Aliidiomarina sedimenti sp. nov., a haloalkaliphilic bacterium in the family Idiomarinaceae

Azadeh Shahinpei,1 Mohammad Ali Amoozegar,2 Seyed Abolhassan Shahzadeh Fazeli,1,3,* Peter Schumann,4 Cathrin Spröer4 and Antonio Ventosa5

Abstract

A novel Gram-staining-negative straight or curved rod-shaped, moderately halophilic and alkaliphilic bacterium, designated strain GBSy1T, was isolated from a sediment sample from the coastal-marine wetland Gomishan in Iran. GBSy1T was motile, and formed non-pigmented, mucoid colonies. Growth occurred with between 1 and 15% (w/v) NaCl and the isolate grew optimally with 5% (w/v) NaCl. The optimum pH and temperature for growth were 8.5 and 34°C, while the strain was able to grow at pH 7.0–10 and 4–40°C. On the basis of the results of 16S rRNA gene sequence analysis, GBSy1T was shown to represent a member of the genus Aliidiomarina within the class Gammaproteobacteria, family Idiomarinaceae and showed closest phylogenetic similarity to Aliidiomarina maris CF12–14T (97.7%). The DNA G+C content of GBSy1T was 51.2 mol%. The cells of GBSy1T contained the isoprenoid ubiquinones Q-8, Q-9 and Q-10 (92, 2 and 2%, respectively). The major cellular fatty acids of the isolate were iso-C15:0 3-OH, iso-C15:0, iso-C17:0 and iso-C17:1ω9c and its polar lipid profile comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and three unknown phospholipids. The level of DNA–DNA relatedness between GBSy1T and Aliidiomarina maris DSM 22154T was 31%. All these features confirmed the placement of GBSy1T within the genus Aliidiomarina. On the basis of evidence from this study, a novel species of the genus Aliidiomarina, Aliidiomarina sedimenti sp. nov., is proposed, with GBSy1T (=IBMC-M 107644=CECT 8340T) as the type strain.

The family Idiomarinaceae, within the order Alteromonadales, embraces the genera Idiomarina [1] and Aliidiomarina [2]. The members of this family are Gram-staining-negative, mesophilic, aerobic or facultatively anaerobic rods, require sodium ions for growth, do not require vitamins or amino acids and show a poor ability to use carbohydrates as sole carbon and energy sources [3]. The major cellular fatty acids present are iso-C15:0 and iso-C17:0 [1, 3, 4] and the major isoprenoid quinone is ubiquinone 8 (Q-8) [5]. At the time of writing, the genus Aliidiomarina accommodates six species with validly published names, Aliidiomarina taiwanensis, the type species, [2], Aliidiomarina maris [6, 7], Aliidiomarina sanyensis [8], Aliidiomarina shirensis [7], Aliidiomarina iranensis [9] and Aliidiomarina minuta [10]. Two additional species ‘Aliidiomarina haloalkalitolerans’ [11] and ‘Aliidiomarina soli’ [12] have been proposed.

The Gomishan wetland is an alkaline thalassohaline, coastal–marine wetland (pH 8.5–9.3, salinity 3–5%), located along the eastern shore of the Caspian Sea in Iran (37° 04’06.3”N 54° 00’24.5”E). It has an area of about 17 000 ha and its height is 27 m below sea level. During a survey of diversity of heterotrophic microorganisms, several halophilic and halotolerant bacteria were isolated. We describe the isolation and polyphasic characterization of a novel, moderately halophilic and slightly alkaliphilic bacterial strain, designated GBSy1T, which is considered to represent a novel species of the genus Aliidiomarina.

The strain was isolated by diluting a sediment sample collected from the shallow coastal region of the Gomishan wetland, in sterile 3.0% (w/v) salt solution, and plating it on modified alkaliphilic halophile agar (MAHA) with 5% (w/v) total salts: (g l−1) NaCl, 30.0; peptone, 5.0; yeast extract, 2.0; meat extract, 1.0; tri-sodium citrate, 0.12; KCl, 0.08; MgSO4.7H2O, 0.04; FeSO4.7H2O, 2.0 mg; MnCl2.4H2O, 0.36 mg; and agar, 15.0 [13]. Sodium sesquicarbonate solution ([g l−1] Na2CO3, 10.6; and NaHCO3, 8.42) was added to obtain alkaline conditions, it was added after sterilization in an autoclave and the plates were incubated at 34°C for the first 48 h and then at 30°C for another 48 h. The plates were observed daily for a total of 10 days.

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Keywords: Aliidiomarina sedimenti; haloalkaliphilic bacterium; new taxon; Idiomarinaceae; Proteobacteria.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain GBSy1T is KU870747.

