

**Salibacterium nitratireducens sp. nov., a haloalkalitolerant bacterium isolated from a
water sample from Sambhar salt lake, India**

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Running title: *Salibacterium nitratireducens* sp. nov.

Subject category: New taxa: Firmicutes and related organisms.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SMB4^T is
LT161881.

Abstract:

A strictly aerobic, haloalkali-tolerant, Gram-positive-staining, non-motile, rod shaped bacterium, designated strain SMB4^T, was isolated from a water sample collected from Sambhar salt lake, Rajasthan, India. Growth occurred at 25-50 °C, 4-12% (w/v) NaCl and pH of 5-9. Strain SMB4^T was positive for β-galactosidase, oxidase, catalase, and urease activities. The fatty acids were dominated by branched forms of fatty acids with iso-, anteiso-, saturated fatty acids with a high abundance of anteiso-C_{15:0}, anteiso-C_{17:0} and C_{18:0}. The cell-wall peptidoglycan of strain SMB4^T contained meso-diaminopimelic acid, while the polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and three unidentified lipids. The DNA G+C content of strain SMB4^T was 49.1 mol%. BLAST sequence similarity search based on 16S rRNA gene sequence indicated that *Salibacterium halochares*, *Salibacterium halotolerans* and *Salibacterium qingdaonense* were the nearest phylogenetic neighbors, with a pair-wise sequence similarity of 98.4, 98.2 and 97.0% respectively. Phylogenetic analysis showed that strain SMB4^T was clustered with *Salibacterium halochares* and together clustered with *Salibacterium halotolerans* and *Salibacterium qingdaonense*. DNA-DNA hybridization of strain SMB4^T with *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium qingdaonense* DSM

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21621^T showed a relatedness of only 39.8, 26.3 and 42.8 %, respectively. Based on its phenotypic characteristics and on phylogenetic inference, strain SMB4^T represents a novel species of the genus *Salibacterium*, for which the name *Salibacterium nitratireducens* sp. nov. is proposed. The type strain is SMB4^T (= MTCC 12633^T = KCTC 33876^T = JCM 32187^T).

Key words: *Salibacterium*; 16S rRNA gene sequence based phylogeny; chemotaxonomy.

Introduction

The genus *Salibacterium* comprises Gram-positve staining, non-endospore forming, non-motile, rod-shaped, cream to white, organoheterotrophic, strictly aerobic to facultatively anaerobic bacteria with MK7 as major isoprenoid quinone; anteiso-C_{15:0} and anteiso-C_{17:0} as major fatty acids; and DNA G + C content ranging between 48-48.9 mol% [1]; the genus belongs to the family *Bacillaceae* of the phylum *Firmicutes*. Species of the genus *Salibacterium* have been isolated from different habitats such as from soil of crystallizer pond of solar saltern, crude sea-salt sample and sediment sample of a salt pan [2, 3, 1]. At the time of writing, the genus *Salibacterium* accommodates 3 recognized species (Euzebay, <http://www.bacterio.net/salibacterium.html>, August, 2017). In the present study, we focused on the characterization and classification of the strain SMB4^T, which was isolated from Sambhar salt lake water sample by a polyphasic taxonomic approach [4] and on the basis of the results assign the novel strain to the genus *Salibacterium*.

Strain SMB4^T was isolated from a water sample collected from Sambhar salt lake, Rajasthan, India. The GPS coordinates of the sampling point is 26° 55.520' N 075° 11.827' E. The temperature and pH of the water sample was 35 °C and 10.0, respectively. For isolation of the strain SMB4^T, the water sample was serially diluted (up to 10 fold dilutions) in 2% (w/v) NaCl solution and 100 µl of each dilution was spread-plated on Zobell's Marine Agar (MA; HIMEDIA) plates, and incubated at 37 °C for 2 days. A number of different colony morphotypes were observed, one that appeared whitish in colour was selected and characterized. Sub-cultivation of the isolate was carried out on Marine Agar (MA; HIMEDIA) at 37 °C. A stock culture of the isolate in Marine broth (MB; HIMEDIA) with 20% glycerol was preserved at -80 °C.

