

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*

Sorokin, Dmitry Y.; Merkel, Alexander Y.; Messina, Enzo; Yakimov, Mikhail M.; Itoh, Takashi; Mesbah, Noha M.; Wiegel, Juergen; Oren, Aharon

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

5
6 Dimitry Y. Sorokin,^{1,2,*} Alexander Y. Merkel,¹ Enzo Messina,³ Mikhail M. Yakimov,³ Takashi
7 Itoh,⁴ Noha M. Mesbah,⁵ Juergen Wiegel⁶ and Aharon Oren⁷

8
9 ¹Winogradsky Institute of Microbiology, Research Centre of Biotechnology, Russian Academy of
10 Sciences, Moscow, Russia

11 ²Department of Biotechnology, TU Delft, The Netherlands

12 ³IAMC-CNR, Messina, Italy

13 ⁴Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan

14 ⁵Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt

15 ⁶Department of Microbiology, University of Georgia, Athens, GA, USA

16 ⁷The Institute of Life Sciences, The Hebrew University of Jerusalem, The Edmond J. Safra
17 Campus, 9190401 Jerusalem, Israel

18

19

20 *Correspondence: Dimitry Y. Sorokin, soroc@inmi.ru

21

22 Running title: Reclassification of the genus *Natronolimnobius*

23 **Abstract**

24

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

41

42

43 **Key words:** *Natronolimnobius*, *Natronolimnohabitans*, *Natrarchaeobaculum*, soda lakes

44 The original description of the genus *Natronolimnobius* was based on three strains of obligately
45 alkaliphilic haloarchaea, isolated from hypersaline soda lakes in Inner Mongolia. It included two
46 species, *Nlb. baerhuensis* (type species) and *Nlb. innermongolicus* with 96.7 % sequence identity
47 of the 16S rRNA genes [1-2]. The genus was classified as a member of the family *Natrialbaceae*,
48 order *Natrialbales* in the class *Halobacteria* [3]. Recently, two more species were added to this
49 genus, *Nlb. aegyptiacus* [4] and *Nlb. sulfurireducens* [5-6] with even lower distances of their 16S
50 rRNA gene from the previously described species from Inner Mongolia (94.5-96% identity).
51 However, with the use of a phylogenomic approach as an alternative for the single conservative
52 molecular marker-based paradigm it is becoming excessively clear that in many fast-evolving
53 lineages, such as the *Natrialbales* in the haloarchaea, the 16S rRNA gene-based phylogeny does
54 not reliably resolve the evolutionary history of their members. A recent example of such
55 inadequacy is the genus *Natrarchaeobius* with its three subgroups clustering polyphyletically in the
56 16S rRNA gene-based reconstruction, while forming a monophyletic group in the tree based on
57 phylogenomic analysis [7].

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

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

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

73 For the phenotypic comparison, in addition to what was already described in the original
74 publications, a cross examination of the four type strains was performed to check for key metabolic
75 properties not tested before. The strains were obtained from the JCM and DSMZ culture
76 collections. The tests for polysaccharide utilization and anaerobic growth with sulfur/DMSO were

77 run as described previously [6, 19]. Additional polar lipid and respiratory quinone analyses were
78 also performed for *Nlb. baerhuensis* and *Nlb. innermongolicus* according to Bale *et al.* [20].

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

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

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

98 The most important phenotypic property of *Nlb. sulfurireducens*, found in hypersaline soda
99 lakes world-wide [5-6], is its ability to grow anaerobically by sulfur respiration using diverse
100 electron donors, including hydrogen, formate, C₄-C₈ fatty acids, pyruvate and peptone/yeast
101 extract. It also accumulates large amounts of poly-β-hydroxyalkanoates during anaerobic growth, a
102 property not reported for any other member of the class *Halobacteria*.

