Sinomicrobium oceani gen. nov., sp. nov., a member of the family Flavobacteriaceae isolated from marine sediment

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A marine bacterium, designated SCSIO 03483T, was isolated from a marine sediment sample collected from the Nansha Islands in the South China Sea. The strain produced roundish colonies with diffusible yellow-coloured pigment on nutrient agar medium or marine agar 2216. Optimal growth occurred in the presence of 0–4 % (w/v) NaCl, at pH 7.0 and a temperature range of 28–37°C. 16S rRNA gene sequence analysis indicated that the isolate belonged to the family Flavobacteriaceae and showed relatively high sequence similarity with Imtechella halotolerans K1T (92.7 %). Phylogenetic analysis based on nearly complete 16S rRNA gene sequences revealed that the isolate shared a lineage with members of the genera Imtechella, Joostella and Zhouia. Phospholipids were phosphatidylethanolamine, two unidentified aminolipids and three unknown polar lipids. The major respiratory quinone was MK-6 and the major fatty acids were iso-C15:0, iso-C17:0 3-OH and summed feature 3 (C16:1ω6c/C16:1ω7c). The DNA G+C content of strain SCSIO 03483T was 38.4 mol%. On the basis of phenotypic, chemotaxonomic and molecular data, strain SCSIO 03483T represents a novel species in a new genus in the family Flavobacteriaceae, for which the name Sinomicrobium oceani gen. nov., sp. nov. is proposed. The type strain of Sinomicrobium oceani is SCSIO 03483T (≡KCTC 23994T=CGMCC 1.12145T).

The family Flavobacteriaceae was established by Jooste (1985) and its description has since been subjected to repeated emendation (Bernardet et al., 1996, 2002). At the time of writing, this family encompassed 106 genera with validly published names, 28 of which have been isolated from different marine environments since 2007. According to the archives, members of the family Flavobacteriaceae represent non-spore-forming, short to moderately long rods that are Gram-negative and display growth at different temperatures. Their major cellular fatty acids are branched or hydroxy fatty acids (high levels of iso-C15:0, iso-C15:1 and iso-C17:0 3-OH) and MK-6 is the respiratory quinone. Their DNA G+C contents range from 27 to 67 mol% (Bernardet et al., 2002; Lee et al., 2008). The abundance of marine members of the family Flavobacteriaceae indicated that this family is an important group of the bacterial community in marine environments and has a specialist role in using high-molecular-mass dissolved organic matter (Kirchman, 2002; Bauer, et al., 2006). In this study, strain SCSIO 03483T was recovered from an abyssal sediment sample. Polyphasic taxonomic data from the present study indicate that the strain represents a novel species and genus of the family Flavobacteriaceae, in the phylum Bacteroidetes.
Strain SCSIO 03483T was isolated from oligotrophic medium (peptone 2 g, seawater 500 ml, distilled water 500 ml, agar 12 g, pH 7.0), after incubation at 28 °C for 7 days. The sediment sample was collected from a depth of 2548 m at 6° 0.733'N 113° 28.842'E, and was processed by using the sucrose density-gradient centrifugation method (Martin & Ames, 1961; Chang et al., 2008). Strain SCSIO 03483T was obtained from the sucrose density gradient S2 fraction. In brief, 1 ml of subsample was layered on top of 9 ml of the working solution (sucrose gradient working solution containing 70%, 50% and 30% sucrose), followed by centrifugation at 2200 r.p.m. and 4 °C for 20 min. After centrifugation, the sample was divided into five fractions (S1 0–4 cm, S2 4–7 cm, S3 7–9 cm, S4 9–10 cm, S5 mud), and each fraction of sucrose was washed by using 1× PBS buffer solution (HyClone SH30256.01B) and then centrifuged at 5000 r.p.m. (Eppendorf 5804R; 805 g) and 4 °C. The supernatant was discarded. The pellet sample was diluted with 1 ml autoclaved seawater and 50 μl sample was transferred to each isolation plate. The isolate was further cultivated on marine agar 2216 (MA; Difco) at 28 °C and maintained as glycerol suspensions (20%, w/v) at −80 °C.

