Sediminicola luteus gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae*

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The taxonomic position of four Gram-negative, rod-shaped, golden-yellow-coloured bacteria isolated from marine sediments was determined. Analysis of the almost complete 16S rRNA gene sequences indicated that these isolates belong to the family *Flavobacteriaceae*. An unclassified bacterium, NBRC 15975, was found to be the closest relative, showing 16S rRNA gene sequence similarity of 93%; other related genera shared only 87.9–90.5% similarity. In contrast, the four isolates shared high levels of 16S rRNA gene sequence similarity (99.3–99.7%) and high DNA–DNA reassociation values (93–104%). The isolates could be differentiated phenotypically from other genera by the abilities to reduce nitrate and to degrade gelatin, casein and starch. The only respiratory quinone was MK-6, and the major fatty acids were iso-C_{15:0}, iso-C_{15:1}, anteiso-C_{15:0}, iso-C_{17:1}ω9c and iso-C_{17:0} 3-OH. The DNA G + C content was 38–40 mol%. Differentiating phenotypic characteristics and large phylogenetic distances between the isolates and previously published genera indicated that the isolates constitute a novel genus, for which the name *Sediminicola* gen. nov. is proposed. The type species is *Sediminicola luteus* sp. nov. (type strain CNI-3^T = NBRC 100966^T = LMG 23246^T).

The family *Flavobacteriaceae*, proposed 20 years ago (Jooste, 1985), is one of the fastest growing families: more than half of the genera have been established during the last 3 years. Members of this family are widely distributed in diverse habitats (Bernardet *et al.*, 2002), including marine environments [e.g. the genera *Aquimarina*, *Gramella*, *Polaribacter*, *Psychroflexus* and *Gelidibacter* (Bernardet *et al.*, 2002; Nedashkovskaya *et al.*, 2005a, b)] in which they may be numerically dominant (Glöckner *et al.*, 1999).

In this study, we report the isolation and characterization of four novel strains from sediments collected on the shores of the Pacific Ocean and the Sea of Japan. On the basis of phylogenetic analyses of these strains, a novel genus of the family *Flavobacteriaceae*, *Sediminicola* gen. nov., is proposed.

Strains CNI-3^T (= NBRC 100966^T = LMG 23246^T), CNI-1-5 (= NBRC 100968) and MNI-85 (= NBRC 100969) were isolated from sediments at Nigata on the shore of the Sea of Japan, whereas strain PMAOS-27 (= NBRC 100967) was isolated at Odawara on the shore of the Pacific Ocean. For routine cultivation and maintenance, marine agar 2216 (MA; Difco) at 20 °C was used; 20% (v/v) glycerol in artificial sea water at –80 °C was used for long-term preservation. Cultures grown for 3 days on MA were used to observe colony shape and colour. Cells grown on MA were observed

under a light microscope equipped with a digital camera (CX41LF; Olympus). Gliding motility was observed by using the hanging drop method (Perry, 1973) under a × 1000 oil-immersion objective. LB medium [2 g Bacto tryptone (Difco) and 1 g Bacto yeast extract (Difco) in 1000 ml water] supplemented with 1, 3, 5, 7 or 10% (w/v) NaCl or with 10, 30, 50 or 70% (v/v) artificial sea water (Naigai Chemicals) was used to test the ability of the bacteria to grow at different salt concentrations. Tolerance of high salt concentrations was also tested in marine broth 2216 (MB; Difco) with a final NaCl concentration adjusted to 5, 8 or 10%. Growth at 4, 10, 20, 30, 37, 40 and 45 °C was assessed on MA and in LBM (LB prepared with artificial sea water). The bathochromic shift test of McCammon & Bowman (2000) was performed to check for the presence of flexirubin-type pigments. For the detection of carotenoid pigments, absorption spectra of acetone extracts from 3-day-old cultures were analysed using a Shimadzu UV-visible spectrophotometer (UV-1650 PC). The production of catalase was assessed by flooding the cultures on agar plates with 3% (v/v) H₂O₂. Cells suspended in water were spotted onto a cytochrome oxidase strip (Nissui Pharmaceuticals), and a change in colour from white to blue was taken as a positive result for the production of oxidase. The degradation of agar and carrageenan (Type I; Sigma) was assessed on MB solidified with 1.5% (w/v) of each polymer, while hydrolysis of carboxymethylcellulose (high viscosity; Sigma) was tested on MB solidified with 3% (w/v) carboxymethylcellulose sodium salt. The degradation of

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CNI-1-5, CNI-3^T, MNI-85 and PMAOS-27 are AB206956, AB206957, AB206958 and AB206959, respectively.

