**Halobacillus alkaliphilus** sp. nov., a halophilic bacterium isolated from a salt lake in Fuente de Piedra, southern Spain

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A Gram-positive, spore-forming, halophilic bacterial strain, FPF<sup>T</sup>, was isolated from a salt lake in southern Spain and subjected to a polyphasic taxonomic study. Strain FPF<sup>T</sup> was strictly aerobic. Cells were coccoidal, occurring singly or in clusters. The cell-wall peptidoglycan type of strain FPF<sup>T</sup> was A4<sub>β</sub> based on L-Orn–D-Asp. Strain FPF<sup>T</sup> was characterized chemotaxonomically by having MK-7 as the major menaquinone and anteiso-C<sub>15</sub>:0, anteiso-C<sub>17</sub>:0, iso-C<sub>15</sub>:0 and iso-C<sub>16</sub>:0 as the main fatty acids. The isolate grew optimally at 37 °C and in presence of 10 % NaCl; no growth was observed in the absence of NaCl. The DNA G+C content was 43.5 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain FPF<sup>T</sup> falls within the evolutionary radiation of species of the genus *Halobacillus*. Levels of 16S rRNA gene sequence similarity between strain FPF<sup>T</sup> and the type strains of nine recognized *Halobacillus* species were in the range 97.0–99.0 %. Levels of DNA–DNA relatedness indicated that strain FPF<sup>T</sup> represents a genomic species that is distinct from recognized *Halobacillus* species. Strain FPF<sup>T</sup> could be differentiated from recognized *Halobacillus* species based on several phenotypic characteristics. On the basis of phenotypic, phylogenetic and genomic data, strain FPF<sup>T</sup> is considered to represent a novel species of the genus *Halobacillus*, for which the name *Halobacillus alkaliphilus* sp. nov. is proposed. The type strain is FPF<sup>T</sup> (= DSM 18525<sup>T</sup> = ATCC BAA-1361<sup>T</sup>).


The genus *Halobacillus* can be differentiated clearly from other related genera based on the cell-wall peptidoglycan type based on L-Orn–D-Asp (Spring *et al.*, 1996; Shida *et al.*, 1997; Yoon *et al.*, 2001), with the exception of that for *H. campisalis*, which is based on *meso*-diaminopimelic acid (Yoon *et al.*, 2007). The aim of the present study was to determine the exact taxonomic status of a halophilic bacterial strain, FPF<sup>T</sup>, by using a polyphasic approach, including phenotypic properties, lipid analyses, phylogenetic analysis based on 16S rRNA gene sequences and levels of genotypic relatedness.

Strain FPF<sup>T</sup> was isolated from samples collected during summer 2003 from Fuente de Piedra saline lake, Malaga province, southern Spain (37° 6’ N 4° 44’ W). It was isolated from a saltern crystallizer pond by the dilution-plating technique. Strain FPF<sup>T</sup> represented the predominant organism in the enrichment and was the only colony-forming organism at the highest dilutions. The enrichment medium (medium A) contained the following components:

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FPF<sup>T</sup> is AM295006.

A scanning electron micrograph of cells of strain FPF<sup>T</sup> and thin-layer chromatographs of total polar lipids of strain FPF<sup>T</sup> are available as supplementary material with the online version of this paper.
Halobacillus alkaliphilus sp. nov.

Cells of strain FP5\textsuperscript{T} were Gram-positive, spore-forming cocci, occurring singly or in bunches, and measured 1.57 μm in diameter (see Supplementary Fig. S1 in IJSEM Online). The coccoid morphology of the cells remained the same in all phases of growth. After incubation for 2 days, colonies of strain FP5\textsuperscript{T} were about 1–2 mm in diameter, circular, smooth and pale orange. Strain FP5\textsuperscript{T} required \( \text{Na}^+ \) and \( \text{Mg}^{2+} \) for growth, but was
also able to grow using K\(^+\) instead of Na\(^+\). Strain FP5\(^T\) grew in media containing 0.5–20\% (w/v) NaCl; optimal growth occurred with 10\% (w/v) NaCl. Strain FP5\(^T\) grew at temperatures of 25–45 °C, with optimum growth at 37 °C. The optimum pH for growth was 9.0. Growth was observed up to pH 10, but no growth occurred below pH 6.0. This optimum alkaline pH for growth was not found for other Halobacillus species (Spring et al., 1996; Claus et al., 1983; Yoon et al., 2003, 2004, 2005; Amoozegar et al., 2003; Liu et al., 2005).

Strain FP5\(^T\) was aerobic and catalase- and oxidase-positive. Acid was produced from glucose, trehalose, maltose, ribose, sucrose, raffinose, fructose and mannose, but not from galactose, cellobiose or xylose. Tyrosine was hydrolysed, but starch, casein, hippurate, gelatin and urease were not. The strain was negative for nitrate reduction and phenylalanine deaminase. Strain FP5\(^T\) was sensitive to (μg per disc) bacitracin (10), tetracycline (30), novobiocin (30), erythromycin (5), ampicillin (25), chloramphenicol (10), fusidic acid (10), penicillin (10 U), lincomycin (10) and vancomycin (30), but resistant to (μg per disc) streptomycin (25), nystatin (100), kanamycin (30) and neomycin (30). Detailed results of morphological analyses and biochemical tests for strain FP5\(^T\) are given in the species description. Differential characteristics between strain FP5\(^T\) and recognized species of the genus Halobacillus are given in Table 1. Of particular note was that only strain FP5\(^T\) was positive for tyrosine hydrolysis and that no growth was observed at <5\% (w/v) NaCl for this strain. This appears to be the first report of an alkaliphilic member of the genus Halobacillus. Cell-wall analysis revealed that the peptidoglycan type of strain FP5\(^T\) was A4β based on L-Orn–D-Asp. The predominant menaquinone was MK-7, as reported for H. halophilus, the type species of the genus. The major fatty acids were anteiso-C\(_{15:0}\) (40.4\%), anteiso-C\(_{17:0}\) (31.0\%), iso-C\(_{15:0}\) (9.6\%) and iso-C\(_{16:0}\) (8.3\%). The polar lipid profile of strain FP5\(^T\) was quite similar to the complex lipid patterns reported for recognized Halobacillus species, with the

