

***Halogeometricum borinquense* gen. nov., sp. nov., a novel halophilic archaeon from Puerto Rico**

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A novel extremely halophilic archaeon was isolated from the solar salterns of Cabo Rojo, Puerto Rico. The organism is very pleomorphic, motile and requires at least 8% (w/v) NaCl to grow. Polar lipid composition revealed the presence of a novel non-sulfate-containing glycolipid and the absence of the glycerol diether analogue of phosphatidylglycerosulfate. The G C content of the DNA is 59 mol%. On the basis of 16S rRNA sequence data, the new isolate cannot be classified in one of the recognized genera, but occupies a position that is distantly related to the genus *Haloferax*. All these features justify the creation of a new genus and a new species for the family *Halobacteriaceae*, order *Halobacteriales*. The name *Halogeometricum borinquense* gen. nov., sp. nov. is proposed. The type strain is ATCC 700274^T.

Keywords: halophilic bacteria, *Halogeometricum borinquense* gen. nov., sp. nov.,
archaea

INTRODUCTION

The family *Halobacteriaceae* (order *Halobacteriales*, domain *Archaea*) presently contains ten recognized genera: *Halobacterium*, *Haloarcula*, *Haloferax*, *Halococcus*, *Natronobacterium*, *Natronococcus*, (Grant & Larsen, 1990; Tindall *et al.*, 1984; Tindall, 1992; Torreblanca *et al.*, 1986), *Halorubrum* (McGenity & Grant, 1995), *Halobaculum* (Oren *et al.*, 1995), *Natrialba* (Kamekura & Dyll-Smith, 1995) and *Natronomonas* (Kamekura *et al.*, 1997). A primary characteristic of all *Halobacteriaceae* is a requirement for high salt concentrations, with a minimum of 1.5 M

sodium chloride needed for growth (Norton, 1992). Most halobacteria grow optimally at 210–270 g NaCl l⁻¹ (Grant & Larsen, 1990; Norton, 1992).

In 1994, we isolated 20 halophilic archaeal strains from the solar salterns of Cabo Rojo, Puerto Rico. Three strains (designated PR3, PR7 and PR19) were similar in morphological and physiological properties and in lipid composition. They showed unique features that did not correspond to any previously described species (Grant & Larsen, 1990; Kamekura & Dyll-Smith, 1995; Tindall, 1992; Kamekura *et al.*, 1997). Strain PR3^T (T = type strain) was chosen for further characterization. Based on 16S rRNA sequences, polar lipid content and physiological analysis the creation of a new genus for the family *Halobacteriaceae*, order *Halobacteriales* appears to be justified. In this paper we present the characteristics of strain PR3^T.

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The GenBank accession number for the 16S rRNA sequence of PR3^T is AF002984.

METHODS

Collection of samples and isolation of halophilic archaea.

Five water samples (400 ml each) were collected in sterile plastic bags (Whirl-Pak) from the solar salterns in Cabo Rojo, Puerto Rico, between May and August of 1994. Each sample was divided into 50 ml aliquots, which were then filtered through 0.45 µm and 0.22 µm nitrocellulose membranes (Millipore). The membranes were transferred onto agar plates containing Seghal–Gibbons medium (Seghal & Gibbons, 1960) (SG) or glycerol solar salt medium (GSS) (250 g solar salt l⁻¹, 10 g glycerol l⁻¹ and 1 g Casamino acids l⁻¹). Inoculated plates were incubated in sealed bags at 40 °C. After 7 d incubation, representative colonies were transferred to SG broth medium. Pure cultures were obtained by the quadrant streak plate method.

The strains *Halobacterium salinarum* ATCC 19700, *Haloferax mediterranei* ATCC 33500^T, *Halorubrum saccharovororum* ATCC 29252^T, *Haloarcula hispanica* ATCC 33960^T, *Haloarcula marismortui* ATCC43049^T, *Haloarcula vallismortis* ATCC 29715^T, *Halomonas elongata* ATCC 33173^T, *Escherichia coli* ATCC 8677 and *Pseudomonas aeruginosa* ATCC 142 were used as reference strains in biochemical tests and in total and polar lipid analysis.

Morphological, cultural and physiological characteristics.

