Actibacter sediminis gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from tidal flat sediment

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A yellow-pigmented, Gram-negative, aerobic bacterial strain comprising rod-shaped cells devoid of flagellar and gliding motility, designated strain JC2129^T, was isolated from tidal flat sediment of Dongmak on Ganghwa Island, South Korea. Results from a 16S rRNA gene sequence analysis indicated that the isolate belonged to the family *Flavobacteriaceae*; the highest level of nucleotide sequence similarity (91.9 %) occurred with *Polaribacter dokdonensis* DSW-5^T. The predominant cellular fatty acids were iso-C_{15:0} (19.8 %), iso-C_{15:1} G (14.0 %), iso-C_{17:0} 3-OH (13.7 %) and iso-C_{13:0} (6.4 %). Flexirubin-type pigments were absent. The major isoprenoid quinone was MK-6. The DNA G + C content was 43–45 mol%. Data from a polyphasic taxonomy study suggested that the isolate represents a novel genus and species in the family *Flavobacteriaceae*, for which the name *Actibacter sediminis* gen. nov., sp. nov. is proposed. The type strain of *Actibacter sediminis* is JC2129^T (=KCTC 12704^T =JCM 14002^T).

Tidal flats in Korea have been known to harbour a highly diverse prokaryotic community, as shown by both culture-dependent and culture-independent surveys (Kim *et al.*, 2004, 2005; Yi & Chun, 2006). In this study, we report the description of a novel member of this bacterial community, a *Flavobacterium*-like bacterium that showed low levels of 16S rRNA gene sequence similarity with respect to members of the family *Flavobacteriaceae* with validly published names.

A bacterial strain, designated JC2129^T, was isolated from a sample of tidal flat sediment from Ganghwa Island, South Korea (37° 36' 22.3" N 126° 22' 59.4" E), using the standard dilution plating method on marine agar 2216 (MA; Conda). The isolate was routinely cultured on MA and maintained at –80 °C as a suspension in marine broth (MB; Conda) supplemented with 20 % (v/v) glycerol.

The 16S rRNA gene was amplified enzymically from a single colony by means of a PCR using AccuPower PCR Premix (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified using an AccuPrep PCR purification kit (Bioneer) and the sequencing of the

16S rRNA gene was performed with an Applied Biosystems ABI3730XL automatic sequencer at Macrogen Corp. (Seoul, South Korea). The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The initial similarity analyses indicated that our isolate belonged to the family *Flavobacteriaceae*. The almost-complete 16S rRNA gene sequence of strain JC2129^T (1374 bp) was aligned manually against those of representatives of the family *Flavobacteriaceae* using the bacterial 16S rRNA secondary structure model and the jPHYDIT program (Jeon *et al.*, 2005). The phylogenetic tree was inferred by using the neighbour-joining method (Saitou & Nei, 1987) on the basis of distance matrix data. Evolutionary distance matrices for the neighbour-joining method were generated according to the model of Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Phylogenetic analyses were carried out using the MEGA3 (Kumar *et al.*, 2004) and PHYLIP (Felsenstein, 2005) programs.

On the basis of the 16S rRNA gene sequence analyses, strain JC2129^T showed low levels of similarity with respect to known species of the family *Flavobacteriaceae*. The highest level of sequence similarity was found with respect

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the strain JC2129^T is EF670651.

A transmission electron micrograph of a negatively stained cell of strain JC2129^T is shown as a supplementary figure available with the online version of this paper.

to *Polaribacter dokdonensis* DSW-5^T (91.9%), followed by *Tenacibaculum litoreum* CL-TF13^T (91.9%) and *Lutibacter litoralis* CL-TF09^T (91.6%). Phylogenetic treeing (Fig. 1) also confirmed that the tidal flat isolate JC2129^T is a member of the family *Flavobacteriaceae* but distantly associated with the aforementioned genera. The tree suggested that strain JC2129^T formed a monophyletic clade with the genera *Lutibacter*, *Polaribacter* and *Tenacibaculum*, with 99% bootstrap support.

The cellular morphology was examined using transmission electron microscopy, after growth of strain JC2129^T on MA at 30 °C for 2 days. Gliding motility was investigated by examining the edge of a hanging drop of a fresh MB culture, as recommended by Bernardet *et al.* (2002). Anaerobic growth was evaluated on MA in an anaerobic-chamber system (Coy Laboratory Products). The pH range (3–10, using increments of 1 pH unit) for growth was determined using MB; the final pH was adjusted with NaOH and HCl solutions after autoclaving. The requirements for NaCl (0–10%, using increments of 1%, w/v) and sea salts (0, 1, 3, 5, 7, 10, 15 and 20%, w/v; Sigma) were tested in R2A medium (Conda). The temperature range for growth was determined on MA at 5–50 °C (using increments of 5 °C). Hydrolysis of casein, starch and Tween 80 was tested using MA, as described by Smibert & Krieg (1994). Chitin hydrolysis was tested on chitin agar as described by Hsu & Lockwood (1975). Cellulase activity was tested by observing the disintegration of filter paper in an MB culture (Smibert & Krieg, 1994; Bernardet *et al.*, 2002). DNase test agar (Difco) supplemented with 2.5% (w/v) NaCl was used for a DNase assay. H₂S production was detected in triple-sugar-iron agar supplemented with 2.5% (w/v) NaCl. Arginine dihydrolase, β -galactosidase,

