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Woodsholea maritima gen. nov., sp. nov., a marine bacterium with a low diversity of polar lipids

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Two cauliform bacteria (CM243^T and CM251) isolated by J. Poindexter from the Atlantic Ocean were characterized by 16S rRNA gene sequencing, *Taq*I restriction fragment length polymorphism and single-strand conformation polymorphism analyses of the internally transcribed 16S–23S rDNA spacer (ITS1) region, analysis of fatty acids from cellular lipids, mass spectrometry of polar lipids and physiological properties. The two strains showed very low diversity of polar lipids with diacyl-sulfoquinovosyl glycerols as the predominant lipids. The two bacterial strains were observed to have nearly identical 16S rRNA gene sequences and could not be differentiated by their ITS1 regions. The isolates differed from species of the genus *Maricaulis* by their 16S rRNA gene sequences, polar lipids and fatty acid patterns. On the basis of the genotypic analyses and estimations of phylogenetic similarities, physiological and chemotaxonomic characteristics, it is proposed that the isolates represent a new genus and species, for which the name *Woodsholea maritima* gen. nov., sp. nov. (type strain CM243^T=VKM B-1512^T=LMG 21817^T) is proposed.

Aquatic habitats, especially the oceans, are still a rich source for unusual bacteria and Jean Poindexter and others isolated a number of marine bacteria that were grouped into the genus *Caulobacter* by their characteristic cell morphology (Poindexter, 1964, 1981b). These bacteria were observed to have dimorphic, prosthecate cells in which reproduction takes place by the separation of two cells that are morphologically and behaviourally different from each other. One cell is sessile, fixed by adhesive material to the substrata and possessing one elongated cylindrical appendage – a prostheca (Staley, 1968). The other cell is motile by a single polar flagellum. The mode of reproduction of the dimorphic prosthecate bacteria helps disperse the population at each generation, thereby minimizing competition for resources. It is consistent with their life style that these bacteria possess tolerance of prolonged nutrient scarcity (Poindexter, 1981a).

Caulobacteria are ubiquitous in aquatic and marine ecosystems and are presumed to be responsible for considerable mineralization of dissolved organic material in aquatic environments, where nutrient concentrations and ambient temperatures are low (Staley *et al.*, 1987). It is consistent with this presumption that practically any type of sea water contains caulobacteria (Jannasch & Jones, 1960; Anast & Smit, 1988).

The ubiquity of this type of bacteria led to their discovery more than a century ago, where the first isolation of a

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Abbreviations: CID, collision-induced dissociation; ECL, equivalent chain-length; ESI-MS, electrospray ionization mass spectrometry; ITS1, internally transcribed spacer region; SSCP, single-strand conformation polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains CM243^T and CM251 are AJ578476 and AJ578477, respectively.

Caulobacter sp. was reported by Loeffler (1890). In 1935, the genus *Caulobacter* was described (Henrici & Johnson, 1935) and, three decades later, Jean Poindexter isolated a large number of caulobacteria from different habitats and described nine species (Poindexter, 1964).

Anast & Smit (1988) were the first to recognize differences between freshwater and marine caulobacteria in a large set of strains. The comparison of 16S rRNA gene sequences of several isolates belonging to *Caulobacter* revealed that the isolates actually form two different lineages (Stahl *et al.*, 1992). A study of the diversity of more than 100 different freshwater and marine *Caulobacter* strains including lipid analysis, immunological profiling, 16S rRNA gene sequencing and physiological data led to the reclassification of many *Caulobacter* species as *Brevundimonas* species and the proposal of the new genus *Maricaulis* (Abraham *et al.*, 1999). In this study, marine isolates were identified as belonging to this new genus, and four additional species of *Maricaulis* have since been proposed (Abraham *et al.*, 2002). However, some of the marine strains isolated by Poindexter and others did not fit into the genus *Maricaulis* or into the newly described genus *Oceanicaulis* (Strömpl *et al.*, 2003), and the aim of this communication is to describe two of them and to place them into a new genus.

