Natranaeroarchaeum

Sorokin, Dimitry Y.; Merkel, Alexander Y.; Yakimov, Michail M.

DOI
10.1002/9781118960608.gbm02091

Publication date
2024

Document Version
Final published version

Published in
Bergey's Manual of Systematics of Archaea and Bacteria, Online

Genus

Halobacteriota/Halobacteria/Halobacteriales/Natronoarchaeaceae/

Natranaeroarchaeum

Sorokin et al. 2022a, VL211

Dimitry Y. Sorokin, *Winogradsky Institute of Microbiology, Federal Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, Russia and Department of Biotechnology, Delft University of Technology, Delft, The Netherlands*

Alexander Y. Merkel, *Winogradsky Institute of Microbiology, Federal Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, Russia*

Michail M. Yakimov, *Institute of Polar Research, ISP-CNR, Messina, Italy*

Edited by: William Whitman, *University of Georgia, Athens, GA, USA*

Natr.an.aer.o.ar.chae'um N.L. neut. n. *natron*, soda; from Arabic n. *natrun*, soda, sodium carbonate; Gr. pref. *an-*, not (here: inseparable prefix); Gr. masc. n. *aêr* air; Gr. masc. adj. *archaîos*, ancient; N.L. neut. n. *Natranaeroarchaeum*, anaerobic natronophilic archaeon.

The genus *Natranaeroarchaeum* is classified as a member of the family *Natronoarchaeaceae*, order *Halobacteriales*, and class *Halobacteria*, according to phylogenomic analyses. It includes extremely halophilic and facultatively aerobic, obligately alkaliphilic, and saccharolytic archaea, capable of anaerobic sulfur respiration with sugars and starch as carbon and energy sources. The genus currently includes two species, the type species *Natranaeroarchaeum sulfidigenes* and *Natranaeroarchaeum aerophilum*, originating from hypersaline soda lakes. The DNA G + C content is 60.8–61.0 (whole-genome sequences). The genus three-letter abbreviation is *Naa*.

DNA G + C content (%): 60.8–61.0 (whole-genome sequences of type strains).

Type species: **Natranaeroarchaeum sulfidigenes** Sorokin et al. 2022a, VL211.

