Halophilic *Archaeca* belonging to the order *Halobacteriales* are found in large numbers in the crystallizer ponds of solar salterns worldwide (Kamekura & Dyall-Smith, 1995; Oren *et al.*, 1997; Grant, 2004; Fendrihan *et al.*, 2006). Several saline and hypersaline environments in Spain have been studied and many halophiles have been isolated from them. A few studies have described halophilic bacteria from the Fuente de Piedra saline lake in the province of Malaga, southern Spain (Martinez-Canovas *et al.*, 2004; Martinez-Checa *et al.*, 2005; Mata *et al.*, 2006), but no extremely halophilic archaea have been described from this lake. In the present study, a novel member of the genus *Haloterrigena* is proposed on the basis of conventional physiological, biochemical and chemical characteristics and through phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA hybridization results. At the time of writing, the genus *Haloterrigena* comprises five species of extremely halophilic archaea, *Haloterrigena turkmenica* (Ventosa *et al.*, 1999), *Haloterrigena thermotolerans* (Montalvo-Rodriguez *et al.*, 2000), *Haloterrigena saccharevitans* (Xu *et al.*, 2005a), *Haloterrigena longa* and *Haloterrigena limicola* (Cui *et al.*, 2006). Owing to taxonomic problems arising within the genera *Haloterrigena* and *Natrinema* (Montalvo-Rodriguez *et al.*, 2000; Xin *et al.*, 2000; Tindall, 2003; Xu *et al.*, 2005b; Wright, 2006; Castillo *et al.*, 2006), a combination of other morphological and chemotaxonomic characters such as lipid analyses have been used to distinguish between species of these two genera.

Strain FP1⁰ was isolated from samples collected during summer 2005 from Fuente de Piedra saline lake, Malaga province, southern Spain (37° 6’ N 4° 44’ W).
Strain FP1T was isolated from a salt pan crystallizer pond by the dilution-plating technique. This strain represented the predominant organism in the enrichment and was the only colony-forming organism at the highest dilutions. Enrichment medium (medium 1; DSM 372) contained the following components (per litre): 5.0 g yeast extract (Oxoid), 5.0 g Casamino acids (Oxoid), 3.0 g trisodium citrate (Applichem), 2.0 g KCl (Applichem), 20 g MgSO4·7H2O (Carlro Erba), 200 g NaCl (Applichem), 0.36 µg MnCl2·4H2O (J.T. Baker) and 0.05 g FeSO4·7H2O (Carlro Erba). The pH of medium 1 was 7.0. Growth on single carbon sources (medium 2) was tested on liquid media containing (per litre) 200 g NaCl (Applichem), 2.0 g KCl (Applichem), 1 g MgSO4·7H2O (Carlro Erba), 16.4 g MgCl2·6H2O (Riedel-de Haën), 0.2 g NaHCO3 (J.T. Baker), 2.29 g CaCl2·2H2O (J.T. Baker), 152 mg NH4Cl (Appli chem), 33 mg K2HPO4 (Appli chem), 0.26 mg FeCl3·4H2O (J.T. Baker) and 10.0 g of the test compound. Solid media were prepared by the addition of 1.8 % (w/v) agar.

*Haloterrigena turkmenica* JCM 9191T, *Haloterrigena thermotolerans* DSM 11552T, *Haloterrigena saccharovorans* JCM 12889T, *Haloterrigena limicola* JCM 13563T and *Natrinema pellirubrum* JCM 10476T (McGenity et al., 1998), obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ) and from the Japan Collection of Microorganisms (JCM), were grown in the media suggested by the culture collections (DSMZ medium no. 372 for *Htg. turkmenica* and *Htg. thermotolerans*; JCM medium no. 168 for *Htg. saccharovorans*, *Htg. limicola* and *N. pellirubrum*). *Halofex mediterranei* CCM 3361T was grown as described by Lanzotti et al. (1988).

