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Chemical structure and function of glycosphingolipids of *Sphingomonas* spp and their distribution among members of the α-4 subclass of *Proteobacteria*

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Sphingomonas **spp are phylogenetically placed in the α-4 subclass of** ***Proteobacteria*****. They have glycosphingolipids (GSL) in their membranes instead of lipopolysaccharide (LPS) as in other Gram-negative bacteria.** ***S. paucimobilis*****, the type species of the genus, has GSL-1, which contains only glucuronic acid (GlcA) as a sugar moiety, and GSL-4A, which contains a tetrasaccharide including GlcA. GSL-1 and GSL-4A form the outer membrane of** ***S. paucimobilis*** **with outer membrane proteins and phospholipids. In the outer membrane, GSLs are assumed to locate and function as does the LPS of other Gram-negative bacteria.** ***Sphingomonas*** **spp closely related to the type species contain both GSL-1 and the oligosaccharide-type GSL such as GSL-4A, but other** ***Sphingomonas*** **spp and other genera in the α-4 subclass of** ***Proteobacteria*** **contain only GSL-1. Structural variations of fatty acids and dihydrosphingosines in the GSL-1 are presented.**

Keywords: *Sphingomonas*; glycosphingolipid; chemical structure; outer membrane; phylogeny

Introduction

It is generally believed that Gram-negative bacteria contain lipopolysaccharide (LPS) in their outer membrane [17]. LPS confers a hydrophilic nature to the surface of the bacteria, and works as a barrier against bactericidal substances. *Sphingomonas* spp belong to the 'Gram-negative bacteria', but our studies on the cellular glycolipid revealed that these bacteria have an unusual cell envelope and lack LPS. Phylogenetically, *Sphingomonas* spp are positioned in the α-4 subclass of *Proteobacteria*. An original member of this group was *Erythrobacter longus* [18], but recently all of the *Sphingomonas* species together with some photosynthetic bacteria have been classified in this group [25].

About 20 years ago, we noticed that the type species of the genus *Sphingomonas*, *S. paucimobilis*, did not have a typical LPS, but contained a certain glycolipid, whose chemical composition was quite different from that of LPS [10]. Some years earlier, the group of Yano and Yabuuchi [36] reported that *Flavobacterium devorans*, later transferred to *S. paucimobilis* [33,34], had a glycosphingolipid (GSL) composed of dihydrosphingosine, a 2-hydroxy fatty acid and glucuronic acid (GlcA). Because of this finding, Yabuuchi *et al* [34] proposed a new genus name, *Sphingomonas*, for this group of bacteria. In 1991 we confirmed the structure of the glycolipid that we had reported earlier and showed that the glycolipid was a GSL with a longer carbohydrate moiety than the GSL reported by Yamamoto *et al* [36]. As a result of those studies, we came to the conclusion that GSLs, usually present in the membrane of eukaryotic cells, are major components of the membrane of sphingomonads.

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Chemical structure of GSL of S. paucimobilis

As described in our first paper [10], the glycolipid of *S. paucimobilis* was thought to be LPS or a lipid A-like molecule. The lipid contained D-mannose (Man), D-galactose (Gal), D-glucosamine (GlcN), D-glucuronic acid (GlcA), and 2-hydroxytetradecanoic acid (2-OH-C14:0). However, no 3-hydroxy fatty acid was present, and the lipid gave a single band in SDS-polyacrylamide gel electrophoresis (SDS-PAGE). These characteristics were different from those of LPS. Furthermore, dihydrosphingosine was detected by ^{1}H- and ^{13}C-NMR and chemical analysis, and finally the glycolipid was proved to be a glycosphingolipid [8].

Detection and determination of sphingosine is not easily done because of the instability of the molecule during hydrolysis or methanolysis. Recently we developed a method of analysis as follows: the carbohydrate portion of GSL is first oxidized by periodate, and sphingosines are liberated from oxidized GSL by methanolysis with 1 M HCl-methanol. Free sphingosines are peracetylated with acetic anhydride/pyridine, and analyzed by gas-liquid chromatography (GLC), or combined gas-liquid chromatography-mass spectrometry (GLC-MS). By this method, and also by analysis with NMR and laser-desorption mass spectrometry (LD-MS), the GSL of *S. paucimobilis* was shown to contain *erythro*-1,3-dihydroxy-2-amino-octadecane (DS18:0) and *erythro*-1,3-dihydroxy-2-amino-*cis*-13,14-methylene-eicosane (DS21cycl) in comparable amounts, and *erythro*-1,3-dihydroxy-2-amino-*cis*-13,14-eicosene (DS20:1) as a minor component (Figure 1). All three sphingosine molecules are dihydrosphingosines (sphinganines),

