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

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Cauliform bacteria are prosthecate bacteria often specialized for oligotrophic environments. A polyphasic approach, comprising 16S rRNA gene sequencing, lipid analysis and salt tolerance characterizations, was used to clarify the taxonomy of one isolate, strain MCS 33^T, obtained from above the hot water plume of a deep-sea hydrothermal vent near Vancouver island, Canada. Cells contained no detectable phospholipids or sulpholipids, but did contain 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 glucoronosyl lipids are replacing, at least partially, the phospholipids in their various tasks in the cell cycle. The G+C content of the genomic DNA of strain MCS 33^T was 62.8 mol%, and Q10 was the predominant respiratory ubiquinone. The 16S rRNA gene sequence of this chemoheterotrophic, aerobic, moderately halophilic strain showed only a low similarity of 94.4 % to that of *Oceanicaulis alexandrii* C116-18^T, and both strains also differed based on their lipids. Although the novel strain was isolated from seawater sampled near a hydrothermal vent, its optimum temperature for growth was 30 °C. The main cellular fatty acids were $C_{18:1}\omega7c$, $C_{18:0}$ and the unknown fatty acid ECL 11.798, and the main hydroxy fatty acid was $C_{12:0}$ 3-OH. The strain is proposed to represent a novel species of a new genus, *Glycocaulis abyssi* gen. nov., sp. nov. The type strain of the type species is MCS 33^T (=LMG 27140^T=CCUG 62981^T).

For decades bacteria having a stalk and reproducing regularly by the separation of two cells that are morphologically and behaviourally different from each other were regarded as members of the genus *Caulobacter* (Poindexter 1964). One descendant is non-motile, 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. Consistent with this is that these bacteria exhibit the physiological properties of oligotrophs (Poindexter, 1981). Henrici and Johnson (1935) placed bacteria possessing these characteristics into the new genus *Caulobacter*. Stahl *et al.* (1992) analysed the phylogeny of a number of caulobacteria and found low levels of similarity between sequences of the 16S rRNA gene. We analysed 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.*, 1994) and *Maricaulis* (Abraham *et al.*, 1999). The marine isolates show particularly 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 described genera.

Abbreviation: FAME, fatty acid methyl ester.

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

Three supplementary figures are available with the online version of this paper.

The strains used in this study were obtained from the American type Culture Collection (ATCC), the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ), the Department of Microbiology and Immunology at the University of British Columbia (MCS strains, *Maricaulis virginensis* VC-5^T, *Woodsholea maritima* CM243^T) and from C. Strömpl, Helmholtz Center for Infection Research, Braunschweig, Germany (*Oceanicaulis alexandrii* C116-18^T). The strains were grown in the marine medium SPYEM: 30 g sea salts (Sigma), 0.5 g NH_4Cl, 1 litre MilliQ-water. After autoclaving and cooling to ambient temperature, 20 ml 50 × PYE, 2 ml 50 % glucose (sterile) and 5 ml riboflavin (0.2 mg ml^{-1}) sterile filtered were added. 50 × PYE includes 100 g peptone and 50 g yeast extract in 1 litre deionized water (autoclaved). The strains were grown in 2-litre 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). Cells of strain MCS 33^T showed morphological features typical of caulobacteria, when analysed by transmission electron microscopy. During cell division two morphotypes of daughter cells were observed: first, the swarmer cell, which is monopolarly and monotrichously flagellated; and, second, the prosthecate daughter cell with its stalk (diameter 85 nm), ending in a spherical holdfast (diameter 140 nm) (Fig. 1a). Typically, strain MCS 33^T formed groups or clusters of cells (Fig. 1b), often bundled by aggregated holdfasts (Fig. 1c, circle). Overall cell shape was vibrioid and cell length ranged from 1.3 to 2.5 μm and cell width from 0.65 to 0.72 μm. The interior part of the stalk appeared only slightly electron-dense and was surrounded by the electron-translucent periplasm. The cell-wall architecture was Gram-negative; an outer membrane, which was intensely undulated, formed 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 enzymically 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. Amplification of the 16S rRNA gene and sequencing was done as described previously (Abraham *et al.*, 2010). The phylogenetic position of strain MCS 33^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 94.2 % similarity to that of both *Maricaulis maris* ATCC 15268^T and *O. alexandrii* C116-18^T and 93.5 % to *W. maritima* CM243^T. Alignment of sequences from the EMBL database (Karsch-Mizrachi *et al.*, 2012) was used to construct a