Cell morphology was determined by phase-contrast microscopy (Zeiss). Colony morphology was analysed on solid medium via a stereomicroscope (M8; Leica). Tolerance to NaCl and MgSO4 and growth at various temperatures and medium via a stereomicroscope (Zeiss). Colony morphology was analysed on solid medium (Fig. 1). 16S rRNA gene sequence similarities

In a phylogenetic tree based on 16S rRNA gene sequences, strain FP1T clustered with recognized species of the genus *Haloterrigena* (Fig. 1). 16S rRNA gene sequence similarities

The sequence of the novel strain was compared with closely related sequences of reference organisms from the FASTA network service. Sequence data were aligned with the CLUSTAL W 1.8 program (Chenna et al., 2003). Phylogenetic analysis was performed by using the PHYLIP package, version 3.6 (Felsenstein, 2004) and with the neighbour-joining method within the MEGA3 program package (Kumar et al., 2004). DNA–DNA hybridizations were performed at 48.5 °C according to Ezaki et al. (1989) and the levels of DNA–DNA relatedness were calculated according to Goris et al. (1998). Hybridizations were carried out between strain FP1T and related species (*Htg. thermotolerans* DSM 11552T, *Htg. saccharovorans* JCM 12889T and *Htg. limicola* JCM 13563T).

Cells of strain FP1T were coccoid, Gram-negative and able to grow over a restricted range of salinities (2.2–4.0 M NaCl). Colonies on agar medium were light red-pigmented. Strain FP1T did not require magnesium for growth and was unable to assimilate sugars (glucose, sucrose or maltose). Detailed results of morphological analyses, antibiotic sensitivity tests and biochemical tests for strain FP1T are given in the species description below.

In a phylogenetic tree based on 16S rRNA gene sequences, strain FP1T clustered with recognized species of the genus *Haloterrigena* (Fig. 1). 16S rRNA gene sequence similarities
between strain FP1<sup>T</sup> and *Htg. limicola* JCM 13563<sup>T</sup>, *Htg. thermotolerans* DSM 11552<sup>T</sup>, *Htg. saccharovorans* JCM 12889<sup>T</sup>, *Htg. turkenica* JCM 13562<sup>T</sup> were 98.9, 96.2, 95.6, 95.5 and 94.6 %, respectively, but the novel strain was also related to *Natrinema versiforme* JCM 10478<sup>T</sup> and *Natrinema altunense* JCM 12890<sup>T</sup> (96.1 and 95.3 % sequence similarity, respectively). The position of *Natrinema ejinorense* in this phylogenetic tree should be resolved in the near future (Fig. 1).

The polar lipid profile of strain FP1<sup>T</sup>, which comprised C<sub>20</sub>C<sub>20</sub> and C<sub>20</sub>C<sub>25</sub> derivatives of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and mannose-6-sulfate(1-2)-glucose glycerol diether (S-DGD), was consistent with that of species of the genus *Haloterrigena* but differed with regard to the glycolipid pattern. The type strains of recognized species of the genus *Haloterrigena* possess mannose-2,6-disulfate(1-2)-glucose glycerol diether (S<sub>2</sub>-DGD) (Ventosa *et al.*, 1999; Montalvo-Rodriguez *et al.*, 2000; Xu *et al.*, 2005a; Cui *et al.*, 2006), whereas strain FP1<sup>T</sup> lacks this component. Species of the genus *Natrinema* have been shown to possess unknown glycolipids and sulfated glycolipids (McGenity *et al.*, 1998; Xin *et al.*, 2000; Xu *et al.*, 2005b) (see Supplementary Fig. S1 in IJSEM Online). Polar lipids were identified by 1H and 13C NMR spectra (see Supplementary Table S1) (De Rosa *et al.*, 1988; Lanzotti *et al.*, 1988, 1989). LC/MS as well as EI/MS analyses of the quinone content of strain FP1<sup>T</sup> revealed the presence of two menaquinones, MK8 and MK8(H<sub>2</sub>). Strain FP1<sup>T</sup> accumulated PHB under optimal growth conditions. The above results indicated that strain FP1<sup>T</sup> was a member of the genus *Haloterrigena*. However, it could be distinguished from recognized species of the genus *Haloterrigena* on the basis of several phenotypic characteristics (Table 1).

The DNA G + C content of strain FP1<sup>T</sup> was determined to be 62.0 mol%. DNA–DNA relatedness values for strain FP1<sup>T</sup> with respect to *Htg. thermotolerans* DSM 11552<sup>T</sup>, *Htg. saccharovorans* JCM 12889<sup>T</sup> and *Htg. limicola* JCM 13563<sup>T</sup> were 22.85, 28.35 and 23.40 %, respectively.

