

**Halanaeroarchaeum sulfurireducens gen. nov., sp. nov., the first obligately anaerobic sulfur-respiring haloarchaeon, isolated from a hypersaline lake**

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## Halanaeroarchaeum sulfurireducens gen. nov., sp. nov., a first obligately anaerobic sulfur-respiring haloarchaeon from hypersaline lakes

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<b>Abstract:</b>	Anaerobic enrichments with acetate as e-donor and carbon source and elemental sulfur as electron acceptor at 4 M NaCl using anaerobic sediments and brines from several hypersaline lakes in Kulunda Steppe (Altai, Russia) resulted in isolation in pure culture of four strains of obligately anaerobic haloarchae growing exclusively by sulfur respiration. Such metabolism has not yet been demonstrated in any known species of Halobacteria and in the whole archaeal kingdom the acetate oxidation with sulfur as acceptor was not previously demonstrated. The four isolates had nearly identical 16S rRNA gene sequences and formed a novel genus-level branch within the family Halobacteraceae. The strains had a restricted substrate range limited to acetate and pyruvate as e-donors and elemental sulfur as e-acceptor. In contrast to aerobic haloarchaea, the biomass of anaerobic isolates completely lacked the typical red pigments. The growth with acetate+sulfur was observed between 3-5 M NaCl and at a pH range from 6.7 to 8.0. The membrane core lipids were dominated by archaeols. On the basis of distinct physiological and phylogenetic data, it is proposed that the sulfur-respiring isolates represent a novel genus and species Halanaeroarchaeum sulfurireducens gen. nov., sp. nov. (type strain HSR2T=JCM 30661T=UNIQEM U935T).

2 ***Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov., a first obligately**  
3 **anaerobic sulfur-respiring haloarchaeon from hypersaline lakes**

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32 Running title: *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov.

33

34 Category: new taxa - Archaea

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36

37 The 16S-rRNA gene sequences of the strains HSR strains described here have been deposited  
38 in the GenBank under the numbers KM875608 and KM875610-KM875612.

39

40 Anaerobic enrichments with acetate as e-donor and carbon source and elemental sulfur  
41 as electron acceptor at 4 M NaCl using anaerobic sediments and brines from several  
42 hypersaline lakes in Kulunda Steppe (Altai, Russia) resulted in isolation in pure culture  
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44 respiration. Such metabolism has not yet been demonstrated in any known species of  
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51 pigments. The growth with acetate+sulfur was observed between 3-5 M NaCl and at a  
52 pH range from 6.7 to 8.0. The membrane core lipids were dominated by archaeols. On  
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55 *sulfurireducens* gen. nov., sp. nov. (type strain HSR2<sup>T</sup>=JCM 30661<sup>T</sup>=UNIQEM U935<sup>T</sup>).

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58 Key words: hypersaline lakes, haloarchaea, sulfur reduction, anaerobic

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64 Our recent study on the microbiology of reductive sulfur cycling in hypersaline habitats  
65 resulted in the discovery of a novel functional group of haloarchaea in anaerobic sediments of  
66 hypersaline lakes growing exclusively by dissimilatory elemental sulfur respiration (Sorokin  
67 *et al.*, 2016). This metabolic type was previously unknown among the haloarchaea, but even  
68 more surprising anaerobic acetate oxidation with a low-potential electron acceptor such as  
69 elemental sulfur has not yet been demonstrated in the whole archaeal kingdom. This makes  
70 the newly discovered group of obligately anaerobic haloarchaea truly unique. The previous  
71 work was mostly focused on the genomic properties of the type strain HSR2<sup>T</sup> and its  
72 functional annotation. Here we provide a formal taxonomic description of the novel taxon as  
73 *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov.

