A novel haloalkaliphilic archaeon, strain B23T was isolated from the former lake Texcoco in Mexico. The strain was Gram-stain-negative, the cells coccoid to ovoid rods, red pigmented and aerobic. Strain B23T grew in 1.7–4.3 M NaCl, at pH 6.5–9.5 and at 25–45 °C with optimal growth at 2.6–3.4 M NaCl, pH 7.5–8.5 and 37 °C. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain B23T was most closely related to Natronobacterium gregoryi SP2 with 97.3 % sequence similarity. The polar lipids of strain B23T were phosphatidylglycerol and several unidentified phospholipids. The G+C content of the DNA of the strain was 62.5 mol%. Levels of DNA–DNA relatedness between strain B23T and Natronobacterium gregoryi DSM 3393T was 32.3%. The name Natronobacterium texcoconense sp. nov. is proposed. The type strain is B23T (= CECT 8068T= JCM 17655T).

The family Halobacteriaceae was proposed by Gibbons (1974). At the time of writing, the family Halobacteriaceae consist of 40 genera and more than 140 species (http://www.bacterio.cict.fr/classigenerafamilies.html#Halobacteriaceae). The genus Natronobacterium (belonging to the family Halobacteriaceae) was described by Tindall et al. (1984). Several micro-organisms were ascribed to this genus, but have been reclassified subsequently and renamed: Natrobacterium magadii to Natratoba magadii (Kamekura et al., 1997), Natronobacterium nitratireducens to Halobiforma nitratireducens (Hezayen et al., 2002), Natronobacterium pharaonis to Natrialba pharaonis (Kamekura et al., 1997) and Natronobacterium vaculatum to Halorubrum vaculatum (Kamekura et al., 1997). Therefore, at the time of writing (April 2013), only Natronobacterium gregoryi (Tindall et al., 1984) belongs to this genus (http://www.bacterio.cict.fr/n/natronobacterium.html).

The former lake Texcoco in Mexico City is considered an extreme saline alkaline environment where the pH in undrained soil can range from 9.8 to 10.5 and electrolytic conductivities (ECs) in saturated extracts from 22 to 150 dS m⁻¹ (Dendooven et al., 2010). The archaeal community in soil of former lake Texcoco was studied by Valenzuela-Encinas et al. (2008). This study showed six novel clades within the family Halobacteriaceae that could not be identified through phylogenetic analysis. They concluded that this soil could be a source of novel species belonging to new archaeal lineages of the family of the Halobacteriaceae.

Strain B23T was isolated from soil of the former lake Texcoco with EC 127 dS m⁻¹ and pH 9.7 (19° 30’ 52” N 98° 59’ 24” W), using a medium with pH 9 that contained (l⁻¹): yeast extract, 5 g; casamino acids, 5 g; CaSO₄, 2H₂O, 0.17 g; MgSO₄.7H₂O, 0.24 g; KCl, 1 g; NH₄Cl, 1 g and sterilized separately KH₂PO₄, 1 g; Na₂CO₃, 5 g; NaCl, 200 g; trace elements, 1 ml. The solution with trace elements contained (l⁻¹): HCl (25%, 7.7 M), 10 ml; FeCl₂.4H₂O, 1.5 g; ZnCl₂, 70 mg; MnCl₂, 4H₂O, 100 mg; H₂BO₃, 6 mg; CoCl₂.6H₂O, 190 mg; CuCl₂.2H₂O, 2 mg; NiCl₂.6H₂O, 24 mg; Na₂MoO₄.2H₂O, 36 mg. Agar plates were prepared by adding 20 g agar l⁻¹. Samples (25 g) of soil were added to 500 ml Erlenmeyer flasks containing 25 ml medium and cultivated at 37 °C with shaking at 120 r.p.m. for several weeks. Serial decimal dilutions were made to 10⁻⁶. Each dilution was inoculated onto culture media plates using a sterile bent glass rod. Colonies that grew on the plates were
seeded by cross-streaking onto new culture media plates. The cross-stria- tions procedure was done twice to ensure the purity of the colonies. The plates were incubated at 37 °C.

Nucleic acids extraction and amplification of the 16S rRNA gene was done as reported by Ruiz-Romero et al. (2013). Comparison of the 16S rRNA gene sequence of strain B23T showed similarity with Natronobacterium gregoryi a member of the family Halobacteriaceae. The sequence of 16S rRNA gene from strain B23T was 1355 bp long. The phylogenetic analysis (multiple alignment analyses and maximum-likelihood phylogenetic analysis) and drawing of the phylogenetic tree was done as reported by Ruiz-Romero et al. (2013). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the isolates are shown in Fig. 1. The sequence similarity between strain B23T and Natronobacterium gregoryi SP2 (NR 028223) was 97.3 %.

The NaCl concentration range for growth was determined between 0 and 30 % (w/v) with 1 % increments at pH 9 and 37 °C. Growth pH was determined at pH 5.0–11.0 with increments of 0.5 units by adding 1 M HCl or 1 M NaOH at 20 % (w/v) NaCl and 37 °C. Growth temperature was determined at 4–60 °C at pH 9 and 20 % (w/v) NaCl. The methods used for the phenotypic tests were in accordance with the proposed minimal standards for the description of new taxa in the order Halobacteriales (Oren et al., 1997). All tests were done in triplicate.

