

**Document Version**

Final published version

**Citation (APA)**

Sorokin, D. Y., Merkel, A. Y., & Yakimov, M. M. (2023). Natrarchaeobaculum. In *Bergey's Manual of Systematics of Archaea and Bacteria, Online* (pp. 1-5). John Wiley & Sons. <https://doi.org/10.1002/9781118960608.gbm02086>

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*Halobacteriota/Halobacteria/Natrialbales/Natrialbaceae/*

# *Natrarchaeobaculum*

Sorokin et al. 2020a<sup>VP</sup>

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*Natr.ar.chae.o.ba'cu.lum*. N.L. neut. n. *natron*, pertaining to soda; Gr. masc. adj. *archaios* ancient; L. neut. n. *baculum*, small stick, rod; N.L. neut. n. *Natrarchaeobaculum* soda-loving archaeal rod.

The genus *Natrarchaeobaculum* is classified as a member of the family *Natrialbaceae*, order *Natrialbales*, class *Halobacteria* according to phylogenomic analyses. It includes extremely halophilic heterotrophic natronoarchaea, some of which can grow anaerobically by sulfur respiration utilizing organic acids, H<sub>2</sub>, and formate as the electron donors. The genus currently includes two species: the facultatively anaerobic type species *Natrarchaeobaculum sulfurireducens* and the aerobic species *Natrarchaeobaculum aegyptiacus*. These species inhabit hypersaline soda lakes. The three letter abbreviation is *Nab*.

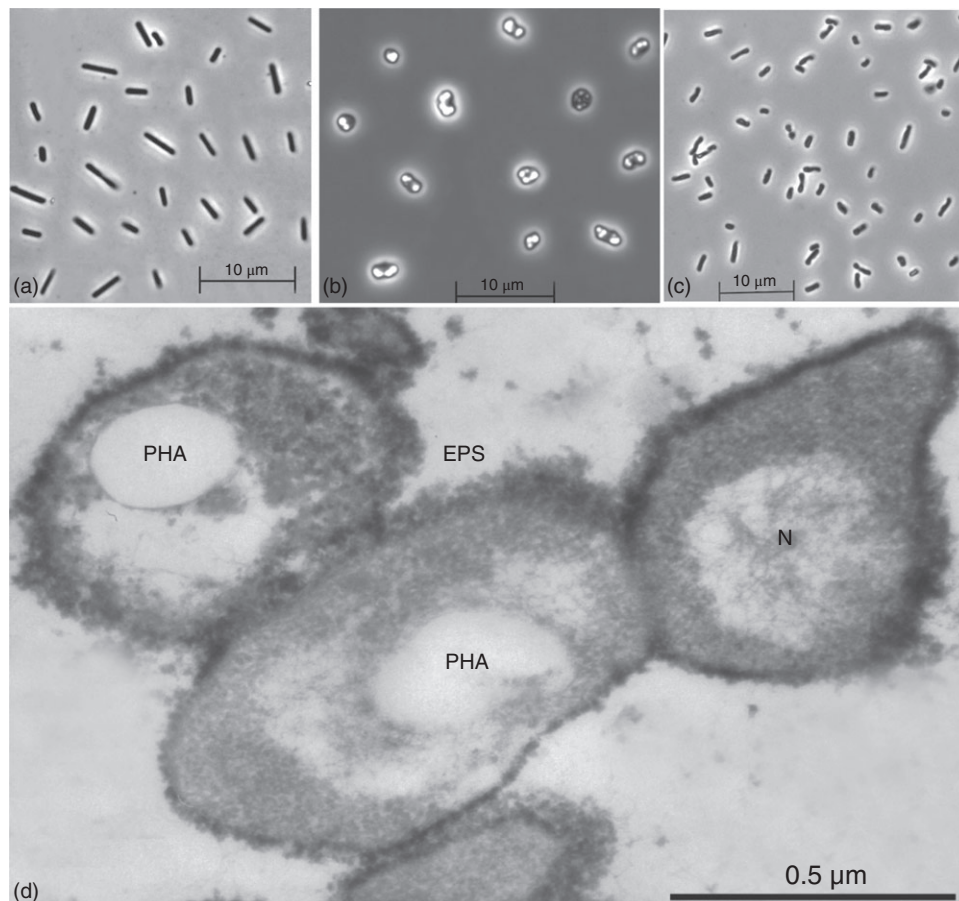
DNA G + C content (mol%): 62.8–64.1 (whole genome sequences).

Type species: ***Natrarchaeobaculum sulfurireducens*** Sorokin et al. 2020a<sup>VP</sup>.

