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Structural determination of the polar glycoglycerolipids from thermophilic bacteria *Meiothermus taiwanensis*

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The polar glycolipids were isolated from the thermophilic bacteria *Meiothermus taiwanensis* ATCC BAA-400 by ethanol extraction and purified by Sephadex LH-20 and silica gel column chromatography. The fatty acid composition of O-acyl groups in the glycolipids was obtained by gas chromatography mass spectroscopy analysis on their methyl esters derived from methanolysis and was made mainly of $C_{15:0}$ (34.0%) and $C_{17:0}$ (42.3%) fatty acids, with the majority as branched fatty acids (over 80%). Removal of O-acyl groups under mild basic conditions provided two glycolipids, which differ only in N-acyl substitution on a hexosamine. Electrospray mass spectroscopy analysis revealed that one has a $C_{17:0}$ N-acyl group and the other hydroxy $C_{17:0}$ in a ratio of about 1 : 3.5. Furthermore, complete de-lipidation with strong base followed by selective N-acetylation resulted in a homogeneous tetraglycosyl glycerol. The linkages and configurations of the carbohydrate moiety were then elucidated by MS and various NMR analyses. Thus, the major glycolipid from *M. taiwanensis* ATCC BAA-400 was determined with the following structure: α-Galp(1-6)-β-Galp(1-6)-β-GalNAcyl(1,2)-α-Glc(1,1)-Gro diester, where N-acyl is $C_{17:0}$ or hydroxy $C_{17:0}$ fatty acid and the glycerol esters were mainly iso- and anteisobranched $C_{15:0}$ and $C_{17:0}$.

Keywords: glycolipid; *Meiothermus taiwanensis*; MS; NMR; thermophilic bacteria.

The thermophilic bacteria such as *Aquifex pyrophilus*, *Thermodesulfotobacterium commune*, *Thermus scotoductus*, *Thermomicrobium roseum* and *Thermodesulfatator indicus* contain unique polar lipids as major membrane components [1–8]. Those lipids are essential for the thermal stability and biological functions of the bacteria in extreme environments [9–11]. The polar lipids found in *Thermus aquaticus*, *Thermus filiformis*, *Thermus scotoductus*, and *Thermus oshimai* were mostly phospholipids and glycolipids [12], and the glycolipids from *Thermus* species examined thus far usually contain three hexoses, one *N*-hexosamine, and one glycerol [7,10,12–15]. Although the sequences of those carbohydrate moieties have been studied by chemical and mass spectroscopic analysis, no complete structure is available as yet due to the lack of information on the linkages and configurations of the carbohydrate moiety. We have been working on a newly discovered species of thermophilic bacteria, *Meiothermus taiwanensis*, recently isolated from the Wu-rai hot spring in Taiwan [16], as part of our program to investigate the immunomodulation activity of the glycolipids and the structure–activity relationship. In this study, we determined the structure of a major glycolipid from the thermophilic bacteria *M. taiwanensis* ATCC BAA-400. The fatty acids were examined by gas chromatography mass spectroscopy (GC-MS) analysis on their methyl esters derived from methanolysis, whereas, the structure of the carbohydrate moiety was elucidated by MS/MS and NMR spectroscopic analyses. To the best of our knowledge this is the first complete glycolipid structure from thermophilic bacteria.

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Abbreviations: HMBC, heteronuclear multiple quantum coherence; HSQC, heteronuclear single quantum coherence; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotational frame nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy; HPAEC-PAD, high performance anion exchange chromatography with pulsed amperometric detection; GC-MS, gas chromatography mass spectroscopy; ES-MS, electrospray mass spectroscopy; CE-MS, capillary electrophoresis mass spectroscopy; MALDI, matrix-assisted laser desorption ionization; FAMEs, fatty acid methyl esters; TMS, trimethylsilylated.