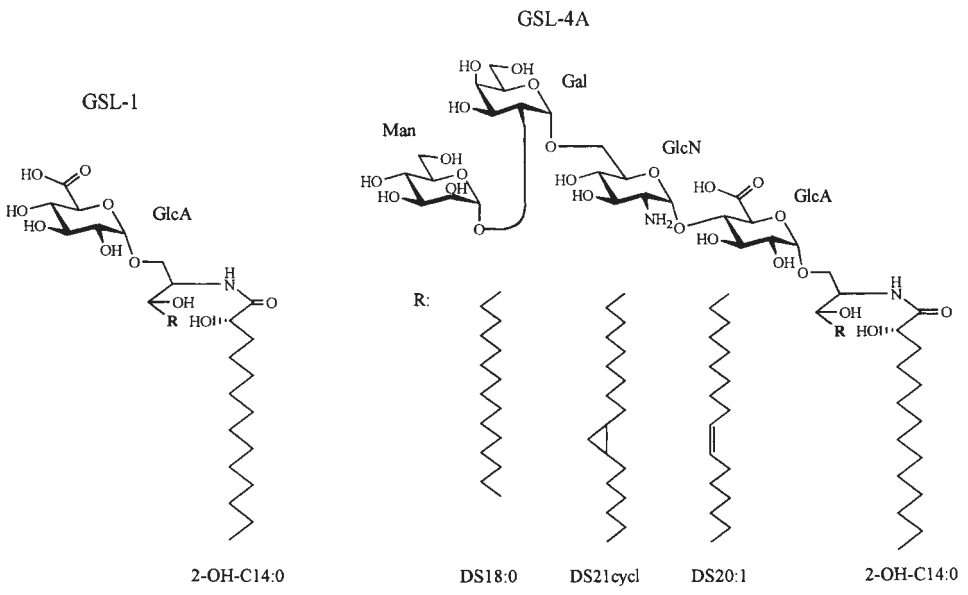

Figure 1 Chemical structure of GSL-1 and GSL-4A isolated from *S. paucimobilis*. Among dihydrosphingosines, DS20:1 was a minor component.

ie, no double bond is present between the C-4 and C-5 positions. Instead, there are other modifications at C-13 and C-14. The position of the cyclopropyl ring or double bond from the terminus of the hydrocarbon chain is identical to that of cellular fatty acids such as palmitoleic acid (C16:1) and *cis*-vaccenic acid (C18:1). Since dihydrosphingosine is biosynthesized through the condensation of serine and a fatty acid in eukaryotic cells [2], we speculated that in the case of bacterial dihydrosphingosine biosynthesis the same condensation reaction takes place.

The difference between the GSL that we purified and the GSL reported by Yamamoto *et al* [36] is the length of the carbohydrate chain. In the membrane of *S. paucimobilis*, GSL with a tetrasaccharide carbohydrate chain (GSL-4A, Figure 1) and that with only GlcA (GSL-1, Figure 1) are present, and the lipid we first purified was GSL-4A; that reported by Yamamoto *et al* was GSL-1. As GSL-1 is more abundant and present in all *Sphingomonas* strains, the base structure of the membrane is assumed to contain GSL-1, and GSL-4A may modify the antigenicity and surface hydrophobicity.

The sequence and the linkage of sugars in the carbohydrate chain of GSL-4A were analyzed by partial hydrolysis, methylation analysis, and ^{1}H- and ^{13}C-NMR analysis. The characteristic feature of the carbohydrate structure is the presence of GlcN with a free amino group adjacent to GlcA. The overall charge of GSL-1 is negative because of the free carboxyl group in GlcA, but that of GSL-4A is neutral because the amino group of GlcN neutralizes the carboxyl group of GlcA. It would be interesting to measure the overall charge of GSL-4A at physiological pH, because in such a condition GSL-1 was measured to carry only 0.5 negative charge [30].

When we compare the carbohydrate structure of *Sphingomonas* GSL with that of mammalian cell membranes, we notice some structural differences. In all mammalian GSL, sugars linked to a C-1 hydroxyl group of sphingosines are neutral sugars, in most cases Glc or Gal [4]. But in the GSL of *S. paucimobilis*, GlcA occupies that position. The configuration of sugars in mammalian GSL is mostly β or a combination of α and β [4]. But in GSL-4A all sugars are in the α-configuration. Also in the ceramide portion, a remarkable difference can be seen. Sphingosines are different as described above. The fatty acid of *S. paucimobilis* GSL is 2-OH-C14:0, but mammalian GSL consists of non-polar or 2-hydroxy fatty acids with longer carbon chains.

GSL of Sphingomonas spp closely related to S. paucimobilis

The distribution of GSL among *Sphingomonas* spp is described below, but here we discuss the distribution of GSL-4A or GSL with similar structures. In the phylogenetic tree based on sequences of 16S ribosomal RNA genes, *S. paucimobilis* forms a cluster with *Sphingomonas* spp isolated from plants, clinical specimens, or the environment [24]. Among them, *S. parapaucimobilis* and *S. sanguis* harbor GSL identical to GSL-4A (Kawahara *et al*, unpublished data). We have isolated many *Sphingomonas* strains from rice and other plants of the family Gramineae, and found that most of them showed the profile of GSL in thin-layer chromatography (TLC) identical to *S. paucimobilis* [7]. These strains are shown to be phylogenetically close to *S. paucimobilis* (Kawahara *et al*, unpublished data). A GSL-4A-like glycolipid was extracted from *S. capsulata*. By chemical and spectroscopic analysis, this GSL was shown to have a structure similar to GSL-4A, but lacked the terminal Man in the carbohydrate chain [9]. A GSL with a different type of oligosaccharide structure was also found. *S. adhaesiva* contained GSL with a different tetrasaccharide; it consists of Glc, Gal and GlcA, but no GlcN. Detailed structures of these GSLs will be published elsewhere. Among the isolates from plants, GSLs with novel oligosaccharide structures were found, and structural analysis is in progress. From these data it can be concluded that strains phylogenetically close to *S. paucimobilis* have the

