Sediminitomix flava gen. nov., sp. nov., of the phylum *Bacteroidetes*, isolated from marine sediment

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A novel bacterium designated Mok-1-85^T was isolated from a marine sediment sample collected from Okinawa Island, Japan. Cells of strain Mok-1-85^T stained Gram-negative, were catalase- and oxidase-positive and were non-motile. In a neighbour-joining tree based on 16S rRNA gene sequences, the novel strain clustered with the genus *Flammeovirga*, a member of the family 'Flammeovirgaceae'. The novel isolate shared low 16S rRNA gene sequence similarities ($\leq 86\%$) with the members of the genus *Flammeovirga* and other related taxa. The major isoprenoid quinone was MK-7 and the predominant fatty acids of this organism were iso-C_{15:0}, C_{16:1} ω 5c and C_{16:0} 3-OH. The G + C content of the DNA was 38 mol%. Combined phylogenetic and physiological data showed that strain Mok-1-85^T represents a novel genus and species for which the name *Sediminitomix flava* gen. nov., sp. nov. is proposed. The type strain is Mok-1-85^T (=NBRC 101625^T=KCTC 12970^T=CIP 109411^T).

The phylum *Bacteroidetes* consists of three large classes, *Bacteroidetes*, *Flavobacteria* and 'Sphingobacteria' each comprising of only one order (Bernardet *et al.*, 2002). They are widely distributed in terrestrial and marine environments and are dominant populations in gastro-intestinal flora (Shewan & McMeekin, 1983; Leser *et al.*, 2002; Bäckhed *et al.*, 2005). Since 2003, the number of recognized genera in the phylum *Bacteroidetes* has increased exponentially, suggesting that the phylum contains many as yet undiscovered members.

In this paper, the isolation of a marine bacterium belonging to the family 'Flammeovirgaceae' is reported. The family 'Flammeovirgaceae' contains four genera (Garrity & Holt, 2001) namely *Flammeovirga* (Takahashi *et al.*, 2006; Nakagawa *et al.*, 1997), *Persicobacter* (Nakagawa *et al.*, 1997), *Flexithrix* (Lewin, 1970) and *Thermonema* (Hudson *et al.*, 1989). With the exception of the genus *Thermonema* which includes thermophilic bacteria, the other genera of the family are mesophilic and share very similar characteristics.

Strain Mok-1-85^T was obtained from a marine sediment sample collected from Okinawa Island, Japan. The sediment sample was serially diluted in sterile artificial seawater (Naigai Chemicals) and an aliquot of each dilution was spread on half-strength marine agar [19 g Bacto Marine Broth 2216 (Difco), 17 g artificial seawater and 15 g agar dissolved in 1 l distilled water; HSMA]. The HSMA plates

were incubated at 30 °C for 7 days. Strain Mok-1-85^T formed large irregular orange-coloured colonies on HSMA plates after 3 days of incubation. The strain was preserved at –80 °C in artificial seawater supplemented with 20 % glycerol (v/v).

Prepman Ultra (Applied Biosystems) was used to prepare a DNA template for 16S rRNA gene amplification. PCR-mediated amplification of the partial sequence of a 16S rRNA gene corresponding to positions 8–1492 of the *Escherichia coli* 16S rRNA gene sequence (Brosius *et al.*, 1978) was carried out using the universal pair of primers 27f and 1492r according to the protocol of Hiraishi *et al.* (1994). Purified PCR product was sequenced directly using an ABI Big Dye Terminator Cycle Sequence kit (version 3.1) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). An almost complete 16S rRNA gene sequence (1415 bp) was obtained. Using the sequence data, a BLAST search (Altschul *et al.*, 1990) of the DNA DataBank of Japan (DDBJ) was performed to detect the closest relatives of strain Mok-1-85^T. *Lishizhenia caseinilytica* (GenBank accession no. AB176674) was found to be the closest relative with a 16S rRNA gene sequence similarity of 86 %. Related sequences were downloaded from DDBJ and aligned with the sequence of strain Mok-1-85^T using the CLUSTAL_X software package (Thompson *et al.*, 1997). Evolutionary trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 2000) methods and the robustness of the phyletic lines was evaluated by using bootstrap analyses (Felsenstein, 1985) with 1000 replicates. The branching patterns of the two

The GenBank/EMBL/DDBJ accession no. for the 16S rRNA gene sequence of *Sediminitomix flava* strain Mok-1-85^T is AB255370.