Supplementary data is available in the online Supplementary Material.
two weeks. The pH of this medium was adjusted to 9.5. The strain was subsequently purified three times by plating on the same medium. In addition, the best media for growth were examined and MH agar [14] with 5.0 % total salts, pH 8.5, and R2A medium with 5 % NaCl [15], pH 8.5 were used. The strain was maintained on these media and also at −80 °C in R2A medium without agar and supplemented with 30 % (v/v) glycerol.

The characterization of the strain was achieved by following a polyphasic approach, including the investigation of phenotypic features, chemotaxonomy (polar lipids profile, fatty acids composition and quinone analyses) and 16S rRNA typic features, chemotaxonomy (polar lipids profile, fatty acids composition and quinone analyses) and 16S rRNA sequence analysis. *Aliidiomarina maris* DSM 22154<sup>T</sup>, *Aliidiomarina shirensis* IBRC-M 10414<sup>T</sup> and *Idiomarina seosinensis* DSM 21922<sup>T</sup> were used as reference strains for comparison in our study. They were cultured according to the recommendations of the culture collection.

Genomic DNA of GBSy1<sup>T</sup> was extracted using the method described by Marmur [16]. The 16S rRNA gene was amplified using the bacterial universal primers 27F and 1492R [17]. The purified PCR product was sequenced in both directions using an automated sequencer (Macrogen). Phylogenetic analysis was performed using the software package MEGA version 6 [18] after obtaining multiple alignments of data available from public databases by CLUSTAL-X [19]. Phylogenetic trees were reconstructed using three different methods, minimum-evolution [20], neighbour-joining [21] and maximum-likelihood [22, 23] algorithms. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings [24].

An almost-complete 16S rRNA gene sequence (1416 bp) of GBSy1<sup>T</sup> was obtained and used for BLAST searches in GenBank and phylogenetic analysis. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA sequence similarity were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; [25]). The 16S rRNA gene sequence analysis revealed that GBSy1<sup>T</sup> represents a member of the genus *Aliidiomarina*. The closest relatives of strain GBSy<sup>1</sup> were *Aliidiomarina maris* CF12-14<sup>T</sup> and *Aliidiomarina shirensis* AIS<sup>T</sup> with sequence similarities of 97.7 and 95.4 %, respectively. Then *Idiomarina seosinensis* CL-SP19<sup>T</sup> and *Idiomarina fontislipidosi* F23<sup>T</sup> were the two most closely related species, with 95.3 and 94.4 % sequence similarities, respectively. The phylogenetic tree obtained using the neighbour-joining algorithm revealed that the novel strain clustered with members of the genus *Aliidiomarina* within the family *Idiomarinaeae* (Fig. 1). The phylogenetic position was completely confirmed in trees generated using the minimum-evolution and maximum-likelihood algorithms (Fig. 1).

Cell morphology was examined with a BX51 microscope (Olympus) equipped with phase-contrast optics using cells from cultures at the exponential growth phase. Gram staining was performed by the Burke method [26]. Motility was analysed by the wet-mount method [26]. Catalase, oxidase, ornithine decarboxylase and arginine dihydrolase activities, nitrate reduction, hydrolysis of aesculin, production of indole and H<sub>2</sub>S were determined as recommended by Smibert and Krieg [27], using media with 5 % (w/v) NaCl. Hydrolysis of Tweens 20, 40 or 80 was examined as described by Harrigan and McCance [28] on media with 5 % (w/v) NaCl. Hydrolysis of gelatin, casein and starch and DNase activity were performed as described by Mata et al. [29]. Tests for the determination of acid production from carbohydrates, as well as utilization of carbon sources, were performed as recommended by Ventosa et al. [14]. Antimicrobial susceptibility tests were performed on Mueller–Hinton agar plus 5 % (w/v) sea salts [14] seeded with a bacterial suspension containing 1.5×10<sup>8</sup> c.f.u. ml<sup>−1</sup> using discs (HiMedia) impregnated with various antimicrobial compounds. The plates were incubated at 34 °C for 48 h and the inhibition zone was interpreted according to the manufacturer's manual. To determine the optimal temperature and pH for growth of the strain, R2A broth with 5 % (w/v) NaCl was incubated at 0, 4, 10, 15, 20, 25–37 (at intervals of 1.0 °C), 40, 45 and 50 °C and at pH 5–10.5 at intervals of 0.5 pH units. The buffers sodium acetate/acetic acid (pH 5–6.5), Tris/HCl (pH 6–8.5) and glycine/sodium hydroxide (pH 9–11.5) were added at a concentration of 50 mM. Growth at different NaCl concentrations (0.5, 1.0, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.5, 10.0, 12.5, 15.0 and 20.0 %, w/v) was tested in R2A broth at pH 8.5. Anaerobic growth was tested in the presence of nitrate by adding 0.1 % KNO<sub>3</sub> to the medium with 5 % (w/v) NaCl in filled stoppered tubes in an anaerobic chamber [30]. Growth was monitored by measuring OD<sub>600</sub> using a spectrophotometric method (model UV–160 A; Shimadzu). Other physiological and biochemical tests were performed as described previously [14, 29, 31].