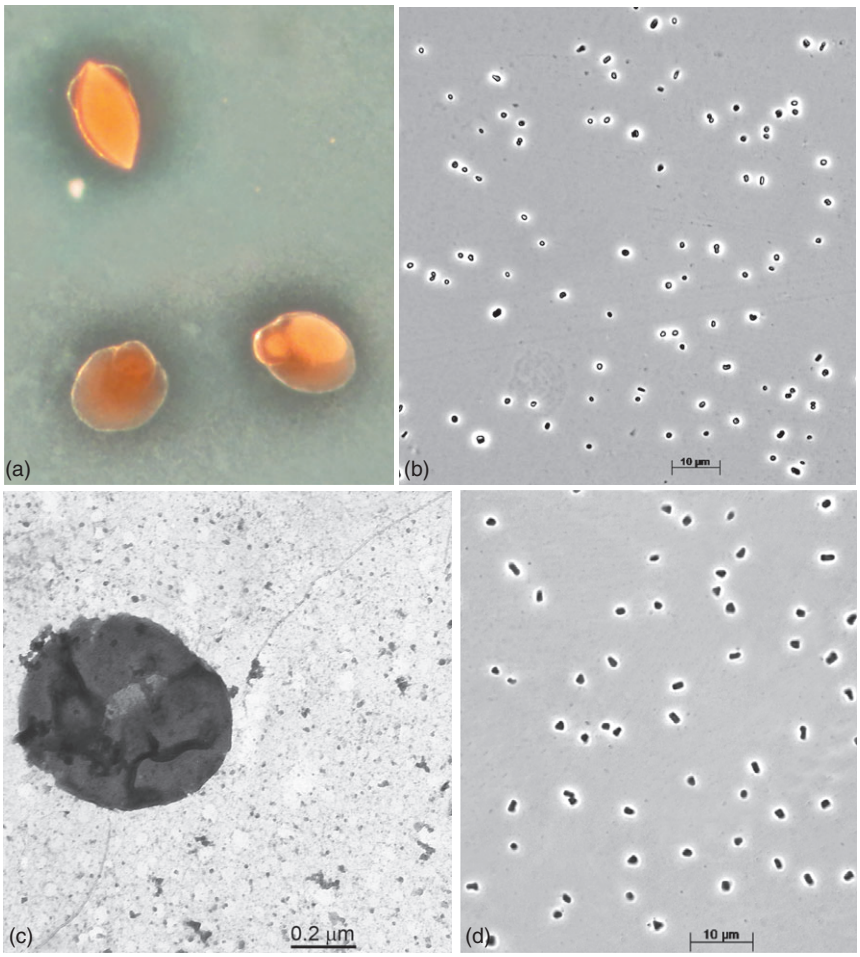
Description

Cells of *Natranaeroarchaeum* are polymorphic flat rods or cocci, 0.5–0.6 × 1–3.0 µm, with a thin monolayer cell wall. Cells grown both anaerobically with sulfur as an electron acceptor and aerobically produce red carotenoids. The **core lipids** are **C_{20}–C_{20} diphytanylglycerol ether (DGE, archaeol) and C_{20}–C_{25} DGE (extended archaeol)** with 1–4 unsaturation with the **polar groups** as phosphatidylglycerol (**PG**) and phosphatidylglycerol phosphate methylether (**PGP-Me**). The dominant **respiratory lipoquinone** is **MK-8:8**. The known species are **saccharolytic heterotrophs growing with sugars and starch either aerobically or anaerobically with sulfur or thiosulfate as the electron acceptor. Fermentative growth was not observed.**

Species are **extremely halophilic** (optimum at 3.5–4.0 M total Na^+), **alkaliphilic** (pH range from 7.2 to 10.2), and **mesophilic** (maximum temperature 45–50°C). The genus includes two species enriched from hypersaline soda lakes in Kulunda Steppe (Altai, Russia). The type species *Natranaeroarchaeum sulfidigenes* is represented by a single isolate enriched from anoxic sulfidic sediments at sulfur-reducing conditions with glucose as the carbon source. *Naa. aerophilum* is represented by four strains enriched from surface oxic sediments

DOI: 10.1002/9781118960608.gbm02091. Published by John Wiley & Sons, Inc., in association with Bergey's Manual Trust.

FIGURE 1. Morphology of *Naa. sulfidigenes* AArc-S^T (a–c) and *Naa. aerophilum* AArc-St1-1^T (d) grown at 4 M total Na^+ and pH 9.5. (a) Anaerobic colonies of AArc-S^T forming polysulfide clearance zones. (b and d) Phase-contrast microphotographs of AArc-S^T cells grown anaerobically with glucose/S_8 and of AArc-St1-1^T grown aerobically with starch, respectively. (c) Electron microphotograph of an AArc-S^T cell with archaella.



and brine at aerobic conditions with starch as the carbon source (Sorokin et al., 2022a,b). The genus is **a member of the *Halobacteriales* order** in the class *Halobacteria*. The genus three-letter abbreviation is *Naa*.

DNA G + C content (%): 60.8–61.0 (whole-genome sequences of type strains).

Type species: **Natranaeroarchaeum sulfidigenes** Sorokin et al. 2022a, VL211.

Number of species with validly published names: 2.

Family classification: The genus *Natranaeroarchaeum* is classified within the family *Natronoarchaeaceae*.

Further descriptive information

The cells of the type species *Naa. sulfidigenes* are mostly coccoid and motile with 1–2 archaella. The colonies formed in sulfur-reducing conditions produce clearance zones in a polysulfide background, which is formed chemically from the release of sulfide and the remaining sulfur. Cells of *Naa. aerophilum* are nonmotile, flattish, and polymorphic (Figure 1). The colonies at both aerobic and anaerobic conditions are pink, which is in contrast to another sulfur-respiring natronoarchaeal genus *Natrarchaeobaculum* whose cells do not produce carotenoids while growing anaerobically with sulfur as the electron acceptor (Sorokin et al., 2018).