74 Sources of inocula were brines and anaerobic sulfidic surface sediments (2-10 cm)  
75 obtained from hypersaline chloride-sulfate lakes (see Sorokin *et al.*, 2012 for a detailed  
76 description) in the Kulunda Steppe (Altai, Russia) in 2009-2013. The enrichment and  
77 isolation procedures, the medium composition and cultivation conditions have been described  
78 previously (Sorokin *et al.*, 2016). Overall, anaerobic enrichments using acetate as *e*-donor/C  
79 source and elemental sulfur as *e*-acceptor at 4 M NaCl, pH 7 and 37°C resulted in isolation of  
80 four strains of haloarchaea designated HSR2<sup>T</sup>, HSR3, HSR4 and HSR5. The cell morphology  
81 of the isolates was typical for haloarchaea, i.e. flat coccoids and board-like rods, non-motile  
82 (**Fig. 1, a-d**). On the other hand, the cell mass lack any detectable red pigments characteristic  
83 of haloarchaea. Flagella were not observed in negatively stained cells. For thin sectioning, the  
84 cell pellets were fixed in 1% (w/v) OsO<sub>4</sub> containing 3.0 M NaCl for 48 h at room temperature,  
85 washed, stained overnight with 1% (w/v) uranyl acetate, dehydrated in an increasing ethanol  
86 series, and embedded in Epon resin. Thin sections were stained with 1% (w/v) lead citrate.  
87 The cells of HSR2<sup>T</sup> had a thin monolayer proteinaceous cell wall and extended nucleoid (**Fig.**  
88 **1, e**) and the cells lyzed immediately when the salt concentration dropped below 1.0 M.

89 The core membrane lipid analysis were performed by a method described in Weijers *et*  
90 *al.* (2009). The core lipids of strain HSR2<sup>T</sup> consisted of two major diether components,  
91 archaeol, and extended archaeol (i.e. C20-C25) in nearly equal proportion (47 and 53%,  
92 respectively), both common in haloarchaea (e.g. Villanueva *et al.*, 2014). The polar  
93 phospholipids were analysed with an LC/MS<sup>n</sup> method described in Sinninghe Damsté *et al.*  
94 (2011). They are dominated by phosphatidylglycerolsulfate (PGS) and  
95 phosphatidylglycerolphosphate methyl ether (PGP-Me), while three other components,  
96 phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), and an unknown complex  
97 phospholipid, were less abundant. All phospholipids were present with an archaeol and an  
98 extended archaeol core.

99 The 16S-rRNA gene sequences of the *Haa. sulfurireducens* strains were aligned with  
100 those of validly named related species of the order *Halobacteriales* (Gupta *et al.*, 2015) using  
101 the SILVA Incremental Aligner (Priësse *et al.*, 2012). The phylogenetic neighbours and  
102 pairwise sequence similarities were determined using EzTaxon-e (Kim *et al.*, 2012) and the  
103 phylogenetic trees were constructed with MEGA5 (Tamura *et al.* 2011) using the neighbour-  
104 joining (NJ) (Saitou & Nei, 1987), maximum-parsimony (MP) (Fitch, 1971) and maximum  
105 likelihood (ML) (Felsenstein, 1981) algorithms with 1,000 randomly selected bootstrap  
106 replicates. Phylogenetic analyses of the 16S rRNA genes of the four isolates revealed that  
107 they are closely related to each other (at least 99% 16S rRNA gene similarity) and, in fact,  
108 represent a single genetic species. These strains were quite distant from the nearest described  
109 members of the family *Halobacteraceae*, forming a separate genus-level lineage together with  
110 some cloned sequences from various hypersaline habitats (**Fig. 2**).

111 The novel isolates were clearly different from all previously described haloarchaea in  
112 respect of their metabolism. First, all strains were obligately anaerobic respirers. Next, their  
113 metabolism was extremely narrow, limited to acetate and pyruvate as *e*-donors/C source and

114 elemental sulfur as *e*-acceptor. The details of anaerobic growth kinetics have been described  
115 previously (Sorokin *et al.*, 2016). In general, the cultures growing with acetate produced more  
116 sulfide (up to 9 mM in one month) and less biomass than the cultures grown on pyruvate.  
117 Apart from sulfide, trace amounts of volatile organic sulfur were detected in stationary culture  
118 of strain HSR2, including carbon disulfide and methanliol. To our knowledge, the formation  
119 of these reduced sulfur compounds had never been previously observed in known sulfur-  
120 reducing prokaryotes. The optimal growth occurred at 4 M NaCl and within the range from 3  
121 to 5 M and at optimal temperature of 37-40°C.

