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**Cauloform bacteria lacking phospholipids from an abyssal hydrothermal vent:
Proposal of *Glycocaulis abyssi* gen. nov., sp. nov. belonging to the *Hyphomonadaceae***

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Running Title: *Glycocaulis abyssi* gen. nov., sp. nov.

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Abbreviations: CID-MS Collision induced dissociation mass spectrum, FAB MS Fast atom bombardment mass spectrometry, FAME Fatty acid methyl ester

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MCS 33^T is AJ227811.

Abstract

Cauloform bacteria are prosthecate bacteria often specialized for oligotrophic environments. A polyphasic approach, comprising 16S rRNA gene sequencing, lipid analysis, and salt tolerance characterizations were used to clarify the taxonomy of one isolate - MCS 33^T - obtained from above the hot water plume of a deep sea hydrothermal vent near Vancouver island, Canada. It contained no detectable phospho- or sulfolipids, but 1,2-di-O-acyl-3-O- α -D-glucopyranosylglycerol, 1,2-di-O-acyl-3-O- α -D-glucopyranuronosylglycerol, and the novel lipid 1,2-di-O-acyl-3-[O- α -D-glucopyranuronosyl]glycerol-6'-N-glycine. It is assumed that the various glucuronosyl lipids are replacing at least partially the phospholipids in their various tasks in the cell cycle. The G+C content of the chromosomal DNA of the strains was determined to be 62.8 mol%, Q10 was found to be the major ubiquinone. The 16S rRNA gene sequence of this chemoheterotrophic, aerobic, and moderate halophilic strain showed only a low similarity of 94.4 % to that of *Oceanicaulis alexandrii* C116-18^T and both species differed also by their lipids. Although the strain was isolated from seawater sampled near a hydrothermal vent its temperature optimum was at 30° C. Main cellular fatty acids were C18: ω 7, C18:0 and the unknown fatty acid ECL 11.798, main hydroxy-fatty acid was C12:0 3-OH. The strain is proposed to comprise a new species of a new genus, *Glycocalis abyssi* gen. nov., sp. nov., the type strain of which is MCS 33^T (= CCUG 62981^T = LMG xxx^T).

For decades bacteria having a stalk and reproducing regularly by the separation of two cells that are morphologically and behaviorally different from each other were regarded as members of the genus *Caulobacter* (Poindexter 1964). One descendant is nonmotile, sessile due to adhesion to the substratum, and prosthecate, possessing a tubular appendage of variable length- a *prostheca* - (Staley 1968). The other descendant is actively motile by

means of one polar flagellum. The mode of reproduction of the dimorphic prosthecate bacteria is regarded as a reflection of an ecological adaptation helping to disperse the population at each generation and thereby minimizing competition between descendants for resources. It is consistent with this that these bacteria exhibit the physiologic properties of oligotrophs (Poindexter, 1981). Henrici and Johnson (1935) placed bacteria of these characteristics into the new genus *Caulobacter*. Stahl *et al.* (1992) analysed the phylogeny of a number of caulobacteria and found a low similarity between the sequences of 16S rRNA. We analyzed a large number of strains for their pattern of proteins, polar lipids and 16S rRNA gene sequences (Abraham *et al.*, 1997) and revealed the paraphyletic nature of marine caulobacteria belonging mainly to the two genera *Brevundimonas* Segers *et al.* and *Maricaulis* Abraham *et al.* (1999). Especially the marine isolates show a high diversity with strains differing so much from the genus *Maricaulis* that they cannot be included in this genus but belong to separate genera, i. e. *Oceanicaulis* (Strömpl *et al.*, 2003) and *Woodsholea* (Abraham *et al.*, 2004). We describe here another of these isolates, MCS 33^T, which does not fit in any of the known genera.

The strains of this study were obtained from the American Type Culture Collection (ATCC), the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSM strains), and from the Dept. of Microbiology and Immunology at the University of British Columbia (MCS strains). The strains were grown in the marine medium SPYEM: 30 g Sea Salts (Sigma), 0.5 g NH₄Cl, 1 l MQ-water. After autoclaving and cooling to ambient temperature 20 ml 50xPYE, 2 ml 50 % glucose (sterile) and 5 ml riboflavin (0.2 mg ml⁻¹) sterile filtrated were added. 50xPYE: 100 g peptone and 50 g yeast extract in 1 l deionized water (autoclaved). The strains were grown in 2 l Erlenmeyer flasks at 30 °C and 100 r. p. m. and the biomass was harvested in the late exponential phase after 72 h.

