Reclassification of the genus Natronolimnobius

Proposal of two new genera, Natronolimnohabitans gen. nov. to accommodate Natronolimnobius innermongolicus and Natrarchaeobaculum gen. nov. to accommodate Natronolimnobius aegyptiacus and Natronolimnobius sulfurireducens

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TAXONOMIC DESCRIPTION

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Reclassification of the genus *Natronolimnobius*: proposal of two new genera, *Natronolimnohabitans* gen. nov. to accommodate *Natronolimnobius innermongolicus* and *Natrarchaeobaculum* gen. nov. to accommodate *Natronolimnobius aegyptiacus* and *Natronolimnobius sulfurireducens*

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Abstract

The genus *Natronolimnobius*, currently including four species, is a member of the order *Natrialbales*, class *Halobacteria,* and consists of obligately alkaliphilic and extremely halophilic members found exclusively in highly alkaline hypersaline soda lakes. The species were classified into this genus mostly based on phylogenetic analysis of the 16S rRNA gene. However, a more advanced phylogenomic reconstruction based on 122 conserved single-copy archaeal protein markers clearly indicates a polyphyletic origin of the species included into this genus, thus warranting its reclassification into three separate genera. We therefore propose to transfer *Nlb. innermongolicus* (type strain N-1311) to a new genus *Natronolimnohabitans* as *Nlh. innermongolicus* comb. nov. and to transfer *Nlb. aegyptiacus* (type strain JW/NM-HA 15) and *Nlb. sulfurireducens* (type strain AArc1) to a new genus *Natrarchaeobaculum* as *Nbl. aegyptiacum* comb. nov. and *Nbl. sulfurireducens* comb. nov. The phylogenomic differentiation of these four species is also supported by the ANI/AAI distances and unique phenotypes. The most important physiological differences includes a previously unreported ability for cellulose and xylan utilization in *Nlb. baerhuensis*, thermophily in *Nbl. aegyptiacus* and anaerobic sulfur respiration in *Nbl. sulfurireducens*. We further present an emended description of *Natronolimnobius baerhuensis*.

The original description of the genus *Natronolimnobius* was based on three strains of obligately alkaliphilic haloarchaea, isolated from hypersaline soda lakes in Inner Mongolia. It included two species, *Nlb. baerhuensis* (type species) and *Nlb. innermongolicus* with 96.7% sequence identity of the 16S rRNA genes [1, 2]. The genus was classified as a member of the family *Natrialbaceae*, order *Natrialbales* in the class *Halobacteria* [3]. Recently, two more species were added to this genus, *Nlb. aegyptiacus* [4] and *Nlb. sulfurireducens* [5, 6] with even lower distances of their 16S rRNA gene from the previously described species from Inner Mongolia (94.5–96% identity). However, with the use of a phylogenomic approach as an alternative for the single conservative molecular marker-based paradigm it is becoming excessively clear that in many fast-evolving lineages, such as the *Natrialbales* in the haloarchaea, the 16S rRNA gene-based phylogeny does not reliably resolve the evolutionary history of their members. A recent example of such inadequacy is the genus *Natrarchaeobius* with its three subgroups clustering polyphyletically in the 16S rRNA gene-based reconstruction, while forming a monophyletic group in the tree based on phylogenomic analysis [7].

Based on phylogenomic data and substantial differences in phenotypes, we here suggest to reclassify three species of the genus *Natronolimnobius* into two new genera, *Natronolimnohabitans* gen. nov. to accommodate *Nlb. innermongolicus* and *Natrarchaeobaculum* gen. nov., to accommodate *Nlb. aegyptiacus* and *Nlb. sulfurireducens*.

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Keywords: *Natronolimnobius*; *Natronolimnohabitans*; *Natrarchaeobaculum*; soda lakes.
Abbreviations: DGE, dialkyl glycerol ether; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PGPGP, phosphatidylglycerolphosphate glycerophosphate; PGP-Me, phosphatidylglycerophosphate methylester.