122 This type of catabolism has not been demonstrated previously in any pure culture of  
123 haloarchaea and the discovery of such haloarchaea has a broad implication on the possible  
124 ecological role of extreme halophiles. Together with the recent demonstration of the ability of  
125 haloarchaea to oxidize CO (King, 2015), to participate in dissimilatory arsenic cycling  
126 (Rascovan *et al.*, 2015) and to actively mineralize such insoluble polymers as chitin and  
127 cellulose (Sorokin *et al.*, 2015), it significantly shifts our perception of haloarchaea as an  
128 important biogeochemical actor in hypersaline habitats.

129  
130 Overall, on the basis of phenotypic and genetic differences, the novel extremely halophilic  
131 and obligately anaerobic sulfur-respiring isolates are suggested to be placed into a new genus  
132 and species within the halobacteria for which a name *Halanaeroarchaeum sulfurireducens* is  
133 proposed.

134  
135 **Description of *Halanaeroarchaeum* gen. nov.**  
136 [hal.an.ae.ro.ar.chae'um Gr.n. *hals*, *halos* salt of the sea; Gr. pref. *an*, not; Gr. n. *aer aeros*,  
137 air; N.L. neut. n. *archaeum* archaeon from Gr. adj. *archaios*-ê-on ancient; N.L. neut. n.  
138 *Halanaeroarchaeum* - anaerobic halophilic archaeon]  
139

140 Obligately anaerobic haloarchaea with the ability to grow by sulfur-dependent respiration on  
141 acetate. Extremely halophilic, neutrophic members of the family *Halobacteraceae*. The cells  
142 are irregularly shaped, flattened, nonmotile. Recommended three-letter abbreviation: Haa.

143

144 **Description of *Halanaeroarchaeum sulfurireducens* sp. nov.**

145 [sul.fu.ri.re.du'cens L. n. *sulfur*, L. part. adj. *reducens* leading back, reducing, N.L. part. adj.  
146 *sulfurireducens* reducing sulfur]

147

148 The cells are angled flattened nonmotile coccoids to board-like rods, 0.5-1.5x1-2  $\mu\text{m}$ . The cell  
149 wall consists of a thin proteinaceous layer. The cells lyze in hypotonic solutions below 1 M  
150 NaCl. Red pigments are absent. The core membrane diether lipids are composed of C20-C20  
151 DGE (archaeol) and C20-C25 DGE (extended archaeol) in equal proportion. The polar  
152 phospholipids included (in the order of abundance) phosphatidylglycerolsulfate (PGS),  
153 phosphatidylglycerolphosphate methyl ether (PGP-Me), phosphatidylglycerol (PG) and  
154 phosphatidylethanolamine (PE). Obligately anaerobic growing by elemental sulfur respiration  
155 with either acetate or pyruvate as *e*-donor/C source. Ammonium is utilized as N-source.  
156 Optimum growth temperature is 37°C (maximum at 46°C). Extremely halophilic with a range  
157 of NaCl for growth from 3 to 5 M (optimum at 4 M) and neutrophilic with a pH range for  
158 growth with acetate and sulfur from 6.5 to 8 (optimum at 7.0-7.5). The G + C content of the  
159 DNA is 62.8 mol% (genome). Habitat - hypersaline lakes. The type strain (HSR2<sup>T</sup>=JCM  
160 30661<sup>T</sup>=UNIQEM U935<sup>T</sup>) was isolated from mixed anaerobic sediments of hypersaline  
161 chloride-sulfate lakes in Kulunda Steppe (Altai, Russia).

