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Halobacteriota/Halobacteria/Halobacteriales/

Natronoarchaeaceae

Sorokin et al. 2022a, VL211

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Na.tro.no.ar.chae.a.ce'ae. N.L. neut. n. *Natronoarchaeum*, the type genus of the family; L. fem. pl. n. suff. *-aceae*, ending to denote a family; N.L. fem. pl. n. *Natronoarchaeaceae*, the family of the genus *Natronoarchaeum*.

The family *Natronoarchaeaceae* is a member of the order *Halobacteriales*, class *Halobacteria*, and is formed on the basis of phylogenomic analyses. It includes extremely halophilic and either aerobic or facultatively anaerobic archaea with variable key functionality, such as anaerobic sulfur respiration and potential to utilize various polysaccharides as growth substrates. The family currently contains four genera: the type genus *Natronoarchaeum* and the genera *Salinarchaeum*, *Halostella*, and *Natranaeroarchaeum* from various hypersaline habitats.

DNA G+C content (mol%): 60.8–68.2 (whole-genome sequences).

Type genus: ***Natronoarchaeum*** Shimane et al. 2010^{VP} emend. Qui et al. 2014.

Based on the results of phylogenomic analysis, the family *Natronoarchaeaceae* was proposed to encompass four genera with an uncertain affiliation in the order *Halobacteriales*, including *Natronoarchaeum*, *Natranaeroarchaeum*, *Salinarchaeum*, and *Halostella* (Sorokin et al., 2022a). In contrast, in the Genome Taxonomy DataBase, the

family *Natronoarchaeaceae* has been divided into three parts—*Natronoarchaeaceae*, “*Salinarchaeaceae*,” and f_QS-9-68-17 that includes the genus *Halostella*. These three “families” have very elevated RED (relative evolutionary divergence) values: 0.880, 0.849, and 0.920, respectively. In contrast, the median RED value for archaeal families in the 08-RS214 GTDB release is 0.727, suggesting that the three families could be combined. Apparently, they were not combined because of the very low bootstrap value (16%) for combining *Halostella*, *Natronoarchaeum*, *Natranaeroarchaeum*, and *Salinarchaeum* into a single family in this release. However, all of our phylogenetic reconstructions yielded 100% bootstrap values for this grouping (see *Natranaeroarchaeum* chapter and Sorokin et al. (2022a)). Similarly, phylogenetic reconstructions of Cui et al. (2023) also have high bootstrap values for the current formulation of the family *Natronoarchaeaceae*.

Comparative properties of the four genera of haloarchaea included in the family are presented in Table 1. The common properties shared by all four members include extreme halophily and the presence of phosphatidylglycerol (PG) and phosphatidylglycerophosphate methyl ester (PGP-Me) as the dominant membrane phospholipids, menaquinone MK-8:8 as the dominant respiratory lipoquinone (although the data are incomplete), and the ability to grow aerobically with starch, sugars, and glycerol.

TABLE 1. Comparative properties of the genera included in the family *Natronoarchaeaceae* (based on the references cited in the text)

Property	<i>Natranaeroarchaeum</i>	<i>Natronoarchaeum</i> ^a	<i>Salinarchaeum</i> ^b	<i>Halostella</i> ^c
Number of species	2	4	2	4
Number of genomes sequences	2	2	2	4
Genome size (Mb)	3.04	3.16	3.26	3.74–4.12
G + C genomic/mol%	60.8	65.2–66.5/63.0–66.7	65.0–66.5/66.4	63.2–68.1
Number of the 16S-rRNA genes	2 (identical)	1–2 (dissimilar)	1	2–3 (dissimilar)
Cell morphology	Irregular cocci, motile	Rods, motility (v)	Rods, motile	Cocci or rods, motile
Core membrane lipids	C ₂₀ –C ₂₀ , C ₂₀ –C ₂₅ DGE	nd	C ₂₀ –C ₂₀ DGE	nd
Identified membrane phospholipids	PG, PGP-Me	PG, PGP-Me, PGP (v)	PG, PGP-Me, PGS	PG, PGP-Me; PA (v)
Sulfo- and glycolipids	–	S ₂ -DGDE Unidentified glycolipid	–	+ (probably sulfated glycosyl-mannosyl glycolipids)
Menaquinones	MK8:8 (major)	nd	nd	MK8:8 and MK8:7 (3 species)
Anaerobic respiration	+ (growth experiments and genomic content)	– (growth experiment and genomic content)	– (growth experiments and genomic content ^d)	(v) (growth experiments)
Electron acceptors for anaerobic respiration	Sulfur and thiosulfate	–	–	DMSO (v); nitrate (full denitrification) (v) (growth experiments)
Fermentative growth	– (growth experiment)	– (growth experiment)	nr	+ Arginine (growth experiment) (v)
Aerobic growth with:	Starch, levan; sugars, glycerol	Galactomannan (v), starch; levan and laminarin (genomic evidence for 2 strain); sugars; glycerol, acetate and lactate (v)	Chitin, laminarin (v), starch; sugars; glycerol, pyruvate, lactate (v)	Starch; sugars; acetate, pyruvate, succinate, malate, fumarate, glycerol, mannitol (v)
pH range (opt.)	8.5–10.2 (9.5–9.7)	5.5–9.5 (7.0–9.0)	6–10 ^d (7.0)	7–9 (7–8)
Salinity range (opt.), M Na ⁺	2.5–4.5 (3.5)	1.4–5.3 (2.6–4.5)	1.4–5.1 (2.6–4.3)	1.4–5.1 (2.6–3.4)
Mg ²⁺ requirement	Low	High	High	v
Temperature range (opt.), °C	25–45 (35–40)	20–55 (37–45)	20–50 (37–40)	20–50 (40)
Amylase/protease/lipase	+/-/-	+/-/-	-/v/-	-/v/-
Catalase/oxidase	+/+(w)	v/v	v/v	+/-
Indole from tryptophane	–	v	–	v
Urease	+ (growth and genomic)	(–) (genomic evidence)	(–) (growth and genomic)	(–) (genomic evidence)
Hypersaline habitat type	Soda lakes		Salt lakes and salterns, salted marine algae	