GBSy1<sup>T</sup> was Gram-staining-negative, motile by means of a polar flagellum and strictly aerobic. Cells were straight or slightly curved rods, with a width of 0.3–0.4 µm and a length of 1.2–2.7 µm. When grown for 24 hours at 34 °C on R2A medium with 5 % NaCl, the non-pigmented colonies were circular, convex, mucoid with entire margins, translucent and 1–2 mm in diameter. This strain was moderately halophilic and slightly alkaliphilic, growing in media containing 1–15.0 % (w/v) NaCl at pH 7.0–10.0 and optimally in media containing 5 % (w/v) NaCl at pH 8.5.

The strain was sensitive to amoxycillin (30 µg), cephoxitin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), gentamicin (10 µg), rifampicin (5 µg), streptomycin (10 µg), cephtazidim (30 µg), polymyxin B (300 U), tobramycin (10 µg), amikacin (30 µg) and carbencillin (100 µg), but resistant to penicillin G (10 U). Other phenotypic features are included in the species description and Table 1.

For determination of the DNA base composition, cells were disrupted by using a Constant Systems TS 0.75 kW (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashon et al. [32]. The DNA G+C content was determined.
by reversed-phase HPLC of nucleosides according to the protocol of Mesbah et al. [33]. The G+C content of the DNA of GBSy1T was 51.2 mol%. This value is within the range reported for members of the family Idiomarinaceae and higher than those of Aliidiomarina maris (50.4 mol%) and Aliidiomarina shirensis (45.8 mol%) [2, 6–9]. DNA–DNA hybridization was carried out as described by De Ley et al. [34] with the modifications described by Huss et al. [35], using a Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostat-equipped 6 × 6 multicell changer and a temperature controller with an in situ temperature probe (Varian). The results of DNA–DNA hybridization experiments between GBSy1T and its closest relative, Aliidiomarina maris IBRC-M 10413T, yielded a relatedness value of 31 %. According to the 70 % threshold proposed by Wayne et al. [36] for the delineation of prokaryotic species using DNA–DNA relatedness, this result confirmed that the novel isolate constitutes a novel species.

Cell biomass for fatty acids, isoprenoid quinone and polar lipids analyses was obtained by cultivation on 2.5 % MH medium at pH 8.5 and 32 °C. Cells were harvested during the mid-exponential growth phase. The whole-cell fatty acid composition of GBSy1T was determined according to the standard protocol of the Microbial Identification System (MIDI, Version 6.1; Identification Library TSBA 5.0; Microbial ID). Extracts were analysed using a model HP6890A gas chromatograph (Hewlett Packard) equipped with a flame-ionization detector as described by Kämpfer and Kroppenstedt [37]. The cellular fatty acid profile of GBSy1T was characterized as having iso-C11:0 3-OH (19.9 %), iso-C15:0 (19.4 %), iso-C17:0 (7.1 %), and iso-C17:1ω9c (6.0 %), as the major fatty acids. This fatty acid profile was consistent with those of other type strains of species of the genus Aliidiomarina, except as regards their relative abundance (Table 1). The profile is available as supplementary material with the online version of this paper.

Isoprenoid quinone analysis was carried out as described by Monciardini et al. [38]. GBSy1T contained Q-8 as the major isoprenoid quinone (92 %) and Q-9 and Q-10 as minor quinones (2 % and 2 %). The major respiratory lipoquinone of GBSy1T was typical of that found in members of the genus Aliidiomarina [2, 6–9], while the isolate could be
Table 1. Characteristics useful for differentiating GBSy1T from Aliidiomarina maris, Aliidiomarina shirensis and Idiomarina seosinensis

