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Description of *Gloeomargarita lithophora* gen. nov., sp. nov., a thylakoid-bearing, basal-branching cyanobacterium with intracellular carbonates, and proposal for *Gloeomargaritales* ord. nov.

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Description of *Gloeomargarita lithophora* gen. nov., sp. nov., a thylakoid-bearing basal-branching cyanobacterium with intracellular carbonates, and proposal for *Gloeomargaritales* ord. nov.
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Abstract:	A unicellular cyanobacterium, strain Alchichica-D10, was isolated from microbialites of the alkaline Lake Alchichica, Mexico. The cells were short rods ($3.9 \pm 0.6 \mu\text{m}$ in length and $1.1 \pm 0.1 \mu\text{m}$ in width) forming biofilms of intense emerald green color. They exhibited red autofluorescence under UV light excitation. UV-visible absorption spectra revealed that they contain chlorophyll a and phycocyanin, and electron microscopy showed the presence of thylakoids. The strain grew within a temperature range of 15-30 °C. Genomic DNA G+C content was 52.2 mol%. The most remarkable feature of this species was its granular cytoplasm, due to the presence of numerous intracellular spherical granules (16-26 per cell) with an average diameter of 270 nm. These granules, easily visible under scanning electron microscopy, were composed of amorphous carbonate containing Ca, Mg, Ba, and Sr. A multi-gene phylogeny based on the analysis of 59 conserved protein markers supported robustly that this strain occupies a deep position in the cyanobacterial tree. Based on its phenotypic characters and phylogenetic position, strain Alchichica-D10 is considered to represent a new genus and novel species of cyanobacteria for which the name <i>Gloeomargarita lithophora</i> gen. nov., sp. nov. is proposed. The type strain is Alchichica-D10 (Culture Collection of Algae and Protozoa CCAP strain 1437/1; Collections de Cyanobactéries et Microalgues Vivantes of the Museum National d'Histoire Naturelle in Paris strain PMC 919.15). Furthermore, a new family, <i>Gloeomargaritaceae</i> , and a new order, <i>Gloeomargaritales</i> , are proposed to accommodate this species under the International Code of Nomenclature for algae, fungi, and plants.

1 **Description of *Gloeomargarita lithophora* gen. nov., sp. nov., a thylakoid-**
2 **bearing basal-branching cyanobacterium with intracellular carbonates, and**
3 **proposal for *Gloeomargaritales* ord. nov.**

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26
27 Contents category: New Taxa (Other Bacteria).

28
29 The GenBank accession number for the sequences of 59 conserved genes of strain Alchichica-
30 D10 is CP017675.

31

32 A unicellular cyanobacterium, strain Alchichica-D10, was isolated from microbialites of the
33 alkaline Lake Alchichica, Mexico. The cells were short rods ($3.9 \pm 0.6 \mu\text{m}$ in length and $1.1 \pm$
34 $0.1 \mu\text{m}$ in width) forming biofilms of intense emerald green color. They exhibited red
35 autofluorescence under UV light excitation. UV-visible absorption spectra revealed that they
36 contain chlorophyll *a* and phycocyanin, and electron microscopy showed the presence of
37 thylakoids. The strain grew within a temperature range of 15-30 °C. Genomic DNA G+C
38 content was 52.2 mol%. The most remarkable feature of this species was its granular
39 cytoplasm, due to the presence of numerous intracellular spherical granules (16-26 per cell)
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41 microscopy, were composed of amorphous carbonate containing Ca, Mg, Ba, and Sr. A multi-
42 gene phylogeny based on the analysis of 59 conserved protein markers supported robustly that
43 this strain occupies a deep position in the cyanobacterial tree. Based on its phenotypic
44 characters and phylogenetic position, strain Alchichica-D10 is considered to represent a new
45 genus and novel species of cyanobacteria for which the name *Gloeomargarita lithophora* gen.
46 nov., sp. nov. is proposed. The type strain is Alchichica-D10 (Culture Collection of Algae and
47 Protozoa CCAP strain 1437/1; Collections de Cyanobactéries et Microalgues Vivantes of the
48 Museum National d'Histoire Naturelle in Paris strain PMC 919.15). Furthermore, a new
49 family, *Gloeomargaritaceae*, and a new order, *Gloeomargaritales*, are proposed to
50 accommodate this species under the International Code of Nomenclature for algae, fungi, and
51 plants.

52

53 Cyanobacteria thrive in a variety of aquatic and terrestrial habitats, where their ability, unique
54 among bacteria, to carry out oxygenic photosynthesis makes them ecologically significant
55 (Castenholz, 2001). They are also important from an evolutionary point of view since they
56 were responsible for the early oxygenation of the Earth's atmosphere (Buick, 2008) and an
57 ancestral cyanobacterium was the endosymbiont that gave rise to the chloroplasts now found
58 in eukaryotic algae and plants (Gray & Doolittle, 1982). In addition, cyanobacteria have a rich
59 fossil record. The massive fossil stromatolites dating back to at least 2.7 billion years ago are
60 considered to have been built by microbial communities dominated by cyanobacteria
61 (Altermann *et al.*, 2006) and unequivocal calcified cyanobacterial fossils are common since
62 the base of the early Cambrian, with *Girvanella* as the first undisputed occurrence at 700
63 million years ago (Riding, 2006). The capacity of several cyanobacteria to precipitate calcium
64 carbonate may have enhanced their preservation and explain, at least partly, their extensive

65 fossil record. In fact, several species induce calcium carbonate precipitation by their
66 photosynthetic activity (Arp *et al.*, 2001; Kamennaya *et al.*, 2012), which increases locally the
67 concentration of CO_3^{2-} by the disproportionation of HCO_3^- to CO_3^{2-} and CO_2 , the latter being
68 fixed by the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO). The
69 export of alkalinity from the intracellular to the extracellular medium, by a mechanism that
70 remains poorly known, raises the saturation index for carbonate minerals in the immediate
71 cell environment and thus leads to mineral precipitation if free cations (e.g., Ca^{2+}) and
72 nucleation sites are present. It has also been proposed that the cell surface, in particular the
73 exopolysaccharidic matrix, may serve as a nucleation site for carbonates (Obst *et al.*, 2009).
74 In all cases, the precipitation of carbonates by cyanobacteria has been regarded as an
75 extracellular uncontrolled process.

76 However, we recently reported the discovery of a cyanobacterial species that contained
77 intracellular carbonate inclusions (Couradeau *et al.*, 2012). It was the first time that this
78 capacity was found in cyanobacteria. More recently, this capability has also been found in a
79 small number of other cyanobacterial taxa (Benzerara *et al.*, 2014; Li *et al.*, 2016).
80 Intracellular carbonates appear to be generally rare in bacteria since, up to the recent
81 discovery in cyanobacteria, their occurrence had been described only in a single species, the
82 proteobacterium *Achromatium oxaliferum* (Head *et al.*, 1996). The new cyanobacterial strain
83 Alchichica-D10, which we provisionally named *Candidatus Gloeomargarita lithophora*
84 (Couradeau *et al.*, 2012), was isolated from microbialite samples collected in the alkaline
85 (~43 mM HCO_3^- , pH ~8.9) Lake Alchichica (Mexico) in 2007 and maintained alive in
86 laboratory aquaria since then. Here, we describe formally this new species and its
87 phylogenetic position within the Cyanobacteria.

