# Structural variation of glycolipids from *Meiothermus taiwanensis* ATCC BAA-400 under different growth temperatures†

Yu-Liang Yang,[a] Feng-Ling Yang,[a] Zih-You Huang,[a] Yu-Hsuan Tsai,[a] Wei Zou[b] and Shih-Hsiung Wu*[a]

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**A major glycolipid, α-Galf(1–3)-α-Galp(1–6)-β-GlcpNAcyl(1–2)-α-Glcp(1–1)-2-acylalkyldiol, is obtained from *Meiothermus taiwanensis*. This novel glycolipid is found only when the bacterium grows above 62 °C, which is significantly different from those from the same bacteria incubated at 55 °C. Terminal galactofuranoside and 1,2-alkyldiol lipids replaced galactopyranoside and glycerol lipids, respectively, under increased growth temperature. This variation is likely necessary for bacteria for keeping the stable outer membrane and surviving under extreme environments.**

## Introduction

*Meiothermus* species are gram-negative thermophilic rods isolated from thermal hot springs, industrial and domestic water traps, and hydrothermal vents with neutral to alkaline pH.[1] They do not contain lipopolysaccharide in the outer membrane, but the polar lipids including glycolipids and phophoglycolipids constitute the majority of the outer cellular membrane. They play an important role in stabilizing the outer membrane, protecting the cell from harsh environments. The structures of polar lipids from *Thermus* and *Meiothermus* species have been investigated using mass spectrometry[2–4] and NMR spectroscopic analysis.[5,6] The glycolipids usually contain three hexoses, one *N*-hexosamine, and one glycerol.[1,3–7] The hydrophobic parts are predominantly *iso*- and *anteiso*-branched fatty acids; straight-chain fatty acids are minor components. The NMR technique is particularly powerful to the structure analysis, in terms of determination of glycosidic configurations and linkages of glycolipids and phosphoglycolipids.[5,6,8,9] Variation of fatty acid compositions has been observed under different growth temperatures, which likely in turn contributes to cell membrane stability at elevated temperatures.[10,11]

We have isolated and determined the structures of major phosphoglycolipids[8] and glycolipids[5,6] from *Thermus* and *Meiothermus* species, and also synthesized the glycolipid of *Meiothermus taiwanensis* by one pot method.[12] In the study of phosphoglycolipids, two types of lipids were found, *i.e.* 1,2-diacyl-*sn*-glycerol and 2-acylalkyldiol.[8] The ratios of two lipids and *iso*- to *anteiso*-branched fatty acids depend significantly on the cultural temperatures. Apparently, when the bacteria was cultured above 62 °C, major glycolipids (GL1 and GL2)[2] found at lower growth temperature almost completely disappeared (see S9 in the ESI†). It seems that the biosynthesis of GL1 and GL2 was hijacked in favor to produce a new polar lipid **1**, whereas, the biosynthesis of phosphoglycolipids were likely not affected. Both **1** and phosphoglycolipid were purified by chromatography on Si-gel and Sephadex LH-20. The structures of the phosphoglycolipids were confirmed to be the same as that obtained at lower growth temperature, containing both 2-acylalkyldiol and 1,2-diacyl-*sn*-glycerol. **1** was found to be a glycolipid. We have elucidated its structure using MS/MS and various NMR techniques in junction with chemical analysis. To the best of our knowledge glycolipid **1** was not known before (Fig. 1).

