**Natronococcus roseus** sp. nov., a haloalkaliphilic archaeon from a hypersaline lake

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A novel halophilic archaeon, strain CG-1\textsuperscript{T}, belonging to the genus *Natronococcus* was isolated from sediment of the soda lake Chagannor in Inner Mongolia, China. The colonies of this strain were pink pigmented, the intensity of the colour decreased when the cells grew at salt saturation levels. The cells were non-motile cocci and strictly aerobic. Hypotonic treatment did not cause cell lysis, even in distilled water. Strain CG-1\textsuperscript{T} grew at 15–30.0 \% (w/v) NaCl and at 30–50 °C and pH 8.0–11.0, with optimal growth occurring at 25–30 \% (w/v) NaCl, 37–45 °C and pH 9–9.5. MgCl\textsubscript{2} was not required for growth. Strain CG-1\textsuperscript{T} was most closely related to the type strains of *Natronococcus amylolyticus* Ah-36\textsuperscript{T}, *Natronococcus jeotgali* B1\textsuperscript{T} and *Natronococcus occultus* SP4\textsuperscript{T}, with which it shared 98.4 \%, 96.2 and 95.7 \% 16S rRNA gene sequence similarity, respectively. The polar lipids consisted of C\textsubscript{20}C\textsubscript{20} and C\textsubscript{20}C\textsubscript{25} derivatives of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and minor phospholipid components. No glycolipids were detected. The DNA G + C content of strain CG-1\textsuperscript{T} was 62.1 mol\%. DNA–DNA hybridization with *N. amylolyticus* DSM 10524\textsuperscript{T}, phylogenetically the most closely related species, was 39 \%; this value showed that strain CG-1\textsuperscript{T} constituted a different genospecies. The comparison of 16S rRNA gene sequences, detailed phenotypic characterization, polar lipid profile and DNA–DNA hybridization studies revealed that strain CG-1\textsuperscript{T} belongs to the genus *Natronococcus* and constitutes a novel species for which the name *Natronococcus roseus* sp. nov. is proposed. The type strain is CG-1\textsuperscript{T} (= CECT 7984\textsuperscript{T} = IBRC-M 10656\textsuperscript{T} = JCM 17958\textsuperscript{T}).

Members of the family *Halobacteriaceae*, the single family recognized within the order *Halobacterales*, have long been identified as the most abundant micro-organisms in hypersaline environments (Oren, 1994). At the time of writing, the family *Halobacteriaceae* comprises 34 genera (Euzèby, 2011). The genus *Natronococcus* was established by Tindall et al. (1984) and currently it contains three species, two of which are haloalkaliphilic: the type species of the genus *Natronococcus occultus* (Tindall et al., 1984) and *Natronococcus amylolyticus* (Kanai et al., 1995), while *Natronococcus jeotgali* is alkalitolerant (Roh et al., 2007).

**Abbreviations:** BPG, bisphophatidylglycerol; PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerol phosphate methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CG-1\textsuperscript{T} is FR877778.

Two supplementary figures are available with the online version of this paper.

The first two species were isolated from the soda lake Magadi and the last one from shrimp jeotgal, a traditional fermented food from Korea. The genus *Natronococcus* is distinguished because the cells are cocci and are found in irregular clusters, pairs or single cells. Members of the genus are non-motile, Gram-variable, strictly aerobic and able to reduce nitrate to nitrite. They grow at salt concentrations between 8 and 30 \% (w/v) NaCl [optimum at 22 \%, (w/v) NaCl], at a pH range of 8.5–11.0 (optimum at pH 9.5) and a temperature range of 20–50 °C (optimum at 40 °C). The DNA G + C content for the major chromosome is 64.0 mol\%, while the minor component has a content of 55.7 mol\% (Tindall et al., 1984).

In the present study, we have characterized strain CG-1\textsuperscript{T}, isolated from sediment of the soda lake Chagannor located in Inner Mongolia, China. Based on the 16S rRNA gene sequence analysis, polar lipid determination and phenotypic...
characteristics, strain CG-1\(^T\) is closely related to members of the genus *Natronococcus*.

Strain CG-1\(^T\) was isolated from a sediment sample from the soda Lake Chagannor (43° 21’ 13° N, 11° 08’ 63° E) located in Inner Mongolia Autonomous Region, China. At the time of sampling, the water of the lake had a temperature of 17.1 °C, the pH was 9.5, the conductivity 21.3 mS cm\(^{-1}\) and the salinity 16% (Grant et al., 2011). Approximately 0.5 g of the sediment sample was dissolved in alkaline saline medium and incubated aerobically at 37 °C; strain CG-1\(^T\) was isolated after 15 days of incubation on alkaline saline solid medium; pure culture was obtained by repeated transfers of separate colonies on agar plates of the same medium. The alkaline saline medium contained (per litre distilled water): peptone (Difco), 5.0 g; meat extract (Difco), 3.0 g; KH\(_2\)PO\(_4\), 3.9 g; MgSO\(_4\), 7H\(_2\)O, 0.78 g; NaCl, 157.0 g; Na\(_2\)CO\(_3\), 21.4 g; NaHCO\(_3\), 17.0 g. The NaCl, Na\(_2\)CO\(_3\) and NaHCO\(_3\) were autoclaved separately and added to the medium prior to incubation (Duckworth et al., 1996). Solid media contained 2.0% (w/v) agar; the strain was maintained on this solid medium and on the same liquid medium with 20% (w/v) glycerol at −80 °C. For comparative purposes, the following culture collection strains were also used in this study: *Natronococcus occultus* DSM 3396\(^T\), *Natronococcus amylyticus* DSM 10524\(^T\) and *Natronococcus jeotgali* DSM 18795\(^T\). These haloarchaea were grown in the media recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and on the same medium used for growing strain CG-1\(^T\).