For electron microscopy growing cells were prepared for negative staining, embedding and ultrathin sectioning as described in detail by Yakimov *et al.* (1998). MCS 33^T cells showed morphological features typical of caulobacteria, when analysed by transmission electron microscopy. During cell division two morphotypes of daughter cells can be observed, first, the swarmer cell, which is monopolarly and monotrichously flagellated and second, the prosthecae daughter cell with its stalk (diameter: 85 nm), ending in a spherical holdfast (diameter: 140 nm) (Fig. 1a). Typically, MCS 33^T forms groups or clusters of cells (Fig. 1b), which often are bundled by aggregated holdfasts (Fig. 1c: circle). The overall cell shape is vibrioid and cell length ranges from 1.3 µm to 2.5 µm, cell width is from 0.65 µm to 0.72 µm. The interior part of the stalk appears only slightly electron dense and is surrounded by the electron translucent periplasm. The cell wall architecture is gram-negative, since an outer membrane, which is intensely undulated, forms the outer boundary of the cell. (Fig. 1c, d).

Genomic DNA was isolated from two loopfuls of bacterial cells using the DNeasy Blood and Tissue kit for purification of total DNA (Qiagen) with the addition of RNase A (Sigma), according to the manufacturers' instructions. DNA was enzymatically digested as described by Gehrke *et al.* (1984) and the mean G+C content was determined by HPLC (Tamaoka & Komagata, 1984). Calculations were carried out according to Mesbah *et al.*, (1989), with non-methylated lambda-phage DNA (Sigma) as a standard. The phylogenetic position of strain MCS33^T was determined by analysis of the 16S rRNA gene sequence (Abraham *et al.*, 1999) using the software CLUSTAL W (Thompson *et al.*, 1997). The 16S rRNA gene sequence showed 94.4% identity to the one of *Oceanicaulis alexandrii* C116-18^T and 93.5% to *Woodsholea maritima* CM243^T. Alignment of sequences from the EMBL database (Karsch-Mizrachi *et al.*, 2012) was used to construct a bootstrap consensus tree

using neighbour joining algorithm (Figure 2) and maximum parsimony (Suppl. Figure S1) with MEGA version 4.1 (Tamura *et al.*, 2007) based on 1000 resamplings and *Caulobacter vibrioides* VKM1496^T as an outgroup.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and analyzed by the method published by Minikin *et al.* (1984). Main ubiquinone of MCS33^T was found to be ubiquinone Q-10. For whole cell fatty acid analysis cells were saponified (15 % (w/v) NaOH, 30 min, 100 °C), methylated to fatty acid methyl esters (FAMES) (methanolic HCl, 10 min, 80 °C) and extracted (hexane/methyl-tert-butyl ether (1:1, v/v)) as described in detail by Osterhout *et al.* (1991). Fatty acid methyl esters were analyzed on a Hewlett-Packard (HP) 5890A gas chromatograph. Separation of fatty acid methyl esters was achieved with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenyl methyl silicone (film thickness 0.33 µm; HP Ultra 2). The computer-controlled parameters were the same as those described by Osterhout *et al.* (1991). The instrument was equipped with a flame ionization detector and an autosampler (HP 7673). H₂ was serving as carrier gas. The cellular fatty acid compositions of MCS 33^T, *Maricaulis*, *Oceanicaulis*, *Woodsholea* and *Hyphomonas* type strains are shown in Table 1. The *Maricaulis* species were the only ones in the *Hyphomonaceae* where 11:0 iso 3OH, 17:0 iso, 17:1 iso ω9c and 18:1ω9c were present and 12:0 3OH and 12:1 3OH absent (Abraham *et al.*, 1999). In MCS 33^T 11:0 iso 3OH, 17:0 iso, 17:1 iso ω9c, 18:1ω9c and 15:0 were all absent and 12:0 3OH and 12:1 3OH present. An unknown fatty acid with ECL 11.798, which is absent in *Maricaulis* strains, was found in considerable amounts in strain MCS 33^T. The main hydroxy fatty acid of this species is 12:0 3OH which is absent in *Maricaulis* strains, while the main hydroxy fatty acid of *Maricaulis* strains, 11:0 iso 3OH, is absent in MCS 33^T.

