Tamilnaduibacter salinus gen. nov., sp. nov., a halotolerant gammaproteobacterium within the family Alteromonadaceae, isolated from a salt pan in Tamilnadu, India

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Two novel Gram-stain-negative, slow-growing, halotolerant strains with rod-shaped cells, designated as strains Mi-7T and Mi-8, which formed pin-point colonies on halophilic media were isolated during a study into the microbial diversity of a salt pan in the state of Tamilnadu, India. Both the strains had an obligate requirement for 1 % (w/v) NaCl for growth and were halotolerant, growing at NaCl concentrations of up to 20 % (w/v) in media. The strains, however, showed an inability to utilize the majority of substrates tested as sole carbon sources for growth and in fermentation reactions. Molecular phylogenetic analyses, based on 16S rRNA gene sequence revealed their closest phylogenetic neighbours to be members of the genus Marinobacter, with whom they showed the highest sequence similarity of 93.6 % and even less with the type strain of the type species, Marinobacter hydrocarbonoclasticus DSM 8798T (91.1 %). Similarities with other genera within the family Alteromonadaceae were below 91.0 %. However, the two strains were very closely related to each other with 99.9 % sequence similarity, and DNA–DNA hybridization analyses confirmed their placement in the same species. The DNA G+C content of both strains was 65 mol%. Using the polyphasic taxonomic data obtained from this study, strains Mi-7T and Mi-8 represent two strains of the same species of a novel genus for which the name Tamilnaduibacter salinus gen. nov., sp. nov., is proposed; the type strain of the novel species is Mi-7T (=MTCC 12009T=DSM 28688T).

The family Alteromonadaceae currently comprises sixteen genera: Aestuariibacter (Yi et al., 2004), Agarivorans (Kurahashi & Yokota, 2004), Aliagarivorans (Jean et al., 2009), Alishewanella (Vogel et al., 2000), Alteromonas (Baumann et al., 1972), Bowmanella (Jean et al., 2006), Catenovulum (Yan et al., 2011), Glaciecola (Bowman et al., 1998), Haliea (Urios et al., 2008b), Marinimicrobium (Lim et al., 2006), Marinobacter (Gauthier et al., 1992), Marinobacterium (González et al., 1997), Melittea (Urios et al., 2008a), Microbulbifer (González et al., 1997), Saccharophagus (Ekborg et al., 2005) and Saliminonas (Jean et al., 2005). The group initially consisted of marine, heterotrophic, Gram-staining-negative, bacteria with one polar flagellum, requiring sodium chloride for their growth (Bowman & McMeekin, 2005). In the last decade, based on rapid advances in phylogenetic and molecular analyses, several revisions have been carried out in the family with numerous genera being carved out into separate families (Ivanova et al., 2004).

Sediment samples collected from the Thamaraikulam solar salt pan in the Kanyakumari district (red pigmented water sample; location 8° 07’ 08.92 N 77° 29’ 04.15’ E) in Tamilnadu were dilution-plated onto halophilic archaea medium (DSMZ medium no. 1184; yeast extract, 5.0 g; KCl, 5.0 g; MgCl₂·6H₂O, 32.5 g; MgSO₄·7H₂O, 50.8 g; CaCl₂·2H₂O, 0.8 g; NaHCO₃, 0.16 g; NaBr, 0.60 g; NaCl, 195.0 g; all 1⁻¹ in distilled water, pH 6.5–7.0) and incubated at 30 °C for 4–6 weeks. Two separate colonies were isolated after about 1 week from one of the dilution plates and subcultured in the same medium. Both the strains (later designated Mi-7T and Mi-8) were purified after growth for 2 weeks on halophilic medium and preserved at −70 °C in 10 % (v/v) glycerol. Initially, sufficient growth of the strains in halophilic archaea

Abbreviation: MALDI-TOF, matrix assisted laser desorption/ionization-time of flight mass spectrometry.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of strains Mi-7T and Mi-8 are HG969252 and HG969253, respectively.

