Salisaeta longa gen. nov., sp. nov., a red, halophilic member of the *Bacteroidetes*

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A rod-shaped, 15–30 µm long, red bacterium, affiliated phylogenetically with the phylum *Bacteroidetes*, was isolated from an experimental mesocosm at Sedom, Israel, filled with a mixture of water from the Dead Sea and the Red Sea. The organism stains Gram-negative and is obligately aerobic, heterotrophic and oxidase- and catalase-positive. Growth is obtained in the presence of 5–20 % NaCl, with an optimum at 10 % NaCl plus 5 % MgCl₂ · 6H₂O. Temperature and pH optima are 37–46 °C and pH 6.5–8.5. Nitrate is not reduced. Glucose, sucrose, maltose and glycerol stimulate growth with acid formation; no growth stimulation is obtained in the presence of fructose, ribose, xylose, mannitol or sorbitol. The G + C content of the DNA is 62.9 mol% (HPLC). Main fatty acids are 16 : 0 iso and 16 : 1 *cis*9, followed by 15 : 0 iso and 15 : 0 anteiso. The isolate is sufficiently different from its closest relatives to be classified within a novel species belonging to a new genus, for which we propose the name *Salisaeta longa* gen. nov., sp. nov. The type strain of *Salisaeta longa* is strain S4-4^T (=DSM 21114^T =CECT 7354^T).

The water level of the Dead Sea (Israel/Jordan) has been decreasing at a rate of nearly 1 m per year over the last decade due to anthropogenic intervention in its water balance (Oren *et al.*, 2004). In the framework of plans to construct a water carrier connecting the Red Sea with the Dead Sea (the 'Peace Conduit'), simulation experiments were performed on the grounds of the Dead Sea Works at Sedom to study the effect of mixing Dead Sea water and Red Sea water. Experimental mesocosms (0.9 m³) filled with 80 % Dead Sea water and 20 % Red Sea water and enriched with low concentrations of phosphate became strongly red due to dense communities of red-pigmented, halophilic micro-organisms, most of them belonging to the domain *Archaea* (Oren *et al.*, 2004; Elevi Bardavid *et al.*, 2007a).

To characterize these communities, we plated samples on different hypersaline growth media. Among the colony types that arose on an agar plate of the high-magnesium, low-nutrient medium developed for cultivation of *Halorubrum sodomense* (Oren, 1983) (in which 0.1 % peptone was replaced by 0.1 % Casamino acids), one presented the typical orange–red colour of *Salinibacter ruber*. This red-pigmented, halophilic member of the *Bacteria* has many physiological characteristics similar to

those of the *Halobacteriales* and is found in saltern crystallizer ponds all over the world (Antón *et al.*, 2002). Based on its 16S rRNA gene sequence, the new isolate, designated strain S4-4^T, was found to be distantly related to *Salinibacter*.

Routine growth was performed at 37 °C in medium containing 10 % NaCl, 5 % MgCl₂ · 6H₂O, 0.5 % K₂SO₄, 0.01 % CaCl₂ · 2H₂O, 0.1 % yeast extract, 0.1 % Casamino acids and 0.2 % soluble starch. The pH was adjusted to 7.0 before autoclaving. In this medium, excellent growth was obtained after 2 days. For solid media, 2 % agar was added. Cell morphology was examined using a Zeiss Axiovert microscope equipped with phase-contrast optics. Gram staining was performed as described by Dussault (1955). Growth in this medium was tested at 25, 30, 37, 46 and 52 °C. To test for effects of pH and salt concentrations on growth, the standard medium was separately modified by addition of buffers (PIPES, pH 5.5 and 6.0; HEPES, pH 7.0, 8.0 and 9.0), NaCl (0, 5, 10, 15, 20 %) and MgCl₂ · 6H₂O (0, 5, 10, 15, 20 %). Physiological tests were performed in 40 ml volumes of the above-described medium in 100 ml Erlenmeyer flasks with shaking (150 r.p.m.) unless specified otherwise. Growth was monitored for 7 days by measuring OD₆₀₀. Most biochemical tests were performed as outlined by Holding & Collee (1971) using media as above. Appropriate positive and negative controls were included in all experiments. Nitrate reduction was tested in liquid medium supplemented with 0.5 % NaNO₃. The formation of nitrite was monitored colorimetrically and formation of gaseous products from

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S4-4^T is EU426570.