oligosaccharide-type GSL in addition to GSL-1. The carbohydrate structure of GSL-4A is most common, but other types of oligosaccharide are also present.

Cell envelope structure of S. paucimobilis and function of GSL

Since *S. paucimobilis* lacks LPS, the questions arise as to whether it has an outer membrane, and if it has, how it is assembled, or what is the role of GSL in the outer membrane. In order to clarify these points we investigated the cell envelope of *S. paucimobilis* [11]. When sucrose-density gradient ultracentrifugation [35] was applied to the membrane fraction, it was separated into two layers as with other Gram-negative bacteria. The high-density layer contained GSL-1 and GSL-4A, and the protein profile of that layer on SDS-PAGE was that of typical outer membrane, ie, a simple profile with several major proteins. The peptidoglycan of this bacterium was also examined. The peptidoglycan preparation contained typical components such as *N*-acetyl-muramic acid, D-alanine or diaminopimelic acid, and these components were identical to those of *Escherichia coli*. Since *S. paucimobilis* has peptidoglycan as in other Gram-negative bacteria, the only difference between *S. paucimobilis* and other Gram-negative bacteria is that the outer membrane contains GSL instead of LPS.

The above conclusion introduces the next question, how GSL and other membrane components assemble. No detailed study has been done on the outer membrane proteins and phospholipids of these bacteria. But the profile of outer membrane proteins on SDS-PAGE and the phospholipid profile on TLC [11,34] indicate that these components of the outer membrane are biochemically similar to those of *E. coli*, and therefore, they should be able to form an outer membrane.

In order to investigate the localization of GSL in the outer membrane, a polyclonal antibody against GSL-4A was prepared. The antibody was adsorbed to GSL-1 to make it specific to the terminal region of the oligosaccharide in GSL-4A. When microscopic immunostaining with a gold particle complex was performed, the gold particles were observed at the outer layer of a thin section of the cell [11] (Figure 2). By the whole mount method, many gold particles were adsorbed on the surface of the cells [11]. Those immunomicroscopic studies showed that the terminal region of the oligosaccharide was exposed to the surface, suggesting that GSL-4A was located at the outer layer of the outer membrane as is LPS in other Gram-negative bacteria. There was no clear evidence concerning the location of GSL-1 in this study, but it is most probable that GSL-1 is also localized at the surface of the outer membrane.

We can consider the role of GSL-1 and GSL-4A in the membrane on the analogy of Re-type and Ra-type LPS of enterobacteria. In fact, there are striking conformational similarities between Re-LPS and GSL-1, if three molecules of GSL-1 are packed together. Re-LPS of *E. coli* has two 3-deoxy-D-*manno*-octulosonic acid (Kdo) moieties, two GlcN (*N*-acylated) moieties, two phosphate groups, and six fatty acids. Three molecules of GSL-1 have three negatively charged sugars, and six hydrocarbon chains. Furthermore, in the system of planar asymmetric lipid bilayers, GSL-1 was shown to behave in a manner similar to Re-LPS for the channel function of porin [30], the activation of complement [12,30], and the interaction with polymyxin B [29]. These conformational and functional similarities of LPS and GSL lead us to how GSL functions in the outer membrane. As illustrated in Figure 3, we propose that GSL covers the outer layer of the outer membrane with the ceramide portion in the membrane as an anchor and the carbohydrate chain exposed to the outside of the cells as with LPS. However, the carbohydrate portion of GSL is much shorter and simpler than that of LPS, and it makes the cell surface of sphingomonads more hydrophobic than that of other Gram-negative bacteria. Such surface hydrophobicity can be noticed even in the wax-like feature of the colonies on agar. The high hydrophobicity allows the influx of hydrophobic substances and makes sphingomonads susceptible to hydrophobic antibiotics [16,21], and able to develop metabolic pathways for aromatic compounds [3,6,13,15,31].

The simple carbohydrate structure of GSL results in a small variation of antigenicity [6] compared with other Gram-negative bacteria, which have a wide variety of core oligosacharides and O-antigenic polysaccharides. However, if we use antibodies specific to the oligosaccharide of GSL, it should be possible to establish a system of GSL-typing for *Sphingomonas* strains.