On the basis of the phylogenetic, genotypic and chemotaxonomic data presented here, we suggest that strain FP1<sup>T</sup> should be classified as the type strain of a novel species within the genus *Haloterrigena*, for which the name *Haloterrigena hispanica* sp. nov. is proposed.

**Description of Haloterrigena hispanica** sp. nov.

*Haloterrigena hispanica* (his.pa’ni.ca. L. fem. adj. *hispanica* of Hispania, from where the organism was originally isolated).

Cells are Gram-negative, coccoid (1.5–2.0 μm in diameter) and become oval in stationary cultures. Colonies on complex agar medium with 3.4 M NaCl are light red, elevated and circular. Growth occurs at NaCl concentrations of 2.2–4.0 M, at Mg<sup>2+</sup> concentrations of 0–0.4 M, at pH values in the range 6.5–8.5 and at temperatures of 37–60 °C. The

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Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain FP1<sup>T</sup> and related taxa. Bootstrap values (%) are based on 1000 replicates and are shown at each node. Bar, 0.01 expected changes per site.
optimal NaCl concentration, Mg$^{2+}$ concentration, pH and temperature for growth are 3.4 M, 0.2 M, pH 7.0 and 50 °C, respectively. Chemo-organotrophic and aerobic. Oxidase- and catalase-positive. Indole formation is positive. Nitrate reduction is observed but nitrite reduction is not. Gelatin, starch, casein, and Tweens 20, 40 and 60 are not hydrolysed. The following substrates are utilized for growth: glycerol, sodium acetate, sodium propionate and sodium citrate. Glucose, mannose, rhamnose, fructose, sucrose, galactose, trehalose, ribose, xylose, cellobiose and arabinose are not utilized for growth. Sensitive to (μg per disc): streptomycin (25), tetracycline (50), nystatin (100), vancomycin (30), lincomycin (10), neomycin (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U). Major polar lipids are C20C20 and C20C25 derivatives (30), ampicillin (25), chloramphenicol (50) and penicillin (10 U).

Table 1. Characteristics that distinguish strain FP1$^T$ from species of the genus Haloterrigena and from Natrinema altunense

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Cocoid</td>
<td>Rods/cocoid</td>
<td>Rods</td>
<td>Cocoid</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td>NaCl range (M)</td>
<td>&gt;2.2</td>
<td>&gt;1.7</td>
<td>&gt;2.0</td>
<td>&gt;2.0</td>
<td>&gt;1.7</td>
<td>&gt;1.7</td>
<td>&gt;1.7</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>62.0</td>
<td>66.6</td>
<td>63.3</td>
<td>59.8</td>
<td>61.9</td>
<td>63.2</td>
<td>65.6</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>1.5–2.0</td>
<td>0.8–7.0</td>
<td>4–13</td>
<td>1.5–2.0</td>
<td>0.7–2.7</td>
<td>0.5–0.6x</td>
<td>1.0–5.0</td>
</tr>
<tr>
<td>Lysis in distilled water</td>
<td>+</td>
<td>NR</td>
<td>+</td>
<td>–</td>
<td>NR</td>
<td>NR</td>
<td>+</td>
</tr>
<tr>
<td>Temperature optimum (°C)</td>
<td>50</td>
<td>42–45</td>
<td>50</td>
<td>45</td>
<td>40–50</td>
<td>41–45</td>
<td>37</td>
</tr>
<tr>
<td>Use of sugars as carbon source</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of Tween 20</td>
<td>–</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major glycolipid</td>
<td>S-DGD</td>
<td>S₂-DGD</td>
<td>S₂-DGD</td>
<td>S₂-DGD</td>
<td>S₂-DGD</td>
<td>S₂-DGD</td>
<td>One major glycolipid: UK</td>
</tr>
<tr>
<td>Sensitivity to tetracycline</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>NR</td>
<td>–</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The type strain, FP1$^T$ (=DSM 18328$^T$ =ATCC BAA-1310$^T$), was isolated from Fuente de Piedra salt lake, southern Spain.

Acknowledgements

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References


