Salterns represent a hypersaline environment and are used for the commercial manufacture of salt from hypersaline water. Bacteria and archaea are the predominant microorganisms in these ecosystems and there exists great microbial diversity within them (Oren, 2002). Most of the bacteria isolated from these ecosystems are moderately halophilic and are adapted to high salt concentrations. Most of the bacterial diversity associated with marine environments has been revealed recently by using conventional cultivation approaches with the application of molecular biology techniques (Thiyagarajan et al., 2010; Eilers et al., 2000). Here, we report a novel bacterium, strain AK4T, isolated from a solar saltern, which was characterized by using a polyphasic taxonomic approach.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain AK4T is closely related to members of the genus Caenispirillum. The genus Caenispirillum was proposed by Yoon et al. (2007) to accommodate a single species, Caenispirillum bisanense, members of which were isolated from sludge of a dye works in Korea.

A novel Gram-negative, vibio-shaped, motile bacterium, designated strain AK4T, was isolated from a sediment sample collected from a solar saltern at Kakinada, Andhra Pradesh, India. Strain AK4T was isolated from a sediment sample collected from a solar saltern at Kakinada, Andhra Pradesh, India. Strain AK4T was positive for oxidase, urease and DNase activities but negative for gelatinase, catalase, ornithine decarboxylase, lysine decarboxylase, nitrate reduction, aesculin, indole and lipase activities. The fatty acids were dominated by unsaturated components, with a high abundance of summed feature 8 (C18:1ω7c and/or C18:1ω6c) and C17:1ω6c. Strain AK4T contained Q-10 as the major respiratory quinone and phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine as major polar lipids. The DNA G+C content of strain AK4T was 71.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain AK4T was most closely related to the type strain of Caenispirillum bisanense of the family Rhodospirillaceae (phylum ‘Proteobacteria’) (96.6% sequence similarity). It shared <93.2% 16S rRNA gene sequence similarity with other members of the family. Based on phenotypic characteristics and phylogenetic inference, strain AK4T is considered to represent a novel species of the genus Caenispirillum, for which the name Caenispirillum salinarum sp. nov. is proposed; the type strain is AK4T (=MTCC 10963T =JCM 17360T).

Strain AK4T was isolated from a sediment sample collected from a solar saltern at Kakinada, Andhra Pradesh, India. The sediment sample had a pH of 8.0. The sample (1 g) was serially diluted (10-fold dilutions) in 2% (w/v) NaCl solution and 100 μl of each dilution was plated on Zobell marine agar (ZMA) medium and incubated at 30 °C. A shiny, creamish to light brown colony, which was observed after 5 days of incubation, was purified and preserved at −70 °C as a glycerol stock for further characterization.

Cell morphology studies of strain AK4T were performed by phase-contrast microscopy (Olympus BX51) and transmission electron microscopy (JEOL 1200 EX II). Physiological properties such as growth at different temperatures (4, 10, 18, 30, 37 and 40 °C) and pH (5, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11 and 12) were determined by using Zobell marine medium, and growth at different NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 8 and 10%, w/v) was tested on Zobell marine medium without NaCl as the basal medium. The different biochemical tests listed in the species description below and in Table 1 were carried out at 30 °C on Zobell marine medium as described by Lányi (1987) and Smibert & Krieg (1994). The ability of strain AK4T to utilize single carbon compounds as the sole carbon source was checked on minimal medium prepared with artificial seawater supplemented with 0.5% (w/v) of each filter-sterilized carbon compound. The sensitivity of
strain AK4T to antibiotics was tested on ZMA by using various antibiotic discs (Hi-Media). Biochemical and enzyme characterization of strain AK4T was performed by using a Vitek 2 GN (bioMérieux) assay with incubation at 30°C, according to the manufacturer’s instructions, except that sterile 2.0 % (w/v) NaCl was used to prepare the inocula (Table S1 available in IJSEM Online).

Fatty acid methyl esters were prepared (classical method) and were analysed by using the Sherlock Microbial Identification System (MIDI-6890 with database TSBA6). For this, strain AK4T and Caenispirillum bisanense JCM 14346T were grown on ZMA at 30°C under aerobic conditions for 3 days and 1 day, respectively. Isoprenoid quinones and polar lipids were analysed by using lyophilized biomass of strain AK4T and C. bisanense JCM 14346T grown on Zobell marine broth at 30°C under aerobic conditions (150 r.p.m.) for 2 days. Polar lipids were extracted according to the method described by Minnikin et al. (1979) and were identified by two-dimensional TLC as described by Collins & Jones (1980). Isoprenoid quinones were extracted as described by Collins et al. (1977) and were analysed by HPLC (LC-10AT VP, Shimadzu liquid chromatography with SPD-M10AVP diode array detector) according to Groth et al. (1997). Genomic DNA was isolated by using the procedure of Marmur (1961) and the G+C content was determined from melting point (Tm) curves (Sly et al., 1986) obtained by using a Lambda 35 spectrophotometer (Perkin Elmer) equipped with Peltier system (PTP-1).