The surface hydrophobicity of sphingomonads can be an

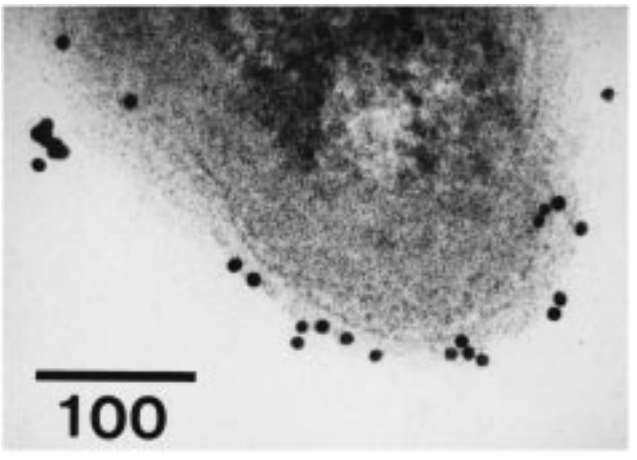

Figure 2 Localization of GSL-4A in the cell of *S. paucimobilis*. GSL-4A was stained by the postembedding immunogold-labeling method. The black bar in the photograph indicates 100 nm.

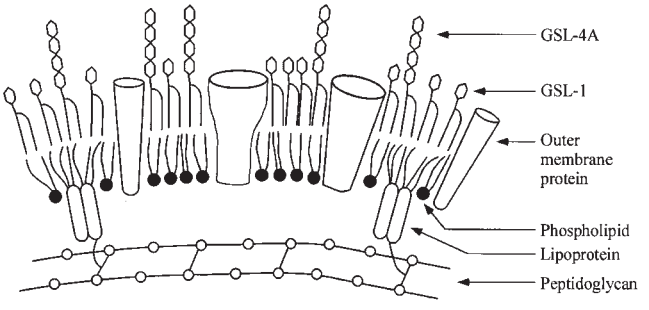

Figure 3 Structural elucidation of the outer membrane of *S. paucimobilis* on the analogy of the enterobacterial outer membrane. Outer membrane proteins and lipoproteins have not yet been investigated biochemically.

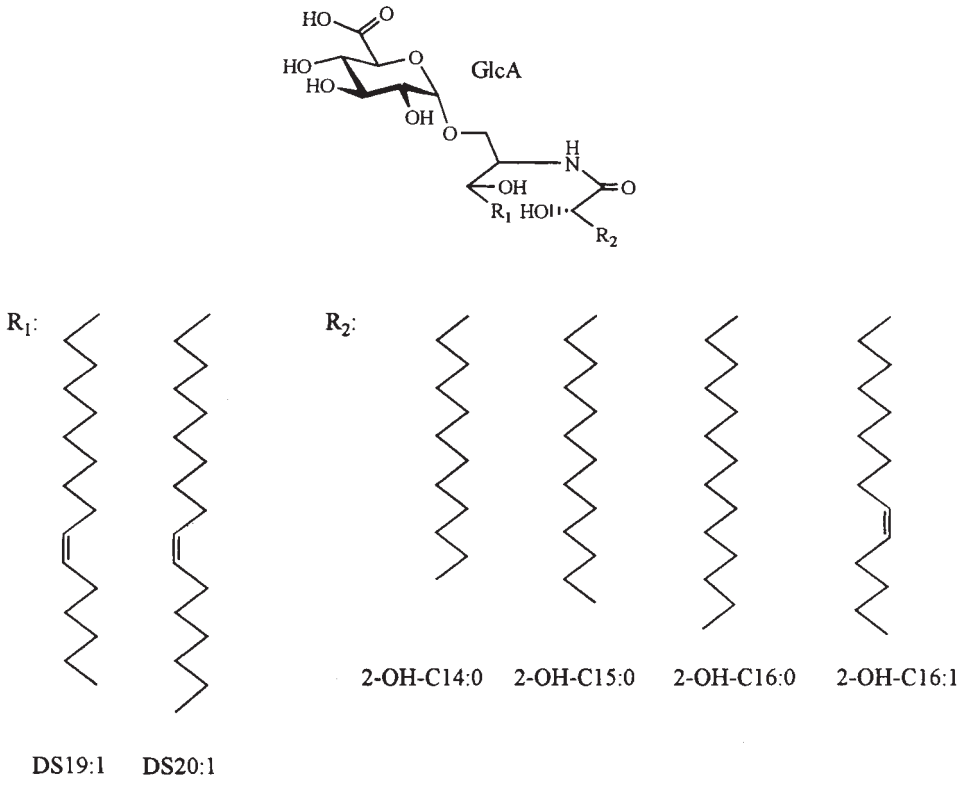

Figure 4 Chemical structure of GSL-1 isolated from *Blastomonas natatoria*. DS20:1 and 2-OH-C14:0 were major components of dihydrosphingosines and fatty acids, respectively. The positions of the double bond in hydrocarbon chains were deduced from the study by Sittig and Hirsch [19].

advantage for the assimilation of hydrophobic compounds, but is it also an advantage for the bacteria to grow or to survive in the environment? We do not have any experimental data for this question, but it can be speculated that such bacteria can adhere easily to animal or plant cells, or to other compounds for colonization, especially in water. We found that *Sphingomonas* strains can be isolated in high frequency from the ears of rice and other plants [7]. The characteristic cell surface of sphingomonads may be advantageous for the symbiosis of the bacteria with such plants.

