Characterization of glycolipids from *Meiothermus* spp.

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Thin-layer chromatographic analysis of the polar lipids of *Meiothermus* strains revealed two glycolipid bands with similar chromatographic mobility to the major glycolipid of *Thermus* strains. In this study the glycolipids from the type strains of *Meiothermus ruber*, *Meiothermus chliarophilus*, *Meiothermus silvanus* and *Meiothermus cerbereus* were characterized using GC, GC/MS, fast atom bombardment MS and chemical methods. All strains contained dihexosyl-(*N*-acyl)hexosaminyglucosyl diacylglycerols, related in structure to the major glycolipid of *Thermus* strains but varying in their fatty acylation pattern. The detection of two glycolipid bands by TLC in *Meiothermus* spp. was attributable to the invariable presence of 2-hydroxyacyl groups *N*-linked to the hexosamine of the polar head group which cause the glycolipids to be more strongly retained on silica TLC plates than 3-hydroxy or non-hydroxylated *N*-acyl glycolipids of similar structure that are also present. *M. silvanus* contained, in addition to these glyceroglycolipids, several glycolipids which were linked to acylated branched octadecanediols rather than to glycerol. The presence of glycolipids containing 2-hydroxyacyl groups *N*-linked to hexosamine appears to be a stable phenotypic marker that distinguishes the genus *Meiothermus* from the genus *Thermus*.

Keywords: thermophiles, *Meiothermus*, glycolipids

INTRODUCTION

The genus *Meiothermus* was proposed to accommodate several moderately thermophilic organisms originally included in the genus *Thermus* (Nobre *et al.*, 1996b). The species *Meiothermus ruber*, *Meiothermus silvanus*, *Meiothermus chliarophilus* and *Meiothermus cerbereus* (Chung *et al.*, 1997; Tenreiro *et al.*, 1995) are distinguished from species of the genus *Thermus* by their 16S rDNA sequences, their lower optimum growth temperatures (50–60 °C compared to about 70 °C for *Thermus* spp.), the occurrence of 2-hydroxy isobranched fatty acids in all strains and a distinctive polar lipid composition.

One of the simplest ways of differentiating *Meiothermus* spp. from *Thermus* spp. is by TLC of their polar lipids, since two prominent glycolipid bands, designated GL-1 bands 1a (GL-1a) and 1b (GL-1b), are always observed

in *Meiothermus* spp., compared to one in *Thermus* spp. (Chung *et al.*, 1997; Donato *et al.*, 1991; Tenreiro *et al.*, 1995). However, the glycolipids from *Meiothermus* have not been characterized before. The similar chromatographic behaviour of GL-1 from *Thermus* strains, and GL-1a and GL-1b from *Meiothermus* strains are consistent with a structural relationship between them, although the different TLC mobility of GL-1a and GL-1b implies some difference in either sugar or fatty acyl composition.

The major glycolipid of strains of the genus *Thermus* has been identified as dihexosyl-(*N*-acyl)hexosaminyglucosyl diacylglycerol (Oshima & Ariga, 1976; Oshima & Yamakawa, 1974) in which, depending on the strain, the *N*-acylated hexosamine is either glucosamine or galactosamine and the hexose residues are three glucose residues, two glucose plus one galactose, or one glucose and two galactose (Carreto *et al.*, 1996; Pask-Hughes & Shaw, 1982). The only known exception is GL-1 from *Thermus aquaticus* 15004 in which the subterminal residue is *N*-acetylgalactosamine

Abbreviation: FAB, fast atom bombardment.

rather than hexose (Carreto *et al.*, 1996). A minor glycolipid detected in most strains of the genus *Thermus* and designated glycolipid 2 (GL-2) lacks the terminal hexose residue found in GL-1 (Carreto *et al.*, 1996). This truncated form is the only glycolipid present in the type 1 colonial variant (t1) of *Thermus scotoductus* strain X-1 (Wait *et al.*, 1997).

Thermus and *Meiothermus* spp. contain predominantly iso- and anteiso-branched fatty acids (Donato *et al.*, 1991; Nobre *et al.*, 1996a). In *T. aquaticus* strains and in the type strain of *Thermus filiformis*, appreciable levels of branched 3-hydroxy fatty acids are also found (Nobre *et al.*, 1996a). The 3-hydroxy fatty acids exclusively *N*-acylate the galactosamine of the major glycolipid and are not O-linked to glycerol. Strains of the genus *Thermus* in which GL-1 contains glucosamine rather than galactosamine have only non-hydroxylated *N*-acyl substituents (Carreto *et al.*, 1996).