nitrate reduction, urease, acid production from glucose and indole-production tests were performed using an API 20NE kit (bioMérieux) according to the manufacturer's instructions; other enzymic activities were determined using an API ZYM kit (bioMérieux). Kits were inoculated with a heavy bacterial suspension in half-strength artificial seawater (24 g NaCl, 5.1 g MgCl₂, 4 g Na₂SO₄, 1.1 g CaCl₂, 0.7 g KCl, 0.2 g NaHCO₃, 0.1 g KBr, 0.027 g H₃BO₃, 0.024 g SrCl₂ and 0.003 g NaF per litre distilled water; Lyman & Fleming, 1940) and AUX media (bioMérieux) supplemented with 2.5% (w/v) NaCl. Carbon-source utilization was tested by incubating strain JC2129^T at 37 °C for 2 weeks on basal agar medium supplemented with yeast extract (0.64 g KCl, 23.6 g NaCl, 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 1.3 g CaCl₂·2H₂O, 0.2 g NH₄Cl, 0.2 g NaNO₃, 15 g Bacto agar and 0.05 g yeast extract per litre distilled water; Choi & Cho, 2006) containing 0.2% carbon source.

A transmission electron micrograph of strain JC2129^T is available as Supplementary Fig. S1 in IJSEM Online. Its biochemical and physiological properties are presented in Table 1 and in the genus and species descriptions.

Chemotaxonomic characteristics were determined from cells grown at 30 °C for 2–3 days on MA or in MB. Analysis of the fatty acid methyl esters was carried out by GLC according to the instructions of the Microbial Identification system (MIDI). Isoprenoid quinones were isolated by using the method of Minnikin *et al.* (1984) and were analysed by HPLC (Varian) as described by Collins (1985). The DNA G+C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). Experiments were performed in triplicate.

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain JC2129^T and representative members of the family *Flavobacteriaceae*. Bootstrap percentages greater than 50% (based on neighbour-joining analyses of 1000 resampled datasets) are shown at nodes. *Flexibacter flexilis* ATCC 23079^T (M62794) (not shown) was used as an outgroup. Bar, 0.05 nucleotide substitutions per position.

Flexirubin-type pigments were sought by using the KOH test as described by Bernardet *et al.* (2002).

The cellular fatty acid profiles of strain JC2129^T and related members of the family *Flavobacteriaceae* are shown in Table 2. The major respiratory quinone was MK-6, but a rather large amount of MK-7 was also found (MK-7/MK-6 ratio, 1:2.8). Flexirubin-type pigments were not detected. The DNA G + C content of strain JC2129^T was found to be 43–45 mol%.

Strain JC2129^T exhibited low levels of 16S rRNA gene sequence similarity (<92%) with respect to all recognized species of the family *Flavobacteriaceae*, and formed a distinct phyletic line. In addition, several phenotypic properties readily served to differentiate the isolate from its phylogenetic neighbours (Table 1). On the basis of the data from this polyphasic study, strain JC2129^T represents a novel genus and species of the family *Flavobacteriaceae*, for which the name *Actibacter sediminis* gen. nov., sp. nov. is proposed.

Description of *Actibacter* gen. nov.

Actibacter (Ac.ti.bac'ter. L. n. *acta* seaside; N.L. masc. n. *bacter* rod; N.L. masc. n. *Actibacter* rod from the seaside).

Cells are rod-shaped with rounded ends, non-flagellated and non-gliding. Gram-negative. Aerobic, chemoheterotrophic and mesophilic. Oxidase- and catalase-positive. Spores are not formed. Flexirubin-type pigments are absent. The major isoprenoid quinone is MK-6. The predominant cellular fatty acids are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and iso-C_{13:0}. As determined by 16S rRNA gene sequence analysis, the genus *Actibacter* is a member of the family *Flavobacteriaceae*. The type species is *Actibacter sediminis*.

Description of *Actibacter sediminis* sp. nov.

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

Table 1. Differential characteristics of strain JC2129^T and related organisms in the family *Flavobacteriaceae*

Taxa: 1, strain JC2129^T; 2, *Polaribacter* (data for six species); 3, *Tenacibaculum* (nine species); 4, *Lutibacter litoralis*. Data are from Gosink *et al.* (1998), Suzuki *et al.* (2001), Frette *et al.* (2004), Yoon *et al.* (2005), Choi *et al.* (2006), Choi & Cho (2006) and this study. +, Positive; –, negative; v, variable among species; NA, not available.