88 Lake Alchichica microbialites are mostly composed of hydromagnesite
89 $[\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4(\text{H}_2\text{O})]$ and aragonite (CaCO_3), and the microbial community inhabiting
90 them is largely dominated by very diverse cyanobacteria (Couradeau *et al.*, 2011; Saghai *et al.*,
91 2015). After several years of growth in laboratory aquaria, the microbialites collected in
92 2007 were still inhabited by a large diversity of cyanobacteria similar to that found in the
93 Lake, which suggests that this community is highly resilient (Couradeau *et al.*, 2011).
94 Microbialites and the aquaria walls were covered by extensive biofilms. These biofilms
95 contained different cyanobacterial morphotypes, with a particularly abundant one consisting
96 of small rod-shaped cells with granular cytoplasm, noticeable under optical microscopy (Fig.
97 1). To enrich this cyanobacterial species, we disrupted a biofilm sample and filtered the

98 detached cells through an isopore filter of 3 μm pore size. We then inoculated a 96-well plate
99 containing BG11 medium with the filtered cells. After one month of incubation at 21 $^{\circ}\text{C}$
100 applying a diel cycle, we observed growth of the targeted morphotype in 6 wells. Sequencing
101 of the 16S rRNA gene from these 6 cultures yielded identical sequences (Couradeau *et al.*,
102 2012; accession number JQ733894). We further purified this cyanobacterium by growth on
103 BG11-agar plates and single colony isolation. This allowed us to obtain cultures with this
104 single cyanobacterial species. However, sequencing of 16S rRNA genes amplified with
105 universal bacterial primers revealed the presence of a contaminant alphaproteobacterium
106 closely related to several species of the genus *Sandarakinorhabdus* (with 97% 16S rRNA
107 gene sequence similarity). We have been unable to eliminate this contaminant from our
108 cultures, partly due to the very slow growth rate of the cyanobacterium. Interestingly, some of
109 these *Sandarakinorhabdus* species have also been reported in association with other
110 cyanobacteria, such as the strain *Sandarakinorhabdus* sp. A14 that is found in cultures of
111 *Microcystis aeruginosa* (Shi *et al.*, 2009). Nevertheless, observations using epifluorescent
112 optical microscopy and scanning electron microscopy (SEM) showed that the cultures were
113 largely dominated by the cyanobacterium and that the contaminant appeared to be rare. Thus,
114 even if non-axenic, the cultures were suitable for the description of the new cyanobacterial
115 species using a variety of techniques.

116 Cyanobacterial cells belonging to the new Alchichica-D10 strain grown in BG11 medium
117 measured $3.9 \pm 0.6 \mu\text{m}$ in length and $1.1 \pm 0.1 \mu\text{m}$ in width. The most conspicuous feature of
118 those cells observed under SEM (using secondary electron mode) was the presence of
119 numerous bright intracellular spherical granules (3-19 per cell) measuring between 60 and
120 380 nm in diameter (Fig. 2a). As previously determined by Benzerara *et al.* (2014) using
121 energy-dispersive x-ray spectrometry (EDXS), these inclusions were composed of Ca
122 carbonate. In addition, cells grown in BG11 contained a relatively small number of larger and
123 darker inclusions rich in P that corresponded to polyphosphate granules (Fig. 2b). When
124 grown in the aquarium water, highly alkaline and rich in Ca, Mg, and other cations but poor in
125 P, the cells contained more carbonate granules (16 to 26 per cell) with an average diameter of
126 270 nm. In contrast with cells grown in BG11, the chemical composition of these inclusions
127 contained Mg, Ba, and Sr as major elements in addition to Ca, and polyphosphate granules
128 were rare (Fig. S1, available in the online Supplementary Material). Interestingly, Ba/Ca and
129 Sr/Ca atomic ratios in the inclusions were 1370 and 86 times higher, respectively, than those
130 measured in the aquaria solution (Couradeau *et al.*, 2012), although Ca, Sr and Ba are usually

131 supposed to be incorporated relatively conservatively by carbonates. This suggested that the
132 cells controlled the chemical composition of the inclusions.

133 The ultrastructure of the cells was studied by transmission electron microscopy (TEM). In
134 addition to the typical Gram negative double cell membrane, an important structural feature
135 was the presence of thylakoids, clearly visible as several concentric layers parallel to the cell
136 periphery (Fig. 3). The occurrence of thylakoids clearly differentiates *Gloeomargarita*
137 *lithophora* from the deep-branching genus *Gloeobacter*, which lacks these endomembrane
138 structures (Rippka *et al.*, 1974). Intracellular structures were observed in the same cells with a
139 contrast different from that of carbonate or polyphosphate inclusions (see arrows in Fig. 3)
140 but with morphological and contrast similarities with carboxysomes found in other
141 cyanobacteria (e.g., Porta *et al.*, 2000).

142 Cells observed by confocal laser scanning microscopy (CLSM) under UV light (405 nm)
143 excitation showed intense red autofluorescence (Fig. S2, available in the online
144 Supplementary Material). The absorption spectrum of pigments extracted with 90% acetone
145 showed peaks at wavelengths of 620 and 664 nm, indicating the presence of phycocyanin and
146 chlorophyll *a* (Fig. S3, available in the online Supplementary Material), which are typical
147 pigments of cyanobacteria.

148 Strain Alchichica-D10 colonies grew very slowly on agar plates. They exhibited an intense
149 emerald color and were surrounded by a thick mucilaginous cover (Fig. S4a, available in the
150 online Supplementary Material). Individual cells appear to be able to glide on the plate
151 surface to initiate the growth of new peripheral colonies (Fig. S4b, available in the online
152 Supplementary Material). This phenomenon can lead to the formation of migration fronts that
153 provide a stratified structure to the margins of mature colonies (Fig. S4a, available in the
154 online Supplementary Material). To determine the optimal growth conditions in the
155 laboratory, we combined 3 different buffered pH values (8.0, 8.5, and 9.0), 5 temperatures
156 (15, 20, 25, 30, and 37 °C), and 3 light intensities (photon flux of 5, 10, and 41 $\mu\text{moles m}^{-2}\text{s}^{-1}$)
157 in both liquid and solid BG11 media buffered with HEPES. Growth was extremely slow at
158 15° C and did not occur at 37 °C. Thus, we focused on the intermediate temperatures. In all
159 cases, growth was slow and took at least 6 weeks to become noticeable by the development of
160 visible colonies on solid medium or by the appearance of green color in the liquid cultures. In
161 these liquid cultures, the highest cell densities were observed at pH 8.0 and 8.5 at 25 and 30
162 °C, whereas in solid medium the optimal condition appeared to be at a pH of 8.5 with low

163 light intensity (photon flux of 5-10 $\mu\text{moles m}^{-2}\text{s}^{-1}$) and a temperature of 25-30 °C (Fig. S5,
164 available in the online Supplementary Material).