Unlike strains of *Thermus*, all *Meiothermus* strains examined contain moderate levels of branched 2-hydroxy fatty acids and most also have lower but significant amounts of 3-hydroxy fatty acids (Chung *et al.*, 1997; Nobre *et al.*, 1996a). In this study we show that the two glycolipid bands in *Meiothermus* spp. are similar to GL-1 from the genus *Thermus* and that the differences in TLC mobility are attributable to their varying *N*-acyl substitution patterns. We also show that in the glycolipids from *Meiothermus* spp., in contrast to *Thermus* spp., 2-hydroxyacyl groups may amidate either the galactosamine or the glucosamine of the polar head group. We also detected long-chain 1,2-diol-linked glycolipids with identical polar head groups to the accompanying glyceroglycolipids in *M. silvanus*.

METHODS

Strains and culture conditions. *Meiothermus ruber* DSM 1279^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; *Meiothermus chliarophilus* ALT-8^T (= DSM 9957^T), *Meiothermus silvanus* VI-R2^T (= DSM 9946^T) and *Meiothermus cerbereus* GY-1^T (= DSM 11376^T) were our isolates.

The cultures were grown to the late exponential phase in 1 litre metal-capped Erlenmeyer flasks containing 200 ml *Thermus* medium (Williams & da Costa, 1992), in a shaking water bath. Each strain was cultured at its optimum growth temperature; 60 °C for *M. ruber*, 55 °C for *M. cerbereus* and *M. silvanus*, and 50 °C for *M. chliarophilus*.

Extraction and purification of polar lipids, and sugar and fatty acid composition. The cultures were harvested by centrifugation, washed twice and lipids were extracted by a modification of the method of Bligh & Dyer as described previously (Prado *et al.*, 1988). Separation of polar and neutral lipids, TLC, analytical methods and hydrolysis of the polar lipids were as described by Prado *et al.* (1988). Purity of the polar lipid fractions was determined by two-dimensional TLC on silica gel G plates (Merck, 10 × 10 cm, 0.25 mm thickness) using chloroform/methanol/water (65:25:4, by vol.) in the first direction and chloroform/acetic acid/methanol/water

(80:15:12:4, by vol.) in the second direction. The sugars liberated by aqueous acid hydrolysis of the purified glycolipids were analysed on cellulose TLC plates (Macherey-Nagel MN-300, 10 × 20 cm, 0.1 mm thickness) developed with pyridine/ethyl acetate/acetic acid/water (36:36:7:21, by vol.) and were visualized by spraying with aqueous silver nitrate (Maclean & Scott, 1976). Total carbohydrate, glucose, galactose, glycerol and hexosamines were determined as described previously (Wait *et al.*, 1997). Sugar standards and sugars hydrolysed under the same conditions as the glycolipids were used as controls.

The total polar lipid extracts and the TLC-purified materials were subjected to acid hydrolysis for 30 min at 100 °C in 6.0 M HCl/methanol (32.5:27.5, v/v) and fatty acid methyl esters were recovered by extraction with hexane/*t*-butyl methyl ether (1.0:1.0, v/v). Trimethylsilylation was achieved by treatment of dried fatty acid methyl esters with 300 µl bis(trimethylsilyl)trifluoroacetamide for 16 h at room temperature. The reagent was evaporated to dryness under N₂ and the derivatives dissolved in hexane/*t*-butyl methyl ether (1.0:1.0, v/v). The fatty acid methyl esters and the trimethylsilylated derivatives were analysed by GC using a Hewlett Packard 5980 chromatograph equipped with 5% phenylmethyl silicone capillary column (0.2 mm × 25 m; Hewlett Packard) as described previously (Wait *et al.*, 1997). Peaks were identified by GC/MS and by comparison with the retention times of authentic standards using MIS Library Generation Software (Microbial ID).

Spectrometry. GC/MS was performed with a Kratos MS80 RFA spectrometer directly interfaced to a Carlo Erba 5160 chromatograph fitted with a BPX-5 fused silica column (25 m × 0.2 mm; SGE) as described previously (Wait *et al.*, 1997).

Fast atom bombardment (FAB) spectra were obtained on the same instrument, using an Ion Tech saddle-field atom gun supplied with high-purity xenon gas. Spectra were recorded in both the positive- and negative-ion modes from approximately 10 µg underivatized glycolipids using a 1:1 mixture (v/v) of glycerol and dithiothreitol/dithioerythritol (5:1, w/w) as liquid matrix. Peracetyl derivatives were prepared according to Dell as described by Carreto *et al.* (1996) and Dell (1990), and 1–5 µg were analysed from 3-nitrobenzyl alcohol matrix, 1 µl 0.05 M sodium iodide solution being added to promote sodium cationization. Terminal residues were identified by collision induced dissociation of B₁ carbenium ions generated by FAB of peracetylated and perdeuteroacetylated glycolipids as described previously (Carreto *et al.*, 1996). Sugar fragment ions are designated according to the nomenclature of Domon & Costello (1988).