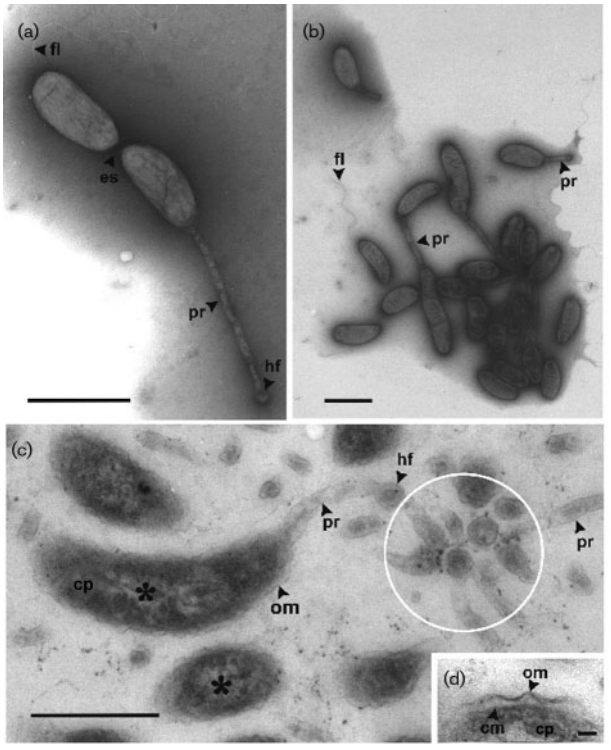

Fig. 1. Ultrastructure of cells of strain MCS 33^T. (a) Negatively stained dividing cells, which show characteristic caulobacterial features. One daughter cell is the swarmer cell and carries a single flagellum. It is still connected to the second prosthecate daughter cell by an extended septum. The stalk is terminated by a characteristic spherical holdfast. (b) Survey view of a cluster of cells, which show prostheca of different developmental states. (c) Ultrathin section. A longitudinal cut shows the vibrioid shape of the cell body, which contains a pronounced chromosome, surrounded by densely packed cytoplasm. Many prostheca end up in a tight cluster of holdfasts and form a typical rosette-like ensemble (white circle). (d) Detailed view of the cell wall, showing the outer membrane and 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; stars, chromosome. Bars 1 μm (a, b), 500 nm (c), 50 nm (d).

bootstrap consensus tree using the neighbour-joining algorithm (Fig. 2), maximum-parsimony (Fig. S1, available in IJSEM Online) and maximum linkage (Fig. S2) with MEGA version 5.0.5 (Tamura *et al.*, 2011), based on 1000 resamplings and with *Caulobacter vibrioides* VKM1496^T as an outgroup.

Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v) and analysed by the method published by Minnikin *et al.* (1984). The main ubiquinone of strain MCS33^T was 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/

