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

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A diverse range of fermentative bacteria have been isolated from the commercial salterns of Salin-de-Giraud (Camargue, France). One of these isolates, strain SG 3902^T, has many of the morphological and physiological characteristics of the genus *Orenia*, as was confirmed by a phylogenetic study based on 16S rRNA gene sequencing. The closest species is *Orenia marismortui*, with a similarity of only 95·1%. However, strain SG 3902^T, unlike *O. marismortui*, does not ferment mannose, glycogen or starch. The G+C contents of the DNA also differ significantly, being 29·6 mol% for *O. marismortui* and 33·7 mol% for strain SG 3902^T. On the basis of these physiological and genetic differences, it is proposed that strain SG 3902^T should be considered as a representative of a new species belonging to the genus *Orenia*, under the name *Orenia salinaria* sp. nov. The type strain is SG 3902^T (= ATCC 700911).

Keywords: halophilic fermentative bacteria, salterns, *Haloanaerobiales*, hypersaline environments, *Orenia*

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 3903^T (Mouné *et al*., 1999). Among the other strains isolated, strain SG 3902^T 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 3902^T 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 3902^T is described here as a new species of the genus, under the name *Orenia salinaria* sp. nov.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain SG 3902^T is Y18485.

METHODS

Source of strains. Strain SG 3902^T 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.

Table 1. Substrates fermented by *Orenia salinaria* strain SG 3902^T grown with 10% (w/v) NaCl under optimal conditions

The following substrates were tested but not utilized: 0·1% starch or glycogen, 6 mM arabinose, xylose, rhamnose, lactose, raffinose, dextrose, arginine or glycerol. +, Substrate utilized; −, substrate not utilized.

Substrate (6 mM)	Utilization
Glucose	+
Fructose	+
Galactose	−
Mannose	−
Trehalose	+
Sucrose	+
Maltose	+
Cellobiose	+
Glucosamine	−
N-Acetylglucosamine	−
Mannitol	+
Pyruvate	−
Lactate	−
Glutamate	−
Glycine-betaine	−
Trimethylamine	−

Media, isolation and culture conditions. The basal synthetic medium used for the growth of strain SG 3902^T contained the following (per litre distilled water): NaCl, 150 g; $MgCl_2.6H_2O$, 15 g; KCl, 3 g; NH_4Cl, 0·5 g; KH_2PO_4, 0·35 g; $CaCl_2.2H_2O$, 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 ($Na_2SeO_3.5H_2O$, 6 mg l^{-1}; $Na_2WO_4.2H_2O$, 8 mg l^{-1}; NaOH, 0·4 g l^{-1}), 1 ml; $NaHCO_3$, 2 g; $Na_2S.9H_2O$, 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 (N_2/CO_2, 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 3902^T were obtained by repeated passage through a deep agar dilution series (Pfennig & Trüper, 1981) in Hungate tubes, with N_2/CO_2 (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 (N_2/CO_2, 90:10) in 60 ml serum bottles stoppered with butyl rubber stoppers, using the Hungate anaerobic technique. The basal medium was supplemented with glucose (6 mM). After growth at 30 °C, the strains were then stored at 4 °C in the dark, for periods of 2–4 months.

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 $MnSO_4$ or 0·5 g yeast extract/soil 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 $MgCl_2$, 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 N_2 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 taurocholate (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, N_2, 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) H_2O_2 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 H_2 and CO_2 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 N_2 (12 ml min^{-1}) or helium