RESULTS

Polar lipid composition

TLC of the polar lipids from *M. ruber*, *M. silvanus*, *M. cerbereus* and *M. chliarophilus* revealed a single phospholipid (PL-2) and two prominent glycolipid bands, the lower and upper of which were designated GL-1a and GL-1b, respectively (Fig. 1). The glycolipids exhibited comparable relative migration (*R_F*) values in all strains except *M. chliarophilus*, in which both bands migrated slightly further from the origin than the corresponding glycolipids from the other strains. The relative proportions of two bands, estimated by the

Fig. 1. TLC of the polar lipids from *Thermus oshimai* strain SPS-14 (lane 1), *M. ruber* (2), *M. chliarophilus* (3), *M. silvanus* (4) and *M. cerbereus* (5). GL-1 (a, b), glycolipid 1 (a, b); MGL, minor glycolipid; PL-2, major phospholipid.

phenol/sulfuric acid procedure, varied with each strain; GL-1a from *M. ruber* contained about 40% of the total lipid-linked carbohydrate and GL-1b accounted for 57%. In *M. cerbereus* GL-1a was also present in lower proportions (37%) than GL-1b (62%), but the converse was the case in *M. silvanus* where GL-1a accounted for 68% of the carbohydrate and GL-1b for 28%. GL-1a of *M. chliarophilus* contained about 35% of the lipid-linked carbohydrate and GL-1b contained 44%. In this organism the remaining carbohydrate was distributed between two minor glycolipids that were not observed in the other strains.

Fatty acid analysis of the polar lipids of *Meiothermus* strains

In all strains iso- and anteiso-branched pentadecanoic acids (i and a15:0) were the major non-hydroxylated components liberated on hydrolysis of the total polar lipid fraction, the iso-branched isomers predominating over the anteiso-branched isomers except in *M. silvanus* (Table 1). Unsaturated fatty acids were vestigial or undetected in polar lipids of the strains, except in *M. cerbereus* where they constituted about 7% of total fatty acids. In this strain unsaturated fatty acids were predominantly detected in phospholipid 2 (PL-2) rather than in the two glycolipids.

All strains investigated contained between 7 and 16% iso-branched 2-hydroxy fatty acids. Moderate amounts of 3-hydroxy fatty acids were also present in *M. ruber* and *M. cerbereus*, the latter of which had higher levels of 3-hydroxy than 2-hydroxy fatty acids. Analysis of the

TLC-separated polar lipids showed that hydroxy fatty acids were not found in hydrolysates of the purified phospholipid fraction. There were, however, significant differences in the distribution of fatty acids between GL-1a and GL-1b. 2-Hydroxy fatty acids, predominantly 2-hydroxy-15-methylhexadecanoate, were largely confined to band 1a where they usually comprised approximately 27% of total fatty acids. In band 1b from all strains 2-hydroxy fatty acids were only detected in trace amounts, though moderate levels of 3-hydroxy fatty acids were present in band 1b from *M. ruber* and *M. cerbereus* (about 10 and 23% of the total, respectively). In hydrolysates of GL-1b from *M. silvanus* and *M. chliarophilus* non-hydroxylated fatty acids predominated; 2- and 3-hydroxy fatty acids were detected only as trace components (Table 1).

Hydrolysis of GL-1a and 1b and the purified phospholipid from *M. silvanus* liberated two late-eluting components, identified as 16-methylheptadecane-1,2-diol and 15-methylheptadecane-1,2-diol, suggesting that a proportion of the polar head groups are linked to long-chain diols rather than to glycerol, as is the case in some *Thermus* strains (Wait *et al.*, 1997).

Sugar composition of *Meiothermus* glycolipids

The compositional data of the polar head group indicate that within a given strain, GL-1a and 1b do not differ in monosaccharide or hexosamine composition (Table 2). *M. cerbereus* and *M. silvanus* GL-1a and 1b contain two glucose residues, one galactose and one galactosamine; *M. chliarophilus* GL-1a and 1b are similar except that galactosamine is replaced by glucosamine. Both glycolipid bands from *M. ruber* contain galactosamine and three glucose residues. The non-stoichiometric amounts of glycerol in the glycolipids from *M. silvanus* is consistent with the detection of long-chain 1,2-diols in their methanolysates.