Growth at different temperatures (4, 10, 28, 37, 40, 45, 50, 55 and 65 °C), pH (4.0–10.0, at intervals of 1.0 pH units, using the following buffer system: pH 5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH2PO4/0.1 M NaOH; and pH 9.0–10.0, 0.1 M NaHCO3/0.1 M Na2CO3) and NaCl concentrations (0, 1, 3, 5, 7, 9, 10, 15, 20 and 25%; w/v) was measured as described by Xu et al. (2005) using nutrient agar (NA) medium as the basal medium and incubation at 28 °C for 7 days. Cell morphology was observed using light microscopy (BH-2; Olympus) and scanning electron microscopy (TEM System-H7650, Hitachi) after 2–7 days growth on NA at 28 °C. Growth under anaerobic conditions was assessed using the GasPak Anaerobic System (BBL) according to the manufacturer’s instructions. Gram staining was performed by using the standard Gram reaction and the KOH lysis test (Cerny, 1978). Methyl red and Voges–Proskauer tests and indole production were determined as recommended by Smibert & Krieg (1994) and Williams et al. (1989). Acid production from carbohydrates was determined using methods described by Gordon et al. (1974). Utilization of different compounds as sole carbon or nitrogen and energy sources was tested as described by Carrasco et al. (2006), using the basal medium recommended by Pridham & Gottlieb (1948). Motility, antibiotic susceptibility, catalase and oxidase activities were observed as described previously Chen et al. (2007). Other phenotypic characteristics were tested by following standard procedures of Tindall et al. (2007).

Biomass for testing polar lipids was obtained by cultivation using NA medium at 28 °C for 4 days. Polar lipids were extracted as described by Xin et al. (2000), and identified by two-dimensional TLC and coloured by spraying with the appropriate detection reagents (Tindall, 1990). Cells for the analysis of cellular fatty acids and isoprenoid quinones were grown on tryptic soy broth (TSB; Difco) in shake flasks at 120 r.p.m. at 28 °C for 4 days and harvested by centrifugation at 5000 r.p.m. (Eppendorf 5804R; 4162 g), washed twice with distilled water and then freeze-dried. The cellular fatty acid composition was determined by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. The fatty acid methyl esters were then analysed by using the Microbial Identification software package (Sherlock Version 6.0). Menaquinones were collected according to the protocol of Collins (1994) and then analysed by HPLC (Tamaoka et al., 1983). DNA G+C contents were determined by using the HPLC method (Mesbah et al., 1989) using the DNA of Escherichia coli DH5 as a standard.

The almost complete 16S rRNA gene sequence of strain SCSIO 03483T was obtained by PCR amplification with the universal primers 27F and 1492R (Lane, 1991). Analysis with 16S rRNA gene sequences of members of the family Flavobacteriaceae was performed by using the software package MEGA version 5.0 (Tamura et al., 2011) after multiple alignments with CLUSTAL_X version 1.83 (Thompson et al., 1997).

Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. The genetic distance matrices were estimated by the Kimura two-parameter model (Kimura, 1980). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

Colonies of strain SCSIO 03483T were non-translucent and shiny with entire edges, and become mucoid on NA medium or marine agar 2216 after incubation for 2–4 days at 30 °C. Cells are Gram-negative, aerobic, non-spore-forming and motile rod-shaped (0.5–0.7 μm wide and 4.0–4.6 μm long) (see Fig. S1, available in IJSEM Online). Strain SCSIO 03483T differed from the type strain of its closest phylogenetic neighbour Imtechella halotolerans K1T in size, as the cells of type strain K1T are more slender and shorter than those of strain SCSIO 03483T. The cells of strain SCSIO 03483T were yellow, non-translucent and motile, while those of I. halotolerans K1T were translucent and not motile after 2 days growth at 30 °C on marine agar.