For the phylogenomic analyses, 122 archaeal single-copy conserved protein markers were obtained from the Genome Taxonomy Data Base [8]. The genes were identified in published genomes using Prodigal v2.6.3 [9], and the amino acids translated sequences were concatenated and aligned in MAFFT v7.427 [10] and automatically trimmed in trimAl 1.2rev59 [11]. The phylogenomic tree was built in PhyML 3.0 [12] with the approximate likelihood-ratio test for branches [13]. A substitution model for phylogenetic reconstruction was automatically selected by the SMS algorithm [14].

For whole genome comparison, four indices were used: Average Nucleotide Identity ANIb (ANI with BLAST); ANIm (ANI with Mummer) [15]; Average Amino acid Identity (AAI) [16–18] and DNA–DNA hybridization (DDH) using the Genome-to-Genome Distance Calculator 2.1 online tool (http://ggdc.dsmz.de/ggdc.php).

For the phenotypic comparison, in addition to what was already described in the original publications, a cross-examination of the four type strains was performed to check for key metabolic properties not tested before. The strains were obtained from the JCM and DSMZ culture collections. The tests for polysaccharide utilization and anaerobic growth with sulfur/DMSO were run as described previously [6, 19]. Additional polar lipid and respiratory quinone analyses were also performed for *Nlb. baerhuensis* and *Nlb. innermongolicus* according to Bale *et al.* [20].

The results of phylogenomic reconstruction based on 122 conserved archaeal marker genes clearly indicated that the genus *Natronolimnobius* is polyphyletic, its four species being distributed in three separate genus-level groups. Group 1 includes *Nlb. baerhuensis* (closest related to the genus *Natrialba*); group 2 includes *Nlb. innermongolicus* (most related to the genus *Haloterrigena*); group 3 includes *Nlb. aegyptiacus* and *Nlb. sulfurireducens* (most related to the genus *Natrarchaeobius*) (Fig. 1). The analysis also hints at the polyphyletic problems with the genus *Halopiger* and the necessity of its taxonomic reevaluation.

The results of whole genome comparison of the four type strains using ANI, AAI and DDH parameters support the conclusion based on phylogenomic analysis, i.e. the necessity for reclassification of the genus *Natronolimnobius* into three different genera. On average, the indices were all in the range of intergenus level within the family *Natrialbaceae* (Table 1).

The phylogenomic splitting of the genus *Natronolimnobius* into three separate genus-level lineages is supported by clear-cut phenotypic differences between the groups. The only obvious common (but probably most ecologically relevant) feature is their obligate alkaliphily, typical for the haloarchaea inhabiting hypersaline soda lakes [21]. But there are substantial differences even in this common parameter: *Nlb. innermongolicus* and *Nlb. sulfurireducens* can grow at a much higher carbonates to chloride ratios in comparison to the other two species, and therefore they are true natronoarchaea (i.e. soda-loving), while *Nlb. baerhuensis* and *Nlb. aegyptiacus* are rather haloalkaliphilic archaea demanding at least 1.5–2 M Cl^- for optimal growth.

The most important phenotypic property of *Nlb. sulfurireducens*, found in hypersaline soda lakes world-wide [5, 6], is its ability to grow anaerobically by sulfur respiration using diverse electron donors, including hydrogen, formate, C_4-C_8