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Materials and methods

Isolation and purification of the glycolipids

M. taiwanensis ATCC BAA-400 (Wu-rai hot spring, Taiwan) was grown aerobically in *Thermus* modified medium [14,16] at 55 °C and harvested until the late exponential phase ($D_{660} = 1.6$). A suspension of wet bacteria in absolute ethanol (1 : 10, w/v, Riedel-de-Haën, Germany) was shaken at room temperature for 2 h. After centrifugation, the supernatant was collected, concentrated, and purified through a Sephadex LH-20 column (Amersham Pharmacia, 80 × 1.1 cm) eluted with methanol. The glycolipids

obtained above were further purified on a silica gel G-60 (Merck, Darmstadt, Germany) chromatography eluted by a chloroform/methanol gradient from 20 : 1 to 3 : 1. The carbohydrate-containing fractions were detected by TLC stained with a molybdate solution [0.02 M ammonium cerium sulfate dihydrate/ammonium molydate tetrahydrate in aqueous 10% (w/v) H_2SO_4] and collected. The glycolipids were still heterogeneous according to the MS analysis due to the variations in lipids, and soluble in neither water nor chloroform.

Chemical modification

De-O-acylation. Glycolipids from silica gel purification were treated with 1% (w/v) NaOMe/MeOH at room temperature for 5 h. The mixture was neutralized by the addition of Dowex 50 (H^+) resin (Acros, NJ, USA) and the filtrate was concentrated. Purification by silica gel G-60 chromatography (MeOH/$CHCl_3$, 1 : 3) gave de-O-acylated glycolipids.

Per-acetylated glycosyl glycerol. The glycolipids were treated with 2 M NaOH at 100 °C for 8 h to remove both O- and N-acyl groups; neutralization of the reaction mixture by acetic anhydride resulted in partial N-acetylation. The precipitates were removed by centrifugation (3000 ***g***, 15 min, room temperature), and the supernatant containing sugar was collected and lyophilized. The above sample was then treated with Ac_2O/pyridine (1 : 2) at room temperature for 1 h. The reaction was quenched by the addition of MeOH, and the mixture was concentrated to a residue. Purification by silica gel G-60 chromatography (EtOAc/hexanes, 2 : 1) gave the per-acetylated glycosyl glycerol.

N-Acetyl glycosyl glycerol. De-O-acetylation was performed on per-acetylated glycosyl glycerol by treatment with 0.01 M NaOMe/MeOH at room temperature for 3 h. The solution was neutralized by the addition of Dowex 50 (H^+) resin and concentrated to a residue. A solution of the above sample in water was passed through a Sephadex G-10 column using water as eluent. The fractions were collected and lyophilized to give *N*-acetyl glycosyl glycerol.

Composition and linkage analyses

The fatty acid composition of the O-acyl groups in the glycolipid was determined by comparing the retention times of FAMEs (fatty acid methyl esters) from glycolipids to the standards in GC-MS analysis. The methyl esters were prepared by treatment of the glycolipids with 0.5 M HCl/MeOH at 80 °C for 1 h. Solvent was removed under a nitrogen stream, and the residue was partitioned between $CHCl_3$ and H_2O. FAMEs in organic phase were analyzed by GC-MS. The fatty acid composition of the N-acyl group in the glycolipid was determined by the MS analysis of de-O-acylated glycolipid.

The sugar composition analysis was determined by either GC-MS or high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex, CA, USA). The GC-MS analyses of glycolipid or *N*-acetyl glycosyl glycerol were performed by methanolysis with 0.5 M methanolic/HCl at 80 °C for 16 h, re-N-acetylation with pyridine/acetic anhydride (in low temperature with equivalent quantity of acetic anhydride), and trimethylsilylation with Sylon HTP (HMDS/TMCS/pyridine, 3 : 1 : 9) trimethylsilylation reagent (Supelco, PA, USA). The final trimethylsilylated (TMS) derivatives were kept in n-hexane for GC-MS analysis. For the HPAEC-PAD analysis, *N*-acetyl glycosyl glycerol was subjected to acidic hydrolysis (2 M trifluoroacetic acid at 100 °C for 5 h) to release monosaccahrides, which were then analyzed by HPAEC-PAD.