Growth occurred at pH 6.0–8.0 and 10–45 °C with 0–10% (w/v) NaCl concentrations. Tests were positive for catalase reaction, hydrolysis of Tween 20, 40 and 80, milk coagulation and peptonization, gelatin, nitrate reduction and casein. Fructose, glucose, glycerol, lactose, D-mannose, D-fructose, xylan and D-xylose can be used as sole carbon sources for growth. This isolate was resistant to the tested antibiotics (μg/disk) including amikacin (30), amoxicillin (10), ampicillin (10), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), lincomycin (2), neomycin (10), netilmicin (30), norfloxacin (10), novobiocin (30), penicillin G (10), rifampicin (5), streptomycin (10), sulfamethoxazole...
(23.75), tetracycline (30), tobramycin (10) and vancomycin (30), but not to erythromycin (15). The morphological, physiological and biochemical differences between strain SCSIO 03483T and its nearest phylogenetic neighbours are shown in Table 1.

The components of polar lipids for this strain are shown Fig. S3. The overall polar lipid pattern included phosphatidylethanolamine (PE), two unidentified amino lipids and three unknown polar lipids. Two unknown polar lipids close to PE on the TLC patterns reacted with ninhydrin, but were negative for molybdenum blue reagent. The result differed slightly from that for the type strain of I. halotolerans K1T (PE, two unidentified aminolipids, four unidentified phospholipids and two unidentified lipids as polar lipids). MK-6 was the predominant menaquinone of strain SCSIO 03483T, while I. halotolerans K1T had MK-6 (64%) as major respiratory quinone along with MK-7 (36%). The cellular fatty acid profiles of strain SCSIO 03483T and related genera are presented in Table S1. The major components in strain SCSIO 03483T were iso-C₁₅:₀ (29.4%), iso-C₁₇:₀-3-OH (19.2%) and summed feature 3 (C₁₆:₀/3·03/C₁₆:₁ω7c (13.2%)). Genomic DNA G+C content of strain SCSIO 03483T was 38.4(±0.5) mol%.

The nearly complete 16S rRNA gene sequences (1371 bp) of strain SCSIO 03483T was obtained. Preliminary sequence comparisons with 16S rRNA gene sequence at EzTaxon-e (Kim et al., 2012) indicated that strain SCSIO 03483T belonged to the family Flavobacteriaceae. The highest level of sequence similarities were found to I. halotolerans K1T (92.7%), Joostella marina En5T (92.3%) and Zhouia amylolytica HN-171T (91.5%). Strain SCSIO 03483T formed a distinct branch and grouped with the clade including species from the genera Intechella, Joostella and Zhouia, which was supported by a high bootstrap probability (99)

Table 1. Differential characteristics of strain SCSIO 03483T and the type strains of related taxa in the family Flavobacteriaceae

<table>
<thead>
<tr>
<th>Characteristic</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>
<th>10</th>
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<tr>
<td>Pigmentation</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow or vivid yellow</td>
<td>Pale yellow</td>
<td>Orange</td>
<td>Yellow</td>
<td>Yellow or yellow-orange</td>
<td>Yellow</td>
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<tr>
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<td>0.8–2.0</td>
<td>1.3–3.0</td>
<td>1.2–4.7</td>
<td>5.0–7.0</td>
<td>1.4–3.2</td>
<td>2.5–4.0</td>
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<td>Motility</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>−</td>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>0–9</td>
<td>1–5</td>
<td>1–8</td>
<td>0–17</td>
<td>3–8.5</td>
<td>0–4</td>
<td>2–6</td>
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<td>10</td>
<td>0–10</td>
<td>0–12</td>
<td>0–15</td>
<td>0–9</td>
<td>1–5</td>
<td>1–8</td>
<td>0–17</td>
<td>3–8.5</td>
<td>0–4</td>
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<td>−</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Catelase</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>−</td>
<td>−</td>
<td>V</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>−</td>
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<td>+</td>
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<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
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<td>Nitrate reduction</td>
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<td>−</td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acid production from:</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Glucose</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Maltose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>38.4</td>
<td>46.1</td>
<td>30–32.3</td>
<td>34.5</td>
<td>32–33</td>
<td>31–33</td>
<td>35–42.5</td>
<td>33–39</td>
<td>42</td>
<td>39.7</td>
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</table>
percentage in the neighbour-joining, maximum-likelihood and maximum-parsimony trees (Figs 1 and S1).