Three supplementary figures are available with the online Supplementary Material.
medium was observed only 3 weeks after incubation at 30 °C. To optimize the growth conditions of these strains, different growth media were tested, i.e. halophilic archaea medium with 10 % (w/v) NaCl (medium M1), marine agar (MA, Hi-Media), tryptic soya broth agar (TSBA, Hi-Media), artificial sea water [ASW; (% w/v) tris base, 0.6; MgSO4, 0.12; KCl, 0.074; (NH4)2PO4, 0.013; NaCl, 0.175; CaCl2·2H2O, 0.014; pH 7.2] supplemented with yeast extract (0.1 % w/v), halophilic media [Ventosa et al., 1982, (% w/v) NaCl, 17.8; MgSO4·7H2O, 0.1; CaCl2·2H2O, 0.036; KCl, 0.2; NaHCO3, 0.006; NaBr, 0.023; FeCl3·6H2O, trace; Proteose-peptone no. 3 (Difco), 0.5; yeast extract (Difco), 1.0; glucose, 0.1; Bacto-agar (Difco), 2.0; pH 7.2 adjusted with KOH] and DSMZ medium no. 73 (% w/v) casamino acids, 1; yeast extract, 1; NaCl, 10; pH 7.2). However, none of the media tested supported growth of the strains, except for medium M1, in which sufficient growth was obtained after 4–5 days at 30 °C.

Morphological tests, Gram staining and endospore staining were performed according to standard methods (Smibert & Krieg, 1994). Motility of cells grown for 4–5 days (on medium M1) was determined by the hanging drop method and observation under a phase-contrast microscope (Olympus model BX 51TRF). Oxidase and catalase activities were determined using oxidase discs (Hi-Media) and 3 % (v/v) H2O2, respectively, according to methods described by Smibert & Krieg (1994). The ability to grow at different temperatures, salt concentrations and pH [using biological buffers, as explained by Breznak & Costilow (1994)] was investigated using M1 broth. Biochemical tests, such as the methyl red test, Voges–Proskauer reaction, indole production and nitrate reduction, were performed according to standard procedures (Länyi, 1987) using M1 as the basal medium. Acid production from sugars, arginine dihydrolase, lysine and ornithine decarboxylase activities, and casein, aesculin, gelatin and starch hydrolysis reactions were examined in M1 medium, using a HI25 Enterobacteriaceae identification and HiCarbo kit according to the manufacturer’s instructions (Hi-Media). Phenotypic characteristics such as utilization of carbohydrates, acid production and various enzymic activities were also determined using API 20NE, API ZYM kits and VITEK systems (bioMérieux) and the Biolog GN2 system according to the manufacturers’ instructions, with the exception that sterile M1 medium (with yeast extract at 0.01 %, w/v) was used to prepare the inoculum for the API NE and API ZYM strips, according to the recommendations of Ventosa et al. (1982).

For analysis of cellular fatty acids and matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF), strains Mi-7T, Mi-8 and Marinobacter hydrocarbonoclasticus DSM 8798T were grown on M1 agar medium for 4 days at 30°C. Fatty acid methyl esters were extracted and analysed as described previously (Krishnamurthi et al., 2009) using the Microbial Identification System (MIDI). For analyses of isoprenoid quinones and polar lipids, the strains were cultivated in M1 broth for 3 days in a rotary shaker (200 r.p.m.) at their respective optimum growth temperatures. Marinobacter hydrocarbonoclasticus DSM 8798T was cultivated on TSB for 2–3 days at 30°C. Lipids and quinones were extracted and analysed as described previously (Krishnamurthi et al., 2009). For MALDI-TOF analyses, the cell extracts were prepared by suspending 5–10 mg of cells in 1 ml 90 % (v/v) absolute ethanol and mixed thoroughly. After centrifuging twice at 16 873 g for 2 min in a microcentrifuge to completely remove the residual ethanol, extraction was carried out with 70 % (v/v) formic acid/acetonitrile (1:1, v/v). After centrifugation, 1 μl of supernatant was placed onto the target, air-dried at room temperature, overlaid with 1 μl of matrix solution and again dried. MALDI-TOF mass spectra measurements were carried out using an Ultraflex III instrument (Bruker Daltonik) operated on default settings. External calibration of the mass spectrometer was performed using Escherichia coli DH5α standard peaks (4346.3, 5095.8, 5380.4, 6254.4, 7273.5 and 10299.1 Da). Mass spectra processing and dendrogram analysis were performed using Flex Analysis (version 3.0; Bruker Daltonik) and BioTyper software (version 1.1; Bruker Daltonik).