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Salibacterium halochares DSM 21373^T and *Salibacterium quigdaonense* DSM 21621^T were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ); strain SMB4^T was characterized simultaneously with *Salibacterium halochares* DSM 21373^T and *Salibacterium quigdaonense* DSM 21621^T.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories Inc.) and sequenced as described previously [5]. The resultant sequence of the 16S rRNA gene was subjected to BLAST sequence similarity searches [6] and the EzBioCloud [7] was used to identify the nearest taxa. All the 16S rRNA gene sequences of closely related species of the genus *Salibacterium* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_W program using MEGA6 [11]. The evolutionary history was inferred using the Neighbor-Joining method [8]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches [9]. The evolutionary distances were computed using the Kimura 2-parameter method [10] and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA6 [11]. Phylogenetic trees were also reconstructed using the maximum-likelihood and maximum parsimony methods using MEGA6 [11].

Colony morphology was examined following growth of the strain on MA at 37 °C for 2 day. Cell morphology was investigated by light microscopy (Nikon) at x 1000 magnification and also by transmission electron microscope (Jeol JEM 2100) at operating voltage of 200 kV. The Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to manufacturer's protocol. Endospore formation was determined by observing under phase contrast microscope and malachite-green staining of isolate grown on MA for a week [12]. Motility was assessed on Motility-Indole-Lysine HiVegTM medium (HIMEDIA) with agar 2 g l⁻¹ and also under light microscopy using hanging drop method. Growth at 4, 10, 15, 20, 25, 30, 37, 42, 45, 50 and 55 °C was ascertained using Marine Broth (MB; HIMEDIA) and salt tolerance [0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30% (w/v) NaCl] was ascertained using Nutrient Broth containing peptone (5 g l⁻¹) and beef extract (3 g l⁻¹). Growth of strain SMB4^T at pH 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11 and 12 was assessed on MB buffered either with acetate or citrate buffer (pH 4-6), [100 mM NaH₂PO₄/Na₂HPO₄] buffer (pH 7-8), 100 mM NaHCO₃/Na₂CO₃ buffer (pH 9-10) or 100 mM Na₂CO₃/NaOH buffer (pH 11-12). Anaerobic growth on MA was determined after incubation in an anaerobic Jar (Anoxomat [USA]), after giving an anaerobic cycle using Anoxomat (Mart, USA). Different biochemical tests listed in description of species and as well as in Table 1 were carried out using cultures grown at 37 °C on MA medium with 10% (w/v) NaCl as described by Lányi [13]

(catalase, oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert & Krieg [14] (H₂S production, gelatin and urea hydrolysis). Extracellular enzymatic activities like amylase, lipase and protease, were studied as described by Srinivas *et al.* [15] except that cultures were grown at 37 °C on MA medium with 10% (w/v) NaCl. Biochemical and enzymatic characterizations, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods [16, 17] except that the cultures were grown at 37 °C on MA medium with 10% (w/v) NaCl.

Standardization of the physiological age of strains SMB4^T, *Salibacterium halochares* DSM 21373^T and *Salibacterium quigdaonense* DSM 21621^T was performed based on the protocol [18] given by Sherlock Microbial Identification System (MIDI, USA). For cellular fatty acids analysis, all three strains were grown on MA plates at 37 °C for 24 h and were of same physiological age (at exponential phase of growth). Cellular fatty acid methyl esters (FAMES) were obtained from cells by saponification, methylation and extraction following the protocol of MIDI. Cellular FAMES were separated by GC (6890) and analyzed using the Sherlock Microbial Identification System (MIDI-6890 with database TSBA6) according to the protocol described by Sherlock Microbial Identification System. Peptidoglycan was prepared and analysed according to the method described by Komagata & Suzuki [19]. Polar lipids were extracted from all strains following the method of Bligh & Dyer and analyzed by two-dimensional TLC after spraying with appropriate detection reagents [20]. Genomic DNA was isolated by using the procedure of Marmur [21] and the mol% G+C content was determined from melting point (T_m) curves [22] obtained by using Lambda 35; Perkin Elmer spectrophotometer equipped with Templab 2.0 software package. For DNA–DNA hybridization experiments, genomic DNA was isolated and purified according to the method of Marmur [21] with additional *RNase* treatment. The relative binding ratio experiment was done by fluorimetry method using a Step One Plus Real-Time PCR system (Applied Biosystems) fitted with 96 well thermal cycling block in 96 well plate as described by Loveland-Curtze *et al.* [23]. DNA suspended in the 2X SSC and 1:10000 SYBR Green was used for the analysis. DNA denaturation at 95.0 °C was carried out followed by the reassociation at optimum temperature 71.5 °C. Fluorescence readings were taken every 10 seconds throughout the protocol and relative binding ratio was calculated according to De Ley *et al.* [24] and Gillis *et al.* [25].