162

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172 **REFERENCES**

- 173  
174  
175 **Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J.*  
176 *Mol. Evol.* **17**, 368–376.  
177  
178 **Fitch, W.M. (1971).** Toward defining the course of evolution: minimum change for a specific tree  
179 topology. *Syst. Zool.* **20**, 406–416.  
180  
181 **Gupta, R.S., Naushad, S. & Baker S. (2015).** Phylogenomic analyses and molecular signatures for  
182 the class *Halobacteria* and its two major clades: a proposal for division of the class *Halobacteria* into  
183 an emended order *Halobacteriales* and two new orders, *Haloferacales* ord. nov. and *Natrialbales* ord.  
184 nov. *Int. J. Syst. Evol. Microbiol.* **65**, 1050-1069.  
185  
186 **Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi,**  
187 **H., Won, S. & Chun, J. (2012).** Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence data-  
188 base with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716–721.  
189  
190 **King, G.M. (2105).** Carbon monoxide as a metabolic energy source for extremely halophilic  
191 microbes: Implications for microbial activity in Mars regolith. *Proc. Nat. Ac. Sci.* **112**, 4465-4470.  
192  
193 **Prüsse, E., Peplies, J. & Glöckner, F.O. (2012).** SINA: accurate high-throughput multiple sequence  
194 alignment of ribosomal RNA genes. *Bioinformatics* **28**,1823–1829.  
195  
196 **Rascovan, N., Maldonado, J., Vazquez, M.P. & Farías M.E. (2016).** Metagenomic study of red  
197 biofilms from Diamante Lake reveals ancient arsenic bioenergetics in haloarchaea. *The ISME J.* **10**,  
198 299-309.  
199

- 200 **Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing  
201 phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.
- 202
- 203 **Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers J.W.H., Foesel, B.U.,**  
204 **Overmann, J. & Dedysh, S.N. (2011).** 13,16-Dimethyl octacosanedioic acid (iso-diabolic acid): A  
205 common membrane-spanning lipid of *Acidobacteria* subdivisions 1 and 3. *Appl. Env. Microbiol.* **77**,  
206 4147–4154.
- 207
- 208 **Sorokin, D.Y., Zacharova, E.E., Pimenov, N.V., Panteleeva, A.N., Tourova, T.P. & Muyzer, G.**  
209 **(2012).** Sulfidogenesis in hypersaline chloride-sulfate lakes of Kulunda Steppe (Altai, Russia). *FEMS*  
210 *Microbiol. Ecol.* **79**, 445-453.
- 211
- 212 **Sorokin D.Y., Kublanov I.V., Toschakov S.V. & Kolganova T.V. (2015).** Halo(natrono)archaea  
213 isolated from hypersaline lakes utilize cellulose and chitin as growth substrates. *Front. Microbiol.* **6**,  
214 article 942.
- 215
- 216 **Sorokin, D.Y., Kublanov, I.V., Gavrilov, S.N., Rojo, D., Roman, P., Golyshin, P.N., Slepak, V.Z.,**  
217 **Smedile, F., Ferrer, M., Messina, E., La Cono, V. & Yakimov, M.M. (2016).** Elemental sulfur and  
218 acetate can support life of a novel strictly anaerobic haloarchaeon. *The ISME J* **10**, 240-252.
- 219
- 220 **Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011).** MEGA5:  
221 molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and  
222 maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739.
- 223
- 224 **Villanueva, L., Sinninghe Damsté, J.S. & Schouten, S. (2014)** A re-evaluation of the archaeal  
225 membrane lipid biosynthetic pathway. *Nature Rev. Microbiol.* **12**, 438–448.
- 226

227 **Weijers, J.W.H., Panoto, E., van Bleijswijk J., Schouten, S., Balk, M., Stams, A.J.M., Rijpstra,**  
228 **W.I.C. & Sinninghe Damsté, J.S. (2009)** Constraints on the biological source(s) of the orphan  
229 branched tetraether membrane lipids. *Geomicrobiol. J.* **26**, 402-414.

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238 **Legends to the figures**

239

240 **Fig. 1** Cell morphology of sulfur-respiring haloarchaea grown anaerobically at 4 M NaCl. (**a-**  
241 **d**), phase contrast microscopy. (**a**), HSR2<sup>T</sup> grown with acetate; (**c-d**), HSR3, HSR4 and HSR5  
242 grown with pyruvate. (**e**), thin section electron microscopy of strain HSR2<sup>T</sup>.