Genomic DNA isolation, PCR amplification, purification and sequencing were performed according to protocols mentioned elsewhere (Krishnamurthi et al., 2009). The identification of phylogenetic neighbours was initially carried out using a program to check against the database of type strains of prokaryotes with validly published names in the EzTaxon server (http://www.ezbiocloud.net/eztaxon) (Kim et al., 2012). The 16S rRNA gene sequence of strains Mi-7T and Mi-8 were aligned with the sequences of other closely related species within the family Alteromonadaceae retrieved from the NCBI database. Sequence alignment, editing and phylogenetic tree reconstruction were performed according to methods outlined by Krishnamurthi et al. (2009).

Genomic DNA for base composition analysis and DNA–DNA hybridization was prepared essentially according to the method of Marmur (1961) except that GES reagent [guanidine thiocyanate/EDTA/Sarkosyl (Pitcher et al., 1989)] was used as the lysis solution. The G+C content of the genomic DNA was determined spectrophotometrically (Lambda 35 spectrophotometer; Perkin Elmer) using the thermal denaturation method (Mandel & Marmur, 1968). DNA–DNA hybridization was carried out between strains Mi-7T and Mi-8 using the membrane filter method (Tourouva & Antonov, 1987), as described by Reddy et al. (2003), except that the probe labelling for DNA–DNA hybridization was conducted by using the nonradioactive DIG-High prime system (Roche). Hybridized DNA was visualized using a DIG luminescent detection kit (Roche) and the level of DNA–DNA relatedness was quantified by measuring the intensity of the spots using densitometry software for gel documentation (Alpha Imager).

Phenotypic properties of the two novel strains (Mi-7T and Mi-8), such as having a Gram-stain-negative cell wall, rod-shaped, non-spore-forming cells, strictly aerobic
Table 1. Comparative biochemical characteristics of strains Mi7<sup>T</sup> and Mi8 and members of the genera *Marinobacter*, *Marinimicrobium*, *Microbulbifer*, *Marinobacterium* and *Melitea*