FAB-MS analysis of the intact glycolipids

The structures of the intact glycolipids were investigated by FAB-MS. Measurement of the molecular mass of native and derivatized glycolipids enables determination of their composition in terms of sugar, glycerol and acyl units (Dell, 1987). In the spectra of the glycolipids from all strains a more or less complex molecular ion distribution was observed, corresponding to molecular species of differing acylation pattern; the negative-ion data obtained from *M. silvanus* are summarized in Table 3. In general, spectra of GL-1a glycolipids were simpler than those of the corresponding GL-1b glycolipids. The acylation heterogeneity of GL-1b glycolipids from *M. ruber* and *M. silvanus* was, however, greater than in the other strains. The quality of the FAB spectra of oligosaccharides is frequently improved by peracetylation or perdeuteroacetylation, enabling determination of the sequence of sugar residues and site-specific localization of acyl substituents, as shown by the spectrum of the perdeuteroacetylated band GL-1a from

Table 1. Fatty acid composition of total polar lipids (TPL), GL-1a and 1b, and the major phospholipid (PL-2) of *Meiothermus* strains

Fatty acid	Fatty acid composition (% of total)															
	<i>M. ruber</i> DSM 1279				<i>M. silvanus</i> VI-R2 ^T				<i>M. chliarophilus</i> ALT-8				<i>M. cerbereus</i> GY-1			
	TPL	GL-1a	GL-1b	PL-2	TPL	GL-1a	GL-1b	PL-2	TPL	GL-1a	GL-1b	PL-2	TPL	GL-1a	GL-1b	PL-2
Straight chain																
14:0	—	0.4	0.3	—	—	0.2	0.3	0.6	—	0.2	0.2	—	—	0.2	0.3	—
15:0	1.3	0.9	1.0	1.6	0.3	0.3	0.5	0.5	2.1	1.8	2.2	3.6	1.6	1.9	1.8	2.0
16:0	1.2	1.0	1.0	3.0	0.6	0.7	2.0	3.8	1.7	0.8	0.9	10.5	1.5	0.8	1.0	3.4
17:0	—	—	0.4	—	—	—	0.2	—	1.4	0.9	1.4	2.9	—	0.3	0.6	1.3
18:0	—	1.5	0.4	0.6	0.5	0.6	1.4	1.3	0.6	0.6	0.2	4.4	—	0.9	0.7	1.1
iso-branched																
i13:0	—	0.5	0.6	—	—	0.3	0.3	0.3	0.5	0.7	0.8	—	—	0.3	0.5	—
i14:0	—	0.5	0.4	—	0.5	0.6	0.6	0.7	1.1	0.9	0.9	—	1.2	1.8	2.6	1.1
i15:0	59.6	53.2	60.0	62.3	16.8	16.7	22.9	18.6	37.0	34.8	35.4	26.7	25.6	28.2	30.3	24.3
i16:0	1.5	1.1	1.1	1.8	2.2	1.8	3.6	2.3	3.5	2.3	4.7	2.9	7.9	6.5	6.9	8.2
i17:0	14.1	5.1	15.2	18.3	8.5	4.4	12.7	16.0	25.7	14.3	32.1	32.7	12.9	6.9	9.2	16.7
i18:0	—	—	0.2	—	—	—	0.3	0.6	—	—	0.4	1.2	3.3	—	2.4	—
i19:0	—	—	—	—	—	—	—	1.4	—	—	—	1.9	—	—	—	—
anteiso-branched																
a15:0	4.8	4.0	4.5	4.6	22.4	22.1	27.2	28.5	9.6	8.3	9.2	7.9	8.3	8.3	10.4	7.6
a17:0	2.1	1.3	1.8	2.7	8.5	5.5	15.8	10.0	6.3	3.6	10.1	5.2	6.8	4.8	5.1	7.2
a19:0	—	—	—	1.1	—	—	—	3.6	—	—	—	—	—	—	—	4.3
Hydroxylated																
3-OH i16:0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.4	—
2-OH i17:0	7.0	24.6	0.4	—	10.5	19.2	1.5	—	7.4	25.1	0.4	—	8.4	24.2	0.6	—
2-OH a17:0	—	—	—	—	5.7	10.4	—	—	—	2.3	—	—	—	1.4	—	—
3-OH i17:0	5.2	0.6	8.9	—	—	—	1.7	—	0.8	—	0.7	—	11.7	1.1	15.9	—
3-OH a17:0	—	—	1.2	—	—	—	0.8	—	—	—	—	—	3.7	0.9	4.4	—
Unsaturated																
i15:1*	1.2	0.6	0.9	1.7	—	—	—	—	—	—	—	—	1.3	0.9	1.3	2.3
16:1 ω 7c alcohol	—	—	—	—	—	—	—	—	—	—	—	—	1.3	1.0	1.1	3.0
i16:1*	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4	0.4	1.4
i17:1 ω 9c	1.1	0.3	0.4	2.0	—	—	—	—	—	—	—	—	4.6	1.9	1.7	9.9
a17:1/i17:1†	—	—	—	—	—	—	—	—	—	—	—	—	—	0.5	0.5	2.0
a17:1 ω 9c	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4	0.4	1.2
17:1 ω 8c	—	—	—	—	—	—	—	—	—	—	—	—	—	0.2	0.2	2.1
Unknown and diols																
Unknown 17:654‡	—	1.1	—	—	2.1	3.7	—	—	1.2	2.2	—	—	—	4.5	—	—
i18:0	0.8	0.3	—	—	12.2	9.1	5.4	6.7	—	—	—	—	—	—	—	—
a18:0	—	—	—	—	4.5	3.2	1.6	3.3	—	—	—	—	—	—	—	—