The strains of this study and the origin of the isolates are listed in Table 1. All strains were grown in marine-*Caulobacter* medium SPYEM: 30 g sea salts (Sigma), 0·5 g NH_4Cl, 1 l Milli-Q water. After autoclaving and cooling, 20 ml 50 × PYE (100 g peptone and 50 g yeast extract in 1 l deionized water, autoclaved), 2 ml 50 % glucose (sterile) and 5 ml filter-sterilized riboflavin (0·2 mg ml^{-1}) were added. The strains were incubated in 2 l flasks at 30 °C and 100 r.p.m. and the biomass was harvested in the late exponential phase after 72 h.

Genomic DNA isolation, determination of DNA base composition, PCR amplification of nearly complete 16S rRNA genes, subsequent sequencing of the amplicons and the mode of analysis have been described elsewhere (Abraham *et al.*, 2002). Resulting sequences were aligned and phylogenetic trees were constructed in the ARB program (http://www.arb-home.de). For analysis of the 16S–23S rDNA interspacer region (ITS1), two PCR primers were used: 16f945, corresponding to positions 927 to 945 of the *Escherichia coli* 16S rDNA (Brosius *et al.*, 1978), and 23r458, corresponding to positions 458 to 473 of the *E. coli* 23S rDNA (Brosius *et al.*, 1980). Fingerprint analysis of the ITS1 PCR products after digestion with *Taq*I and single-strand conformation polymorphism (SSCP) analysis (Orita *et al.*, 1989) of the resulting restriction fragments were performed as described elsewhere (Abraham *et al.*, 2002).

After an incubation period of 48 h on SPYEM agar plates at 28 °C, a loopful of biomass was harvested for whole-cell fatty acid analysis and fatty acid methyl esters were prepared as described previously (Abraham *et al.*, 2002). Fatty acid methyl esters were separated and identified using the Microbial Identification System (MIDI). Lipids were extracted using a modified Bligh–Dyer procedure (Bligh & Dyer, 1959) as described previously (Vancanneyt *et al.*, 1996). This total lipid fraction was fractionated by column chromatography and the phospholipid fraction was analysed by electrospray ionization mass spectrometry (ESI-MS). ESI-MS in the negative mode was performed in a QTOF-MS. Neon served as the collision gas for high-energy collision-induced dissociation (CID). ^{1}H NMR spectra were recorded in 7 : 3 d-chloroform/d_3-methanol at 300 K on a Bruker ARX-400 NMR spectrometer relative to internal tetramethylsilane.

Strains were grown with different concentrations of NaCl

Table 1. Strains used in this study

Abbreviations: LMG, BCCM/LMG Bacteria Collection, Universiteit Gent, Belgium; ATCC, American Type Culture Collection, Manassas, VA, USA; DSM, Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany; VKM, All-Russian Collection of Microorganisms of the Academy of Sciences, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia.

Strain	Origin
CM243^T (=VKM B-1512^T=LMG 21817^T)	Estuarine water, Woods Hole, MA, USA, J. Poindexter
CM251 (=LMG 21818)	Estuarine water, Woods Hole, MA, USA, J. Poindexter
Maricaulis maris ATCC 15268^T	Filtered sea water
Maricaulis parjimensis MCS 25^T (=LMG 19863^T)	Filtered sea water, Indian Ocean off Goa, India
Maricaulis salignorans MCS 18^T (=LMG 19864^T)	Sea water, Salsbury Point County Park, adjacent to Hood Canal, Bridge, WA, USA
Maricaulis virginensis VC-5^T (=VKM B-1513^T)	Deep-sea, Virgin Islands, J. Poindexter
Maricaulis washingtonensis MCS 6^T (=LMG 19865^T)	Sea water, Inner marina, Edmonds, WA, USA
Oceanicaulis alexandrii C116-18^T	Marine dinoflagellate
Hyphomonas jannaschiana ATCC 33883^T	Mussel bed in Galapagos thermal vent
Hyphomonas polymorpha DSM 2665^T	Human nasal sinus infection

