Sandarakinotalea sediminis gen. nov., sp. nov., a novel member of the family Flavobacteriaceae

Shams Tabrez Khan, Yasuyoshi Nakagawa and Shigeaki Harayama

The family Flavobacteriaceae currently accommodates around 43 different genera and hence is one of the major families of the phylum Bacteroidetes (the Cytophaga–Flavobacterium–Bacteroides group). Although the family is mostly composed of halophilic and/or mesophilic bacteria, it also includes halotolerant members, exemplified by Salegentibacter salegens, and psychrophilic members, such as Psychroflexus torquis and Psychroserpens burtonensis (Bowman et al., 1998; McCammon & Bowman, 2000). In this paper, we report the characterization of four isolates that form a monophyletic and distinct cluster in a phylogenetic tree based on 16S rRNA gene sequences. Polyphasic taxonomic analyses demonstrated that these strains belong to a novel genus and species of the family Flavobacteriaceae.

Strains CKA-5T (＝NBRC 100970T＝LMG 23247T), CKA-11 (＝NBRC 100971) and CKA-36 (＝NBRC 100973) were isolated from a marine-sediment sample collected at the city of Katsuura, while strain CTO-75 (＝NBRC 100972) was isolated from a marine-sediment sample collected at the city of Toyohashi. The strains were maintained at 20 °C on 1-2 % (w/v) agar plates or slants made with half-strength Bacto marine agar 2216 (Difco) diluted in artificial sea water (Naigai Chemicals) unless otherwise stated. For long-term preservation, cells were stored at −80 °C in artificial sea water supplemented with 20 % (v/v) glycerol.

Colony colour and shape were examined using 3-day-old cells grown on half-strength Bacto marine agar. Cell morphology was observed under an Olympus light microscope (CX41LF) without staining. Gliding motility of bacterial cells grown on half-strength Bacto marine agar was observed by using the hanging drop method under a ×1000 oil-immersion objective (Perry, 1973). The absorption spectra of carotenoids in acetone extracts of cells were determined between 260 and 700 nm using a Shimadzu UV-visible spectrophotometer (UV-1650 PC). The bathochromic shift test with 20 % KOH (w/v) was performed to detect flexirubin-type pigments, as described by McCammon & Bowman (2000).

Bacterial growth at different temperatures was checked in half-strength Bacto marine broth 2216 (Difco) and on Bacto marine agar at 4, 10, 20, 30, 37, 40 and 45 °C. Growth at different salt concentrations was checked by growing the strains in 1/5-strength LB [2 g Bacto tryptone (Difco) and 1 g Bacto yeast extract (Difco) dissolved in 1000 ml water] supplemented with different concentrations (1, 3, 5, 7 and 10 %, w/v) of NaCl. Growth in 1/5-strength LB supplemented with 0, 10, 30, 50 and 70 % (v/v) artificial sea water was also checked. The inorganic nutrients required for growth were determined by growing the strains in 1/5-strength LB supplemented with NaCl (2-8 %) and one of the following:...
plates were incubated at 20°C for 1 month. Stabs of 3% (w/v) CM-cellulose (high viscosity; Sigma) in 1/5-strength LBM were used to test the liquefaction of CM-cellulose. The depolymerization of starch, gelatin, chitin, casein and DNA was tested for as described by Cowan & Steel (1993). Nitrate reduction, denitrification, indole production from tryptophan, acid production from glucose and hydrolysis of urea, aesculin and gelatin were tested by using API 20NE strips (bioMérieux) according to the manufacturer’s instructions (except that inocula were prepared in artificial sea water). Biolog GN2 microplates were used to test the utilization of 95 different carbon sources. Inocula were prepared according to Rüger & Krambeck (1994) and microplates were incubated at 20°C for 24–60 h after inoculation. Fatty acid methyl esters were analysed using the MIDI Microbial Identification system. Isoprenoid quinones were extracted and analysed by using the method of Nakagawa & Yamasato (1993).

