Sediminivirga luteola gen. nov., sp. nov., a member of the family Brevibacteriaceae, isolated from marine sediment

Gaiyun Zhang,1,2 Shuang Wang1,2 and Lina Wang1,2

1Key Laboratory of Marine Biogenic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, Fujian, PR China
2South China Sea Bio-Resource Exploitation and Utilization Collaborative Innovation Center, Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, Fujian, PR China

A Gram-stain-positive actinobacterial strain, designated F23T, was isolated from marine sediment collected from the western Pacific. Strain F23T showed less than 94.5% 16S rRNA gene sequence similarity with type strains of species with validly published names. Phylogenetic analysis, based on 16S rRNA gene sequences, revealed that the novel isolate formed a distinct monophyletic clade within the family Brevibacteriaceae and clustered distantly with the genera Brevibacterium and Spelaeicoccus. Cells of strain F23T were non-motile, rod-shaped and aerobic to microaerophilic. Optimal growth occurred at 35–37 °C, at pH 8.0–9.0 and in the presence of 1% NaCl (w/v). The isolate contained meso-diaminopimelic acid as the characteristic cell-wall diamino acid, MK-8(H2) and MK-7(H2) as the predominant menaquinones and anteiso-C15 : 0 and anteiso-C17 : 0 as the major fatty acids. The DNA G+C content of strain F23T was 69.0 mol%. On the basis of phylogenetic analysis, phenotypic and chemotaxonomic characteristics and 16S rRNA gene signature nucleotide patterns, strain F23T represents a novel species in a novel genus in the family Brevibacteriaceae, for which the name Sediminivirga luteola gen. nov., sp. nov. is proposed. The type strain is F23T (=JCM 19771T =CGMCC 1.12785T =MCCC 1A09945T).

The family Brevibacteriaceae was first described by Breed (1953), and emended by Stackebrandt et al. (1997) and Zhi et al. (2009). At the time of writing, only two genera Brevibacterium and Spelaeicoccus, are considered to be members of the family Brevibacteriaceae, and the genus Brevibacterium comprises approximately 30 species with validly published names isolated from diverse habitats (http://www.bacterio.net/brevibacterium.html), while the genus Spelaeicoccus contains a single species, Spelaeicoccus albus, isolated from a natural cave (Lee, 2013), which is also the type species. All strains of species of both genera were chemotaxonomically characterized by having meso-diaminopimelic acid as the diamino acid in the cell-wall peptidoglycan, MK-9(H2) or MK-8(H2), or MK-7(H2) and MK-8 (H2) as the major menaquinone(s), no mycolic acids and DNA G+C contents of 55–71 mol% (Cui et al., 2013, Kim et al., 2013, Kumar et al., 2013, Lee, 2013). Here, we describe the taxonomic properties of a novel actinobacterial strain, F23T, belonging to the family Brevibacteriaceae using a polyphasic approach.

Strain F23T was isolated from a marine sediment sample collected from the western Pacific at site WP1 (10° 42.0’ N 142° 19.5’ E; 5233 m). The sediment sample was decimally diluted with sterile seawater, spread onto the isolation medium (10 g glucose, 5 g peptone, 5 g yeast extract, 0.2 g MgSO4 . 7H2O, 10 g NaHCO3, 27 g Na2CO3 . 10H2O, 20 g agar; all 1% natural seawater, pH 10) and incubated at 28 °C for three weeks. The isolate was then cultured routinely on modified Zobell 2216E agar (MZ2: 1.0 g yeast extract, 5.0 g tryptone, 34 g NaCl, 15 g agar; all 1% distilled water, pH 7.4–7.6) and maintained as glycerol suspensions (20%, v/v) at −80 °C.

