Mariniflexile fucanivorans sp. nov., a marine member of the *Flavobacteriaceae* that degrades sulphated fucans from brown algae

Tristan Barbeyron,¹ Stéphane L'Haridon,² Gurvan Michel¹ and Mirjam Czjzek¹

Correspondence

Tristan Barbeyron
barboun@sb-roscoff.fr

¹Centre National de la Recherche Scientifique, Université Pierre et Marie Curie-Paris 6, Unité Mixte de Recherche 7139 'Végétaux Marins et Biomolécules', Station Biologique, F-29682 Roscoff Cedex, Bretagne, France

²Centre National de la Recherche Scientifique, Université de Bretagne Occidentale, IFREMER, Unité Mixte de Recherche 6197 'Laboratoire de Microbiologie des Environnements Extrêmes', IFREMER, F-29280 Plouzané, Bretagne, France

A rod-shaped, Gram-negative, chemo-organotrophic, heterotrophic, strictly aerobic, gliding bacterial strain, SW5^T, capable of degrading sulphated fucans from brown algae was isolated from a water-treatment facility that recycles the effluent of an alginate-extraction plant in Landerneau (Brittany, France). Its taxonomic position was investigated by a polyphasic approach. Strain SW5^T formed dark-yellow colonies, was oxidase-negative and catalase-positive and grew optimally at 25 °C and pH 7.5 and in the presence of 2.5 % (w/v) NaCl. The DNA G + C content was 34.5 mol%. Phylogenetic analysis based on the sequence of the 16S rRNA gene allocated strain SW5^T to the genus *Mariniflexile* in the family *Flavobacteriaceae*, with a similarity of 98.4 % to the type strain of *Mariniflexile gromovii*, the only recognized *Mariniflexile* species. Its low level of DNA–DNA relatedness (<25 %) with the type strain of this species and differentiating phenotypic characteristics demonstrated that strain SW5^T constitutes a novel *Mariniflexile* species, for which the name *Mariniflexile fucanivorans* sp. nov. is proposed. Strain SW5^T (=CIP 109502^T =DSM 18792^T) is the type strain.

Sulphated fucans are matrix polysaccharides from the cell wall of marine fucal brown algae, consisting of an α -L-fucose backbone substituted by sulphate ester groups and branched with other monosaccharide residues (Mabeau & Kloareg, 1987; Kloareg & Quatrano, 1988; Mabeau *et al.*, 1990). In fucal brown algae such as *Pelvetia canaliculata*, *Ascophyllum nodosum* and *Fucus spiralis*, the repetitive unit of sulphated fucans is a disaccharide consisting of α -1,3-L-fucopyranose 2-sulphate- α -1,4-L-fucopyranose 2,3-disulphate, with the 3-linked residue at the non-reducing end (Descamps *et al.*, 2006). A recently isolated bacterial strain, SW5^T, produced an extracellular enzyme that specifically hydrolysed the α -1,4 linkage of sulphated fucans from fucal brown algae (Descamps *et al.*, 2006). The gene encoding this fucanase has been cloned (Colin *et al.*, 2006) and the enzyme was shown to belong to family 107 of glycoside hydrolases (<http://www.cazy.org>).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SW5^T is AJ628046.

A photograph of colonies of strain SW5^T on ZoBell agar and a phase-contrast micrograph of a ZoBell broth culture are available as supplementary material with the online version of this paper.

In this study, we report the polyphasic characterization of strain SW5^T as a representative of a novel species of the genus *Mariniflexile*. The genus *Mariniflexile*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 1996, 2002; Bernardet & Nakagawa, 2006) of the phylum *Bacteroidetes*, initially contained only *Mariniflexile gromovii*, isolated from the sea urchin *Strongylocentrotus intermedius* (Nedashkovskaya *et al.*, 2006).