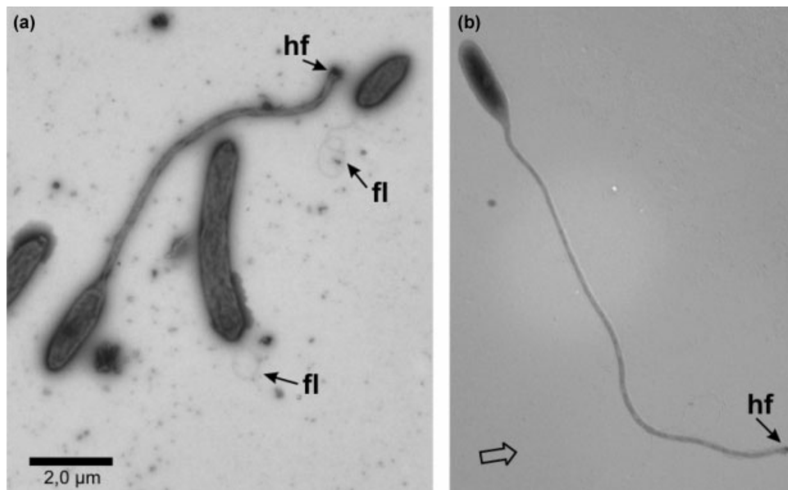

Fig. 1. Cells of the marine isolate CM243^T, growing exponentially, are depicted as negatively stained (a) and Pt shadow-cast (b) preparations. Typical stalks, terminated by a holdfast (hf), can be recognized. Motile swarming cells of different length are characterized by a monotrichous, monopolar flagellum (fl). Open arrow in (b) indicates the direction of shadow-cast.

and at different temperatures, for phenotypic characterizations, as described elsewhere (Abraham *et al.*, 1999). Enzyme activity tests were conducted with the use of API ZYM test strips (bioMérieux), according to the protocol supplied by the manufacturer. Substrate specificity tests were conducted with the use of API 20NE test strips (bioMérieux) using a protocol supplied by the manufacturer. The test strips were incubated at 30 °C for 7 days and monitored after 1, 2 and 7 days. A test was considered positive when the interface between sample well and air was visibly turbid due to bacterial growth after a 7 day incubation period.

Morphology and ultrastructure of mid-exponentially growing cells of CM243^T were analysed as negatively stained and shadow-cast samples with the transmission electron microscope as described previously (Golyshina *et al.*, 2000; Yakimov *et al.*, 1998). As seen in Fig. 1, these cells show the typical features of *Caulobacter*-type Gram-negative bacteria. Both morphologies can be recognized (Fig. 1). Bacteria with a characteristic stalk, ranging from 2·5 µm up to more than 12 µm in length, a median diameter of 135 nm ($\pm$13 nm; $n=43$), and terminated by a distinct holdfast, are often found. They are associated with planktonic motile variants (Fig. 1), which are monotrichously and monopolarly flagellated (Fig. 1a; fl). Mid-exponentially growing cells show cell lengths from 1·51 to 5·4 µm and a mean cell diameter of 690 nm ($\pm$13 nm; $n=30$).

Nearly complete 16S rRNA genes were amplified and sequenced using internally binding sequencing primers. Strains CM243^T and CM251 exhibited identical sequences over the 1421 nt analysed, except for a G→C exchange at base position 306. Results from initial FASTA database searches indicated affiliation of the two strains with the '*Alphaproteobacteria*' (Garrity *et al.*, 2001). Detailed alignments in ARB showed that strains CM243^T and CM251 clustered within the *Rhodobacteraceae* and were most closely related to the genera of marine caulobacteria, *Maricaulis* and *Oceanicaulis* (Fig. 2). Estimated evolutionary distances of strain CM243^T from the type strains of closely related species and relevant taxa were: 7·48 % to *Maricaulis maris*, 7·79 % to *Maricaulis washingtonensis*, 7·87 % to *Maricaulis salignorans*, 6·55 % to *Maricaulis parjimensis*, 6·32 % to *Maricaulis virginensis*, 7·16 % to *Oceanicaulis alexandrii*, 10·58 % to *Hyphomonas polymorpha*, 11·60 % to *Hirschia baltica* and 13·76 % to *Caulobacter vibrioides*.