<table>
<thead>
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<th>Characteristic</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>Cell size (µm)</td>
<td>2.0–3.0 0.3–0.6/1.7–3.7</td>
<td>1.6–3.6 × 0.1–0.4 0.5–0.8 × 0.9–1.1</td>
<td>1.1–1.7 × 0.3–0.5</td>
<td>1.6–2.3 × 0.5–0.7</td>
<td>1.6–2 × 0.5–0.9</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Temperature range for growth (optimum) (°C)</td>
<td>20–45 (25–30) 10–45 (32)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10–45 (35–40)</td>
<td>10–41 (37)</td>
<td>4–41 (37)</td>
<td>15–37 (30)</td>
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<tr>
<td>NaCl range for growth (optimum) (%)</td>
<td>1–20 (8–10) 0.5–20 (3–6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5–15 (1–3)</td>
<td>0.5–6 (0.5–3)</td>
<td>0.7–7 (4)</td>
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<tr>
<td>pH range for growth (optimum)</td>
<td>6–9 (7–7.5)/5–9 (6–7)</td>
<td>6.0–9.5 (7.0–7.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6–10.5 (7–7.5)</td>
<td>6–10 (7.5)</td>
<td></td>
<td></td>
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<tr>
<td>Nitrate reduced to nitrite</td>
<td>±</td>
<td>±</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
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<tr>
<td>Nitrate reduced to N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>±</td>
<td>±</td>
<td>–</td>
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<td>Hydrolysis of:</td>
<td>+/–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Starch</td>
<td>+/–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Assimilation of:</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
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<td>Potassium gluconate</td>
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<td>ND</td>
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<td>Trisodium citrate</td>
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<td>+</td>
<td>ND</td>
<td>ND</td>
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<td>Production of:</td>
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<td>+</td>
<td>±</td>
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<td>N-Acetyl-β-glucosaminidase</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
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<td>Alkaline phosphatase</td>
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<td>ND</td>
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<td>α-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>±</td>
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<td>Cystine arylamidase</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>Esterase lipase (C8)</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>w/w</td>
<td>w/w</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Trypsin</td>
<td>w/w</td>
<td>w/w</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:0, C&lt;sub&gt;16&lt;/sub&gt;:1ω7c, C&lt;sub&gt;16&lt;/sub&gt;:1ω9c, C&lt;sub&gt;16&lt;/sub&gt;:2ω7c, C&lt;sub&gt;10&lt;/sub&gt;:0, C&lt;sub&gt;10&lt;/sub&gt;:1ω7c, C&lt;sub&gt;10&lt;/sub&gt;:1ω9c</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;:0ω9c, C&lt;sub&gt;18&lt;/sub&gt;:0ω7c, C&lt;sub&gt;18&lt;/sub&gt;:1ω9c, C&lt;sub&gt;18&lt;/sub&gt;:1ω7c</td>
<td>C&lt;sub&gt;19&lt;/sub&gt;:1ω9c, C&lt;sub&gt;19&lt;/sub&gt;:1ω7c, C&lt;sub&gt;19&lt;/sub&gt;:1ω5c, C&lt;sub&gt;19&lt;/sub&gt;:1ω3c</td>
<td>iso-C&lt;sub&gt;15&lt;/sub&gt;:0ω9c, iso-C&lt;sub&gt;15&lt;/sub&gt;:0ω7c, iso-C&lt;sub&gt;15&lt;/sub&gt;:0ω5c, iso-C&lt;sub&gt;15&lt;/sub&gt;:0ω3c</td>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:0ω9c, iso-C&lt;sub&gt;16&lt;/sub&gt;:0ω7c, iso-C&lt;sub&gt;16&lt;/sub&gt;:0ω5c, iso-C&lt;sub&gt;16&lt;/sub&gt;:0ω3c</td>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0ω9c, iso-C&lt;sub&gt;17&lt;/sub&gt;:0ω7c, iso-C&lt;sub&gt;17&lt;/sub&gt;:0ω5c, iso-C&lt;sub&gt;17&lt;/sub&gt;:0ω3c</td>
</tr>
<tr>
<td>Predominant phospholipids</td>
<td>DPG, PG, PE, PL</td>
<td>DPG, PE, APL1, PL2</td>
<td>PE, PG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>DPG, PG, AL</td>
<td></td>
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<tr>
<td>Isoprenoid quinone</td>
<td>Q-9</td>
<td>Q-9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Q-8</td>
<td>ND</td>
<td>Q-8&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>65.0</td>
<td>58.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>57</td>
<td>58</td>
<td>55</td>
<td>57</td>
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</tbody>
</table>