Isolated strains were examined for motility and morphological features in wet mounts (Vreeland, 1993). Optimal conditions for growth were determined by growing each isolate in SG broth supplemented with 5, 8, 10, 15, 25 and 30% (w/v) NaCl and temperatures of 4, 22, 28, 37, 40 and 50 °C. The pH tolerance of each isolate was also tested in SG medium with pH values 4, 6, 7, 8 and 10. The cultures were inoculated with 100 µl of a late-exponential-phase culture grown at 37 °C without shaking. All of the optimal conditions tests were incubated for 7 d. The extent of growth was determined visually (Gonzalez *et al.*, 1978; Vreeland, 1993). Gram stains were prepared as described by Dussault (1955). Spore and capsule stains were performed according to the methods described by Murray *et al.* (1994). Minimal magnesium requirement for growth was determined using the optimal growth conditions determined above with variable amounts of MgSO₄ (Ihara *et al.*, 1997; Takashina *et al.*, 1990).

Antibiotic sensitivity was tested by spreading bacterial suspensions on plates of SG medium and applying antibiotic discs (penicillin 10 IU, bacitracin 10 µg, novobiocin 30 µg, kanamycin 30 µg, ampicillin 30 µg, vancomycin 30 µg, sulfamethazone and trimethoprim 25 µg, tetracycline 30 µg, erythromycin 15 µg and chloramphenicol 30 µg) (Bonelo *et al.*, 1984; Colwell *et al.*, 1979; Torreblanca *et al.*, 1986). The results were recorded as sensitive or resistant after 14 d incubation at 40 °C, with sensitive being defined as the appearance of a zone of inhibition extending at least 2 mm beyond the antibiotic disc.

Lipid chromatography. Total lipid analysis was performed as described by Ross *et al.* (1981), without freeze-drying the cells. Three hundred milligrams of wet cells were used for lipid extraction. Polar lipid extraction and TLC was performed as described by Torreblanca *et al.* (1986) using single or double development. Plates were stained for glycolipids and other lipids using the α-naphthol stain (Torreblanca *et al.*, 1986) or the CeSO₄ stain (Kates, 1972). The presence of sulfate groups on the separated lipids was determined using Azure A stain as described by Matsubara *et al.* (1994).

Biochemical tests. Nitrate reduction was tested in liquid SG

medium supplemented with 0.1% (w/v) KNO₃ (Tindall *et al.*, 1984). Formation of gaseous products from nitrate was detected by the presence of bubbles in Durham tubes (Vreeland, 1993). Anaerobic growth in the presence of either nitrate or arginine was tested as described previously (Hartmann *et al.*, 1980; Hochstein & Tomlinson, 1985; Mancinelli & Hochstein, 1986). Production of acids from sugars was examined in phenol red broth base (BBL) supplemented with 25% (w/v) NaCl, 0.2% (w/v) KCl and 2% (w/v) MgSO₄·7H₂O (Thongthai *et al.*, 1992). To determine the ability to use sugars as energy sources, the minimal medium of Rodríguez-Valera *et al.*, (1980) was supplemented with 1% of the test sugar and 0.01% yeast extract. Control plates with 1% (w/v) or 0.01% yeast extract were also inoculated. The result was considered positive when growth appeared on the control medium with 1% yeast extract and on the medium with the sugar being tested, but not on the 0.01% yeast extract control (Rodríguez-Valera *et al.*, 1983).

Indole production was detected by adding the Kovacs' reagent to the SG broth supplemented with 1% (w/v) tryptone (Holding & Collee, 1971; Takashina *et al.*, 1990). To determine starch hydrolysis, the strains were streaked onto SG with 1% (w/v) soluble starch and 2% (w/v) Bacto-agar, and the plates were flooded with iodine solution after growth was obtained (Takashina *et al.*, 1990). Catalase was determined by adding a 1% (w/v) H₂O₂ solution to colonies on SG agar medium. Gelatin hydrolysis was detected in SG supplemented with 1% (w/v) gelatin. After 14 days, the tubes were examined for the presence of liquefaction (Holding & Collee, 1971; Tomlinson & Hochstein, 1976). The presence of oxidase was determined using the DRY SLIDE (Difco) biochemical test.

