Orenia salinaria sp. nov., a fermentative bacterium isolated from anaerobic sediments of Mediterranean salterns

Sophie Mouné, Claire Eatock, Robert Matheron, John C. Willison, Agnès Hirschler, Rodney Herbert and Pierre Caumette

INTRODUCTION

Several halophilic, obligately anaerobic, fermentative bacteria belonging to the domain Bacteria have been isolated from sediments of hypersaline environments. Most of these bacteria were isolated from athalassohaline environments such as the Dead Sea, inland salt lakes or subterranean waters in oilfields (Oren, 1992; Ollivier et al., 1994; Rainey et al., 1995; Ravot et al., 1997). Few of them have been isolated from thalassohaline environments such as solar salterns (Liaw & Mah, 1992; Zhilina et al., 1991, 1992; Simankova et al., 1993).

During ecological investigations in solar salterns of the French Mediterranean Coast (Salin-de-Giraud, Camargue, Rhone Delta), we isolated several strains of fermentative halophilic bacteria from the sediments of hypersaline lagoons with total salinities ranging from 13 to 34%. These bacteria co-exist with halophilic, phototrophic and sulfate-reducing bacteria described previously by Caumette et al. (1994). Most of the fermentative bacterial isolates belong to the family Halobacteroidaceae. One of these isolates has been described as a new species with the name Haloanaerobacter salinarius, strain SG 3903T (Mouné et al., 1999). Among the other strains isolated, strain SG 3902T is phylogenetically related to the genus Orenia according to 16S rDNA similarities. This genus is currently represented by a single species, Orenia marismortui, isolated from the Dead Sea (Oren et al., 1987; Rainey et al., 1995). Isolate SG 3902T showed sufficient physiological and genetic differences from the species O. marismortui to be considered as a representative of a new member of the genus Orenia. Thus, strain SG 3902T is described here as a new species of the genus, under the name Orenia salinaria sp. nov.

METHODS

Source of strains. Strain SG 3902T was isolated from the sediment of hypersaline ponds (20–34% total salinity) in the Salin-de-Giraud salterns (Camargue, France). The sulfide-rich black sediment was covered by a thin layer of gypsum; at the highest salinities, a deposit of halite was present.

The GenBank/EMBL/DBJ accession number for the 16S rRNA sequence of strain SG 3902T is Y18488.
Table 1. Substrates fermented by *Orenia salinaria* strain SG 3902T grown with 10% (w/v) NaCl under optimal conditions

<table>
<thead>
<tr>
<th>Substrate (6 mM)</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
</tr>
<tr>
<td>Mannose</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetylgallosamine</td>
<td>–</td>
</tr>
<tr>
<td>Mannitol</td>
<td>–</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>–</td>
</tr>
<tr>
<td>Lactate</td>
<td>–</td>
</tr>
<tr>
<td>Glutamate</td>
<td>–</td>
</tr>
<tr>
<td>Glycine-betaine</td>
<td>–</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>–</td>
</tr>
</tbody>
</table>

The following substrates were tested but not utilized: 0.1% starch or glycogen, 6 mM arabinose, xylose, rhamnose, lactose, raffinose, dextrose, arginine or glyceral. +, Substrate utilized; –, Substrate not utilized.

Media, isolation and culture conditions. The basal synthetic medium used for the growth of strain SG 3902T contained the following (per litre distilled water): NaCl, 150 g; MgCl₂·6H₂O, 15 g; KCl, 3 g; NH₄Cl, 0.5 g; KH₂PO₄, 0.35 g; CaCl₂·2H₂O, 0.05 g; yeast extract, 0.1 g; 0.1% (w/v) resazurin solution, 1 ml; trace-element solution SL12 (Overmann *et al*., 1992), 1 ml; selenite/tungstate solution (Na₃SeO₃·5H₂O, 6 mg l⁻¹; Na₂WO₄·2H₂O, 8 mg l⁻¹; NaOH, 0.4 g l⁻¹), 1 ml; NaHCO₃, 2 g; Na₂S·9H₂O, 0.5 g; vitamin V7 solution (Pfennig *et al*., 1981), 1 ml; pH 7.2–7.4.

The medium was prepared under a gas mixture (N₂/CO₂, 90:10) according to the method of Pfennig *et al*., (1981). Prior to inoculation, the medium was supplemented with organic substrates as carbon and energy sources (see Table 1 for substrate utilization).