Cells of *Natrarchaeobaculum* are mostly flat rods or cocci, 0.5–0.6 × 1–3.5 μm, and occasionally motile with a thin

monolayer cell wall. Strains of the **type species accumulate polyhydroxyalkanoates (PHA)** during growth with fatty acids. The dominant **core lipids** are C<sub>20</sub>–C<sub>20</sub> **diphytanylglycerol ether (DGE, archaeol)** and C<sub>20</sub>–C<sub>25</sub> **DGE (extended archaeol)** in equal proportion with the **polar groups** dominated by phosphatidylglycerol (**PG**) and phosphatidylglycerophosphate methylether (**PGP-Me**). The dominant **respiratory lipoquinone** is **MK-8:8**. The members of *Natrarchaeobaculum sulfurireducens* are **heterotrophs growing aerobically with C<sub>4</sub>–C<sub>8</sub> fatty acids and peptone** or by **anaerobic sulfur respiration** with the same substrates and, in addition, **utilizing H<sub>2</sub> or formate as the electron donors**. *Natrarchaeobaculum aegyptiacum* is an **obligate aerobe utilizing** a range of **sugars** and **also some organic acids** for growth. Both species are **extremely halophilic** (optimum at 3.5–4.6 M total Na<sup>+</sup>), **obligately alkaliphilic** (pH optimum at 9.1–9.5), and **mesophilic** (maximum temperature 43–56°C). The genus includes two species: the type species *Nab. sulfurireducens* containing multiple closely related isolates from Central Asia and Egypt (Sorokin et al., 2018a, 2019, 2020a) and a single-strain species from Egypt *Nab. aegyptiacus* (Zhao et al., 2018). The genus is **a member of the Natrialbales order** in the class *Halobacteria*.

**FIGURE 1.** Cell morphology of *Nab. sulfurireducens* AArc1<sup>T</sup> (a, b, d) and *Nab. aegyptiacum* (c) grown at 4M total NaCl and pH 9.5. (a and b) phase contrast microphotographs of cells grown anaerobically with formate/S<sub>8</sub> and aerobically with butyrate, respectively; (c) phase contrast micrograph of *Nab. aegyptiacum* grown aerobically with pyruvate; (d) thin section microphotograph of cells grown anaerobically with butyrate/S<sub>8</sub>. PHA, polyhydroxyalkanoate granule; N, nucleoid; EPS, extracellular polysaccharide layer.



*DNA G + C content (mol%)*: 62.8–64.1 (whole genome sequences).

*Type species*: ***Natrarchaeobaculum sulfurireducens*** Sorokin et al. 2020a<sup>VP</sup>.

Number of species with validly published names: 2.

Family classification: The genus *Natrarchaeobaculum* is classified within the family *Natrialbaeae*.

#### Further descriptive information

The cells are flat and polymorphic. Cells of *Nab. sulfurireducens* growing with fatty acids are coccoid and often have large inclusions of polyhydroxyalkanoates (PHA), while the cells growing anaerobically with formate or H<sub>2</sub> are mostly rod-shaped and without inclusions (Figure 1). The biomass

and the colonies grown aerobically are pink or yellowish. In contrast, the cells of *Nab. sulfurireducens* grown under sulfur-reducing conditions lack the carotenoid pigments, and the biomass pellet is black due to accumulation of FeS.

The most characteristic feature of *Nab. sulfurireducens* is its ability to grow anaerobically by elemental sulfur and DMSO respiration (Sorokin et al., 2018a). Especially interesting is its potential to utilize ether H<sub>2</sub> or formate as the electron donor for sulfur respiration, although yeast extract is still required as a carbon source (i.e. lithoheterotrophy). Such catabolism has only been demonstrated so far for two other members of *Halobacteria* – the neutrophilic, obligately anaerobic *Halodesulfurarchaeum* (Sorokin et al., 2017, 2018b) and the alkaliphilic, facultatively anaerobic *Halalkaliarchaeum* (Sorokin et al., 2018a, 2019, 2020b).

### Habitat, enrichment, and isolation

Genus *Natrarchaeobaculum* represents a dominant group of sulfur-reducing natronoarchaea found in hypersaline soda lakes. Most of the strains of *Nab. sulfurireducens* (in total seven) were obtained from anoxic sulfidic sediments. The pure culture isolation was achieved by several rounds of dilution series under anaerobic conditions, and the final purity of the isolate was confirmed by 16S rRNA gene sequencing.