Distribution of GSL among bacteria in the α-4 subclass of Proteobacteria

In the phylogenetic study of Woese [32] and Stackebrandt *et al* [22], only *Erythrobacter longus*, an aerobic marine bacterium with bacteriochlorophyll *a* [18], was a member of the α-4 subclass of *Proteobacteria*. But recent studies demonstrated that the α-4 subclass contains all *Sphingomonas* spp, *Rhizomonas suberifaciens*, *Blastomonas natatoria* and several photosynthetic bacteria including *E. longus* [1,37]. The most characteristic taxonomic feature of this group is the profile of their cellular fatty acids. All members of this group contain 2-hydroxy fatty acids as the sole polar fatty acid and no 3-hydroxy fatty acid is present. As we see in the present review, this profile of hydroxy fatty acid suggests the presence of GSL. In our laboratory many trials have been done to extract GSL from various strains for structural analysis. All *Sphingomonas* spp so far examined contain GSL-1, and strains or species in the phylogenetic group of *S. paucimobilis* additionally contain GSL with an oligosaccharide chain such as GSL-4A. As pointed out by Yabuuchi *et al* [34], *S. yanoikuyae* contains GSL that shows as a spot moving slightly slower than GSL-1 on TLC. In our preliminary experiments, *S. macrogoltabidus* and *S. terrae* gave the same spot of the GSL as *S. yanoikuyae*. All other *Sphingomonas* spp and species belonging to the α-4 subclass have only GSL-1, as far as we have examined. However, the composition of dihydrosphingosine and 2-hydroxy fatty acid varies depending on species. We have analyzed the composition of dihydrosphingosine and 2-hydroxy fatty acid by GLC, total molecular weight by fast atom bombardment-mass spectrometry (FAB-MS), and using these data, the chemical structure of each GSL was elucidated.

R. suberifaciens, a plant pathogen [26,27], contained GSL-1 similar to that of *S. paucimobilis* (Figure 1), but the content of DS20:1 was higher, and an unknown dihydrosphingosine was additionally present as a minor component. The major fatty acid was 2-OH-C14:0, but 2-hydroxypentadecanoic acid (2-OH-C15:0) was also present in a ratio of 4:1.

GSL-1 from a budding bacterium, *Blastomonas natatoria* [19,20], contained DS20:1 as a major dihydrosphingosine and DS19:1 as minor one (less than 10%). The profile of 2-hydroxy fatty acids of this GSL-1 was very characteristic. As shown in Figure 4, it contained 2-OH-C14:0 as a major component (about 60%), and 2-OH-C15:0, 2-hydroxyhexadecanoic acid (2-OH-C16:0), and 2-hydroxyhexadecenoic acid (2-OH-C16:1) as minor ones.

The components of GSL-1 from *E. longus* were simpler than those described above. As shown in Figure 5, the dihydrosphingosine in this GSL-1 was DS20:1, and no minor

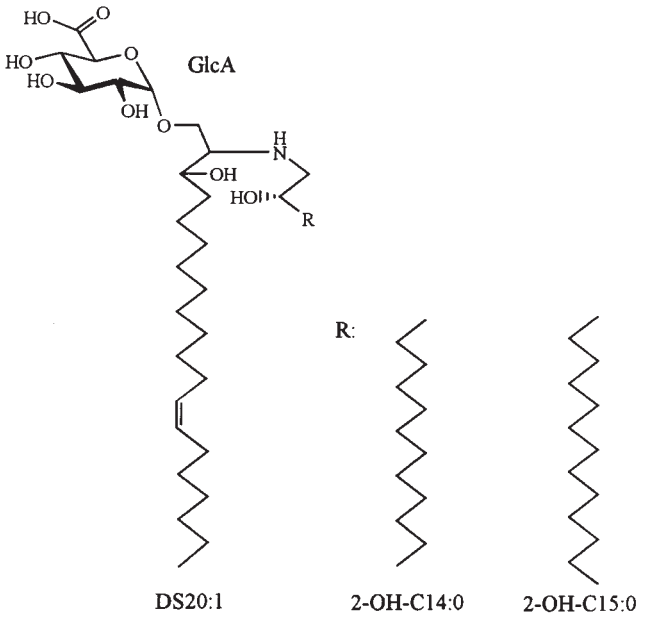

Figure 5 Chemical structure of GSL-1 isolated from *Erythrobacter longus*. In addition to the fatty acids shown in the figure, 2-OH-C16:0 was present as a minor fatty acid.

component was detected. As fatty acid components, 2-OH-C14:0 and 2-OH-C15:0 were present in about equal amounts. As a minor fatty acid, 2-OH-C16:0 was measured in an amount of 5%.