For the carbohydrate linkage analysis, the Hakomori methylation analysis [17] was carried out. The glycolipid or *N*-acetyl glycosyl glycerol was per-O-methylated with methyl iodide and dimethylsulfoxide anion in dimethylsulfoxide, and then hydrolyzed by 2 M trifluoroacetic acid at 100 °C for 5 h. The solvent was evaporated by compressed air, the residue was reduced with 0.25 M $NaBD_4$ in 1 M NH_4OH for 40 min. The reaction was quenched with 20% HOAc and coevaporated with MeOH. The residue was then per-acetylated with Ac_2O/pyridine (1 : 1, v/v) overnight, dried with toluene, and finally analyzed by GC-MS.

Analytical methods

GC-MS was carried out on a Hewlett Packard Gas Chromatography HP6890 connected to an HP5973 Mass Selective Detector. The HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., Hewlett Packard) at 60 °C was used. The programs for analyses of TMS and FAMEs were set up at 60 °C for 1 min, increasing to 140 °C at 25 °C·min^{-1}, to 200 °C at 5 °C·min^{-1}, and finally to 300 °C at 10 °C·min^{-1}. For partial methylated aditol acetates derivatives, the oven was programmed at 60 °C for 1 min before increasing to 290 °C at 8 °C·min^{-1}, and finally to 300 °C at a rate of 10 °C·min^{-1}. Peaks were analyzed by GC-MS and compared with the database. Also, the arabitol derivative was used as an internal standard.

HPAEC-PAD analysis was used to determine the sugar composition. The hydrolysates from *N*-acetyl glycosyl glycerol were analyzed by HPAEC-PAD in a DX-500 BioLC system, which included a GP40 gradient pump, an ED40 electrochemical detector (PAD detection) with a working gold electrode, an LC30 column oven, and an AS3500 autosampler. The Dionex Eluant Degas Module was employed to purge and pressurize the eluants with helium. The monosaccharides were separated on Carbopac PA10 analytical column (4 × 250 mm) with Carbopac PA10 Guard (4 × 50 mm) column, flowing at a rate of 1 mL·min^{-1} at 30 °C, and detected by following pulse potentials and durations: $E_1 = 0.05$ V (0.4 ms); $E_2 = 0.75$ V (0.2 ms); and $E_3 = -0.15$ V (0.4 ms). The integration was recorded from 0.2 to 0.4 ms during the E_1 application.

NMR analysis

NMR analytic conditions for carbohydrate analysis were carried out based on approaches reported previously [18,19]. NMR spectra were recorded in D_2O (0.6 mL) with a Varian INOVA-500 spectrometer at 298 K with standard pulse sequences provided by Varian. Chemical shifts ^1H and ^{13}C were given in p.p.m. relative to HDO (4.75 p.p.m.) and external methanol-d_4 (49.15 p.p.m.), respectively. 1D total

correlation spectroscopy (TOCSY) spectra were recorded with mixing times (20 ms, 100 ms, and 180 ms) which allowed the assignment of the protons H-1 to H-4 for Gal and GalNAc, and H-1 to H-6 for Glc. Four anomeric protons were selected in respective 1D TOCSY experiments. 2D heteronuclear multiple quantum coherence (gradient HMBC) and heteronuclear single quantum coherence (gradient HSQC) spectra were performed with H-C coupling constants at both 8 Hz/140 Hz and 5 Hz/150 Hz. Rotational frame nuclear Overhauser effect spectroscopy (ROESY) spectrum was obtained with mixing time 200 ms 2D nuclear Overhauser effect spectroscopy (NOESY) spectra were obtained with mixing time 300 ms and 500 ms.

Mass analysis

MALDI mass spectroscopy. Glycolipids from silica gel purification were dissolved in CH_3OH and analyzed by a MALDI-TOF mass spectrometer (MALDI^{TM}; Micromass, Manchester, UK). Mass spectra were acquired for the mass range of 600–2000 Da under a pulsed nitrogen laser of wavelength 337 nm. Cyano-4-hydroxycinnamic acid was used as matrix.