Taxa: 1, GBSy1T; 2, A. maris DSM 22154T; 3, A. shirensis IBRC-M 10414T; 4, I. seosinensis DSM 21922T. +, Positive; –, negative. Unless otherwise indicated all data are from this study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tr>
<td>Cell size (µm)</td>
<td>0.3–0.4×1.2–2.7</td>
<td>0.3–0.6×0.8–2.4</td>
<td>0.4–0.5×0.8–1.7</td>
<td>0.3–0.6×1.0–1.9</td>
</tr>
<tr>
<td>Salinity range (% (w/v))</td>
<td>1–15</td>
<td>0.1–15</td>
<td>0.5–15</td>
<td>1–20</td>
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<tr>
<td>Optimum NaCl</td>
<td>5</td>
<td>2–3</td>
<td>1–10</td>
<td>7–10</td>
</tr>
<tr>
<td>Growth temperature range (°C)</td>
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<td>4–42</td>
<td>1–45</td>
<td>4–40</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>7–10</td>
<td>6–11.5</td>
<td>5–9.5</td>
<td>6–10</td>
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<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrolysis of:</td>
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<tr>
<td>Aesculin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>DNA</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Utilization of:</td>
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<tr>
<td>L-Leucine</td>
<td>–</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Susceptibility to:</td>
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<tr>
<td>Ampicillin (30 µg)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Kanamycin (30 µg)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)*</td>
<td>51.2</td>
<td>50.4</td>
<td>45.8</td>
<td>45.0</td>
</tr>
<tr>
<td>Major fatty acids (&gt;6 %)</td>
<td>iso-C15:0 3-OH, iso-C15:0 3-OH &amp; iso-C17:0 3-OH, iso-C15:0 3-OH &amp; iso-C17:0 3-OH, iso-C17:0 3-OH, iso-C17:0 3-OH</td>
<td>iso-C15:0 3-OH, iso-C15:0 3-OH, iso-C17:0 3-OH &amp; iso-C17:0 3-OH</td>
<td>iso-C15:0 3-OH, iso-C15:0 3-OH</td>
<td>iso-C15:0 3-OH, iso-C15:0 3-OH, iso-C17:0 3-OH, iso-C17:0 3-OH &amp; iso-C17:0 3-OH</td>
</tr>
</tbody>
</table>

*Data for the DNA G+C content of the reference species were obtained from Zhang et al. [6], Chiu et al. [7] and Choi and Cho [40].

distinguished by the presence of Q-10 as minor quinone unlike the other species of the genus Aliidiomarina. Polar lipids were analysed as described by Groth et al. [39]. The major polar lipids of GBSy1T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and three unknown phospholipids (Fig. 2), whereas the closest relatives, A. maris CF12-14T and A. shirensis AIS1T, contained only diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as polar lipids, like the type species A. taiwanensis AIT1T [2, 7]. However, the polar lipid profile of GBSy1T was more similar to that of A. haloalkali-tolerans [6], which also contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and four unknown phospholipids [11].

In summary, the results of 16S rRNA gene sequence analysis (Fig. 1) revealed that GBSy1T represents a member of the genus Aliidiomarina but represents a branch separate from closely related species. GBSy1T shared some phenotypic features and similar chemotaxonomic characteristics, such as quinone composition and fatty acid composition, with species of the genus Aliidiomarina. However, several phenotypic features, such as NaCl concentration range and optimum for growth, temperature range and optimum for growth, pH range and optimum for growth, acid production from carbohydrates, nitrate reduction and genomic DNA G+C content, can be used to distinguish this strain from phylogenetically related taxa (Table 1). Therefore, on the basis of data from this polyphasic taxonomic study, we propose that strain GBSy1T represents a novel species of the genus Aliidiomarina, for which the name Aliidiomarina sedimenti sp. nov. is proposed.

Fig. 2. Polar lipids of GBSy1T after two-dimensional TLC and detection with molybdophosphoric acid and heating at 200°C for 10 min. PG, Phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PL1–3, three unknown phospholipids.
DESCRIPTION OF ALIDIOMARINA SEDIMENTI SP. NOV.

Alidiomarina sedimenti (se.di-men’ti. L. gen. n. sedimenti, of sediment).

Cells are Gram-staining-negative, motile, straight or slightly curved rods. Cells are approximately 0.3–0.4 μm wide and 1.2–2.7 μm long. Heterotrophic and strictly aerobic. Colonies grown for 24 h at 34 °C on R2A medium with 5 % NaCl are circular with entire margins (1–2 mm in diameter), convex, mucoid, non-pigmented and translucent. Growth occurs at 4–40 °C (optimum 34 °C), at pH 7.0–10.0 (optimum pH 8.5) and with 1–15 % NaCl (optimum 5 %). Positive for oxidase and catalase activities, but negative for arginine dihydrolase and ornithine decarboxylase. Indole negative for oxidase and catalase activities, but negative for arginine dihydrolase and ornithine decarboxylase. Indole

The type strain is GBSY1 = (9), isolated from the Pacific Ocean, including descriptions of two species, Alidiomarina abyssalis sp. nov. and Alidiomarina zabelii sp. nov. Int J Syst Evol Microb 2000;50:901–907.


Funding information
This work was supported by grants from Iranian Biological Resource Centre (IBRC) (MI-1391-15) (to M.A. Amoozegar), from Spanish Ministerio de Economía y Competitividad (CGL2013-46941-P) and Junta de Andalucía (P10-CVI-6226), both include European funds (FEDER) (to A. Ventosa).

Acknowledgement
We thank B. Schink for his help with the etymology of the new taxon.

Conflicts of interest
The author declare that there is no conflicts of interest.

References

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