Application of neighbour-joining and parsimony methods resulted in identical tree topologies. Diagnostic sequence stretches serving to distinguish strain CM243^T from all currently recognized species of *Maricaulis* and *Oceanicaulis* can be found, for example, in helices 9, 19, 25 and 45 (Neefs *et al.*, 1991) (data not shown). Identification of distinguishing sequence patterns, together with the evolutionary distances estimated from sequence similarity values, and the dendrogram topologies, suggest that strains CM243^T and CM251 are equally distant from *Maricaulis* and *Oceanicaulis*.

Molecular typing analysis of the 16S–23S rDNA ITS1 has been applied as a rapid bacterial identification tool for strains CM243^T and CM251 in order to confirm the close relationship between the strains. The use of the ITS1 region has allowed discrimination at, approximately, the species level, with correlation to DNA–DNA relatedness data (Guasp *et al.*, 2000). For both strains, equivalent single, 1·6 kb ITS1 PCR products were obtained (data not shown). These PCR fragments contained approximately 0·6 kb of the 5′-region of the 16S rDNA and nearby 0·5 kb of the 3′-region of the 23S rDNA, with 0·5 kb corresponding to the ITS1 region. *Taq*I restriction fingerprints obtained from the PCR products of CM243^T and CM251 were identical, consisting of six bands of between 155 and 396 bp. Furthermore, no differences between the strains were detected in the resulting single-strand profiles analysed by SSCP. Consequently, significant sequence heterogeneities that would result in different conformations and, consequently, differences in the mobilities of single strands were assumed to be absent. These results confirmed

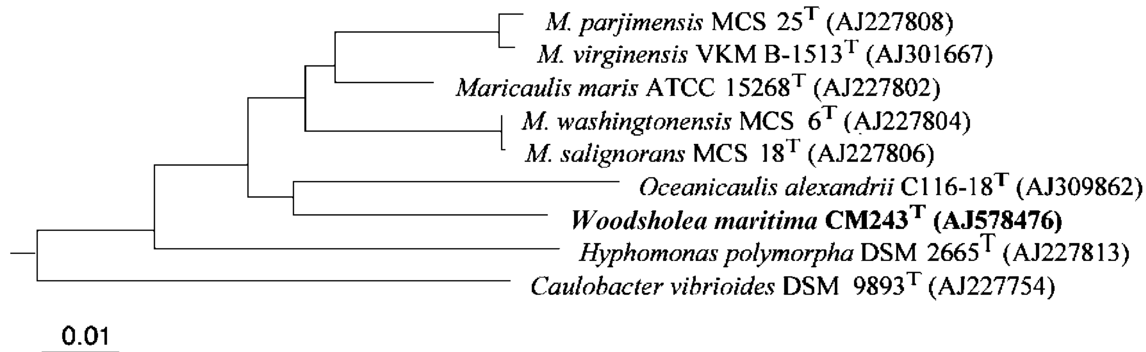

Fig. 2. Unrooted dendrogram of phylogenetic relationships with strain CM243^T, based on comparisons of 16S rDNA sequences of the type strains of all species of the genera *Maricaulis* and *Oceanicaulis*, as well as the type strains of the type species of *Hyphomonas* and *Caulobacter*. The 16S rDNA sequences of different bacterial species, within and without the alphaproteobacteria, were used as outgroup. Strain numbers and database accession numbers of all sequences used for calculation of the distance matrix are listed. A distance matrix was calculated from only unambiguously determined, homologous positions, using DNADIST (Jukes–Cantor corrections; Jukes & Cantor, 1969) and dendrograms of estimated phylogenetic relationships were calculated, using the FITCH program of the PHYLIP package (Felsenstein, 1989) as implemented in the ARB program (Ludwig *et al.*, 2003). Bar, 1 nt substitution per 100 bases.

unequivocally a close phylogenetic association between the two strains.