### Genome analysis of *Natrarchaeobaculum sulfurireducens*

Two genomes were sequenced, from the type strain AArC1<sup>T</sup> and from AArC-Mg isolated from hypersaline soda lakes in southwestern Siberia and northeastern Mongolia, respectively. Both are represented by circular chromosomes of 3.5–3.6 Mb and plasmids (two in AArC1<sup>T</sup> and one in AArC-Mg). The detailed genome analysis was published previously (Sorokin et al., 2018a). Here are the most important functional features that were confirmed physiologically:

1. The genomes encode a single periplasmic membrane-bound polysulfide reductase PsrABCD (with the coding genes accompanied by four sulfurtransferases genes) and a DMSO reductase DmsABCD. These complexes are responsible for the observed sulfur- and DMSO-dependent anaerobic respiration.
2. Both genomes have two operons encoding membrane-bound, periplasmic Mo-formate dehydrogenase, and Ni, Fe-uptake hydrogenase responsible for the observed anaerobic growth with formate and H<sub>2</sub> as the electron donors.
3. The archaeal type III PHA synthase is represented by PhaCE.
4. The aerobic respiration in both strains uses two quinol oxidases from the heme-copper superfamily: *bo*<sub>3</sub> (CyoABCD) and *ba*<sub>3</sub> (CbaABCD).
5. Genomes contain multiple copies of membrane transporters responsible for potassium trafficking employed mostly in an inorganic mode of osmoprotection, including TrkAH, KefB, NhaP, and Kch. For the alkaliphilic adaptation, two types of Na<sup>+</sup>/H<sup>+</sup> antiporters are present – a single subunit NhaA and a multisubunit complex MnhABCDEF.

### Maintenance and preservation

Active liquid cultures of *Natrarchaeobaculum* are viable at 4°C for up to 3 months. Long-term preservation by deep freezing is possible with 15% glycerol as a cryoprotectant.

### Taxonomy

Two *Natrarchaeobaculum* species were originally classified on the basis of 16S rRNA gene sequences as distant species of the genus *Natronolimnobius* (Sorokin et al., 2019; Zhao et al., 2018). However, according to phylogenomic analysis based on the 122 conserved single-copy archaeal protein markers, *Natronolimnobius* was reclassified into three different genera, one of which was *Natrarchaeobaculum*, currently incorporating the type species *Nab. sulfurireducens* and *Nab. aegyptiacus* as the second species (Sorokin et al., 2020a) (Figure 2). The latter, in contrast to the type species of the genus, is an obligate aerobe incapable of sulfur respiration (Zhao et al., 2018; Sorokin et al., 2019).

### List of species of the genus *Natrarchaeobaculum*

*Natrarchaeobaculum aegyptiacum*  
Sorokin et al. 2020a<sup>VP</sup> (basonym: *Natronolimnobius aegyptiacus* Zhao et al. 2018<sup>VP</sup>)

.....  
ae.gyp.ti'a.cum. L. neut. adj. *aegyptiacum* Egyptian.

Cells are nonmotile flattened polymorphic rods, 0.5–0.8 × 1.5–2.5 μm. Colonies are from pale yellow to pink. Cells are obligately aerobic organoheterotroph utilizing sugars including D-fructose, D-glucose, D-galactose, D-mannose, cellobiose, maltose, trehalose, D-raffinose; organic acids including acetate, pyruvate, and C<sub>4</sub>–C<sub>8</sub> fatty acids. Can also grow with starch and inulin. Lipase and proteolytic activity are absent. Does not accumulate PHA. Catalase and oxidase are positive. Indole formation from tryptophan is negative. Obligately alkaliphilic and extremely halophilic natronoarchaea, with a pH range for growth from 8.5–9 to 10.1 (optimum at 9.2–9.5), a salt range from 2.5 to 5 M total Na<sup>+</sup> (optimum at 3.2–4.6 M), and a temperature range of 38–56°C (optimum at 52°C). The minimal Cl<sup>-</sup> requirement for growth at 4 M total Na<sup>+</sup> is 1.2 M. Cells lyse in hypotonic conditions at less than 0.5 M NaCl. The only core lipid is C<sub>20</sub>–C<sub>20</sub> DGE (archaeol). The intact polar lipids include PG and PGP-Me with a lesser proportion of PGP. The only respiratory quinone is MK-8:8. The type strain was isolated from sediment of Lake Fazda in Wadi an Natrun (Egypt).



DNA G + C content (mol%): 62.8–62.9 (from two genome sequences).

EMBL/GenBank accession number (16S rRNA gene): DQ834966 (type strain).

EMBL/GenBank accession number (genome assembly): CP024047 and CP024045/CP024046.

### Acknowledgment

The work was supported by the Gravitation-SIAM Program of the Dutch Ministry of Education and Science (grant 24002002).

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