### Table 1. Differential characteristics between strain FP5\(^T\) and recognized Halobacillus species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>+</td>
<td>(V)</td>
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<td>Spore shape*</td>
<td>S</td>
<td>S</td>
<td>E or S</td>
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<tr>
<td>Colony colour†</td>
<td>POW</td>
<td>O</td>
<td>O</td>
<td>POW</td>
<td>CW</td>
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<td>43</td>
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<tr>
<td>NaCl concentration for growth (% w/v)</td>
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<td>0.5–25</td>
<td>0.23</td>
<td>1–24</td>
<td>&gt;0.5–24</td>
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<td>0.5–21</td>
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<td>DNA G+C content (mol%)</td>
<td>43.5</td>
<td>40.1–40.9</td>
<td>43</td>
<td>42</td>
<td>45</td>
<td>41.3</td>
<td>44</td>
<td>41.4</td>
<td>42.2</td>
<td>42.9</td>
<td>43.3</td>
<td>42.1</td>
</tr>
</tbody>
</table>

*E, Ellipsoidal; S, spherical.
†CW, Cream or white; LOW, light orange–yellow; LY, light yellow; O, orange; POW, pale orange–yellow; PY, pale yellow; YO, yellow–orange.
presence of two phospholipids, phosphatidylglycerol, diphasphatidylglycerol and an unidentified glycolipid as major lipids. By using two-dimensional TLC, eluted with chloroform/methanol/water (65:25:4 by volume) for the first dimension and with chloroform/methanol/acetic acid/water (85:15:12:4 by volume) for the second dimension, the lipid profile of strain FP5T revealed the presence of two minor phospholipids and one minor glycolipid (Supplementary Fig. S2).

The complete 16S rRNA gene sequence of strain FP5T determined in this study comprised 1484 nt. Comparative 16S rRNA gene sequence analyses showed that strain FP5T was phylogenetically most closely affiliated to members of the genus Halobacillus (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain FP5T fell within the radiation of the cluster comprising Halobacillus species (Fig. 1). The 16S rRNA gene sequence of strain FP5T showed similarity levels of 97.0–99.0 % with respect to sequences of the type strains of recognized Halobacillus species (Fig. 1).

The above results indicated that strain FP5T was a member of the genus Halobacillus. However, it could be distinguished from recognized species of the genus Halobacillus on the basis of several phenotypic characteristics (Table 1). The DNA G+C content of strain FP5T was 43 mol%, Mean levels of DNA–DNA relatedness between strain FP5T and the type strains of recognized Halobacillus species were in the range 4.5–35 % (H. trueperi, 4.5%; H. salinus, 10.5%; H. karajensis, 18.2%; H. yeomjeoni, 22.5%; H. dabanensis, 30.3%; H. halophilus, 35.0%). Therefore, on the basis of the data presented, strain FP5T should be placed in the genus Halobacillus as a member of a novel species, for which the name Halobacillus alkaliphilus sp. nov. is proposed.

**Description of Halobacillus alkaliphilus sp. nov.**

*Halobacillus alkaliphilus* (al.ka.li.phi’lus. N.L. n. alkali alkali; Gr. adj. philos loving; N.L. masc. adj. alkaliphilus loving alkaline conditions).

Cells are Gram-positive, spore-forming coccì (1.57 µm in diameter). Colonies on agar medium are circular (1–2 mm in diameter), smooth and pale orange. Growth occurs at NaCl concentrations of 0.5–20 % (w/v), with optimal growth at 10 % (w/v), at temperatures of 25–45 °C, with optimum growth at 37 °C, and at pH 6.0–10.0. Aerobic. Catalase- and oxidase-positive. Acid is produced from xylose, glucose, trehalose, maltose, ribose, sucrose, raffinose, fructose and mannose, but not from galactose or cellobiose. Hydrolyses tyrosine, but not starch, casein, hippurate, gelatin or urease. Negative for nitrate reduction and phenylalanine deaminase. The peptidoglycan type is A4b based on L-Orn–D-Asp. The major menaquinone is MK-7. The major fatty acids are anteiso-C15 : 0 (40.4 %), anteiso-C17 : 0 (31.0 %), iso-C15 : 0 (9.6 %) and iso-C16 : 0 (8.3 %). Major lipids are two phospholipids (phosphatidylglycerol and diphasphatidylglycerol) and one unidentified glycolipid. The DNA G+C content of the type strain is 43.5 mol% (Tm).

The type strain, FP5T (=DSM 18525T =ATCC BAA-1361T), was isolated from Fuente de Piedra salt lake, southern Spain.
Acknowledgements
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References