For 16S rRNA gene sequencing, DNA was prepared by using a bacterial DNA isolation kit (Genomic DNA kit; Qiagen). The 16S rRNA gene was amplified by PCR with universal bacterial primers 27f (5’-AGAGTTTGATCC-TGGCTCAG-3’) and 1492r (5’-TACGGYTACCTTGTTACGACTT-3’). The PCR product was purified via a QIAquick PCR purification kit (Qiagen) and it was sequenced by using an ABI PRISM model 3700 automatic DNA sequencer and Big Dye Terminator cycle sequencing.

### Table 1. Differential characteristics between strain AK4T and the type strain of Caenispirillum bisanense

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width (µm)</td>
<td>0.8–1.2</td>
<td>0.5–0.7</td>
</tr>
<tr>
<td>Length (µm)</td>
<td>2–6</td>
<td>0.7–7.0</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Vibrio</td>
<td>Spiral</td>
</tr>
<tr>
<td>NaCl range (%)</td>
<td>2–8</td>
<td>0–7</td>
</tr>
<tr>
<td>Growth in the absence of NaCl</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Optimum growth conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl concentration (%)</td>
<td>2–4</td>
<td>0–4</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30–35</td>
<td>32–37</td>
</tr>
<tr>
<td>pH</td>
<td>7–8</td>
<td>7.5–8.5</td>
</tr>
<tr>
<td>Growth on trypticase soy agar</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 40</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Vitek 2 GN results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate alkalization</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lipase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Malate assimilation</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine arylamidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Histidine assimilation</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>γ-Glutamyl-transferase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>Q-10, Q-11</td>
<td>Q-10</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PE, PG, PC, AL1–2, UL1–3</td>
<td>DPG, PE, PG, PC, AL1–2, UL1–2</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>71.0</td>
<td>72.0</td>
</tr>
</tbody>
</table>

*AL, Unknown aminolipid; DPG, diphasphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, unknown phospholipid; UL, unknown lipid.
Cells of strain AK4<sup>T</sup> were vibrioid (0.8–1.2 × 4.0–6.0 μm) (Fig. S1) and multiplied by binary fission. Cells were Gram-negative and motile by means of a single monopolar flagellum. Colonies were circular, 2–4 mm in diameter, smooth, creamish to light brown, translucent and convex when grown on ZMA. Chemoheterotrophic growth was observed. The strain grew at 15–45 °C (optimum at 30–37 °C) and at pH 6–10 (optimum at pH 7.5–8.5). Growth occurred at salinities of 2–8% (w/v) NaCl, with optimum at 2–4% (w/v) NaCl. The fatty acid profile was dominated by unsaturated components: summed feature 8 (C<sub>16:1</sub>ω7c and/or C<sub>18:1</sub>ω6c, 34.84%) and C<sub>17:1</sub>ω6c (20.63%) (Table 2). The ubiquinones present in strain AK4<sup>T</sup> were Q-10 (90%) and Q-11 (10%). The polar lipid profile of strain AK4<sup>T</sup> comprised phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, diphosphatidylglycerol, and two unidentified amnolipids and three unidentified unidentified lipids (Fig. S2). The DNA G+C content of strain AK4<sup>T</sup> was 71.0 mol% (T<sub>M</sub>) (Table 1).

The phylogenetic relationship of strain AK4<sup>T</sup> was analysed based on levels of 16S rRNA gene sequence similarity with related species by using BLAST (EzTaxon). The results indicated that strain AK4<sup>T</sup> was related most closely to <i>C. bisanense</i> JCM 14346<sup>T</sup> with a pairwise 16S rRNA gene sequence similarity of 96.6%. Phylogenetic analysis based on maximum-likelihood and neighbour-joining trees also indicated that strain AK4<sup>T</sup> clustered with <i>C. bisanense</i> (Fig. 1).