The mass spectra of the polar lipids of strains CM243^T and CM251 showed surprisingly few peaks. The spectrum of strain CM243^T was dominated by two peaks at *m*/*z* 797 and 847, with two minor peaks at 795 and 845. A fifth peak was detected at *m*/*z* 904 (Fig. 3). With the aid of CID MS, the main lipid compounds were elucidated. The ions at *m*/*z* 795 and 797 were identified as α-D-glucopyranosyl diacylglycerol and α-D-glucopyranuronosyl diacylglycerols known from many other *Caulobacter*, *Brevundimonas*, *Maricaulis* and *Hyphomonas* strains (Abraham *et al.*, 1997, 1999). The ions at *m*/*z* 845 and 847 were identified as sulfoquinovosyl diacylglycerols and the ion at *m*/*z* 904 as α-D-glucuronopyranosyl diacylglycerol taurine amide also described in *Maricaulis* and *Hyphomonas* strains. The fatty acids and their position on the glycerol backbone can be determined by the more frequent loss of those fatty acids positioned at *sn*-2 as free fatty acid as well as substituted ketene (Murphy & Harrison, 1994). With this method, the structures of the anions and hence the structure of the glyco- and sulfolipids were identified (Table 2). For the ions at *m*/*z* 795 and 904, because of their low intensities, no CID spectra were obtained. Instead, their compositions were assumed to be similar to identical ions observed in the MS spectra of *Maricaulis* and *Hyphomonas* species (Abraham *et al.*, 1997).

The main glycolipids in CM243^T and CM251 were also found commonly in all *Maricaulis*, *Oceanicaulis* and *Hyphomonas* species. The situation was different for the main polar lipids, where phosphatidylglycerol was absent in CM243^T and CM251, was found only in some species of *Maricaulis* and was present in all *Hyphomonas* and *Oceanicaulis* species. The occurrence of the sulfolipid

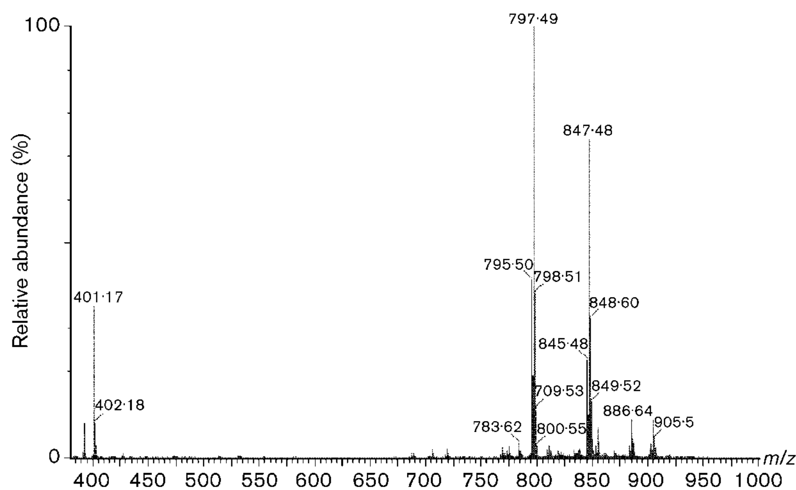

Fig. 3. ESI-MS of the polar lipid extract of strain CM243^T. The prominent peaks at *m*/*z* 795 and 797 are glucopyranosyl- and glucopyranuronosylglycerol glycolipids and those at *m*/*z* 845 and 847 are sulfoquinovosyl lipids. A small peak at *m*/*z* 903 suggests a glucuronopyranosyl-*sn*-glycerol taurineamide.