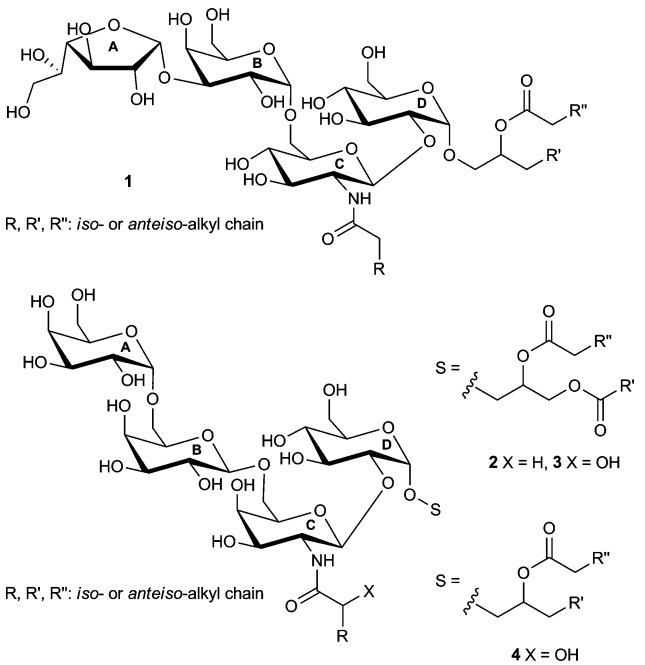


**Fig. 1** Structures of glycolipids **1**–**4**.

## Results and discussion

Analysis of fatty acid compositions showed that *iso*-$C_{15:0}$, -$C_{16:0}$, and -$C_{17:0}$ were the major lipids and *iso*-$C_{14:0}$, *anteiso*-$C_{15:0}$, -$C_{17:0}$, and unsaturated $C_{18:1}$ were the minor ones (see S6 in the ESI†). In comparison to the major glycolipids from the same species,[6] **1** contains significantly more *iso*-$C_{15:0}$ fatty acid (46.3%). The sugar composition analysis by GC-MS indicated that **1** consists of one glucose, two galactoses, and one glucosamine. Meanwhile, four anomeric protons of **1** were observed in $^1$H NMR spectrum (see S10 in the ESI†) at 5.02, 4.71, 4.68, and 4.52 ppm, with $^3J_{H-H}$ 0, 3.7, 3.7, and 8.4 Hz; and $^1J_{H-C}$ 175, 175, 175, 169 Hz, respectively

[a]*Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan. E-mail: shwu@gate.sinica.edu.tw; Fax: +886-2-2653-9142; Tel: +886-2-2785-5696*
[b]*Institute for Biological Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ontario, K1A 0R Canada*
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(see S16 in the ESI†). The coupling constants suggest three α and one β anomeric configurations presented in **1**. The low-field singlet anomeric signal is an indicative of a furanose. Additionally, the doublet methyl group signal observed in $^1$H NMR confirms that *iso*- and *anteiso*-branched fatty acids are the major compositions. No acetyl group was observed.

In order to facilitate the structural assignments, **1** was per-acetylated to afford glycolipid (**1a**) which was dissolved in $C_6D_6$ for NMR analysis. An α-glucopyranose (D), a β-glucosamine (C), and an α-galactopyranose (B) were easily confirmed by 1D TOCSY (mixing times from 80–120 ms, see S22 in the ESI†). Their chemical shifts ($^1$H and $^{13}$C) were assigned based on 2D-HSQC (see S13–S14 in the ESI†). In respect to residue A, the anomeric resonances at $\delta_H$ 5.55 and $\delta_C$ 107.5, H-5 at $\delta_H$ 5.87 (down-field due to acetylation), and H-4 at $\delta_H$ 4.87, together with the $^1$H–$^{13}$C correlation between H-1 and C-4 observed in HMBC experiment indicate residue A to be an α-galactofuranose (see S17–S18 in the ESI†). The configuration assignment was further supported by 2D-ROESY experiment (Fig. 2 and S19–S20 in the ESI†).

