Sediminibacter furfurosus gen. nov., sp. nov. and *Gilvibacter sediminis* gen. nov., sp. nov., novel members of the family *Flavobacteriaceae*

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Two Gram-negative, chemoheterotrophic, non-motile strains, Mok-1-36^T and MAOS-86^T, were isolated from marine-sediment samples collected from the coasts of Okinawa island and the city of Odawara in Japan, respectively. Phylogenetic studies based on 16S rRNA gene sequences indicated that Mok-1-36^T and MAOS-86^T were members of the family *Flavobacteriaceae*, clustering with members of the genera *Ulvibacter* and *Vitellibacter*, respectively. Strains Mok-1-36^T and MAOS-86^T shared pairwise 16S rRNA gene sequence similarities of 93.5 and 89.1 % with the type strains of *Ulvibacter litoralis* and *Vitellibacter vladivostokensis*, respectively. Phylogenetic distinctiveness and phenotypic differences from their phylogenetic neighbours indicated that these strains represent two novel species and genera within the family *Flavobacteriaceae*, for which the names *Sediminibacter furfurosus* gen. nov., sp. nov. (MAOS-86^T) and *Gilvibacter sediminis* gen. nov., sp. nov. (Mok-1-36^T) are proposed. The type strain of *Sediminibacter furfurosus* is MAOS-86^T (=NBRC 101622^T=CIP 109285^T) and the type strain of *Gilvibacter sediminis* is Mok-1-36^T (=NBRC 101626^T=CIP 109286^T).

Since the description of the family *Flavobacteriaceae* (Reichenbach, 1992; Bernardet *et al.*, 2002), a number of novel genera have been included. This family is one of the major branches of the phylum *Bacteroidetes*, and includes many marine species. They are chemoorganotrophs and play an important role in the carbon cycles of marine ecosystems (Abell & Bowman, 2005).

This paper describes the characterization of two strains, Mok-1-36^T and MAOS-86^T, isolated from marine-sediment samples collected from the coasts of Okinawa island and the city of Odawara, Japan, respectively. An analysis of their physiological, chemotaxonomic and morphological properties indicated that these two strains represent two novel species and genera within the family *Flavobacteriaceae*.

Marine-sediment samples were serially diluted with sterile artificial seawater (Naigai Chemicals) and spread on half-strength marine agar (HSMA) plates [18.7 g marine broth 2216 (Difco) dissolved in 1 l artificial seawater and solidified with 1.6 % agar]. The plates were incubated at 30 °C for 5 days. Single colonies from the plates were picked and purified on plates containing the same medium at the same temperature. For routine cultivation and for most investigations, the same agar or the corresponding broth (HSMB)

was used at the same incubation temperature. Purified strains were stored at –80 °C in artificial seawater supplemented with 20 % (v/v) glycerol.

Template DNA for 16S rRNA gene analysis was prepared by using Prepman Ultra (Applied Biosystems). PCR-mediated amplification of 16S rRNA genes was carried out with primers 27f and 1492r, as described by Hiraishi *et al.* (1994). Amplicons were directly sequenced using the BigDye Terminator version 3.1 cycle sequencing kit and analysed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Sequences were edited and assembled using the ATGC program (Genetyx). The almost-complete 16S rRNA gene sequences thus obtained were compared with those in the DDBJ database by means of BLASTN searches (Altschul *et al.*, 1990). Alignment with related sequences was performed using the CLUSTAL X software package (Thompson *et al.*, 1997). Phylogenetic trees were then constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 2000) methods; tree topologies were evaluated and compared using the bootstrap resampling method of Felsenstein (1985), with 1000 replicates. 16S rRNA gene sequence analysis indicated that both strains were members of the family *Flavobacteriaceae*. The results of BLASTN searches against sequences in DDBJ showed *Gelidibacter algens* IC147^T (GenBank accession no. AF001367) and *Psychroserpens* sp. 18III/A01/061 (AY576714) to be the closest relatives of MAOS-86^T and Mok-1-36^T, respectively showing 16S rRNA gene sequence

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain MAOS-86^T and strain Mok-1-36^T are respectively AB255369 and AB255368.

similarity levels of 92 and 93 %. In the neighbour-joining tree, however, strain Mok-1-36^T formed a monophyletic clade with *Ulvibacter litoralis* KMM 3912^T (93.5 % identity) and strain MAOS-86^T formed a monophyletic clade with *Vitellibacter vladvostokensis* KMM 3516^T (89.1 % identity) (Fig. 1); both results were supported by high bootstrap values. The maximum-parsimony tree produced a very similar topology (data not shown).