For polar lipid fatty acid analysis lipids were extracted using a modified Bligh-Dyer

procedure (Blight and Dyer 1959) as described previously (Vancanneyt *et al.* 1996) and analysed by Fast Atom Bombardment Mass Spectrometry (FAB-MS) (Abraham *et al.* 1997). The polar lipids of MCS 33^T consisted entirely of glycolipids. No phospholipids or sulfolipids were detected (Figure 3). The glycolipids were α -D-glucopyranosyl- and α -D-glucopyranuronosyl-diacylglycerols, also common in *Caulobacter*, *Brevundimonas*, *Maricaulis* and some other alpha-Proteobacteria. Using MS/MS the structures of twenty-one of these glycolipids could be identified as listed in Table 2. Additionally to these glycolipids the (-)-FAB MS showed two ions with higher masses. Their masses were even, requiring odd-numbered molecular masses pointing to the presence of nitrogen in the molecules. To elucidate the structure of these lipids intensive MS studies were performed. The (-)-FAB CID mass spectrum of the ion at m/z 854 showed the loss of two different fatty acids, octadecenoic acid and octadecanoic acid, as ions corresponding to the neutral loss of free fatty acids (m/z 572 and 570) and the corresponding ketenes (m/z 590 and 588) were observed (Suppl. Fig. S2). Furthermore there were a number of fragments in the upper mass region at intervals of about 14 mass units. These correspond to fragmentations along the fatty acid acyl chains and represent charge remote fragmentation similar to that observed in other polar lipids (Jensen *et al.* 1987). After the neutral losses of one fatty acid and the ketene of the other one from the molecular ion at m/z 854 an ion of m/z 306 amu was formed. This diagnostically important daughter ion at m/z 306 fragmented further by the neutral loss of dehydro-glycerol giving the even-numbered and hence N-containing ion at m/z 232. A similar series of fragmentation leads via the neutral loss of the two fatty acids as ketenes to the ion m/z 324 which loses dehydro-glycerol to the pyranosyl ion at m/z 250. The other minor component at m/z 852 in the lipid fraction behaved in a similar way. The polar lipid therefore seemed to be a glucuronosyl-glycerol which is esterified at the 6-position to glycine. The observed daughter

ions are in full accord with the postulated partial structure as indicated in the fragmentation scheme. The relative abundance of the carboxylate anions provides evidence for the relative positions of the two acyl functions. By analogy to phospholipids the loss of the sn-2-acyl position may be favoured, thus yielding a more abundant carboxylate anion (Murphy & Harrison, 1994). The same seemed to be true here. MS data led to the identification of the main component as 1-octadecenoyl-2-octadecanoyl-3-[O- α -D-glucopyranuronosyl]glycerol-6'-N-glycine and the minor compound as 1,2-di-octadecenoyl-3-[O- α -D-glucopyranuronosyl]glycerol-6'-N-glycine.

For phenotypic characterization strains were grown in 20 ml medium PYEM (2 g peptone, 2 g yeast extract, 0.5 g ammonium chloride, 1 l deionized water. After autoclaving and cooling to ambient temperature 5 ml riboflavin (0.2 mg ml⁻¹) sterile filtrated, 2 ml 50 % glucose (sterile), 1 ml 20 % magnesium sulphate (sterile) and 1 ml 10 % calcium chloride (sterile) were added and amended with 0, 5, 10, 20, 30, 40, 60, 80 or 100 g l⁻¹ NaCl. OD₆₀₀ of the cell suspension was determined at the beginning of the experiment and after 2 days. The differences between these two measurements were used to determine salt tolerances. The growth was tested at different temperatures in SPYEM medium with the same OD protocol. In a medium without sodium chloride weak growth of MCS 33^T was observed and a salt concentration of 100 g l⁻¹ also allowed only weak growth of this species. Good growth occurred between 20-60 g l⁻¹. For comparison *Maricaulis maris* ATCC 15268^T showed a similar behaviour but tolerated also 80-100 g l⁻¹ for at least moderate growth. Temperature below 15°C and above 40°C inhibited growth of MCS 33^T which had its growth optimum at 30°C. While *Maricaulis maris* could not grow below 15° C and at 50° C it showed its growth optimum at 40°C.