The template for 16S rRNA gene amplification was prepared by using the InstaGene matrix (Bio-Rad). The pair of universal primers, 27f and 1492r, was used to amplify the portion of the 16S rRNA gene corresponding to positions 8–1492 in *Escherichia coli* 16S rRNA (Brosius *et al.*, 1978). Amplified fragments were sequenced directly using a BigDye Terminator version 1.1 cycle sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The ATGC program (Genetyx) was used for sequence editing and assembly. Assembled sequences were then compared with the 16S rRNA gene sequences in the DDBJ database by using BLAST (Altschul *et al.*, 1990) and aligned with related sequences using CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were inferred by using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 2000) algorithms. The topology was evaluated by bootstrap resampling analysis of 1000 replicates (Felsenstein, 1985). For G+C content determination and DNA–DNA hybridization, DNA was extracted from cells grown in Bacto marine broth to late-exponential growth phase according to the protocol of Minamisawa (1990). The fluorometric hybridization method of Ezaki *et al.* (1989) was used for quantitative DNA–DNA hybridization at 50°C in 25% (v/v) formamide. The G+C contents were determined according to the HPLC method of Mesbah *et al.* (1989).

Comparison of almost complete 16S rRNA gene sequences (1448 bp) showed that all of the strains studied (except CKA-36) shared 100% similarity, although their repetitive extragenic palindromic PCR profiles (Vinuesa *et al.*, 1998) differed (data not shown). Strain CKA-36 shared 99–9% 16S rRNA gene sequence similarity with the other three strains. The four isolates shared only 90–91% sequence similarity with their closest neighbours with validly published names, *Psychroflexus torquis* and *Algibacter lectus*, respectively, and showed 88–90% similarity with the other related genera in the family *Flavobacteriaceae* (Fig. 1). The tree topologies obtained from the neighbour-joining and maximum-parsimony algorithms were similar (not shown).

Almost identical fatty acid profiles were obtained for all the strains. The mean percentage values for the various fatty acids were as follows (fatty acids amounting to less than 1% of the total fatty acid content in all strains are not listed): i-C15:0 (27%), i-C17:0 3-OH (13%), a-C15:0 (10%), i-C16:0 (10%), i-C17:0 9c (7%), i-C16:0 3-OH (6%), summed feature 4 (C16:1ω7c/i-C15:0 2-OH) (6%), i-C16:0 (3%), i-C15:0 3-OH (3%), C17:1ω6c (3%), a-C17:1ω9c (2%), i-C14:0 (2%) and C17:0 2-OH (2%). Notably, one of the major fatty acids found in most genera within the family *Flavobacteriaceae*, pentadecenoic acid (C15:1ω7c), was not detected. The only respiratory quinone was MK-6. The DNA G+C content was in the range 35–37 mol%.

The present polyphasic study strongly suggests that the four strains studied can be classified within a novel genus of the family *Flavobacteriaceae*, for which the name *Sandarakinotalea* gen. nov. is proposed. With the exception of some differences in carbon-source utilization, no significant phenotypic differences were found among the strains. These results, along with the high DNA–DNA reassociation values among the strains (67–99%), strongly suggests that the four strains studied can be classified as a single species of the proposed genus, for which the name *Sandarakinotalea sediminis* sp. nov. is proposed. The features useful for the differentiation of the novel genus from related genera in the family *Flavobacteriaceae* are given in Table 1.

**Description of *Sandarakinotalea* gen. nov.**

*Sandarakinotalea* (San.da.rai.ko.ta.ile.a. Gr. adj. sandarakinos -e-on of orange colour; L. fem. n. tala a slender staff, a rod; N.L. fem. n. *Sandarakinotalea* an orange-coloured rod).