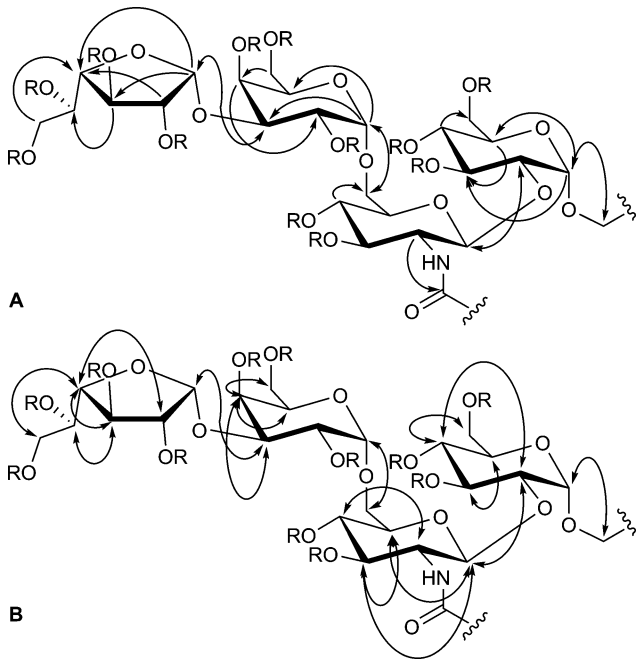


**Fig. 2** Key $^3J_{H\text{-}C}$ (A) and ROE (B) correlations of **1** (R = H) and **1a** (R = Ac).

Due to *O*-acetylation resulting in down-field shift of proton signals, the relative up-field resonances of H-3 in residue B, H-6 in residue C, and H-2 in residue D suggested that those are the glycosidic linkage positions. The methylation analysis by GC-MS confirmed a terminal α-galactofuranose (A), a 3-*O*-linked α-galactopyranose (B), a 6-*O*-linked β-glucosamine (C), and a 2-*O*-linked α-glucopyranose (D). Correlations observed in 2D-HMBC between A1 and B3, B1 and C6, and C1 and D2 as well as ROEs between H-1 in A and H-3 in B, H-1 in B and H-6 in C, together with H-1 in C and H-2 in D (Fig. 2) confirmed the tetrasaccharide structure of **1a**.

The lipid structure of **1** was solved by NMR (HMBC and TOCSY) and tandem mass spectroscopic analysis. The aglycon lipid is a 1,2-alkyldiol where the tetrasaccharide is connected to its 1-*O* position and an acyl group is linked to 2-*O* position by an ester bond. In addition, there is a long chain fatty acid linked to amino group of glucosamine through an amide bond. Tandem MS analysis of **1** gave a major peak at *m/z* 1433 $[M+Na]^+$ and minor peaks with an *m/z* difference of 14 Da, indicating that the aliphatic chains on 1,2-alkyldiol or *N*-acylglucosamine were heterogeneous. In the $MS^2$ spectrum (Fig. 3), deduced from *m/z* 1433, three Y ions and one B ion indicated that a $C_{17:0}$ fatty acid linked to the glucosamine. It is also evidenced by the B and C ions in $MS^4$ spectrum (deduced from *m/z* 886). Two ion peaks at *m/z* 1190 and 866 in $MS^2$, the loss of $C_{15:0}$ fatty acid, confirmed the majority of fatty acid composition ($C_{15:0}$ > 51%). In the $MS^3$ spectrum (deduced from *m/z* 1109), two ion peaks and minor peaks with an *m/z* difference of 14 suggested the length of 1,2-alkyldiol is $C_{16}$~$C_{20}$. The $^1$H and $^{13}$C NMR data of **1** and its per-acetylated derivative **1a** are listed in S7 in the ESI.†

It is interesting that **1** only exists as part of membrane of *M. taiwanensis* grown at temperatures above 62 °C. When we repeated a previous experiment by growing *M. taiwanensis* at 55 °C, we found, in addition to two major glycolipids (**2** and **3**) reported previously (Fig. 1) with a terminal sugar as α-galactopyranoside and glycerol aglycon, another glycolipid (**4**) with 1,2-alkyldiol in aglycon and glucosamine substituted with *N*-2-hydroxyl fatty acid (S8 in the ESI†).

Variations of fatty acids in thermophilic bacteria under different temperatures have been reported previously.[8,10] The changes are associated with the maintenance of an adequate liquid-crystalline balance, which contributes to an ideal physical state of the membrane.[13] But variation of sugar configuration has never been reported although two glycolipids containing terminal galactofuranose were found in *T. thermophilus* Samu-SA1 at 75 °C.[9] Both glycolipids have the same sugar compositions as **1** but with different glycosidic linkages, in which the aglycons are 2-acylalkyldiol and 1,2-diacyl-*sn*-glycerol. The optimal cultural temperature of *T. thermophilus* Samu-SA1 is 75 °C while that of *M. taiwanensis* is 55 °C, suggesting their membrane structures function most effectively at these temperatures in terms of stability and nutrition permeability.