*Data from: a, Gauthier et al. (1992); b, Yoon et al. (2003); c, Yoon et al. (2007); d, Nishijima et al. (2009); e, Sproer et al. (1998); f, Satomi et al. (2002).
metabolism, requirement for Na\textsuperscript{+} ions for growth, positive reactions for oxidase and catalase and negative reactions for indole production, indicated typical properties of species of Alteromonas-like gammaproteobacteria (Ivanova et al., 2004). Comparative biochemical properties of the two novel strains along with Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} indicated that both strains differed from the latter in their physiological characteristics, such as optimum temperature, NaCl concentration and pH for growth (Table 1). In terms of enzyme activities, both strains showed positive responses for alkaline phosphatase, cystine arylamidase, esterase, esterase lipase, leucine arylamidase, lipase and valine arylamidase and negative responses for N-acetyl-\(\beta\)-glucosaminidase, whereas Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} was negative for the former substrates and positive for the latter (Table 1). Both strains, however, were not able to utilize a wide range of substrates either as the sole carbon source in assimilation tests or in fermentation reactions (Table 1). In this respect the strains showed some similarity in their biochemical patterns to Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T}, which was also not able to utilize most of the carbon sources, except for malic acid and trisodium citrate in the basal medium M1 (Table 1), utilized by strain Mi-8 but not Mi-7\textsuperscript{T}. Moreover strain Mi-7\textsuperscript{T} was able to hydrolyse starch, which was not the case for Marinobacter hydrocarbonoclasticus (Table 1). In this respect the strains showed some similarity in their biochemical patterns to Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T}, which was also not able to utilize most of the carbon sources, except for malic acid and trisodium citrate in the basal medium M1 (Table 1), utilized by strain Mi-8 but not Mi-7\textsuperscript{T}. Moreover strain Mi-7\textsuperscript{T} was able to hydrolyse starch, which was not the case for strain Mi-8 (Table 1). In the Biolog GN2 and VITEK systems, both strains were unable to oxidize the majority of the substrates and there were few differences between them and Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T}.

The results of fatty acid analysis indicated that the composition of the two strains, Mi-7\textsuperscript{T} and Mi-8, was qualitatively and quantitatively different from that of Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} (Table 2). Both the strains showed the presence of fatty acid C\textsubscript{16:0} (about 20 % of total fatty acids), which is one of the major fatty acids of the family Alteromonadaceae and is present in the majority of the genera of the family, i.e. Alteromonas, Agarivorans, Marinobacter, Salinimonas, Aliagarivorans, Bowmanella, Catenovulum, Glaciecola, etc. Both strains contained summed features 3 (C\textsubscript{16:1\~v\,7c}/C\textsubscript{16:1\~c\,6c}) and 9 (C\textsubscript{16:0} 10-methyl/iso-C\textsubscript{17:1\~c\,9c}) as major components, whereas Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} showed these in smaller amounts (<5 %, Table 2). Additionally Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} had C\textsubscript{18:1\~c\,9c} as the predominant fatty acid (>30 %), whereas the two novel strains had minute quantities of this fatty acid (<2 %; Table 2).

Analyses of two-dimensional lipid profiles indicated that strains Mi-7\textsuperscript{T} and Mi-8 had identical lipid profiles (Fig. S1, available in the online Supplementary Material) with diphosphatidylglycerol (DPG), phosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholid present in major amounts and two aminophospholipids (APL1 & 2) occurring in minor quantities (Fig. S1). Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} differed from the two novel strains in not containing DPG and had two APLs (with different Rf values) and one unknown lipid (Fig. S1, Table 1). MALDI-TOF analyses indicated the presence of some unique peaks in Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} which were absent in strains Mi-7\textsuperscript{T} and Mi-8 (marked by arrows in Fig. S2). Therefore, data from polar lipid profiles and mass spectral analyses supported the phylogenetic analyses and justifies the placement of the two strains within a novel genus.

Phylogenetic analyses of strains Mi-7\textsuperscript{T} and Mi-8 revealed that they belong to the class Gammaproteobacteria within the family Alteromonadaceae (Figs 1 and S3, the latter shows the full tree). Both strains showed high 16S rRNA gene sequence similarities to each other (99.9 %). The highest sequence similarity was with Marinobacter pelagius HS225\textsuperscript{T} (93.6 %); Marinobacter hydrocarbonoclasticus DSM 8798\textsuperscript{T} had a similarity of 91.1 %, with an overall similarity of 91.1–93.6 % with the genus Marinobacter. Pairwise sequence similarity of the two strains to other species of the genera within the family Alteromonadaceae was less than 91 % in the following decreasing order; Marinimicrobium (89.6–90.5 %), Microbulbifer (86.9–90.0 %), and <90 % to the following genera: Marinobacterium, Melitea, Halicea, Alteromonas, Aliagarivorans, Agarivorans, Aliishewanna, Bowmanella, Glaciecola, Aestuariibacter, Catenovulum, Salinimonas and Saccharophagus. This low level of similarity clearly indicates that the two strains might