243

244 **Fig. 2.** Phylogenetic position of novel anaerobic sulfur-respiring haloarchaeae based on the  
245 16S rRNA gene within the order *Halobacteriales* (Gupta *et al.*, 2015). The numbers on the  
246 nodes indicate the bootstrap values (>75%) calculated using the NJ algorithm probabilities.  
247 The tree was rooted with *Natronomonas moolapensis* (AB576127), *Natronomonas pharaonis*  
248 (CR936257) and *Halomarina oriensis* (AB519798) sequences. *Methanohalophilus halophilus*  
249 (FN870068) sequence served as the outgroup. The bar represents 0.05 accumulated changes  
250 per nucleotide.

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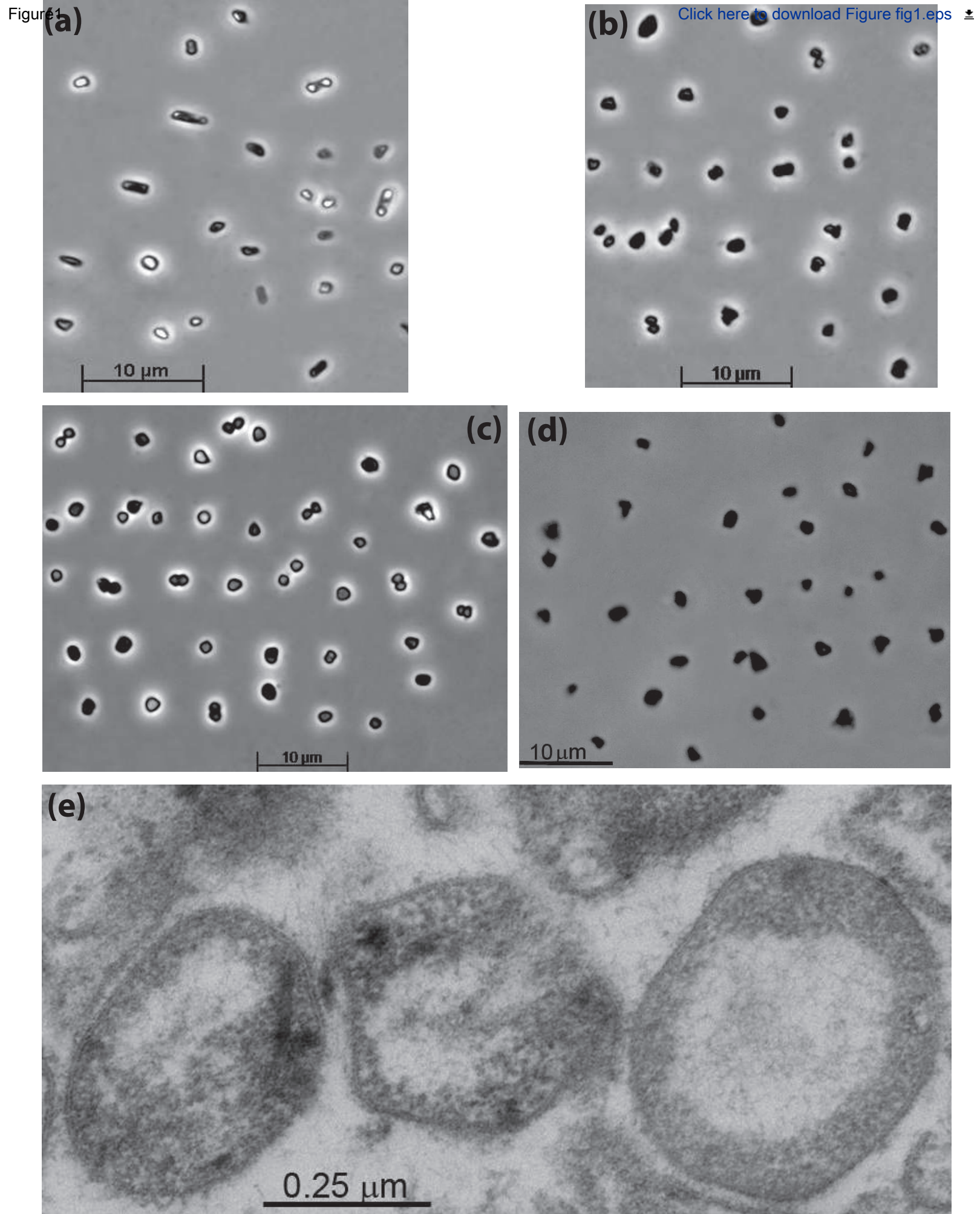
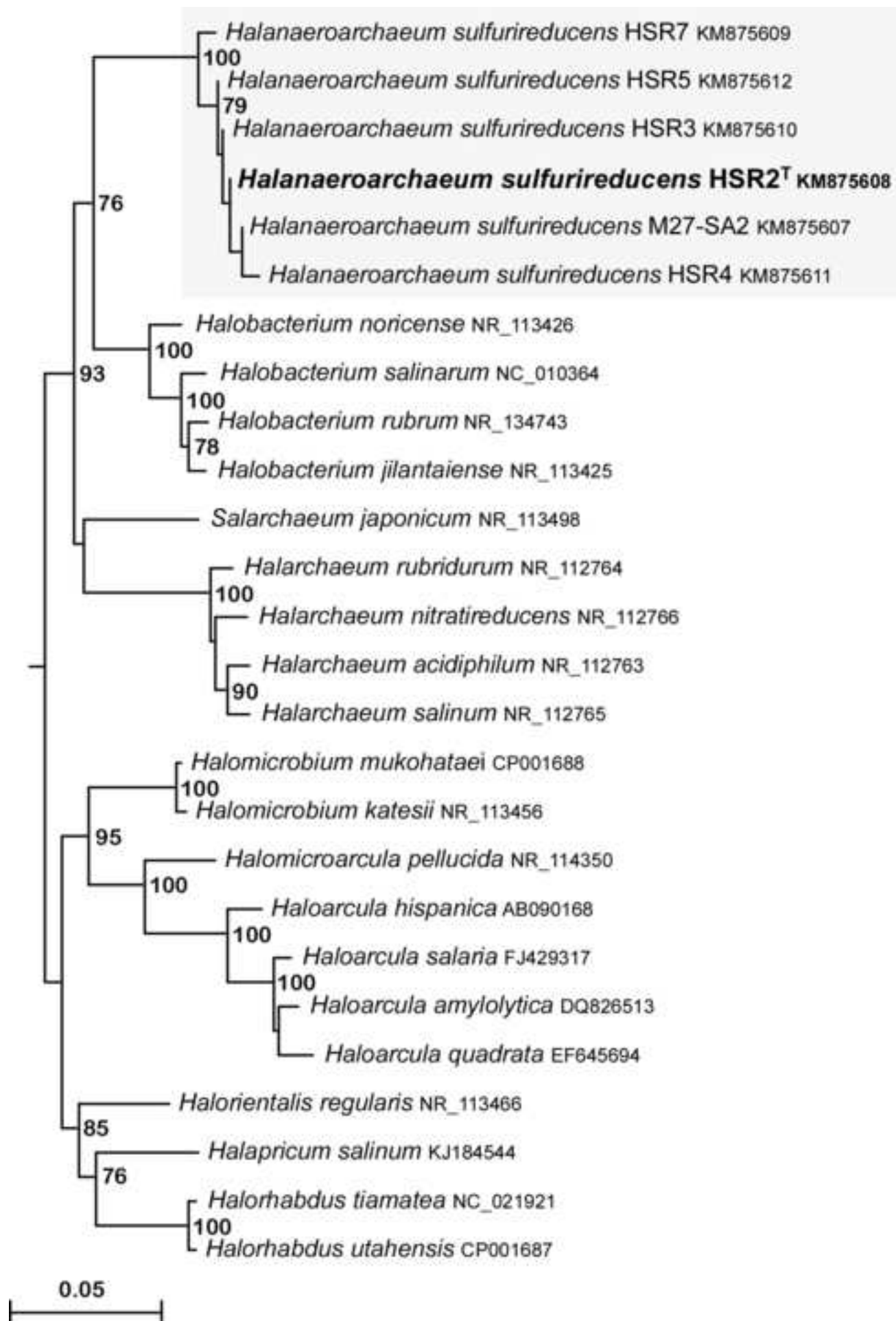
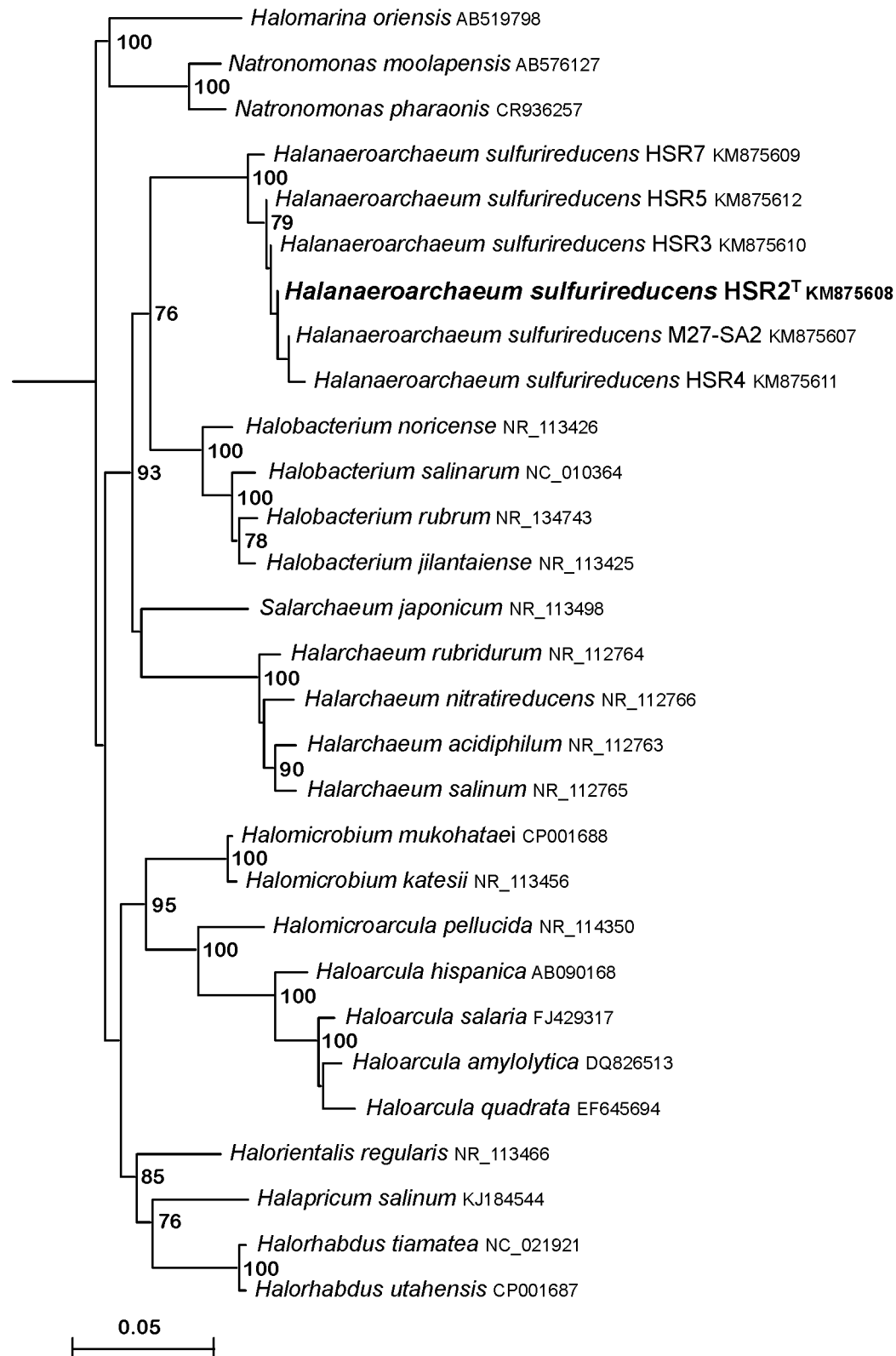
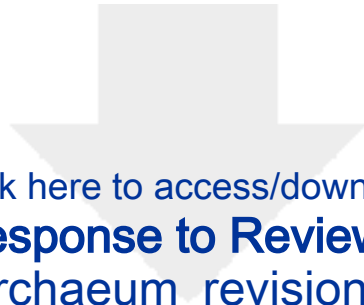


fig.1







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