CE-MS and MS/MS. A crystal Model 310 CE instrument (ATI Unicam, Boston, MA, USA) was coupled to an API 3000 mass spectrometer (MDS/Sciex, Concord, ON, Canada) via a microionspray interface. A sheath solution (isopropanol/methanol, 2 : 1) was delivered at a flow rate of 1 $\mu L \cdot min^{-1}$ to a low dead volume tee (250 μm i.d., Chromatographic Specialities, Brockville, ON, Canada). All aqueous solutions were filtered through a 0.45-μm filter (Millipore, Bedford, MA, USA) before use. An electrospray stainless steel needle (27 gauge) was butted against the low dead volume tee and enabled the delivery of the sheath solution to the end of the capillary column. The separation was obtained on about 90 cm length bare fused-silica capillary using 10 mM ammonium acetate/ammonium hydroxide in deionized water, pH 9.0, containing 5% (v/v) methanol. A voltage of 20 kV was typically applied at the injection. The outlet of the capillary was tapered to ≈ 15 μm i.d. using a laser puller (Sutter Instruments, Novato, CA, USA). Mass spectra were acquired with dwell times of 3.0 ms per step of 1 *m/z* unit in full-mass scan mode. For capillary electrophoresis mass spectroscopy (CE-ESMS) experiments, about 30 nL sample was introduced using 4.35 PSI for 0.1 min. The MS/MS data were acquired with dwell times of 3.0 ms per step of 1 *m/z* unit. Fragment ions formed by collision activation of selected precursor ions with nitrogen in the RF-only quadrupole collision cell, were analyzed by scanning the third quadrupole.

Results and Discussion

Sugar/fatty acid compositions and sugar linkage analysis

The fatty acid composition of the O-acylated groups linked on glycerol part of the glycolipid was determined by GC-MS analysis on FAMEs derived from glycolipid by methanolysis in 0.5 M HCl/MeOH. Quantitative analysis indicated that the glycolipid contains mainly isobranched (61.7%) and

Table 1. The O-acylated fatty acids present in the glycoglycerolipids from *Meiothermus taiwanensis* ATCC BAA-400.

Fatty acids	Composition (%)
Straight chain	
15:0	3.5
16:0	2.4
17:0	6.6
Isobranched	
14:0	1.3
15:0	22.8
16:0	12.3
17:0	23.7
18:0	1.6
Anteisobranched	
15:0	7.7
17:0	12.0
Unsaturated	
17:1	1.0
Unknown	4.1