Enzyme activity tests were conducted with API ZYM test strips (bioMérieux), according to the manufacturer's instructions. Substrate specificity tests were conducted with API 20NE test strips (bioMérieux) and BIO Typ 100 (bioMérieux), according to the manufacturers' instructions, at 30 °C for 4 days. A test was considered positive if a colour change was noticeable. The results are given in the species description. The characters that discriminate between strain MCS33^T and its closest phylogenetic neighbours are summarized in Table 2.

Conclusions

The entire lack of phospholipids in strain MCS 33^T deserves some discussion. Minnikin *et al.* (1974) reported on a reduction of the phospholipid content of *Brevundimonas diminuta* under phosphate limitation, and high levels of glycolipids and sulfolipids were reported from marine caulobacter strains, including *Maricaulis maris* (De Siervo, 1985) reaching amounts as low as 0.3% of the total lipids. We also found considerable amounts of sulfolipids in *Maricaulis* spp. but they were not detected in MCS 33^T. Taking the various functions of phospholipids identified in the cell cycle into account (Dowhan, 1997) it is puzzling how these functions can be replaced by glycolipids in MCS 33^T. One possibility is that the glycine lipids can replace some of the phospholipids. A deeper understanding of the function of these glycolipids in MCS 33^T should give us a much broader insight into the role of polar lipids beyond their involvement in the cell membrane. Results of such studies can probably reveal some mechanisms which are valid for many eubacterial cells.

The ecological niche of strain MCS 33^T is worth mentioning. The deep sea is influenced by three parameters: low temperature, high hydrostatic pressure and low nutrient concentration. This last feature is the consequence of the total darkness of the deep sea

preventing any photosynthesis. Consequently, deep-sea bacteria depend on the sinking of material that has escaped degradation in the upper layers of the ocean (Jannasch & Tylor, 1984; Witte *et al.*, 2003). This situation is dramatically changed around deep-sea hydrothermal vents. Here a productive ecosystem almost independent from solar energy is located which uses oxygen only as the product of photosynthesis. It is driven by hydrothermal fluids resulting from seawater penetrating into the ocean crust and heated by magma chambers leaching minerals. A shell of warm water is found around these hydrothermal vents which cools down to 2°C, the temperature typical for the deep sea, within a few hundred meters around the vent (Baross *et al.*, 1982). Although strain MCS 33^T was isolated from seawater sampled near a hydrothermal vent it did not differ significantly from the temperature requirements known from the other cauloform bacteria. The species probably lives in a narrow zone around the hydrothermal vent where the temperature just fits the requirement of the species, neither too high nor reaching the usual +2°C of the deep sea. Together with the oligotrophic properties of the strain this points to an interesting specialization of this microorganism. The lack of phospholipids reduces the essential amount of phosphorus required by this strain (Van Mooy *et al.* 2009). This can be an additional advantage for the bacteria to occupy a niche in a thin shell around the hydrothermal vents where the temperatures are optimal to support growth of this microorganism. It remains to be determined whether this kind of bacteria can also be found at other hydrothermal vents and what their specific roles are in the food web of hydrothermal vent microbial communities.

Concerning the rather low 16S rRNA gene sequence similarity of MCS 33^T with its closest neighbours it became obvious that this strain may belong to a novel genus. The analysis of the whole cell fatty acids of MCS 33^T revealed the same picture seen from the 16S rRNA gene sequence analysis. A number of fatty acids, 11:0 iso 3OH, 17:0 iso, 17:1 iso ω9c

and 18:1 ω 9c, characteristic for the *Maricaulis* group, were absent while 12:0 3OH and 12:1 3OH were found which were absent in *Maricaulis* strains. In terms of its fatty acids profile MCS 33^T is closer to species of the genus *Hyphomonas* than to the genus *Maricaulis* (Table 1) although the multiplication of the cells of MCS 33^T is the same found for *Maricaulis* species. Strain MCS 33^T differed from *Maricaulis* spp. and *Oceanicaulis* spp. by the lack of sulpho- and phospholipids and from *Woodsholea* spp. by the lack of sulpho- and taurine lipids (Abraham *et al.*, 1997). Within the *Hyphomonadaceae* strain MCS 33^T has chemotaxonomically a rather unique position because of the lack of phospho- and sulpholipids. The 16S rRNA gene sequence and the lipid pattern of this isolate from the deep sea identifies it as a member of a novel genus for which we propose the name *Glycocalis* with *Glycocalis abyssi* as type species and MCS 33^T as type strain.

New genus and species

Glycocalis gen. nov.