165 Preliminary phylogenetic analyses based on 16S rRNA gene sequences suggested the
166 proximity of strain Alchichica-D10 to the basal order Gloeobacterales (Couradeau *et al.*,
167 2012). However, this relationship was not strongly supported and was based on unrooted
168 phylogenetic trees. In fact, a 16S rRNA rooted tree published later showed that *G. lithophora*
169 did not branch as sister of the *Gloeobacter* species but as the second branch to diverge within
170 the Cyanobacteria after *Gloeobacter*, though still with weak statistical support (Saw *et al.*,
171 2013). To resolve this uncertainty, we carried out a multi-gene phylogenetic analysis. For this
172 purpose, we extracted *G. lithophora* genomic DNA that was sequenced using the Illumina
173 Genome Analyzer II technology, which yielded 2.1 Gbp of DNA sequences (with a G + C
174 content of 52.2). Among these sequences, we fetched 59 conserved genes involved in
175 transcription and translation (Table S1, available in the online Supplementary Material). Their
176 translated protein sequences were aligned with the respective homologous sequences found in
177 all completely sequenced cyanobacterial genomes and several other bacteria included as
178 outgroups. Alignments were trimmed to eliminate ambiguously aligned regions and
179 concatenated to build a 7,220 amino acids-long concatenation, which was analyzed using
180 Bayesian inference to reconstruct a phylogenetic tree. The resulting tree was highly supported
181 and placed *G. lithophora* in an early-diverging position, as the third most basal cyanobacterial
182 branch, just after the two available *Gloeobacter* species and a group containing the strain
183 *Synechococcus* sp. PCC 7336 and the two thermophilic strains *Synechococcus* sp. JA-2-
184 3B'a(2-13) and JA-3-3Ab isolated from Yellowstone (Fig. 4 and Fig. S6, available in the
185 online Supplementary Material). Therefore, *Gloeobacter* and *Gloeomargarita* are not sister
186 groups, contradicting our previous single gene-based suggestion that strain Alchichica-D10
187 might be a divergent species belonging to the order Gloeobacterales. Indeed, the presence of
188 thylakoids in strain Alchichica-D10 (see above) constituted a major difference with the
189 thylakoid-lacking *Gloeobacter* genus (Rippka *et al.*, 1974), in agreement with their placement
190 in independent branches in the multi-gene phylogenetic tree.

191 Although *G. lithophora* is the only species available in culture for this new genus, a large
192 diversity of related environmental 16S rRNA gene sequences has been detected, indicating
193 that it belongs to a diverse clade found in various environments, in particular freshwater
194 microbialites and microbial mats (Ragon *et al.*, 2014). Interestingly, several sequences have
195 been retrieved from microbial mats thriving in continental hot springs from various locations,

196 such as Yellowstone, central Tibet, and Algeria (Amarouche-Yala *et al.*, 2014; Lau *et al.*,
197 2009; Turner *et al.*, 1999). Moreover, cells with morphological characteristics similar to those
198 of *G. lithophora*, including the presence of numerous carbonate inclusions in the cytoplasm,
199 were observed by electron microscopy in the Algerian hot spring samples (Ragon *et al.*,
200 2014). These results indicate that the different lineages related to *Gloeomargarita* have
201 adapted to a wide range of temperatures.

202 As *G. lithophora* does not belong to the Gloeobacterales, the erection of a new genus, family
203 and order to accommodate this new cyanobacterial species is required (see below). As far as
204 we know, the genus name *Gloeomargarita* has never been used in botanical literature, so it
205 can be validly published as a new cyanobacterial genus under the International Code of
206 Nomenclature for algae, fungi and plants (McNeill *et al.*, 2012).

207

208 **Description of *Gloeomargarita* gen. nov.**

209 *Gloeomargarita* (Gloe.o.mar.ga.ri'ta. Gr. n. *gloios* glutinous substance; L. fem. n. *margarita*
210 pearl; N.L. fem. n. *Gloeomargarita* glutinous cells containing pearls).

211 Unicellular rods with oxygenic photoautotrophic metabolism and gliding motility. Contain
212 chlorophyll *a* and phycocyanin and photosynthetic thylakoids located peripherally.
213 Reproduction by transverse binary fission in a single plane. Do not produce well-defined
214 sheath layers. Contain spherical inclusions of earth alkaline carbonates in the cytoplasm. The
215 type species is *Gloeomargarita lithophora* sp. nov.

216

217 **Description of *Gloeomargarita lithophora* sp. nov.**

218 *Gloeomargarita lithophora* (li.tho'pho.ra. Gr. masc. n. *lithos* stone; Gr. masc. n. *phoros*
219 carrier; N.L. fem. n. *lithophora* carrier of stones).

220 Exhibits the following properties in addition to those given in the genus description. Cells are
221 1.1 µm wide and 3.9 µm long in average. Growth occurs at 15-30 °C (optimum 25 °C) in
222 alkaline freshwater and BG11 medium. The G + C content of the genomic DNA of the type
223 strain is 52.2 mol%. The type strain, Alchichica-D10 (=CCAP 1437/1, =PMC 919.15), was
224 isolated from microbialites of the alkaline Lake Alchichica (Mexico) preserved in laboratory
225 aquaria at Orsay (France). The 16S rRNA gene sequence of the type strain is available in
226 GenBank under accession number JQ733894.

227 The holotype of *G. lithophora* is the specimen PueAl-43a in the FCME Herbarium of the
228 Faculty of Sciences at the UNAM. Type locality: Alchichica Lake (Mexico). Living cultures
229 CCAP 1437/1 and PMC 919.15 are ex-holotypes.

230

231 **Description of *Gloeomargaritaceae* fam. nov.**

232 *Gloeomargaritaceae* (Gloe.o.mar.ga.ri.ta.ce'ae. N.L. fem. n. *Gloeomargarita* type genus of
233 the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Gloeomargaritaceae* the
234 family of the genus *Gloeomargarita*).

235 The description is the same as for the genus *Gloeomargarita*.

236 Type genus is *Gloeomargarita* gen. nov.

237

238 **Description of *Gloeomargaritales* ord. nov.**

239 *Gloeomargaritales* (Gloe.o'mar.ga.ri.ta'les. N.L. fem. n. *Gloeomargarita* type genus of the
240 order; suff. *-ales* ending denoting an order; N.L. fem. pl. n. *Gloeomargaritales* the order of
241 the genus *Gloeomargarita*).

242 The description is the same as for the genus *Gloeomargarita*.

243

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249

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331

332 **Figure legends**

333 **Fig. 1.** Differential interference contrast (DIC) image of several *Alchichica*-D10 cells
334 collected from an aquarium biofilm. Bar, 10 μm .

335

336 **Fig. 2.** Electron microscopy analyses of *Alchichica*-D10 cells grown in BG11. (a) Scanning-
337 transmission electron microscopy image in high angle annular dark field (STEM-HAADF)
338 mode: Ca-carbonates appear as brighter round-shaped inclusions, while polyphosphate
339 granules are darker, sometimes bigger globules. (b) Scanning-transmission electron
340 microscopy energy dispersive X-rays spectrometry (STEM-EDX) map of the same area:
341 Calcium is in red, phosphorus in green and carbon in blue; as a result, Ca-carbonates appear
342 in red and polyphosphate granules in green. Bars, 1 μm .

343

344 **Fig. 3.** Bright-field TEM image of a thin section of *Alchichica*-D10 cells embedded in EPON
345 resin. Several concentric thylakoid membranes are visible under the cell membrane.
346 Arrowheads indicate structures that may correspond to carboxysomes. Bar, 1 μm .