Strain AK4<sup>T</sup> could be differentiated phenotypically from <i>C. bisanense</i> JCM 14346<sup>T</sup> (Table 1). It differed with respect to cell size and shape, NaCl requirement, salt tolerance and growth temperature range. Differences were also observed in the fatty acid, polar lipid and quinone profiles and in the DNA G+C content (Table 1). Thus, based on phenotypic, chemotaxonomic and phylogenetic characteristics, we consider that strain AK4<sup>T</sup> represents a novel species of the genus <i>Caenispirillum</i>, for which the name <i>Caenispirillum salinarum</i> sp. nov. is proposed.

### Description of Caenispirillum salinarum sp. nov.

*Caenispirillum salinarum* (sa.li.na’rum. L. pl. n. salinae saltpits, saltworks; N.L. gen. pl. n. salinarum of saltworks, from which the type strain was isolated).

Cells are Gram-negative, non-spore-forming and vibrio-shaped (0.8–1.2 μm wide, 2.0–6.0 μm long). Cells form chains, multiply by binary fission and are motile by means of a single monopolar flagellum. Colonies on marine agar are circular, 2–4 mm in diameter, smooth, creamish to light brown, translucent and convex. Grows well at 30–35 °C, and optimally at pH 7–8. Optimal growth occurs in the presence of 2–4% (w/v) NaCl; growth does not occur in the absence of NaCl or in the presence of greater than 8% (w/v) NaCl. Tewens 20, 40 and 60 are not hydrolysed. DNase and oxidase activities are present but catalase and nitrate reduction activities are absent. The following substrates are not utilized: sorbitol, adonitol, melibiose, sucrose, trehalose, lactose, dulcitol, salicin, raffinose, inulin, cellobiose, adonitol and galactose. Positive reactions (from VITEK 2 GN) in tests for urease and l-proline arylamidase but not for N-acetyl-β-glucosaminidase, N-acetyl-β-galactosaminidase, phosphatase, citrate utilization, lysine decarboxylase, ornithine decarboxylase, l-pyrolidonyl-arylamidase, Ala-Phe-Pro-arylamidase, tyrosine arylamidase, glutamyl arylamidase, pNA, β-glucosidase, β-xilosidase, glycine arylamidase, Glu-Gly-Arg-arylamidase, α-galactosidase, β-glucuronidase, α-glucosidase, lipase, palatinose, β-alanine arylamidase pNA, γ-glutamyl-transferase or β-galactosidase. Susceptible to (μg per disc) ampicillin (25), neomycin (30), chloramphenicol (30), kanamycin (30), gentamicin (10), penicillin (20), polymyxin (300), lincomycin (2), tetracycline (30), novobiocin (30), chlorotetracycline (30), vancomycin (30), spectinomycin (100), cefadroxil (30), bacitracin (8), amoxicillin (30), streptomycin (30), cephalothin (30), ciprofloxacin (10) and cephadoxil (30). The fatty acid profile is dominated by unsaturated components, such as summed feature 8 (C<sub>18:1</sub>ω7c and/or C<sub>18:1</sub>ω6c) and

### Table 2. Fatty acid composition of strain AK4 and Caenispirillum bisanense JCM 14346<sup>T</sup>

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;12:0&lt;/sub&gt;</td>
<td>6.77</td>
<td>2.14</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>3.27</td>
<td>6.78</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:1ω8c&lt;/sub&gt;</td>
<td>5.06</td>
<td>0.49</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:1ω6c&lt;/sub&gt;</td>
<td>20.63</td>
<td>0.85</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1ω2OH&lt;/sub&gt;</td>
<td>6.33</td>
<td>5.89</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>6.09</td>
<td>5.79</td>
</tr>
<tr>
<td>Summed feature 8*</td>
<td>34.84</td>
<td>68.29</td>
</tr>
</tbody>
</table>

*Summed feature 3 comprised C<sub>16:1ω7c/C16:1ω6c. Summed feature 8 comprised C<sub>18:1ω7c and/or C<sub>18:1ω6c.}

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On: Sat, 29 Dec 2018 11:58:16
C17:1ω6c (Table 2). The ubiquinones present are Q-10 (90%) and Q-11 (10%). The polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, diphosphatidylglycerol, two unidentified aminolipids and three unidentified lipids.

The type strain, AK4T (MTCC 10963T=JCM 17360T), was isolated from a sediment sample collected from a solar saltern at Kakinada, Andhra Pradesh, India. The G+C content of the genomic DNA of the type strain is 71.0 mol%.

Acknowledgements

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References