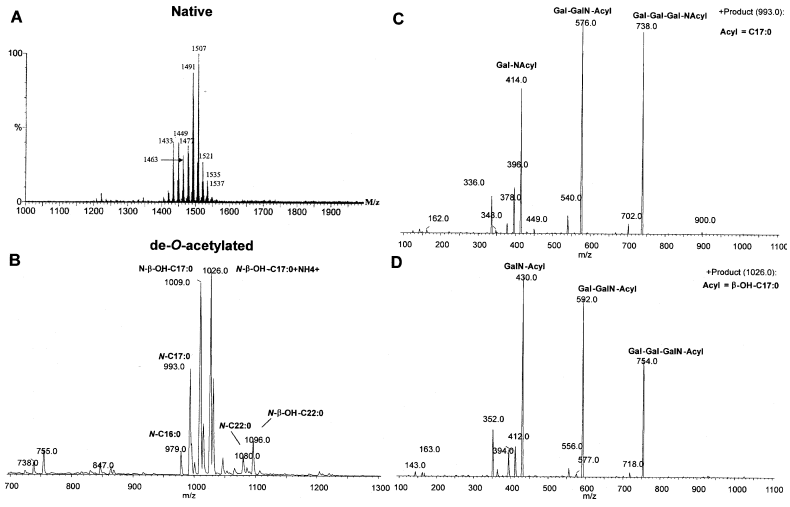

Fig. 1. MS Spectra of native and de-O-acetylated glycolipids. (A) MALDI-TOF MS (+ev) of native glycolipids from *Meiothermus taiwanensis* ATCC BAA-400. A cluster of peaks was observed due to the fatty acid heterogeneity. The peak at *m/z* 1491 ($M + Na^+$) represents a glycolipid with three hexoses, one hexosamine, one glycerol, and three fatty acids (two $C_{17:0}$ and one $C_{15:0}$ lipids), and the glycolipid at *m/z* 1507 ($M + Na^+$) contains one $C_{17:0}$, one hydroxy-$C_{17:0}$ and one $C_{15:0}$. (B) ES-MS (+ev) spectra of de-O-actylated glycolipids, *m/z* 993 ($M_1 + H^+$) and 1009 ($M_2 + H^+$) and *m/z* 1026 ($M_2 + NH_4^+$). (C) MS/MS analysis of peak 1026 (in B) and (D) MS/MS analysis of peak 993 (in B).

anteisobranched (19.7%) fatty acids. Over 80% of fatty acids were $C_{15:0}$ and $C_{17:0}$ (Table 1). The fatty acid composition of the N-acylated group will be discussed later.

Compositional analysis of sugar was independently performed using two methods. One was based on HPAEC-PAD analysis on the acid hydrolyzates of the *N*-acetyl glycosyl glycerol. Glucose, galactose, and galactosamine were found to be in a ratio of 1 : 2 : 1. The other followed a standard methanolysis/trimethyl-silylation procedure, by which we analyzed the TMS methylated sugar alditol acetates by GC-MS and compared with the standard profiles for quantitative and qualitative measurement.

In addition, to confirm the sugar composition, the sugar linkage analysis also indicated that the glycolipid contains one terminal galactopyranose (t-Gal-1-), one 1,6-linked galactopyranose (-6-Gal-1-), one 1,6-linked galactopyranosamine (-6-GalNAc-1-), and one 1,2-linked glucopyranose (-2-Glc-1-). All sugar residues in the glycolipids are pyranoses.

N-Amide and sugar sequence

MALDI-TOF mass spectroscopic analysis of the glycolipids showed a cluster of peaks at *m/z* (+ev) 1433, 1449, 1463, 1477, 1491, and 1507 with mass differences of 14 and 16 (Fig. 1A), which probably resulted from the heterogeneity of fatty acids. On the other hand, the ES-MS spectrum (Fig. 1B) obtained from the de-O-acylated glycolipid (see above) was simpler, showing major peaks at *m/z* (+ev) 993, 1009 and 1026. In fact *m/z* 1009 and 1026 were derived from the same molecule but only differently ionized as they provided identical fragmentation in MS/MS experiments. One (*m/z* 1009) represents ($M + H^+$), and the other (*m/z* 1026) probably added an ammonium ion ($M + NH_4^+$) from the buffer used in MS analysis (Fig. 1B). A comparison of the daughter ions from MS/MS analysis on *m/z* 1026 and 993 revealed a difference of *m/z* 16 on all major fragments as indicated in Fig. 1C,D. Two ions, *m/z* 414 and 430, from *N*-acyl hexosamine are indicative that the hexosamine was acylated by two major fatty acids, $C_{17:0}$ (*m/z* 414) and hydroxy (presumably 3-hydroxy) $C_{17:0}$ (*m/z* 430). The ratio of the $C_{17:0}$ and hydroxy $C_{17:0}$ is approximately 1 : 3.5 according to relative peak heights in MS spectrum. Small amounts of other N-acyl lipids in glycolipid were also detected, e.g. $C_{16:0}$ (*m/z* 979), $C_{22:0}$ (*m/z* 1080), and hydroxy $C_{22:0}$ (*m/z* 1096) (Fig. 1B). The lack of adequate detection of N-acyl lipids was due to the relative stability of the amide bond under methanolysis conditions. The significant amount of hydroxy fatty acids presented in this glycolipid as amide linked to galactosamine is similar to those of *T. filiformis* and *T. aquaticus* [15].