103 The most important distinguishing property of *Nlb. aegyptiacus* is its thermophily.
104 Although the original publication reported that it can grow at temperatures as low as 30°C, it did
105 not grow even at 37°C in the cross check experiments. Thermophily at extremely high pH is a
106 unique adaptation since, normally, proteins are hydrolyzed at such a combination of conditions,
107 and only a few thermoalkaliphilic haloarchaea have been obtained in culture. *Nlb. baerhuensis*
108 prefers a carbohydrate diet, while *Nlb. innermongolicus* mostly utilizes organic acids as growth
109 substrates. Our comparative study yielded further important physiological information on these
110 species, not reported in the original descriptions. Our enrichments from various hypersaline soda

111 lakes with amorphous cellulose as substrate resulted for two out of eight samples in binary cultures
112 of cellulolytic natronoarchaea: a dominant type with a strong cellulolytic capacity has recently
113 been described as *Natronobiforma cellulositropha* gen. nov., sp. nov. [22-23]. The minor
114 component of such cultures with a weak cellulolytic activity was represented by isolates AArce11
115 (from Siberian soda lakes) and AArce18-2 (from the soda lake Owens in California), both closely
116 affiliated with *Nlb. baerhuensis* (99.2% 16S rRNA gene sequence identity) [19]. Apart from
117 cellulose and xylan, these two isolates also utilized soluble alpha- (starch, dextrin) and beta-
118 (barley glycan and laminarin) glycans. Therefore, we also checked the potential of the type strain
119 of *Nlb. baerhuensis* to grow with various celluloses and xylan. It grew well only with xylan, while
120 growth with celluloses was limited to amorphous alpha-cellulose (Sigma). This example also
121 demonstrates the importance of having multiple isolates which might comprise the same genomic
122 species and, yet, be different in important functions. Despite the fact that strongly cellulolytic
123 *Natronobiforma* also grew well with xylan, in enrichments from soda lakes in four different
124 geographic areas using xylan as carbon source it lost the competition to AArce11-like organisms
125 (100% partial 16S rRNA gene sequence identity). This confirmed our belief that *Nlb. baerhuense*
126 represents a generalistic type of polysaccharide decomposers utilizing mostly soluble
127 (oligo)saccharides, in contrast to the specialized cellulotrophs represented in soda lakes by the
128 genus *Natronobiforma*.

129 Another previously unreported functionality of two *Natronolimnobius* species emerged
130 from our olive oil enrichments for lipolytic organisms from hypersaline soda lakes (unpublished
131 data). These resulted in domination of two lipolytic natronoarchaeal isolates: one from Siberian
132 soda lakes was closely related *Nlb. innermongolicus* (99.7 % 16S rRNA sequence identity) and
133 another from the Wadi an Natrun alkaline lakes was related to *Nlb. aegyptiacus* isolated from the
134 same area (99.4 % 16S rRNA sequence identity). Cross-examination of the type strains of these
135 two species confirmed the ability of *Nlb. innermongolicus* to use olive oil as growth substrate,
136 while *Nlb. aegyptiacus* was negative. This again shows the importance of having multiple isolates
137 for comprehensive characterization of prokaryotic species.

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

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

145 **Description of *Natronolimnohabitans* gen. nov.**

146
 147 *Natronolimnohabitans* (Na.tro.no.lim.no.ha'bi.tans. Arabic n. *natrun* or *natron* soda, sodium
 148 carbonate; Gr. fem. n. *limne* lake; L. masc. n. *habitans* an inhabitant; N.L. masc. n.
 149 *Natronolimnohabitans* an organism living in soda lakes)

150
 151 The genus description is based on that of *Natronolimnobius innermongolicus* [1]. The cells are
 152 nonmotile rods. They are aerobic organoheterotrophs, utilizing mostly organic acids, and are
 153 obligately alkaliphilic and extremely halophilic. Core membrane polar lipids include archaeol (C₂₀-
 154 C₂₀ DGE) and extended archaeol (C₂₀-C₂₅). The main respiratory quinone is MK-8:0. The genus is
 155 a member of the family *Natrialbaceae*, order *Natrialbales*, class *Halobacteria*. The recommended
 156 three-letter abbreviation is *Nlh*. The type species is *Natronolimnohabitans innermongolicus*.