Fig. 1. Neighbour-joining phylogenetic tree of *Sediminotomix flava* sp. nov. Mok-1-85^T and related genera based on 16S rRNA gene sequence comparisons. Bootstrap values are indicated at the branches from 1000 replications. Only significant bootstrap values (>500) are shown. The sequence of *Chlorobium limicola* M1^T was used as an outgroup. GenBank accession numbers are given in parentheses. Closed circles at the nodes represent branches that were also recovered in maximum-parsimony analysis. Bar, 0.02 K_{nuc} .

trees obtained by the neighbour-joining (Fig. 1) and maximum-parsimony (data not shown) methods were slightly different from each other. However, the novel isolate formed a cluster, supported by high bootstrap values, with members of the genus *Flammeovirga* in both trees (Fig. 1). Pairwise 16S rRNA gene similarities with other related genera were also checked. Strain Mok-1-85^T shared $\leq 86\%$ sequence similarity with other related genera.

Exponentially growing cells of strain Mok-1-85^T taken from HSMA plates after 2–3 days of incubation at 30 °C were used for the following tests unless specified otherwise. Cell morphology was observed under a light microscope equipped with a digital camera (CX41LF; Olympus). Cells were 0.5–0.7 μm in width and 10–35 μm in length. Gliding motility was checked on HSMA plates and by the hanging drop method described by Perry (1973) under a $\times 1000$ oil immersion objective. No gliding motility was observed on the HSMA plates or by the hanging drop method. The presence of flexirubin-type pigments was checked by the bathochromic shift test (Fautz & Reichenbach, 1980) with 20% (w/v) KOH. Flexirubin-type pigments were absent. Extracts of cells for the carotenoid analysis were prepared as described by Schmidt *et al.* (1994) and the absorption spectrum (260–700 nm) was recorded using a UV-visible spectrophotometer (UV-1650 PC; Shimadzu).

The absorption spectrum obtained for strain Mok-1-85^T was similar to that of *Flammeovirga aprica* NBRC 15941^T, indicating the presence of saxoxyanthin (Takahashi *et al.*, 2006). The production of oxygen bubbles by the cells when mixed with 3% (v/v) H_2O_2 on a glass slide was examined to check for the presence of catalase. The presence of oxidase activity was judged by the development of blue colour when cells suspended in sterile water were spotted on to a cytochrome oxidase strip (Nissui Pharmaceuticals). Strain Mok-1-85^T tested positive for both catalase and oxidase activities.

Tolerance to high concentrations of NaCl was checked by growing the novel strain in half-strength marine broth (HSMB) supplemented with 4, 5, 6, 7, 8, 9 and 10% (w/v) NaCl. Growth on one-fifth-strength Luria–Bertani (LB) medium [2 g Bacto tryptone (Difco) and 1 g Bacto yeast extract (Difco) in 1 l water] supplemented with 0, 1, 2 and 3% (w/v) NaCl was also checked. The novel strain was able to grow on one-fifth-strength LB containing 2 and 3% (w/v) NaCl and in HSMB containing 4% (w/v) NaCl. However, no growth was observed in one-fifth-strength LB with 0% NaCl or in HSMB supplemented with 6, 7, 8, 9 and 10% (w/v) NaCl. Weak growth was observed in HSMB with 5% (w/v) NaCl and in one-fifth-strength LB with 1% (w/v) NaCl. The ability of strain Mok-1-85^T to grow at different temperatures was checked on HSMA plates

incubated at 4, 10, 15, 20, 25, 30, 37, 40 and 42 °C. Strain Mok-1-85^T was able to grow at 15, 20, 25, 30, 37 and 40 °C and optimal growth took place at 25–30 °C. No growth was observed on HSMA plates incubated at 4, 10 or 42 °C.