Three-day-old colonies of Mok-1-36^T on HSMA were yellow and irregular. While the colonies of MAOS-86^T appeared circular and colourless for the first 3–5 days incubation, confluent growth turned brownish after 5–6 days. The cell morphology of 3- to 5-day-old HSMA cultures was observed under an Olympus light microscope (CX41LF) without staining. The cells of both strains were rod-shaped and 0.5–0.7 µm wide. The cell length for Mok-1-36^T was 3.0–5.0 µm and that for MAOS-86^T was 1.0–2.5 µm. The hanging drop method of Perry (1973) (using a 1000 × oil-immersion objective) was used to check for the presence of gliding motility; neither strain showed gliding motility. The presence of flexirubin-type pigments was assessed using the bathochromic shift test with 20 % (w/v) KOH, as described by Fautz & Reichenbach (1980). Flexirubin-type pigments were absent from both strains. To detect carotenoid-type pigments, the absorption spectra (260–700 nm) of acetone extracts of the cells were measured using a Shimadzu UV-visible spectrophotometer (UV-1650

PC). Carotenoid pigments were detected only in strain Mok-1-36^T. Catalase activity was revealed by the production of oxygen bubbles when cells harvested from colonies grown on HSMA plates were mixed with a drop of 3 % (v/v) hydrogen peroxide on a glass slide. Oxidase activity was assessed using a cytochrome oxidase test strip (Nissui Pharmaceuticals) on which cells from 2- to 3-day-old cultures suspended in distilled water were spotted. Both strains were positive for catalase and oxidase.

The ability to grow at different temperatures was examined on HSMA plates at 4, 10, 15, 20, 25, 30, 37, 40 and 42 °C. Tolerance of high NaCl concentrations was assessed in HSMB with final NaCl concentrations adjusted to 4, 5, 6, 7, 8, 9 and 10 % (w/v). Growth in one-fifth-strength Luria–Bertani medium [12 g Bacto tryptone (Difco) and 1 g Bacto yeast extract (Difco) per litre distilled water] supplemented with 0, 1, 2 and 3 % (w/v) NaCl was also tested. Cellulose degradation was tested by placing cellulose-paper strips (Whatman No. 1 filter paper) in cultures in one-fifth-strength Luria–Bertani medium made up in artificial seawater (LBM medium). CM-cellulose degradation was tested by inoculating cells deeply into one-fifth-strength LBM medium solidified with 3 % (w/v) CM-cellulose (high viscosity; Sigma). Degradation of agar and carrageenan (Type I; Sigma) was assessed, in each case, on plates of HSMB solidified with 1.5 % (w/v) of the polymer. Degradation of starch, casein, chitin, DNA and gelatin was tested according to the protocol of Cowan & Steel (1993). Other biochemical tests (reduction of nitrate and nitrite, production of indole from tryptophan, production of acid from glucose, presence of urease, β-galactosidase and β-glucosidase) were carried out using API 20NE strips (bioMérieux) according to the manufacturer's instructions, except that cultures were suspended in artificial seawater. Biolog GN2 MicroPlates (Biolog) were used to test for the utilization of 95 different carbon sources; the plates were inoculated as suggested by Rüger & Krambeck (1994). The results from the API 20NE strips and the Biolog GN2 MicroPlates were recorded after incubation at 30 °C for 12–36 h. Additionally, utilization of several carbon sources (peptone, sucrose, maltose, raffinose, D-mannose, D-arabinose, D-galactose, L-rhamnose, L-aspartic acid, pyruvic acid, L-glutamic acid, meso-erythritol, adonitol, glycerol and xylitol) was tested using the basal medium described by Suzuki *et al.* (2001). The morphological and physiological characteristics of the two novel strains are given in the genus and species descriptions and in Table 1. Strains Mok-1-36^T and MAOS-86^T were distinguishable from their phylogenetic neighbours on the basis of a number of phenotypic characteristics (Table 1).

Genomic DNA was prepared by using the method of Minamisawa (1990), and the DNA G+C contents of Mok-1-36^T and MAOS-86^T were determined using the HPLC method of Mesbah *et al.* (1989); G+C contents of 43 and 39 mol%, respectively, were found. The method of Nakagawa & Yamasato (1993) was used to extract and

Fig. 1. Neighbour-joining phylogenetic tree, based on the analysis of almost-complete 16S rRNA gene sequences, showing the positions of strains Mok-1-36^T and MAOS-86^T in the family *Flavobacteriaceae*. *Flexibacter flexilis* ATCC 23079^T was used as an outgroup. Numbers at nodes show bootstrap values (when more than 500) calculated from 1000 resampling units. Filled circles indicate nodes that were also recovered in the maximum-parsimony tree. Bar, 0.02 K_{nuc}.