One- and two-dimensional chromatograms of the polar lipids of strain S4-4^T are available as supplementary material with the online version of this paper.

nitrate was detected by the presence of gas bubbles in Durham tubes. Hydrolysis of starch was examined on agar plates of the standard growth medium, which contains 0.2 % soluble starch. Hydrolysis was detected by flooding the plates with iodine solution. Gelatin hydrolysis was determined by growing colonies on agar plates amended with 0.4 % gelatin and flooding the plates with a solution of 15 % (w/v) HgCl_2 in 15 % (w/v) HCl after growth was obtained. Indole production was detected with Kovács' reagent after growing the cells in medium supplemented with 0.01 % L-tryptophan. Growth stimulation by sugars and other compounds (0.2–0.5 %) was tested in medium without starch in which the concentrations of yeast extract and Casamino acids were lowered to 0.01 % each, and 20 mM HEPES was added as buffer (pH 7). To test for acid production from sugars (0.5 %), the same medium was used without buffer and the pH of the cultures was measured periodically with a pH electrode. The presence of catalase was tested by adding a 1 % (v/v) H_2O_2 solution to colonies on plates. The presence of oxidase was determined with tetramethyl *p*-phenylenediamine hydrochloride (Holding & Collee, 1971). To test for sensitivity to antibiotics and antibacterial compounds, the following inhibitors were used at concentrations of 40 $\mu\text{g ml}^{-1}$: penicillin G, ampicillin, streptomycin, novobiocin, bacitracin, rifampicin, anisomycin and neomycin.

The isolate consists of very long (15–30 μm), Gram-negative, rod-shaped cells (Fig. 1). Optimal growth was obtained at 10–12 % NaCl (range 5–20 %), 5 % $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (range 5–20 %), pH 6.5–8.5 (range 6.0–9.0) and 37–46 °C (range 25–50 °C). When 5 % (0.25 M) $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was replaced by an equivalent concentration of MgSO_4 , growth was inhibited. In the absence of NaCl and MgCl_2 , no growth was obtained. Further properties are given in the species description.

Polar lipids were extracted and analysed according to Oren *et al.* (1996). One- and two-dimensional TLC of the polar

Fig. 1. Phase-contrast micrograph of cells of strain S4-4^T, grown in the standard medium described in the text. Bar, 10 μm .

lipid fraction revealed the presence of three glycolipids and four major phospholipids, one of which contains an amino group, as well as a number of minor lipids. The lipid pattern differs significantly from that of *Salinibacter ruber*, especially in the glycolipid fractions (see Supplementary Fig. S1, available in IJSEM Online). Fatty acid analysis was performed as described by Elevi Bardavid *et al.* (2007b). The main fatty acids of strain S4-4^T are 16:0 iso (26–30 %) and 16:1 *cis*9 (35–38 %). 15:0 iso and 15:0 anteiso correspond to 8.5 and 3.8 %, respectively, of the total fatty acid composition, while 17:0 iso, 17:0 anteiso and 17:1 iso together represent approximately 10 %. Other fatty acids were found at less than 1 % each. This composition is considerably different from that of *Salinibacter ruber*, which has a predominance of 15:0 iso, 16:1 *cis*9 and 18:1 *cis*11, each representing 25–30 % of the total fatty acids. 15:0 anteiso and 16:0 are also present, corresponding to 4–5 and 7–10 %, respectively (Elevi Bardavid *et al.*, 2007b).

Pigments were extracted from cell pellets with methanol/acetone (1:1, v/v) and absorption spectra were recorded against the solvent in a Hewlett Packard model 8452A diode array spectrophotometer. Extracts showed maximum absorption at 478 nm and a shoulder at 506–510 nm, similar to spectra from *Salinibacter ruber* (Antón *et al.*, 2002).