^aShimane et al. (2010, 2013), Qiu et al. (2014), Naghoni et al. (2017).

^bCui et al. (2011), Dominova et al. (2013), Minegishi et al. (2017).

^cSong et al. (2016), Han et al. (2019), Han and Cui (2020).

^dThe highest values are not proven properly because the final pH values at alkaline range are not reported. Lipids: PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PGP-Me, phosphatidylglycerophosphate methyl ester; S₂-DGDE, disulfated diglycosyl diether; DGE, dialkyl glycerol ether; PA, phosphatidic acid; v, variable in different species; w, weak reaction; nd, no data.

The genus *Natronaeroarchaeum* is the only one in which the species are obligately alkaliphilic and found exclusively in hypersaline soda lakes. The closest related genus *Natronoarchaeum* can tolerate alkaline conditions but is still a neutrophile with optimum growth at neutral pH (Shimane et al., 2010, 2013; Qiu et al., 2014; Naghoni et al., 2017). Although tolerance to pH up to 9.5–10 has also been reported for two species of the genus *Salinarchaeum* (Cui et al., 2011; Minegishi et al., 2017), the results were apparently not verified by checking the final pH values. Our results with the neutrophilic chitinotrophic *Salinarchaeum* sp. HArchT-Bsk (Sorokin et al., 2015) and the pH-neutral nature of the hypersaline habitats from which the two described species of *Salinarchaeum* were isolated suggest that this genus, as well as the genus *Halostella*, represents typical neutrophilic haloarchaea.

The unique discriminating feature of the genus *Natronaeroarchaeum* is the ability to grow by sulfur respiration with sulfur or thiosulfate, but not DMSO, with sugars or starch as the electron donor and carbon source. In contrast, two out of four described species of the genus *Halostella* (*H. litorea* and *H. limicola*) are capable of DMSO-dependent anaerobic respiration, and, furthermore, *H. limicola* can also ferment arginine (Han et al., 2019; Han and Cui, 2020).

A unique feature of the type species in the genus *Natronoarchaeum* is its ability to use a heteropolysaccharide galactomannan (arabic gum) as growth substrate. Likewise, a unique feature of all three known strains in the genus *Salinarchaeum* is their capacity to utilize chitin as the growth substrate. Furthermore, *Salinarchaeum* sp. strain HArchT-Bsk can grow with soluble barley beta-glucan. Its genome encodes five copies of the GH18-family endochitinase, four copies of endo-beta-1,4-glucanase from the GH5 family, and one copy of cellulase GH9 (Dominova et al., 2013). In contrast, the genome of *S. laminariae* encodes only two GH18 and one of each from GH5 and GH9 families. On the other hand, the genus *Natronaeroarchaeum* is specialized on utilization of soluble alpha-glucans as substrates, and the two sequenced genomes of its type strains encode multiple copies of extra- and intracellular alpha-amylases from the GH families 13 and 15 (Sorokin et al., 2021, 2022a,b).

Finally, a prominent discriminating structural feature of the genus *Natronoarchaeum* is the presence of disulfated glycolipid S2-DGDE as one of the dominant membrane lipids in all four of its known species.

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