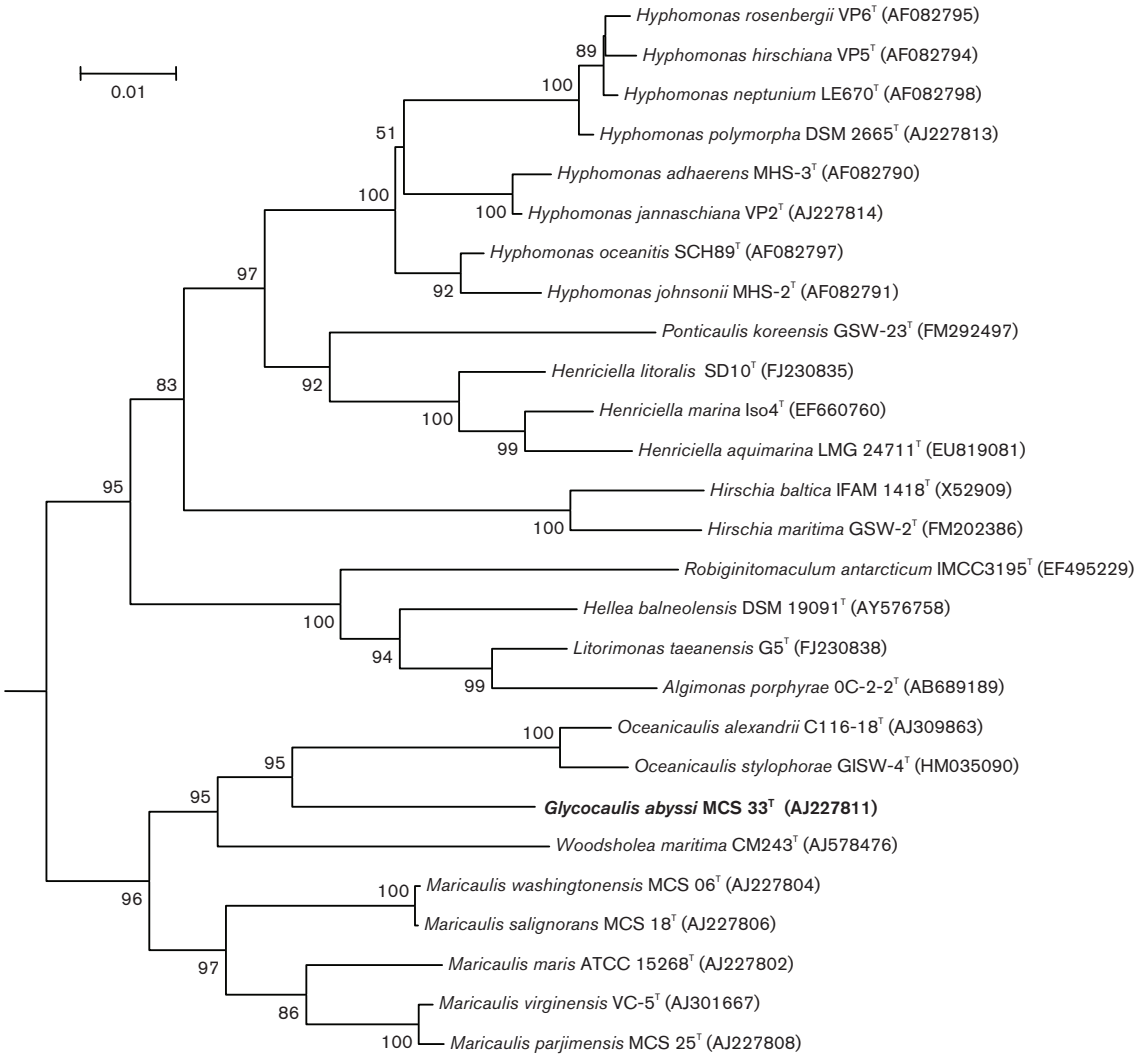

Fig. 2. Neighbour-joining tree constructed with 16S rRNA gene sequences, showing the phylogenetic position of strain MCS 33^T within the family *Hyphomonadaceae*. Evolutionary distances were computed using the method of Jukes & Cantor (1969). The sequence of *Caulobacter vibrioides* VKM 1496^T (GenBank 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.