347

348 **Fig. 4.** Bayesian phylogenetic tree based on the analysis of a concatenation of 59 conserved
349 proteins (7220 amino acids) reconstructed using PhyloBayes MPI (Lartillot *et al.*, 2013) with
350 the CAT GTR model. Numbers at branches are posterior probabilities (only those >0.50 are
351 shown). For space constraints, the outgroup and the *Synechococcus/Prochlorococcus* group
352 have been replaced by triangles (for the complete tree see Fig. S6, available in the online
353 Supplementary Material). The scale bar indicates the number of substitutions per position.

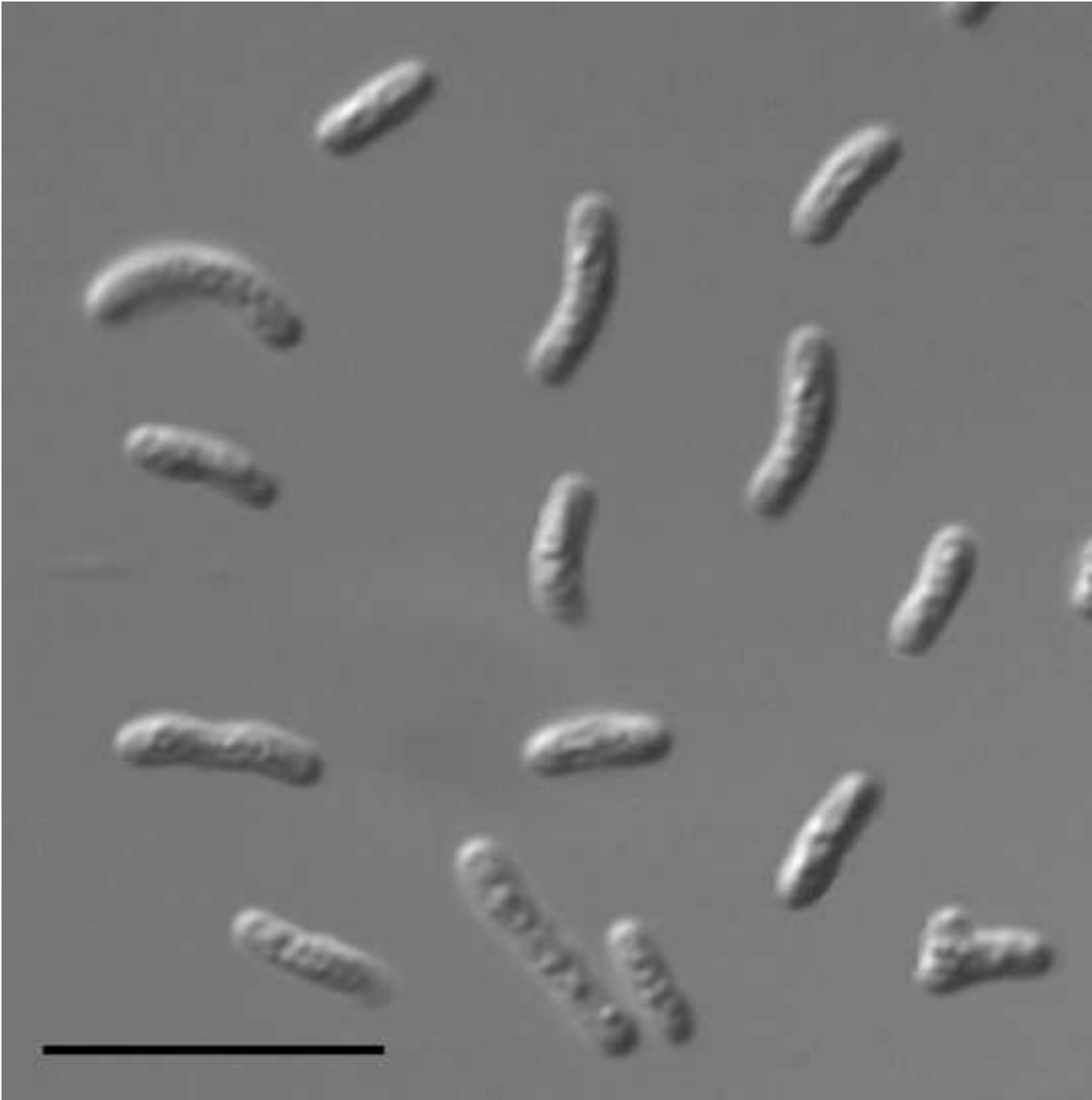
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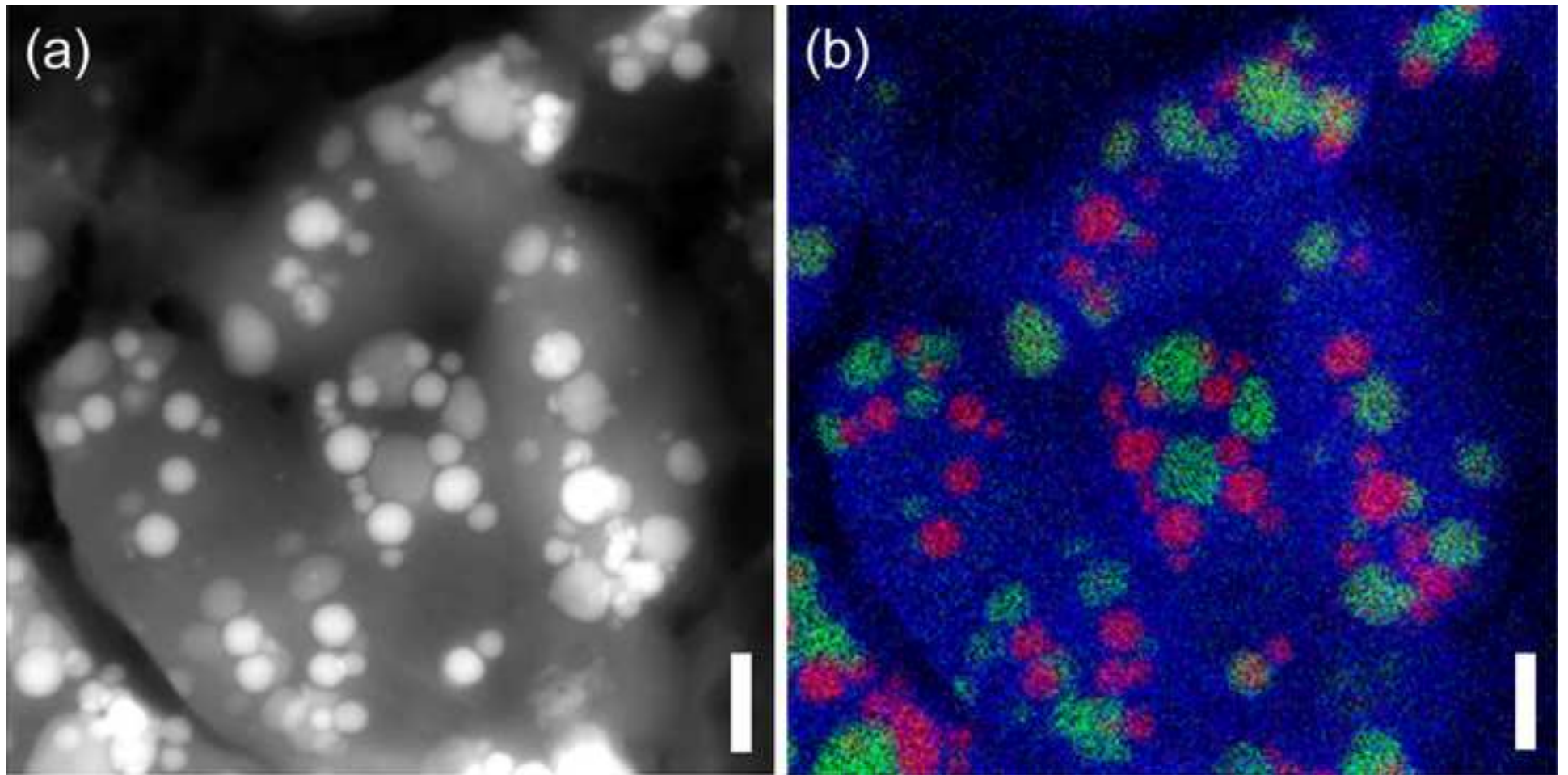
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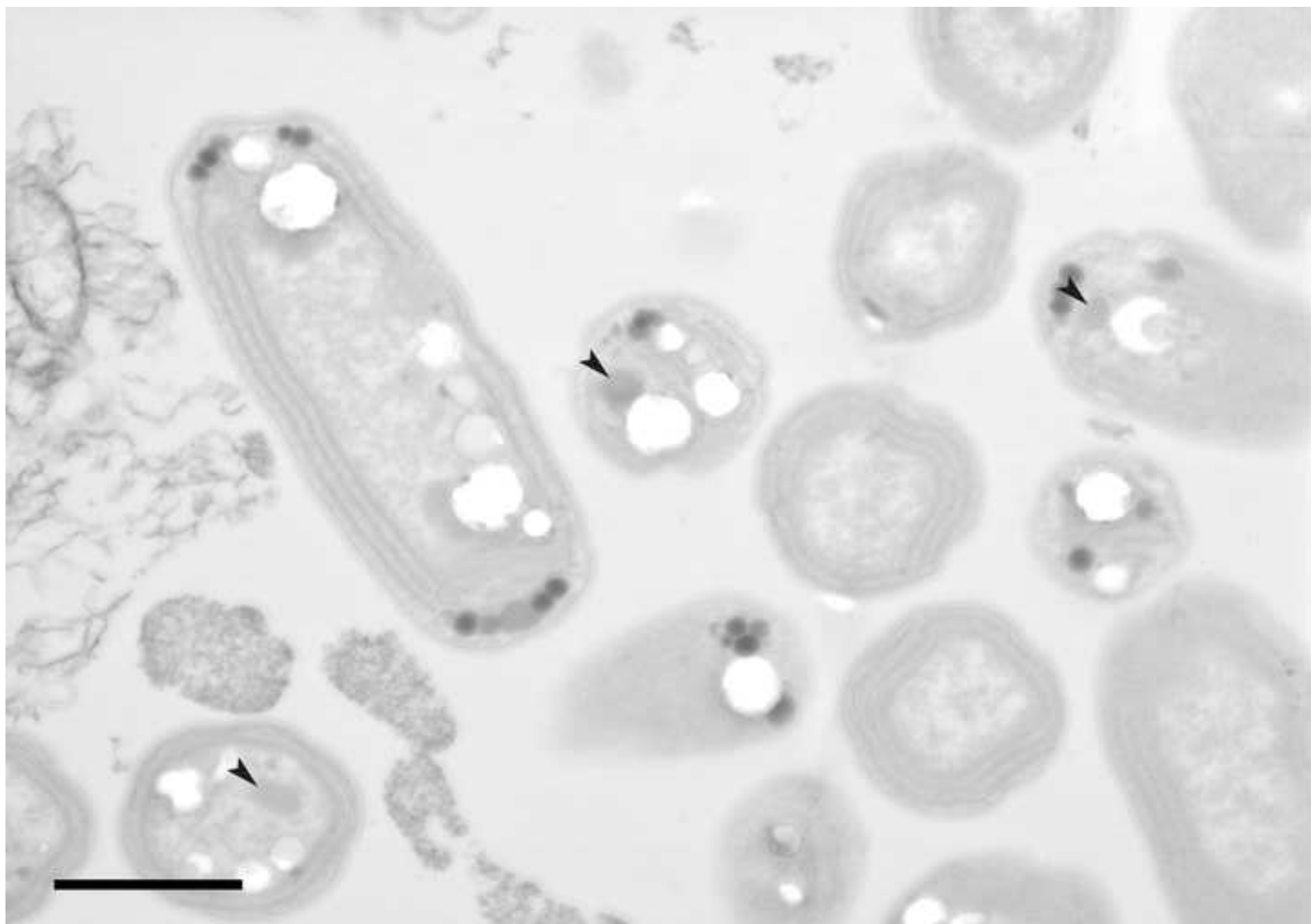
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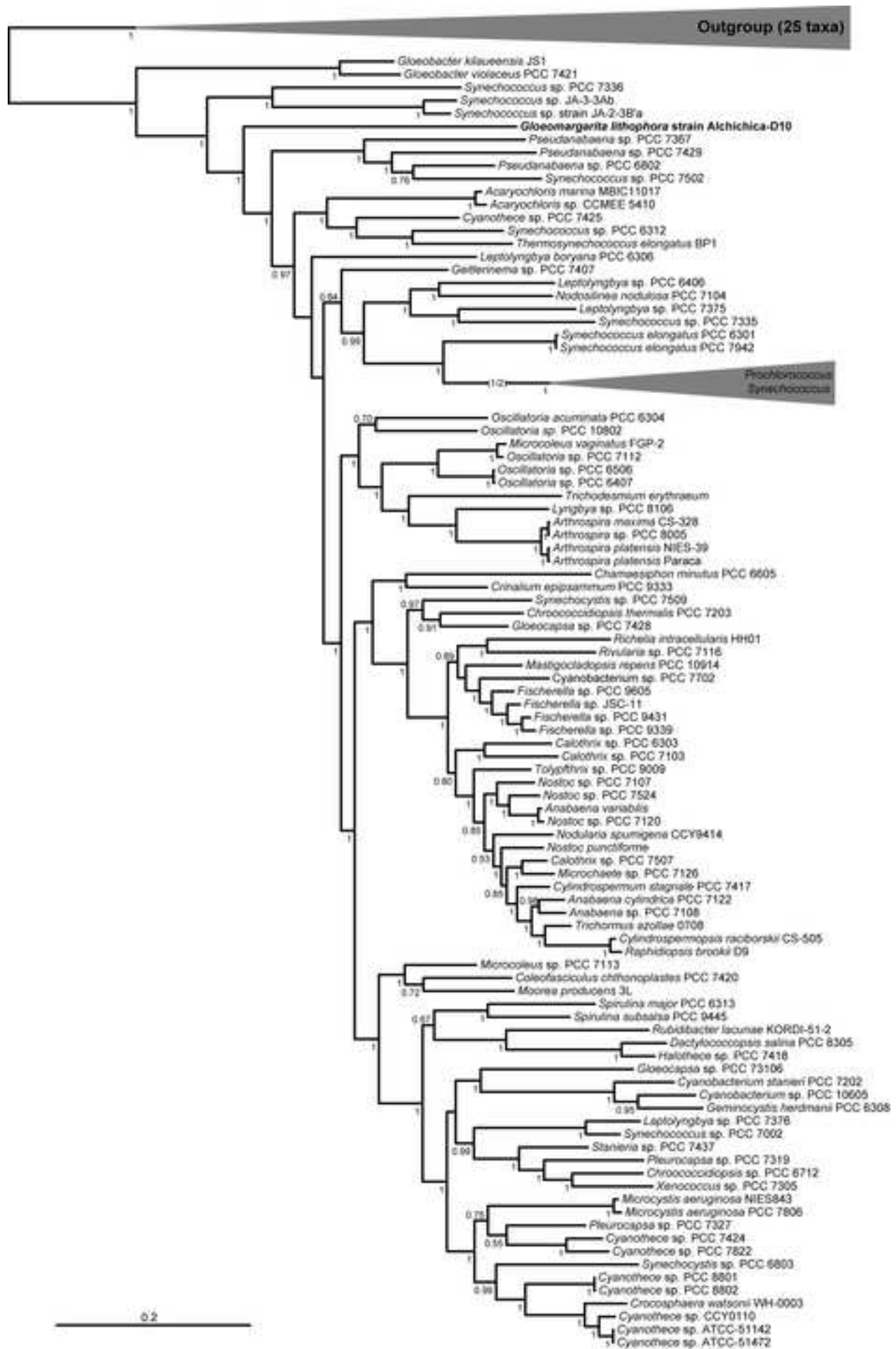
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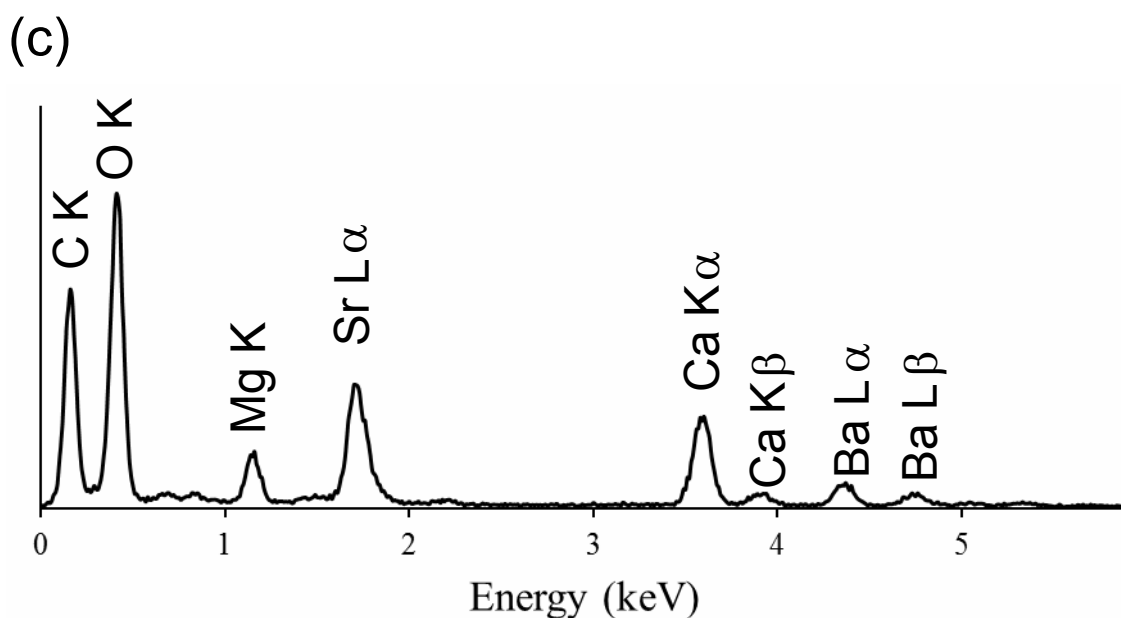
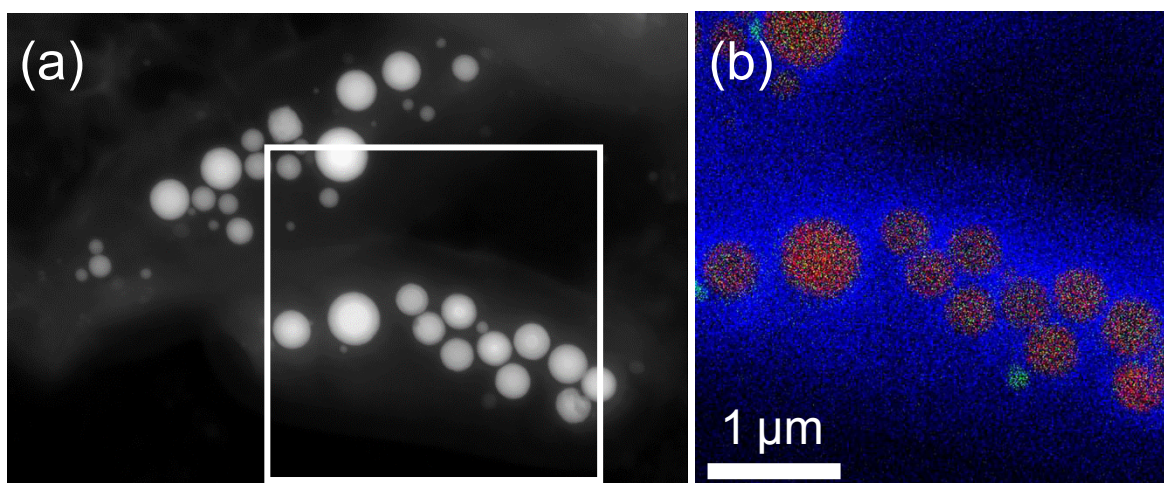