* The position of the double bond is not known.

† The two fatty acids have the same retention time on GC.

‡ Unknown fatty acid or fatty alcohol with an equivalent chain length of 17:654.

M. chliarophilus (Fig. 2). Abundant B-type glycosidic fragmentations yielded fragment ions at m/z 343 (B_1), 640 (B_2), 1204 (B_3) and 1501 (B_4), which established the sugar sequence as hexose-hexose-*N*-acylhexosamine-hexose. Glycosidic cleavage is particularly favoured at hexosamine, resulting in the prominent B_3 ion at m/z 1204 which identified the *N*-acyl substituent as 2-hydroxy-i17:0; a weak fragment at m/z 1180 indicated that a small amount of 3-hydroxy-i15:0 was also

present. The fragments at m/z 523, 537 and 551 originate by cleavage of the *sn*-3 $\text{CH}_2\text{-O}$ bond of glycerol and (in conjunction with GC/MS data) confirm that the O-acyl substituents are predominantly i15:0 and i17:0, together with about 5% of 16:0. There was no evidence for the acylation of glycerol by hydroxy fatty acids. The greater complexity of the spectra of *M. ruber* and *M. silvanus* GL-1b was largely attributable to the presence of a wider range of amide-linked fatty acids. In the spectrum

Table 2. Composition of the polar head group of the major glycolipids of *Meiothermus* spp.

Strain	Composition (ratio)*				
	Glc	Gal	GlcN	GalN	Gro
<i>Meiothermus ruber</i> DSM 1279 ^T					
GL-1a	2.8	0.0	ND	1.0	1.0
GL-1b	2.9	0.0	ND	1.0	1.0
<i>Meiothermus silvanus</i> VI-R2 ^T					
GL-1a	2.0	1.0	ND	1.0	0.6
GL-1b	2.2	1.0	ND	1.0	0.8
<i>Meiothermus chliarophilus</i> ALT-8 ^T					
GL-1a	1.9	1.0	1.0	ND	0.9
GL-1b	1.7	1.0	1.0	ND	0.9
<i>Meiothermus cerbereus</i> GY-1 ^T					
GL-1a	2.1	1.0	ND	1.0	1.1
GL-1b	2.1	1.0	ND	1.0	1.0

* Glc, glucose; Gal, galactose; GlcN, glucosamine; GalN, galactosamine; Gro, glycerol; ND, not detected.

Table 3. Negative-ion FAB-MS data from *M. silvanus* GL-1a and GL-1b

Composition*	Acyl substituents	[M-H] ⁻ (observed) [†]	[M-H] ⁻ (calculated) [‡]
GL-1a			
Hex-Hex-HexNAcyl-Hex-LCD	15:0; 2-OH 17:0	1424.8	1424.9608
Hex-Hex-HexNAcyl-Hex-Gro	(15:0) ₂ ; 2-OH 16:0	1441.0	1440.9194
Hex-Hex-HexNAcyl-Hex-Gro	(15:0) ₂ ; 2-OH 17:0	1454.9	1454.9351
Hex-Hex-HexNAcyl-Hex-Gro	15:0; 16:0; 2-OH 17:0	1469.0	1468.9507
Hex-Hex-HexNAcyl-Hex-Gro	15:0; 17:0; 2-OH 17:0	1482.8	1482.9664
GL-1b			
Hex-Hex-HexNAcyl-Hex-LCD	(15:0) ₂	1381.0	1380.9347
Hex-Hex-HexNAcyl-Hex-LCD	15:0; 17:0	1409.1	1408.9660
Hex-Hex-HexNAcyl-Hex-Gro	(15:0) ₃	1411.1	1410.9089
Hex-Hex-HexNAcyl-Hex-Gro	(15:0) ₂ ; 16:0	1425.0	1424.9225
Hex-Hex-HexNAcyl-Hex-Gro	(15:0) ₂ ; 17:0	1439.1	1438.9404
Hex-Hex-HexNAcyl-Hex-Gro	15:0; (17:0) ₂	1467.1	1466.9715