crystalline cellulose was assessed by cultivating the bacteria in 1/5-strength LBM medium containing Whatman (No. 1) paper strips at 20 °C for 1 month. The oxidation of different carbon sources was tested using Biolog GN2 Microplates (Biolog) according to Rüger & Krambeck (1994). The Sherlock Microbial Identification System (MIDI) was used for fatty acid methyl ester analysis. Isoprenoid quinones were extracted and analysed by using the method of Nakagawa & Yamasato (1993). Nitrate reduction, indole production from tryptophan, acid production from glucose and hydrolysis of urea, aesculin and gelatin were tested using API 20 NE strips (bioMérieux) according to the instructions of the supplier, except that inocula were prepared in artificial sea water. The degradation of starch, chitin, casein, gelatin and DNA was assessed by using the protocols of Cowan & Steel (1993). The 16S rRNA gene was amplified by using the set of universal primers 27f and 1492r (Brosius *et al.*, 1978), and a DNA template prepared by using InstaGene matrix (Bio-Rad). PCR products purified using a QIAquick® (Qiagen) kit were sequenced using an ABI BigDye Terminator v1.1 Cycle Sequencing kit and an ABI PRISM 3100 genetic analyser (Applied Biosystems). Sequences were edited and assembled

using the ATGC program (Genetyx) and were compared with 16S rRNA gene sequences from public databases by using the BLAST program (Altschul *et al.*, 1990). Related sequences were aligned using the CLUSTAL X program (Thompson *et al.*, 1997). A phylogenetic tree was inferred using the neighbour-joining method (Saitou & Nei, 1987) and the topology was evaluated by using a bootstrap resampling analysis of 1000 replicates (Felsenstein, 1985). DNA was extracted from cells grown to late exponential growth phase by using the protocol of Minamisawa (1990). The fluorometric hybridization method of Ezaki *et al.* (1989) was used to determine the DNA–DNA reassociation values at a hybridization temperature of 50 °C in 25 % formamide. The DNA G + C content was determined using the HPLC method of Mesbah *et al.* (1989).

The almost complete 16S rRNA gene sequences (1440 bp) of the isolates showed the highest level of similarity (93 %) with an unclassified bacterium, NBRC 15957. Other valid genera in the family *Flavobacteriaceae* shared rather low levels of sequence similarity (87.9–90.5 %) with the isolates. In contrast, the novel isolates had high levels of 16S rRNA gene

Fig. 1. Rooted tree, based on 16S rRNA gene sequences, showing the position of the novel isolates in the family *Flavobacteriaceae*. The tree was generated by using the neighbour-joining method (Saitou & Nei, 1987); bootstrap values indicated at branches were calculated from 1000 resamplings (Felsenstein, 1985). Values below 500 are not shown. Bar, 0.02 K_{nuc} . *Weeksella virosa* was used as an outgroup.

sequence similarity (99.3–99.7 %) and high DNA–DNA reassociation values (93–104 %) with each other. Phylogenetic analysis of the 16S rRNA gene sequences revealed that the four isolates formed a distinct lineage within the family *Flavobacteriaceae* (Fig. 1). These data suggest that the novel monophyletic clade constitutes a novel genus in the family *Flavobacteriaceae*.

The phenotypic and biochemical features of the isolates are given in the genus and species descriptions and in Table 1. The following fatty acids were detected in all of the strains (percentage values in the type strain CNI-3^T are shown in parentheses, and fatty acids amounting to less than 1 % are not listed): i-C_{15:0} (12 %), i-C_{17:0} 3-OH (12 %), a-C_{15:0} (11 %), i-C_{15:1} (11 %), summed feature 3 (C_{16:1}ω7*c*/i-C_{15:0} 2-OH) (14 %), i-C_{17:1}ω9*c* (9 %), i-C_{16:0} (5 %), i-C_{16:0} 3-OH (5 %), i-C_{16:1} (3 %), i-C_{15:0} 3-OH (4 %) and i-C_{14:0} (1 %). Pentadecanoic acid (C_{15:0}) was absent from all of the novel strains, but present in all the related genera shown in Table 1. The DNA G + C contents of the four strains were in the range 38–40 mol%.

The strains differed from other *Flavobacteriaceae* genera with validly published names in terms of a number of features listed in Table 1. On the basis of the results of 16S rRNA gene analysis and the differences in phenotypic characteristics, we propose that these strains should be classified as a novel genus of the family, for which the name *Sediminicola* gen. nov. is proposed; the type species is *Sediminicola luteus* sp. nov.

Description of *Sediminicola* gen. nov.