methyl-tert-butyl ether (1 : 1, v/v)] as described by Osterhout *et al.* (1991). FAMEs were analysed on a Hewlett Packard (HP) 5890A gas chromatograph. Separation of FAMEs 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). H_2 served as carrier gas. The cellular fatty acid compositions of strain MCS 33^T and the type strains of 10 recognized species of *Hyphomonadaceae* are shown in Table 1. In strain MCS 33^T, iso-$C_{11:0}$ 3-OH, iso-$C_{17:0}$, iso-$C_{17:1}\omega9c$, $C_{18:1}\omega9c$ and $C_{15:0}$ were all absent but $C_{12:0}$ 3-OH and $C_{12:1}$ 3-OH were present while sopceis of the genera *Maricaulis* were the only ones in the *Hyphomonadaceae* where iso-$C_{11:0}$ 3-OH, iso-$C_{17:0}$, iso-$C_{17:1}\omega9c$ and $C_{18:1}\omega9c$ were present but $C_{12:0}$ 3-OH and $C_{12:1}$ 3-OH were absent (Abraham *et al.*, 1999). An unknown fatty acid with ECL 11.798, which was absent in members of the genera *Maricaulis*, *Woodsholea* and *Oceanicaulis*, was found in considerable amounts in strain MCS 33^T. The main hydroxy fatty acid of strain MCS 33^T was $C_{12:0}$ 3-OH, which was also found in species of the genera *Oceanicaulis* and *Woodsholea* but absent in members of the genus *Maricaulis*. In addition to $C_{12:0}$ 3-OH, strain MCS 33^T contained also small amounts of $C_{12:1}$ 3-OH, which was not found in species of the genera *Maricaulis*,

Table 1. Fatty acid content (mean % of total) of whole-cell hydrolysates of strain MCS 33^T in comparison with members of the genera *Maricaulis*, *Woodsholea*, *Oceanicaulis* and *Hyphomonas*

Strains: 1, MCS 33^T; 2, *M. 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, *W. maritima* CM243^T; 8, *O. alexandrii* C116-18^T; 9, *O. stylophorae* GISW-4^T (data from Chen *et al.*, 2012); 10, *H. polymorpha* DSM 2665^T; 11, *H. jannaschiana* ATCC 33833^T. Only fatty acids amounting to more than 1.0 % (mean) are shown. The following strains also contained significant amounts (>1.0 %) of additional fatty acids: *H. polymorpha* DSM 2665^T also contained summed feature 9 ($C_{19:0}$ cyclo $\omega 10c$, ECL 18.846 and/or ECL 18.858; 4.5 %); and *O. alexandrii* C116-18^T contained 4.6 % and *O. stylophorae* GISW-4^T 1.1 % $C_{19:0}$. tr, Trace amount (<1.0 %); −, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
iso-$C_{11:0}$ 3-OH	−	2.6	tr	tr	tr	5.4	−	−	−	−	
ECL 11.798*	7.4	−	−	−	−	−	−	−	−	−	−
$C_{12:0}$ 3-OH	3.0	−	−	−	−	−	3.5	1.3	3.0	tr	tr
$C_{12:1}$ 3-OH	tr	−	−	−	−	−	−	−	−	1.2	1.5
$C_{15:0}$	−	tr	tr	tr	−	−	−	−	−	1.9	1.7
ECL 15.275	−	−	−	−	−	−	6.1	−	−	−	−
$C_{16:0}$	4.6	17.0	11.2	8.9	3.6	9.8	1.4	2.2	2.3	1.9	10.4
Summed feature 4†	−	6.6	3.0	2.6	2.2	2.4	−	−	−	−	tr
$C_{16:1}\omega 9c$	−	1.0	tr	tr	−	−	−	−	−	−	−
$C_{17:0}$	2.9	5.3	9	8.7	7	15.3	2.2	18.0	6.9	18.3	9.7
$C_{17:1}\omega 6c$	1.8	tr	1.0	1.1	1.8	1.3	tr	3.9	−	15.3	4.2
$C_{17:1}\omega 8c$	−	4.0	10.2	10.0	4.7	9.6	−	1.0	−	10.9	4.8
iso-$C_{17:0}$	−	7.7	9.6	10.8	1.7	6.9	−	−	−	−	−
iso-$C_{17:1}\omega 9c$	−	17.4	22.4	28.0	3.9	13.8	−	−	−	−	−
$C_{18:0}$	10.8	1.1	tr	tr	7.9	4.2	16.9	18.2	29.3	tr	3.7
$C_{18:1}\omega 7c$	68.7	24.5	16.2	12.9	47.9	13.0	65.4	29.1	27.8	21.7	48.4
$C_{18:1}\omega 9c$	−	6.4	10.7	7.7	6.0	3.4	−	−	−	−	−
11 Methyl $C_{18:1}\omega 5$	tr	1.6	tr	1.0	tr	2.9	−	18.7	25.8	1.1	7.0
ECL 18.424	−	1.3	1.3	1.8	2.3	5.7	−	−	−	−	−
ECL 18.797	−	tr	tr	tr	4.9	2.6	−	−	−	20.3	5.0