Pure cultures of strain SG 3902T were obtained by repeated passage through a deep agar dilution series (Pfennig & Trüper, 1981) in Hungate tubes, with N₂/CO₂ (90:10) in the gas phase and glucose as the organic substrate. The tubes were incubated at 30°C in the dark. The purity of the cultures was checked microscopically and by inoculation into different media specific for aerobic bacteria and sulfate-reducing bacteria.

Pure strains were grown in liquid cultures under a gas phase (N₂/CO₂, 90:10) in 60 ml serum bottles stopped with butyl rubber stoppers, using the Hungate anaerobic technique. The basal medium was supplemented with glucose (6 mM).

Microscopy. Microscopic observations and photomicrographs were made with an Olympus OM2 photomicroscope, according to the method of Pfennig & Wagener (1986). Flagella were observed by transmission electron microscopy with a JEOL 1200 ES electron microscope after negative staining with 1% (v/v) tungstic acid neutralized to pH 7.2. The ultrastructure of the cells was studied by transmission electron microscopy after fixation of a cell pellet with osmic acid and ultrathin sectioning of the cells according to the method of Glazer *et al*., (1971).

Physiological tests. The ability to produce endospores was checked by growth in liquid medium supplemented with 1 mM glucose and 0.18 mM MnSO₄ or 0.5 g yeast extract/soybean extract for spore induction, after exposure of the cells to a temperature of 80°C for 20 min.

Utilization of carbon sources and electron donors was tested in triplicate in basal liquid medium amended with substrates at the concentrations given in Table 1. Growth tests for utilizable substrates, optimum concentrations of NaCl and MgCl₂, optimum pH, optimum temperature and sulfide tolerance were assessed in completely filled 25 ml screw-cap tubes, as described by Caumette *et al*., (1988). The glucose fermentation test was carried out in synthetic medium lacking sodium bicarbonate and buffered with 0.4 M Tris/HCl. The medium was prepared under an N₂ gas phase.

For aerobic growth tests, the basal growth medium without sodium bicarbonate and sulfide was buffered with 0.4 M Tris/HCl and supplemented with glucose as the substrate. Growth was checked in test tubes open to the air and plugged with a cotton-wool stopper. Antibiotic- and antibacterial susceptibility were tested in completely filled screw-cap tubes with the growth medium used for maintenance of strains, supplemented with the following substances: anisomycin (40 µg ml⁻¹), chloramphenicol (20 µg ml⁻¹), tetracycline (40 µg ml⁻¹), erythromycin (40 µg ml⁻¹), kanamycin (40 µg ml⁻¹) and sodium taururonolate (50 µg ml⁻¹), according to the method described by Oren (1990). Growth was measured by following the increase in optical density of the cultures at 450 nm (Spectronic 20; Bausch and Lomb) over a period of 10 d.

The utilization of nitrogen sources (ammonia, nitrate, N₃, cysteine) was checked by growth in liquid medium lacking organic or mineral nitrogen compounds, buffered with MOPS (3 g l⁻¹) and prepared under an argon gas phase with an adequate nitrogen source. The growth was checked through five consecutive transfers.

Vitamin requirements were determined in 60 ml serum bottles by means of growth tests involving five consecutive transfers in synthetic medium lacking vitamins and yeast extract. Catalase was tested by adding a few drops of 3% (v/v) H₂O₂ to a cell pellet on a microscope slide. Oxidase was checked by using the oxidase kit (bioMérieux). The utilization of sulfate, sulfite, thiosulfate or nitrate was tested in Postgate’s Medium B (Postgate, 1984), modified by sulfate omission, in 60 ml serum bottles, with glucose as the electron donor. After growth, the utilization of sulfur compounds was determined by means of sulfide production, as revealed by the formation of a black precipitate of FeS in the liquid culture; nitrate reduction was determined by nitrite production, as revealed by the formation of a red colour following the addition of Griess reagent.

Analytical procedures. The presence of H₂ and CO₂ among the fermentation products was determined using a Chrompack CP 9001 gas chromatograph equipped with a thermal conductivity detector (135°C) and a semi-capillary Poraplot Q column (25 m long; 0.53 mm internal diameter) operated at 35°C with either N₂ (12 ml min⁻¹) or helium.
(12 ml min⁻¹) as the carrier gas for H₂ or CO₂ detection, respectively.

A flame-ionization detector (220 °C) was used for alcohol determination, using the same column operated at 150 °C with helium (12 ml min⁻¹) as the carrier gas.