Growth at various temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50, 55 and 60 °C), at pH 5–13 (at intervals of 1 pH unit) and in the presence of 0–15% (w/v) NaCl (at intervals of 1%) was determined using MZ2 as the basal medium. Cell morphology was observed with optical microscope (BH-2; Olympus) and transmission electron microscopy (H-600; Hitachi). Gram staining was carried out by the standard Gram reaction as described by Cerny (1978). Cell motility was determined by the semi-solid...
puncture method with a straight needle. Cultures were incubated after the puncture inoculation in semi-solid agar [MZ2 broth plus 0.5 % (w/v) agar in this study] at 35 °C for 6 days. Motility was manifested macroscopically by a diffuse zone of growth spreading from the line of inoculation. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MZ2 at 35 °C for approximately 14 days. The activities of catalase and oxidase were determined using methods described by Yin et al. (2015). Hydrolysis of cellulose, starch and casein, milk coagulation and peptonization, nitrate reduction, urease activity, the methyl red test and production of H₂S and indole were performed as described by Xu et al. (2007). Further physiological and biochemical characteristics were determined using the API 20E and API ZYM systems (bioMérieux) and Biolog GP2 MicroPlate panels, according to the instructions of the manufacturer. The detailed morphological, physiological and biochemical properties of strain F23T are given in Tables 1 and S1 (available in the online Supplementary Material) and in the species description.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene of strain F23T were performed as described by Li et al. (2007). The near-complete 16S rRNA gene sequence was obtained with primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GTTACCTTGTAAGAGTTTACGACTT-3′) (Lane, 1991). The sequence was compared with 16S rRNA gene sequences of valid species from GenBank via the BLAST program and the EzTaxon-e server (Kim et al., 2012). All sequence alignments were analysed with the MEGA 6 software package (Tamura et al., 2013). Phylogenetic trees were reconstructed using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The stability of relationships was evaluated by performing a bootstrap analysis based on 1000 replications (Felsenstein, 1985). The DNA G+C content was determined as described by Mesbah et al. (1989) using reversed phase HPLC. The almost-complete 16S rRNA gene sequence for strain F23T (1498 nt) was determined. The isolate was most closely related to Brevibacterium luteolum CF87T (94.2 % sequence similarity) and Cellulomonas iranensis OT (94.2 %), followed by Brevibacterium massiliense 5401308T (94.0 %) and Spelaeicoccus albus D3-40T (94.0 %). Sequence similarities between strain F23T and the type strains of

Table 1. Phenotypic characteristics that differentiate strain F23T from related members of the family Brevibacteriaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Motility</td>
<td>–</td>
<td>V(–)</td>
<td>–</td>
</tr>
<tr>
<td>Indole production</td>
<td>–</td>
<td>NA(–)</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>V(–)</td>
<td>+</td>
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<tr>
<td>Oxidase</td>
<td>–</td>
<td>V(–)</td>
<td>–</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>V(+)</td>
<td>+</td>
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<tr>
<td>Growth in NaCl (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range 0–10</td>
<td>0–20</td>
<td>0–6</td>
<td></td>
</tr>
<tr>
<td>Optimum 1</td>
<td>0–5</td>
<td>0–3</td>
<td></td>
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<tr>
<td>Growth pH:</td>
<td></td>
<td></td>
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<tr>
<td>Range 6–12</td>
<td>5–12.5</td>
<td>5–9</td>
<td></td>
</tr>
<tr>
<td>Optimum 8–9</td>
<td>6–10</td>
<td>7</td>
<td></td>
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<tr>
<td>Growth temperature (°C)</td>
<td></td>
<td></td>
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<tr>
<td>Range 4–55</td>
<td>4–45</td>
<td>10–37</td>
<td></td>
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<tr>
<td>Optimum 35–37</td>
<td>25–40</td>
<td>28–30</td>
<td></td>
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<tr>
<td>Major cellular fatty acids</td>
<td>anteiso-C15 &lt; 0 anteiso-C17 &lt; 0 anteiso-C15 &lt; 0 anteiso-C17 &lt; 0 anteiso-C15 &lt; 0 anteiso-C17 &lt; 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>MK-7(H2) and MK-8(H2) MK-7(H2) or MK-8(H2) or MK-7(H2) and MK-8(H2) MK-9(H2) MK-9(H2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor menaquinones*</td>
<td>MK5(H2), MK6(H2), MK7, MK8, MK9(H2) MK-6, MK-6(H2), MK-7, MK-8, MK-9(H2) NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major polar lipids</td>
<td>DPG, PG, GL, PL DPG, PG, Pl,GL,PL PG, PI, GL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69</td>
<td>55.8–71.4</td>
<td>64.3</td>
</tr>
</tbody>
</table>

*Data for minor menaquinones for the genus Brevibacterium were from some species only, as no data are available for most species.
other species with validly published names were less than 94.0 %. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain F23\textsuperscript{T} fell within the radius of the suborder Micrococccinae, forming a distinct monophyletic clade within the family Brevibacteriaceae and clustered distantly with the genera Brevibacterium and Spelaeicoccus (Figs 1, S1 & S2). This relationship was also supported by the other tree-making methods used in this study (data not shown). The DNA G + C content of strain F23\textsuperscript{T} was determined to be 69.0 mol%.