The characterization of strain CG-1\(^T\) was performed according to the proposed minimal standards for the description of new taxa of the order *Halobacteriales* (Oren et al., 1997). Cell motility and the morphology of exponentially growing liquid cultures were examined using an Olympus BX41 microscope equipped with phase-contrast optics. Colony morphology, pigmentation and size were observed by growth of strain CG-1\(^T\) on agar plates of the same liquid medium with 20% (w/v) glycerol at −80 °C. For comparative purposes, the following culture collection strains were also used in this study: *Natronococcus occultus* DSM 3396\(^T\), *Natronococcus amylyticus* DSM 10524\(^T\) and *Natronococcus jeotgali* DSM 18795\(^T\). These haloarchaea were grown in the media recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and on the same medium used for growing strain CG-1\(^T\).

Polar lipids were extracted with chloroform/methanol as previously described (Corcelli et al., 2000). TLC was carried out using Merck TLC silica gel 60 plates (Art. 5626), the plates were eluted in the solvent system: chloroform: methanol: water: formic acid = 90:30:1 (by volume). The TLC plate was stained by spraying with 5% sulfuric acid in water, followed by heating in an oven at 180 °C. The spots were observed after 20 min, the glycolipids appeared in the first five or ten minutes as red–purple spots. TLC of the polar lipids (see Fig. S1 available at IJSEM online) showed that strain CG-1\(^T\) contained phosphatidyldiglycerol (PG) and phosphatidyldiglycerol phosphate methyl ester (PGP-Me) with different chain length C\(_{20}\)C\(_{20}\) and C\(_{20}\)C\(_{25}\). In addition, a minor band comigrating with bisphosphatidylglycerol (BPG) was present; BPG is the archaeal cardiolipin and is typically present in membrane domains involved in bioenergetics functions (Corcelli et al., 2007). Phospholipid PL2, phosphatidyldiglycerol-(cyclo)-phosphate which is characteristic of *Natronococcus occultus* (Lanzotti et al., 1989) was not present in this organism. Other minor phospholipids not identified yet were present, these lipids were not stained by Azure-A (which specifically stains sulfolipids). Glycolipids were not detected. This polar lipid profile corresponded to that of species of the genus *Natronococcus* (Tindall et al., 1984).

Chromosomal DNA of strain CG-1\(^T\) was isolated and purified according to the method described by Marmur (1961). The G + C content of genomic DNA was determined from the melting point (T\(_{m}\)) of the thermal denaturation profile (Marmur & Doty, 1962) using the equation of Owen & Hill (1979) as described previously (Ventosa et al., 2004). The DNA G + C content of strain CG-1\(^T\) is 62.1 mol%. This value is within the range described for species of the genus *Natronococcus*, 51.9–61.2 mol% (Tindall et al., 1984). The 16S rRNA gene of strain CG-1\(^T\) was amplified by PCR using universal primers as described previously (López-Garcia et al., 2001; Arahal et al., 1996). The almost complete nucleotide sequence of strain CG-1\(^T\) (1541 bp) was aligned by ChromasPro software. The ARB software package (Ludwig et al., 2004) was used for 16S rRNA gene sequence analysis. Following the recommendations of Ludwig et al. (1998), alternative treeing methods (maximum-parsimony, neighbour-joining and maximum-likelihood) were used (Saitou & Nei, 1987). Base-frequency filters were applied in the sequence-comparison analysis and the effects on the results were evaluated. Topologies of phylogenetic trees inferred/reconstructed using the neighbour-joining and maximum-likelihood algorithms were highly similar to that of the tree constructed by maximum-parsimony, so only this is shown.
The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012). The 16S rRNA gene sequence data showed that strain CG-1 T was closely related to *N. amylolyticus* Ah-36T, *N. jeotgali* B1T and *N. occultus* SP4T, showing 16S rRNA gene sequence similarities of 98.4 %, 96.2 % and 95.7 %, respectively. Lower similarities were obtained with the type strains of species of the genus *Halostagnicola* and other haloarchaea. The phylogenetic tree (Fig. 1) constructed by maximum-parsimony confirmed that strain CG-1 T was related to *N. amylolyticus* Ah-36T, *N. jeotgali* B1T and *N. occultus* SP4T, showing 16S rRNA gene sequence similarities of 98.4 %, 96.2 % and 95.7 %, respectively. Lower similarities were obtained with the type strains of species of the genus *Halostagnicola* and other haloarchaea. The phylogenetic tree (Fig. 1) constructed by maximum-parsimony confirmed that strain CG-1 T was related to *N. amylolyticus* Ah-36T, *N. jeotgali* B1T and *N. occultus* SP4T and it was within the cluster constituted by the three species of the genus *Natronococcus*.