Organic acids were determined with HPLC with a Shimadzu LC 6A pump, a PYE Unicam UV detector (at 210 nm) and a Rezex organic acid (Phenomenex) column (300 mm x 7.8 mm); 5 mM H₂SO₄ was used as the solvent at a flow rate of 0.5 ml min⁻¹. The volume of the injection loop was 20 µl.

DNA base composition and sequence of the 16S rDNA
Isolation of genomic DNA. Strain SG 3902T was grown in liquid culture with glucose, lyophilized and stored under 2-propanol. Before use, the cells were centrifuged to remove the 2-propanol then resuspended and washed in 0.1% (w/v) SDS/10 mM Tris-HCl/1 mM EDTA, pH 8.0 (Vargas et al., 1995). Genomic DNA was then prepared as described by Ausubel et al. (1989).

Determination of G+C content. The G+C content of genomic DNA from SG 3902T was determined by HPLC as described by Mesbah et al. (1989), using bacteriophage lambda DNA as the standard.

Amplification of 16S rDNA by PCR. A 1.4 kb fragment of the gene encoding 16S rRNA was amplified by the PCR, using the following primers specified by Amann et al. (1995): sense primer, 5'-AGAGTTTGATCCTGGCTCA-3'; Bacteria, positions 8–26 (Escherichia coli numbering); antisense primer, 5'-ACGGGCGGTGTGTA(G)C-3'; Universal, positions 1406–1392 (E. coli numbering). The PCR reaction mixture contained the following (in 100 µl): 0.5 µg genomic DNA; 200 pmol each primer; 50 mM KCl; 10 mM Tris/ HCl, pH 9.0; 0.1% (v/v) Triton X-100; 1.25 mM MgCl₂; 0.2 mM each dNTP; 5% (v/v) DMSO; and 2.5 U Tag DNA polymerase (Promega). An initial cycle comprised 3 min denaturation at 94 °C, 2 min annealing at 50 °C and 3 min extension at 72 °C followed by 34 cycles of 1 min at 94 °C, 2 min at 50 °C and 3 min at 72 °C.

Sequencing of the PCR product. The PCR product was purified after electrophoresis on 1% (w/v) agarose gel, using a SephaGlas BandPrep kit (Pharmacia Biotech). The 1.4 kb PCR product was sequenced directly on an Applied Biosystems Automatic Sequencer (Genome Express), using the PCR and the following two internal primers: 16S-a, 5'-ACTCTTACGGAGGCAGC-3' (E. coli numbering 338–355) and 16S-b, 5'-CGTCAATTCCTTTGAGGTTC-3' (E. coli numbering 626–906).

Genetic analysis. The 16S rRNA/rDNA sequences of 11 species of fermentative, halophilic, anaerobic bacteria were obtained from the EMBL database. The following strains were used (accession nos are in parentheses): Haloanaerobium praevalens DSM 2288T (M59123), Halocella cellulolytica DSM 7362T (X89072), Halothermothrix orenii DSM 544T (L22016), Haloanaerobacter chitinovorans OGC 229T (X89076), Haloanaerobacter lacuarius DSM 6640T (X89075), Haloanaerobacter saliniarius DSM 121467T (Y14212), Halobacteroides halobius DSM 5150T (X89074), O. marismortui DSM 5156T (X89073), Sporohalobacter lortetii DSM 3070T (M59122), Acetohalobium arabaticum DSM 5501T (X89077) and Megaplastera elsdoni ATCC 17752 (M26493). These sequences were aligned with the sequence of strain SG 3902T by the CLUSTAL W method, using the MEGALIGN program of the DNASTAR software package.

The alignment was then modified to remove regions containing unidentified bases or gaps of more than three nucleotides. The subsequent analysis was based on a comparison of approximately 1256 nucleotides.

Analysis of compatible solutes by 13C-NMR spectroscopy. Strain SG 3902T was grown in 8 l batch cultures in the basal medium supplemented with 6 mM glucose and 15% (w/v) NaCl. Cultures were incubated at room temperature (25 °C) and continuously sparged with oxygen-free nitrogen to maintain anaerobic conditions. Mid-exponential phase (OD₅₅₀ = 0.3) cultures were harvested, extracted and analysed by natural abundance spectroscopy according to the methods of Welsh & Herbert (1994b).

RESULTS

Enrichment and isolation

Different types of fermentative bacteria were enriched from the black anoxic sediment below the thin gypsum and halite crust in hypersaline ponds of the Salin-de Giraud (Camargue, France) where the total salinity ranged between 20 and 34% (w/v). Several strains were purified and isolated by using glucose as the fermentable substrate. One of these, strain SG 3902T, was selected for further characterization.