Sediminicola [se.di.mi.ni.co'la. L. n. *sedimen* -inis sediment; L. masc. suff. -cola (from L. n. *incola*) an inhabitant; N.L. masc. n. *Sediminicola* an inhabitant of sediment, referring to the source of the strains].

Gram-negative, aerobic, rod-shaped, non-motile cells. Produces golden-yellow-coloured carotenoid pigments. Flexirubin-type pigments are not produced. Predominant cellular fatty acids are i-C_{15:0}, a-C_{15:0}, i-C_{15:1}, i-C_{17:1}ω9*c*, i-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7*c*/i-C_{15:0} 2-OH). Sequence analysis of 16S rRNA genes shows that *Sediminicola* is a member of the family *Flavobacteriaceae*. The DNA G + C content of the type species is in the range 38–40 mol%. The type species is *Sediminicola luteus*.

Description of *Sediminicola luteus* sp. nov.

Sediminicola luteus (lu.te'us. L. masc. adj. *luteus* golden yellow, because the colony colour is golden yellow).

In addition to possessing the characteristics given in the description of the genus, the cells are 0.5–0.7 μm in width and 3–5 μm in length; 7-day-old cells form longer filaments of 5–10 μm. Growth occurs at 10–30 °C; the optimal growth temperature is 20 °C. Growth occurs in 10–100 % strength artificial sea water. NaCl alone is not sufficient to support growth. Positive for catalase and weakly positive for oxidase. Positive for degradation of gelatin, casein and starch and for nitrate reduction. Urea, chitin, cellulose, CM-cellulose

Table 1. Differentiating phenotypic characteristics for the novel isolates and other related genera

Genera: 1, *Sediminicola* gen. nov. (this study); 2, *Robiginitalea* (Cho & Giovannoni, 2004); 3, *Arenibacter* (Nedashkovskaya *et al.*, 2004a); 4, *Muricauda* (Yoon *et al.*, 2005); 5, *Vitellibacter* (Nedashkovskaya *et al.*, 2003); 6, *Maribacter* (Nedashkovskaya *et al.*, 2004b); 7, *Zobellia* (Nedashkovskaya *et al.*, 2004c); 8, *Pibocella* (Nedashkovskaya *et al.*, 2005c); 9, *Ulvibacter* (Nedashkovskaya *et al.*, 2004d). Abbreviations: Y, yellow; O, orange; Y–O, yellow to orange; ND, not determined; +, positive; –, negative; w, weak; v, variable.

Characteristics	1	2	3	4	5	6	7	8	9
Pigment (colour/flexirubin)	Y/–	O/–	O/–	Y/ND	Y–O/+	Y–O/–	v/+	Y–O/–	Y–O/+
Gliding motility	–	–	–	–	–	+	+	+	–
Catalase/oxidase	+/w	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Hydrolysis of:									
Gelatin	+	–	v	–	+	v	+	+	+
Casein	+	–	–	–	+	–	v	+	–
DNA	–	–	v	ND	+	v	v	–	–
Starch	+	+	–	–	–	v	v	+	–
Urease activity	–	–	v	–	–	–	–	–	–
Nitrate reduction	+	–	+	–	–	v	+	–	+
Growth with/at:									
8–10 % NaCl	–	+	v	+	–	–	v	+	–
4 °C	–	–	–	–	+	+	v	+	+
37 °C	–	+	+	+	+	–	v	–	–
40–45 °C	–	+	v	v	+	–	v	–	–
Pentadecanoic acid (C _{15:0})	–	+	+	+	+	+	+	+	+
DNA G + C content (mol%)	38–40	55–56	37–40	41–45	41	35–39	36–43	35	36–38

and DNA are not depolymerized. Indole is not produced from tryptophan. Acid is not produced from glucose, α -Cyclodextrin, dextrin, glycogen, cellobiose, D-fructose, D-galactose, gentiobiose, α -D-glucose, α -D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β -D-glucoside, raffinose, sucrose, trehalose, turanose, DL-lactic acid, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-ornithine, L-proline, L-threonine and glucose 1-phosphate are oxidized. Tweens 40 and 80, *N*-acetyl-D-galactosamine, *N*-acetyl D-glucosamine, adonitol, D-arabitol, i-erythritol, L-fucose, *myo*-inositol, L-rhamnose, D-sorbitol, xylitol, methyl pyruvic acid, mono-methyl succinic acid, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -, β - and γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-histidine, hydroxy-L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, DL-carnitine, γ -aminobutyric acid, inosine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate and glucose 6-phosphate are not oxidized. The DNA G + C content of the type strain is 38 mol%.