The most characteristic feature of the genus is its capacity to grow by anaerobic sulfur/thiosulfate-dependent respiration using sugars or starch as the electron donor. In this, it is similar to its neutrophilic counterpart, *Halapricum desulfuricans* (Sorokin et al., 2021a,b). However, there is a significant difference: while the latter ferments sugars first and then

utilizes produced H_2 as the actual electron donor for sulfur respiration, *Natranaeroarcheum* species are strictly respiratory, and their genomes lack membrane-bound and cytoplasmic hydrogenases (Sorokin et al., 2022a). There is also a substantial difference between the two species inside the genus. The type species is more active anaerobically and utilizes both sulfur and thiosulfate as the electron acceptors. The second species can only grow anaerobically with sulfur, and growth is much less active.

Habitat, enrichment, and isolation

Both species of the genus were obtained from the same hypersaline soda lakes in Kulunda Steppe (Altai, Russia). Only a single strain of *Naa. sulfidigenes* was enriched from sulfidic sediments anaerobically with sulfur as acceptor and glucose as carbon, energy, and electron donors. The four strains of *Naa. aerophilum* were enriched as aerobic amylotrophs from surface-oxygenated sediment layer and brines. The final isolation of pure cultures was achieved by plating maximal serial dilutions either inside soft agar (for the type species) or by surface spreading (in case of the *Naa. aerophilum* strains).

Genome analysis of *Natranaeroarchaeum species*

The closed genome of *Naa. sulfidigenes* AArc-S^T is 3.04 Mb and contains 3,176 genes encoding 3,120 proteins. The draft genome of *Naa. aerophilum* AArc-St1-1^T is 3.29 Mb and contains 3,382 genes encoding 3,239 proteins. The detailed genome analyses were published previously (Sorokin et al., 2022a,b). The most important physiological features of the two species were confirmed by analyses of the genomes. Sulfur- and thiosulfate-dependent respiration is enabled by polysulfide reductase PsrABC and thiosulfate reductase PhsABC; the ability to use starch and other alpha-glucans is reflected in the presence of multiple genes coding for intra- and extracellular alpha-glucanases from the GH families 13 and 15.

Maintenance and preservation

Active liquid cultures of *Natranaeroarchaeum* 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

According to the 16S rRNA gene sequence comparison, the anaerobically enriched type strain AArc-S and the four strains enriched aerobically with starch or inulin form a monophyletic group of a separate genus with the genus *Natronoarchaeum* as the closest relative (Figure 2a). Furthermore, the whole-genome comparison (AAI, ANI, and DDH) allowed separation of the type strain AArc-S and the four amylotrophic isolates into two distinct species within the new genus *Natranaeroarchaeum*.

Currently, the genus *Natranaeroarchaeum* is classified into a new family *Natronoarchaeaceae* on the basis of phylogenomic reconstruction based on 122 conserved single-copy archaeal protein markers (Sorokin et al., 2022a). Apart from the *Natranaeroarchaeum,* this family includes three more genera: *Natronoarchaeum*, the type genus (Shimane et al., 2010); *Salinarchaeum* (Cui et al., 2011); and *Halostella* (Song et al., 2016) (Figure 2b). The family is a member of the order *Halobacteriales*, which, until recently, was one of the three orders in the class *Halobacteria* and phylum *Halobacteriota*. All of them have been recently merged into a single order *Halobacteriales* according to the Genome Taxonomy Database (GTDB) classification (Cui et al., 2023).

In the recent 08-RS214 version of the GTDB, the genera *Natronoarchaeum* and *Natranaeroarchaeum* were merged into a single genus. However, merging *Natronoarchaeum* and *Natranaeroarchaeum* causes the relative evolutionary divergence (RED) value for this combined genus to become 0.880. However, the median RED value for archaeal genera in the 08-RS214 GTDB release is 0.909. Thus, the RED value for this combined genus falls well below the median value. We are confident that the addition of more genome sequences will confirm the separation of the two genera in the future versions of the GTDB. Moreover, the 16S rRNA sequence identity between any two *Natronoarchaeum* and *Natranaeroarchaeum* species ranges from 94.57 to 93.09%, well below the genus separation level for archaea. Recent phylogenomic reconstructions in Cui et al. (2023) also confirm the separation of these two genera.

