Natrarchaeobaculum

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Genus

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Natrarchaeobaculum

Sorokin et al. 2020a[VP]

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Natr.ar.chae.o.ba'cu.lum. N.L. neut. n. *natron,* pertaining to soda; Gr. masc. adj. *archaîos* 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_2, 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[VP].

Cells of *Natrarchaeobaculum* are mostly flat rods or coccids, 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_{20}–C_{20} diphytanylglycerol ether (DGE, archaeol) and C_{20}–C_{25} 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_4–C_8 fatty acids and peptone** or by **anaerobic sulfur respiration** with the same substrates and, in addition, **utilizing H_2 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^+), **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.*

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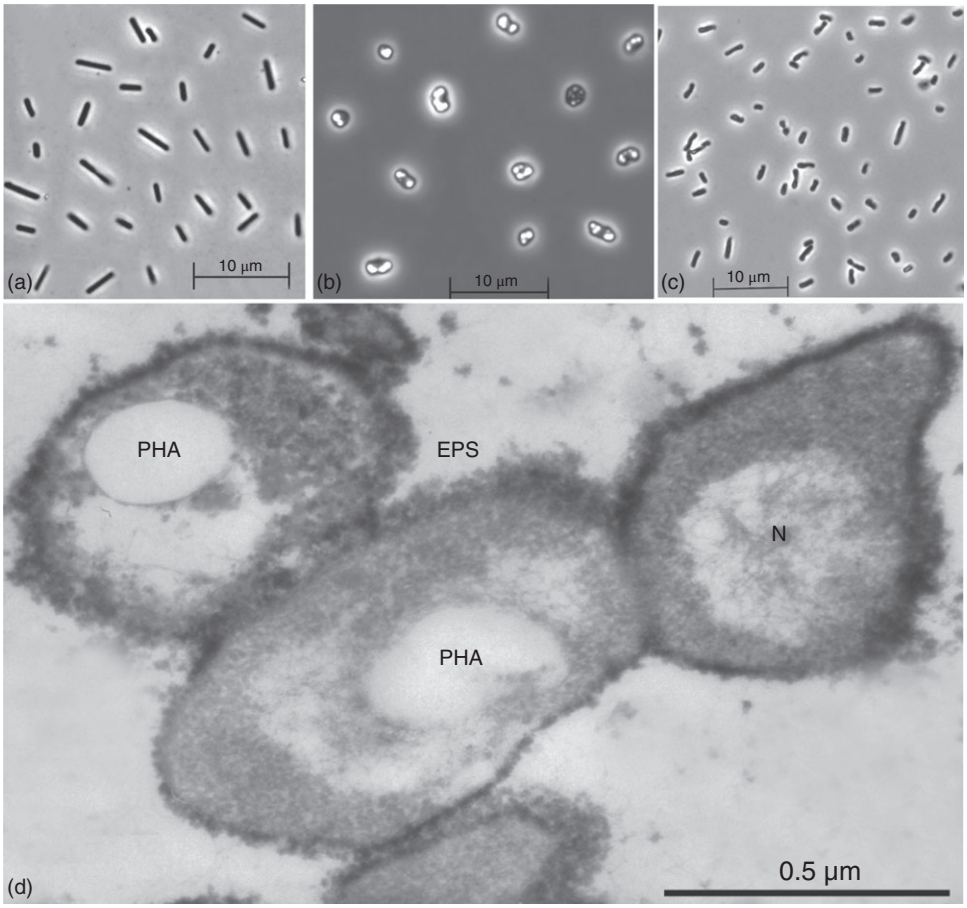
FIGURE 1. Cell morphology of *Nab. sulfurireducens* AArc1^T (a, b, d) and *Nab. aegyptiacum* (c) grown at 4 M total NaCl and pH 9.5. (a and b) phase contrast microphotographs of cells grown anaerobically with formate/S_8 and aerobically with butyrate, respectively; (c) phase contrast micrograph of *Nab. aegyptiacum* grown aerobically with pyruvate; (d) thin section microphotograph of cells grown anaerobically with butyrate/S_8. 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^{VP}.

Number of species with validly published names: 2.

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

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_2 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_2 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^T 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^T 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_2 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_3 (CyoABCD) and ba_3 (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^+/H^+ antiporters are present – a single subunit NhaA and a multisubunit complex MnhABCDEFG.

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).