Besides relatively low 16S rRNA sequence similar values (92.7%), strain SCSIO 03483T can also be distinguished from members of the most closely related species identified, *I. halotolerans*, the type species of the genus *Imtechella*, by chemotaxonomic properties, including the components of polar lipids, respiratory quinones and DNA G+C content (Table 1). Furthermore, strain SCSIO 03483T can be distinguished from members of the most closely related genera, such as *Imtechella*, *Joostella*, *Zhouia*, *Krokinobacter*, *Leptobacterium*, *Lutaonella* etc., by many obvious differences in physiological characteristics including intensity of colony colour, cell size, pH, temperature and NaCl tolerance range for growth, oxidase activity, hydrolysis of gelatin and starch, antibiotic susceptibility, production of acid by using sole carbon sources and hydrolysis of Tweens 20, 40 and 80, etc. The detailed different characteristics of strain SCSIO 03483T and the type strains of related taxa in the family *Flavobacteriaceae* are shown in Table 1. Thus, based on the above data from polyphasic studies, we propose a novel genus and species in the family *Flavobacteriaceae*, *Sinomicrobium oceani* gen. nov., sp. nov., to accommodate strain SCSIO 03483T.

**Description of Sinomicrobium gen. nov.**

*Sinomicrobium* (Si.no.mi.cro’bium. M.L. *Sinus* of China; N.L. neut. *microbium* a microbe; N.L. neut. *Sinomicrobium* a microbe from China).

Cells are Gram-reaction-negative, aerobic, non-spore-forming and rod-shaped. Produce diffusible yellow pigments. Catalase-positive and oxidase-negative. Reduces nitrate to nitrite. Phospholipids are PE, two unidentified aminolipids and three unknown polar lipids. The predominant menaquinone is MK-6 and the major fatty acids are iso-C15 : 0, iso-C17 : 0 3-OH and summed feature 3 (C16 : 1ω6c/C16 : 1ω7c). The type species is *Sinomicrobium oceanii*.

**Description of Sinomicrobium oceanii sp. nov.**

*Sinomicrobium oceanii* (o.ce.a’ni. L. gen. *oceanii* of an ocean, referring to its optimal growth under marine conditions).

The description is as for the genus with the following additional properties. Cells are usually 0.5–0.7 μm wide and 4.0–4.6 μm long. Growth occurs at pH 6.0–8.0 and 10–45 °C with 0–10% (w/v) NaCl and optimal growth at
pH 7.0, 28–37 °C with 0–4 % (w/v) NaCl concentrations. Production of H₂S and utilization of urea do not occur. Positive for hydrolysis of gelatin, Tweens 20, 40 and 80, casein and milk coagulation and peptonization, negative for hydrolysis of starch, cellulose, methyl red, Voges–Proskauer tests and indole production. Fructose, glucose, glycerol, lactose, D-mannose, L-rhamnose, xylan and D-xylose can be used as sole carbon sources, while D-arabinose, cellobiose, galactose, galactoside, mannotol, inositol, raffinose, ribose, D-sorbitol, sucrose, trehalose and xylitol are not utilized. Histidine can be used as sole nitrogen source, but not alanine, L-arginine, asparagine, cysteine, glutamic acid, glycine, hypoxanthine, methionine, phenylalanine, proline, serine, threonine, L-tyrosine, tryptophan or valine. Resistant to most antibiotics tested except erythromycin. The polar lipids include PE, two unidentified aminolipids and three unknown polar lipids. The major fatty acids are iso-C₁₅ : 0, iso-C₁₇ : 0 3-OH and summed feature 3 (C₁₆ : 1v₀/C₁₆ : 1v₁/7c). The predominant respiratory quinone is menaquinone 6 (MK-6).

The type strain, SCSIO 03483T (=KCTC 23994T=CGMCC 1.12145T), was isolated from deep-sea sediment sample at a depth of 2548 m, collected from the Nansha Islands sea area in the South China Sea. The DNA G+C content of strain SCSIO 03483T is 38.4 mol%.

Acknowledgements

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