Morphology and fine structure

The individual cells of strain SG 3902T were long rods, 1 µm in width and 6–10 µm in length, in young cultures grown with glucose and 10% (w/v) NaCl (Fig. 1a). After the end of the exponential growth phase, they rapidly formed sphaeroplasts and large irregular cells (Fig. 1b). In old cultures, in media appropriate for spore induction, spherical subterminal spores appeared in the cells (Fig. 1c). The cells were motile. Negatively stained cells showed peritrichous flagella (Fig. 2). Electron-microscopic examination of thin sections of strain SG 3902T revealed a typical Gram-negative, layered cell envelope (data not shown). Colonies were glossy in surface agar and opaque in deep agar shake tubes. They were white to slightly yellow with entire edges; their diameter ranged from 1 to 2 mm.

Growth and physiology

Strain SG 3902T grew over a wide salinity range. Growth was observed at NaCl concentrations between 2 and 30% (w/v) NaCl, with optimal growth at 5–10%. Below 5% (w/v) NaCl and above 10% (w/v) NaCl, the cells were distorted and irregular (Fig. 3). At 2% (w/v) NaCl, the cells were elongated and rapidly formed sphaeroplasts. Above 15% (w/v) NaCl, very large cells appeared and their number increased with increasing salinity (Fig. 3). At the optimal NaCl concentration, the optimal temperature for growth of strain SG 3902T was 40–45 °C and growth occurred at temperatures between 10 and 50 °C. The optimum pH was 7.2–7.4 and growth was possible between pH 5.5 and pH 8.5. For optimal growth, strain SG 3902T

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required a minimum of 1 mM Mg$^{2+}$. No growth was obtained in the presence of oxygen. Under optimal conditions, the growth rate of strain SG 3902$^T$ was 0.452 h$^{-1}$.

The strain showed a high sulfide tolerance, up to 20 mM. Neither growth factors nor vitamins were required.

Strain SG 3902$^T$ was not capable of dissimilatory reduction of inorganic nitrogen (NO$_3^-$) or sulfur compounds (SO$_4^{2-}$, SO$_3^{2-}$, S$_2$O$_3^{2-}$) thus showing that it has a strictly fermentative metabolism. The strain did not use nitrate or cysteine as a nitrogen source but could use dinitrogen and ammonia when grown through five consecutive transfers in liquid medium lacking other nitrogen sources.

The strain was sensitive to chloramphenicol, erythromycin and tetracycline. However, growth was not inhibited by anisomycin, kanamycin or Na-taurocholate.

Strain SG 3902$^T$ used the following fermentable substrates: glucose, fructose, trehalose, sucrose, maltose, cellobiose and mannitol (Table 1). The fermentation products are listed in Table 2. With glucose as the substrate, the major products obtained were ethanol, acetate, formate, lactate, CO$_2$ and H$_2$. Catalase- and oxidase tests were negative.

**Genomic characteristics and 16S rDNA gene sequencing**

The G+C content of the DNA of strain SG 3902$^T$ was 33.7 mol %.

A partial sequence (1337 nucleotides) of the 16S rRNA gene of strain SG 3902$^T$ was determined. The sequence was aligned and a genetic analysis was performed with
16S rRNA gene sequences of 14 representatives of the closest phyla in the domain Bacteria. A distance matrix based on the Jukes & Cantor method (1969) showing the percentage similarities and divergences between the different sequences has been constructed. The sequence from M. elsdenii was included as the outgroup for rooting the dendrogram. The dendrogram derived from the distance matrix is shown in Fig. 4. Only the type species of the genus Haloanaerobium (H. praevalens, DSM 2228T) is shown. Strain SG 3902T was most closely related to O. marismortui (95.1% similarity). The next closest species in the neighbouring genus is H. halobius (90.0% similarity).

**Identification of compatible solutes**

At supra-optimal concentrations, the growth of strain SG 3902T was progressively inhibited resulting both in an increase in the lag phase of growth and a decrease in the growth rate (data not shown). Natural abundance 13C spectra of extracts of strain SG 3902T when grown in media containing 15% (w/v) NaCl showed strong signals at 55-15, 67-90 and 171-56 ppm. These signals correspond to authentic glycine-betaine.