Zymomonas mobilis, a facultative anaerobic bacterium known for its ethanol-producing ability, is placed in the α-group of *Proteobacteria* [22], and its close relation to the α-4 subclass was recently described by several groups [1,3,14]. One of the cellular lipids of this bacterium was reported by Tahara and Kawazu [23] as a glycosphingolipid. The lipid contains 1,3-dihydroxy-2-amino-hexadecane (DS16:0), GlcA, and hexadecanoic or tetradecanoic acid as fatty acid components (Figure 6). Compared with GSL-1 of *Sphingomonas* spp, the chain length of dihydrosphingosine is shorter, and the fatty acids have no hydroxyl group, although the sugar moiety is identical.

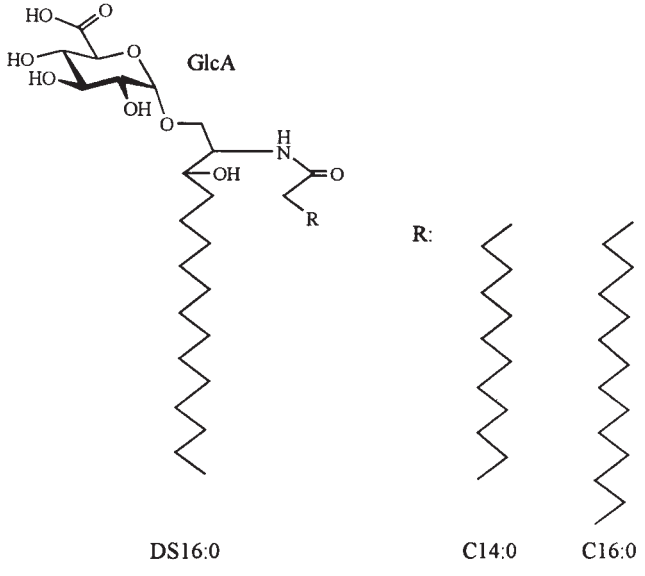

Figure 6 Chemical structure of GSL isolated from *Zymomonas mobilis* [23].

Chlorobium limicola, a phototrophic green sulfur bacterium, was reported by Jensen *et al* [5] to contain aminoglycosphingolipid (Figure 7). This GSL is present in the plasma membrane of the bacterium and may function as a membrane anchor for bacteriochlorophyll *a*-protein. As shown in Figure 7, the GSL contains neuraminic acid, dihydrosphingosine (DS18:0), and tetradecanoic acid. Fatty acid and sugar moieties are different from GSL-1 of *Sphingomonas* spp, but a common structural principle between *Sphingomonas* and *Chlorobium* GSL can be seen in the sugar portion. In the GSL of *C. limicola*, neuraminic acid possesses free carboxyl and amino groups in one molecule. In GSL-4A of *S. paucimobilis* (Figure 1), GlcA with a carboxyl group is linked by the amino group containing GlcN. The phylogenetic relationship of *C. limicola* with the bacteria in the α-4 subclass is not clear [28,32], but besides the phylogenetic relation, horizontal transfer of genes needed for the biosynthesis of GSL could explain the presence of GSL in the bacteria outside of the α-4 subclass.

Conclusions

As described in the present review, *Sphingomonas* spp and other members of the α-4 subclass of *Proteobacteria* have GSL and form a characteristic cell envelope different from other Gram-negative bacteria. The hydrophobic cell surface of these bacteria is convenient for the uptake of aromatic hydrocarbons. Moreover, this unique cell surface might also be convenient for survival of the bacteria in their ecological niches. The natural function of GSL would be clarified through an ecological study on sphingomonads and taxonomically related bacteria as well as by a biochemical study on GSL and GSL-containing membranes.

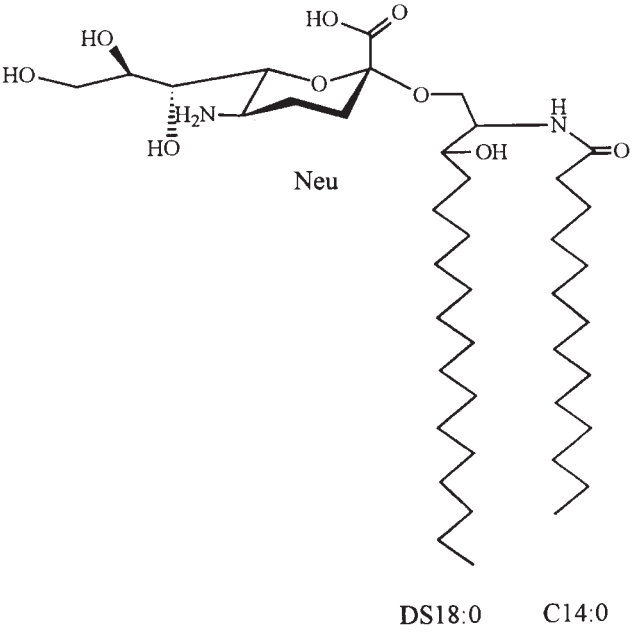

Figure 7 Chemical structure of GSL isolated from *Chlorobium limicola* f *thiosulfatophilum* [5].