Table 1. Differentiating characteristics for strains Mok-1-36^T and MAOS-86^T

Strains: 1, Mok-1-36^T; 2, *U. litoralis* KMM 3912^T (data from Nedashkovskaya *et al.*, 2004); 3, MAOS-86^T; 4, *V. vladivostokensis* KMM 3516^T (Nedashkovskaya *et al.*, 2003).

Characteristic	1	2	3	4
Gliding motility	–	+	–	–
Pigmentation	Yellow	Yellow	Light brown	Yellow–orange
Flexirubin-type pigments	–	+	–	+
Growth at:				
37 °C	+	–	+	+
42 °C	–	–	–	+
Hydrolysis of:				
Starch	–	–	+	–
DNA	+	–	+	+
CM-cellulose	+	–	–	–
DNA G+C content (mol%)	43	36–38	39	41

analyse the respiratory quinones from freeze-dried cells. Menaquinone-6 was detected as the sole quinone in both strains. For fatty acid analysis, cells of strains MAOS-86^T and Mok-1-36^T were grown under the same conditions as their respective phylogenetic neighbours, *Vitellibacter vladivostokensis* and *Ulvibacter litoralis*, had been grown under for their original descriptions, i.e. at 28 °C for 72 h on medium A (Nedashkovskaya *et al.*, 2003) and on marine agar 2216 (Nedashkovskaya *et al.*, 2004), respectively. As the growth temperature and duration of incubation for *U. litoralis* were not specified in the latter study, strain Mok-1-36^T was also grown at 28 °C for 72 h. The standard protocol of the Microbial Identification system (MIDI; Microbial ID) was used. The major fatty acids of strain Mok-1-36^T were iso-15:0, iso-15:1, iso-16:0 3-OH and iso-17:0 3-OH, very similar to those determined (using the MIDI system) for *U. litoralis* (Nedashkovskaya *et al.*, 2004). The predominant fatty acids of MAOS-86^T were iso-15:0, iso-15:1, iso-17:0 3-OH and summed feature 3 (16:1 ω 7c and/or iso-15:0 2-OH). This fatty acid profile was notably different from that determined for *V. vladivostokensis* using a different chromatography procedure (Nedashkovskaya *et al.*, 2003). The detailed fatty acid profiles for the four taxa are given in Table 2.

Hence, the novel taxonomic status of the two isolates is supported by their unique phenotypic traits (Tables 1 and 2), and it is concluded that strains Mok-1-36^T and MAOS-86^T represent novel species of two novel genera within the family *Flavobacteriaceae*. The names *Sediminibacter furfurosus* gen. nov., sp. nov. and *Gilvibacter sediminis* gen. nov., sp. nov. are proposed for strains MAOS-86^T and Mok-1-36^T, respectively.

Table 2. Fatty acid compositions (%) of strains Mok-1-36^T and MAOS-86^T, *U. litoralis* KMM 3912^T and *V. vladivostokensis* KMM 3516^T

Strains: 1, Mok-1-36^T; 2, *U. litoralis* KMM 3912^T (data from Nedashkovskaya *et al.*, 2004); 3, MAOS-86^T; 4, *V. vladivostokensis* KMM 3516^T (Nedashkovskaya *et al.*, 2003). The fatty acid compositions of Mok-1-36^T, *U. litoralis* KMM 3912^T and MAOS-86^T were determined using the MIDI system, whereas that of *V. vladivostokensis* KMM 3516^T was determined using another GLC method (Nedashkovskaya *et al.*, 2003). tr, Trace (<1% of the total fatty acid content); NR, not reported; ND, not detected.

Fatty acid	1	2	3	4
iso-14:0	1	2	tr	NR
iso-15:0	19	21	39	68
anteiso-15:0	1	2	2	8
iso-15:1	15	13	25	2
15:0	ND	4	ND	4
15:1 ω 6c	ND	1	tr	NR
16:0	2	NR	2	2
iso-16:0	7	3	tr	3
iso-16:1	4	4	tr	tr
iso-17:0	ND	NR	ND	3
iso-17:1	ND	NR	ND	4
iso-17:1 ω 9c	ND	4	3	NR
17:1 ω 6c	tr	2	tr	NR
15:0 2-OH	1	1	tr	NR
iso-15:0 3-OH	5	3	6	2
iso-16:0 3-OH	9	9	1	NR
iso-17:0 3-OH	16	17	9	tr
17:0 2-OH	tr	2	tr	NR
Summed feature 3*	7	6	9	NR

*Summed feature 3 contains 16:1 ω 7c and/or iso-15:0 2-OH.