Phase-contrast microscopy. One millilitre of stationary phase cells was centrifuged into a pellet and resuspended in 0.5 ml filter-sterilized brine containing 20% (w/v) NaCl. The cells were then mixed with an equal volume of melted 2% (w/v) agarose containing 20% (w/v) NaCl. A single drop of this cell suspension was placed onto a clean microscope slide and allowed to harden. A cover slip was placed onto the drop of agar and the slide was placed into a 45 °C oven and allowed to melt to form a thin (10 µm) layer. The slide was then cooled and viewed using an Olympus BH phase-contrast microscope. Photographs of individual cells were obtained using an MTK-1 television camera fitted with a 20× ocular lens. Images were digitized and electronically enhanced using the Paint Shop Pro and Power Point editing programs. To enhance the view of the gas vesicles, selected images were digitally treated to a special effect of hot waxing using the Paint Shop Pro editing program. These digital images were printed using an Hewlett Packard 870Cse Deskjet printer system or a Techtronix Phaser II sublimation printer.

Electron microscopy. A 4 µl drop of the culture was applied to a carbon-coated collodion-stabilized copper grid for 30 s. The drop was washed away with 4–5 drops of 1% (w/v) aqueous uranyl acetate and the grid allowed to air dry. Specimens were examined in a Philips CM12 transmission electron microscope operating at 100 kV. Micrographs were recorded at a nominal magnification of ×60000 on Kodak emulsion SO-163 (Oren, 1983).

DNA base composition and 16S rRNA analysis. The G + C content of the DNA and the 16S rRNA phylogenetic analyses were performed by the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ). The DNA for the G + C content was

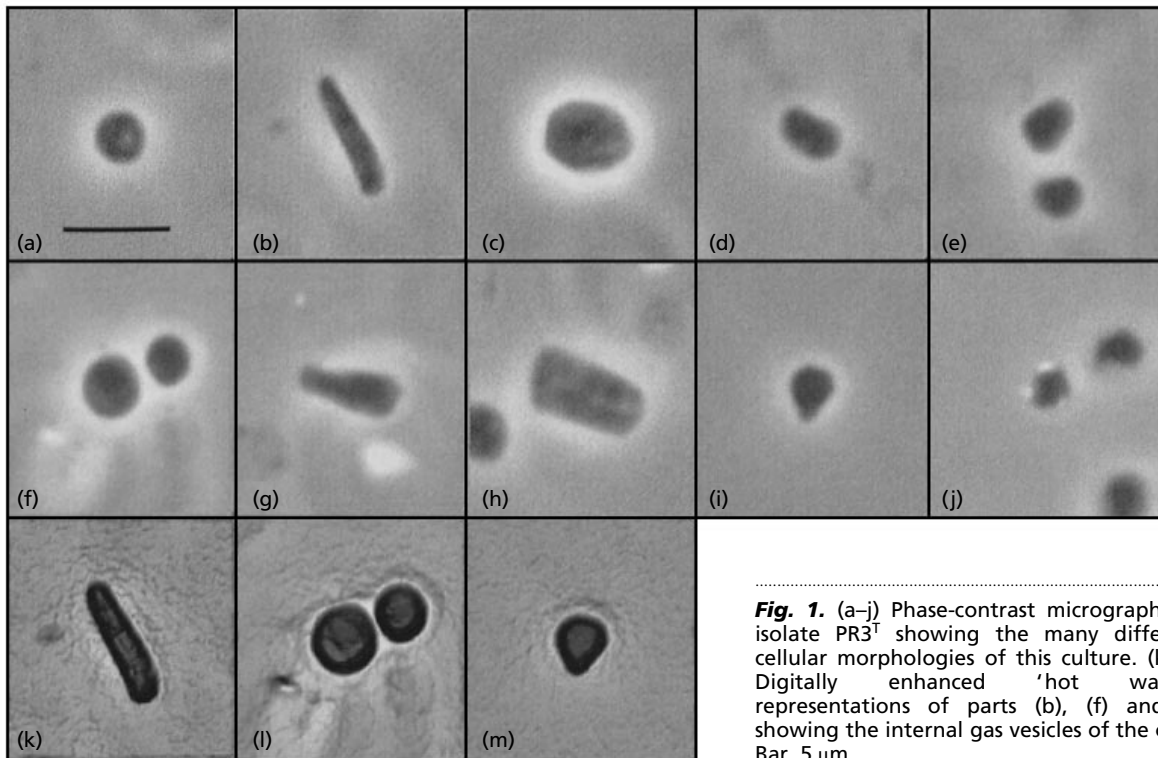


Fig. 1. (a–j) Phase-contrast micrographs of isolate PR3^T showing the many different cellular morphologies of this culture. (k–m) Digitally enhanced 'hot waxed' representations of parts (b), (f) and (i) showing the internal gas vesicles of the cells. Bar, 5 μm.

isolated and purified by chromatography on hydroxyapatite. The G + C content was determined by HPLC.

Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and purification of the PCR products was carried out as described previously (Barns *et al.*, 1994; Rainey *et al.*, 1996). Purified PCR products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the Applied Biosystems 373A DNA Sequencer. A total of 1451 bases corresponding to positions 19–1469 of the *Haloferax mediterranei* sequence (accession no. D11107) were identified during these analyses. Distance analysis of the resulting sequence of PR3^T was performed using the PHYLIP program (version 3.5.1) (Felsenstein, 1993). A multiple-sequence alignment was made by using the CLUSTAL W program with 16S rRNA gene sequences of organisms belonging to the extremely halophilic archaea (McGenity & Grant, 1995; Kamekura & Dyll-Smith, 1995; Oren *et al.*, 1995; Maidak *et al.*, 1996). The halophiles used and their corresponding accession numbers in parentheses were *Halobacterium salinarum* Y12 (D14127), *Haloferax mediterranei* (D11107), *Halorubrum saccharovororum* (X82167), *Haloarcula vallismortis* (D50851), *Haloferax denitrificans* (D14128), *Haloferax gibbonsii* (D13378), *Haloferax volcanii* (K00421), *Halobaculum gomorrense* (L37444), *Halorubrum sodomense* (D13379) and *Natrialba asiatica* BIT (D14124). *Methanospirillum hungatei* (M60880) served as the outgroup. The 16S rRNA gene similarity values were calculated by pairwise comparison of the sequences within the alignment. SEQBOOT was used to generate 100 bootstrapped data sets. Distance matrices were calculated with DNADIST. One hundred trees were inferred by using NEIGHBOR. Any bias introduced by the order of sequence addition was minimized by randomizing the input order. CONSENSE

was used to determine the most frequent branching order. The final tree was drawn using TREEVIEW (Page, 1996).

RESULTS

Cell and colony morphology

Three strains (designated PR3, PR7 and PR19) all isolated from Cabo Rojo proved to have very similar morphology, physiology and lipid compositions. Strain PR3 was studied in further detail and was designated the type strain of the new genus and species described below. Cells of isolate PR3^T were extremely pleomorphic showing different shapes (short and long rods, squares, triangles, ovals) when grown on SG broth (Fig. 1). Mean cell dimensions of the rod forms were 1–3 × 1–2 μm. Motility in SG broth was due to peritrichous flagella (Fig. 2). Cells stained Gram-negative. Spores or capsules were not observed.

Colonies of PR3^T on SG medium were 0.5–1 mm in size, pink, very mucoid, circular in size, convex, with an entire margin after 1 week incubation at 40 °C, and had a milky appearance due to the presence of gas vesicles (Fig. 1k–m, and Fig. 2). On plates of GSS medium, growth was slower, and colonies were less pigmented.

Cultural and biochemical characteristics

Strain PR3^T required at least 8% (w/v) NaCl to grow in SG medium. Optimum growth occurred at 20–25% (w/v) NaCl at 40 °C and a pH of 7. No growth was