*Unidentified fatty acid with equivalent chain-length of 11.798.
†Summed feature 4 comprised $C_{18:1}\omega 7c/C_{18:1}\omega 9t/C_{18:1}\omega 12t/C_{18:1}\omega 7t$ (ECL 17.798).

Oceanicaulis or *Woodsholea* but was the main hydroxylated fatty acid of species of the genus *Hyphomonas*.

Polar lipids were extracted using a modified Bligh-Dyer procedure (Bligh & Dyer 1959) as described previously (Vancanneyt *et al.* 1996) and analysed by fast atom bombardment MS (Abraham *et al.* 1997). The polar lipids of strain MCS 33^T consisted entirely of glycolipids. No phospholipids or sulpholipids were detected (Fig. S3). The glycolipids were α-D-glucopyranosyl- and α-D-glucopyranuronosyl-diacylglycerols, also common in members of the genera *Caulobacter*, *Brevundimonas*, *Maricaulis* and some other *Alphaproteobacteria*. Using MS/MS, the structures of 21 of these glycolipids could be identified as listed in Table 2. Additionally to these glycolipids, fast atom bombardment MS revealed two ions with higher masses. Their masses were even, requiring odd-numbered molecular masses, suggesting the presence of nitrogen in the molecules. To elucidate the structure of these lipids intensive MS studies were performed. The fast atom bombardment collision-induced dissociation 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 (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 Da 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 agreement with the

Table 2. Polar lipids identified in strain MCS 33^T by fast atom bombardment ionization and collision-induced dissociation MS

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.

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

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 PYEM medium (2 g peptone, 2 g yeast extract, 0.5 g ammonium chloride, 1 litre deionized water). After autoclaving and cooling to ambient temperature, 5 ml riboflavin (0.2 mg ml^{-1}, sterile filtered), 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 NaCl l^{-1}. OD_{600} 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. Growth was tested at different temperatures in SPYEM medium with the same OD protocol. In a medium without sodium chloride weak growth of strain MCS 33^T was observed and a salt concentration of 100 g l^{-1} also allowed only weak growth. Good growth occurred between 20 and 60 g NaCl l^{-1}. By comparison, *M. maris* ATCC 15268^T showed a similar behaviour and at least moderate growth with 80–100 g NaCl l^{-1}. Temperature below 15 °C and above 40 °C inhibited growth of strain MCS 33^T, which had its growth optimum at 30 °C. *M. maris* could not grow below 15 °C or at 50 °C; it showed its growth optimum at 40 °C.

Enzyme activity tests were conducted with API ZYM test strips (bioMérieux), used 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 manufacturer's 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 MCS 33^T and its closest phylogenetic neighbours are summarized in Table 2.

The entire lack of phospholipids in strain MCS 33^T warrants 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 sulpholipids were reported from marine caulobacter strains, including *M. maris* (De Siervo, 1985), reaching amounts as low as 0.3 % of the total lipids. We also found considerable amounts of sulpholipids in *Maricaulis* spp. but they were not detected in strain 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 strain 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 strain 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 may reveal mechanisms that are valid for many eubacterial cells.