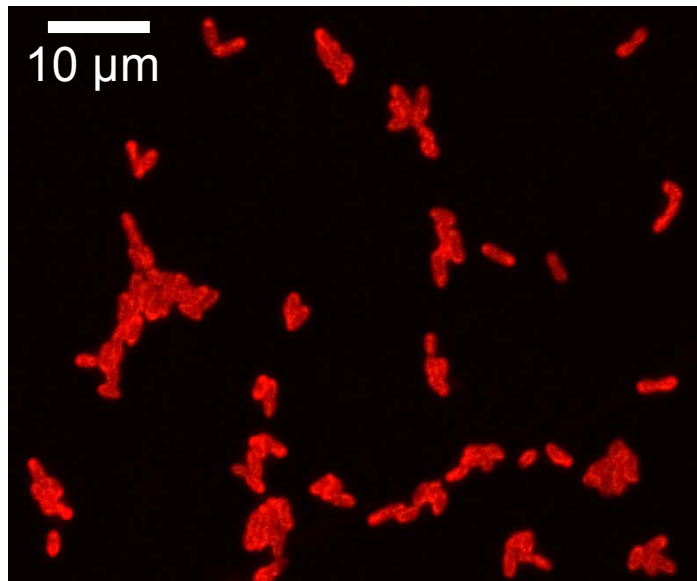
Supplementary Table S1, Proteins used for phylogenetic reconstruction