Strain SW5^T was isolated from mud of a water-treatment facility (Descamps *et al.*, 2006) at Landerneau (Brittany, France) that recycled the effluent of an alginate-extraction plant. For direct comparison, *M. gromovii* KMM 6038^T was studied in parallel with strain SW5^T in all phenotypic tests (except for growth at different temperatures, pH and NaCl concentrations) and whole-cell protein analysis. The two strains were routinely cultivated in ZoBell medium 2216E (ZoBell, 1941), solidified when necessary with 1.5 % (w/v) agar. Pure cultures were stored at –80 °C in culture medium containing 20 % (v/v) glycerol. Growth was monitored by the increase of optical density at 600 nm, using a UV1601 spectrophotometer (Shimadzu). All growth experiments were performed in triplicate. The temperature range for growth was evaluated at 4, 10, 15,

20, 25, 32 and 37 °C. The optimal pH was determined at 25 °C in *Cytophaga* marine broth (DSMZ medium 172) with the pH adjusted using 10 mM MES for pH 5.0, 5.5 and 6.0, 10 mM PIPES for pH 6.5 and 7.0, 10 mM HEPES for pH 7.5, 10 mM Tris for pH 8.0 and 8.5 and no buffer for pH 9.0. The effect of NaCl on growth was determined at 25 °C in the same medium containing 0–9 % (w/v) NaCl at 1 % intervals.

Genomic DNA was extracted by using the procedure described by Barbeyron *et al.* (1984) and purified on a caesium chloride gradient. The Chargaff's coefficient of the genomic DNA, expressed as the molar percentage of G + C, was determined by the DSMZ using the HPLC method. Genomic DNA extracted from strain SW5^T displayed a Chargaff's coefficient (DNA G + C content) of 34.5 mol%. The 16S rRNA gene sequence from strain SW5^T was amplified by PCR using purified genomic DNA as template and the bacterial domain-specific primers 8F (Hicks *et al.*, 1992) and 1492R (Kane *et al.*, 1993). PCRs were typically carried out in a volume of 50 µl containing 10–100 ng template, 0.4 µM of each specific primer, 250 µM each dNTP, 0.1 mg BSA, 1 × GoTaq buffer (Promega) and 1.25 U GoTaq DNA Polymerase (Promega). The thermal profile of the PCR was 2 min at 95 °C followed by 30 cycles of 0.5 min at 95 °C, 0.5 min at 50 °C and 1.5 min at 72 °C and a final polymerization step of 4 min at 72 °C. PCR products were purified with the MinElute PCR purification kit (Qiagen), cloned in vector pCRII2.1 and sequenced using Big Dye-labelled dideoxynucleotides, the Thermosequenase kit (Amersham) and an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems/Hitachi). The 16S rRNA gene sequence of strain SW5^T was aligned using the MAFFT software (Katoh *et al.*, 2002, 2005) with a

representative set of sequences obtained from GenBank (Benson *et al.*, 1999) and the alignment was then refined manually. The secondary structure was used as a guide to ensure that only homologous regions were compared. Of the 1474 nucleotides that were sequenced, 1286 were used in the phylogenetic analysis. The neighbour-joining algorithm (Saitou & Nei, 1987) was used with Kimura's distance correction (Kimura, 1980) and bootstrap analysis was performed to provide confidence estimates for the phylogenetic tree topologies (Felsenstein, 1985). The maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods were also applied, as implemented in the PHYLO_WIN package (Galtier *et al.*, 1996). Although some differences were observed between trees generated by the neighbour-joining, maximum-likelihood and maximum-parsimony methods, all phylogenetic trees based on 16S rRNA gene sequences showed that SW5^T branched with *M. gromovii* KMM 6038^T (Fig. 1) with a bootstrap value of 100 %. Pairwise comparisons of 16S rRNA gene sequences were made using the FASTA software (Pearson & Lipman, 1988). The 16S rRNA gene sequence of strain SW5^T shared 98.4, 94.5, 94.1 and 94 % similarity, respectively, with those of *M. gromovii* KMM 6038^T (Nedashkovskaya *et al.*, 2006), *Gaetbulibacter saemankumensis* SMK-12^T (Jung *et al.*, 2005), *Yeosuana aromatorans* GW1-1^T (Kwon *et al.*, 2006) and *Algibacter lectus* KMM 3902^T (Nedashkovskaya *et al.*, 2004). Sequence similarity with *Winogradskyella* species (Nedashkovskaya *et al.*, 2005), *Tamlana crocina* HST1-43^T (Lee, 2007) and *Olleya marilimosa* CAM030^T (Nichols *et al.*, 2005) was 93 % and similarity to other species used in the phylogenetic analysis was <93 %. DNA relatedness between strain SW5^T and *M. gromovii* KMM 6038^T was

