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Intracellular amorphous Ca-carbonate and magnetite biomineralization by a magnetotactic bacterium affiliated to the Alphaproteobacteria

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Abstract

Bacteria synthesize a wide range of intracellular submicrometer-sized inorganic precipitates of diverse chemical compositions and structures, called biominerals. Their occurrences, functions and ultrastructures are not yet fully described despite great advances in our knowledge of microbial diversity. Here, we report bacteria inhabiting the sediments and water column of the permanently stratified ferruginous Lake Pavin, that have the peculiarity to biomineralize both intracellular magnetic particles and calcium carbonate granules. Based on an ultrastructural characterization using transmission electron microscopy (TEM) and synchrotron-based scanning transmission X-ray microscopy (STXM), we showed that the calcium carbonate granules are amorphous and contained within membrane-delimited vesicles. Single-cell sorting, correlative fluorescent *in situ* hybridization (FISH), scanning electron microscopy (SEM) and molecular typing of populations inhabiting sediments affiliated these bacteria to a new genus of the Alphaproteobacteria. The partially assembled genome sequence of a representative isolate revealed an atypical structure of the magnetosome gene cluster while geochemical analyses indicate that calcium carbonate production is an active process that costs energy to the cell to maintain an environment suitable for their formation. This discovery further expands the diversity of organisms capable of intracellular Ca-carbonate biomineralization. If the role of such biomineralization is still unclear, cell behaviour suggests that it may participate to cell motility in aquatic habitats as magnetite biomineralization does.

45 # Introduction

46 Biomineralization is a process whereby organisms trigger the formation of minerals by sequestering
47 diverse chemical elements such as Fe, Mn, Ca, As, O, S, or P into relatively stable solid phases [1, 2].
48 Produced minerals are chemically diverse including oxides, oxy-hydroxides, carbonates, phosphates,
49 sulphates and sulphides, some of which involve metals and metalloids [2]. This process can have a
50 significant impact on the geochemical cycles of these diverse chemical species. Two types of
51 biomineralization pathways are distinguished: (i) biologically-induced mineralization, which refers to
52 all cases where mineral precipitation results from chemical shifts in the environment of the
53 organisms induced by their metabolic activity and (ii), biologically controlled mineralization, for
54 which specific genes and cell structures are involved [3].

55 Biomineralization is not restricted to eukaryotes; many prokaryotes developed mechanisms
56 to nucleate and grow diverse minerals as well. However, only few cases of controlled
57 biomineralization are reported in prokaryotes. The most emblematic case of prokaryotic controlled
58 biomineralization is that of magnetotactic bacteria (MTB), which produce intracellular magnetic
59 crystals enclosed within compartments delimitated by a lipid bilayer and called magnetosomes [4].
60 The underlying genetic determinism, molecular machinery, ecologic role and evolution of this
61 biomineralization pathway have been the focus of interdisciplinary investigations and represent the
62 most documented case of biomineralization in prokaryotes [5, 6].

63 A much less documented case is provided by bacteria biomineralizing intracellular calcium
64 carbonate inclusions. Two phylogenetically distinct groups have been shown to be capable of this
65 biomineralization: (i) several species of the Cyanobacteria phylum and (ii), some *Achromatium*
66 species in the Gammaproteobacteria class. The large sulphur bacterium *Achromatium oxaliferum* was
67 first described by Schewiakoff in 1893 [7]. It accumulates numerous calcium carbonate inclusions
68 filling most of the cell volume [8–11]. This group of Proteobacteria was observed worldwide in the
69 microoxic-anoxic zone of the sediment-water interface of both freshwater and marine environments.
70 Population densities can be as high as 10^5 cells/mL and account for over 90% of the total local
71 bacterial volume [9, 10, 12]. Despite the large biomass represented by *Achromatium* populations,
72 their impact on biogeochemical cycles is not well understood. However, it has been shown that
73 calcium carbonates accumulating within *Achromatium* cells can represent most of the solid calcium
74 in some sediments, especially when (extracellular) pore waters are undersaturated with calcium
75 carbonate phases [12, 13]. No cultured representative strain is available, which limits the
76 physiological characterization of cells to culture-independent field and microcosm analyses. Only

some *Achromatium* populations have been shown to be able to fix inorganic carbon, while others are not able to do so. Since all *Achromatium* populations can form intracellular Ca-carbonates, this suggests that inorganic C fixation is not key to intracellular Ca-carbonate biomineralization. Moreover, they gain energy from sulphur oxidation and may use oxygen or nitrate as potential electron acceptors [15–19]. Transmission electron microscopy (TEM) analyses suggested the presence of membranes surrounding the calcium carbonate inclusions of *Achromatium* [12, 13].