Protein	Protein name
EFG	elongation factor G
EFTs	elongation factor Ts
EFTu	elongation factor Tu
IF-1	initiation factor 1
IF-2B	initiation factor 2B
IF-3	initiation factor 3
rpL1	ribosomal protein L1
rpL10	ribosomal protein L10
rpL11	ribosomal protein L11
rpL13	ribosomal protein L13
rpL14	ribosomal protein L14
rpL15	ribosomal protein L15
rpL16	ribosomal protein L16
rpL17	ribosomal protein L17
rpL18	ribosomal protein L18
rpL19	ribosomal protein L19
rpL2	ribosomal protein L2
rpL20	ribosomal protein L20
rpL21	ribosomal protein L21
rpL22	ribosomal protein L22
rpL23	ribosomal protein L23
rpL24	ribosomal protein L24
rpL27	ribosomal protein L27
rpL28	ribosomal protein L28
rpL29	ribosomal protein L29
rpL3	ribosomal protein L3
rpL31P	ribosomal protein L31P
rpL32	ribosomal protein L32
rpL33	ribosomal protein L33
rpL34	ribosomal protein L34
rpL35	ribosomal protein L35
rpL4	ribosomal protein L4
rpL5	ribosomal protein L5
rpL6	ribosomal protein L6
rpL7-L12	ribosomal protein L7-L12
rpL9	ribosomal protein L9
rpPSRP3	ribosomal protein PSRP3
rpS10	ribosomal protein S10
rpS11	ribosomal protein S11
rpS12	ribosomal protein S12
rpS13	ribosomal protein S13
rpS14	ribosomal protein S14
rpS15	ribosomal protein S15
rpS16	ribosomal protein S16
rpS17P	ribosomal protein S17P
rpS18	ribosomal protein S18
rpS19	ribosomal protein S19
rpS1P	ribosomal protein S1P
rpS2	ribosomal protein S2

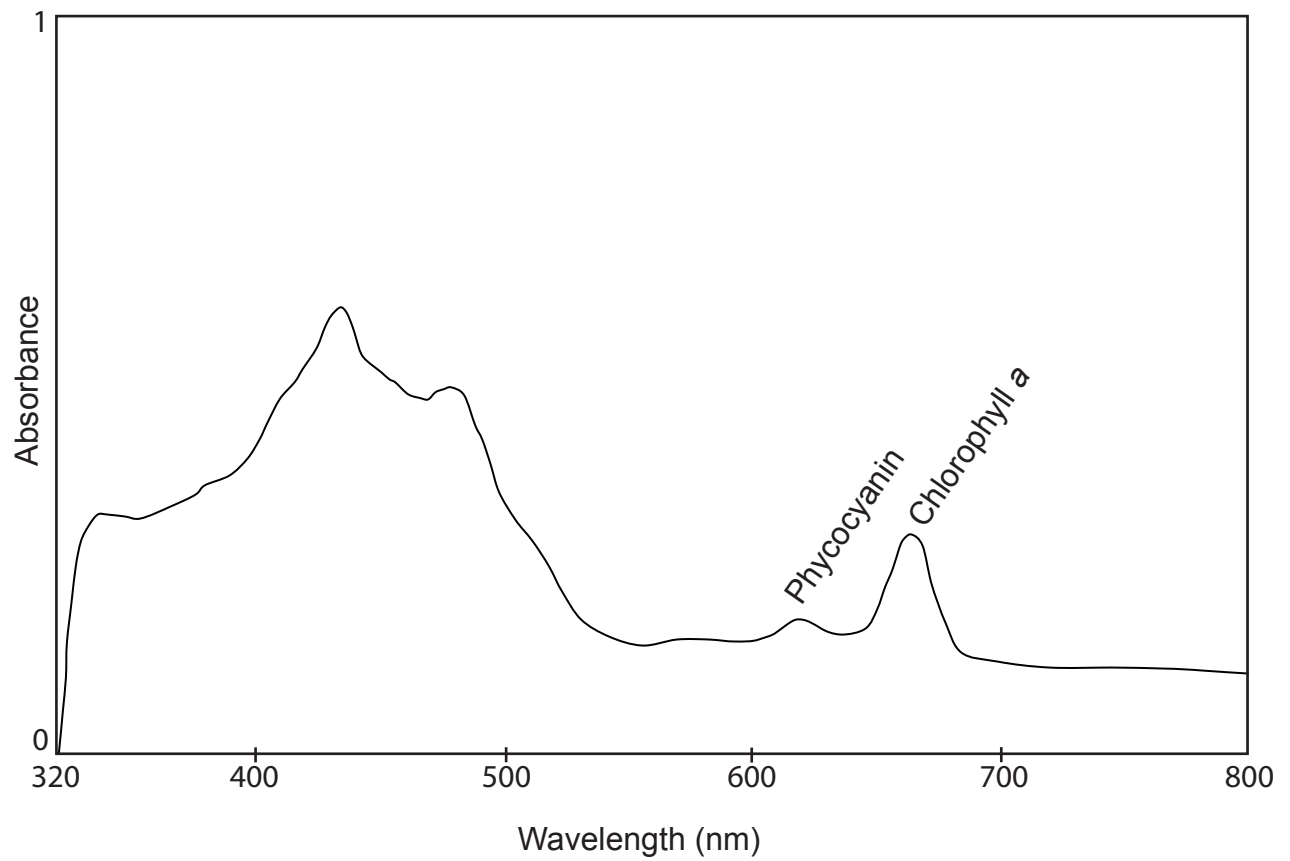
rpS20	ribosomal protein S20
rpS21	ribosomal protein S21
rpS3	ribosomal protein S3
rpS30EA	ribosomal protein S30EA
rpS4	ribosomal protein S4
rpS5	ribosomal protein S5
rpS6	ribosomal protein S6
rpS7	ribosomal protein S7
rpS8	ribosomal protein S8
rpS9	ribosomal protein S9



Supplementary Fig. S1. Electron microscopy analyses of Alchichica-D10 cells grown in aquarium water. (a) Scanning-transmission electron microscopy high angle annular dark field (STEM-HAADF) image: Ca-carbonates appear as brighter round-shaped inclusions. (b) Energy dispersive X-ray spectrometry (EDXS) map of the area outlined in (a): Calcium is in red, phosphorus in green and carbon in blue; as a result, Ca-carbonates appear in red and PolyP granules in green. (c) EDXS spectrum of a carbonate inclusion showing the presence of Ca, Sr, Mg and Ba.