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain SW5^T, *M. gromovii* KMM 6038^T and representatives of other genera of the family Flavobacteriaceae. GenBank accession numbers are given in parentheses. Numbers at branching points are bootstrap percentages (from 500 replicates) in distance, maximum-likelihood and maximum-parsimony analyses, respectively; dashes indicate that the node was not observed in the corresponding analysis. *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

investigated by slot-blot DNA–DNA hybridization, using the ECF random-prime labelling and signal amplification system (Amersham Life Sciences), following the procedure described by Kristjánsson *et al.* (1994). Amounts of 25, 50, 75 and 100 ng target DNA denatured in 0.4 M NaOH were slotted onto a nylon hybridization membrane (Bio-Rad) and probed with 200 ng fluorescein-labelled tracer DNA. For each triplicate of DNA–DNA association, the hybridization temperature was chosen in the optimal range of the hybridization buffer (Johnson, 1984; Ivanova *et al.*, 1988). Final high-stringency washes and signal amplification were performed according to the manufacturer's instructions (Amersham Life Sciences). Hybridization signals were detected with a Typhoon 640 fluorescence scanner (Molecular Dynamics) and analysed with the IMAGE-QUANT program. The signal (maximum peak area) produced by hybridization of the probe with homologous target DNA was set as 100 % and compared with the signal generated by heterologous DNA. When the DNA from SW5^T was used to probe the DNA of *M. gromovii* KMM 6038^T or vice versa, the level of DNA–DNA reassociation was ≤ 25 %. This value is well below that observed at the species level (Stackebrandt & Goebel, 1994), suggesting that SW5^T represents a novel species of the genus *Mariniflexile*.

Similar to *M. gromovii* KMM 6038^T, strain SW5^T formed circular, shiny, dark-yellow colonies, 2 mm in diameter, on ZoBell agar within 5 days at 20 °C. No spreading occurred at the margins of colonies of either strain (see Supplementary Fig. S1 available in IJSEM Online). Cell morphology and gliding motility were investigated on wet mounts of an exponential ZoBell broth culture at 20 °C by phase-contrast microscopy (Olympus BH2 microscope; $\times 1000$). Cells of strain SW5^T were round-ended rods, about 0.3–0.5 μm in diameter and 3.5–4.0 μm long, occurring singly or in pairs (Supplementary Fig. S2). Under the same growth conditions, *M. gromovii* KMM 6038^T formed shorter cells (~ 2.0 μm). Stationary-phase cultures of strain SW5^T also contained shorter cells (~ 1.0 μm) and non-refractive spherical bodies. Cells were non-flagellated, adhered to the slide and to the coverslip, displayed active gliding motility and stained Gram-negative using the method described by Doetsch (1981).

To determine the type of respiratory metabolism, strain SW5^T was inoculated in Veillon's tubes containing ZoBell medium solidified with 0.6 % (w/v) agar. Oxygen was removed from the medium by boiling. To determine whether metabolism was oxidative or fermentative, strain SW5^T was inoculated in a modified Leifson O-F medium (Hugh & Leifson, 1953; Smibert & Krieg, 1981) containing 0.5 % (w/v) glucose. Cytochrome oxidase activity was assayed by vigorous shaking of cells from a 2.5 ml broth culture for 5 min in 0.5 ml natural seawater in the presence of 20 μl dimethylparaphenylene diamine oxalate (bioMérieux). The presence of the enzyme was revealed by a blue coloration of the cellular suspension. Catalase activity was assayed by mixing a colony from a ZoBell agar plate with a drop of 10 % (v/v) hydrogen peroxide.