The biomineralization of intracellular amorphous calcium carbonate (ACC) by phylogenetically and geographically widespread cyanobacteria species, has been discovered more recently [19]. They include deep-branching species, suggesting the possibility that intracellularly calcifying cyanobacteria may have thrived on the early Earth. Similarly to *Achromatium*, it was shown that they can form intracellular ACC even in extracellular solutions undersaturated with respect to ACC, again demonstrating some energy cost which has been attributed to active uptake of Ca [20]. Also similar with *Achromatium*, ACC biomineralization in cyanobacteria occurs within a microcompartment but in this case, it is delimited by an envelope that is not composed of a lipid bilayer but of a yet-to-be-determined nature [21]. Two distinct patterns of ACC distribution were described within the cyanobacterial cells: one with ACC inclusions scattered throughout the cell cytoplasm and another one in which inclusions start forming at the septum and lie at the cell poles after cell division [22, 23]. In contrast to *Achromatium*, ACC-producing cyanobacteria were shown to live in oxic environments where they perform oxygenic photosynthesis [23]. These cyanobacteria can be abundant in some habitats. For example, they reach up to 10% of all the operational taxonomic units (OTUs) identified in a geothermal pool at Little Hot Creek in California [24]. Due to its ability to sequester abundant alkaline earth elements within ACC, the cyanobacterium *Gloeomargarita lithophora* [19, 25], was shown to be particularly interesting for the remediation of radionuclides such as ^{90}Sr and Ra [20, 26]. Moreover, this cyanobacterium is the closest modern relative of plastids, questioning the possibility that this capability to sequester Ca was transferred to the first photosynthetic eukaryotes [27].

The biological role of intracellular calcium carbonate biomineralization is still unclear, but some functions have been hypothesized: intracellular carbonates may be involved in i) the buffering of intracellular pH [10, 28], ii) a buoyancy-regulating mechanism [13, 17, 19, 29] or iii) the storage of inorganic carbon that may serve in some cases as an electron acceptor in carbonate respiration [13, 17]. However, in both Cyanobacteria and *Achromatium*, the biochemical pathways and the genetic determinants involved in calcium carbonate formation remain unknown. Moreover, the evolutionary history of this process has yet to be determined to test whether it appeared independently in distinct

110 phyla by convergent evolution, or derived from a common ancestor, and/or spread by transfer. For
111 this purpose, a better assessment of the biology and ecology of intracellular calcium carbonate
112 biomineralizing bacteria as well as the phylogenetic distribution of this capability is crucial.
113 Considering that this process was detected only very recently in cyanobacteria, it is likely that we do
114 not have an exhaustive view of the phylogenetic distribution of intracellular calcium carbonate
115 biomineralization, and much less of the ecological niches where such a biomineralization is
116 performed.

117 Recent observations in the ferruginous and permanently stratified (meromictic) Lake Pavin,
118 Massif Central, France [30], support this assumption by revealing intracellular calcium carbonates
119 biomineralizing microorganisms within a broad uncharacterized diversity of biomineralizing
120 microorganisms. Here, we report a magnetotactic bacterium in the water column and sediments of
121 Lake Pavin that biomineralizes both intracellular magnetite and calcium carbonate. Molecular typing
122 of single cells sorted from sediments revealed that the double biomineralization is performed by
123 members of an undescribed genus within Alphaproteobacteria with no close MTB relatives.
124 Hereafter, we characterize the ultrastructure and intracellular mineral phases biomineralized by
125 these bacteria to finally give some insight into magnetite biomineralization using a partial genome
126 sequence.