The 16S rRNA gene sequence of 1516 bp was determined for strain SMB4^T. The phylogenetic relationships of strain SMB4^T was ascertained based on the 16S rRNA gene sequence similarity compared with other strains identified using BLAST sequence similarity search. The 16S rRNA gene sequence analysis placed strain SMB4^T within the genus *Salibacterium*. The results with

type strains using Ezbiocloud indicated that at the 16S rRNA gene sequence level, *Salibacterium halochares*, *Salibacterium halotolerans* and *Salibacterium qingdaonense* were the nearest phylogenetic neighbors, with a pair-wise sequence similarity of 98.4, 98.2 and 97.0% respectively. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 28 sequences. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1339 positions in the final dataset. Phylogenetic analysis based on neighbour-joining tree further revealed clear affiliations of the novel isolate with the genus *Salibacterium* and clustered with *Salibacterium halochares* and together clustered with *Salibacterium halotolerans* and *Salibacterium qingdaonense* (Fig. 1).

Cells of strain SMB4^T were Gram-stain positive, non-motile rods, 0.5-0.6 µm wide and 1.1-1.3 µm long (Supplementary Fig. S1). Endospores are not formed. Colonies were circular, 1.2 mm in diameter, shiny, smooth, opaque, whitish and convex with entire margin after 48 h growth at 37 °C. Growth was observed at 25-50 °C with optimum growth at 37 °C, at 4-12 % (w/v) NaCl, with optimum growth at 10 % and at pH 5-9, with optimum growth at pH 7. Susceptible to (µg/disc unless indicated), cefazolin (30), novobiocin (30), polymixin B (300), cephadroxil (30), cefalexin (30), chloramphenicol (30) and bacitracin (8 units); resistant to kanamycin (30), pencillin G (2 units), spectinomycin (100), cefprozil (30), amoxicilin (30), chlortetracycline (30), vancomycin (30), ceftazidime (30), sulfamethaxazole (300) and streptomycin (300); and intermediately sensitive to ampicillin (25), tetracycline (30), neomycin (30), gentamycin (10) and lincomycin (2). Other characteristics are presented in Table 1.

The cellular fatty acid composition of strain SMB4^T showed a spectrum of 18 fatty acids with a pronounced dominance of (>10 %) of anteiso-C_{15:0}, anteiso-C_{17:0} and C_{18:0} (Table 2). The fatty acids were dominated by saturated and branched (iso and anteiso) fatty acids. The strain SMB4^T differed from *Salibacterium halochares* DSM 21373^T based on the presence of major fatty acid C_{16:0} and absence of some minor fatty acids (Table 2); it differed with *Salibacterium qingdaonense* DSM 21621^T based on the presence of major fatty acid iso-C_{16:0} and C_{18:0} is minor fatty acid (Table 2); and it also differed with *Salibacterium halotolerans* S7^T based on the presence of major fatty acid C_{16:0} and absence of C_{18:0} (Table 2). The cell-wall peptidoglycan of strain SMB4^T contained meso-diaminopimelic acid as the diagnostic diamino acid. The polar lipids of strain SMB4^T comprised diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), one unidentified phospholipid (PL1) and three unidentified lipids (L1, L2, L3) (Supplementary Fig. S2). The strain SMB4^T differed from *Salibacterium halochares* DSM 21373^T based on the presence of a

phosphatidic acid [2]; it also differed with *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T based on the absence of unknown lipids [1]. The DNA base composition of strain SMB4^T was 49.1 mol% G+C (*Tm*). Results of DNA–DNA hybridization between the strain SMB4^T and the type strains of closely related phylogenetic neighbours, *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T, revealed the DNA-DNA relatedness of 39.8, 26.3 and 42.8 %, respectively. These values were below 70%, confirming that the novel strain was not a member of *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T [26].