The ecological niche of strain MCS 33^T is of note. 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 sinking of material that has escaped degradation in the upper layers of the ocean (Jannasch & Taylor, 1984; Witte *et al.*, 2003). This situation is dramatically changed around deep-sea hydrothermal vents. Here a productive ecosystem almost independent of solar energy exists, one 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 that leach minerals from the rocks. A shell of warm water is found around these hydrothermal vents, and this cools to 2 °C, the temperature typical for the deep sea, within a few hundred metres around the vent (Baross *et al.*, 1982). Strain MCS 33^T

was isolated from a water sample taken at 1762 m depth at 47° 57′ N 129° 05′ W above a black smoker of the Juan de Fuca ridge (Yurkov & Beatty, 1998). Although strain MCS 33^T was isolated from seawater sampled near a hydrothermal vent its temperature requirements did not differ significantly from those of other known cauliform bacteria. The species represented by this strain probably lives in a narrow zone around the hydrothermal vent where the temperature just fits its requirement, 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 micro-organism. The lack of phospholipids reduces the essential amount of phosphorus required by this strain (Van Mooy *et al.* 2009), which can be an additional advantage for these bacteria to occupy a niche in a thin shell around the hydrothermal vents where the temperatures are optimal to support their growth. It remains to be determined whether this species can also be found at other hydrothermal vents and what its specific roles are in the food web of hydrothermal vent microbial communities.

The low 16S rRNA gene sequence similarity between strain MCS 33^T and its closest neighbours indicated that this strain may belong to a new genus. The analysis of the whole-cell fatty acids of strain MCS 33^T supported this view. A number of fatty acids, namely iso-$C_{11:0}$ 3-OH, iso-$C_{17:0}$, iso-$C_{17:1}\omega9c$ and $C_{18:1}\omega9c$, characteristic for the *Maricaulis* group, were absent while $C_{12:0}$ 3-OH and $C_{12:1}$ 3-OH were found which were absent in *Maricaulis* strains. While $C_{12:0}$ 3-OH is the main hydroxylated fatty acid in *Woodsholea* and *Oceanicaulis* species, the $C_{12:1}$ 3-OH found in strain MCS 33^T is absent in these two genera. In terms of its fatty acid profile, strain MCS 33^T is closer to species of the genus *Hyphomonas* than to those of the genus *Maricaulis* (Table 1), although cell multiplication of strain 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 sulpholipids and phospholipids and from *Woodsholea* spp. by the lack of sulpholipids and taurine lipids (Abraham *et al.*, 1997). Within the *Hyphomonadaceae*, strain MCS 33^T has a unique chemotaxonomic position based on its lack of phospholipids and sulpholipids. Also of note is the β-glucosidase activity of strain MCS 33^T, which has not been found in any of the type strains of *Maricaulis*, *Oceanicaulis* and *Woodsholea* species. The assimilation of malate also distinguished strain MCS 33^T from these genera. The differential phenotypic characteristics between strain MCS 33^T and *Maricaulis* and *Oceanicaulis* species are summarized in Table 3. The 16S rRNA gene sequence, cellular fatty acids, enzyme activity and lipid pattern of this isolate from the deep sea identify it as a member of a novel species of a new genus, for which we propose the name *Glycocaulis abyssi* gen. nov., sp. nov.

Description of *Glycocaulis* gen. nov.

Glycocaulis [Gly.co.cau′lis. Gr. adj. *glukus* sweet (used to coin the noun glucose); L. masc. n. *caulis* stalk; N.L. masc. n. *Glycocaulis*, sweet (sugar) stalk].

Cells are Gram-reaction-negative, rod-shaped, fusiform or vibriod, and 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. Multiplies 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. Chemo-organotrophic, strict aerobes. Catalase-, alkaline phosphatase- and leucine arylamidase-positive but oxidase-negative; requires organic growth factors not satisfied by mixtures of B vitamins and amino acids. Requires NaCl for growth. Growth is inhibited or cells become deformed in media containing 1 % (w/v) or more organic material. The genus is characterized by two major fatty acids, $C_{18:1}\omega7c$ and $C_{18:0}$, and the hydroxy fatty acid $C_{12:0}$ 3-OH. Polar lipids are α-D-glucopyranosyl diacylglycerol and α-D-glucopyranuronosyl diacylglycerol, while sulfoquinovosyl diacyl glycerols and phospholipids are absent. Isolated from seawater. The type species is *Glycocaulis abyssi*.

Description of *Glycocaulis abyssi* sp. nov.