N-Acetyl glycosyl glycerol was obtained by the total deacylation, full acetylation and de-O-acetylation of the glycolipid (see above). The ES-MS spectrum of *N*-acetyl

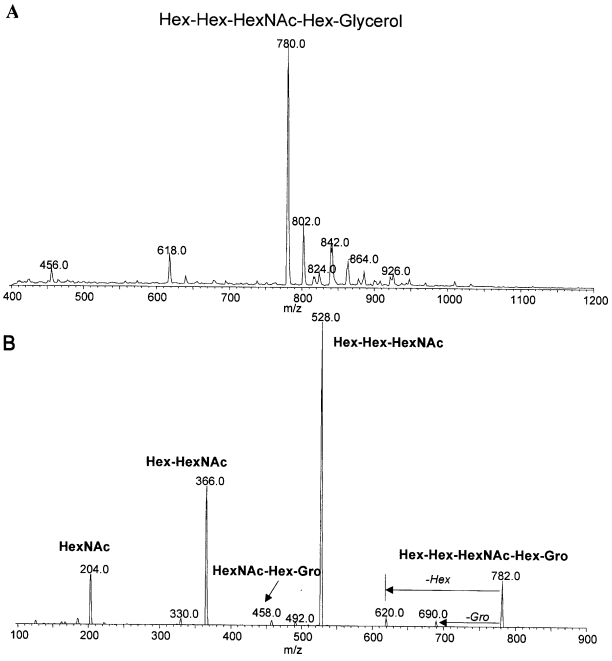

Fig. 2. Mass spectra of the N-acetyl glycolipids. (A) ES-MS spectrum (–ev) of the *N*-acetyl tetraglycosyl glycerol derived from the major glycolipids of *Meiothermus taiwanensis* ATCC BAA-400. Both O- and N-acyl groups were removed and the amino group was acetylated. (B) MS/MS spectra (+ev) revealed the sugar sequence of the tetraglycosyl glycerol.

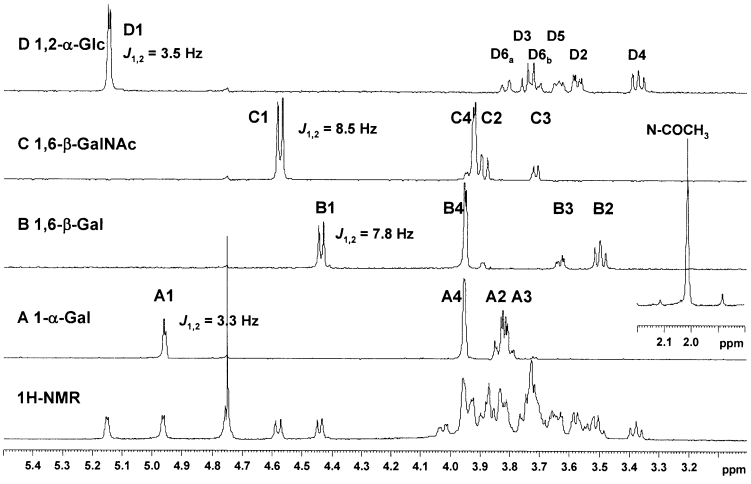

Fig. 3. The 500 MHz spectra of ^{1}H NMR and 1D TOCSY of the *N*-acetyl tetraglycosyl glycerol. Four anomeric protons were irradiated in respective 1D TOCSY experiments. Chemical shifts of the anomeric protons were assigned as following: t-α-Gal at δ 4.96 ($J_{1,2}$ = 3.3 Hz), 1,6-β-Gal at 4.44 ($J_{1,2}$ = 7.8 Hz), 1,6-β-GalNAc at δ 4.58 ($J_{1,2}$ = 8.5 Hz), and 1,2-α-Glc at 5.15 ($J_{1,2}$ = 3.5 Hz) p.p.m.