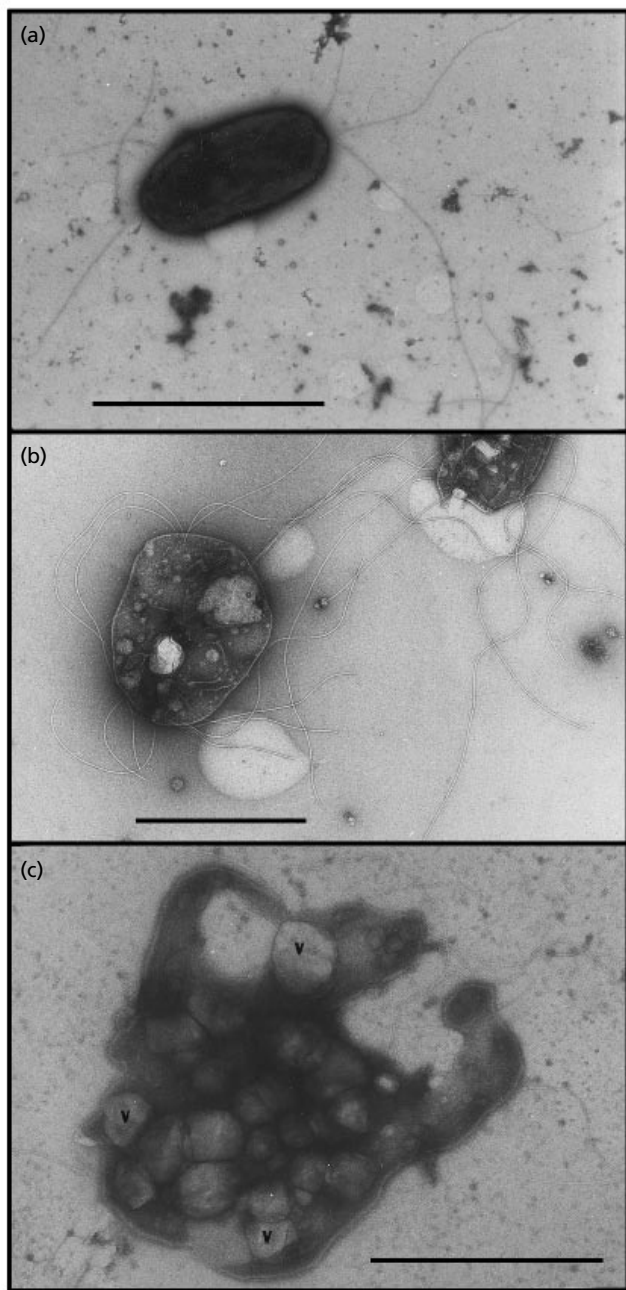


Fig. 2. Electron micrographs of negatively stained strain PR3^T showing (a and b) peritrichous flagella and (c) gas vesicles (V). Bars, 1 µm.

observed below 22 °C or above 50 °C and at pH values below 6 and above 8. At optimal conditions of salinity, temperature and pH, strain PR3^T could grow at very low magnesium concentrations. The best growth was obtained with 40–80 mM Mg²⁺.

The isolate showed positive catalase and oxidase reactions. Nitrite was produced from nitrate with gas production. PR3^T could grow anaerobically in the presence of nitrate. Strain PR3^T strongly hydrolysed

gelatin. Starch was not hydrolysed. Indole was formed from tryptophan. Glucose, mannose, fructose, xylose, maltose, trehalose, cellobiose, raffinose and glycerol were used as carbon sources. Acid was produced from fructose, arabinose, ribose, xylose, lactose and sucrose. The organism was unable to grow anaerobically on arginine.

The strain was resistant to penicillin, kanamycin, ampicillin, vancomycin, tetracycline, erythromycin and chloramphenicol. Isolate PR3^T showed susceptibility to novobiocin, bacitracin and sulfamethazone plus trimethoprim.

Lipid analyses

Total lipid analysis of PR3^T revealed spots attributable to glycerol diether moieties, characteristic of the *Archaea* (Ross *et al.*, 1981).

TLC of polar lipids showed that isolate PR3^T contains the glycerol diether analogues of phosphatidylglycerol (PG), phosphatidylglycerophosphate (PGP) and a single glycolipid (Fig. 3). This glycolipid runs somewhat faster on the TLC plate than TGD-2, the glycolipid marker of *Haloarcula*. Phosphatidylglycerol-sulfate (PGS), which is present in halophilic archaeal species of *Haloarcula*, *Halobacterium*, *Halorubrum* and *Halococcus*, was not detected in isolate PR3^T.

DNA base composition

The G + C content of the DNA of strain PR3^T was 59 mol %. The G + C content of the two similar strains designated PR7 and PR19, was 59.6 and 60.5 mol % respectively.

Phylogeny

The data obtained from the 16S rRNA 1451 bases and the phylogenetic analysis reveal that strain PR3^T occupies a position that is distantly related to the members of the genus *Haloferax* (Fig. 4). The highest similarity value (93.7%) exists with *Haloferax volcanii* ATCC 29605^T (Table 1).