<table>
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<th>Fatty acid</th>
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<td>C\textsubscript{12:0} 3-OH</td>
<td>9.0</td>
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<td>C\textsubscript{14:0}</td>
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<td>C\textsubscript{16:0}</td>
<td>26.1</td>
<td>22.5</td>
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<td>Summed features*</td>
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<tr>
<td>3</td>
<td>24.7</td>
<td>21.0</td>
<td>6.9</td>
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<td>9</td>
<td>9.8</td>
<td>14.7</td>
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<td>8</td>
<td>4.8</td>
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<tr>
<td>C\textsubscript{16:1~v,9c}</td>
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<td>C\textsubscript{18:0,9c}</td>
<td>&lt;1.0</td>
<td>1.1</td>
<td>31.6</td>
</tr>
<tr>
<td>iso-C\textsubscript{16:0}</td>
<td>1.8</td>
<td>1.7</td>
<td>0.2</td>
</tr>
<tr>
<td>iso-C\textsubscript{17:0}</td>
<td>&lt;1.0</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>iso-C\textsubscript{17:0}</td>
<td>1.8</td>
<td>3.1</td>
<td>0.5</td>
</tr>
<tr>
<td>C\textsubscript{17:1~v,8c}</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>3.5</td>
</tr>
<tr>
<td>C\textsubscript{18:0}</td>
<td>2.5</td>
<td>2.6</td>
<td>2.9</td>
</tr>
</tbody>
</table>

*Summed features 3 (C\textsubscript{16:1\~v\,7c} and C\textsubscript{16:1\~c\,6c}), 8 (C\textsubscript{18:0\,7c}/C\textsubscript{18:1\~c\,6c}) and 9 (C\textsubscript{16:0} 10-methyl/iso-C\textsubscript{17:1\~c\,9c}) consist of a mixture of fatty acids which are not separated by the MIDI-GC Sherlock system.
belong to a novel genus within the family *Alteromonadaceae*. A tree reconstructed using the neighbour-joining method placed strains Mi-7^T^ and Mi-8 in a clade well separated from the genus *Marinobacter* and other genera within the family (Fig. 1). This tree topology was well supported using the maximum-parsimony and maximum-
likelihood algorithms (Fig. 1). It is interesting to note that in the tree, *Marinobacter lutaoensis* T5054\(^T\) branches off quite early in the evolutionary line of descent from the clade containing all species of the genus *Marinobacter* indicating that this species might be wrongly placed in the genus. However, further debating of that point is beyond the scope of this manuscript. The low sequence similarity (<95% 16S rRNA gene sequence similarity was proposed as a threshold value for proposing a novel genus by Ludwig *et al.* (1998)) along with phylogenetic evidence (Figs 1 and S3) clearly indicates that the two strains represent a novel species of a novel genus within the family *Alteromonadaceae*.

Whole genome DNA–DNA hybridization of strains Mi-7\(^T\) and Mi-8 indicated a high genomic relatedness of >70%. Genome relatedness was 100% when Mi-7\(^T\) DNA was used as a probe and it was 78% when Mi-8 was used as a probe. The DNA of *Marinobacter hydrocarbonoclasticus* DSM 8798\(^T\) showed a relatedness of 41% and 33% when Mi-7\(^T\) and Mi-8 were used as probes, respectively. This indicates that both strains have high genomic relatedness and according to the recommendations of Stackebrandt & Goebel (1994) belong to the same species. The DNA G+C content of the two strains was 65%, which is higher than that reported for any species of the genus *Marinobacter* [range of 54.1–63.5% with the lowest value for *Marinobacter koreensis* (Kim *et al.*, 2006) and the highest value for *Marinobacter lutaoensis* (Gauthier *et al.*, 1992)] and reasonably above that of the type species *Marinobacter hydrocarbonoclasticus* DSM 8798\(^T\) (Table 1). Therefore, based on the phenotypic, chemotaxonomic and phylogenetic properties strains Mi-7\(^T\) and Mi-8 represent the same novel species of a novel genus for which the name *Tamilnaduibacter salinus* gen. nov., sp. nov. is proposed.