(12 ml min^{-1}) as the carrier gas for H_2 or CO_2 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 using HPLC with a Shimadzu LC 6A pump, a PYE Unicam UV detector (at 210 nm) and a Rezex organic acid (Phenomenex) column (300 mm × 7·8 mm); 5 mM H_2SO_4 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 3902^T 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 3902^T was determined by HPLC as decribed 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 $MgCl_2$; 0·2 mM each dNTP; 5 % (v/v) DMSO; and 2·5 U *Taq* 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′-ACTCCTACGGGAGGCAGC-3′ (*E. coli* numbering 338–355) and 16S-b′, 5′-CGTCAATTCCTTTGAGTTTCA-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 2228^T (M59123), *Halocella cellulolytica* DSM 7362^T (X89072), *Halothermothrix orenii* OCM 544^T (L22016), *Haloanaerobacter chitinovorans* OGC 229^T (X89076), *Haloanaerobacter lacunaris* DSM 6640^T (X89075), *Haloanaerobacter salinarius* DSM 12146^T (Y14212), *Halobacteroides halobius* DSM 5150^T (X89074), *O. marismortui* DSM 5156^T (X89073), *Sporohalobacter lortetii* DSM 3070^T (M59122), *Acetohalobium arabaticum* DSM 5501^T (X89077) and *Megasphaera elsdenii* ATCC 17752 (M26493). These sequences were aligned with the sequence of strain SG 3902^T 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 ^{13}C-NMR spectroscopy. Strain SG 3902^T 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 (OD_{450} = 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 3902^T, was selected for further characterization.

Morphology and fine structure

The individual cells of strain SG 3902^T 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 3902^T 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 3902^T 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 3902^T 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 3902^T

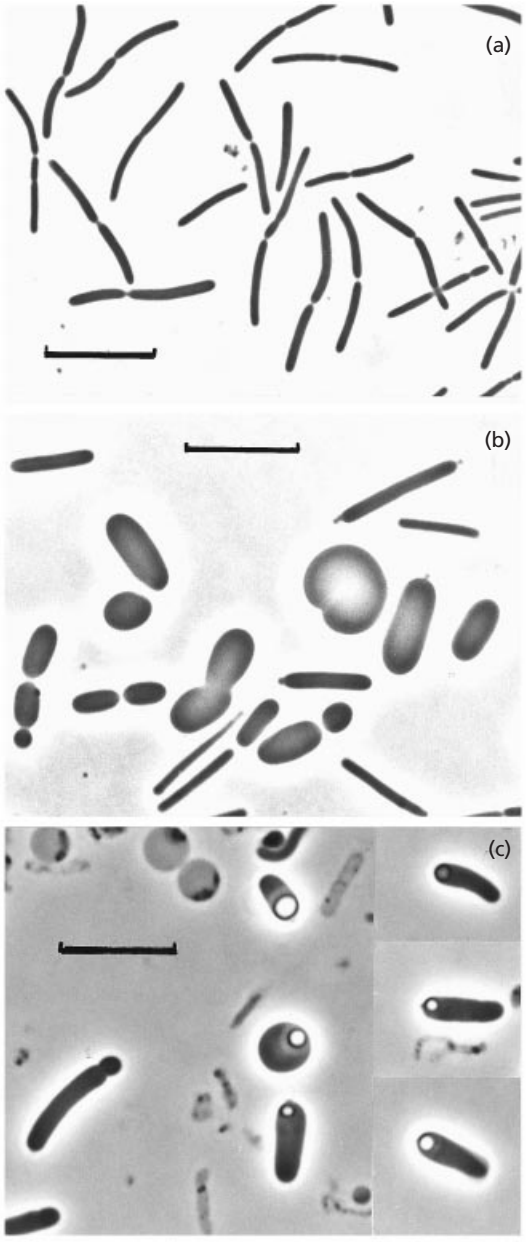

Fig. 1. Microphotographs of *O. salinaria* strain SG 3902^T grown with glucose in the exponential phase of growth (a) and in old cultures with irregular cells (b) or with sporulating cells at different stages of sporulation (c). Bars, 10 μm.

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-}, $S_2O_3^{2-}$) 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.