DISCUSSION

The presence of glycerol diether moieties indicates that isolate PR3^T is an extremely halophilic archaeon (Ross *et al.*, 1981; Torreblanca *et al.*, 1986). High salt requirement, resistance to kanamycin, chloramphenicol and penicillin (which inhibit growth of halophilic eubacteria) and susceptibility to bacitracin and novobiocin also place this strain into the *Halobacteriales* (Tindall, 1992; Torreblanca *et al.*, 1986).

The polar lipid chromatogram of strain PR3^T showed a novel pattern (Fig. 3). The sole glycolipid of PR3^T behaved similar to the glycolipid of *Haloarcula* species (1-*O*-[β-D-glucose-(1' → 6')-α-D-mannose-(1' → 2')-α-D-glucose]-2,3-di-*O*-phytanyl-*sn*-glycerol [TGD-2])

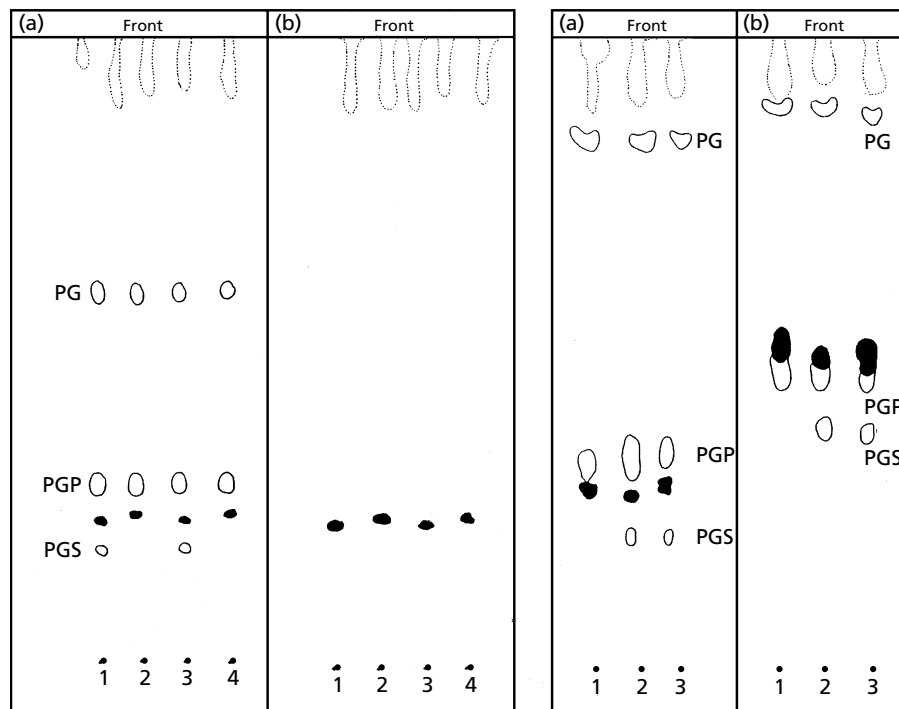


Fig. 3. Left-hand parts: thin-layer chromatogram of polar lipids extracted from strain PR3 (lanes 2 and 4), *Haloarcula marismortui* (lane 1) and *Haloarcula vallismortis* (lane 3). The silica gel plate was developed once with chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) and was stained with CeSO_4 (a) or with α -naphthol (b). Dark spots indicate the position of glycolipids and clear spots indicate phospholipids. Right-hand parts: single development chromatography of polar lipids extracted from strain PR3 (lane 1), *Haloarcula vallismortis* (lane 2), and a mixture of strain PR3 and *Haloarcula vallismortis* (lane 3). The solvents used were chloroform/methanol/acetic acid/water [80:12:15:4, by vol. for (a); 85:22.5:10:4 for (b)], and plates were stained with CeSO_4 to reveal all lipids. In all parts, dark spots indicate the position of glycolipids and clear spots indicate phospholipids. PGP, phosphatidylglycerophosphate; PG, phosphatidylglycerol; and PGS, phosphatidylglycerosulfate.

