Aliidiomarina soli sp. nov., isolated from saline–alkaline soil

Lian Xu,† Ji-Quan Sun,¹,²,* Li-Juan Wang,¹ Xiao-Zhen Liu,¹ Yin-Yao Ji,³ Zhong-Qiu Shao⁴ and Xiao-Lei Wu¹,*

Abstract

A Gram-stain-negative, motile, non-spor-forming bacterium, designated strain Y4G10-17T, was isolated from the saline-alkali farmland top soil, Inner Mongolia, northern China. Strain Y4G10-17T could grow at 4–45 °C (with 30 °C as the optimal temperature), pH 6.0–12.0 (optimal at pH 9.0) and in the presence of 1.0–12.0 % (w/v) NaCl (optimal at 4.0–6.0 %). Phylogenetic analysis based on the eight different copies of the 16S rRNA gene sequences revealed that strain Y4G10-17T shared the highest sequence similarity with Aliidiomarina maris CF12-14T, 97.93–98.66 %, and lower than 97.0 % sequence similarity with all other type strains. Its major cellular fatty acids contained iso-C₁₅:0, iso-C₁₇:0 3-OH and summed feature 3 (iso-C₁₅:0 2-OH and/or C₁₆:1 ω7c). Q-8 was the predominant ubiquinone. The major polar lipids of strain Y4G10-17T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unknown lipids and one unknown aminolipid. The genomic DNA G+C content was 49.3 mol%. DNA–DNA hybridization revealed that strain Y4G10-17T showed 20.2±5 % genomic DNA relatedness with its close relative A. maris CF12-14T. Based on the phenotypic, phylogenetic and genotypic characteristics, strain Y4G10-17T represents a novel species within the genus Aliidiomarina, for which the name Aliidiomarina soli sp. nov. is proposed. The type strain is Y4G10-17T (=CGMCC 1.15759T=KCTC 52381T).

The genus Aliidiomarina, belonging to Idiomarinaceae, was first described by Huang et al. [1]. The members of this genus are mesophilic and halophilic, with iso-C₁₇:1ω9c as a major cellular fatty acid component, in addition to exhibiting the characteristics of Idiomarinaceae. At the time of writing, the genus Aliidiomarina consisted of seven published species: Aliidiomarina taiwanensis [1], A. haloalkalitoleraus [2], A. iranensis [3], A. shirensis [4], A. maris CF12-14T [4, 5], A. minuta [6] and A. sanyensis [7]. Most of the Aliidiomarina strains were isolated from the saline conditions, such as seawater [1, 2], saline lake [4, 6], coastal-marine wetlands [3] and pool of Spirulina platensis cultivation [7].

During an investigation into the bacterial diversity of a saline-alkaline farmland, one bacterial strain, designated Y4G10-17T, was isolated from the top saline-alkaline soil sampled from Hangjin Banner (107° 49′21″E 40° 04′12″N), Ordos, Inner Mongolia, northern China, by using the 10-fold dilution method on Luria–Bertani agar (LB: 10 g tryptone L⁻¹, 5 g yeast extract L⁻¹, 10 g NaCl L⁻¹, 20 g agar L⁻¹, pH 7.0) at 30 °C for 2–3 days in dark. The strain was purified by repeated streaking on LB agar. It was then cultured routinely in LB broth and shaken at 150 r.p.m. for 24 h. Its genomic DNA was prepared using the DNA extraction kit (Biotech) following the manufacturer’s instruction. The 16S rRNA gene was amplified with the primer set 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGG(C/T)ACCTTGTTACGACTT-3′) with the protocol described earlier [8]. The PCR amplicon was purified with a PCR purification kit (Biotech) and sequenced after it was ligated into the pMD19-T vector (TaKaRa), according to the manufacturer’s instructions. Eight different copies of 16S rRNA gene sequences were obtained from strain Y4G10-17T. The nearly complete (1151–1513 nt) 16S rRNA gene sequences of strain Y4G10-17T were first compared with the available 16S rRNA gene sequences using the BLAST program (http://www.ncbi.nlm.nih.gov/blast) to determine its approximate phylogenetic affiliation. Sequences were aligned by using CLUSTAL X software [9]. Phylogenetic trees were then reconstructed with the neighbour-joining [10], maximum-likelihood [11] and minimum-evolutionary [12, 13] algorithms. The neighbour-joining tree used the maximum composite likelihood distance [14], the maximum-likelihood tree used the Tamura–Nei model [14].
and the minimum-evolutionary tree used the Kimura 2-parameter model [14]; each of them were reconstructed with the complete deletion option. Bootstrap analysis (1000 replications) was used to assess the stability of the tree topology [15]. The 16S rRNA gene sequence similarities between strain Y4G10-17T and A. minuta MLST1T and A. iranensis GBPy7T were calculated by using MEGA 6.0 with the Tamura–Nei model and with pairwise deletion option, while the similarities with the other related strains were calculated using the EzTaxon-e server (http://www.ezbiocloud.net; [16]). The eight copies of the 16S rRNA gene of strain Y4G10-17T were clustered together but in different branches, sharing 98.38–99.91% sequence similarities (Fig. 1 and Table S1, available in the online Supplementary Material). The lowest similarity between the eight copies of the 16S rRNA gene was even below the threshold of