Description of *Glycocalis* gen. nov. *Glycocalis* (Gly-co-'cau-lis. Gr. n. *glycos* sugar; L. n. *caulis* stalk; M. L. masc. n. *Glycocalis*, sugar stalk). Gram-negative cells, rod shaped, fusiform or vibrioid, cells possess a prostheca varying in length depending on environmental conditions, extending from one pole as a continuation of the long axis of the cell. Adhesive material is present at the distal end of the prostheca. Multiplication by binary fission. At the time of division one cell possesses a prostheca and the other a single polar flagellum. Each appendage occurs at the cell pole opposite the one formed during fission. The flagellated cell secretes adhesive material at the base of the flagellum, develops a prostheca at this site, and enters the immotile vegetative phase. Chemoorganotrophic, strict aerobes. Catalase, alkaline phosphatase and leucine arylamidase-positive and oxidase-negative; requirements for organic

growth factors not satisfied by mixtures of B vitamins and amino acids. For growth NaCl is required. Growth is inhibited or cells become deformed in media containing 1% (w/v) or more of organic material. The genus is characterized by two major fatty acids 18:1 ω 7, 18:0 and the hydroxy fatty acid 12:0 3-OH. Polar lipids are α -D-glucopyranosyl diacylglycerol and α -D-glucopyranuronosyl diacylglycerol, while sulfoquinovosyl diacyl glycerols and phospholipids are absent. Isolated from sea water. The G+C content is 63 %. The type species is *Glycocalis abyssi*.

Glycocalis abyssi sp. nov. (a-bys'si. L. n. *abyssus* depth; *abyssi* from the depth). The description of *Glycocalis abyssi* is the same as the one given for the genus. Cells are rod shaped, 0.3-0.4 by 0.8-1 μ m, prostheca is ca. 0.05 μ m in diameter. Colonies circular, convex, colorless. Can store carbon as poly- β -hydroxybutyric acid. No acid is produced from carbohydrates and nitrate is not reduced. With API ZYM system, the species is positive in esterase, esterase-lipase, valine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, weakly positive in cystine arylamidase and β -glucosidase but negative in lipase, α - and β -galactosidase, β -glucuronidase, α -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, and α -fucosidase. In the BIOTYP 100 system is uses esculin, myo-inositol, L(+)-tartrate and L-tyrosine but not the other substrates. The species grows on peptone-yeast extract media with 30 g l⁻¹ NaCl with optimal growth at 20-60 g l⁻¹ NaCl. For growth NaCl is required, salt concentrations above 100 g l⁻¹ NaCl are not tolerated. *G. abyssi* grows fairly well between 15°C and 35°C, while the optimal growth temperature is 30°C. optimal pH for growth is around neutrality, pH range is 6.0-8.0. Polar lipids are α -D-glucopyranosyl diacylglycerol, α -D-glucopyranuronosyl diacylglycerol and α -D-glucopyranosyl diacylglycerol-6'-*N*-glycine, while sulfoquinovosyl diacyl glycerols and

phospholipids are absent. Isolated from water obtained from above the hot water plume of a deep sea hydrothermal vent near Vancouver island, Canada. The type strain is MCS 33^T (= LMG ...^T = CCM ...^T). The G+C content is 62.8 %.

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Table 1: Fatty acid content (mean percentage of total) of whole-cell hydrolysates of *Glycoaulis abyssi* in comparison with *Maricaulis*, *Woodsholea*, *Oceanicaulis* and *Hyphomonas* species^a

1 = MCS 33^T, 2 = *Maricaulis maris* ATCC 15268^T, 3 = *M. washingtonensis* MCS 6^T, 4 = *M. salignorans* MCS 18^T, 5 = *M. parjimensis* MCS 25^T, 6 = *M. virginensis* VC-5^T, 7 = *Woodsholea maritima* CM243^T, 8 = *Oceanicaulis alexandrii* C116-18^T, 9 = *O. stylophorae* GISW-4^T (data from Chen *et al.*, 2011), 10 = *Hyphomonas polymorpha* DSM 2665^T, 11 = *H. jannaschiana* ATCC 33833^T