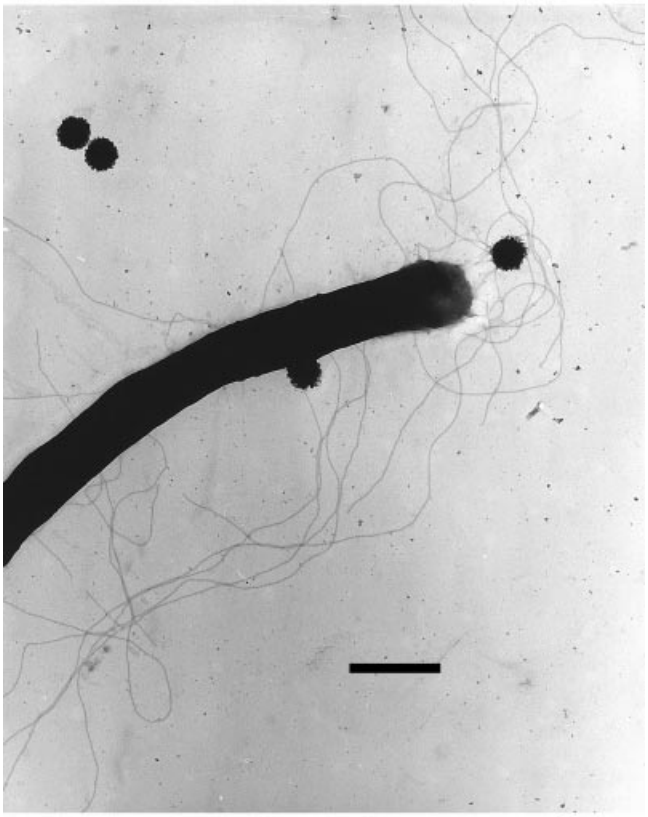

Fig. 2. Electron micrograph of negatively stained cells of *O. salinaria* strain SG 3902^T showing peritrichous flagella. Bar, 1 μm.