Strain SMB4^T clusters with the genus *Salibacterium* demonstrating a close phylogenetic relationship with *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T which were chosen for comparative characterization. The characteristics that differentiate strain SMB4^T from *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T are given in Tables 1 and 2. Strain SMB4^T differed from *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T with respect to different phenotypic and chemotaxonomic characteristics (Tables 1 and 2). For instance, strain SMB4^T differed from *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium quigdaonense* DSM 21621^T in cell size, motility, colony colour, salinity growth range and optimum, temperature growth range, pH growth range and optimum, biochemical characteristics, hydrolysis of complex substrates, utilization of various carbon sources and fatty acid composition (Tables 1 and 2). Thus, the cumulative differences that strain SMB4^T exhibits from the *Salibacterium halochares* DSM 21373^T and *Salibacterium quigdaonense* DSM 21621^T unambiguously support the creation of a new species to accommodate strain SMB4^T, for which the name *Salibacterium nitratireducens* sp. nov. is proposed.

Description of *Salibacterium nitratireducens* sp. nov.

Salibacterium nitratireducens (ni.tra.ti.re.du'cens. N.L. n. *nitras* -atis nitrate; L. pres. part. *reducens* leading back, bringing back and in chemistry converting to a different oxidation state; N.L. part. adj. *nitratireducens* reducing nitrate).

Cells are Gram-stain-positive, rod shaped, 0.5-0.6 µm wide and 1.1-1.3 µm long, non-motile, divide by binary fission. Endospores are not observed. Colonies on Marine agar were circular, 1.2 mm in diameter, shiny, smooth, opaque, whitish and convex with entire margin. Growth at 25-50 °C

with an optimum temperature of 37 °C and tolerates up to 12% (w/v) NaCl with optimum growth at 10% (w/v) NaCl. NaCl is required for growth. Growth occurs at pH 5-9, with optimum growth at pH 7. Cells grow aerobically, with a respiratory, chemoorganotrophic mode of metabolism. Oxidase, catalase, β -galactosidase and urease activities are present but lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, aesculinase, caseinase, gelatinase, amylase and lipase activities are absent. Nitrate is reduced. Indole and H₂S gas are not produced. The methyl red reaction is positive and Voges-Proskauer's reaction is negative. Utilizes mannitol, citrate, glucose, maltose, galactose, trehalose and sucrose but not inulin, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, adonitol, arabitol, erythritol, α -methyl-D-glucoside, rhamnose, cellobiose, melizitose, α -methyl-D-mannoside, xylitol, D-arbinose, malonate, sorbose, arabinose, lactose, xylose, fructose, raffinose, melibiose, L-arabinose and mannose. The major cellular fatty acid is anteiso-C_{15:0}, anteiso-C_{17:0} and C_{18:0}. The cell-wall peptidoglycan of strain SMB4^T contained *meso*-diaminopimelic acid, while the polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified phospholipid and three unidentified lipids.

The type strain, SMB4^T (= MTCC 12633^T = KCTC 33876^T = JCM 32187^T) was isolated from a water sample collected from Sambhar salt lake, Rajasthan, India. The DNA base composition of strain SMB4^T was 49.1 mol% G+C (*Tm*).

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Author statements

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Legends to Figures

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain SMB4^T and closely related species of the genus *Salibacterium*. Numbers at nodes are bootstrap values (>60%) based on 10,000 replications. *Tumebacillus permanentifrigoris* Eur1 9^T (DQ444975) is used as an out group. Bar, 0.01 substitutions per nucleotide position.

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Supplementary Fig. S1. Electron micrograph of negatively stained cells of strain SMB4^T. Bar, 1.0 μm.

Supplementary Fig. S2. Two-dimensional thin layer chromatography of the total lipid of strain SMB4^T, developed by molybdophosphoric acid (5% w/v) in absolute ethanol. Diphosphatidylglycerol (DPG), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), unidentified Phospholipid (PL1), unidentified Lipid (L1, L2 and L3).

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Table 1. Features that distinguish strain SMB4^T from the closely related type strains of the genus *Salibacterium*

Data from the present study. All strains were Gram-staining-positive, rod shaped, optimum temperature for growth, grows aerobically, positive for catalase activity; utilizes mannitol and sucrose. All strains were negative for endospore formation, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase activities; H₂S and indole production; Voges Proskauer's reaction; hydrolysis of casein, gelatin and Tween 80; utilization of inulin, sodium gluconate, salicin, dulcitol, inositol, sorbitol, adonitol, arabitol, erythritol, α -methyl-D-glucoside, rhamnose, melizitose, α -methyl-D-mannoside, xylitol, D-arabinose, malonate, sorbose, arabinose, raffinose, melibiose and L-arabinose. +, Positive; -, negative; w, weak positive.