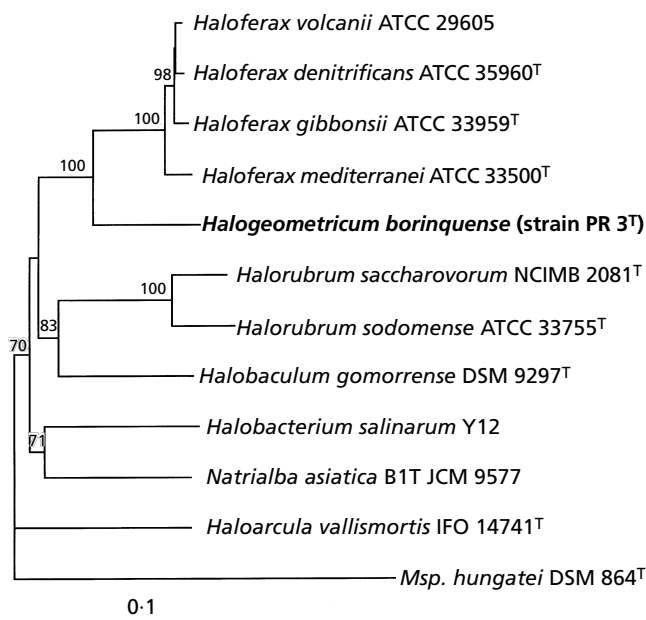


Fig. 4. Neighbour-joining distance tree of 16S rRNA sequences. Bar represents 10 substitutions per 100 nucleotides. Bootstrap values higher than 70% are shown. *Methanospirillum hungatei* DSM 864^T was used as the outgroup.

(Torreblanca *et al.*, 1986), but it ran somewhat faster, indicating that they are not identical. The chromatogram and Azure A staining revealed that strain PR3^T lacks PGS. Other genera that lack PGS are *Natrialba* (Kamekura & Dyll-Smith, 1995), *Haloferax* (Torreblanca *et al.*, 1986), *Halobaculum* (Oren *et al.*, 1995), *Natronobacterium*, *Natronococcus* (Tindall *et al.*, 1984) and *Natronomonas* (Kamekura *et al.*, 1997). Strain PR3^T does not possess any of the common marker lipids associated with the more closely related halophilic genera such as *Haloferax* (Torreblanca *et al.*, 1986), *Halobaculum* (Oren *et al.*, 1995) or *Natrialba* (Kamekura & Dyll-Smith, 1995). Based upon its TLC migration behaviour and staining characteristics, the glycolipid present in isolate PR3^T would appear to be a novel glycolipid which may be useful as a marker for the new genus represented by this strain (Table 1).

Analysis of the 16S rRNA phylogenetic tree (Fig. 4) revealed that strain PR3^T belongs to a separate phylogenetic branch, distantly related to *Haloferax*. The highest similarity existed between strain PR3^T and *Haloferax volcanii* ATCC 29605 (93.7%). Similarity values between PR3^T and *Haloarcula* species are very low (86.2–87.4%), showing that this organism is not phylogenetically close to this genus. Some physio-

Table 1. Main differences between strain PR3^T (*Halogeometricum*) and known genera of neutrophilic haloarchaeaData were obtained from Kamekura & Dyal-Smith (1995), Tindall (1992) and Torreblanca *et al.* (1986).

Characteristic	<i>Haloarcula</i>	<i>Halobacterium</i>	<i>Halobaculum</i>	<i>Halococcus</i>	<i>Haloferax</i>	<i>Halogeometricum</i>	<i>Halorubrum</i>	<i>Natrialba</i>
Cell shape	Irregular rods, triangles, rectangles	Rods	Rods	Cocci	Irregular rods, discs	Short and long rods, squares, triangles, ovals	Rods	Rods
Motility	D	+	±	–	D	+	+	+
Glycolipid present	TDG-2	S-TDG-1, S-TeGD	S-DGD-1	S-DGD-1	S-DGD-1	NG†	S-DGD-3	S ₂ -DGD-1
PGS present	+	+	–	–	–	–	+	–
G + C content (mol%)	62.7–68	66–70.9	70	57–66.6	59.5–64	59.1	65.3–71.2	60.3–63.1

* D, different reactions in different species.

† NG, novel glycolipid not identified.

logical properties of PR3^T also indicated that this organism did not belong to the genus *Haloarcula*. Characteristics such as the presence of gas vesicles, growth at 8% (w/v) NaCl, and the mucoid aspect of the colony do not fit into the description of the genus *Haloarcula*. These features are more typical of *Haloferax* species (Grant & Larsen, 1990; Torreblanca *et al.*, 1986).