**Description of *Tamilnaduibacter salinus* sp. nov.**

*Tamilnaduibacter salinus* (sa.li’n us. L. masc. adj. salinus of or belonging to salt).

Cells take 4–5 days to grow in M1 medium at 30 °C and form pin-point cream colonies. Very specific for requirements with respect to growth medium; does not grow in any marine water simulated media (marine agar, artificial seawater supplemented media, etc.) except for M1 medium. The size of cells is 1.5–3.7 × 0.1–0.6 μm. The temperature and pH ranges for growth are 20–45 °C and pH 6.0–9.0, respectively. Requires a minimum of 1% (w/v) NaCl for growth, is extremely halotolerant and has the ability to grow in M1 medium supplemented with up to 20% (w/v) NaCl. Unable to utilize the majority of substrates for growth and in fermentation reactions as shown in Table 1 and described below. In addition to the characteristics given in the genus description, produces the enzymes lipase and tyrosine arylamidase and is negative for the utilization of adonitol, L-arabitol, cellobiose, d-glucose, 5-keto-d-glucanote, malonate, maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, D-tagatose and sodium citrate in the VITEK system. Negative for the assimilation of L-histidine, L-lactate and L-malate. Negative for H2S production and the enzymes acetylglycosaminidase, alanine arylamidase, alpha-3,4-pro-arylamidase, alpha-galactosidase, beta-galactosidase, alpha-glu- cosidase, beta-glucosidase, glutamyl arylamidase, gamma-glutamyltransferase, glycine arylamidase, gly-gly-ary-arylamidase, lysine decarboxylase, ornithine decarboxylase, phosphatase, proline arylamidase, L-tryptophan arylamidase, tyrosine arylamidase, urease and xylosidase. In the Biolog GN2 system, the type strain is positive for oxidation of L-arabinose and Tween 80 and negative for the rest of the substrates.

The type strain Mi-7\(^T\) (=MTCC 12009\(^T\)=DSM 28688\(^T\)) and strain Mi-8 were isolated from a salt pan in Tamilnadu, India. The DNA G+C content of the two strains is 65 mol%.

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**References**


Int J Syst Bacteriol
sea-ice habitats.

Pseudoalteromonadaceae
fam. nov., sp. nov.: psychrophilic bacteria from Antarctic

2653–2656.

Syst Evol Microbiol
sp. nov., a

19567569

International Journal of Systematic and Evolutionary Microbiology
Marinimicrobium koreense

Agarivorans albus

Krishnamurthi, S., Bhattacharya, A., Mayilraj, S., Saha, P., Schumann,

Microbulbifer hydrolyticus
Gonza´lez, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman,

56


Oceanospirillum jannaschii

Sporosarcina
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comb. nov. and

Oceanospirillum marinus gen. nov., sp. nov. and

Marinobacter hydrocarbonoclasticus

Physiological study of the genus Oceanospirillum based on 16S rRNA and gyrB genes: emended description of the genus Oceanospirillum, description of Pseudospirillum gen. nov., Oceanobacter gen. nov. and Terasakiella gen. nov. and transfer of Oceanospirillum jannaschii and Pseudomonas staniieri to Marinobacterium as Marinobacterium jannaschii comb. nov. and Marinobacterium staniieri comb. nov.


Sporosarcina macrumdoromus sp. nov., from a cyanobacterial mat sample from a pond in the McMurdo Dry Valleys, Antarctica.


Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA.


Pitarch, A. Verma and others

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