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-3T (=NBRC 100966T=LMG 23246T), 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
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 Rueger & 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 typtophan, 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 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 (87–90–95%) 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.](image-url)
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-3T 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}ω7c/i-C_{15:0} 2-OH) (14 %), i-C_{17:1}ω9c (9 %), i-C_{16:0} (5 %), i-C_{16:1} 3-OH (5 %), i-C_{16:1}ω7c (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.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tbody>
<tr>
<td>Pigment (colour/flexirubin)</td>
<td>Y/−</td>
<td>O/−</td>
<td>O/−</td>
<td>Y/Nd</td>
<td>Y−O/+</td>
<td>Y−O/−</td>
<td>Y+/−</td>
<td>Y−O/−</td>
<td>Y−O/+</td>
</tr>
<tr>
<td>Gilding motility</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Catalase/oxidase</td>
<td>+/W</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
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<td>+/+</td>
<td>+/+</td>
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<td>Hydrolysis of:</td>
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<td>V</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Casein</td>
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<td>−</td>
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<td>+</td>
<td>−</td>
<td>V</td>
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<td>+</td>
</tr>
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<td>−</td>
<td>V</td>
<td>ND</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>−</td>
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<tr>
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<td>+</td>
<td>−</td>
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<td>−</td>
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<td>V</td>
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<td>Urease activity</td>
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<td>−</td>
<td>V</td>
<td>−</td>
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<td>−</td>
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<td>−</td>
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<td>Nitrate reduction</td>
<td>+</td>
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<td>+</td>
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<td>−</td>
<td>V</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>Growth with/at:</td>
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<td>8–10 % NaCl</td>
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<td>V</td>
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<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
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</tr>
<tr>
<td>4 °C</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
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<td>37 °C</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>40–45 °C</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Pentadecanoic acid (C_{15:0})</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>38–40</td>
<td>55–56</td>
<td>37–40</td>
<td>41–45</td>
<td>41</td>
<td>35–39</td>
<td>36–43</td>
<td>35</td>
<td>36–38</td>
</tr>
</tbody>
</table>

**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}ω9c, i-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c/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...
and DNA are not depolymerized. Indole is not produced from tryptophan. Acid is not produced from glucose. α-Cyclodextrin, dextrin, glycogen, cellulobiose, 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-ascorbic acid, l-glutamic acid, glycyl L-ascorbic acid, glycol L-glutamic acid, L-ornithine, L-proline, L-threonine and glucose 1-phosphate are oxidized. Tewens 40 and 80, α-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-galactonate acid lactone, D-galacturonic acid, β-glucuronic acid, D-glucuronic acid, D-gluconic acid, D-glucosamine acid, D-glucuronic acid, α-β- and γ-hydroxybutyric acid, p-hydroxyphenylactic acid, itaconic acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, glycerol, DL-glycerol phosphate and glucose 6-phosphate are not oxidized. Acid is not produced from glucose. Acid is not produced from glucose. Acid is not produced from glucose. Acid is not produced from glucose.

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Acknowledgements

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References