Devereux *et al.* (1990) and Fry *et al.* (1991) have proposed that a similarity of less than 98% in a 16S rRNA sequence should be considered evidence for separate species, and that with a similarity of less than 93–95% strains should be classified in different genera. A similarity matrix constructed from a comparison of the 16S rRNA sequences showed that the closest relative to strain PR3 was the genus *Haloferax* at only 92.7–93.3%. The similarity of this strain to the other halophilic genera ranged between 86 and 89%. The similarity of the PR3 16S rRNA to that from both *Halorubrum* and *Halobacterium* was only 88%. Therefore, our proposal that isolate PR3^T represents a new genus that is remotely related to *Haloferax* appears justified on this basis.

Recently, minimal standards for the description of the *Halobacteriales* have been established (Oren *et al.*, 1997). One of the analyses proposed is the characterization of polar lipid patterns, since this property has been shown to be particularly important in classification at the generic level. Based on polar lipid analysis, PR3^T could not be considered a species of *Haloferax*, which has S-DGD-1 as its major glycolipid. Similarity values of 16S rRNA between PR3^T and members of the *Haloferax* are too low to consider PR3^T a member of this genus. In addition, the GC content (59.1 mol%) is not in the range reported for *Haloferax* species (59.5–64 mol%; Table 1).

These data strongly suggest that PR3^T represents a new genus and species. We propose that this strain be named *Halogeometricum borinquense*. Strain PR3^T has been designated the type strain of this group and has been deposited in the American Type Culture Collection as strain ATCC 700274^T.

Description of *Halogeometricum* gen. nov.

Halogeometricum Montalvo-Rodríguez, Vreeland, Oren, Kessel, Betancourt and López-Garriga (Ha.lo.ge.o.me'tri.cum. Gr. n. *hals*, *halos* the sea, salt. L. adj. *geometricum* geometrical; M.L. neut. n. *Halogeometricum* salty geometric shape).

Gram-negative pleomorphic cells (short and long rods, squares, triangles, and ovals). Colonies are pigmented (pink), small, circular, opaque, convex with an entire margin. Oxidase and catalase-positive. Chemo-organotrophic and aerobic. Grows anaerobically in the presence of nitrate. Arginine not fermented. Halophilic, requiring at least 8% (w/v) NaCl for growth. The polar lipids are glycerol diether analogues of phosphatidylglycerol, phosphatidylglycerophosphate, and a single, as-yet-unidentified glycolipid. Phosphatidylglycerosulfate is absent. Certain carbohydrates can stimulate growth with acid production. The G + C content of the type species is 59.1 mol%. The type species is *Halogeometricum borinquense*.

Description of *Halogeometricum borinquense* sp. nov.

Halogeometricum borinquense (bo.rin.quen'se. M.L. fem. adj. *borinquense* of Borinquen, the native Indian name for Puerto Rico).

Cells are extremely pleomorphic (rods are 1–3 × 1–2 µm), showing different shapes (short and long rods, squares, triangles and ovals). Motile by peritrichous flagella. Gas vesicles are present creating a milky appearance. The colonies are pink, small (0.5–1 mm) and very mucoid. Chemo-organotrophic and aerobic. Anaerobic growth occurs with nitrate. Arginine not fermented. Requires at least 8% (w/v) NaCl for growth. The optimal NaCl concentration range is 20–25% (w/v) at 40 °C; the optimal Mg²⁺ concentration range is 40–80 mM. The optimal temperature is 40 °C (in medium containing 20–25% (w/v) NaCl and 40–80 mM Mg²⁺). Nitrate is reduced to nitrite with the production of gas. Indole is produced from tryptophan. Gelatin is strongly hydrolysed.

Starch is not hydrolysed. Resistant to penicillin, kanamycin, ampicillin, vancomycin, tetracycline, erythromycin and chloramphenicol. Susceptible to novobiocin, bacitracin and sulfamethazone plus trimethoprim. Glucose, mannose, fructose, xylose, maltose, trehalose, cellobiose, raffinose and glycerol are used as carbon sources. Acid is produced from fructose, arabinose, ribose, xylose, lactose and sucrose. Isolated from the solar salterns of Cabo Rojo, Puerto Rico. The G + C content of the type strain is 59.1 mol% (as determined by HPLC). The type strain is ATCC 700274^T.

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