![Phylogenetic tree reconstructed by neighbour-joining method based on the 16S rRNA gene sequences. Bootstrap values (numbers on branch nodes expressed as percentages of 1000 replications) >50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.](image)

**Fig. 1.** Phylogenetic tree reconstructed by neighbour-joining method based on the 16S rRNA gene sequences. Bootstrap values (numbers on branch nodes expressed as percentages of 1000 replications) >50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.
98.65%, which is required to define a novel species of prokaryotes, as proposed by Kim et al. [17]. The neighbour-
joining tree also revealed that strain Y4G10-17T was clustered with A. maris CF12-14T (=DSM 22154T; [5]) (Fig. 1). All eight copies of 16S rRNA genes of strain Y4G10-17T shared the highest 16S rRNA gene sequence similarities with A. maris CF12-14T, 97.93–98.66% (Table S2) and lower than 97% sequence similarities with other type strains. This affiliation was supported by the phylogenetic trees reconstructed with two other algorithms (Figs. S1 and S2). Therefore, A. maris DSM 22154T was selected as reference strain for further biochemical and chemotaxonomic analyses. The reference strain was obtained from DSMZ culture collection.

The genomic DNA G+C content was determined by the thermal denaturation method [18] using DNA from Escherichia coli K12 as a standard reference. DNA–DNA hybridization was carried out to assess the genomic DNA relatedness between strain Y4G10-17T and its closely related strain A. maris DSM 22154T by using the optical renaturation rate method described by De Ley et al. [19] with the modifications described by Huss et al. [20]. For determining the cellular fatty acids, cells of strain Y4G10-17T and its closely related strain A. maris DSM 22154T were grown on trypticase soy agar (TSA; Difco) at 30°C and were harvested at roughly the same growth stage in the exponential growth phase (24 h). The fatty acids were prepared and identified following the instructions of the Microbial Identification system (MIDI) as described previously [21]. Polar lipids were extracted with chloroform/methanol system, examined by two-dimensional TLC and sprayed with molybdenum blue (for the detection of total lipids), ninhydrin (for the detection of aminolipids) and ammonium molybdate (for the detection of phospholipids), as described by Kates [22]. Menaquinones were extracted with chloroform/methanol (2:1, v/v) and analysed as described by Komagata and Suzuki [23] using HPLC. The G+C content of genomic DNA was 49.3 mol% (Table 1), which was within the range recorded for all Aliidiomarina strains (45.8–54.7 mol%) [1–4, 6, 7]. DNA–DNA hybridization revealed that genomic DNA relatedness between strain Y4G10-17T with its closest relative A. maris DSM 22154T is 20.2±5%, which is below the threshold value (70%) recommended for defining a novel species [24, 25]. The cellular fatty acid profile of strain Y4G10-17T was characterized by iso-C15:0 (30.8%), iso-C17:0 (18.8%), summed feature 9 (iso-C17:1ω9c and/or C16:1ω7c 10-methyl, 13.9%), iso-C15:1 F (8.7%), iso-C11:0 3-OH (6.1%) and summed feature 3 (iso-C15:0 2-OH and/or C16:1ω7c, 5.7%) as the major fatty acids. This fatty acid profile was in agreement with those of the other Aliidiomarina type strains, although the relative abundance of some fatty acid were different [2–4, 6, 7] (Table S3). Ubiquinone Q-8 (98.2%) was the predominant isoprenoid quinone, in accordance with those of Aliidiomarina strains, while minor amounts of ubiquinone Q-9 (1.8%) was also detected in strain Y4G10-17T. The major polar lipids of strain Y4G10-17T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unknown lipids and one unknown aminolipid (Fig. S3). The polar lipid and main quinone profiles both supported the affiliation of strain Y4G10-17T to genus Aliidiomarina.