Semi-quantitative estimation of the intracellular glycine-betaine content was carried out when the cells were grown at a salinity of 15%. This estimation,
**Table 2.** Main characteristics of halophilic fermentative bacteria physiologically or phylogenetically close to *Orenia salinaria* strain 3902T

<table>
<thead>
<tr>
<th>Character</th>
<th><em>O. salinaria</em> SG 3902T</th>
<th><em>O. marismortui</em> DSM 5156T</th>
<th><em>S. lortetii</em> DSM 3070T</th>
<th>Halobacteroides halobius†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (width x length, μm)</td>
<td>1 x 6–10</td>
<td>0.6 x 3–13</td>
<td>0.5 x 2.5–10</td>
<td>0.5 x 10–20</td>
</tr>
<tr>
<td>Morphology</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Long rod</td>
</tr>
<tr>
<td>Spore formation</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Gas vacuole</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>NaCl range (%)</td>
<td>2–25</td>
<td>3–18</td>
<td>4–15</td>
<td>8–30</td>
</tr>
<tr>
<td>NaCl optimum (%)</td>
<td>5–10</td>
<td>3–12</td>
<td>8–9</td>
<td>9–15</td>
</tr>
<tr>
<td>Temp. range (°C)</td>
<td>10–50</td>
<td>25–50</td>
<td>25–52</td>
<td>30–47</td>
</tr>
<tr>
<td>Temp. optimum (°C)</td>
<td>40–45</td>
<td>36–45</td>
<td>37–45</td>
<td>37–42</td>
</tr>
<tr>
<td>pH range</td>
<td>5.5–8.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>pH optimum</td>
<td>7.2–7.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>G + C content of DNA (mol%)</td>
<td>33.7 (HPLC)</td>
<td>29.6 (mp)</td>
<td>31.5 (mp)</td>
<td>30.7 (mp)</td>
</tr>
<tr>
<td>Utilization of N₂</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Substrates fermented:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Glutamate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Glycogen</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation products</td>
<td>Lactate, formate, acetate, ethanol, CO₂, H₂</td>
<td>Formate, acetate, ethanol, CO₂, H₂</td>
<td>Acetate, propionate, butyrate, isobutyrate, isovalerate, H₂</td>
<td>Acetate, ethanol, CO₂, H₂</td>
</tr>
</tbody>
</table>

*From Oren et al. (1987).
† From Oren (1983).
‡ From Oren et al. (1984).

Based on peak height of the NMR spectra (with acetate as the internal standard), yielded a value of 1.9–2.2 μmol (mg protein)⁻¹.

**DISCUSSION**

Strain SG 3902T is a Gram-negative, rod-shaped, carbohydrate-fermenting, halophilic, obligate anaerobe that grows optimally at NaCl concentrations of 50–100 g l⁻¹. These characteristics are consistent with the assignment of strain SG 3902T to the family Halobacteroidaceae (Rainey et al., 1995). The genetic relatedness study based on comparison of 16S rDNA sequences showed that strain SG 3902T is a member of this family. In the Halobacteroidaceae cluster, strain SG 3902T is included with the group composed of the genera *Orenia* and Halobacteroides (see Fig. 4). In this cluster, strain SG 3902T is most closely related to the species *O. marismortui* (95.1% sequence identity). On the basis of this phylogenetic relatedness (only 5% difference), our strain SG 3902T should be considered as a new representative of the genus *Orenia*. However, with respect to its DNA base composition (Table 2), *Orenia* strain SG 3902T (G + C content 33.7 mol%) is closer to *S. lortetii* (G + C content 31.5 mol%) than to *O. marismortui* (G + C content 29.6 mol%) or *H. halobius* (G + C content 30.7 mol%).

Like *O. marismortui* and *S. lortetii*, *Orenia* strain SG 3902T is capable of sporulation. However, the spore formation in this strain was not evident when the strain was grown in the defined medium. Spores could be produced in high numbers only after growth in media supplemented with MnSO₄ or yeast/salt extract.

*Orenia* strain SG 3902T uses a rather limited number of carbohydrates compared to its relatives (Table 2). In particular, it is unable to use polysaccharides (glycogen, starch), in contrast to *O. marismortui*, *H. halobius* and *S. lortetii*, all of which are able to metabolize such molecules. Physiologically, *S. lortetii* is rather different, having a capacity to degrade amino acids and to use a different metabolic pathway for...
Orenia salinaria sp. nov.

An interesting property of Orenia strain SG 3902^T was the capacity to use N\textsubscript{2} as the sole nitrogen source, thus suggesting the presence of a nitrogenase activity in this strain. This property has not been demonstrated in the Haloanaerobiales or in O. marismortui.