The degradation of cellulose was tested by placing cellulose paper strips (Whatman No. 1 filter paper) in cultures in one-fifth-strength LBM medium (one-fifth-strength LB prepared with artificial seawater). To test the degradation of carboxymethylcellulose (CM-cellulose), using an inoculating needle, the cells were stabbed deeply into one-fifth-strength LBM solidified with 3 % (w/v) CM-cellulose (high viscosity; Sigma). The degradation of agar and carrageenan (Sigma, type I) was assessed on plates of HSMB solidified with 1.5 % (w/v) of either of the polymers. The abilities of the novel strain to degrade starch, chitin, DNA, gelatin, Tweens 20, 40 and 80 and alginic acid and to produce H₂S were tested according to the protocols of Lewin & Lounsbury (1969), Smibert & Krieg (1981) and Cowan & Steel (1993). Casein degradation was tested on casein plates [one fifth-strength LBM medium supplemented with 1.2 % (w/v) casein and solidified with 1.5 % (w/v) agar]. The ability to grow on 1 % (w/v) peptone and tryptic soya agar (TSA) prepared with artificial seawater was tested on plates solidified with 1.5 % (w/v) agar. The survival of cells after treatment at 55 °C for 10 min was tested using cells suspended in sterile artificial seawater. The oxidation or fermentation of glucose (O/F test; Hugh & Leifson, 1953) was checked by using O/F basal medium (Eiken Kizai) prepared with the artificial seawater. The reduction of

nitrate and nitrite, the production of indole from tryptophan, the production of acids from glucose and the activities of urease, β -galactosidase and β -glucosidase were checked using API 20 NE strips (bioMérieux) according to the manufacturer's instructions, except that the cells were suspended in artificial seawater. Cells suspended in artificial seawater were also used to test the production of acids from various carbon sources (API 50CH strips; bioMérieux) and the activities of several enzymes (API ZYM strips; bioMérieux). The results of these phenotypic analyses are given in the genus and species descriptions and in Table 1.

The protocol of Minamisawa (1990) was used for the preparation of genomic DNA and the G+C content was determined using the standard HPLC method of Mesbah *et al.* (1989). The G+C content of the novel strain was 38 mol%. For the fatty acid analysis, strain Mok-1-85^T was grown on marine agar plates at 25 °C for 24–36 h. The standard protocol of the Microbial Identification System (Microbial ID) was used for the preparation and analysis of fatty acid methyl esters. The predominant fatty acids of this organism were iso-C_{15:0}, C_{16:1} ω 5c and C_{16:0} 3-OH. Two of the three predominant fatty acids (iso-C_{15:0} and C_{16:0} 3-OH) were also found as the major fatty acids in members of the genus *Flammeovirga* (Takahashi *et al.*, 2006), the closest phylogenetic neighbour to the novel strain. However the fatty acid profile of the novel strain was different from that of members of the genus *Flammeovirga* (Tables 1, 2). The protocol of Nakagawa & Yamasato (1993) was used for

Table 1. Characteristics that differentiate members of the genus *Sediminitomix* gen. nov. from related genera

Taxa: 1, *Sediminitomix* gen. nov.; 2, *Flammeovirga* (data from Takahashi *et al.*, 2006); 3, *Persicobacter* (Nakagawa *et al.*, 1997; Reichenbach, 1989); 4, *Flexithrix* (Lewin, 1970). +, Positive; –, negative; v, variable; NA, not available.

Characteristic	1	2	3	4
Catalase/oxidase	+ / +	v/v	– / +	– / –
Pigment:				
Type	Saproxanthin	Saproxanthin	Saproxanthin	Zeaxanthin
Colony colour	Orange	Orange reddish	Pink to orange	Golden yellow
Gliding motility	–	+	+	+
Cell size:				
Width (μ m)	0.5–0.7	0.4–0.9	0.5	0.4–0.6
Length (μ m)	10–35	1.7–96	4–30	5–15*
Degradation of:				
Alginic acid	+	+	+	–
Gelatin	+	v	+	–
Starch	+	+	+	–
H ₂ S production	+	+	–	–
O/F test with glucose	–	Fermentative	NA	NA
Growth at 37 °C	+	–	+	–
Nitrate reduction	+	+	v	–
Major fatty acids	iso-C _{15:0} , C _{16:1} ω 5c and C _{16:0} 3-OH	iso-C _{15:0} , C _{20:4} ω 6c, 9c, 12c, 15c and C _{16:0} 3-OH	NA	NA
DNA G+C content (mol%)	38	31–36	40–42	37

*Cells are often 30–60 μ m long.

Table 2. Fatty acid profiles of *Sediminitomix flava* sp. nov. Mok-1-85^T and *Flammeovirga aprica* NBRC 15941^T, the closest phylogenetic neighbour

Data for *Flammeovirga aprica* NBRC 15941^T are from Takahashi *et al.* (2006). Fatty acids amounting to <1 % of the total fatty acids are not shown.