For analysis of polar lipids and menaquinones, biomass was obtained from cells cultured in modified ZoBell 2216E broth at 35 °C for 3 days in a rotary shaker and harvested by centrifugation. Polar lipids were extracted and analysed according to the procedures described by Kates (1986). Extracted lipids were separated by two-dimensional TLC using chloroform/methanol/water (65 : 25 : 4, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (85 : 12 : 15 : 4, by vol.) for the second dimension. Total lipids were detected by spraying the plate with 10 % ethanolic molybdophosphoric acid. Phospholipids, glycolipids and phosphatidylcholine were detected by spraying the plates with molybdenum blue, α-naphthol-sulphuric acid and Dragendorff reagents, respectively. Isoprenoid quinones were extracted according to the method of Collins \textit{et al.} (1977) and analysed using ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS) as described by Kaiser \textit{et al.} (2012). The cellular fatty acid composition of strain F23\textsuperscript{T} was analysed by using cells grown at 35 °C for 3 days on tryptone soy agar (TSA; Difco). Cellular fatty acid methyl esters were prepared and analysed by GC (GC6850, Agilent) according to the instructions given for the Microbial Identification System version 6.1 and were compared using the database TSBA6 (MIDI). The diamino acid of the peptidoglycan, in line with all members of the family Brevibacteriaceae contained the only genus Brevibacterium \textit{Micrococcineae} (Kim \textit{et al.}, 2013, Kumar \textit{et al.}, 2013, Lee, 2013). In addition, anteiso-C\textsubscript{15 : 0} and anteiso-C\textsubscript{17 : 0} were also typically found as the major fatty acids in members of the genera \textit{Brevibacterium} and \textit{Spelaeicoccus} and phosphatidylglycerol has been determined to be a major polar lipid (Lee, 2013, Trujillo & Goodfellow, 2012). Although strain F23\textsuperscript{T} possessed chemical features similar to those of the genus \textit{Brevibacterium}, significant differences in the proportions of several fatty acids and in the composition of minor menaquinones were found between the isolate and members of the genus \textit{Brevibacterium} (Tables 1 & S2). Furthermore, strain F23\textsuperscript{T} could be readily differentiated from members of the genus \textit{Spelaeicoccus} by the absence of cyclohexyl-C\textsubscript{17 : 0} as a major fatty acid and phosphatidylninositol as a major polar lipid (Lee, 2013). Phenotypic features that distinguished strain F23\textsuperscript{T} from members of the closely related genera, \textit{Brevibacterium} and \textit{Spelaeicoccus}, are given in Tables 1 and S1.

Affiliation of strain F23\textsuperscript{T} to the suborder Micrococccinae was supported by possession of all the 16S rRNA gene signature nucleotides that had been defined for the suborder (Zhi \textit{et al.}, 2009). The family \textit{Brevibacteriaceae} was described and emended mainly based on 16S rRNA gene signature sequences (Breed, 1953, Stackebrandt \textit{et al.}, 1997, Zhi \textit{et al.}, 2009). At the time of the last emendation, the family \textit{Brevibacteriaceae} contained the only genus \textit{Brevibacterium}, which is also the type genus. However, a novel genus, \textit{Spelaeicoccus}, and many novel species of the genus \textit{Brevibacterium} have been proposed since the emendation. In the present study, all type strains of species belonging to the genera \textit{Brevibacterium}, \textit{Spelaeicoccus} and \textit{Sediminivirga} gen. nov. were characterized for signature

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig1.png}
\caption{Neighbour-joining tree showing the phylogenetic relationship of strain F23\textsuperscript{T} and representatives of some related taxa in the suborder Micrococccinae. Bootstrap values (>50 %) based on 1000 resampled datasets are shown at branch nodes. \textit{Streptomyces albus} NBRC 13014\textsuperscript{T} was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.}
\end{figure}
nucleotides of the family *Brevibacteriaceae*. The following corrections should be noted: W instead of A at position 120, K instead of A at position 196, W instead of A at position 444, U instead of C at position 843 and Y instead of U at position 1210. In addition, strain F23<sup>T</sup> possessed a unique set of signature nucleotides that placed it in a novel lineage within the family *Brevibacteriaceae* (Table S3), such as 129 : 232 (T–A), 157 : 164 (T–G), 199 : 218 (G–C), 200 : 217 (G–C), 202 : 215 (T–C) and 207 : 212 (G–A), with low levels of 16S rRNA gene sequence similarities (<94.5 %) to members of the family *Brevibacteriaceae*. From the combination of morphological, chemotaxonomic and phylogenetic data presented, it is suggested that strain F23<sup>T</sup> represents a novel species and genus of the family *Brevibacteriaceae*, for which the name *Sediminivirga luteola* gen. nov., sp. nov. is proposed.