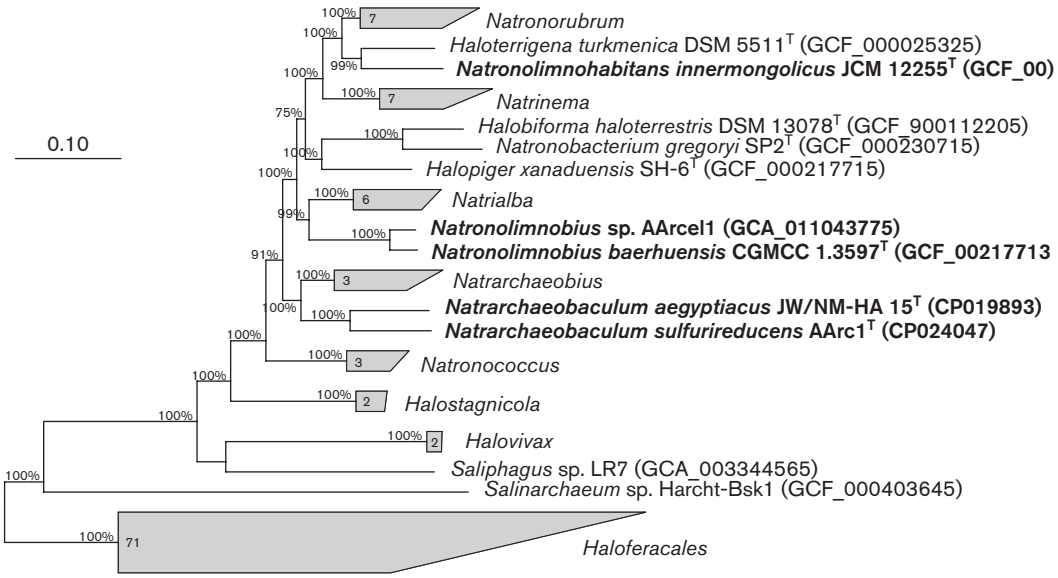

Fig. 1. The phylogenomic position of four type strains of the genus *Natronolimnobius* within the order *Natrialbales* based on concatenated partial amino acid sequences of 122 single-copy archaeal conserved protein markers. The tree was built using the PhyML 3.0 program and the approximate likelihood-ratio test for branches. Values in the collapsed clusters indicate the number of analysed genomes. Bootstrap values above 70% are shown at the nodes. Bar, 0.10 changes per position.

Table 1. Full genome comparison of the type strains of the genus *Natronolimnobius*

	***Nlb. sulfurireducens* AArc1**	***Nlb. aegyptiacus* JW/NM-HA 15**	***Nlb. innermongolicus* N-1311**	***Nlb. baerhuensis* IHC-005**
ANIb				
N. sulfurireducens		78.7	77.8	76.0
N. aegyptiacus	78.4		77.6	75.7
N. innermongolicus	77.3	77.1		
N. baerhuensis	75.8	75.8	76.9	
ANIm				
N. sulfurireducens AArc1^T		85.4	84.8	84.0
N. aegyptiacus	85.4		85.0	84.1
N. innermongolicus	84.8	85.0		85.0
N. baerhuensis	84.0	84.1	85.1	
GGDC (DDH formula 2)				
N. sulfurireducens		24	24	22
N. aegyptiacus	24		24	22
N. innermongolicus	24	24		23
N. baerhuensis	22	22	23	
AAI (Two-way) [SD] percentage				
N. sulfurireducens		75.4	72.1	71.2
N. aegyptiacus	75.4		71.1	70.1
N. innermongolicus	72.1	71.1		71.4
N. baerhuensis	71.2	70.1	71.4	

fatty acids, pyruvate and peptone/yeast extract. It also accumulates large amounts of poly-β-hydroxyalkanoates during anaerobic growth, a property not reported for any other member of the class *Halobacteria*.