127

128

129 # Materials and methods

130 ***Site description and sample collection***

131 Sediment samples were collected from the shore of Lake Pavin. This was done by fully filling one litre
132 glass bottles with 300-400 mL of sediments and 600-700 mL of water that overlaid the sediments. Air
133 bubbles were excluded. Once in the laboratory, bottles were stored with their cap closed, under dim
134 light and at room temperature (*ca.* 25 °C). Two locations were sampled at Lake Pavin during the
135 different sampling campaigns that took place between May 2015 and October 2019: (i) 45.499107°N,
136 2.886273°E and (ii) 45.499101°N, 2.889239°E.

137 Water column samples were collected in October 2018 at depths ranging between 45 and 65
138 m from a platform located near the centre of Lake Pavin (45.495792°N, 2.888117°E). Water was
139 collected using a Niskin bottle at the targeted depths and was then transferred in one litre glass
140 bottles filled to their capacity and tightly closed. Bacteria of interest collected from the water column
141 could be conserved for a few days only when stored at 4°C, whereas those from sediments could be
142 observed and magnetically sorted up to 5 years after their sampling and storage in the laboratory, at
143 room temperature. This is the reason why the majority of our study focused on bacteria from
144 sediments.

145 ***Solution chemistry analyses***

146 Solutions in the sampling bottles were filtered at 0.22 µm before analyses. The pH was measured
147 with a CyberScan pH 5500 pH-meter (Eutech instruments) using a combined pH microelectrode
148 (Fisherbrand) calibrated with Hanna pH standard buffer solutions (4.010, 7.010, 10.010) and an
149 uncertainty of 0.01 pH unit. Elemental analyses of [Ca^{2+}] were performed on samples acidified with
150 HNO_3 (2%) by ICP-AES (iCAP6200 Thermofisher) with an uncertainty of 0.7%. All measurements were
151 far above the quantification limit (*ca*. 0.1 ppb).

152 ***Dissolved orthophosphate concentration and alkalinity of sediment pore water (Margot)***

153 Alkalinity measurements were carried out according to the protocol previously described [31, 32].
154 The standard range was tested with Evian® water before sample alkalinity measurements. Here,
155 alkalinity was approximately equivalent to the concentration of HCO_3^- under the present conditions
156 [33].

157 The saturation index was defined as: SI = log (IAP/K_{sp}), where IAP corresponds to the ion
158 activity product and K_{sp} to the thermodynamic solubility product for a mineral phase. A solution was
159 supersaturated with respect to a mineral phase when SI > 0. Here Ca^{2+} and HCO_3^- activities were
160 approximated to the measured concentrations. The solubility constant (pK_{sp}) at 25°C of amorphous
161 Ca carbonate (ACC) used in the present study was 6.278, as determined by Purgstaller *et al.* [34].

162 ***Magnetic enrichment and light microscope observation***

163 North-seeking magnetic cells were concentrated by placing the south pole of a neodymium-iron-
164 boron magnet (disc magnet with a diameter of 5 mm and a height of 5 mm) for 3 hours next to the
165 bottles, above the sediment-water interface (for the sediment samples) or at mid-height against the
166 bottle (for water column samples). Examination of magnetically concentrated cells was carried out
167 using the hanging drop technique [35] under a Zeiss Primo Star light microscope equipped with
168 phase-contrast and differential interference contrast optics. Magnetotaxis was evidenced by rotating
169 a stirring bar magnet at 180° on the microscope stage to reverse the local magnetic field. Motility
170 and the magnetotactic behaviour were also observed and recorded under a Leica LMD6000 light
171 microscope equipped with a Leica DMC 4500 camera.