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

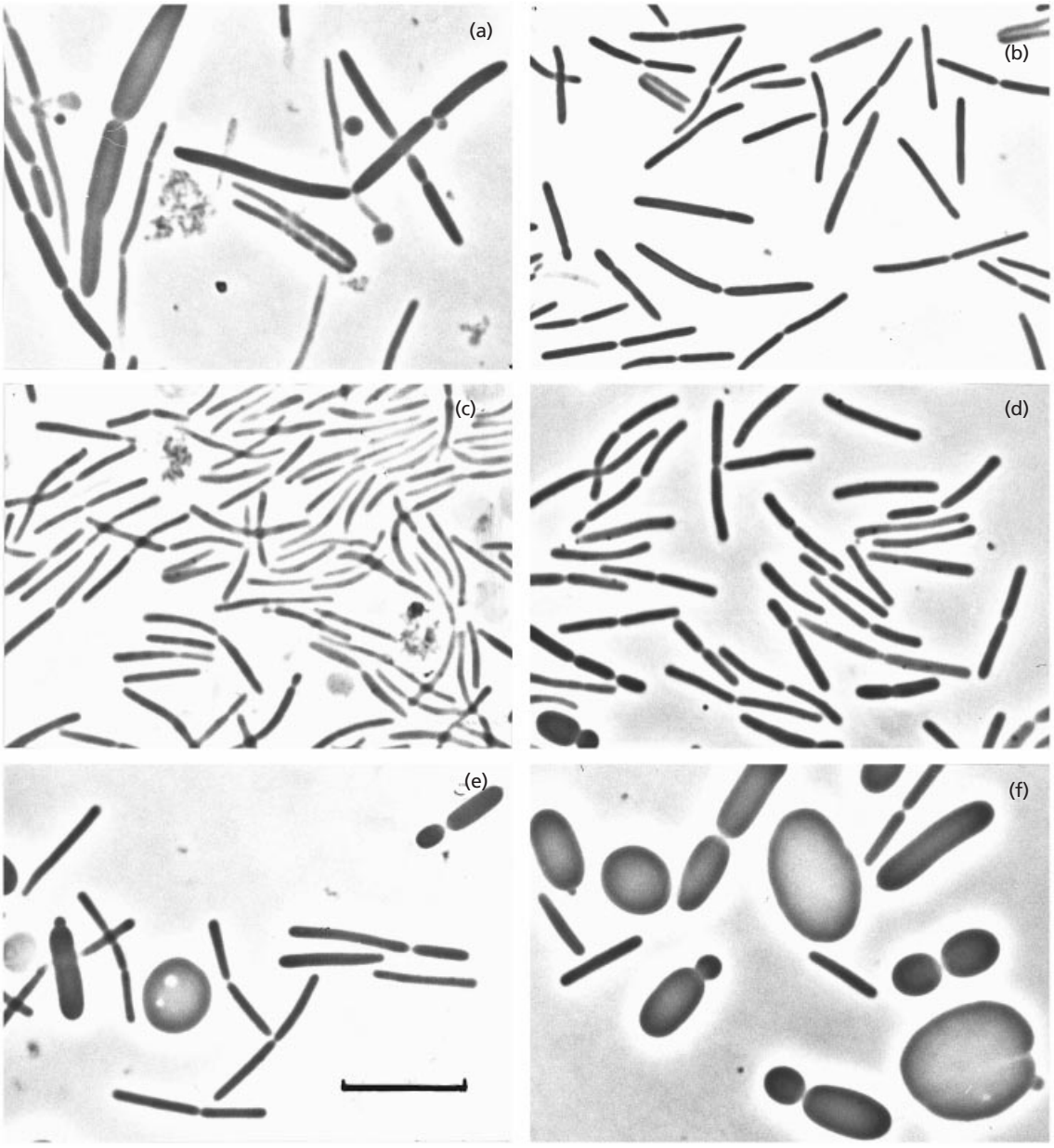

Fig. 3. Photomicrographs of *O. salinaria* strain SG 3902^T grown at different salinities, showing changes in cell morphology: 2 (a), 5 (b), 10 (c), 15 (d), 20 (e) and 25% NaCl (f). All of the photomicrographs are at the same magnification. Bar, 10 μm.

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 out-group 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 2228^T) is shown. Strain SG 3902^T 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 3902^T 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 ^{13}C spectra of extracts of strain SG 3902^T 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 3902^T

+, Positive test result; −, negative test result; ND , not determined.

Character	*O. salinaria* SG 3902^T	*O. marismortui** DSM 5156^T	*S. lortetii*† DSM 3070^T	*Halobacteroides halobius*‡
Cell size (width × length, μm)	1 × 6–10	0·6 × 3–13	0·5 × 2·5–10	0·5 × 10–20
Morphology	Rod	Rod	Rod	Long rod
Spore formation	−	+	+	−
Gas vacuole	−	ND	+	−
NaCl range (%)	2–25	3–18	4–15	8–30
NaCl optimum (%)	5–10	3–12	8–9	9–15
Temp. range (°C)	10–50	25–50	25–52	30–47
Temp. optimum (°C)	40–45	36–45	37–45	37–42
pH range	5·5–8·5	ND	ND	ND
pH optimum	7·2–7·4	ND	ND	ND
G+C content of DNA (mol%)	33·7 (HPLC)	29·6 (mp)	31·5 (mp)	30·7 (mp)
Utilization of N_2	+	ND	ND	ND
Substrates fermented:				
Galactose	−	−	−	+
Sucrose	+	+	ND	+
Mannose	−	+	−	+
Glutamate	−	−	+	ND
Glycogen	−	+	ND	ND
Starch	−	+	+	+
Amino acids	−	−	+	−
Pyruvate	−	−	ND	+
Glucose fermentation products	Lactate, formate, acetate, ethanol, CO_2, H_2	Formate, acetate, ethanol, CO_2, H_2	Acetate, propionate, butyrate, isobutyrate, isovalerate, H_2	Acetate, ethanol, CO_2, H_2

* 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 μ mol (mg protein)$^{-1}$.