<u>Characteristic</u>	<u><i>Salibacterium nitrireducens</i> SMB4^T</u>	<u><i>Salibacterium halochares</i> DSM 21373^T</u>	<u><i>Salibacterium qingdaonensis</i> DSM 21621^T</u>	<u><i>Salibacterium halotolerans</i> S7^T</u>
<u>Cell size (μm)</u>	<u>1.1-1.3 x 0.5-0.6</u>	<u>1.7-3.5 x 0.5-0.7</u>	<u>1.8-3.2 x 0.3-0.4</u>	<u>1.5-2.8 x 0.1-0.3</u>
<u>Motility</u>	<u>=</u>	<u>±</u>	<u>=</u>	<u>=</u>
<u>Colour of colony</u>	<u>White</u>	<u>Cream</u>	<u>Cream-white to yellow</u>	<u>Cream to yellow</u>
<u>Salinity growth range (% w/v)</u>	<u>4-12</u>	<u>6-24</u>	<u>2.5-20</u>	<u>2-25</u>
<u>Optimum salinity growth (% w/v)</u>	<u>10</u>	<u>15</u>	<u>12</u>	<u>12</u>
<u>Temperature growth range (°C)</u>	<u>25-50</u>	<u>25-45</u>	<u>25-45</u>	<u>25-45</u>
<u>pH growth range</u>	<u>5-9</u>	<u>6-9</u>	<u>6.5-10.5</u>	<u>6.5-10.5</u>
<u>pH for growth optimum</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>9</u>
<u>Biochemical:</u>				
<u>Oxidase</u>	<u>±</u>	<u>±</u>	<u>=</u>	<u>±</u>
<u>Nitrate reduction</u>	<u>±</u>	<u>=</u>	<u>±</u>	<u>=</u>
<u>Methyl Red reaction</u>	<u>±</u>	<u>=</u>	<u>=</u>	<u>=</u>
<u>Hydrolysis of:</u>				
<u>Aesculin</u>	<u>=</u>	<u>±</u>	<u>=</u>	<u>=</u>
<u>ONPG</u>	<u>±</u>	<u>=</u>	<u>=</u>	<u>=</u>
<u>Starch</u>	<u>=</u>	<u>±</u>	<u>=</u>	<u>=</u>
<u>Urea</u>	<u>±</u>	<u>=</u>	<u>±</u>	<u>=</u>
<u>Utilization of:</u>				
<u>Glycerol</u>	<u>=</u>	<u>±</u>	<u>=</u>	<u>=</u>
<u>Cellobiose</u>	<u>=</u>	<u>±</u>	<u>±</u>	<u>±</u>
<u>Citrate</u>	<u>±</u>	<u>=</u>	<u>=</u>	<u>±</u>
<u>Glucose</u>	<u>±</u>	<u>=</u>	<u>±</u>	<u>±</u>

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Lactose	-	±	±	-
Xylose	-	±	±	±
Maltose	±	±	-	±
Fructose	-	-	±	-
Galactose	+	-	+	-
Trehalose	±	-	-	±
Mannose	-	-	±	±
DNA G+C content (mol%)*	49.1	47.2	48	48.4
Source*	Water sample from Sambhar lake	Soil of crystallizer pond of solar saltern	Crude sea-salt sample	Salt pans of Kutch

