

Delft University of Technology

#### 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, Dimitry 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, <sup>1,2,*</sup> Alexander Y. Merkel, <sup>1</sup> Enzo Messina, <sup>3</sup> Mikhail M. Yakimov, <sup>3</sup> Takashi
7	Itoh, <sup>4</sup> Noha M. Mesbah, <sup>5</sup> Juergen Wiegel <sup>6</sup> and Aharon Oren <sup>7</sup>
8	
9	<sup>1</sup> Winogradsky Institute of Microbiology, Research Centre of Biotechnology, Russian Academy of
10	Sciences, Moscow, Russia
11	<sup>2</sup> Department of Biotechnology, TU Delft, The Netherlands
12	<sup>3</sup> IAMC-CNR, Messina, Italy
13	<sup>4</sup> Japan Collection of Microorganisms, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan
14	<sup>5</sup> Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt
15	<sup>6</sup> Department of Microbiology, University of Georgia, Athens, GA, USA
16	<sup>7</sup> The 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
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22 Running title: Reclassification of the genus *Natronolimnobius* 

- 23 Abstract
- 24

The genus Natronolimnobius, currently including four species, is a member of the order 25 Natrialbales, class Halobacteria, and consists of obligately alkaliphilic and extremely halophilic 26 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.

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

44 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 45 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].

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*.

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 amoni 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**).

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<sup>-</sup> for optimal growth.

9898The most important phenotypic property of *Nlb. sulfurireducens*, found in hypersaline soda99lakes world-wide [5-6], is its ability to grow anaerobically by sulfur respiration using diverse100electron donors, including hydrogen, formate,  $C_4$ - $C_8$  fatty acids, pyruvate and peptone/yeast101extract. It also accumulates large amounts of poly-β-hydroxyalkanoates during anaerobic growth, a102property 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 not grow even at 37°C in the cross check experiments. Thermophily at extremely high pH is a 105 106 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 furher important physiological information on these 110 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* 

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(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*.

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.

The result of the comparison of the phenotypic properties of the four *Natronolimnobius*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.

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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)

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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

156 three-letter abbreviation is *Nlh*. The type species is *Natronolimnohabitans innermongolicus*.

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## 159 Description of Natronolimnohabitans innermongolicus comb. nov.

160 Basonym: Natronolimnobius innermongolicus Itoh et al. 2005

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

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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<sup>+</sup> 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<sup>-</sup> requirement for growth at 4 M total Na<sup>+</sup> is 0.5 M. The predominant polar lipids are phosphatidylglycerol (PG) and 175 176 phosphatidylglycerolphosphate methyl ester (PGP-Me); minor components are 177 phosphatidylglycerolphosphate (PGP) and phosphatidylglycerolphosphate glycerophosphate 178 (PGPGP). Glycolipids are not detected. The respiratory guinones 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.

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

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*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* sodaloving archaeal rod)

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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 ( $C_{20}$ - $C_{20}$  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.

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## 199 Description of Natrarchaeobaculum sulfurireducens comb. nov.

200 Basonym: Natronolimnobius sulfurireducens Sorokin et al. 2019

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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)

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205 The cells are flattened polymorphic motile rods and in some conditions nonmotile coccoids, 0.5-206  $0.6 \ge 1-3.5 \ \mu\text{m}$ . 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<sup>+</sup>. Cells grown aerobically are bright red 207 due to high concentrations of carotenoids and a proton-pumping bacteriorhodopsin (genomic data). 208 209 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 H<sub>2</sub> or formate (in the presence of 212 acetate or yeast extract as the C source),  $C_4$ - $C_9$  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  $H_2$ /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 negative. They are obligately alkaliphilic, growing at pH from 8.5-9 to 10.5 (optimum at 9.5-10), 217 218 extremely halophilic with a range from 2.5 to 5 M total Na<sup>+</sup> (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<sup>+</sup>). The 220 optimum growth temperature at pH 9.5 is 40-43°C (maximum is 48°C at pH 9). Archaeol (C<sub>20</sub>-C<sub>20</sub> 221 DGE) and extended archaeol (C<sub>20</sub>-C<sub>25</sub> DGE) are the dominant core membrane lipids. The major phospholipids 222 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 62.8-62.9 mol% (genomes). Habitat - hypersaline alkaline lakes. The species description is based 225 226 on seven closely related strains isolated from various soda lakes in Central Asia, Africa and USA. The type strain is  $AArc1^{T}$  (=JCM 30663<sup>T</sup> = UNIQEM U932<sup>T</sup>). The genome of the type strain 227 228 consists of a chromosome and 2 plasmids with the GenBank accession numbers CP024047 and 229 CP024045/CP024046, respectively.