Description of *Sediminibacter* gen. nov.

Sediminibacter (Se.di.mi.ni.bac' ter. L. n. *sedimen* -inis sediment; N.L. masc. *bacter* rod; N.L. masc. n. *Sediminibacter* a rod from sediment).

Cells are non-motile, Gram-negative, aerobic, rod-shaped and lack carotenoid and flexirubin-type pigments. Catalase- and oxidase-positive. The main fatty acids are iso-15:0, iso-15:1, iso-17:0 3-OH and summed feature 3 (16:1 ω 7c and/or i-15:0 2-OH). The predominant quinone is menaquinone-6. The type species is *Sediminibacter furfurosus*.

Description of *Sediminibacter furfurosus* sp. nov.

Sediminibacter furfurosus (fur.fu.ro'sus. L. masc. adj. *furfurosus* brownish, because cells in confluent growth have a brownish tinge).

Exhibits the following properties in addition to those described for the genus. Cells are 0.5–0.7 μ m wide and

1.0–2.5 µm long. Colonies on marine agar are colourless, but confluent colonies more than 5 days old turn a brownish colour. Growth occurs at temperatures in the range 10–37 °C, with an optimum at 25–30 °C. Growth occurs at NaCl concentrations of 1–6 % (w/v), with optimal growth at 3–4 %. Growth does not occur in the absence of NaCl or at NaCl concentrations above 6 % (w/v). Starch, gelatin and DNA are degraded. Chitin, agar, carrageenan, cellulose, CM-cellulose and urea are not degraded. Casein degradation, indole production and β -glucosidase and β -galactosidase activities are weakly positive. Acid is not produced from glucose. Nitrate and nitrite are not reduced. α -Cyclodextrin, dextrin, glycogen, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α -D-glucose, α -D-lactose, lactulose, maltose, D-mannose, melibiose, methyl β -D-glucoside, D-psicose, raffinose, D-sorbitol, sucrose, trehalose, turanose, D-galacturonic acid, D-glucosaminic acid, DL-lactic acid, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-ornithine, L-serine, L-threonine, urocanic acid, inosine, uridine, thymidine, glucose 1-phosphate and glucose 6-phosphate are utilized. All other substrates tested are not utilized. The DNA G+C content of the type strain is 39 mol%.

The type strain, MAOS-86^T (=NBRC 101622^T=CIP 109285^T) was isolated from a marine-sediment sample collected from the coast at Odawara, Japan.

Description of *Gilvibacter* gen. nov.

Gilvibacter (Gil.vi.bac' ter. L. adj. *gilvus* yellow; N.L. masc. n. *bacter* rod; N.L. masc. n. *Gilvibacter* yellow rod).

Gram-negative, non-motile, yellow-coloured, rod-shaped cells. Catalase- and oxidase-positive. The main fatty acids are iso-15:0, iso-15:1, iso-16:0 3-OH and iso-17:0 3-OH. The main respiratory quinone is menaquinone-6. Flexirubin-type pigments are absent. The type species is *Gilvibacter sediminis*.

Description of *Gilvibacter sediminis* sp. nov.

Gilvibacter sediminis (se.di.mi' nis. L. gen. n. *sediminis* of a sediment).

In addition to the characteristics given in the genus description, the following properties are displayed. Colonies are irregular and are yellow-coloured due to the presence of carotenoid pigments. Cells are 0.5–0.7 µm wide and 3.0–5.0 µm long. Nitrate is reduced, but nitrite is not reduced. Gelatin, DNA and CM-cellulose are degraded. Chitin, agar, casein, starch, carrageenan, cellulose and urea are not degraded. Acid is not produced from glucose and indole is not produced from tryptophan. Weakly positive for β -galactosidase and positive for β -glucosidase. Growth occurs at 15–37 °C and optimally at 25–30 °C. The optimal NaCl concentration for growth is 3–4 % (w/v); growth occurs with NaCl at concentrations up to 6 % (w/v). Of the 95 different carbon sources tested on Biolog GN2

microplates, only L-arabinose is utilized. Peptone, sucrose, maltose and D-raffinose are utilized when tested with the protocol of Suzuki *et al.* (2001). The DNA G+C content of the type strain is 43 mol%.

The type strain, Mok-1-36^T (=NBRC 101626^T=CIP 109286^T), was isolated from a marine-sediment sample collected from the coast of Okinawa island, Japan.

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