Amylase activity was assayed on 0.2 % (w/v) soluble starch ZoBell agar plates. DNase activity was detected in DNA agar (Difco), prepared with seawater. Amylase and DNase activities were revealed by flooding the plates with Lugol solution or 1 M HCl, respectively. Agarase, κ -carrageenase and ι -carrageenase activities were tested by inoculating ZoBell medium solidified with 15 g agar, 10 g κ -carrageenan or 20 g ι -carrageenan l^{-1} , respectively. Strains were considered positive when colonies were located in craters in the substrate. Intracellular and extracellular fucanase activities of strain SW5^T and *M. gromovii* KMM 6038^T were assayed by incubating sulphated fucans with protein extracts from bacterial cells cultivated in ZoBell broth supplemented by 0.2 % (w/v) sulphated fucans (intracellular activity) and with a cell-free supernatant from the same medium (extracellular activity). The degradation products were revealed by carbohydrate-PAGE (C-PAGE; Zablackis & Perez, 1990). The presence of oligofucans in the polyacrylamide gels revealed the presence of a fucanase activity. Extraction of sulphated fucan from the brown alga *Pelvetia canaliculata* and C-PAGE staining were performed as described previously (Descamps *et al.*, 2006). Production of flexirubin-type pigments was assessed by flooding a 4 day plate culture with 20 % (w/v) KOH, followed by the observation of changes in colony colour from yellow to red or brown (Reichenbach *et al.*, 1974).

Strain SW5^T was strictly aerobic, chemo-organotrophic and heterotrophic, with an oxidative metabolism that used oxygen as the electron acceptor. Nitrate could not be used as an electron acceptor. Strain SW5^T and *M. gromovii* KMM 6038^T were cytochrome oxidase-negative and catalase-positive and did not synthesize flexirubin. Unlike *M. gromovii* KMM 6038^T, strain SW5^T hydrolysed the sulphated fucoidan from *Pelvetia canaliculata* (Fig. 2). The ability to use simple carbohydrates as carbon sources was tested in artificial seawater supplemented with 2 g ammonium nitrate, 250 mg Casamino acids and 150 mg yeast extract l^{-1} and containing the carbohydrate under investigation at a concentration of 0.5 % (w/v). Other tests were performed by using the identification strips API 20 NE, API ZYM and API 50CH (bioMérieux). The latter were used in both the acidification (with the API 50CHB/E medium) and assimilation modes using a medium containing 2 g ammonium sulphate, 100 mg yeast extract and 1.5 g agar l^{-1} in 40 mM phosphate buffer, pH 8. All culture media were supplemented to 25 g NaCl l^{-1} . Biolog GN2 microplates were inoculated with bacterial suspensions in a medium that contained 25 g NaCl, 8 g MgCl_2 and 0.5 g KCl l^{-1} . API galleries and GN2 microplates were incubated at 20 °C for 2 weeks. Sensitivity to ($\mu\text{g ml}^{-1}$) chloramphenicol (10), penicillin G (200), streptomycin (200), kanamycin (200), ampicillin (200), rifampicin (10) and tetracycline (10) (all from Sigma-Aldrich) was tested at 20 °C in *Cytophaga* marine broth. The phenotypic characteristics of strain SW5^T are compared with those of *M. gromovii* KMM 6038^T in Table 1. Overall, the results obtained in this study for the latter strain are in agreement

Fig. 2. C-PAGE of oligofucans generated by cell extracts (lanes 2 and 3) and culture supernatants (lanes 4 and 5) from strain SW5^T (lanes 2 and 4) and *M. gromovii* KMM 6038^T (lanes 3 and 5). The substrate fucan from *Pelvetia canaliculata* is shown as a control (lane 1).

with previously published data (Nedashkovskaya *et al.*, 2006) except that we could not confirm the presence of cytochrome oxidase activity.