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

232 Basonym: Natronolimnobius aegyptiacus Zhao et al. 2018

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

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236 Cells are non-motile flattened rods,  $0.5-0.8 \times 1.5-2.5 \mu m$ . Colonies are from pale vellow 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  $C_4$ - $C_8$  fatty acids. Soluble starch and inulin can support growth, lipase and proteolytic activity are 240 241 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 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 243 244 total Na<sup>+</sup> (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<sup>-</sup> requirement for growth at 4 M total Na<sup>+</sup> is 1.2 M. Cells lyse in 245 hypotonic conditions at less than 0.5 M NaCl. The core lipids are represented solely by  $C_{20}$ - $C_{20}$ 246

DGE (archaeol). The dominant polar lipids include phosphatidylglycerol (PG) and methylated phosphatidylglycerolphosphate (PGP-Me) and the minot 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<sup>T</sup> =DSM 23470<sup>T</sup>), 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

- 252 GenBank genome accession number of the type strain is CP019893.
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# 254 Emended description of Natronolimnobius baerhuensis Itoh et al. 2005

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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 distiguished 259 refractive coccoid motphology. The cell are negative for PHA accumulation. Starch and casein are 260 hydrolyzed. The maximum pH for growth is 9.8 and the minimal Cl<sup>-</sup> 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 261 262 polar lipids include phosphatidylglycerol (PG) and methylated phosphatidylglycerolphosphate 263 (PGP-Me), and the minor components are identified as phosphatidylglycerolphosphate (PGP) and phosphatidylglycerolphosphate glycerophosphate (PGPGP). The respiratory quinone pool consist 264 265 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.

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## 275 **Conflict of interests**

- The authors declare that there is no conflict of interests.
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- 279 **REFERENCES**
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	Nlb.	Nlb. aegyptiacus	Nlb.	Nlb.
	sulfurireducens	JW/NM-HA 15	innermongolicus	baerhuensis
	AArc1		N-1311	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		85.4	84.8	84.0
AArc1 <sup>T</sup>				
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	

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

- 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	Natronolimnobius	Natronolimnohabitans	Natrarchaeobaculum	
	Nlb. baerhuensis	gen.nov. Nlh. innermongolicus	gen. nov. Nab. sulfurireducens Nab. aegyptiacus	
Number of isolates	2	1	7	1
Cell morphology	pleomorphic, motile; during growth on cellulose - cocci with a thick cell wall <sup>*</sup>	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	* -
<i>e</i> -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 <sup>*</sup> , raffinose, lactose;	acetate, propionate, pyruvate, butyrate <sup>*</sup> , 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 <sup>*</sup>
Hydrolytic activity Amylase Esterase/lipase Protease Polymer utilization for growth* Catalase/oxidase Indole from tryptophane Salinity range (opt.) M Na <sup>+</sup>	+* +(Tween 80) -(gelatin )/+(casein )* Starch, dextrin, xylane, alpha-cellulose +/+ + 1.6-4.2 (2.5-3.2)	- + (Tween 80) + (gelatin) Olive oil +/+ + 2.5-4.5 (3.5)	- - (tributyrin/olive oil) - (casein; gelatin) - +/w - (for the type strain) 3.0-5.0 (4.0)	+ - (Tween 80) - (gelatin) Starch, inulin +/+ - 2.5-5.0 (3.2-4.6)
Cl <sup>-</sup> dependence (minimal, M)*	2.0	0.5	0.2	1.5
pH range (opt.) Temperature (°C) Core lipids Intact membrane polar lipids	PGP-M	7.5-10.0 (9.5) max. $52^*$ (opt. 45) C <sub>20</sub> -C <sub>20</sub> , C <sub>20</sub> -C <sub>25</sub> DGE <sup>*</sup> Me, PG GP <sup>*</sup>		7.5-10.1 <sup>*</sup> (9.2-9.5) max. 56 (opt. 52) $C_{20}$ - $C_{20}$ DGE GP-Me, PG r: PGP
Cardiolipins Respiratory quinones DNA G+C (mol%; genome)	PGI	PGP* MK-8(H2) 30%* 64.3		-8:0* 64.1

353

<sup>\*</sup>Determined in the cross examination of the type strains; <sup>\*\*</sup>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.

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