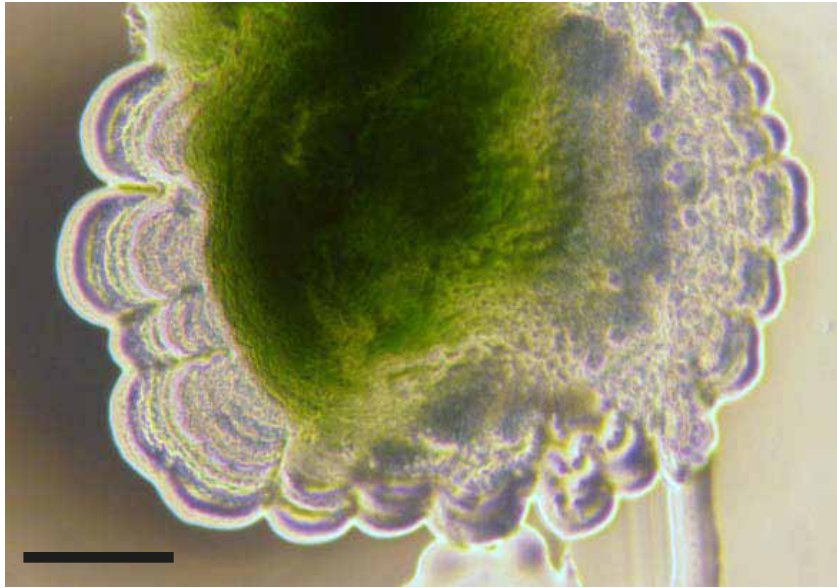


Supplementary Fig. S2. Autofluorescence of Alchichica-D10 cells observed under confocal laser scanning microscopy with UV light illumination (405 nm). Bar, 10 μm .

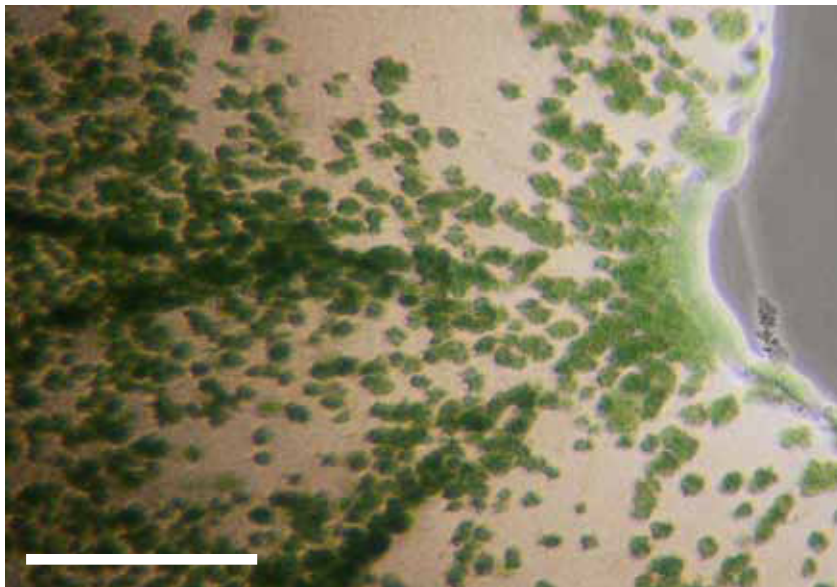


Supplementary Fig. S3. Absorption spectra of strain Alchichica-D10 pigments extracted with 90% acetone.

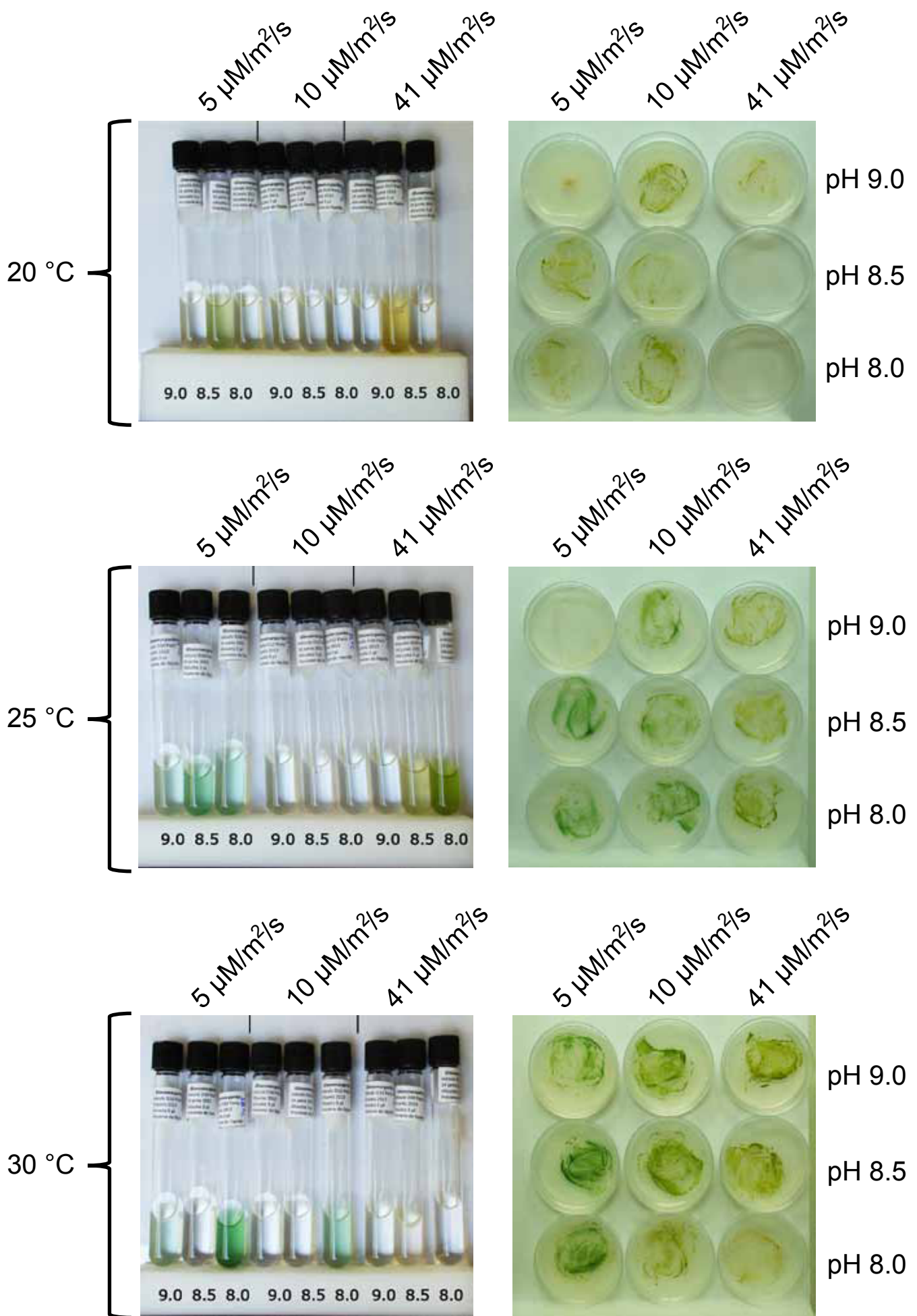
(a)



(b)



Supplementary Fig. S4. (a) Alchichica-D10 strain colony growing on a BG11-agar plate. (b) Magnification of the colony border. Bars, 1 mm (a) and 100 μ m (b).



Supplementary Fig. S5. Growth of strain Alchichica-D10 in liquid and solid BG11 medium under different temperature, pH, and light intensity conditions.



Supplementary Fig. S6. Bayesian phylogenetic tree based on the analysis of a concatenation of 59 proteins (7220 amino acids) reconstructed using PhyloBayes MPI with the CAT GTR model. Numbers at branches are posterior probabilities (only those >0.50 are shown). The scale bar indicates the number of substitutions per position.