Fatty acid (%)	Mok-1-85 ^T	NBRC 15941 ^T
Straight chain fatty acids:		
C _{14:0}	5	8
C _{15:0}	—	1
C _{16:0}	6	—
Branched fatty acids:		
i-C _{13:0}	—	3
i-C _{14:0}	—	2
i-C _{15:0}	23	37
a-C _{15:0}	1	1
Unsaturated fatty acids:		
C _{16:1} ω5c	21	2
C _{20:4} ω6c,9c,12c,15c	—	24
Hydroxy fatty acids:		
C _{15:0} 3-OH	—	1
i-C _{15:0} 3-OH	2	4
C _{16:0} 3-OH	18	9
i-C _{17:0} 3-OH	2	—
Summed features*:		
2	2	—
3	10	—

*Summed feature 2, C_{14:0} 3-OH and/or iso I-C_{16:1}; summed feature 3, iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

the analysis of isoprenoid quinones. Strain Mok-1-85^T contained MK-7 as the only quinone.

Very low 16S rRNA gene sequence similarities with any of the sequences available in DDBJ and the formation of a single clade with the genus *Flammeovirga* in the neighbour-joining and parsimony evolutionary trees indicated that strain Mok-1-85^T represents a novel genus in the family 'Flammeovirgaceae'. This phylogenetic conclusion was also supported by the fatty acid profile and other phenotypic characteristics of the novel strain (Table 1). Therefore, strain Mok-1-85^T is proposed as a member of a new genus and novel species, *Sediminitomix flava* gen. nov., sp. nov.

Description of *Sediminitomix* gen. nov.

Sediminitomix (Se.di.mi.ni.to'mix. L. n. *sedimen* -inis sediment; L. fem. n. *tomix* a thread; N.L. fem. n. *Sediminitomix* a thread isolated from sediment).

Gram-negative, non-motile, long rods (10–35 µm long and 0.5–0.7 µm in width), chemo-organotrophic, catalase- and oxidase-positive. NaCl is required for growth. Major cellular fatty acids are iso-C_{15:0}, C_{16:1}ω5c and C_{16:0} 3-OH. The main respiratory quinone is MK-7. Growth occurs at 15–40 °C, negative result in the O/F test for glucose. The

DNA G+C content is 38 mol%. The type species of the genus is *Sediminitomix flava*.

Description of *Sediminitomix flava* sp. nov.

Sediminitomix flava (fla.va. L. fem. adj. *flava* reddish yellow, the colour of the colonies).

Displays the following properties in addition to those given in the genus description. Colonies are large and orange-coloured with non-entire margins on HSMA plates. The colour of the colonies is due to saproxanthin. Growth occurs at 15–40 °C and is optimal at 25–30 °C. Growth occurs at 2–4 % (w/v) NaCl and weakly at 1 % (w/v) NaCl. Positive for the degradation of starch, gelatin, DNA, aesculin, alginate, Tweens 20, 40 and 80 and for the production of H₂S. Weakly positive result in tests for the degradation of agar. Negative in tests for the degradation of cellulose, CM-cellulose, chitin, carrageenan, urea and for indole production. Grows on 1 % (w/v) peptone agar prepared with artificial seawater, but does not grow on 1 % (w/v) tryptic soya agar prepared with artificial seawater or on casein plates [0.2 % (w/v) Bacto tryptone, 0.1 % (w/v) Bacto yeast extract, 1.2 % (w/v) casein and 1.5 % (w/v) agar dissolved in artificial seawater]. Sensitive to treatment at 55 °C for 10 min. Nitrate is reduced to nitrite, but nitrite is not reduced to N₂. Positive result in tests for β-glucosidase and β-galactosidase. Positive result for alkaline phosphatase, acid phosphatase, leucine arylamidase and valine arylamidase activities in the API ZYM test. Acids are produced from D-xylose, galactose, glucose, amygdalin, aesculin, cellobiose, starch, glycogen, gentiobiose and 2-ketoglucuronate in API 50CH tests. The DNA G+C content of the type strain is 38 mol%.

The type strain, Mok-1-85^T (=NBRC 101625^T=KCTC 12970^T=CIP 109411^T), was isolated from marine sediment from Okinawa Island, Japan.

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