* Hex, hexose; HexN, hexosamine; LCD, long-chain diol; Gro, glycerol.

[†] Measured masses of deprotonated molecules in negative-ion spectra of underivatized glycolipids.

[‡] Calculated monoisotopic masses of deprotonated molecules of underivatized glycolipids.

of peracetylated GL-1b from *M. ruber*, B₃ ions were observed at m/z 1088, 1116, 1146 and 1174 (Fig. 3), showing that i15:0, i17:0, 3-hydroxy-i15:0 and 3-hydroxy-i17:0 *N*-acyl groups were present. The abundant fragment at m/z 523 showed that the O-linked acyl chains, however, were predominantly i15:0. The intensities of these and the B₃ ions enabled calculation of the approximate proportions of *N*- and O-acyl groups in the *Meiothermus* spp. glycolipids (Fig. 4). The FAB spectra did not distinguish between 2- and 3-hydroxy fatty acyl groups, but the GC and GC/MS experiments described above indicated that 2-hydroxy fatty acids

were almost exclusively present in GL-1a, whereas GL-1b contained the 3-hydroxy isomers.

Several signals were observed in the spectra of GL-1a and 1b from *M. silvanus*, which had no counterparts in spectra of glycolipids from the other strains. In the negative-ion spectra the most abundant of these (Table 3) were observed at m/z 1424.8 (GL-1a) and m/z 1409.1 and 1381.0 (GL-1b), and were assigned to deprotonated molecules of glycolipids in which the polar head groups are linked to 16-methylheptadecane-1,2-diol rather than to glycerol. This is supported by the positive-ion spectra

Fig. 2. Positive-ion FAB mass spectrum of perdeuteroacetylated GL-1a from *M. chliarophilus*. The structure depicts the species with $[M+Na]^+$ at m/z 2092. The signals at m/z 2064 and 2078 represent $[M+Na]^+$ ions of glycolipids in which the *O*-acyl groups are 15:0/15:0 and 15:0/16:0, respectively. The absence of significant B_3 fragments other than m/z 1204 indicates that there is little *N*-acylation heterogeneity (the signal at m/z 1078 represents sequential loss of two deuterioacetate groups).

of their acetyl derivatives in which abundant fragments at m/z 493, originated by loss of the glycan head group from the lipid moiety, show that the diol was *O*-acylated with (mostly) i15:0. The masses of their molecular ions and B_3 carbenium fragments indicate that the diol-linked GL-1a glycolipids were predominantly *N*-acylated with 2-hydroxy-i17:0, whereas the GL-1b compounds had a mixture of i17:0 and 3-hydroxy-i17:0.

The terminal monosaccharides of the glycolipids were identified by collisional activation of the B_1 carbenium ions at m/z 331 and 343 in the acetyl and deuterioacetyl derivatives, respectively (Carreto *et al.*, 1996; Richter *et al.*, 1990). All the resulting daughter ion spectra were identical to those from authentic standards of glucopyranose and distinct from galactopyranose and galactofuranose reference spectra.

Since the FAB spectra fix the position of hexosamine, the sugar sequence of the glycolipids from *M. ruber* could be completely defined (Fig. 4). In the compounds from *M. cerbereus*, *M. silvanus* and *M. chliarophilus*, however, only the positions of the hexosamine and the terminal glucose were unambiguously specified, since the FAB data do not establish the relative locations of the remaining glucose and galactose residues. In the related glycolipids from *Thermus* spp., whenever the identity of

the innermost residue (i.e. that linking hexosamine to the *sn*-3 position of glycerol) has been determined, it has proved to be glucose (Carreto *et al.*, 1996; Oshima & Yamakawa, 1974) and we have provisionally assumed that this is also true for *Meiothermus* glycolipids.