Cellular proteins were extracted from the cell pellet of 2.0 ml of a 48 h ZoBell broth culture at 20 °C washed twice with artificial seawater. Cells were disrupted by vigorous shaking in 375 µl BugBuster protein extraction reagent (Novagen) and left on ice for 10 min. The proteins were denatured for 5 min at 100 °C in 125 µl 4 × loading buffer (60 mM Tris/HCl, pH 6.8, 2 % SDS, 0.005 % (w/v) bromophenol blue, 1.4 M β-mercaptoethanol). Amounts of protein loaded on the SDS-PAGE gel (20 × 20 cm, 1.5 mm thick; 12 % (w/v) polyacrylamide in running gel; 4 % (w/v) in stacking gel) were standardized with respect to the strain that had the lowest optical density. The whole-cell protein profiles of strain SW5^T and *M. gromovii* KMM 6038^T showed significant differences (Fig. 3). The fatty acid composition of strain SW5^T was determined by the DSMZ, according to the standard protocol of the MIDI Microbial Identification System (Sasser, 1990), with cells grown on TSBA40 (30 g trypticase soy broth and 15 g agar l⁻¹) at 28 °C for 48 h. The fatty acid profile of strain SW5^T contained five major fatty acids: iso-C_{15:0} (15.3 %), iso-C_{16:0} 3-OH (10 %), C_{15:1} (8.7 %), iso-C_{17:0} 3-OH (6.8 %) and anteiso-C_{15:0} (6.7 %). The detailed fatty acid compositions of strain SW5^T and *M. gromovii* KMM 6038^T are given in Table 2. While both strains contained similar amounts of iso-C_{17:0} 3-OH and iso-C_{15:0}, the proportions of some other compounds differed significantly, probably as a result of the use of different growth media (marine agar 2216 was used for *M. gromovii* KMM 6038^T; Nedashkovskaya *et al.*, 2006) (Table 2).

Table 1. Phenotypic characteristics of strain SW5^T and *M. gromovii* KMM 6038^T

Both strains were Gram-negative, non-flagellated rods, positive for the following characteristics: respiratory metabolism; gliding motility; presence of catalase, β-galactosidase, esterase (C4), esterase lipase (C8), alkaline and acid phosphatases, leucine, valine and cystine arylamidases, trypsin and α-chymotrypsin activities; hydrolysis of aesculin and alginic acid; utilization of D-glucose, D-fructose, L-fucose, D-lactose, D-mannose and glucuronic acid; acid production from D-glucose, D-galactose, D-fructose, D-mannose, D-xylose, L-fucose, D-lactose and mannitol (weakly). Both strains were negative for the following characteristics: presence of cytochrome oxidase, α-galactosidase, α-mannosidase and β-glucuronidase activities; hydrolysis of agar and κ-carrageenan; production of flexirubin pigments; nitrate reduction; utilization of Tweens 40 and 80, L-arabinose, trehalose, glycerol, adonitol, D-arabitol, L-erythritol, inositol, D-sorbitol, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine; production of acid from melibiose, melezitose, turanose, D-lyxose, D-tagatose, D-fucose, L-xylose, L-sorbose, glycerol, erythritol, D-adonitol, dulcitol, inositol, D-sorbitol, D- and L-arabitol, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, N-acetylglucosamine and inulin. Both strains were susceptible to (µg ml⁻¹) ampicillin (200), tetracycline (10) and chloramphenicol (10) but not to streptomycin (200) or kanamycin (200). +, Positive; –, negative; w, weakly positive.

Characteristic	Strain SW5 ^T	<i>M. gromovii</i> KMM 6038 ^T
Cell length (µm)	3.5	2.0
Growth at/with:		
6 % (w/v) NaCl	–	+*
37 °C	–	+*
Presence of:		
Lipase (C14)	w	–
α-Glucosidase, β-glucosidase, α-fucosidase	+	–
Hydrolysis of:		
ι-Carrageenan, dextrin, glycogen, starch	+	–
Fucoidan from <i>Pelvetia canaliculata</i>	+	–
Gelatin	–	+
DNA	+	w
Utilization of:		
D-Galactose, maltose, cellobiose, gentiobiose	+	–
L-Rhamnose, sucrose, raffinose, D-mannitol	–	+
Acid production from:		
D-Arabinose, L-arabinose, L-rhamnose, sucrose, raffinose	–	+
D-Ribose, cellobiose, maltose, gentiobiose, amygdalin, starch, glycogen, arbutin, salicin	+	–
Trehalose, xylitol	w	–
DNA G + C content (mol%)	34.5	35.7

*Data from Nedashkovskaya *et al.* (2006).

