 ± 0.8 (2)	1.5

* α -Naphthol-positive carotenoid.

Table 4. *Effect of growth temperature and phase of growth on the glycolipid and phospholipid compositions of Thermus SPS 11*

Refer to Table 3 for details.

Component	50 °C		60 °C		70 °C		75 °C	
	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.	Exp.	Sta.
Glycolipids								
GL ₁	93.9	93.6	95.4	94.7 ± 0.1 (2)	94.8	95.4	95.6 ± 1.1 (3)	95.1
GL ₂	4.6	4.5	3.3	3.0 ± 0.3 (2)	3.0	3.5	2.6 ± 0.7 (3)	4.9
GL ₃	1.4	0.6	0.5	0.8 ± 0.2 (2)	0.7	0.4	0.6 ± 0.2 (3)	—
CA*	1.4	1.3	0.8	1.4 ± 0.3 (2)	1.5	0.6	1.3 ± 0.7 (3)	—
Phospholipids								
PL ₁	1.7	0.8	2.8	2.1 ± 1.6 (2)	6.4	6.0	7.8 ± 0.3 (3)	7.0 ± 0.9 (2)
PL ₂	95.7	96.9	94.4	94.9 ± 1.7 (2)	90.7	89.7	89.0 ± 1.2 (3)	88.0 ± 0.7 (2)
PL ₃	1.2	1.5	1.5	1.7 ± 0.6 (2)	1.2	3.0	1.2 ± 0.4 (3)	2.3 ± 0.4 (2)
Origin	1.4	0.8	1.4	1.3 ± 0.4 (2)	1.7	1.3	1.2 ± 0.5 (3)	2.0 ± 0.1 (2)

* α -Naphthol-positive carotenoid.Table 5. *Effect of growth temperature on the major fatty acid composition of the polar lipids of SPS 11 and SPS 17 in late exponential phase, and the fatty acid composition of GL₁ at the optimum growth temperature*

The values are expressed as a percentage of the total.

Fatty acid*	Strain . . . Growth temp. . . .	Polar lipids				GL ₁	
		SPS 11		SPS 17		SPS 11	SPS 17
		50 °C	73 °C	50 °C	73 °C	73 °C	75 °C
i-C ₁₃		2.72	2.84	1.86	1.30	1.12	2.34
UN		ND	0.83	ND	ND	ND	ND
i-C ₁₄		3.48	0.54	4.61	0.76	0.75	0.58
n-C ₁₄		0.94	0.90	0.78	1.16	0.80	ND
i-C ₁₅		35.11	49.42	31.23	43.86	47.60	40.18
a-C ₁₅		12.58	6.81	14.00	6.91	6.15	6.26
n-C ₁₅		4.11	1.79	3.43	2.10	2.36	3.03
i-C ₁₆		6.65	2.37	13.22	3.27	3.39	3.61
a-C ₁₆		ND	0.46	ND	1.14	ND	ND
UN		0.31	ND	2.19	ND	ND	ND
n-C ₁₆		1.39	3.67	1.37	1.61	1.13	1.09
i-C ₁₇		15.57	25.32	11.04	30.50	31.10	35.89
a-C ₁₇		6.89	4.23	5.00	4.96	4.44	5.83
UN		6.20	ND	7.51	ND	ND	ND
n-C ₁₇		3.51	0.83	2.69	1.38	1.18	1.83
UN		0.62	ND	0.79	0.67	ND	ND

ND, not detected.

* UN, Unidentified; i, iso-branched; a, anteiso-branched; n, normal.

fairly high levels (6.20–7.51% of the total) in both strains grown at 50 °C, but was not detected at 75 °C. There also appeared to be no preferential distribution of acyl chains in GL₁ at the optimum growth temperature (Table 5).

Analysis of the major glycolipids of SPS 11 and SPS 17

The analysis of GL₁ of strain SPS 11 indicated a glucose:glucosamine:glycerol:fatty acyl ratio of 2.86:1.01:1.00:2.88. The molecular proportion of the long-chain fatty acids was determined by GLC. Galactose and galactosamine were not detected by paper chromatography and all the phenol/sulphuric-acid-positive carbohydrate was confirmed as glucose by the glucose

oxidase method. The presence of amide bands in the infrared spectrum indicated *N*-acylation of the glucosamine; furthermore the glycolipid gave a negative ninhydrin reaction on TLC. GL₁ of strain SPS 17 proved identical, with a glucose:glucosamine:glycerol:fatty acyl ratio of 2.98:1.06:1.00:2.82.

DISCUSSION

The total extractable lipid of *Thermus* sp. strains SPS 11 and SPS 17 increased only slightly as the growth temperature was raised from 50 °C to 75 or 78 °C, respectively. For *T. aquaticus* strain YT-1 Ray *et al.* (1971*b*) showed a very large increase over the temperature range for growth; nevertheless, the results presented here are in agreement with the values (8–12% of the dry weight) reported by Pask-Hughes & Shaw (1982) at the optimum growth temperature for five strains of *Thermus* sp. We also failed to detect the increase in phosphorus and carotenoid contents at higher growth temperatures described by Ray *et al.* (1971*b*). In the present study, only an increase in the carbohydrate content was observed with increasing growth temperature.

The polar lipid patterns on TLC, as well as the major phospholipid and major glycolipid contents of strains SPS 11 and SPS 17 agree with the results of Pask-Hughes & Shaw (1982) and Oshima (1978).

The relative increase in GL₁ of strain SPS 17 as the growth temperature was increased was not as pronounced as that in strain HB-8 (Oshima, 1978) and the relative concentration of GL₁ of strain SPS 11 was unaffected by the growth temperature.

We have also shown that an increase in the growth temperature caused a decrease in the major phospholipid (PL₂) and an increase in PL₁ of both strains. Similar alterations have been described in other thermophiles such as *Bacillus caldovenax* (Hasegawa *et al.*, 1980) and *Bacillus stearothermophilus* (Souza *et al.*, 1974).

The major fatty acyl chains of SPS 11 and SPS 17 proved identical to those described for other *Thermus* strains (Ray *et al.*, 1971*a*; Oshima, 1978; Pask-Hughes & Shaw, 1982). GL₁ of strain SPS 17 is similar to the major glycolipid of *Thermus* strain H (Pask-Hughes & Shaw, 1982), since galactose and/or galactosamine are not present.

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