glycosyl glycerol (Fig. 2A) showed a major peak at *m*/*z* (–ev) 780.0 with minor peaks at 618.0 (- Hex) and 456.0 (-2Hex). MS/MS analysis on the major ion, *m*/*z* (+ev) 782, provided more detailed information on the sequence of the carbohydrate moiety (Fig. 2B). Because the breakup of the HexNAc glycosidic bond often produces a relatively stable positive-charged oxazoline-like fragment, the high intensity peaks at *m*/*z* 204, 366 and 528 were indicative that those fragments contain HexNAc at the reducing end. On the other hand, the observation of *m*/*z* 620 (M-Hex) and 458 (M-2Hex, HexNAc-Hex-Gro) as daughter ions suggested Hex-Hex at the nonreducing end. Further ES-MS/MS analysis of these daughter ions (*m*/*z* 620 and 458) was performed and the results were consistent with the following tetraglycosyl glycerol sequence: Hex-Hex-HexNAc-Hex-Gro. This sequence is similar to the ones previously reported with some strains of thermophilic eubacterial genus *T. aquaticus* and *T. filiformis* [15].

Glycosyl linkage and anomeric configuration

With the solid results of the sugar composition and sequence, the linkages and configurations of glycosidic bonds would be investigated by NMR to determine the complete structure of the glycolipid. A clean ^{1}H-NMR spectrum of the tetraglycosyl glycerol is shown in Fig. 3. Four H-1 anomeric proton signals were observed as expected and their configurations could be identified by their coupling constants. 1D-TOCSY experiments further indicated that they represent the anomeric protons of α-Glc (5.15 p.p.m., $J_{1,2} = 3.5$ Hz), α-Gal (4.96 p.p.m., $J_{1,2} = 3.3$ Hz), β-GalNAc (4.58 p.p.m., $J_{1,2} = 8.5$ Hz) and β-Gal (4.44 p.p.m., $J_{1,2} = 7.8$ Hz), respectively [19]. Based on 1D-TOCSY spectra, the chemical shifts of Glc residue from H-1 to H-6 and those of Gal and GalNAc residues from H-1 to H-4 were able to be assigned (Fig. 3). The H-5 protons of β-Gal and β-GalNAc were assigned based on NOE interaction to H-1 by NOESY or ROESY experiments (data not shown). ^{13}C chemical shifts were obtained from HSQC experiment and both ^{1}H and ^{13}C chemical shifts are summarized in Table 2. Six methylene carbons (-CH_2-O-) were detected as negative peaks in the HSQC experiment.

Table 2. NMR data of the tetraglycosyl glycerol derived from the major glycolipid from *Meiothermus taiwanensis* ATCC BAA-400. In p.p.m. from the HSQC spectrum obtained in D2O at 25 °C.

Residue	Atom	δ_H	δ_C
A	1	4.96	98.5
α-Gal(1→}	2	3.82	68.5
	3	3.83	69.6
	4	3.96	69.4
	5	3.96	71.2
	6	3.72	61.3
B	1	4.44	103.5
6)-β-D-Galp(1→}	2	3.51	70.9
	3	3.64	72.9
	4	3.96	68.9
	5	3.87	73.1
	6	3.87, 3.71	66.5
C	1	4.58	103.4
6)-β-GalNAc(1→}	2	3.90	52.8
	3	3.72	71.1
	4	3.93	68.0
	5	3.84	73.8
	6	4.02, 3.87	69.5
	NAc	2.01	22.4
D	1	5.15	98.5
2)-α-Glc(1→}	2	3.59	80.9
	3	3.75	71.8
	4	3.38	70.1
	5	3.65	71.9
	6	3.82, 3.72	60.7
E	1	3.53, 3.74	69.2
1)-Glycerol	2	3.94	70.5
	3	3.58, 3.68	62.8