157
 158
 159 **Description of *Natronolimnohabitans innermongolicus* comb. nov.**

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

161
 162 *Natronolimnohabitans innermongolicus* (in.ner.mon.go'li.cus. N.L. masc. adj. *innermongolicus*
 163 pertaining to Inner Mongolia)

164
 165 The cells are flat polymorphic rods, 0.6 x 3-6 µm. Colonies are red, translucent and with a smooth
 166 surface. Cells lyse at low-salt conditions below 0.5 M NaCl. They are aerobic organoheterotrophs,
 167 utilizing the following organic compounds as substrates: organic acids, including acetate,
 168 propionate, pyruvate, butyrate, lactate, malate, fumarate, citrate; glycerol; sugars, including
 169 glucose, galactose, arabinose, raffinose, sorbitol. They also can grow with olive oil and hydrolyze
 170 gelatin but not starch or casein. Cells do not accumulate PHA while growing with fatty acids.
 171 Catalase and oxidase are positive. Indole formation from tryptophan is positive. They are
 172 obligately alkaliphilic, with a pH range for growth from 7.5 to 10.0 (optimum at 9.5), extremely
 173 halophilic with a Na⁺ range from 2.5 to 4.5 M (optimum at 3.5 M), and mesophilic, with a
 174 temperature range between 19 and 52°C (optimum at 45°C). The minimal Cl⁻ requirement for
 175 growth at 4 M total Na⁺ is 0.5 M. The predominant polar lipids are phosphatidylglycerol (PG) and
 176 phosphatidylglycerolphosphate methyl ester (PGP-Me); minor components are
 177 phosphatidylglycerolphosphate (PGP) and phosphatidylglycerolphosphate glycerophosphate
 178 (PGPGP). Glycolipids are not detected. The respiratory quinones consist of MK-8:0 as the major

179 compound with a smaller fraction of and MK-8:2. The G + C content of the type strain is 64.3
 180 mol% (genome). The type strain is N-1311 (=CGMCC 1.2124 = JCM 12255). The GenBank
 181 accession number for the genome assembly of the type strain is GCA_000337215.

182

183 **Description of *Natrarchaeobaculum* gen. nov.**

184

185 *Natrarchaeobaculum* (Natr.ar.chae.o.ba'cu.lum. N.L. n. *natron* (arbitrarily derived from Arabic n.
 186 *natrun* or *natron*) soda, sodium carbonate; N.L. pref. *natro-* pertaining to soda; Gr. masc. adj.
 187 *archaios* ancient; L. neut. n. *baculum* small stick, rod; N.L. neut. n. *Natrarchaeobaculum* soda-
 188 loving archaeal rod)

189

190 The genus description is based on two members of the genus *Natronolimnobius*, *Nlb. aegyptiacus*
 191 [4] and *Nlb. sulfurireducens* [6]. The cells are polymorphic and mostly flat. They are mostly
 192 aerobic organoheterotrophs, utilizing organic acids, but some strains can also grow anaerobically
 193 by sulfur respiration. They are obligately alkaliphilic and extremely halophilic; some members are
 194 moderately thermophilic. Archaeol (C₂₀-C₂₀ DGE) is the predominant core membrane lipid. The
 195 only respiratory quinone is MK-8:0. The genus is a member of the family *Natrialbaceae*, order
 196 *Natrialbales*, class *Halobacteria*. The recommended three-letter abbreviation is *Nbl*. The type
 197 species is *Natrarchaeobaculum sulfurireducens*.

198

199 **Description of *Natrarchaeobaculum sulfurireducens* comb. nov.**

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

201

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

204

205 The cells are flattened polymorphic motile rods and in some conditions nonmotile coccoids, 0.5-
 206 0.6 x 1-3.5 μm. The cell wall consists of a thin monolayer covered with an extracellular matrix.
 207 The cells lyse in hypotonic solutions below 1-1.5 M Na⁺. Cells grown aerobically are bright red
 208 due to high concentrations of carotenoids and a proton-pumping bacteriorhodopsin (genomic data).
 209 They accumulate large amounts of PHA during growth with fatty acids, both aerobically and
 210 anaerobically. They are facultatively anaerobic; anaerobic respiratory growth is possible either
 211 with elemental sulfur or DMSO as the electron acceptors and H₂ or formate (in the presence of
 212 acetate or yeast extract as the C source), C₄-C₉ fatty acids, pyruvate, lactate, glycerol and peptone