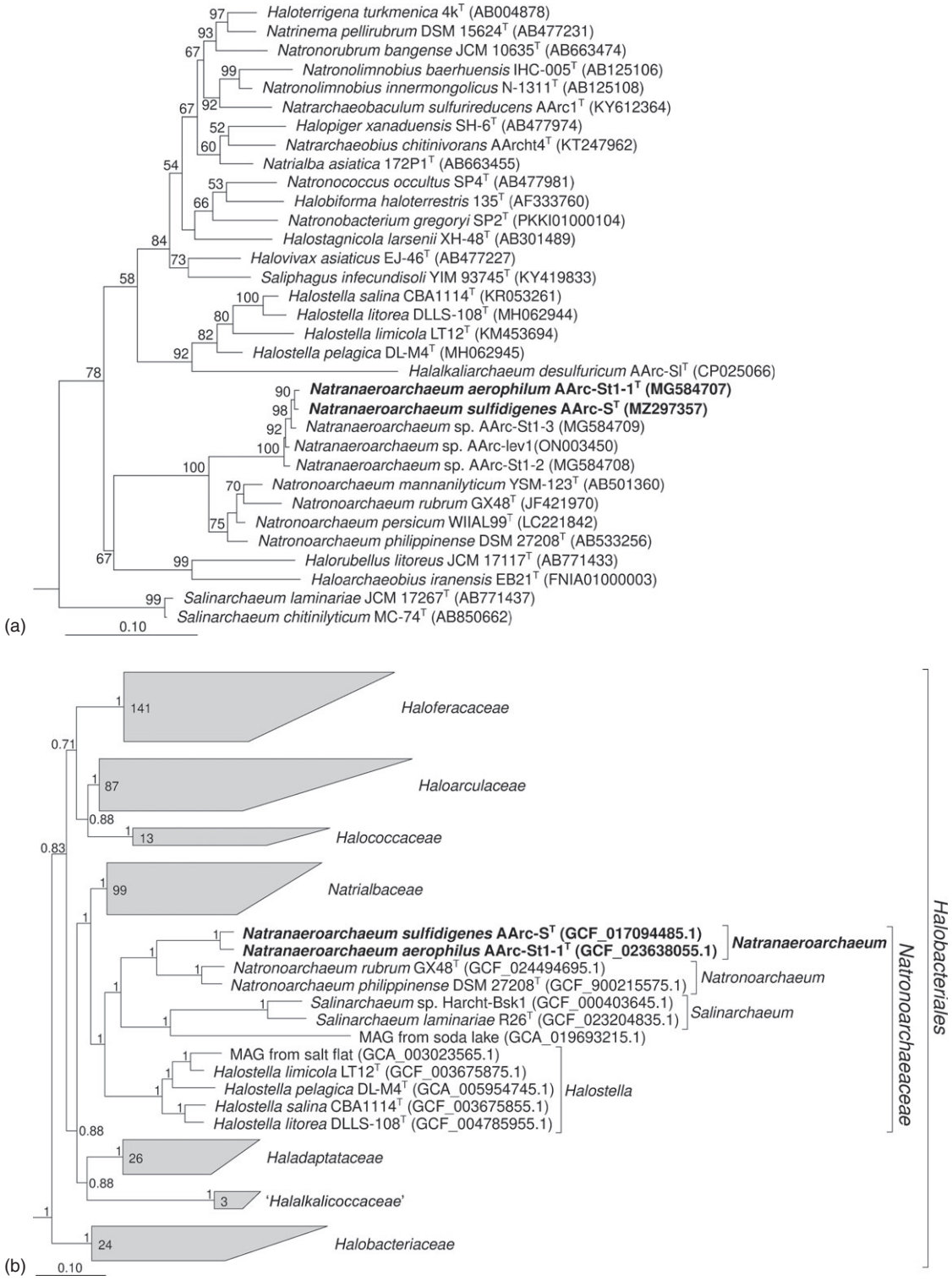
List of species of the genus *Natranaeroarchaeum*

Natranaeroarchaeum aerophilum Sorokin et al. 2022b, VL211

a.e.ro'phi.lum. N.L. neut. adj. suff. *-philum*, loving; N.L. neut. adj. *aerophilum*, air-loving.