Fig. 3. SDS-PAGE of protein extracts from strain SW5^T (lane 1) and *M. gromovii* KMM 6038^T (lane 2). Protein bands were visualized by staining with Coomassie blue.

The phylogenetic and fatty acid data showed that strain SW5^T belongs to the genus *Mariniflexile*. Phenotypic characteristics, DNA–DNA relatedness values and protein profiles support the description of strain SW5^T as a representative of a novel species, for which the name *Mariniflexile fucanivorans* sp. nov. is proposed.

Description of *Mariniflexile fucanivorans* sp. nov.

Mariniflexile fucanivorans [fu.ca'ni.vo'rans. N.L. n. *fucanum* fucan (polyfucose); L. part adj. *vorans* devouring from L. v. *vorare* to devour; N.L. part. adj. *fucanivorans* fucan-devouring].

Cells are non-flagellated, Gram-negative rods, 0.3–0.5 µm in diameter and 3.0–4.0 µm long, occurring singly or in pairs, with rounded ends. Cells are non-motile in the liquid phase but show active gliding motility on solid surfaces. Colonies on ZoBell 2216E agar are circular, shiny, dark yellow and 2.0–3.0 mm in diameter after incubation for 5 days. Spreading does not occur at the colony margins. Optimal growth temperature is 25 °C; growth occurs between 4 and 32 °C. Optimal pH for growth is 7.5; growth does not occur at pH 5.0 or 10.0. Optimal NaCl concentration for growth is 2.5 % (w/v) and no growth occurs in the absence of NaCl or at concentrations >6 % (w/v). Under optimal growth conditions, the doubling time is around 3.0 h. Catalase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, alkaline and acid phosphatases, β-galactosidase, α-glucosidase, α-fucosidase, trypsin and α-chymotrypsin activities are present, but cytochrome

Table 2. Whole-cell fatty acid profiles of strain SW5^T and *M. gromovii* KMM 6038^T

Data for *M. gromovii* KMM 6038^T were taken from Nedashkovskaya *et al.* (2006). Values are percentages of total fatty acids. –, Not detected; NR, not reported; tr, trace (<1 %). Fatty acids that amounted to <1 % in both strains are not listed, therefore the totals do not reach 100 %. The two strains were cultivated under different conditions.

Fatty acid	Strain SW5 ^T	<i>M. gromovii</i> KMM 6038 ^T
Straight-chain		
C _{15:0}	4.6	13.8
C _{16:0}	tr	1.4
Branched-chain		
iso-C _{14:0}	2.6	–
iso-C _{15:0}	15.3	15.0
iso-C _{15:1}	5.4	16.9
anteiso-C _{15:0}	6.7	5.4
anteiso-C _{15:1}	tr	1.6
iso-C _{16:0}	1.8	1.0
iso-C _{16:1}	3.1	1.5
iso-C _{17:1ω9c}	1.0	NR
Unsaturated		
C _{15:1}	8.7	3.1
C _{17:1}	4.2	1.1
Hydroxy		
C _{15:0} 2-OH	2.0	1.4
C _{15:0} 3-OH	3.9	1.6
iso-C _{15:0} 3-OH	8.2	4.3
C _{16:0} 3-OH	tr	1.1
iso-C _{16:0} 3-OH	10.0	2.5
C _{17:0} 2-OH	1.9	–
iso-C _{17:0} 3-OH	6.8	8.9
Summed feature 3*	5.5	8.4
Unknown†	2.7	NR

*Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1ω7c}, which could not be separated by GLC with the MIDI system.