Characteristic	<i>Salibacterium nitratireducens</i> SMB4 ^F	<i>Salibacterium halochares</i> DSM 21373 ^F	<i>Salibacterium qingdaonensis</i> DSM 21621 ^F	<i>Salibacterium halotolerans</i> S7 ^F
Cell size (µm)	1.1-1.3 x 0.5-0.6	1.7-3.5 x 0.5-0.7	1.8-3.2 x 0.3-0.4	1.5-2.8 x 0.1-0.3
Motility	-	+	-	-
Colour of colony	White	Cream	Cream-white to yellow	Cream to yellow
Salinity growth range (% w/v)	4-12	6-24	2.5-20	2-25
Optimum salinity growth (% w/v)	10	15	12	12
Temperature growth range (°C)	25-50	25-45	25-45	25-45
pH growth range	5-9	6-9	6.5-10.5	6.5-10.5
pH for growth optimum	7	8	9	9
Biochemicals:				
Oxidase	+	+	-	+
Nitrate reduction	+	-	+	-
Methyl Red reaction	+	-	-	-
Hydrolysis of:				
Aesculin	-	+	-	-
ONPG	+	-	-	-
Starch	-	+	-	-
Urea	+	-	+	-
Utilization of:				
Glycerol	-	+	-	-
Cellobiose	-	+	+	+
Citrate	+	-	-	+
Glucose	+	-	+	+
Lactose	-	+	+	-
Xylose	-	+	+	+
Maltose	+	+	-	+
Fructose	-	-	+	-
Galactose	+	-	+	-
Trehalose	+	-	-	+
Mannose	-	-	+	+
DNA G+C content (mol%)*	49.1	47.2	48	48.4

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Characteristic	<i>Salibacterium nitratireducens</i> SMB4 ^T	<i>Salibacterium halochares</i> DSM 21373 ^T	<i>Salibacterium qingdaonensis</i> DSM 21621 ^T	<i>Salibacterium halotolerans</i> S7 ^T
Source*	Water sample from Sambhar lake	Soil of crystallizer pond of solar saltern	Crude sea-salt sample	Salt pans of Kutch

*Data for type strains taken from [1].

Table 2. Comparison of the fatty acid composition of strain SMB4^T with the closely related type strains of the genus *Salibacterium*

Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 10% or more of the total fatty acids are in bold. ND, not detected. Values of less than 0.5% for all strains are not shown. For cellular

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fatty acids analysis, strains SMB4^T, *Salibacterium halochares* DSM 21373^T, *Salibacterium halotolerans* S7^T and *Salibacterium qingdaonense* DSM 21621^T were grown on MA plates at 37 °C for 24 h and were of same physiological age (at exponential phase).

Fatty acid composition	<i>Salibacterium nitratireducens</i> SMB4 ^T	<i>Salibacterium halochares</i> DSM 21373 ^T	<i>Salibacterium qingdaonense</i> DSM 21621 ^T	<i>Salibacterium halotolerans</i> S7 ^T
C _{14:0}	ND	ND	1.1	9.5
iso-C _{14:0}	1.1	0.9	1.7	ND
C _{15:0}	ND	ND	1.0	ND
iso-C _{15:0}	4.7	3.8	6.9	8.5
anteiso-C _{15:0}	27.7	36.5	31.8	37.0
iso-C _{15:1} I	ND	ND	0.6	ND
C _{16:0}	4.2	15.1	8.7	31.7
iso-C _{16:0}	3.2	3.6	11.0	ND
C _{16:1} ω7c	ND	ND	0.8	ND
C _{17:0}	0.6	ND	0.7	ND
iso-C _{17:0}	8.1	5.1	5.6	ND
anteiso-C _{17:0}	22.4	11.9	23.4	13.4
anteiso-C _{17:1} A	0.7	ND	ND	ND
C _{18:0}	10.6	20.5	2.4	ND
iso-C _{18:0}	1.3	0.5	0.4	ND
C _{18:1} 2OH	0.9	ND	ND	ND
C _{18:1} ω7c	ND	ND	1.9	ND
C _{18:1} ω9c	0.8	0.3	0.6	ND
iso-C _{19:0}	3.6	ND	ND	ND
anteiso-C _{19:0}	4.8	1.1	0.2	ND
C _{20:0}	2.2	ND	ND	ND
C _{20:1} ω7c	0.9	ND	ND	ND
*Summed feature 8	0.7	ND	ND	ND

*Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 8 comprised C_{18:1} ω7c and/or C_{18:1} ω6c.