References

1 Balkwill DL, GR Drake, RH Reeves, JK Fredrickson, DC White, DB Ringelberg, DP Chandler, MF Romine, DW Kennedy and CM Spadoni. 1997. Taxonomic study of aromatic-degrading bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp nov, *Sphingomonas subterranea* sp nov, and *Sphingomonas stygia* sp nov. Int J Syst Bacteriol 47: 191–201.

2 Braun P and EE Snell. 1968. Biosynthesis of sphingolipid bases. J Biol Chem 243: 3775–3783.

3 Fredrickson JK, DL Balkwill, GR Drake, MF Romine, DB Ringelberg and DC White. 1995. Aromatic-degrading *Sphingomonas* isolates from the deep subsurface. Appl Environ Microbiol 61: 1917–1922.

4 Hakomori S. 1983. Chemistry of glycosphingolipids. In: Handbook of Lipid Research Vol 3 (Kanfer JN and Hakomori S, eds), pp 1–150, Prenum Press, New York.

5 Jensen MT, J Knudsen and JM Olson. 1991. A novel aminoglycosphingolipid found in *Chlorobium limicola* f *thiosulfatophilum* 6230. Arch Microbiol 156: 248–254.

6 Karlson U, F Rojo, JD van Elsas and E Moore. 1995. Genetic and serological evidence for the recognition of four pentachlorophenol-degrading bacterial strains as a species of the genus *Sphingomonas*. Syst Appl Microbiol 18: 539–548.

7 Kawahara K, I Mizuta, W Katabami, M Koizumi and S Wakayama. 1994. Isolation of *Sphingomonas* strains from ears of rice and other plants of family Gramineae. Biosci Biotech Biochem 58: 600–601.

8 Kawahara K, U Seydel, M Matsuura, H Danbara, ET Rietschel and U Zähringer. 1991. Chemical structure of glycosphingolipids isolated from *Sphingomonas paucimobilis*. FEBS Lett 292: 107–110.

9 Kawahara K, U Seydel and U Zähringer. 1992. Chemical structure of glycosphingolipids isolated from *Sphingomonas capsulata* and *S. paucimobilis*. Abstracts of XVIth Int Carbohydr Sym, p 603.

10 Kawahara K, K Uchida and K Aida. 1982. Isolation of an unusual 'lipid A' type glycolipid from *Pseudomonas paucimobilis*. Biochim Biophys Acta 712: 571–575.

11 Kawasaki S, R Moriguchi, K Sekiya, T Nakai, E Ono, K Kume and K Kawahara. 1994. The cell envelope structure of the lipopolysaccharide-lacking Gram-negative bacterium *Sphingomonas paucimobilis*. J Bacteriol 176: 284–290.

12 Münstermann M, A Wiese, K Brandenburg, U Zähringer, L Brade, K Kawahara and U Seydel. 1999. Complement activation by bacterial surface glycolipids: a study with planar bilayer membranes. J Membr Biol 167: 223–232.

13 Nagata Y, T Nariya, R Ohtomo, M Fukuda, K Yano and M Takagi. 1993. Cloning and sequencing of a dehalogenase gene encoding an enzyme with hydrolase activity involved in the degradation of γ-hexachlorocyclohexane in *Pseudomonas paucimobilis*. J Bacteriol 175: 6403–6410.

14 Nohynek LJ, E-L Nurmiaho-Lassila, EL Suhonen, H-J Busse, M Mohammadi, J Hantula, F Rainey and MS Salkinoja-Salonen. 1996. Description of chlorophenol-degrading *Pseudomonas* sp strains KF1^T, KF3, and NKF1 as a new species of the genus *Sphingomonas*, *Sphingomonas subarctica* sp nov. Int J Syst Bacteriol 46: 1042–1055.

15 Nohynek LJ, EL Suhonen, E-L Nurmiaho-Lassila, J Hantula and M Salkinoja-Salonen. 1995. Description of four pentachlorophenol-degrading bacterial strains as *Sphingomonas chlorophenolica* sp nov. Syst Appl Microbiol 18: 527–538.

16 Reina J, A Bassa, I Llompart, D Portela and N Borrell. 1991. Infections with *Pseudomonas paucimobilis*: report of four cases and review. Rev Infect Dis 13: 1072–1076.

17 Rietschel ET, L Brade, B Lindner and U Zähringer. 1992. Biochemistry of lipopolysaccharides. In: Bacterial Endotoxic Lipopolysaccharides Vol 1 (Morrison DC and Ryan JL, eds), pp 3–41, CRC Press, Boca Raton.

18 Shiba T and U Simidu. 1982. *Erythrobacter longus* gen nov, sp nov, an aerobic bacterium which contains bacteriochlorophyll *a*. Int J Syst Bacteriol 32: 211–217.

19 Sittig M and P Hirsch. 1992. Chemotaxonomic investigation of budding and/or hyphal bacteria. Syst Appl Microbiol 15: 209–222.