DISCUSSION

The observation of two glycolipid bands on TLC of the polar lipids is characteristic of all the *Meiothermus* strains examined (Chung *et al.*, 1997; Donato *et al.*, 1991; Tenreiro *et al.*, 1995) and was one of the features originally used to establish *Meiothermus* as a genus separate from *Thermus* (Nobre *et al.*, 1996b). The results presented here show that both glycolipid bands contain diglycosyl-(*N*-acyl)glycosaminylglucosyl diacylglycerols, which are structurally related to the major glycolipid of *Thermus* strains. Within a given strain the monosaccharide compositions and sequences of GL-1a and 1b were identical, the only detectable differences being the pattern of *N*-acylation of the hexosamine. Since the monosaccharide sequence of the polar head group of *M. ruber* is identical to that of GL-1 of *T. aquaticus* strain YT-1 (Carreto *et al.*, 1996) and that of *M. chliarophilus* to those of GL-1 from *T. scotoductus* strains X-1 and CG-2 (Carreto *et al.*, 1996; Wait *et al.*, 1997), it follows that the distinct TLC mobilities of the

Fig. 3. Positive-ion FAB mass spectrum of peracetylated GL-1b from *M. ruber*. The insets show expansions of the $[M+Na]^+$ signals (m/z 1939–2025) and the B_3 carbenium ions (m/z 1088, 1116 and 1174). The structure depicts the species with $[M+Na]^+$ at 2025. The signals at m/z 1939 and 1967 are the $[M+Na]^+$ ions of glycolipids *N*-acylated with 15:0 and 17:0, respectively. The low abundance of the fragment at m/z 551 indicates that there is little *O*-linked 17:0.

Meiothermus glycolipids must be attributable to differences in the lipid domains of the molecules. Nevertheless, the slightly greater R_F of both glycolipid bands from *M. chliarophilus* was presumably due to the presence of glucosamine rather than galactosamine in the polar head group which was otherwise identical in sequence to the glycolipids from *M. silvanus* and *M. cerbereus*. Different hexose or hexosamine compositions also influence the TLC behaviour of GL-1 from *Thermus* strains (unpublished results).

We have shown previously that hydroxy fatty acids are constituents of the major glycolipid of strains of *T. aquaticus* and the type strain of *T. filiformis* (Carreto *et al.*, 1996; Nobre *et al.*, 1996a). These are invariably 3-hydroxy isomers and are exclusively amide-linked to galactosamine, while glycolipids from strains of the genus *Thermus* in which glucosamine replaces galactosamine in the polar head group contain only non-hydroxylated *N*-acyl groups.

In this study we have shown that glycolipids from *Meiothermus* strains contain both 2- and 3-hydroxyacyl groups and that 2-hydroxy-acid-containing glycolipids can be separated from their 3-hydroxy-acid-containing counterparts by TLC. However, under the analytical conditions used, the latter co-migrate with glycolipids in which hexosamine is *N*-acylated with non-hydroxylated substituents and cannot be recovered in pure form.

Thus, the slower-migrating GL-1a glycolipids have hexosamine exclusively *N*-acylated with 2-hydroxy fatty acids, but depending on the strain, GL-1b may comprise glycolipids *N*-acylated with 3-hydroxy fatty acids, non-hydroxylated fatty acids or a mixture of both. In *M. cerbereus*, for example, 3-hydroxy fatty acids are the sole amide-linked acyl groups of GL-1b, whereas GL-1b glycolipids from *M. silvanus* and *M. chliarophilus* have, almost exclusively, non-hydroxylated *N*-acyl groups, and in *M. ruber* a mixture of 3-hydroxy and non-hydroxylated 15:0 and 17:0 acyl components are present.

The GL-1a from *Meiothermus* spp. have 2-hydroxy-*N*-acyl groups, irrespective of whether they contain glucosamine or galactosamine, suggesting that the enzyme which adds 2-hydroxyacyl groups is less stringent in its acceptor requirements than the one responsible for addition of 3-hydroxyacyl moieties in *Thermus* strains, which seems to have an absolute specificity for galactosamine. Interestingly, 3-hydroxy-*N*-acyl chains are largely absent from the only glucosamine-containing glycolipids detected, those from *M. chliarophilus*, suggesting that the enzyme responsible for 3-hydroxyacylation in *Meiothermus* spp. has the same restrictive acceptor specificity as in *Thermus* strains. Thus it seems that in *Meiothermus* glycolipids, 2-hydroxy, 3-hydroxy and non-hydroxylated fatty acids