The most important distinguishing property of *Nlb. aegyptiacus* is its thermophily. Although the original publication reported that it can grow at temperatures as low as 30 °C, it did not grow even at 37 °C in the cross check experiments. Thermophily at extremely high pH is a unique adaptation since, normally, proteins are hydrolysed at such a combination of conditions, and only a few thermoalkaliphilic haloarchaea have been obtained in culture. *Nlb. baerhuensis* prefers a carbohydrate diet, while *Nlb. innermongolicus* mostly utilizes organic acids as growth substrates. Our comparative study yielded furher important physiological information on these species, not reported in the original descriptions. Our enrichments from various hypersaline soda lakes with amorphous cellulose as substrate resulted (for two out of eight samples) in binary cultures of cellulolytic natronoarchaea: a dominant type with a strong cellulolytic capacity has recently been described as *Natronobiforma cellulositropha* gen. nov., sp. nov [22, 23]. The minor component of such cultures with a weak cellulolytic activity was represented by isolates AArcel1 (from Siberian soda lakes) and AArcel8-2 (from the soda lake Owens in California), both closely affiliated with *Nlb. baerhuensis* (99.2% 16S rRNA gene sequence identity) [19]. Apart from cellulose and xylan, these two isolates also utilized soluble alpha- (starch, dextrin) and beta- (barley glycan and laminarin) glycans. Therefore, we also checked the potential of the type strain of *Nlb. baerhuensis* to grow with various celluloses and xylan. It grew well only with xylan, while growth with celluloses was limited to amorphous alpha-cellulose (Sigma). This example also demonstrates the importance of having multiple isolates which might comprise the same genomic species and, yet, be different in important functions. Despite the fact that strongly cellulolytic *Natronobiforma* also grew well with xylan, in enrichments from soda lakes in four different geographic areas using xylan as carbon source it lost the competition to AArcel1-like organisms (100% partial 16S rRNA gene sequence identity). This confirmed our belief that *Nlb. baerhuense* represents a generalistic type of polysaccharide decomposers utilizing mostly soluble (oligo)saccharides, in contrast to the specialized cellulotrophs represented in soda lakes by the genus *Natronobiforma*.

Another previously unreported functionality of two *Natronolimnobius* species emerged from our olive oil enrichments for lipolytic organisms from hypersaline soda lakes (unpublished data). These resulted in domination of two

lipolytic natronoarchaeal isolates: one from Siberian soda lakes was closely related *Nlb. innermongolicus* (99.7% 16S rRNA sequence identity) and another from the Wadi an Natrun alkaline lakes was related to *Nlb. aegyptiacus* isolated from the same area (99.4% 16S rRNA sequence identity). Cross-examination of the type strains of these two species confirmed the ability of *Nlb. innermongolicus* to use olive oil as growth substrate, while *Nlb. aegyptiacus* was negative. This again shows the importance of having multiple isolates for comprehensive characterization of prokaryotic species.

The result of the comparison of the phenotypic properties of the four *Natronolimnobius* species is presented in Table 2.

On the basis of phylogenomic analysis and phenotypic differentiation we propose to transfer *Nlb. innermongolicus* to a new genus *Natronolimnohabitans* as *Nlh. innermongolicus* comb. nov. and to transfer *Nlb. aegyptiacus* and *Nlb. sulfurireducens* to a new genus *Natrarchaeobaculum* as *Nbl. aegyptiacum* comb. nov. and *Nbl. sulfurireducens* comb. nov. We further present an emended description of *Natronolimnobius baerhuensis*.

DESCRIPTION OF *NATRONOLIMNOHABITANS* GEN. NOV.

Natronolimnohabitans (Na.tro.no.lim.no.ha'bi.tans. Arabic n. *natrun* or *natron* soda, sodium carbonate; Gr. fem. n. *limne* lake; L. masc. n. *habitans* an inhabitant; N.L. masc. n. *Natronolimnohabitans* an organism living in soda lakes)

The genus description is based on that of *Natronolimnobius innermongolicus* [1]. The cells are nonmotile rods. They are aerobic organoheterotrophs, utilizing mostly organic acids, and are obligately alkaliphilic and extremely halophilic. Core membrane polar lipids include archaeol (C_{20}-C_{20} DGE) and extended archaeol (C_{20}-C_{25}). The main respiratory quinone is MK-8:0. The genus is a member of the family *Natrialbaceae*, order *Natrialbales*, class *Halobacteria*. The recommended three-letter abbreviation is *Nlh*. The type species is *Natronolimnohabitans innermongolicus*.

DESCRIPTION OF *NATRONOLIMNOHABITANS INNERMONGOLICUS* COMB. NOV.