	1	2	3	4	5	6	7	8	9 ^c	10	11
iC11:0 3-OH	-	2.6	tr ^b	tr	tr	5.4	-	-	-	-	
ECL 11.798 ^a	7.4	-	-	-	-	-	-	-	-	-	-
C12:0 3-OH	3.0	-	-	-	-	-	3.5	1.3	3.0	tr	tr
C12:1 3-OH	tr	-	-	-	-	-	-	-	-	1.2	1.5
C15:0	-	tr	tr	tr	-	-	-	-	-	1.9	1.7
ECL 15.275	-	-	-	-	-	-	6.1	-	-	-	-
C16:0	4.6	17.0	11.2	8.9	3.6	9.8	1.4	2.2	2.3	1.9	10.4
Sum4	-	6.6	3.0	2.6	2.2	2.4	-	-	-	-	tr
C16:1 ω 9c	-	1.0	tr	tr	-	-	-	-	-	-	-
C17:0	2.9	5.3	9	8.7	7	15.3	2.2	18.0	6.9	18.3	9.7
C17:1 ω 6c	1.8	tr	1.0	1.1	1.8	1.3	tr	3.9	-	15.3	4.2
C17:1 ω 8c	-	4.0	10.2	10.0	4.7	9.6	-	1.0	-	10.9	4.8
iC17:0	-	7.7	9.6	10.8	1.7	6.9	-	-	-	-	-
iC17:1 ω 9c	-	17.4	22.4	28.0	3.9	13.8	-	-	-	-	-
C18:0	10.8	1.1	tr	tr	7.9	4.2	16.9	18.2	29.3	tr	3.7
C18:1 ω 7c	68.7	24.5	16.2	12.9	47.9	13.0	65.4	29.1	27.8	21.7	48.4
C18:1 ω 9c	-	6.4	10.7	7.7	6.0	3.4	-	-	-	-	-
11Me-C18:1 ω 5	tr	1.6	tr	1.0	tr	2.9	-	18.7	25.8	1.1	7.0
ECL18.424	-	1.3	1.3	1.8	2.3	5.7	-	-	-	-	-
ECL18.797	-	tr	tr	tr	4.9	2.6	-	-	-	20.3	5.0

^aOnly the fatty acids counting for more than 1.0% (mean amount) were indicated. The following strains contained also significant amounts (>1.0%) of additional fatty acids: *Hyphomonas polymorpha* DSM 2665^T also contained Sum9 (19:0 cyclo ω 10c and/or ECL 18.846 and/or ECL 18.858; 4.5%); *Oceanicaulis alexandrii* contained 4.6% and *O. stylophorae* 1.1% C19:0; Sum4 = c18:1 ω 7/t18:1 ω 9/t18:1 ω 12/t18:1 ω 7 (ECL 17.798); ^bUnidentified fatty acid with equivalent chain length of 11.798; ^ctr, Trace amount (< 1.0%); ^ddata from Chen *et al.*, 2011

Table 2: Polar lipids identified in *G. abyssi* MCS 33^T by FAB ionization and CID-MS

Mass	Type	Fatty acids	
		sn-1	sn-2
726	MGD	16:1	16:1
728	MGD	16:1	16:0
768	MGD	18:1	17:1
768	MGD	19:1	16:1
770	MGD	18:1	17:0
770	MGDOx	18:1	16:0
772	MGDOx	18:0	16:0
784	MGDOx	18:1	17:0
784	MGDOx	18:0	17:1
796	MGDOx	18:1	18:1
798	MGDOx	18:1	18:0
798	MGDOx	19:1	17:0
810	MGDOx	19:1	18:1
810	MGDOx	19:2	18:0
810	MGDOx	20:2	17:0
812	MGDOx	19:1	18:0
822	MGDOx	20:1	18:2
822	MGDOx	20:2	18:1
822	MGDOx	20:3	18:0
824	MGDOx	20:1	18:1
824	MGDOx	20:2	18:0
853	GGG	18:1	18:1
855	GGG	18:1	18:0

MGD=1,2-di-O-acyl-3-O- α -D-glucopyranosylglycerol, MGDOx=1,2-di-O-acyl-3-O- α -D-glucopyranuronosylglycerol, GGG = 1,2-di-O-acyl-3-[O- α -D-glucuronopyranosyl]glycerol-6'-N-glycine

Table 3: Differential phenotypic characteristics among MCS 33^T and its closest relatives *Woodsholea maritima* CM243^T, *Oceanicaulis alexandrii* C116-18^T, *O. stylophorae* GIS-W4^T, *Maricaulis salignorans* MCS 18^T and *M. washingtonensis* MCS 6^T.