Table 1. Differentiating characteristics of strain Y4G10-17T and the other type strains of Aliidiomarina.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (µm)</td>
<td>0.1–0.3×1.2–2.4</td>
<td>0.3–0.6×0.8–2.4*</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td>Range 4–45</td>
<td>4–40</td>
</tr>
<tr>
<td></td>
<td>Optimum 30</td>
<td>30</td>
</tr>
<tr>
<td>Growth at salinity (w/v, %)</td>
<td>Range 1–14</td>
<td>1–15</td>
</tr>
<tr>
<td></td>
<td>Optimum 1.0–5.0</td>
<td>2.0–3.0</td>
</tr>
<tr>
<td>Growth at pH</td>
<td>Range 6–12</td>
<td>6–11</td>
</tr>
<tr>
<td></td>
<td>Optimum 9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Indole production</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adipic acid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Malic acid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>DNA G+C content (mol%)</td>
<td>49.8</td>
</tr>
<tr>
<td></td>
<td>Quinone</td>
<td>Q-8, Q-9</td>
</tr>
</tbody>
</table>

*Data from Zhang et al. [5].

After cells of strain Y4G10-17T were grown on LB agar at 30°C for 24 h, they were harvested, negatively stained with 1% (w/v) phoshotungstic acid and observed using transmission electron microscopy (Hitachi-7000). Gram-staining and endospore formation were investigated as described by Smibert and Krieg [26]. The growth of strain Y4G10-17T was assessed at various temperatures (4, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C) by using LB broth as the basal medium. Growth at different pH values (pH 4.0–13.0 in 1.0 unit interval) was assessed in LB medium adjusted with 10% HCl or NaOH solutions. NaCl tolerance was tested using a modified LB broth containing 0–18% (w/v) NaCl.
(at an interval of 1%). Antibiotic sensitivity tests were performed using the diffusion method [27] on Mueller–Hinton agar and LB agar with filter-paper discs (8 mm diameter) containing one of the following antibiotics: ampicillin (10 µg), amoxicillin (10 µg), bacitracin (0.04 U), carbenicillin (100 µg), cefradine (30 µg), chloromycetin (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (2 µg), novobiocin (30 µg), penicillin G (10 U), polymyxin (300 I), rifampicin (5 µg), roxithromycin (15 µg), spectinomycin (100 µg), streptomycin (10 µg), trimethoprim (5 µg), tetracycline (30 µg) and vancomycin (30 µg). Oxidase and catalase activities were evaluated by adding oxidase reagent (bioMérieux) and 3 % hydrogen peroxide solution, respectively, to a fresh colony [28]. Other biochemical characteristics were tested using API 20NE and API ZYM kits (bioMérieux), according to the manufacturer’s instructions. Positive results obtained from the API 20NE test were reconfirmed by using traditional cultivation methods [28].

The type strain, Y4G10-17T (=CGMCC 1.15759T=KCTC 52381T), was isolated from saline–alkaline soil collected from a farmland in Ordos, Inner Mongolia, northern China. The genomic DNA G+C content of the type strain is 49.3 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


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**DESCRIPTION OF ALIDIOMARINA SOLI SP. NOV.**

*Alidiomarina soli* (so’li. L. neut. gen. n. soli of soil, the isolation source of the type strain).

Cells are strictly aerobic, Gram-stain-negative, motile, non-spore-forming, straight or curved rods, 0.1–0.3 µm in width and 1.2–2.4 µm in length, with single or two polar flagella (Fig. S4). The cells of strain Y4G10-17T were narrower than those of the other type strains (Table 1). Phenotypic features of strain Y4G10-17T are listed in the species description and Table 1.

The phylogenetic, phenotypic, chemotaxonomic and morphological data all supported that strain Y4G10-17T represents a novel species of the genus *Alidiomarina*, for which the name *Alidiomarina soli* sp. nov. is proposed.

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