†Sum of unknown fatty acids with an ECL (equivalent chain-length) of 11.543, 13.565 and 16.582.

oxidase, α-galactosidase, α-mannosidase and β-glucuronidase activities are absent. Aesculin, DNA, dextrin, glycogen, starch, alginic acid, ι-carrageenan and sulphated fucan are hydrolysed, but Tweens 40 and 80, agar, κ-carrageenan and gelatin are not. D-Glucose, D-galactose, D-fructose, L-fucose, D-mannose, D-lactose, maltose, cellobiose, gentiobiose and glucuronic acid are used as carbon sources, but L-arabinose, L-rhamnose, trehalose, sucrose, raffinose, glycerol, D-mannitol, adonitol, D-arabitol, L-erythritol, inositol, D-sorbitol, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine are not. Acid is produced from D-glucose, D-galactose, D-fructose, D-mannose, trehalose, D-xylose, D-ribose, L-fucose, maltose, D-lactose, cellobiose, gentiobiose, amygdalin, mannitol, xylitol, starch, glycogen, arbutin and salicin, but not from D- or L-arabinose, L-rhamnose,

melibiose, melezitose, turanose, D-lyxose, D-tagatose, D-fucose, L-xylose, L-sorbose, sucrose, raffinose, glycerol, erythritol, D-adonitol, dulcitol, inositol, D-sorbitol, D- or L-arabitol, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-xyloside, N-acetylglucosamine or inulin (API 50CH). Growth occurs in the presence of ($\mu\text{g ml}^{-1}$) streptomycin (200) and kanamycin (200), but not in presence of penicillin (200), ampicillin (200), rifampicin (10), tetracycline (10) or chloramphenicol (10). Other characteristics are shown in Table 1. The DNA G+C content of the type strain is 34.5 mol%.

The type strain is SW5^T (=CIP 109502^T =DSM 18792^T), isolated from mud collected from a water-treatment facility recycling the effluent of an alginate-extraction plant at Landerneau, Brittany, France.

Acknowledgements

This work was performed in the framework of 'Marine Genomics Europe' NoE 7 (EC contract no. GOCE-CT-2004-505403). The authors thank Nolwenn Calvez for technical assistance and Olga I. Nedashkovskaya (Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia) for providing the type strain of *Mariniflexile gromovii* used in this study.