Characteristic	1	2	3	4
Isolation source(s)	Tidal flat	Seawater or saprophytic	Seawater, tidal flat, saprophytic or parasitic	Tidal flat
Gliding motility	–	–	v*	–
Production of flexirubin-type pigments	–	v	v	–
Sea-salt requirement	–	v	v	+
Growth at:				
pH 6	+	NA	+	–
25 °C	+	–	+	+
37 °C	+	–	v	–
Hydrolysis of:				
Starch	+	+	v	+
Gelatin	+	v	+†	+
Aesculin	+	v	–	+
Nitrate reduction	–	v‡	v	–
Oxidase activity	+	v	+	–
Utilization of:				
Citrate	+	–	v	+
Glycerol	–	v	–§	–
L-Leucine	–	–	v	+
Pyruvate	+	–	–§	+
Succinate	–	–	–§	+
Sucrose	+	v	v	–
L-Proline	–	–	v	–
L-Glutamate	+	v	v	–
DNA G + C content (mol%)	43–45	31–33	29–33	33.9

*All species except *Tenacibaculum skagerrakense* are positive for this characteristic.

†Data not available for *T. skagerrakense*.

‡All species except *Polaribacter glomeratus* are negative for this characteristic.

§Data not available for *Tenacibaculum lutimaris*.

Table 2. Cellular fatty acid compositions of strain JC2129^T and type strains of related organisms in the family *Flavobacteriaceae*

Strains: 1, JC2129^T; 2, *Polaribacter filamentus* 215^T (data from Gosink *et al.*, 1998); 3, *Tenacibaculum maritimum* JCM 8137^T (Yoon *et al.*, 2005); 4, *L. litoralis* JCM 13034^T (Choi & Cho, 2006). Strain JC2129^T, *T. maritimum* JCM 8137^T and *L. litoralis* JCM 13034^T were grown on MA at 30 °C for 1–2 days, whereas *P. filamentus* 215^T was grown on seawater–cytrophaga medium at 5 °C for 3–4 weeks. Values are percentages of total fatty acids; components amounting to less than 1% of the total fatty acids in all strains are not included. –, Not detected/not reported.

Fatty acid	1	2	3	4
iso-C _{13:0}	6.4	5	1.8	–
iso-C _{15:0}	19.8	22	16.8	16.7
iso-C _{15:1} G	14.0	12	–	4.2
iso-C _{16:0}	1.2	–	0.3	–
anteiso-C _{15:1} A	1.2	–	–	1.6
anteiso-C _{15:0}	3.7	6	0.8	15.1
C _{15:1} ω6c	5.2	9	2.2	1.5
C _{17:1} ω6c	2.3	–	0.3	1.0
C _{17:1} ω8c	1.7	–	–	–
C _{15:0} 2-OH	2.2	–	1.1	2.1
C _{15:0} 3-OH	2.1	2	3.8	1.5
C _{17:0} 2-OH	1.6	–	–	–
C _{17:0} 3-OH	2.9	–	0.6	–
iso-C _{16:0} 3-OH	1.2	–	5.0	13.4
iso-C _{17:0} 3-OH	13.7	–	13.7	3.9
iso-C _{17:1} ω9c	3.7	–	–	–
Summed feature 3*	4.8	–	17.9	1.0

*Summed features represent groups of fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprised iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

Cells are 0.7–0.9 µm wide and 2.4–3.2 µm long. Colonies on MA are circular, smooth, convex, entire and yellow-pigmented. Growth occurs at 5–45 °C (optimum, 37 °C), at pH 5–8 (optimum, pH 6) and in the presence of 1–10% NaCl (optimum, 1–3%) or 1–15% sea salts (optimum, 1%). Growth also occurs on R2A medium in the absence of NaCl and sea salt. Nitrate is not reduced to nitrite. Indole and H₂S are not produced. Aesculin, gelatin, starch and Tween 80 are degraded, but DNA, agar, casein, crystalline cellulose (filter paper) and chitin are not. Alkaline phosphatase, esterase (C4), esterase lipase (C8), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present, but lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-fucosidase and α-mannosidase activities are absent. Acid is not produced from glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate or phenylacetate. Growth occurs on peptone, tryptone and yeast extract.

Acetate, citrate, pyruvate, sucrose and L-glutamate are utilized. Glycerol, L-leucine, L-proline, succinate, benzoate, L-proline and *p*-toluic acid are not utilized. The predominant cellular fatty acids are iso-C_{15:0} (19.8%), iso-C_{15:1} G (14.0%), iso-C_{17:0} 3-OH (13.7%) and iso-C_{13:0} (6.4%). The DNA G + C content is 43–45 mol%.

The type strain, JC2129^T (=KCTC 12704^T =JCM 14002^T), was isolated from sediment of getbol (a Korean word for tidal flat) on Ganghwa Island, South Korea.

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