		Fatty acids (% of total)			
		GL-1a		GL-1b	
		R ¹	R ²	R ¹	R ²
<i>M. ruber</i>					
$ \begin{array}{c} \text{CH}_2 - \text{R}^2 \\ \\ \text{CH} - \text{R}^2 \\ \\ \text{Glc} - \text{Glc} - \text{GalNR}^1 - \text{Glc} - \text{O} - \text{CH}_2 \end{array} $	15:0	—	89	30	91
	16:0	—	3	—	3
	3-OH 15:0	—	—	13	—
	17:0	—	8	27	8
	2-OH 17:0	>90	—	—	—
	3-OH 17:0	—	—	30	—
<i>M. cerbereus</i>					
$ \begin{array}{c} \text{CH}_2 - \text{R}^2 \\ \\ \text{CH} - \text{R}^2 \\ \\ \text{Glc} - \text{Gal} - \text{GalNR}^1 - \text{Glc} - \text{O} - \text{CH}_2 \end{array} $	15:0	—	72	—	76
	16:0	—	10	—	10
	17:0	—	18	—	14
	2-OH 17:0	>90	—	—	—
	3-OH 17:0	—	—	>90	—
<i>M. silvanus</i>					
$ \begin{array}{c} \text{CH}_2 - \text{R}^2 \\ \\ \text{CH} - \text{R}^2 \\ \\ \text{Glc} - \text{Gal} - \text{GlcNR}^1 - \text{Glc} - \text{O} - \text{CH}_2 \end{array} $	15:0	—	75	43	77
	16:0	—	5	10	6
	17:0	—	20	42	17
	2-OH 16:0	12	—	—	—
	2-OH 17:0	83	—	—	—
	3-OH 17:0	—	—	5	—
<i>M. chliarophilus</i>					
$ \begin{array}{c} \text{CH}_2 - \text{R}^2 \\ \\ \text{CH} - \text{R}^2 \\ \\ \text{Glc} - \text{Gal} - \text{GlcNR}^1 - \text{Glc} - \text{O} - \text{CH}_2 \end{array} $	15:0	—	74	—	72
	16:0	—	5	—	4
	17:0	—	20	>90	23
	2-OH 17:0	>90	—	—	—
	3-OH 17:0	—	—	—	—

Fig. 4. Structures of the glycolipids from *Meiothermus* spp., showing the acylation patterns deduced from the FAB-MS results.

can be N-linked to galactosamine, whereas glucosamine can be N-acylated only by 2-hydroxy and non-hydroxylated fatty acids.

The mechanism of glycolipid biosynthesis in *Thermus* and *Meiothermus* has not been investigated; however, because the different N-acylation patterns observed are stable and reproducible, it seems unlikely that they represent an adaptive response to varying environmental conditions.

In contrast to the other strains investigated in this study, a proportion of the glycolipids from *M. silvanus* were linked to 1,2-alkanediols, rather than to glycerol. Recently, we identified similar compounds in *T. scotoductus* X-1 and *T. filiformis* Tok4 A2 and showed that they had similar polar head groups to the glyceroglycolipids from these strains (Wait *et al.*, 1997). In all other *Thermus* spp. examined 1,2-diol-containing glycolipids were minor components or were undetectable. Long-chain 1,2-diols were first described as constituents of polar lipids of undefined structure from *Thermomicrobium roseum* (Pond *et al.*, 1986), where they apparently completely replace glycerolipids. In *Thermus* strains however, 1,2-diol-linked glycolipids are always accompanied by glycerolipids, from which they are difficult to separate by TLC. The discontinuous dis-

tribution of 1,2-diol-linked glycolipids within the genera *Thermus* and *Meiothermus* and their lack of correlation to optimum growth temperature suggest that they do not represent a specific adaptation to growth at high temperatures. The occurrence of diols in the *Thermus/Meiothermus* line of descent and also in *Thermomicrobium roseum* is thus consistent with the distant phylogenetic affinity between these organisms which has been inferred from 16S rDNA studies (Embley *et al.*, 1993; Hartmann *et al.*, 1989; Van de Peer *et al.*, 1994).

In this study we have characterized the glycolipids from all four currently recognized species of the genus *Meiothermus*. The presence of two TLC-resolvable glycolipid bands is attributable to molecular species containing 2-hydroxyacyl substituents N-linked to hexosamine, which are more strongly retained by silica TLC plates than 3-hydroxy- and non-hydroxylated N-acylated glycolipids of otherwise identical structure, enabling chromatographic separation of this subpopulation of the total glycolipid pool.

Although no distinct physiological role has been assigned to these compounds, the presence of 2-hydroxy-N-acyl substituted glycolipids appears to be a stable phenotypic marker that distinguishes the genus *Meiothermus* from *Thermus*.

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