The cells are angular, flat, polymorphic cocci or rods, mostly nonmotile, varying in size from 1 to 3 µm. The cells lyse in hypotonic solutions below 1 M NaCl. Produces red carotenoids. The core membrane diether lipids are C_{20}–C_{20} DGE (archaeol) and C_{20}–C_{25} DGE (extended archaeol). The polar lipid head groups include PGP-Me and PG. Glyco- and

FIGURE 2. Phylogenetic position of *Natranaeroarchaeum* based on sequence analyses of 16S rRNA gene (a) and concatenated alignment of 122 single-copy conserved bacterial protein markers of its two type strains (Parks et al., 2020) within the class *Halobacteria* (b). The trees were 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 (Minh et al., 2013) as well as approximate likelihood-ratio test for branches (Anisimova and Gascuel, 2006). The bootstrap consensus tree is shown with values placed at the nodes. Bar, 0.10 changes per position.



sulfolipids are absent. The dominant respiratory quinone is MK-8:8. The members are facultatively anaerobic and saccharolytic, with a limited substrate spectrum including several starch-like alpha-glucans, levan (beta-fructan), maltose, trehalose, and cellobiose. Capable of anaerobic sulfidogenic growth with glucose and maltose as the electron donors and carbon sources and sulfur as the electron acceptor. Thiosulfate, DMSO, nitrate, and fumarate do not support anaerobic respiration. Ammonium, urea, and yeast extract can serve as nitrogen sources. Oxidase- and catalase-positive. Indole production from tryptophan is negative. Mesophilic, with a maximum growth temperature of 50°C. Extremely halophilic, with a range of total Na^+ for growth from 3 to 5 M (optimum at 4 M), and moderately alkaliphilic, with a pH range for growth from 7.2 to 9.3 (optimum at 8.0–8.8). The G + C content of the DNA is 61.0% (genome sequence of the type strain). The species description is based on cumulative properties of four closely related isolates enriched as in aerobic amylolytic cultures from surface oxic sediments and brines of hypersaline soda lakes in Kulunda Steppe (Altai, Russia). The species also includes three other closely related strains (AArc-St1-2, AArc-St1-3, and AArc-lev1) isolated from soda lakes in the same area.

Type strain: AArc-St1-1 (JCM 32519 = UQM 41561).

EMBL/GenBank accession number (16S rRNA gene): MG 584707 (type strain).

EMBL/GenBank accession number (genome assembly): GCF_023638055 (type strain).

Natranaeroarchaeum sulfidigenes Sorokin et al. 2022a, VL211

sul.fi.di'ge.nes. N.L. neut. n. *sulfidum*, sulfide; Gr. suff. *-genes*, producing, from Gr. ind. v. *gennaô*, to produce; N.L. neut. part. adj. *sulfidigenes*, sulfide-producing.

The cells are motile, polymorphic, from flat rods to cocci, 0.5–1 × 0.8–2.5 µm. The cells lyse below 1.5 M NaCl. Red carotenoids are produced during both aerobic and anaerobic (less intense) growth. The core membrane diether lipids are dominated by the C_{20}–C_{20} DGE (archaeol) and the C_{20}–C_{25} DGE (extended archaeol) with 0–4 double bonds. The polar lipid head groups are PGP-Me and PG. The dominant respiratory lipoquinone is MK-8:8. Glyco- and sulfolipids were not detected. Facultatively anaerobic with strictly respiratory metabolism. Anaerobic respiration is possible with either sulfur or thiosulfate as the electron acceptor. Thiosulfate is reduced partially to sulfide and sulfite. DMSO, nitrate, and fumarate are not respired. Aerobic growth was observed only at microoxic conditions. It is heterotrophic and saccharolytic, utilizing hexoses (glucose, fructose, mannose, galactose, raffinose, trehalose, and maltose), glycerol, and starch. The substrates tested but not utilized include arabinose, rhamnose, sucrose, lactose, raffinose, melibiose, melezitose, ribose, xylose, cellobiose, glucuronic and galacturonic acids, mannitol, arabitol, inositol, inulin, levan, acetate, lactate, pyruvate, glycine, glutamate, and aspartate. Ammonium and urea but not nitrate can serve as nitrogen sources. Oxidase is weakly positive, and catalase is positive. Extremely halophilic, with a range of total Na^+ for growth from 3 to 5 M (optimum at 3.5–4 M), and obligately alkaliphilic, with a pH range from 8.5 to 10.2 (optimum at 9.5–9.7). Mesophilic, with the temperature range for aerobic growth at 25–45°C (optimum at 35–40). The G + C content of the genomic DNA is 60.8%. The type strain was isolated following enrichment at sulfur-reducing conditions with glucose as substrate from sulfidic sediments of hypersaline soda lakes in Kulunda Steppe (Altai, Russia).