Cell motility and morphology were determined under a phase-contrast light microscope (Carl Zeiss) at ×63 magnification of cultures grown at 37 °C for 10 days (Fig. S1 available in IJSEM Online). The procedure for scanning electron microscopy was reported previously by Ruiz-Romero et al. (2013) (Fig. S2).

Tests for catalase, casein hydrolysis, indole, mobility, nitrate reduction, oxidase, starch hydrolysis and Tween 80 hydrolysis were performed as described by Barrow & Feltham (2004). The use of different carbon sources by the strain was determined in Bergersen’s synthetic medium (Subba-Rao, 1999) with carboxymethyl cellulose, glucose, lactose, maltose, mannose and sucrose.

The susceptibility to antibiotics was determined using agar plates of a culture medium with the following antimicrobial compounds (50 µg ml⁻¹): erythromycin, neomycin, novobiocin, penicillin, rifampicin. Additionally, the susceptibility to other antibiotics was determined by placing a sample of B23T on agar medium plates with concentrations of the antibiotic per disc: amikacin (30 µg), ampicilin (10 µg), bacitracin (10 U), chloramphenicol (30 µg), cefalotin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), gentamicin (30 µg), levofloxacin (5 µg) netilmicin (30 µg), nitrofurantoin (300 µg), pefloxacin (5 µg) and trimetoprim sulfametoxazol (25 µg).

DNA–DNA hybridization was done by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) Identification Service as described by De Ley et al. (1970) with consideration of the modifications described by Huss et al. (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer.
equipped with a Peltier-thermostat-regulated $6 \times 6$ multicell changer and a temperature controller with in-situ temperature probe (Varian). DNA–DNA hybridization was necessary for the description of the new species as strain B23T shared more than 97 % 16S rRNA gene sequence similarity with Natronobacterium gregoryi SP2T (Stackebrandt & Goebel, 1994; Tindall et al., 2010). The cells were disrupted using a Constant Systems TS 0.75 kW (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite for hybridization experiments (Cashion et al., 1977). DNA G+C content and polar lipids (by TLC) were determined at the DSMZ Identification Service.

Colonies of strain B23T were circular, elevated, entire, small, opaque and red pigmented after incubation at 37 °C for 10 days. Cells of strain B23T were coccoid to ovoid rods (approximately 0.8–1.0 $\times$ 0.3–1.4 µm).

Phylogenetic analysis based on the 16S rRNA gene and maximum-likelihood (Guindon et al., 2010) indicated that strain B23T was closely related to Natronobacterium gregoryi, Halobiforma lacisalis, Halobiforma haloterrestris and Halobiforma nitratireducens (Fig. 1) with 16S rRNA gene sequence similarities to these species of 97.3, 94.9, 94.7 and 94.9 %, respectively. Phenotypic characteristics, biochemical tests and G+C content of the DNA are given in Table 1. Biochemical tests used were based on Cowan and Steel's manual for the identification of medical bacteria (Barrow & Feltham, 2004). The level of DNA–DNA relatedness between strain B23T and Natronobacterium gregoryi DSM 3393T (used as the source of the labelled probes) was 32.3 %. Polar lipid analysis indicated that strain B23T contained phosphatidylglycerol (Fig. S3) and several unidentified phospholipids. The DNA G+C content of strain B23T was 62.5 mol%.

Based on these results, it is concluded that strain B23T represents a novel species of the genus Natronobacterium, for which the name Natronobacterium texcoconense sp. nov. is proposed.

Description of Natronobacterium texcoconense sp. nov.

Natronobacterium texcoconense (tex.co.co.nen’se. N.L. neut. adj. texcoconense of or belonging to Texcoco).

Cells were Gram-stain-negative, coccoid to ovoid rods approximately 0.8–1.0 $\times$ 0.3–1.4 µm. Colonies were red, circular and 0.5–1.0 mm in diameter at 37 °C after 10 days.
of growth. They were aerobic and oxygen was used as the final electron acceptor. Growth occurred in NaCl concentrations of 1.7–4.3 M with an optimum of 2.6–3.4 M and between pH 6.5 and pH 9.5 with an optimum at 7.5–8.5. The temperature range for growth was between 25–45 °C, with optimum at 37 °C. The organism was catalase-positive and indole, oxidase, nitrate reduction, casein hydrolysis, starch hydrolysis, ascusulin hydrolysis, gelatin hydrolysis and Tween 80 hydrolysis were negative. Anaerobic growth with arginine did not occur. The following substrates were used as sole carbon and energy sources: sucrose, glucose, mannose and lactose. Carboxymethyl cellulose and maltose were not used. Fermentation of glucose and ascusulin hydrolysis was positive. Polar lipids of the organism were phosphatidylylglycerol and several unidentified phospholipids. The organism was sensitive to nitrofurantoin, penicillin, rifampicin and trimethoprim-sulfamethoxazole and resistant to amikacin, netilmicin, novobiocin and pefloxacin.

The type strain is *Natronobacterium texcoconense* B23T (≡CECT 8068≡JCM 17655T) isolated from soil of the former lake Texcoco in Mexico. The G+C content of the DNA of the type strain was 62.5 mol%.

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**References**