Basonym: *Natronolimnobius innermongolicus* Itoh *et al.* 2005

Natronolimnohabitans innermongolicus (in.ner.mon.go'li.cus. N.L. masc. adj. *innermongolicus* pertaining to Inner Mongolia)

The cells are flat polymorphic rods, 0.6×3–6 µm. Colonies are red, translucent and with a smooth surface. Cells lyse at low-salt conditions below 0.5 M NaCl. They are aerobic organoheterotrophs, utilizing the following organic compounds as substrates: organic acids, including acetate, propionate, pyruvate, butyrate, lactate, malate, fumarate, citrate; glycerol; sugars, including glucose, galactose, arabinose, raffinose, sorbitol. They also can grow with olive oil and hydrolyse gelatin but not starch or casein. Cells do not accumulate PHA while growing with fatty acids. Catalase and oxidase are positive. Indole formation from tryptophan is positive. They are obligately alkaliphilic, with a pH range for growth from 7.5 to 10.0 (optimum at 9.5), extremely halophilic with a Na^+ range from 2.5 to 4.5 M (optimum at 3.5 M), and mesophilic, with a temperature range between 19 and 52 °C (optimum at 45 °C). The minimal Cl^- requirement for growth at 4 M total Na^+ is 0.5 M. The predominant polar lipids are phosphatidylglycerol (PG) and phosphatidylglycerolphosphate methyl ester (PGP-Me); minor components are phosphatidylglycerolphosphate (PGP) and phosphatidylglycerolphosphate glycerophosphate (PGPGP). Glycolipids are not detected. The respiratory quinones consist of MK-8:0 as the major compound with a smaller fraction of and MK-8:2. The G+C content of the type strain is 64.3 mol% (genome). The type strain is N-1311 (=CGMCC 1.2124=JCM 12255). The GenBank accession number for the genome assembly of the type strain is GCA_000337215.

DESCRIPTION OF *NATRARCHAEOBACULUM* GEN. NOV.

Natrarchaeobaculum (Natr.ar.chae.o.ba'cu.lum. N.L. n. *natron* (arbitrarily derived from Arabic n. *natrun* or *natron*) soda, sodium carbonate; N.L. pref. *natro-* 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 description is based on two members of the genus *Natronolimnobius*, *Nlb. aegyptiacus* [4] and *Nlb. sulfurireducens* [6]. The cells are polymorphic and mostly flat. They are mostly aerobic organoheterotrophs, utilizing organic acids, but some strains can also grow anaerobically by sulfur respiration. They are obligately alkaliphilic and extremely halophilic; some members are moderately thermophilic. Archaeol (C_{20}-C_{20} DGE) is the predominant core membrane lipid. The only respiratory quinone is MK-8:0. The genus is a member of the family *Natrialbaceae*, order *Natrialbales*, class *Halobacteria*. The recommended three-letter abbreviation is *Nbl*. The type species is *Natrarchaeobaculum sulfurireducens*.

DESCRIPTION OF *NATRARCHAEOBACULUM SULFURIREDUCENS* COMB. NOV.

Basonym: *Natronolimnobius sulfurireducens* Sorokin *et al.* 2019

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

The cells are flattened polymorphic motile rods and in some conditions nonmotile coccoids, 0.5–0.6×1–3.5 µm. The cell wall consists of a thin monolayer covered with an extracellular matrix. The cells lyse in hypotonic solutions below 1–1.5 M Na^+. Cells grown aerobically are bright red due to high concentrations of carotenoids and a proton-pumping bacteriorhodopsin (genomic data). They accumulate large amounts of PHA during growth with fatty acids, both aerobically and

Table 2. Comparative properties of the species of *Natronolimnobius*, *Natronolimnohabitans* and *Natrarchaeobaculum* based on literature data [1, 2, 4–6, 19] and cross-examination of the type strains