Aerobic, Gram-negative and rod-shaped cells. Produces light-orange-coloured, non-diffusible, carotenoid pigment. Flexirubin-type pigments are not formed. Catalase- and oxidase-positive. Growth occurs at 10–40°C; optimal temperature range for growth is 15–20°C. Sea salts are required for growth. Predominant fatty acids are i-C15:0 9c, a-C15:0 5c, i-C16:0 and i-C17:0 3-OH. The only quinone present is MK-6.
Table 1. Phenotypic characteristics that differentiate the novel isolates from related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Pigment colour/type*</td>
<td>O/C</td>
<td>O/ND</td>
<td>O/C</td>
<td>Y/ND</td>
<td>Y-O/ND</td>
<td>Y/C</td>
<td>Y/F</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>Gliding motility</td>
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<td>+</td>
<td>-</td>
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<td>+</td>
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<td>Hydrolysis of:</td>
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<td>Gelatin</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>DNA</td>
<td>+</td>
<td>-</td>
<td>V</td>
<td>-</td>
<td>V</td>
<td>ND</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Growth in/at:</td>
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<td>8–10% NaCl (w/v)</td>
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<td>V</td>
<td>+</td>
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<td>-</td>
<td>+</td>
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<td>37°C</td>
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<td>V</td>
<td>-</td>
<td>V</td>
<td>ND</td>
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<td>4°C</td>
<td>-</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Fatty acids (%)</td>
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<tr>
<td>i-C15:1</td>
<td>0</td>
<td>13</td>
<td>0–12</td>
<td>8</td>
<td>12–18</td>
<td>0</td>
<td>10–13</td>
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<tr>
<td>a-C15:1</td>
<td>0</td>
<td>0</td>
<td>13–18</td>
<td>tr</td>
<td>4</td>
<td>0</td>
<td>0</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>35–37</td>
<td>31–33</td>
<td>32–36</td>
<td>32–34</td>
<td>37–38</td>
<td>34</td>
<td>31–33</td>
</tr>
</tbody>
</table>

*O, Orange; Y, yellow; Y-O, yellow to orange; C, carotenoid; F, flexirubin.
The DNA G+C content of the type species ranges between 35 and 37 mol%. The genus is a member of the family Flavobacteriaceae. The type species is *Sandarakinotalea sediminis*.

**Description of Sandarakinotalea sediminis sp. nov.**

*Sandarakinotalea sediminis* (se.dì’mi.nis. L. gen. n. sediminis of sediment).

Shows the following characteristics in addition to those given for the genus. Cells are non-motile, 0.5–0.7 μm in width and 3–5 μm in length. Cells may form longer filaments of 5–10 μm after longer incubation (6–7 days). Colonies are circular or irregular. Growth is supported by 10, 30, 50 and 70% (v/v) artificial sea water. Growth in 1/5-strength LB requires NaCl and either potassium or magnesium salt. Starch, DNA and gelatin are depolymerized. Agar, cellulose, CM-cellulose, chitin, aesculin and urea cannot be degraded. Indole from tryptophan and acid from glucose are not produced. Nitrate is not reduced. Dextrin, xycelodextrin, glycogen, maltose, mannotol, monomethyl succinate, α-ketobutyric acid, α-ketovaleric acid, l-alanyl glycine, l-aspartic acid, l-glutamic acid, glycy1 l-aspartic acid, glycy1 l-glutamic acid, l-ornithine, l-proline, l-serine and l-threonine are oxidized. TWEEN 40 and 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adenyl, l-arabinose, D-arabitol, cellubiose, i-erythritol, D-fructose, l-fucose, D-galactose, gentiobiose, α-D-glucose, myoinositol, α-D-lactose, lactulose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, methylpyruvic 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-cellobionic acid, D-glucose, d-glucuronic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinimide acid, glucuronamide, alaminide, D-alanine, L-alanine, l-asparagine, glycy1 l-aspartic acid, glycy1 l-glutamic acid, l-histidine, hydroxy-l-proline, l-leucine, D-phenylalanine, α-pyrogulamic acid, α-serine, D-carnitine, γ-amino butyric acid, urocanic acid, uridine, thymidine, phenylalanine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, D-l-α-ketoglutaric acid, glucose l-phosphate and glucose 6-phosphate are not oxidized. Mannose is oxidized only by strain CKA-5T and inosine only by strain CKA-36. The DNA G+C content of the type strain is 37 mol%.

The type strain, CKA-5T (=NBRC 100970T =LMG 23247T), was isolated from marine sediment collected on the Pacific Ocean coast at Katsuura, Japan. Reference strains are strains CKA-11 (=NBRC 100971), CKA-36 (=NBRC 100973) and CTO-75 (=NBRC 100972), isolated from marine sediment.

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**References**