Glycocaulis abyssi (a.bys′si. L. n. *abyssus* depth; L. gen. n. *abyssi* of/from the depth).

The description is as given for the genus with the following additions. Cells are rod-shaped, 0.3–0.4 by 0.8–1 μm; the prostheca is about 0.05 μm in diameter. Colonies are circular, convex and colourless. Can store carbon as poly-β-hydroxybutyric acid. No acid is produced from carbohydrates and nitrate is not reduced. With the API ZYM system, positive for esterase, esterase lipase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, weakly positive for cystine arylamidase and β-glucosidase, but negative for lipase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In the BIOTYP 100 system, utilizes aesculin, *myo*-inositol, L(+)-tartrate and L-tyrosine but not the other substrates. Grows on peptone-yeast extract media with 30 g NaCl l^{-1} with optimal growth at 20–60 g NaCl l^{-1}. No growth above 100 g NaCl l^{-1}. Grows fairly well between 15 and 35 °C, while the optimal growth temperature is 30 °C. The optimal pH for growth is around neutrality; pH range for growth is 6.0–8.0. Polar lipids also include α-D-glucopyranosyl diacylglycerol-6′-*N*-glycine.

The type strain is MCS 33^T (=LMG 27140^T=CCUG 62981^T), which was isolated from water obtained from 1762 m depth above the hot water plume of a deep-sea hydrothermal (black smoker) vent near Vancouver island, Canada. The DNA G+C content of the type strain is 62.8 mol%.

Table 3. Differential phenotypic characteristics among strain MCS 33^T and its closest relatives

Strains: 1, MCS 33^T; 2, *Woodsholea maritima* CM243^T; 3, *Oceanicaulis alexandrii* C116-18^T; 4, *O. stylophorae* GIS-W4^T; 5, *Maricaulis salignorans* MCS 18^T; 6, *M. washingtonensis* MCS 6^T. All data from this study except where indicated. +, Positive; −, negative; ++, strongly positive; +/−, variable; ND, not determined.

Characteristic	1	2	3	4†	5	6
Growth temperature range (°C)	15–35	10–40	4–35	15–45	10–40	4–30
Salt tolerance range (g l^{-1})	20–60	5–100	20–100	0–90	0–80	0–80
Valine arylamidase	++	+	+/−	+	+	+/−
Cystine arylamidase	+/−	+	+/−	+	+	+/−
Trypsin	++	+	++	++	++	++
α-Chymotrypsin	++	+	++	++	++	++
Acid phosphatase	+	++	+/−	+	+/−	-
Naphthol-AS-BI-phosphohydrolase	++	++	+	+	+	+
β-Glucosidase	+	−	−	−	−	−
N-Acetyl-β-glucosaminidase	−	+	−	−	−	−
Oxidase	−	+	+	+	+	+
Protease	−	−	−	+	−	−
Nitrate reduction	−	−	+	−	+	+
Assimilation of:						
Malate	+	−	−	−	−	−
Citrate	−	−	−	+	−	−
Arabinose	−	−	−	+	−	−
Adipate	+	−	−	+	−	−
Hydroxy-fatty acid(s)	$C_{12:0}$ 3-OH, $C_{12:1}$ 3OH	$C_{12:0}$ 3-OH	$C_{12:0}$ 3-OH	$C_{12:0}$ 3-OH	iso-$C_{11:0}$ 3-OH	iso-$C_{11:0}$ 3-OH
Polar lipids*	MGD, MGDOx, GGG	MGD, MGDOx, SQD, Taur	MGD, MGDOx, PG, SQD	ND	MGD, MGDOx (PG), SQD	MGD, MGDOx (PG), SQD
DNA G+C content (mol%)	62.8	65.2	61.8	61.6	63.3	63.0

*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; PG, phosphatidylglycerol; Taur, 1,2-diacyl-3-α-D-glucuronopyranosyl-sn-glycerol taurinamide; SQD, 1,2-diacyl-3-O-sulfoquinovosylglycerol.
†Data for *O. stylophorae* GIS-W4^T from Chen *et al.*, 2012.

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