DISCUSSION

Strain SG 3902^T 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 3902^T to the family *Halobacteroidaceae* (Rainey *et al.*, 1995). The genetic relatedness study based on comparison of 16S rDNA sequences showed that strain SG 3902^T is a member of this family. In the *Halobacteroidaceae* cluster, strain SG 3902^T is included with the group composed of the genera *Orenia* and *Halobacteroides* (see Fig. 4). In this cluster, strain SG 3902^T 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 3902^T should be considered as a new representative of the genus *Orenia*. However, with respect to its DNA base composition (Table 2), *Orenia* strain SG 3902^T (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 3902^T 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 $MnSO_4$ or yeast/soil extract.

Orenia strain SG 3902^T 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

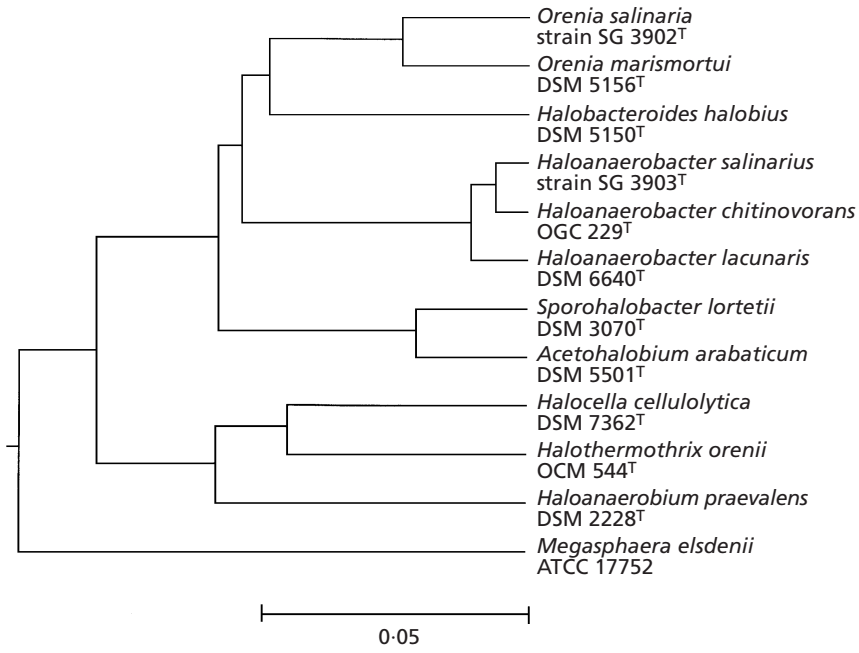

Fig. 4. Phylogenetic tree based on similarities of 16S DNA sequences of *O. salinaria* strain SG 3902^T and its relatives. The bar represents the distance (K_{nuc}) corresponding to 5 nucleotide differences per 100 nucleotides.

glucose fermentation (Table 2). According to the products obtained from glucose fermentation, both *Orenia* strains use the mixed acid pathway, whereas *S. lortetii* probably has a fermentative pathway similar to that for butyric acid fermentation.

An interesting property of *Orenia* strain SG 3902^T was the capacity to use N_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 μmol (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 sphaeroplasts are common in old cultures, as are spherical subterminal spores. Surface colonies are circular, translucid, 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 7·2 –7·4. Cells are susceptible to chloramphenicol, erythromycin and tetracycline; they are resistant to anisomycin, kanamycin and Na-taurocholate. 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, CO_2 and H_2. Growth occurs with N_2 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 salterns. The type strain is strain SG 3902^T (= ATCC 700911^T), isolated from salt ponds in the salterns of Salin-de-Giraud (Camargue, France). The EMBL 16S rRNA gene sequence accession number is Y18485.

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