References

- Barbeyron, T., Kean, C. & Forterre, P. (1984). DNA adenine methylation of GATC sequences appeared recently in the *Escherichia coli* lineage. *J Bacteriol* **160**, 586–590.
- Benson, D. A., Boguski, M. S., Lipman, D. J., Ostell, J., Ouellette, B. F., Rapp, B. A. & Wheeler, D. L. (1999). GenBank. *Nucleic Acids Res* **27**, 12–17.
- Bernardet, J.-F. & Nakagawa, Y. (2006). An introduction to the family *Flavobacteriaceae*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 7, pp. 455–480. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatidis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.
- Bernardet, J. F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.
- Colin, S., Deniaud, E., Jam, M., Descamps, V., Chevolut, Y., Kervarec, N., Yvin, J.-C., Barbeyron, T., Michel, G. & Kloareg, B. (2006). Cloning and biochemical characterization of the fucanase FcnA: definition of a novel glycoside hydrolase family specific for sulfated fucans. *Glycobiology* **16**, 1021–1032.
- Descamps, V., Colin, S., Lahaye, M., Jam, M., Richard, C., Potin, P., Barbeyron, T., Yvin, J.-C. & Kloareg, B. (2006). Isolation and culture of a marine bacterium degrading the sulfated fucans from marine brown algae. *Mar Biotechnol* **8**, 27–39.
- Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Galtier, N., Gouy, M. & Gautier, C. (1996). SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* **12**, 543–548.
- Hicks, R. E., Amann, R. I. & Stahl, D. A. (1992). Dual staining of natural bacterioplankton with 4',6-diamino-2-phenylindole and fluorescent oligonucleotide probes targeting kingdom-level 16S rRNA sequences. *Appl Environ Microbiol* **58**, 2158–2163.
- Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various negative bacteria. *J Bacteriol* **66**, 24–26.
- Ivanova, T. L., Turova, T. P. & Antonov, A. S. (1988). DNA-DNA hybridization studies on some purple non-sulfur bacteria. *Syst Appl Microbiol* **10**, 259–263.
- Johnson, J. L. (1984). DNA reassociation and RNA hybridization of bacterial nucleic acids. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 8–11. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Jung, S.-Y., Kang, S.-J., Lee, M.-H., Lee, S.-Y., Oh, T.-K. & Yoon, J.-H. (2005). *Gaetbulibacter saemankumensis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a tidal flat sediment in Korea. *Int J Syst Evol Microbiol* **55**, 1845–1849.
- Kane, M. D., Poulsen, L. K. & Stahl, D. A. (1993). Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide hybridization probes designed from environmentally derived 16S rRNA sequences. *Appl Environ Microbiol* **59**, 682–686.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* **30**, 3059–3066.
- Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* **33**, 511–518.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kloareg, B. & Quatrano, R. S. (1988). Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanogr Mar Biol Ann Rev* **26**, 259–315.
- Kristjánsson, J. K., Hjörleifsdóttir, S., Marteinson, V. T. & Alfredsson, G. A. (1994). *Thermus scotoductus*, sp. nov., a pigment-producing thermophilic bacterium from hot tap water in Iceland and including *Thermus* sp. X-1. *Syst Appl Microbiol* **17**, 44–50.
- Kwon, K. K., Lee, H.-S., Jung, H.-B., Kang, J.-H. & Kim, S.-J. (2006). *Yeosuana aromativorans* gen. nov., sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae*, isolated from estuarine sediment of the South Sea, Korea. *Int J Syst Evol Microbiol* **56**, 727–732.
- Lee, S. D. (2007). *Tamlana crocina* gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae*, isolated from beach sediment in Korea. *Int J Syst Evol Microbiol* **57**, 764–769.
- Mabeau, S. & Kloareg, B. (1987). Isolation and analysis of the cell walls of brown algae: *Fucus spiralis*, *F. ceranoides*, *F. vesiculosus*, *F. serratus*, *Bifurcaria bifurcata* and *Laminaria digitata*. *J Exp Bot* **38**, 1573–1580.

- Mabeau, S., Kloareg, B. & Joseleau, J.-P. (1990). Fractionation and analysis of fucans from brown algae. *Phytochemistry* **29**, 2441–2445.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Rhee, M.-S., Lysenko, A. M., Rohde, M., Zhukova, N. V., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2004). *Algibacter lectus* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from green algae. *Int J Syst Evol Microbiol* **54**, 1257–1261.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K.-O., Lysenko, A. M., Rohde, M. & other authors (2005). *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **55**, 49–55.
- Nedashkovskaya, O. I., Kim, S. B., Kwak, J., Mikhailov, V. V. & Bae, K. S. (2006). *Mariniflexile gromovii* gen. nov., sp. nov., a gliding bacterium isolated from the sea urchin *Strongylocentrotus intermedius*. *Int J Syst Evol Microbiol* **56**, 1635–1638.
- Nichols, C. M., Bowman, J. P. & Guezennec, J. (2005). *Olleya marilimosa* gen. nov., sp. nov., an exopolysaccharide-producing marine bacterium from the family *Flavobacteriaceae*, isolated from the Southern Ocean. *Int J Syst Evol Microbiol* **55**, 1557–1561.
- Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.
- Reichenbach, H., Kleinig, H. & Achenbach, H. (1974). The pigments of *Flexibacter elegans*: novel and chemosystematically useful compounds. *Arch Microbiol* **101**, 131–144.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 409–443. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Zabackis, E. & Perez, J. (1990). A partially pyruvated carrageenan from Hawaiian *Grateloupia filicina* (Cryptonemiales, Rhodophyta). *Bot Mar* **33**, 273–276.
- ZoBell, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* **4**, 41–75.