213 as the electron donors. Aerobic growth occurs with acetate and the above-mentioned organic acids
214 and peptone/yeast extract, but not with H₂/formate. Sugars are not utilized under any conditions.
215 Ammonium serves as the N-source. Can not grow with polymers, including starch, olive oil and
216 casein. Oxidase is weakly positive, catalase is positive. Indole formation from tryptophan is
217 negative. They are obligately alkaliphilic, growing at pH from 8.5-9 to 10.5 (optimum at 9.5-10),
218 extremely halophilic with a range from 2.5 to 5 M total Na⁺ (optimum at 3.5-4 M), and
219 natronophilic, with a low level of minimal chloride requirement (0.2 M and 4 M total Na⁺). The
220 optimum growth temperature at pH 9.5 is 40-43°C (maximum is 48°C at pH 9). Archaeol (C₂₀-C₂₀
221 DGE) and extended archaeol (C₂₀-C₂₅ DGE) are the dominant core membrane lipids. The major
222 phospholipids include phosphatidylglycerolphosphate methyl ester (PGP-Me) and
223 phosphatidylglycerol (PG); phosphatidylglycerophosphate (PGP) is a minor component.
224 Glycolipids are absent. The only respiratory quinone is MK-8:0. The G + C content of the DNA is
225 62.8-62.9 mol% (genomes). Habitat - hypersaline alkaline lakes. The species description is based
226 on seven closely related strains isolated from various soda lakes in Central Asia, Africa and USA.
227 The type strain is AArc1^T (=JCM 30663^T = UNIQEM U932^T). The genome of the type strain
228 consists of a chromosome and 2 plasmids with the GenBank accession numbers CP024047 and
229 CP024045/CP024046, respectively.

230

231 **Description of *Natrarchaeobaculum aegyptiacum* comb. nov.**

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

233

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

235

236 Cells are non-motile flattened rods, 0.5–0.8 x 1.5–2.5 μm. Colonies are from pale yellow to pink,
237 depending on illumination and age. Cells are obligately aerobic organoheterotrophs utilizing the
238 following compounds as substrates: sugars, including D-fructose, D-glucose, D-galactose, D-
239 mannose, cellobiose, maltose, trehalose, D-raffinose; organic acids, including acetate, pyruvate and
240 C₄-C₈ fatty acids. Soluble starch and inulin can support growth, lipase and proteolytic activity are
241 absent. Cells grown with fatty acids do not accumulate PHA. Catalase and oxidase are positive.
242 Indole formation from tryptophan is negative. They are obligately alkaliphilic, with a pH range for
243 growth from 8.5-9 to 10.1 (optimum at 9.2-9.5), extremely halophilic with a range from 2.5 to 5 M
244 total Na⁺ (optimum at 3.2-4.6 M), and moderately thermophilic, with a range of 38-56 °C and an
245 optimum at 52 °C. The minimal Cl⁻ requirement for growth at 4 M total Na⁺ is 1.2 M. Cells lyse in
246 hypotonic conditions at less than 0.5 M NaCl. The core lipids are represented solely by C₂₀-C₂₀

247 DGE (archaeol). The dominant polar lipids include phosphatidylglycerol (PG) and methylated
248 phosphatidylglycerolphosphate (PGP-Me) and the minor component is represented by
249 phosphatidylglycerolphosphate (PGP). The only respiratory quinone is MK-8:0. The type strain,
250 JW/NM-HA 15^T (=ATCC BAA-2088^T =DSM 23470^T), was isolated from sediment of Lake Fazda
251 in Wadi an Natrun (Egypt). The genomic DNA G + C content of the type strain is 64.1 mol%. The
252 GenBank genome accession number of the type strain is CP019893.

253

254 **Emended description of *Natronolimnobius baerhuensis* Itoh et al. 2005**

255

256 In addition to the properties reported earlier [1, 2], the type strain of *Nlb. baerhuensis* and several
257 closely related isolates from soda lakes are able to utilize cellobiose, starch, xylan and insoluble
258 alpha-cellulose as growth substrates. During growth on cellulose the cells have a distinguished
259 refractive coccoid morphology. The cells are negative for PHA accumulation. Starch and casein are
260 hydrolyzed. The maximum pH for growth is 9.8 and the minimal Cl⁻ requirement is 2 M. The core
261 membrane lipids are identified as C₂₀-C₂₀ and C₂₀-C₂₅ dialkyl glycerol ethers (DGE). The major
262 polar lipids include phosphatidylglycerol (PG) and methylated phosphatidylglycerolphosphate
263 (PGP-Me), and the minor components are identified as phosphatidylglycerolphosphate (PGP) and
264 phosphatidylglycerolphosphate glycerolphosphate (PGPGP). The respiratory quinone pool consist
265 of MK-8:0 as the major compound with a smaller fraction of and MK-8:2. The GenBank accession
266 number for the genome assembly of the type strain is GCA_002177135.