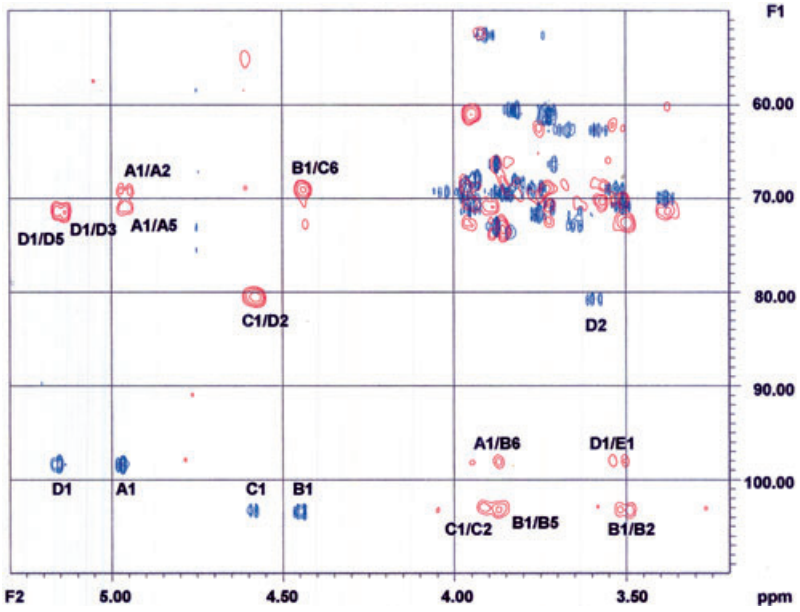

Fig. 4. 2D gHSQC (red) and gHMBC (blue) spectra of the *N*-acetyl tetraglycosyl glycerol were used to assign the glycosyl linkages and configurations.

Table 3. HMBC and NOE correlations observed in the tetraglycosyl glycerol.

Residue	From proton	NOE to protons	HMBC to carbons
α-Gal1- (A)	A1	A2, B6a, 6b	A2, A5, B6
-6-β-Gal1- (B)	B1	B2, B5, C6a, 6b	C6
-6-β-GalNAc-1- (C)	C1	C2, D2, D3	D2
-2-α-Gal1- (D)	D1	D2, E1a,1b	D3, D5, E1
$OCH_2CH(OH)CH_2OH$ (E)	E1		E2, E3
	E2		E1, E3

Three of them (δ_C 66.5, 69.2, and 69.5) were in residues in which a glycosyl substituent was present at O_6, and the other three (δ_C 60.7, 61.3, and 62.8) were in residues in which an unsubstituted hydroxyl group was present at O_6. The interglycosidic linkages were determined based on the HMBC (Fig. 4, Table 3) and NOE interactions (Table 3), the terminal Gal (A) was α-(1-6)-linked to Gal (B) because of the NOE and HMBC correlations observed between H-1 of Gal (A) and H-6 and C-6 of Gal (B). Similarly, the Gal (B) was assigned to be β-(1-6)-linked to GalNAc (C), which was then β-(1-2)-linked to Glc (D) based on both NOE and HMBC correlations. Finally, the Glc (D) at the reducing end was then α-(1-1)-linked to glycerol (E).

Based on all the information obtained from sugar and fatty acid composition analyses and MS and NMR experiments, we are able to report the major glycolipid from thermophilic bacteria *M. taiwanensis* ATCC BAA-400 having the following structure: α-Galp(1-6)-β-Galp(1-6)-β-GalNAcyl(1-2)-α-Glc(1-1)Gro diester, where, the N-acyl lipids were mainly $C_{17:0}$ and hydroxy $C_{17:0}$ fatty acids, and the glycerol diester was mainly made of branched $C_{15:0}$ and $C_{17:0}$ fatty acids.

The monosacchrides in the major glycoglycerolipids of *Meiothermus ruber*, *M. silvanus*, *M. chliarophilus*, and *M. cerbereus* are two or three glucoses, one galactose, either one galactosamine or one glucosamine, and glycerol [15]. In *M. taiwanensis* ATCC BAA-400 glycoglycerolipids, there are two galactoses, one glucose, one galactosamine, and one glycerol, which is different from other *Meiothermus*, but similar to its relative genus *Thermus* spp. 3-Hydroxy fatty acids linked to *N*-acyl galactosamine is specific, which is quite different from *Thermus* glycolipids [11]. This study is the first to determine the full structure of glycoglycerolipid in thermophilic bacteria *Meiothermus* spp. The structural information will be very useful for further investigations of the mechanisms of glycoglycerolipid biosynthesis *in vivo*, and even for chemical synthesis *in vitro* and their physiological roles.

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