Property	*Natrarchaeobaculum* gen. nov.		*Natronolimnohabitans* gen.nov.	*Natronolimnobius*
	Nab. sulfurireducens	***Nab. aegyptiacus***	***Nlh. innermongolicus***	***Nlb. baerhuensis***
Number of isolates	7	1	1	2
Cell morphology	motile flat rods and coccoids	rods, nonmotile	rods, nonmotile	pleomorphic, motile;during growth on cellulose - cocci with a thick cell wall*
Pigmentation	pink (aerobic and anaerobic on DMSO)	yellow or pink	red	red
PHA accumulation	+, with fatty acids (anaerobic and aerobic)†	–*	–*	–*
Aerobic growth	+	+	+	+
Anaerobic growth	respiratory with sulfur and DMSO as acceptor	–*	–*	–*
e-donors for anaerobic growth	H_2, formate, pyruvate,lactate, glycerol, C_4-C_9 fatty acids, peptone	–	–	–
Substrates for aerobic growth	acetate, pyruvate, lactate, glycerol, butyrate, peptone	pyruvate, glucose, fructose, mannose, galactose, maltose, cellobiose raffinose; acetate and C_4-C_9 fatty acids*	acetate, propionate, pyruvate, butyrate*, lactate, malate, fumarate, citrate glycerol; glucose, galactose, arabinose, raffinose, sorbitol	acetate, fumarate,pyruvate, glycerol; glucose, arabinose, fructose, mannose, galactose, rhamnose, xylose*, maltose, cellobiose*, raffinose, lactose;
Hydrolytic activity				
Amilase Esterase/lipase Protease	– – (tributyrin/olive oil) – (casein; gelatin)	+ – (Tween 80) – (gelatin)	- +* (Tween 80) + (gelatin)	+* +(Tween 80) gelatin (–)/casein (+)*
Polymer utilizationfor growth*	–	Olive oil (variable); starch, inulin	Olive oil	Starch, dextrin, xylane, alpha-cellulose
Catalase/oxidase	+/w	+/+	+/+	+/+
Indole from tryptophane	– (for the type strain)	–	+	+
Salinity range (opt.) M Na^+	3.0–5.0(4.0)	2.5–5.0 (3.2–4.6)	2.5–4.5 (3.5)	1.6–4.2 (2.5–3.2)
Cl^- dependence (minimal, M)*	0.2	1.5	0.5	2.0
pH range (opt.)	7.0–10.0 (9.1–9.3)	7.5–10.1* (9.2–9.5)	7.5–10.0(9.5)	7.0–9.8* (9.0)
Temperature (°C)	max. 48 (opt. 40–43)	max. 56 (opt. 52)	max. 52* (opt. 45)	max. 46 (opt. 37)
Core lipids	C_{20}-C_{20}, C_{20}-C_{25} DGE	C_{20}-C_{20} DGE	C_{20}-C_{20}, C_{20}-C_{25} DGE*	C_{20}-C_{20}, C_{20}-C_{25} DGE*
Intact membrane polar lipids	major: PGP-Me, PG minor: PGP	PGP-Me, PG	PGP-Me, PG PGP*	
Cardiolipins			PGPGP†	
Respiratory quinones	MK-8:0	MK-8:0*	MK-8:0 70%; MK-8(H2) 30%*	
DNA G+C (mol%; genome)	62.8–62.9	64.1	64.3	60.1

*Determined in the cross examination of the type strains.
†PHA synthetase type IIIA operon *phaCE* is present in the genome. Lipids: (PG) phosphatidylglycerol, (PGP-Me) phosphatidylglycerophosphate methylester, (PGP) phosphatidylglycerophosphate, (PGPGP) phosphatidylglycerolphosphate glycerophosphate, (DGE) dialkyl glycerol ether.