20 Sly LI and MM Cahill. 1997. Transfer of *Blastobacter natatorius* (Sly 1985) to the genus *Blastomonas* gen nov as *Blastomonas natatoria* comb nov. Int J Syst Bacteriol 47: 566–568.

21 Smalley DL, VR Hansen and VS Baselski. 1983. Susceptibility of *Pseudomonas paucimobilis* to 24 antimicrobial agents. Antimicrob Agents Chemother 23: 161–162.

22 Stackebrandt E, RGE Murray and HG Trüper. 1988. *Proteobacteria* classis nov, a name for the phylogenetic taxon that includes the purple bacteria and their relatives. Int J Syst Bacteriol 38: 321–325.

23 Tahara Y and M Kawazu. 1994. Isolation of glucuronic acid-containing glycosphingolipid from *Zymomonas mobilis*. Biosci Biotech Biochem 58: 586–587.

24 Takeuchi M, T Sakane, M Yanagi, K Yamamoto, K Hamana and A Yokota. 1995. Taxonomic study of bacteria isolated from plants: proposal of *Sphingomonas rosa* sp nov, *Sphingomonas pruni* sp nov, *Sphingomonas asaccharolytica* sp nov, and *Sphingomonas mali* sp nov. Int J Syst Bacteriol 45: 334–341.

25 Takeuchi M, H Sawada, H Oyaizu and A Yokota. 1994. Phylogenetic evidence for *Sphingomonas* and *Rhizomonas* as nonphotosynthetic members of the alpha-4 subclass of the *Proteobacteria*. Int J Syst Bacteriol 44: 308–314.

26 van Bruggen AHC, KN Jochimsen and PR Brown. 1990. *Rhizomonas suberifaciens* gen nov, sp nov, the causal agent of lettuce corky root. Int J Syst Bacteriol 40: 175–188.

27 van Bruggen AHC, KN Jochimsen, EM Steinberger, P Segers and M Gillis. 1993. Classification of *Rhizomonas suberifaciens*, an unnamed *Rhizomonas* species, and *Sphingomonas* spp in rRNA superfamily IV. Int J Syst Bacteriol 43: 1–7.

28 Van den Eynde H, Y Van de Peer, J Perry and R De Wachter. 1990. 5S rRNA sequences of representatives of the genera *Chlorobium*, *Prosthecochloris*, *Thermomicrobium*, *Cytophaga*, *Flavobacterium*, *Flexibacter* and *Saprospira* and a discussion of the evolution of eubacteria in general. J Gen Microbiol 136: 11–18.

29 Wiese A, M Münstermann, T Gutsmann, B Lindner, K Kawahara, U Zähringer and U Seydel. 1998. Molecular mechanisms of polymyxin B-membrane interactions: direct correlation between surface charge density and self-promoted transport. J Membr Biol 162: 127–138.

30 Wiese A, JO Reiners, K Brandenburg, K Kawahara, U Zähringer and U Seydel. 1996. Planar asymmetric lipid bilayers of glycosphingolipid or lipopolysaccharide on one side and phospholipids on the other: membrane potential, porin function, and complement activation. Biophys J 70: 321–329.

31 Wittich R, H Wilkes, V Sinnwell, W Francke and P Fortnagel. 1992. Metabolism of dibenzo-*p*-dioxin by *Sphingomonas* sp strain RW1. Appl Environ Microbiol 58: 1005–1010.

32 Woese C. 1987. Bacterial evolution. Microbiol Rev 51: 221–271.

33 Yabuuchi E, E Tanimura, A Ohyama, I Yano and A Yamamoto. 1979. *Flavobacterium devorans* ATCC 10829; a strain of *Pseudomonas paucimobilis*. J Gen Appl Microbiol 25: 95–107.

34 Yabuuchi E, I Yano, H Oyaizu, Y Hashimoto, T Ezaki and H Yamamoto. 1990. Proposals of *Sphingomonas paucimobilis* gen nov and comb nov, *Sphingomonas parapaucimobilis* sp nov, *Sphingomonas yanoikuyae* sp nov, *Sphingomonas adhaesiva* sp nov, *Sphingomonas capsulata* comb nov, and two genospecies of the genus *Sphingomonas*. Microbiol Immunol 34: 99–119.

35 Yamada H, H Tokuda and S Mizushima. 1989. Proton motive force-dependent and -independent protein translocation revealed by an efficient *in vitro* assay system of *Escherichia coli*. J Biol Chem 264: 1723–1728.

36 Yamamoto A, I Yano, M Masui and E Yabuuchi. 1978. Isolation of a novel sphingoglycolipid containing glucuronic acid and 2-hydroxy fatty acid from *Flavobacterium devorans* ATCC 10829. J Biochem 83: 1213–1216.

37 Yrjälä K, S Suomalainen, EL Suhonen, S Kilpi, L Paulin and M Romantschuk. 1998. Characterization and reclassification of an aromatic- and chloroaromatic-degrading *Pseudomonas* sp, strain HV3, as *Sphingomonas* sp HV3. Int J Syst Bacteriol 48: 1057–1062.