Orenia strain SG 3902^T was isolated from one of the most hypersaline ponds in the Salin-de-Giraud; the water salinity ranged between 20 and 34\%. It was isolated from the sediment immediately underlying a photosynthetic microbial mat composed of cyanobacteria and purple bacteria. When the salinity increases above 30\%, the microbial mats start to decompose and the coloured layers due to the presence of the photosynthetic micro-organisms are no longer visible. However, most of these bacteria are still present, coexisting with the fermentative bacteria.

The salinity range of Orenia strain SG 3902^T suggests adaptation to this environment, although its optimum salinity (5–10\%, w/v, NaCl) was lower than the usual salinity range in the water. The strain is able to adapt to such salinities (up to 25\%, w/v, NaCl) and subsequent osmotic pressures by the synthesis or uptake of osmotically compatible solutes, principally glycine-betaine. The strain was grown with yeast extract in the culture medium. Since yeast extract contains significant amounts of glycine-betaine (Dulaney et al., 1968), Orenia strain SG 3902^T may possess a functional glycine-betaine-uptake system. Whilst osmotically regulated glycine-betaine transport systems are widespread amongst halotolerant and many halophilic eubacteria (Galinski & Trüper, 1994; Welsh & Herbert, 1994a, b), they have not been reported previously for anaerobic halophiles (Galinski, 1995). Recently, Oren (1999) reported that osmoregulatory processes in the Haloanaerobiales are mainly mediated via mineral salt accumulation and that compatible solutes were never detected in the cells of the described species. As stated by Oren (1999), the synthesis of compatible solutes like glycine-betaine is energetically expensive and difficult for a fermentative bacterium that obtains only a small amount of energy from its substrate. Thus, the accumulation of mineral salts in the cells is a ‘cheaper’ solution for balancing the external salinity. However, Orenia strain SG 3902^T is the first strain of the Haloanaerobiales that is known to accumulate glycine-betaine in the cells in rather large amounts [up to 2.2 \mu mol (mg protein)^{-1}]. probably via an uptake metabolism.

From an ecological standpoint, the ability to accumulate compatible solutes such as glycine-betaine is advantageous since it is energetically more favourable than synthesis. In hypersaline environments such as the Salin-de-Giraud salterns, a diverse range of oxygenic and anoxygenic phototrophs synthesize glycine-betaine as a compatible solute (Caumette, 1993; Caumette et al., 1994) and hence it is likely to be readily available, in the sediment, to fermentative bacteria such as strain 3902^T.

Thus, according to all the genetic and physiological characteristics discussed above, and particularly in view of the 16S rDNA differences relative to the existing species, strain SG 3902^T can be considered as a representative of a new species of the genus Orenia, under the name Orenia salinaria sp. nov.
Description of Orenia salinaria sp. nov.

Orenia salinaria (sa.li.na’ria. L. adj. salinaria pertaining to salinae salterns, salt-works).

Cells are Gram-negative, colourless, sporulating rods, motile by peritrichous flagella. The cells are long (1 μm wide × 6–10 μm long) in young cultures; large degenerate cells and sphaero-plasts are common in old cultures, as are spherical subterminal spores. Surface colonies are circular, translucent, glossy and are white to slightly yellow with entire edges. Colony diameters range from 1 to 2 mm. Obligately halophilic. Growth occurs at NaCl concentrations between 2 and 25% (w/v) NaCl; optimal growth is at 5–10%. The cells accumulate glycine-betaine as the compatible solute. The temperature range for growth is 10–50 °C, with an optimum at 40–45 °C. The pH range is 5.5–8.5, with an optimum at pH 7–7.4. Cells are susceptible to chloramphenicol, erythromycin and tetracycline; they are resistant to anisomycin, kanamycin and Na-cloramphenicol, erythromycin and tetracycline. Obligately anaerobic fermentative; catalase- and oxidase-negative. Glucose, fructose, trehalose, sucrose, maltose, cellobiose and mannitol are fermented. The major products of glucose fermentation are ethanol, formate, acetate, lactate, CO₂ and H₂. Growth occurs with N₂ as the sole nitrogen source. The G+C content of the DNA is 33.7 mol% (HPLC). The habitat is anoxic organic sediment from solar salt-mans. The type strain is strain SG 3902 (HPLC). The source. The G+C content of the DNA is 33.7 mol% (HPLC).

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REFERENCES