Table 2. Polar lipids identified in *Woodsholea maritima* CM243[T]

MGD, 1,2-di-*O*-acyl-3-*O*-α-D-glucopyranosylglycerol; MGDOx, 1,2-di-*O*-acyl-3-*O*-α-D-glucopyranuronosylglycerol; SQDG, 1,2-diacyl-3-*O*-sulfoquinovosylglycerol; TAU, 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol taurineamide.

Glycolipids			Sulfolipids		
Mass	Type	Fatty acids	Mass	Type	Fatty acids
796	MGDOx	18:1–18:1	846*	SQDG	18:1–18:1
798*	MGD	19:0–18:1	848*	SQDG	18:1–18:0
798*	MGDOx	18:1–18:0	904	TAU	18:1–18:0

*Compounds analysed by CID.

Table 3. Polar lipids as biomarkers in *Woodsholea maritima* and related genera (Abraham *et al.*, 1999)

1,2-di-*O*-acyl-3-*O*-α-D-glucopyranosylglycerol and 1,2-di-*O*-acyl-3-*O*-α-D-glucopyranuronosylglycerol were present in all genera. PG, Phosphatidylglycerol; SQDG, 1,2-diacyl-3-*O*-sulfoquinovosylglycerol; TAU, 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol taurineamide. +++, Present in all strains; +, present in some species; −, absent.

Genus	PG	SQDG	TAU
Woodsholea	−	+++	+++
Maricaulis	+	+++	+
Oceanicaulis	+++	+++	−
Hyphomonas	+++	−	+++

1,2-diacyl-3-*O*-sulfoquinovosylglycerol is restricted to species of the genera *Maricaulis* and *Oceanicaulis* as well as CM243[T] and CM251, while it was lacking in *Hyphomonas* species. Another sulfolipid, 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol taurineamide, also found in CM243[T] and CM251, is known from *Hyphomonas* species but was found only in those *Maricaulis* strains that are closely related to *M. maris* (Abraham *et al.*, 2002) (Table 3). Strains CM243[T] and CM251 showed a low diversity of fatty acids and phospholipids, in marked contrast to the phylogenetically related genera *Maricaulis* and *Hyphomonas*. Such a low diversity of fatty acids was recently reported, as well, for *Parvularcula bermudensis*, a marine bacterium

Table 4. Fatty acid content (mean percentage of total) of whole-cell hydrolysates of *Woodsholea maritima* strains and those of related genera

Strains: 1, *W. maritima* CM243[T]; 2, *W. maritima* CM251; 3, *M. parjimensis* MCS 25[T]; 4, *M. washingtonensis* MCS 6[T]; 5, *M. maris* ATCC 15268[T]; 6, *M. salignorans* MCS 18[T]; 7, *M. virginensis* VKM B-1513[T]; 8, *H. polymorpha* DSM 2665[T]; 9, *H. jannaschiana* ATCC 33833[T]. Those fatty acids for which the amount for all taxa was less than 1 % are not given. tr, Trace amount (less than 1 %); –, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
11:0 iso 3-OH	–	–	tr	tr	2·6	tr	5·4	–	–
12:0 3-OH	3·5	3·9	–	–	–	–	–	tr	tr
12:1 3-OH	–	–	–	–	–	–	–	1·2	1·5
Summed feature 3*	2·7	2·9	–	–	–	–	–	–	–
15:0	–	–	–	tr	tr	tr	–	1·9	1·7
ECL 15·275†	6·1	6·7	–	–	–	–	–	–	–
16:0	1·4	1·8	3·6	11·0	17·0	8·9	9·8	1·9	10·0
Summed feature 4	–	–	2·2	3·0	6·6	2·6	2·4	–	tr
16:1ω9*c*	–	–	–	tr	1·0	tr		–	–
17:0	2·2	2·3	7·0	9·0	5·3	8·7	15·3	18·0	9·7
17:1ω6*c*	tr	–	1·8	1·0	tr	1·1	1·3	15·0	4·2
17:1ω8*c*	–	–	4·7	10·0	4·0	10·0	9·6	11·0	4·8
17:0 iso	–	–	1·7	9·6	7·7	10·8	6·9	–	–
17:1 iso ω9*c*	–	–	3·9	22·4	17·4	28·0	13·8	–	–
18:0	16·9	18·9	7·9	tr	1·1	tr	4·2	tr	3·7
18:1ω7*c*	65·4	63·5	47·9	16·2	24·5	12·9	13·0	21·7	48·4
18:1ω9*c*	–	–	6·0	10·7	6·4	7·7	3·4	–	–
11-Me 18:1ω5*t*	–	–	tr	tr	1·6	1·0	2·9	1·1	7·0
ECL 18·424	–	–	2·3	1·3	1·3	1·8	5·7	–	–
ECL 18·797	–	–	4·9	tr	tr	tr	2·6	20·3	5·0

*Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 3: 14:0 3-OH, 16:1 iso I, ECL 10·928 and/or 12:0 ALDE. Summed feature 4: 15:0 iso 2-OH, 16:1ω7*c* and/or 16:1ω7*t*.
†Unidentified fatty acids with equivalent chain-length (ECL) given.

that comprises a deep phylogenetic branch in the '*Alphaproteobacteria*' (Cho & Giovannoni, 2003).

The total cellular fatty acid compositions of strains CM243^T and CM251 were determined (Table 4). The predominant fatty acids present in both strains were 12 : 0 3-OH, 16 : 0, 17 : 0, 18 : 0, summed feature 3, 18 : 1ω7*c* and the unidentified fatty acid ECL 15·275. Strains CM243^T and CM251 differed from *Maricaulis* species in their fatty acid profiles by the presence of 12 : 0 3-OH, summed feature 3 and the unidentified fatty acid ECL 15·275, and by the absence of 11 : 0 iso 3-OH, 17 : 1ω6*c*, 17 : 1ω8*c*, iso-17 : 0, iso-17 : 1ω9*c*, 18 : 1ω9*c*, 11-Me 18 : 1ω5*t*, summed feature 4 and the unidentified fatty acids ECL 18·424 and ECL 18·797 (Table 3). *Hyphomonas* species were observed to differ from strains CM243^T and CM251 by the absence of summed feature 3 and the unidentified fatty acid ECL 15·275, and by the presence of 12 : 1 3-OH, 15 : 0, 17 : 1ω6*c*, 17 : 1ω8*c* and the unidentified fatty acid ECL 18·797 (Table 3). Poly-β-hydroxybutyrate was detected in the ^{1}H NMR spectra of total lipid extracts of CM243^T and CM251.

Strains CM243^T and CM251 were able to grow on peptone/yeast extract media with NaCl concentrations between 5 and 100 g l^{-1}, showing optimal growth with 40 g NaCl l^{-1} and no growth without NaCl addition. In contrast to *Maricaulis* species, strain CM243^T was unable to reduce nitrate to nitrite (Anast & Smit, 1988). The growth requirements and enzyme activities observed are given in detail in the species description. In Table 5, the enzymic activities of strains CM243^T and CM251 are compared with those of *Maricaulis*, *Oceanicaulis* and *Hyphomonas* species.

Strains CM243^T and CM251 are considerably different from all species of *Maricaulis* so far described and the proposal of a species in a new genus is justified.

Description of *Woodsholea* gen. nov.

Woodsholea (Woods.hol'e.a. N.L. fem. n. *Woodsholea* named in honour of the Woods Hole Oceanographic Institution, Massachusetts, USA).

Gram-negative cells, rod-shaped, vibriod. Cells possess a stalk, varying in length depending on the strain and 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 stalk. Occur singly. Multiplication by binary fission. Colonies circular, convex, colourless. Chemo-organotrophic, aerobes, cells can store carbon as poly-β-hydroxybutyric acid. Requirement for organic growth factors is complex and not satisfied by mixtures of B vitamins and amino acids. Grows on peptone/yeast extract media with 40 g NaCl l^{-1}. Growth is inhibited or cells become deformed in media containing 1 % (w/v) or more organic material. Growth temperature range is 20–40 °C and optimal pH for growth is approximately neutral. Do not reduce nitrate, oxidize tryptophan to indole or hydrolyse arginine, urea, aesculin, gelatin or *p*-nitrophenyl-3-D-galactopyranoside. Do not use glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate or phenylacetate as carbon sources. Cells show no catalase activity, are positive for alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, acid phosphatase, esterase (C_4), esterase lipase (C_8), oxidase and trypsin but negative for α- and β-galactosidase, α-glucuronidase, α- and β-glucosidase, α-mannosidase and α-fucosidase. The