Type strain: AArc-S (=JCM 34033 = UNIQEM U1000).

EMBL/GenBank accession number (16S rRNA gene): MZ 297357 (type strain).

EMBL/GenBank accession number (closed genome): CP064786.

Acknowledgment

Sorokin D.Y. was supported by the Gravitation-SIAM Program of the Dutch Ministry of Education and Science (grant 24002002).

References

Anisimova M & Gascuel O (2006) Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst Biol* **55**: 539–552.

Cui C, Han D, Hou J, & Cui H-L (2023) Genome-based classification of the class *Halobacteria* and description of *Haladaptataceae* fam. nov. and *Halorubellaceae* fam. nov. *Int J Syst Evol Microbiol* **73**: 005984.

Cui HL, Yang X, & Mou YZ (2011) *Salinarchaeum laminariae* gen. nov., sp. nov.: a new member of the family *Halobacteriaceae* isolated from salted brown alga *Laminaria*. *Extremophiles* **15**: 625–631.

Kalyaanamoorthy S, Minh BQ, Wong T, von Haeseler A, & Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* **14**: 587–589.

Minh BQ, Minh ATH, von Haeseler A (2013) Ultrafast Approximation for Phylogenetic Bootstrap. *Mol Biol Evol* **30**: 1188–1195.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A et al. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* **37**: 1530–1534.

Parks DH, Chuvochina M, Chaumeil PA, Rinke C, Mussig AJ, & Hugenholtz P (2020) A complete domain-to-species taxonomy for Bacteria and Archaea. *Nat Biotechnol* **38**: 1079–1086.

Shimane Y, Hatada Y, Minegishi H, Mizuki T, Echigo A, Miyazaki M et al. (2010) *Natronoarchaeum mannanilyticum* gen. nov., sp. nov., an aerobic, extremely halophilic archaeon isolated from commercial salt. *Int J Syst Evol Microbiol* **60**: 2529–2534.

Song HS, Cha IT, Rhee JK, Yim KJ, Kim AY, Nam SW et al. (2016) *Halostella salina* gen. nov., sp. nov., an extremely halophilic archaeon isolated from solar salt. *Int J Syst Evol Microbiol* **66**: 2740–2746.

Sorokin DY, Messina E, La Cono V, Ferrer M, Ciordia S et al. (2018) Sulfur respiration in a group of facultatively anaerobic natronoarchaea ubiquitous in hypersaline soda lakes. *Front Microbiol* **9**: 2359.

Sorokin DY, Messina E, Smedile F, La Cono V, & Hallsworth JE (2021a) Carbohydrate-dependent sulfur respiration in halo(alkali)philic euryarchaea from hypersaline lakes. *Environ Microbiol* **23**: 3779–3808.

Sorokin DY, Yakimov MM, Messina E, Merkel AY, Koenen M, Bale NJ et al. (2021b) *Halapricum desulfuricans* sp. nov., carbohydrate-utilizing sulfur-reducing haloarchaea from hypersaline lakes. *Syst Appl Microbiol* **44**: 126249.

Sorokin DY, Yakimov MM, Messina E, Merkel AY, Koenen M, Bale NJ et al. (2022a) *Natranaeroarchaeum sulfidigenes* gen. nov., sp. nov., carbohydrate-utilizing sulfur-respiring haloarchaeon from hypersaline lakes and proposal of a new family *Natronoarchaeaceae* fam. nov. in the order *Halobacteriales*. *Syst Appl Microbiol* **45**: 126356.

Sorokin DY, Elcheninov AG, Khijhniak TV, Koenen M, Bale NJ, Sinninghe Damsté JS et al. (2022b) *Natronocalculus amylovorans gen.* nov., sp. nov., and *Natranaeroarchaeum aerophilus* sp. nov., dominant culturable amylolytic natronoarchaea from hypersaline soda lakes in southwestern Siberia. *Syst Appl Microbiol* **45**: 12.

Validation List No. 211 (2023) List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* **73**: 5845. Ⓑ