In order to survive at higher temperature, microorganisms develop their membrane structures to adapt to the environment. In thermophilic bacteria and archaea, this homeoviscous adaptation is achieved by adjusting the chemical composition of lipids in order to keep a favorable membrane phase likely with a higher phase transition temperature ($T_c$).[14–16] High-growth temperature adaptation increases heat resistance of the cells, presumably due to decreases in membrane fluidity. Galactofuranosyl moiety may stabilize the membrane structure by interglycosyl head group hydrogen bonding.[17] In the new glycolipids, the terminal sugar is replaced by furanosyl unit; it may possible help the outer cell membrane to decrease its membrane fluidity in higher temperatures through interglycosyl head group hydrogen bonding. And, the changes of intermolecular interactions and dynamically fluctuating hydrogen-bonded network may make the self-assembling/organizing systems to be exhibited supermesomorphic thermotropic phase behaviors.[18]

In addition, bulky head groups would enhance the steric protection,[19] possibly by stabilizing the membrane through hydrogen bonding *via* glycosyl head groups.[20] Structurally, compounds **2–4** consist of two β-anomeric configurations and two

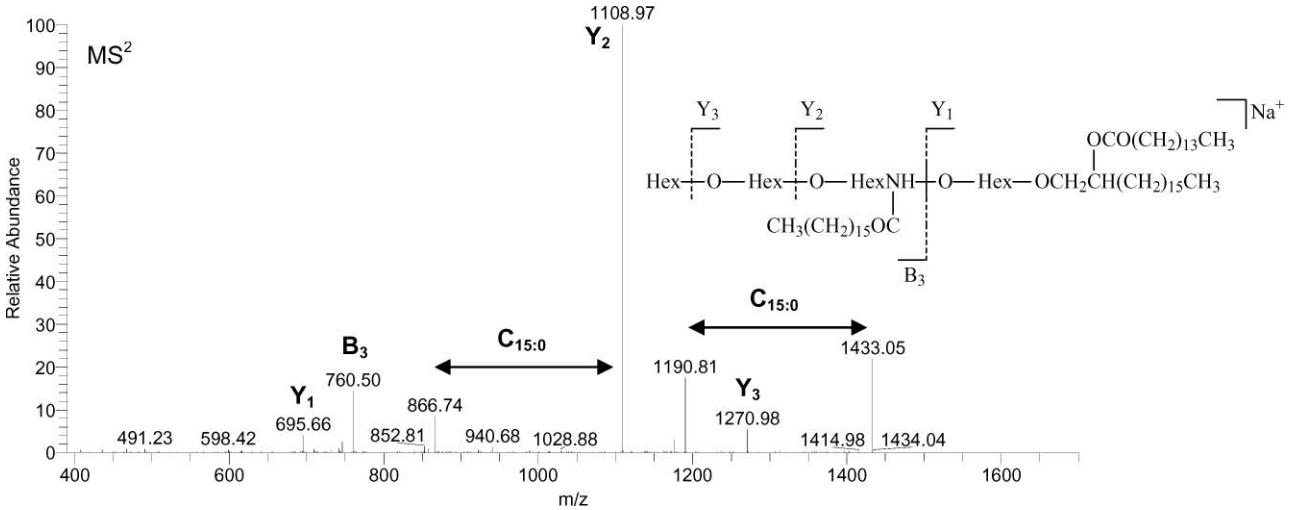


**Fig. 3** $MS^2$ spectrum and fragmentations of **1**.

1,6-glycosidic linkages, which enhances the flexibility of carbohydrate linkages. Conversely, the structure of **1** could form more intramolecular hydrogen bonds and therefore is more rigid than **2–4**. The conformational investigations of these glycolipids are ongoing. Very few studies have been reported that elucidate the relationships between the molecular structure of monomeric glycolipids and the architecture of their supramolecuar aggregates. This is in part due to the difficulty of obtaining sufficient amounts of chemically pure compounds from natural sources or by synthetic methods.[21]

In terms of biosynthetic pathway, there may be two enzymes including galactopyranose mutase and galactofuranose transferase involved for the addition of terminal galactofuranose similar to those found in *Mycobacterium tuberculosis*.[22] The activity of the enzyme(s) is likely temperature dependent, which become significant when the temperature is elevated above 62 °C. It is also possible that the same galactosyltransferase may be switched to transfer galactofuranose at higher temperature.

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