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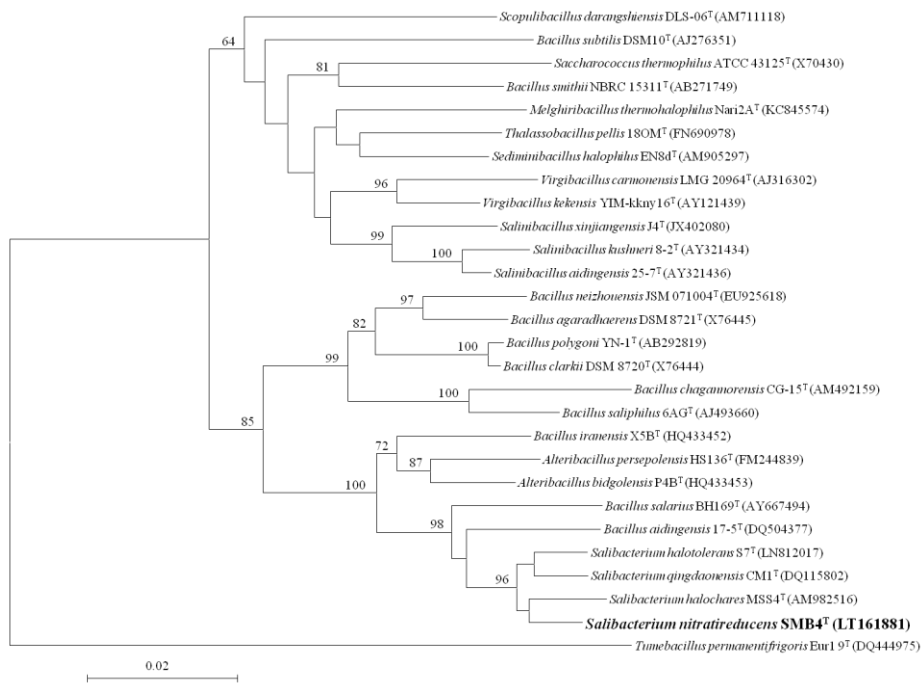
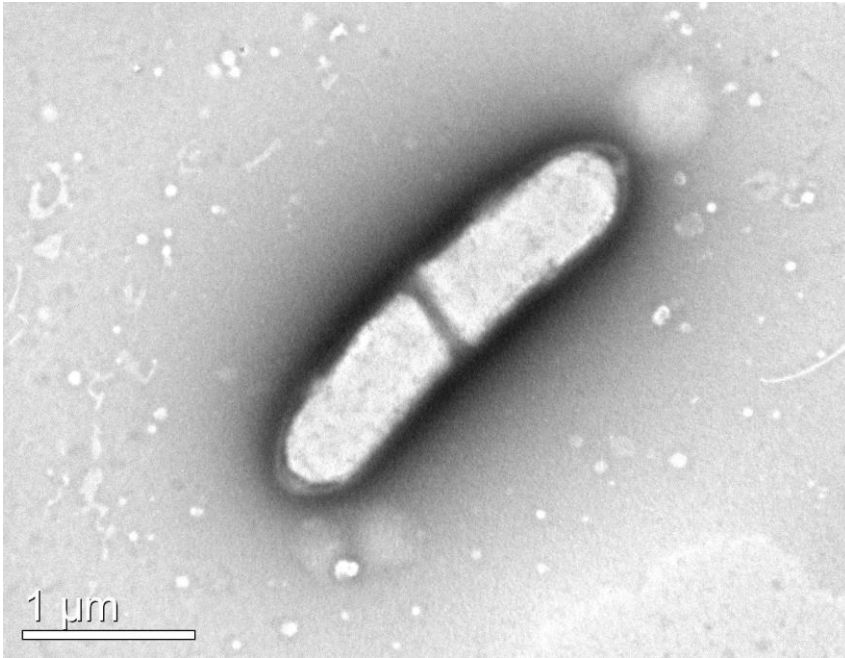
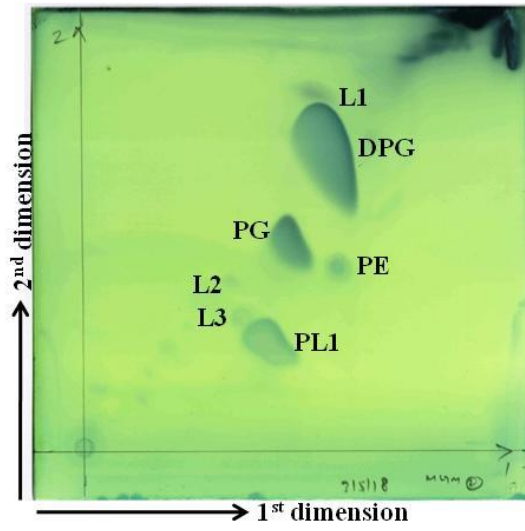


Fig. 1

Supplementary Figures



Supplementary Fig. S1



Supplementary Fig. S2