**Description of *Sediminivirga* gen. nov.**

*Sediminivirga* (se.di.mi.ni.vir’ga. L. n. sedimen -inis sediment; L. fem. n. virga a rod; N.L. fem. n. sediminivirga a rod living in sediment)

Cells are Gram-stain-positive, non-motile, rod-shaped and aerobic to microaerophilic. The diagnostic diaminoc acid in the cell wall is *meso*-diaminopimelic acid. The major menaquinones are MK-7(H<sub>2</sub>) and MK-8(H<sub>2</sub>). The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unknown glycolipid and an unknown phospholipid. The predominant cellular fatty acids are anteiso-C<sub>15 : 0</sub> and anteiso-C<sub>17 : 0</sub>. The type species is *Sediminivirga luteola*. Phylogenetically, the genus belongs to the family *Brevibacteriaceae*, suborder *Micrococccineae*.

**Description of *Sediminivirga luteola* sp. nov.**

*Sediminivirga luteola* (lu.te.o’la. L. adj. luteus yellow; L. dim. fem. adj. luteola yellowish)

Displays the following characteristics in addition to those of the genus. Cells are approximately 0.3–0.4 µm in diameter and 1.3–1.8 µm in length. Colonies on MZ2 are approximately 1–2 mm in diameter, opaque, circular with regular margins, convex and pale cream to pale yellow after 3 days at 35 °C. Growth occurs in 0–10 % (w/v) NaCl (optimum, 1 %), at pH 6–12 (optimum, pH 8–9) and at 4–55 °C (optimum, 35–37 °C). No growth occurs at 60 °C or in 11 % (w/v) NaCl. Catalase-positive and oxidase-negative. Positive for casein hydrolysis and negative for nitrate reduction, milk coagulation and peptonization, hydrolysis of cellulose and starch, the methyl red test, oxidase, activity of arginine dihydrolase, tryptophan decarboxylase and gelatinase, Voges–Proskauer reaction, fermentation of mannitol, inositol and sucrose, but negative for the production of H<sub>2</sub>S and indole, activity of β-galactosidase, N-acetyl-β-glucosaminidase, β-glucosidase, α-mannosidase and β-fucosidase. Analysis using the API 20E system revealed that cells are positive for activity of arginine dihydrolase, tryprophan decarboxylase and gelatinase, Voges–Proskauer reaction, fermentation of mannitol, inositol and sucrose, but negative for the production of H<sub>2</sub>S and indole, activity of β-galactosidase, lysisine decarboxylase, ornithine decarboxylase and urea, fermentation of glucose, sorbitol, rhamnose, melibiose, amygdalin and arabinose. In the Biolog GP2 Micro-Plate, positive for the oxidation of dextrin, Tween 40, Tween 80, D-fructose, α-D-glucose, maltose, maltotriose, D-mannitol, D-mannose, sucrose, trehalose, turanose, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketovaleric acid, D-lactic acid methyl ester, L-lactic acid, succinic acid, mono-methyl ester, pyruvic acid, L-asparagine, glycerol, adenosine and thymidine and weakly positive for the oxidation of D-sorbitol, lactamide, L-glutamic acid, glycidyl-L-glutamic acid, D-glucose-6-phosphate, D-L-α-glyceraldehyde phosphate and propionic acid, but negative for the oxidation of the remaining 63 Biolog GP2 substrates. In addition to anteiso-C<sub>15 : 0</sub> and anteiso-C<sub>17 : 0</sub>, significant amounts of iso-C<sub>15 : 0</sub> and iso-C<sub>16 : 0</sub> are also present. The type strain, F23<sup>T</sup> (=JCM 19771<sup>T</sup> =CGMCC 1.12785<sup>T</sup> = MCCC 1A09945<sup>T</sup>), was isolated from marine sediments collected from the western Pacific. The genomic DNA G+C content of the type strain is 69.0 mol%.

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**References**