DNA–DNA hybridization studies between strain CG-1 T and the type strains of the phylogenetically most closely related species of the genus *Natronococcus* were performed by the competition procedure of Johnson (1994), as described in detail by Gutiérrez et al. (2002). The percentages of DNA–DNA hybridization between strains CG-1 T and *N. amylolyticus* DSM 10524T and *N. occultus* DSM 3396T were 39 % and 48 %, respectively. These levels of DNA–DNA hybridization were low enough for this strain to be classified as a genotypically distinct species (Wayne et al., 1987; Stackebrandt & Goebel, 1994; Stackebrandt et al., 2002), within the genus *Natronococcus*. Differences in phenotypic characteristics, pH range and optimum NaCl concentration for growth, hydrolysis of different compounds and utilization of several substrates (Table 1), polar lipids and 16S rRNA gene sequences, together with the DNA–DNA hybridization data, justify the creation of a novel species within the genus *Natronococcus* for which we propose the name *Natronococcus roseus* sp. nov.

**Description of *Natronococcus roseus* sp. nov.**

*Natronococcus roseus* (ro’se.us. L. masc. adj. *roseus* rose-coloured, referring to the colour of the colonies).

Cells are non-motile cocci, with a diameter of 1–2 μm, occur in irregular clusters, in pairs or as single cells (Fig. S2) and stain Gram-variable. The colonies are circular, entire, smooth, pink pigmented, with a size of 1–2 mm in diameter in alkaline saline medium after 7 days at 37 °C. Cell lysis does not occur in distilled water. Growth occurs in the presence of 15–30 % NaCl, with optimum growth at 25 % NaCl. MgCl₂ is not required. Growth occurs between 30 and 50 °C (optimum 37–45 °C) and pH 8.0–11.0 (optimum

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**Table 1. Differential features of strain CG-1T from other closely related species of the genus *Natronococcus***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Sediment soda lake</td>
<td>Soda lake</td>
<td>Soda lake</td>
<td>Fermented food</td>
</tr>
<tr>
<td>Colony pigmentation</td>
<td>Pink</td>
<td>Orange–red</td>
<td>Brown pale</td>
<td>Orange–red</td>
</tr>
<tr>
<td>NaCl optimum range (%)</td>
<td>25–30</td>
<td>15–20</td>
<td>15–20</td>
<td>23–25</td>
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<tr>
<td>pH optimum</td>
<td>9</td>
<td>9</td>
<td>9.5</td>
<td>7.5</td>
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<tr>
<td>Mg²⁺ requirement</td>
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<td>+</td>
<td>–</td>
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<td>Citrate</td>
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<td>+</td>
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<td>Starch hydrolysis</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<td>Gelatin hydrolysis</td>
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<td>–</td>
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<td>–</td>
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<td>Oxidase</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<td>Utilization of:</td>
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<tr>
<td>d-Fructose</td>
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<td>Lactose</td>
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<td>Acetate</td>
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<td>–</td>
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<td>Presence of PL2 lipid</td>
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<tr>
<td>Sensitivity to erythromycin (15 μg)</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>62.1</td>
<td>63.5*</td>
<td>64.0†</td>
<td>62.1‡</td>
</tr>
</tbody>
</table>

*From Kanai et al. (1995).†Major component (Bd), from Tindall et al. (1984).‡From Roh et al. (2007).
pH 9.0–9.5). Strictly aerobic, nitrate- and nitrite-reduction positive, but gas is not produced, oxidase-negative and catalase-positive. Chemo-organotrophic. Casein, DNA, glycine, asparagine and lysine. Isoleucine is not used as a sole source of carbon, nitrogen and energy. Susceptible to novobiocin (10 μg), bacitracin (10 U), anisomycin (5 μg), erythromycin (15 μg) and streptomycin (10 μg); resistant to rifampicin (5 μg), ampicillin (10 μg), neomycin (30 μg), chloramphenicol (30 μg) and nalidixic acid (30 μg). The polar lipid pattern consists of C20C20 and C20C25 derivatives of PG and PGP-Me and BPG. Phospholipid PL2, phosphatidylglycerol-(cyclo)-phosphate, which is characteristic of Natronococcus occultus, is not present. Other minor phospholipids not identified are present, these lipids were not stained by Azure-A. Glycolipids are not detected. The type strain is CG-1 T (= CECT 7984 = IBRC-M 10656 T = JCM 17958 T). This strain was isolated from sediment of the soda lake Chagannor in Inner Mongolia, China. The DNA G+C content of the type strain is 62.1 mol%.

Acknowledgements

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References