Table 5. Enzymic assays and indicator cellular fatty acids of CM243^T and type species of related genera

Strains: 1, *Maricaulis washingtonensis* MCS 6^T; 2, *M. salignorans* MCS 18^T; 3, *M. parjimensis* MCS 25^T; 4, *M. maris* ATCC 15268^T; 5, CM243^T; 6, CM251; 7, *Oceanicaulis alexandrii* C116-18^T; 8, *Hyphomonas polymorpha* DSM 2665^T. No activity of α-galactosidase, β-galactosidase, α-glucuronidase, α-mannosidase or α-fucosidase was found in any of the species.

Enzyme assay	1	2	3	4	5	6	7	8
Alkaline phosphatase	++++	++++	+++++	+++++	+++++	+++++	+++++	+++++
Esterase (C4)	++++	++++	+++	+++	+++	++	++++	++++
Esterase lipase (C8)	++++	++++	+++	+++	+++	++	++++	++
Lipase (C14)	+	+	+	+	+	−	+	−
Leucine arylamidase	+++++	+++++	++++	+++++	++++	+++++	+++++	+++
Valine arylamidase	+	++	+++	++	++	+	+	−
Cystine arylamidase	+	+++	++	++	++	++	+	+
Trypsin	++++	+++++	+++++	++	+++	++++	+++++	−
α-Chymotrypsin	+++++	+++++	++	+++++	++	−	++++	−
Acid phosphatase	−	+	+++++	−	++++	++	+	++++
Naphthol-AS-BI-phosphohydrolase	++	++	+++++	+++	+++++	++	++	++
α-Glucosidase	−	−	++	−	−	−	−	+
β-Glucosidase	−	−	++	−	−	−	−	+
N-Acetyl-β-glucosaminidase	−	−	−	−	++	−	−	−

genus is characterized by two major fatty acids, 18 : 0 and 18 : 1ω7*c*, and minor amounts of 12 : 0 3-OH, 16 : 0, 17 : 0, summed feature 3 and the unidentified fatty acid ECL 15·275. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, sulfoquinovosyl diacylglycerol and α-D-glucuronopyranosyl diacylglycerol taurine amide. Isolated from sea water. The G + C content is 65 mol%. The type species is *Woodsholea maritima*.

Description of *Woodsholea maritima* sp. nov.

Woodsholea maritima (L. fem. adj. *maritima* marine).

The description is as that of the genus with the following additions. Cells are 1·5–5·4 × 0·7 μm with a 2·5–12 × 0·14 μm stalk; optimal growth occurs between 5 and 100 g NaCl l^{-1}. No growth without salt. Optimal growth temperature is 20–40 °C; some growth is observed at 10 °C. pH range for growth is 6·0–8·0. Main polar lipids are 1-nonadecanoyl-2-octadecenoyl-3-*O*-α-D-glucopyranosylglycerol, 1-octadecenoyl-2-octadecanoyl-3-*O*-α-D-glucopyranuronosylglycerol and 1,2-di-octadecenoyl-3-*O*-sulfoquinovosylglycerol. Strains have no to weak α-chymotrypsin and *N*-acetyl-β-glucosaminidase activities; some isolates including the type strain have very weak lipase activity. Isolates have been obtained from sea water at Woodshole, USA. The G + C content of the type strain is 65·2 mol%.

The type strain is $CM243^T$ (= VKM $B-1512^T$ = LMG 21817^T); a further strain is CM251 (= LMG 21818).

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