Halorubrum litoreum sp. nov., an extremely halophilic archaeon from a solar saltern

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An extremely halophilic archaeon, strain Fa-1T, was isolated from a marine solar saltern in Fujian, China. Strain Fa-1T required Mγ2+ and at least 2.0 M NaCl for growth. It was able to grow at pH 6.5–9.0 (optimally at pH 7.0–7.5) and at 20–55 °C (optimally at 37–42 °C). The major polar lipids of strain Fa-1T were phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and a sulfated diglycosyl diether. On the basis of a 16S rRNA gene sequence analysis, strain Fa-1T was closely related to nine species of the genus Halorubrum, showing sequence similarities of 97.4–98.4 %. The G+C content of the DNA of strain Fa-1T is 64.9 mol% (Tm). DNA–DNA hybridization values between strain Fa-1T and the most closely related members of the genus Halorubrum were below 51 %. On the basis of the data from this study, strain Fa-1T represents a novel species of the genus Halorubrum, for which the name Halorubrum litoreum sp. nov. is proposed. The type strain is Fa-1T (=CGMCC 1.5336T =JCM 13561T).

The genus Halorubrum is the largest genus in terms of numbers of species, within the family Halobacteriaceae. At the time of writing, this genus contains 17 species with validly published names, including three halophilic species and 14 neutrophilic species (Fig. 1). Most of these species were isolated from salt or soda lakes, but three species, namely Halorubrum saccharovorum (Tomlinson & Hochstein, 1976), Halorubrum coriense (Nuttall & Dyall-Smith, 1993) and Halorubrum trapanicum (McGenity & Grant, 1995; Trüper, 2003), were isolated from marine solar salters. Here, we describe the taxonomy of a novel halophilic strain, Fa-1T, isolated from a marine solar saltern.

Strain Fa-1T was isolated from sediment from the Fuqing solar saltern (25° 18’ 25”–25° 51’ 41” N 119° 3’ 41”–119° 40’ 41” E) in Fujian Province, China. The medium and method used for isolation were as described previously (Feng et al., 2004). The strain was routinely grown aerobically at 37 °C in a complex medium containing the following ingredients (l−1): Casamino acids (Difco), 7.5 g; yeast extract (Difco), 10.0 g; trisodium citrate, 3.0 g; MgSO4·7H2O, 20.0 g; KCl, 2.0 g; FeSO4·7H2O, 0.05 g; NaCl, 200 g (pH 7.0–7.5).

Phenotypic tests were performed according to the proposed minimal standards for the description of novel taxa of the order Halobacteriales (Oren et al., 1997). Colony morphology was observed on salt-milk agar medium (Kocur & Hodgkiss, 1973) after incubation at 37 °C for 7–10 days. Production of H2S was tested by growing the isolate in a tube with the above-described liquid complex medium supplemented with 0.5 % (w/v) Na2S2O3; a filter-paper strip impregnated with lead acetate was used for H2S detection. Various tests relating to cell morphology and growth, biochemistry and nutrition, sensitivity to antimicrobial agents, polar lipids and nucleic acids were performed as described by (or cited by) Cui et al. (2006). The DNA G+C content was determined by means of thermal denaturation (Marmur & Doty, 1962).

The cells of strain Fa-1T were found to be motile, rod-shaped (0.3–0.5 × 2.0–5.0 μm) and Gram-negative and were able to grow over a wide range of salinities (2.0–5.1 M NaCl; optimal growth at 3.4 M). Colonies on salt-milk agar medium were red. Strain Fa-1T utilized glucose, galactose and sucrose, but not mannose, fructose or lactose, as carbon sources for growth. It reduced nitrate to nitrite under anaerobic conditions but was not able to grow with nitrate under anaerobic conditions. More-detailed results from the phenotypic and nutritional tests performed with strain Fa-1T are given in the species description. These physiological test results, when combined, serve to distinguish strain Fa-1T from members of the genus Halorubrum (Table 1).
Polar lipid analysis (Kates, 1986) (see Supplementary Fig. S1 available in IJSEM Online) indicated that strain Fa-1T contains phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and a sulfated diglycosyl diether, a profile that is similar to those of the neutrophilic species of Halorubrum. The DNA G+C content of strain Fa-1T is 64.9 mol%.