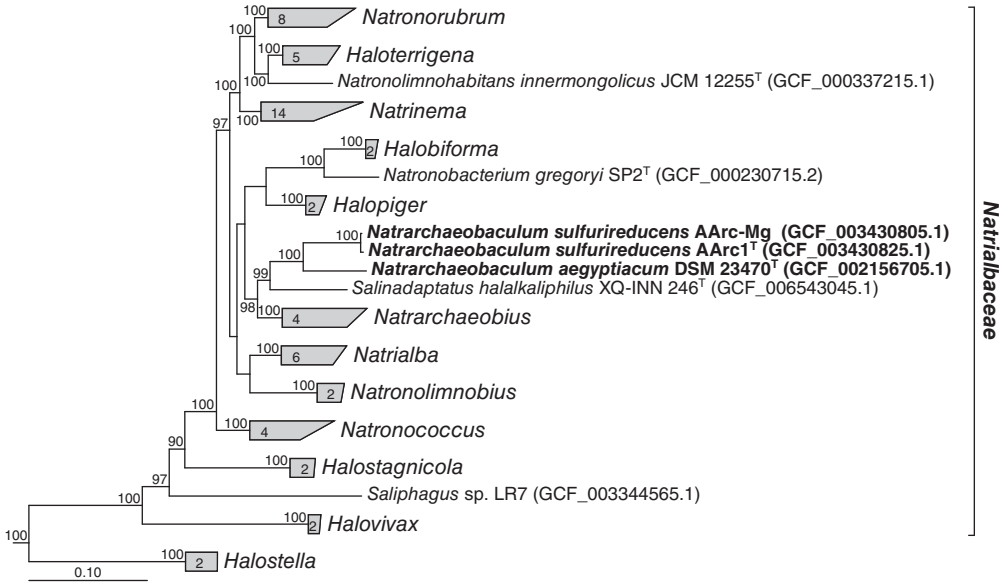
List of species of the genus *Natrarchaeobaculum*

Natrarchaeobaculum aegyptiacum Sorokin et al. 2020a^{VP} (basonym: *Natronolimnobius aegyptiacus* Zhao et al. 2018^{VP})

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_4–C_8 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^+ (optimum at 3.2–4.6 M), and a temperature range of 38–56°C (optimum at 52°C). The minimal Cl^- requirement for growth at 4 M total Na^+ is 1.2 M. Cells lyse in hypotonic conditions at less than 0.5 M NaCl. The only core lipid is C_{20}–C_{20} 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).

FIGURE 2. Phylogenetic position of *Natrarchaeobaculum* based on sequence analyses of concatenated alignment of 122 single copy conserved bacterial protein markers (Parks et al., 2020) within the class *Halobacteria*. The tree was built using the IQ-TREE 2 program (Minh et al., 2020) with fast model selection via ModelFinder (Kalyaanamoorthy et al., 2017) and ultrafast bootstrap approximation as well as approximate likelihood-ratio test for branches (Anisimova and Gascuel, 2006). Bootstrap consensus tree is shown with values above 90% placed at the nodes. Bar, 0.10 changes per position.



Type strain: JW/NM-HA 15 (ATCC BAA-2088 =DSM 23470).

DNA G+C content (mol%): 64.1 (from genome sequence).

EMBL/GenBank accession number (16S rRNA gene): KX857214 (type strain).

EMBL/GenBank accession number (genome assembly): CP019893.

Natrarchaeobaculum sulfurireducens Sorokin et al. 2020a^{VP} (basonym: *Natronolimnobius sulfurireducens* Sorokin et al. 2019^{VP})

sul.fu.ri.re.du'cens L. neut. n. *sulfur*, sulfur (S); L. pres. part. *reducens*, leading back, reducing; N.L. part. adj. *sulfurireducens*, reducing sulfur.

The cells are flattish and polymorphic, from rods to cocci, 0.5–0.6 × 1–3.5 µm, depending on the growth condition, and occasionally motile. The cell wall consists of a thin *S*-monolayer covered with an exopolysaccharide (EPS) matrix. The cells lyse in hypotonic solutions below 1–1.5 M Na^+. Red pigments are produced during aerobic growth. The membrane diether lipids are composed of C_{20}–C_{20} DGE (archaeol) and C_{20}–C_{25} DGE (extended archaeol) cores in equal proportion with the PGP-Me, PG, and PGP polar heads. The dominant respiratory menaquinone is MK-8:8. The cells are facultatively anaerobic. Anaerobic respiratory growth is possible with either elemental sulfur or DMSO as the electron acceptor and the following electron donors: H_2 and formate (in presence of acetate or yeast extract as carbon sources), C_4–C_9 fatty acids, pyruvate, lactate, glycerol, and peptone. Aerobic growth is possible with acetate and the above-mentioned substrates except for H_2 and formate. Sugars are not utilized. Ammonium or amino acids serve as the nitrogen source. Oxidase is weakly positive, and catalase is positive. Optimum growth temperature is 40–43°C (maximum is 48°C at pH 9). It is extremely halophilic with a range of total Na^+ for growth from 2.5 to 5 M (optimum at 3.5–4 M) and obligately alkaliphilic, with a pH range for growth from 8.5–9 to 10.5 (optimum at 9.5–10). It is found in hypersaline soda lakes. The type strain was isolated from anaerobic sediments of a hypersaline soda lake in Kulunda Steppe (Altai, Russia). The species also includes other six closely related strains isolated from various hypersaline soda lakes in Central Asia, Africa, and North America. The genome of the type strain consists of a circular chromosome and two plasmids with the GenBank accession numbers CP024047 and CP024045/CP024046, respectively.

Type strain: AArc1 (=JCM 15733=UNIQEM U237).

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

EMBL/GenBank accession number (16S rRNA gene): DQ 834966 (type strain).

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

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