	MCS 33 ^T	CM243 ^T	C116-18 ^T	GIS-W4 ^T	MCS 18 ^T	MCS 6 ^T
Growth temperature [°C]	15-35	10-40	4-35	15-45	10-40	4-30
Salt tolerance [g l ⁻¹]	20-60	5-100	20-100	0-90	0-80	0-80
Valine arylamidase	++	+	+/-	+	+	+/-
Cystine arylamidase	+/-	+	+/-	+	+	+/-
Trypsin	++	+	++	++	++	++
α -Chymotrypsin	++	+	++	++	++	++
Acid phosphatase	+	++	+/-	+	+/-	-
Naphtol-AS-BI-phosphohydrolase	++	++	+	+	+	+
β -glucosidase	+	-	-	-	-	-
<i>N</i> -acetyl- β -glucosaminidase	-	+	-	-	-	-
oxidase	-	+	+	+	+	+
protease	-	-	-	+	-	-
nitrate reduction	-	-	+	-	+	+
Assimilation of						
Malate	+	-	-	-	-	-
Citrate	-	-	-	+	-	-
Arabinose	-	-	-	+	-	-
Adipate	+	-	-	+	-	-
Hydroxy-fatty acids	C12:0 3OH C12:1 3OH	C12:0 3OH	C12:0 3OH	C12:0 3OH	iC11:0 3OH	iC11:0 3OH
Polar lipids	MGD MGDO _x GGG	MGD MGDO _x SQD Taur	MGD MGDO _x PG SQD	n. d.	MGD MGDO _x (PG) SQD	MGD MGDO _x (PG) SQD
G+C %	62.8	65.2	61.8	61.6	63.3	63.0

Legends:

Figure 1: Ultrastructure of *Glycocalyx abyssi* MCS 33^T. (a) Negatively stained dividing cells, which show characteristic caulobacterial features. One daughter cell is the swarmer cell and carries a single flagellum. It is still connected to the second prosthecae daughter cell by an extended septum. The stalk is terminated by characteristic spherical holdfast. (b) Survey view of a cluster of cells, which show prosthecae of different developmental states. (c) Ultrathin section of *G. abyssi* MCS 33^T. A longitudinal cut shows the vibrioid shape of the cell body, which contains a pronounced chromosome, surrounded by densely packed cytoplasm. Many prosthecae end up in a tight cluster of holdfasts and form a typical rosette-like ensemble (white circle). (d) Detailed view of the cell wall, which presents the outer membrane and the cytoplasmic membrane. The periplasm appears as an electron translucent matrix. (fl: flagellum; es: extended septum; pr: prosthecum; hf: holdfast; CP: cytoplasm; om: outer membrane; cM cytoplasmic membrane; ★: chromosome) Bars 1 μm (a, b); 500 nm (c); 50 nm (d).

Figure 2: Neighbour-joining tree constructed with 16S rRNA gene sequences, showing the phylogenetic position of strain MCS 33^T within the family *Hyphomonadaceae*. Evolutionary distances were computed using the method of Jukes & Cantor (1969). The sequence of *Caulobacter vibrioides* VKM 1496^T (GenBank accession no. AJ227754; not shown) was used as an outgroup. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

Figure 3: Fast Atom Bombardment mass spectrum (FAB) recorded in the negative mode of the total lipids from *Glycocalyx abyssi* MCS 33^T. In the range between m/z 725-823 1,2-di-O-acyl-3-O- α -D-glucopyranosyl glycerols (MGD) and 1,2-di-O-acyl-3-O- α -D-glucuronopyranosyl glycerols (MGDOx), and finally around m/z 850 1,2-di-O-acyl-3-[O- α -D-

glucuronopyranosyl]glycerol-6'-N-glycines (GGG) are seen. Insert shows the chemical structure of the novel lipid GGG.

Supplementary Figure S1: Maximum parsimony tree constructed with 16S rRNA gene sequences, showing the phylogenetic position of strain MCS 33^T within the family *Hyphomonadaceae*. The sequence of *Caulobacter vibrioides* VKM 1496^T (GenBank accession no. AJ227754; not shown) was used as an outgroup. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes.

Figure 1: Morphology of strain MCS 33^T

Figure 2:

Figure 3: FAB-neg MS of the phospholipid fraction

Supplementary Figure S1: Phylogenetic tree MP