The type strain, CNI-3^T (=NBRC 100966^T = LMG 23246^T), was isolated from marine sediment on the shore of the Sea of Japan.

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References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- 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.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Cho, J.-C. & Giovannoni, S. J. (2004). *Robiginitalea biformata* gen. nov., sp. nov., a novel marine bacterium in the family *Flavobacteriaceae* with a higher G+C content. *Int J Syst Evol Microbiol* **54**, 1101–1106.
- Cowan, S. T. & Steel, K. J. (1993). *Manual for the Identification of Medical Bacteria*, 3rd edn. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Glöckner, F. O., Fuchs, B. M. & Amann, R. (1999). Bacterio-plankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl Environ Microbiol* **65**, 3721–3726.
- Jooste, P. J. (1985). *The taxonomy and significance of Flavobacterium–Cytophaga strains from dairy sources*. PhD thesis, University of the Orange Free State, South Africa.
- McCammon, S. A. & Bowman, J. P. (2000). Taxonomy of Antarctic *Flavobacterium* species: description of *Flavobacterium gillisiae* sp. nov., *Flavobacterium tegetincola* sp. nov., and *Flavobacterium xanthum* sp. nov., nom. rev. and reclassification of [*Flavobacterium*] *salegens* as *Salegentibacter salegens* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 1055–1063.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minamisawa, K. (1990). Division of rhizobitoxine-producing and hydrogen-uptake positive strains of *Bradyrhizobium japonicum* by *nifDKE* sequence divergence. *Plant Cell Physiol* **31**, 81–89.
- Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Vitellibacter vladivostokensis* gen. nov., sp. nov., a new member of the phylum *Cytophaga–Flavobacterium–Bacteroides*. *Int J Syst Evol Microbiol* **53**, 1281–1286.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, M., Mikhailov, V. V. & Bae, S. K. (2004a). *Arenibacter certesii* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 1173–1176.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K. & 7 other authors (2004b). *Maribacter* gen. nov., a new member of the family *Flavobacteriaceae*, isolated from marine habitats, containing the species *Maribacter sedimenticola* sp. nov., *Maribacter aquivivus* sp. nov., *Maribacter orientalis* sp. nov. and *Maribacter ulvicola* sp. nov. *Int J Syst Evol Microbiol* **54**, 1017–1023.
- Nedashkovskaya, O. I., Suzuki, M., Vancanneyt, M., Cleenwerck, I., Lysenko, A. M., Mikhailov, V. V. & Swings, J. (2004c). *Zobellia amurskyensis* sp. nov., *Zobellia laminariae* sp. nov. and *Zobellia russellii* sp. nov., novel marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **54**, 1643–1648.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Rhee, M. S., Lysenko, A. M., Falsen, E., Frolova, G. M., Mikhailov, V. V. & Bae, S. K. (2004d). *Ulvibacter litoralis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 119–123.
- Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Frolova, G. M., Mikhailov, V. V., Lee, K. H. & Bae, S. K. (2005a). Description of *Aquimarina muelleri* gen. nov., sp. nov., and proposal of the reclassification of [*Cytophaga*] *latercula* Lewin 1969 as *Stanierella latercula* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **55**, 225–229.
- Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Frolova, G. M., Mikhailov, V. V., Bae, S. K., Lee, D. H. & Kim, I. S. (2005b). *Gramella echinicola* gen. nov., sp. nov., a novel halophilic bacterium of the family *Flavobacteriaceae* isolated from the sea urchin *Strongylocentrotus intermedius*. *Int J Syst Evol Microbiol* **55**, 391–394.
- Nedashkovskaya, O. I., Kim, S. B., Lee, K. H., Bae, S. K., Frolova, G. M., Mikhailov, V. V. & Kim, I. S. (2005c). *Pibocella ponti* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae*

isolated from the green alga *Acrosiphonia sonderi*. *Int J Syst Evol Microbiol* **55**, 177–181.

Perry, L. B. (1973). Gliding motility in some non-spreading flexibacteria. *J Appl Bacteriol* **36**, 227–232.

Rüger, H.-J. & Krambeck, H.-J. (1994). Evaluation of the BIOLOG substrate metabolism system for classification of marine bacteria. *Syst Appl Microbiol* **17**, 281–288.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Yoon, H.-J., Li, M.-H., Oh, T.-K. & Park, Y.-H. (2005). *Muricauda flavescens* sp. nov. and *Muricauda aquimarina* sp. nov., isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea, and emended description of the genus *Muricauda*. *Int J Syst Evol Microbiol* **55**, 1015–1019.