267

268

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274

275 **Conflict of interests**

276 The authors declare that there is no conflict of interests.

277

278

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

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

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

Property	<i>Natronolimnobius</i>	<i>Natronolimnohabitans</i> gen.nov.	<i>Natrarchaeobaculum</i> gen. nov.	
	<i>Nlb. baerhuensis</i>	<i>Nlh. innermongolicus</i>	<i>Nab. sulfurireducens</i>	<i>Nab. aegyptiacus</i>
Number of isolates	2	1	7	1
Cell morphology	pleomorphic, motile; during growth on cellulose - cocci with a thick cell wall*	rods, nonmotile	motile flat rods and coccoids	rods, nonmotile
Pigmentation	red	red	pink (aerobic and anaerobic on DMSO)	yellow or pink
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, fumarate, pyruvate, glycerol; glucose, arabinose, fructose, mannose, galactose, rhamnose, xylose, maltose, cellobiose*, raffinose, lactose;	acetate, propionate, pyruvate, butyrate*, lactate, malate, fumarate, citrate glycerol; glucose, galactose, arabinose, raffinose, sorbitol	acetate, pyruvate, lactate, glycerol, butyrate, peptone	pyruvate, glucose, fructose, mannose, galactose, maltose, cellobiose raffinose; acetate and C ₄ -C ₈ fatty acids*
Hydrolytic activity				
Amylase	+	-	-	+
Esterase/lipase	+(Tween 80)	+(Tween 80)	-(tributylin/olive oil)	-(Tween 80)
Protease	-(gelatin)/(casein)*	+(gelatin)	-(casein; gelatin)	-(gelatin)
Polymer utilization for growth*	Starch, dextrin, xylane, alpha-cellulose	Olive oil	-	Starch, inulin
Catalase/oxidase	+/+	+/+	+/w	+/+
Indole from tryptophane	+	+	-(for the type strain)	-
Salinity range (opt.) M Na ⁺	1.6-4.2 (2.5-3.2)	2.5-4.5 (3.5)	3.0-5.0 (4.0)	2.5-5.0 (3.2-4.6)
Cl ⁻ dependence (minimal, M)*	2.0	0.5	0.2	1.5
pH range (opt.)	7.0-9.8* (9.0)	7.5-10.0 (9.5)	7.0-10.0 (9.1-9.3)	7.5-10.1* (9.2-9.5)
Temperature (°C)	max. 46 (opt. 37)	max. 52* (opt. 45)	max. 48 (opt. 40-43)	max. 56 (opt. 52)
Core lipids	C ₂₀ -C ₂₀ , C ₂₀ -C ₂₅ DGE*	C ₂₀ -C ₂₀ , C ₂₀ -C ₂₅ DGE*	C ₂₀ -C ₂₀ , C ₂₀ -C ₂₅ DGE	C ₂₀ -C ₂₀ DGE
Intact membrane polar lipids		PGP-Me, PG PGP*		major: PGP-Me, PG minor: PGP
Cardiolipins		PGPGP*		
Respiratory quinones		MK-8:0 70%; MK-8(H ₂) 30%*		MK-8:0*
DNA G+C (mol%; genome)	60.1	64.3	62.8-62.9	64.1

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

357 **Legend to the figure**

358
359 **Fig. 1.** The phylogenomic position of four type strains of the genus *Natronolimnobius* within the
360 order *Natrialbales* based on concatenated partial amino acid sequences of 122 single-copy archaeal
361 conserved protein markers. The tree was built using the PhyML 3.0 program and the approximate
362 likelihood-ratio test for branches. Values in the collapsed clusters indicate the number of analyzed
363 genomes. Bootstrap values above 70% are shown at the nodes. Bar, 0.10 changes per position.
364