anaerobically. They are facultatively anaerobic; anaerobic respiratory growth is possible either with elemental sulfur or DMSO as the electron acceptors and H_2 or formate (in the presence of acetate or yeast extract as the C source), C_4-C_9 fatty acids, pyruvate, lactate, glycerol and peptone as the electron donors. Aerobic growth occurs with acetate and the above-mentioned organic acids and peptone/yeast extract, but not with H_2/formate. Sugars are not utilized under any conditions. Ammonium serves as the N-source. Can not grow with polymers, including starch, olive oil and casein. Oxidase is weakly positive, catalase is positive. Indole formation from tryptophan is negative. They are obligately alkaliphilic, growing at pH from 8.5 to 9 to 10.5 (optimum at 9.5–10), extremely halophilic with a range from 2.5 to 5 M total Na^+ (optimum at 3.5–4 M), and natronophilic, with a low level of minimal chloride requirement (0.2 M and 4 M total Na^+). The optimum growth temperature at pH 9.5 is 40–43 °C (maximum is 48 °C at pH 9). Archaeol (C_{20}-C_{20} DGE) and extended archaeol (C_{20}-C_{25} DGE) are the dominant core membrane lipids. The major phospholipids include phosphatidylglycerolphosphate methyl ester (PGP-Me) and phosphatidylglycerol (PG); phosphatidylglycerophosphate (PGP) is a minor component. Glycolipids are absent. The only respiratory quinone is MK-8:0. The G+C content of the DNA is 62.8–62.9 mol% (genomes). Habitat - hypersaline alkaline lakes. The species description is based on seven closely related strains isolated from various soda lakes in Central Asia, Africa and USA. The type strain is AArc1^T (=JCM 30663^T=UNIQEM U932^T). The genome of the type strain consists of a chromosome and two plasmids with the GenBank accession numbers CP024047 and CP024045/CP024046, respectively.

DESCRIPTION OF *NATRARCHAEOBACULUM AEGYPTIACUM* COMB. NOV.

Basonym: *Natronolimnobius aegyptiacus* Zhao *et al.* 2018

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

Cells are non-motile flattened rods, 0.5–0.8×1.5–2.5 µm. Colonies are from pale yellow to pink, depending on illumination and age. Cells are obligately aerobic organoheterotrophs utilizing the following compounds as substrates: sugars, including D-fructose, D-glucose, D-galactose, D-mannose, cellobiose, maltose, trehalose, raffinose; organic acids, including acetate, pyruvate and C_4-C_8 fatty acids. Soluble starch and inulin can support growth, lipase and proteolytic activity are absent. Cells grown with fatty acids do not accumulate PHA. Catalase and oxidase are positive. Indole formation from tryptophan is negative. They are obligately alkaliphilic, with a pH range for growth from 8.5 to 9 to 10.1 (optimum at 9.2–9.5), extremely halophilic with a range from 2.5 to 5 M total Na^+ (optimum at 3.2–4.6 M), and moderately thermophilic, with a range of 38–56 °C and an 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 core lipids are represented solely by C_{20}-C_{20} DGE (archaeol). The dominant polar lipids include phosphatidylglycerol (PG) and methylated phosphatidylglycerolphosphate (PGP-Me) and the minor component is represented by phosphatidylglycerophosphate (PGP). The only respiratory quinone is MK-8:0. The type strain, JW/NM-HA 15^T (=ATCC BAA-2088^T =DSM 23470^T), was isolated from sediment of Lake Fazda in Wadi an Natrun (Egypt). The genomic DNA G+C content of the type strain is 64.1 mol%. The GenBank genome accession number of the type strain is CP019893.

EMENDED DESCRIPTION OF *NATRONOLIMNOBIUS BAERHUENSIS* ITOH *ET AL.* 2005

In addition to the properties reported earlier [1, 2], the type strain of *Nlb. baerhuensis* and several closely related isolates from soda lakes are able to utilize cellobiose, starch, xylan and insoluble alpha-cellulose as growth substrates. During growth on cellulose the cells have a distiguished refractive coccoid motphology. The cell are negative for PHA accumulation. Starch and casein are hydrolysed. The maximum pH for growth is 9.8 and the minimal Cl^- requirement is 2 M. The core membrane lipids are identified as C_{20}-C_{20} and C_{20}-C_{25} dialkyl glycerol ethers (DGE). The major polar lipids include phosphatidylglycerol (PG) and methylated phosphatidylglycerolphosphate (PGP-Me), and the minor components are identified as phosphatidylglycerolphosphate (PGP) and phosphatidylglycerolphosphate glycerophosphate (PGPGP). The respiratory quinone pool consist of MK-8:0 as the major compound with a smaller fraction of and MK-8:2. The GenBank accession number for the genome assembly of the type strain is GCA_002177135.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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