The DNA G+C content, as determined by HPLC of deoxyribonucleosides (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984), was 62.9 mol%. The 16S rRNA gene was amplified by PCR, using primers 27F and 1492R (Martínez-Murcia *et al.*, 1995). Sequencing was performed using the Big Dye Terminator reagent and purified PCR products were electrophoresed on an ABI 373A DNA sequencer. The sequences obtained were aligned with sequences deposited in the GenBank database through BLAST software (Altschul *et al.*, 1997). The nearly complete 16S rRNA gene presented a similarity of 88 % with that of *Salinibacter ruber* M31^T (Fig. 2). Alignment of the 16S rRNA gene sequence with that of *Salinibacter ruber* M31^T using EzTaxon Server 2.0 (Chun *et al.*, 2007) gave 90.5 % similarity. In addition, we amplified part of the 16S rRNA gene using primers EHB4F (Antón *et al.*, 2002) and EHB9R (Peña *et al.*, 2005), designed as 'specific' primers for *Salinibacter*. The partial 16S rRNA gene sequence obtained was 91 % similar to the corresponding sequence of *Salinibacter ruber* M31^T. Thus, this primer pair can no longer be considered specific for *Salinibacter ruber*; the discovery of a new genus related to *Salinibacter* requires re-evaluation of the probes/primers previously designed for this genus.

The main differences between strain S4-4^T and its closest relative *Salinibacter ruber* are shown in Table 1. The data presented show that strain S4-4^T is sufficiently different from its closest relative to be classified as the type strain of a novel species belonging to a new genus. We propose the name *Salisaeta longa* gen. nov., sp. nov. for this organism.

Fig. 2. 16S rRNA gene sequence-based phylogeny reconstructed from distance values by using the minimum-evolution method. Bootstrap values (>50 %) are given at nodes. The sequence of *Chlorobium limicola* DSM 245^T served as the outgroup. Bar, 0.02 inferred substitutions per nucleotide position.

Description of *Salisaeta* gen. nov.

Salisaeta (Sa.li.sae'ta. L. masc. n. *sal*, *salis* salt; L. fem. n. *saeta* a bristle; N.L. fem. n. *Salisaeta* a salt bristle).

Long rod-shaped bacteria, affiliated phylogenetically with the phylum *Bacteroidetes*. Gram-reaction negative. Aerobic, heterotrophic. Oxidase- and catalase-positive. Halophilic, requiring at least 5 % NaCl for growth. Known habitat: hypersaline water bodies. The DNA G+C content of the type strain of the type species is 62.9 mol%. The type species is *Salisaeta longa*.

Description of *Salisaeta longa* sp. nov.

Salisaeta longa (lon'ga. L. fem. adj. *longa* long).

Displays the following properties in addition to those described for the genus. Straight or slightly curved rods, 15–30 µm long. Colonies on agar are red, circular and convex with an entire margin. Pigment extracts in methanol/acetone (1:1, v/v) show an absorption maximum at 478 nm and a shoulder at 506–510 nm. Grows optimally at 10 % NaCl plus 5 % MgCl₂·6H₂O. Tolerates 20 % MgCl₂·6H₂O. The optimal pH for growth is 6.5–8.5; no growth below pH 6.0 or above pH 9.0. Temperature optimum is 37–46 °C; no growth above 50 °C or below 25 °C. Nitrate is not reduced. Glucose, sucrose, maltose and glycerol stimulate growth with acid formation. No growth stimulation or acid formation is obtained in the presence of ribose, xylose, mannitol or sorbitol; fructose

causes acidification of the medium, but does not stimulate growth. Starch and gelatin are weakly hydrolysed. Indole is produced from tryptophan. Sensitive to penicillin G, ampicillin, novobiocin and rifampicin. Insensitive to streptomycin, neomycin, bacitracin and anisomycin. Polar lipids include three glycolipids and four major phospholipids, one of which contains an amino group. Main fatty acids are 16:0 iso and 16:1 *cis*9, followed by 15:0 iso and 15:0 anteiso.

The type strain is strain S4-4^T (=DSM 21114^T =CECT 7354^T), isolated from an experimental mesocosm filled with a mixture of water from the Dead Sea and the Red Sea, Israel.

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Table 1. Characteristics that differentiate strain S4-4^T and *Salinibacter ruber* M31^T

Data for *Salinibacter ruber* M31^T are from Antón *et al.* (2002).

Characteristic	Strain S4-4 ^T	<i>Salinibacter ruber</i> M31 ^T
Cell length (µm)	15–30	0.4–2.6
NaCl range (% w/v)	5–20	15–30
Indole production	–	+
Sensitivity to streptomycin	–	+
DNA G+C content (mol%)	62.9	66.5*

*The range of DNA G+C content among different strains of *Salinibacter ruber* was reported to be 66.3–67.7 mol% (Antón *et al.*, 2002).

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