Phylogenetic analysis (Fig. 1) based on 16S rRNA gene sequences and performed using the neighbour-joining method (Kumar et al., 2004) showed that strain Fa-1T is closely related to the type strains of Halorubrum distributum (Zvyagintseva & Tarasov, 1987; Oren & Ventosa, 1996), Halorubrum xinjiangense (Feng et al., 2005), Hrr. trapanicum (McGenity & Grant, 1995), Hrr. coriense (Nuttall & Dyall-Smith, 1993), Halorubrum sodomense (Oren, 1983), Halorubrum terrestre (Ventosa et al., 2004), Halorubrum tebenquichense (Lizama et al., 2002), Halorubrum arcis (Xu et al., 2007), and Halorubrum ezzemoulense (Kharroub et al., 2006), with sequence similarities ranging from 97.4 to 98.4 %. DNA–DNA hybridization was carried out between strain Fa-1T and the type strains of the four most closely related species, namely Hrr. distributum JCM 9100T, Hrr. xinjiangense BD-1T, Hrr. trapanicum JCM 10477T and Hrr. coriense JCM 9275T: the results revealed levels of DNA relatedness of 50.2, 42.2, 28.2 and 31.5 %, respectively.

On the basis of these results, strain Fa-1T represents a novel species of the genus Halorubrum, for which the name Halorubrum litoreum sp. nov. is proposed.

### Table 1. Differentiation of strain Fa-1T with respect to related members of the genus Halorubrum

<table>
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<th>7</th>
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<td>DNA G+C content (mol%)</td>
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<td>64.2</td>
<td>63.6</td>
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<td>64.4</td>
<td>63.2</td>
<td>66</td>
<td>61.9</td>
<td>65.7</td>
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</table>

The DNA G+C content of strain Fa-1T is 64.9 mol%.
Description of *Halorubrum litoreum* sp. nov.

*Halorubrum litoreum* (lit.to’re.um. L. neut. adj. litoreum of, or belonging to, the seashore).

Cells are motile, rod-shaped (0.3–0.5 × 2.0–5.0 μm) and Gram-negative. Colonies on agar plates containing 3.4 M NaCl are red, elevated and round. Chemoheterotrophic and aerobic. Growth occurs at NaCl concentrations of 2.0–5.1 M, at Mg$^{2+}$ concentrations of 0.03–0.7 M, at pH values in the range 6.5–9.0 and at temperatures in the range 20–55 °C. The optimal NaCl concentration, pH and temperature for growth are 3.4 M, pH 7.0–7.5 and 37–42 °C. Catalase- and oxidase-positive. Does not grow under anaerobic conditions with nitrate, arginine or DMSO. Nitrate reduction to nitrite is observed. H$_2$S is produced from Na$_2$S$_2$O$_3$. Negative for indole formation. Tween 80 is hydrolysed weakly; negative for caseinase, amylase and gelatinase. The following substrates are utilized as carbon sources for growth: glucose, galactose, maltose, sucrose, lactose, glycerol, acetate, pyruvate, malate, fumarate, L-alanine, L-arginine, L-glutamate and L-ornithine. Mannose, fructose, sorbose, D-ribose, xylose, starch, mannotol, D-sorbitol, lactate, succinate, citrate, glycerol, L-aspartate and L-lysine are not utilized as carbon sources. Sensitive to the following antibiotics (μg per disc): rifampicin (5), novobiocin (30). Resistant to the following antibiotics (μg per disc, unless otherwise indicated): ampicillin (10), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), kanamycin (30), neomycin (30), tetracycline (30), vancomycin (30), norfloxacin (10), streptomycin (10), bacitracin (0.04 IU) and penicillin G (10 IU). Major polar lipids are phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate. The DNA G+C content of the type strain is 64.9 mol% (Tm).

The type strain, Fa-1$^T$ (=CGMCC 1.5336$^T$ = JCM 13561$^T$), was isolated from the Fuying solar saltern in Fujian Province, China.

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References