172 ***In situ and in microcosms measurements of chemical and cell count profiles***

173 Dissolved oxygen profiles were measured in the sediments, using a fiber-optic oxygen sensor (50-µm
174 tip diameter, REF OXR50) and a FireStingO_2 meter, both from Pyroscience. High-resolution profiles
175 (100 µm steps, from 10 mm above the sediment to -25 mm below the sediment) were achieved with
176 a Pyroscience MU1 motorized micromanipulator. Sensor calibration was made against saturated
177 humid air (O_2 sat.=100%) and a water solution flushed with N_2. Magnetotactic cells were highly
178 concentrated and diverse in sediment samples, those with large refractive granules could be easily
179 distinguishable from other bacteria and sediment particles; they were counted using a Malassez
180 counting chamber. Measurements were systematically carried out on three replicates of three
181 different samples. For each replicate, the number of cells was counted at three different locations in
182 the sediment sample. Cell counts were reported as the means of triplicate counts for each sample.

183 In the water column, dissolved oxygen and redox potential were measured *in situ* using a CTD
184 O_2-pH-redox probe (YSI 6600). Three 1 L bottles of water were collected every meter above and
185 below the oxycline in order to determine the depth distribution of magnetotactic bacteria. For each
186 depth, three drops of 40 µL were observed using the hanging drop technique [35]. Magnetotactic
187 cells accumulating at the edge of the drops when a magnetic field was applied from one side of the

188 drop were counted under a ZEISS Primo Star light microscope. The number of cells counted in each
189 drop was multiplied by a factor of 25 to obtain the concentration of cells per millilitre. Cell counts
190 were reported as the means of triplicate counts for each depth.

191 ***Cell sorting and whole genome amplification (WGA)***

192 Cell sorting was carried out on sediment samples collected in Lake Pavin with an InjectMan® NI2
193 micromanipulator and a CellTram® vario, hydraulic, manual microinjector from Eppendorf mounted
194 on a Leica DM IL LED microscope equipped with a 63x/0.70 PH objective. The microscope and
195 micromanipulator were placed inside a clean chamber, sterilized beforehand by 1 h germicidal
196 irradiation with ultraviolet (wavelength of the lamp: 254 nm). A 10-µL drop containing magnetically
197 concentrated cells was gently added to a 30-µL drop of filtered Lake Pavin water on a hydrophobic
198 coverslip in order to magnetically transfer magnetotactic cells toward the filtered water. One to ten
199 cells with bright granules were transferred with a sterile microcapillary (TransferTip® (ES), 4 µm inner
200 diameter, Fig. S1) into a 4 µL drop of phosphate buffer saline (PBS). This drop was stored at -20 °C
201 before WGA. To obtain sufficient gDNA for 16S rRNA gene and shotgun metagenomic sequencing,
202 WGA was carried out using the multiple displacement amplification (MDA) technique with the REPLI-
203 g Single Cell Kit (QIAGEN) following the manufacturer's instructions. The concentration of double
204 strand gDNA was measured using a QuBit™ 4 fluorimeter (ThermoFisher Scientific).

205 ***Cloning and sequencing of the 16S rRNA genes of magnetically concentrated and sorted cells***

206 The 16S rRNA gene of all processed DNA samples was amplified using the Phusion® Hot Start Flex
207 DNA Polymerase following the manufacturer's recommendations, a DNA template of 50 - 70 ng/µL
208 and the 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-TACGGHTACCTTGTTACGACTT-3' primers
209 [36]. Blunt-end fragments of 16S rRNA gene sequences were cloned using a Zero Blunt® TOPO® PCR
210 Cloning Kit with One Shot® TOP10 chemically competent *E. coli* cells. The inserts of the resulting
211 clones were digested using the restriction enzyme EcoRI to select five operational taxonomic units
212 (OTUs) representative of the sediment-sorted-cell populations and were sent for sequencing
213 (Eurofins Genomics Germany GmbH). Sequences were compared to data from the NCBI nucleotide
214 database with Basic Local Alignment Search Tool [37]. The 16S rRNA gene sequences of the sorted
215 MTB OTUs were checked using the UCHIME2 chimera detection algorithm [38] and deposited in
216 GenBank under accession numbers MT021453 to MT021457.

217 ***Phylogenetic analyses***

218 All the 16S rRNA gene sequences of Rhodospirillaceae and Acetobacteraceae type strains (NCBI
219 taxonomy) available in the public databases NCBI in January 2020 were downloaded and completed
220 with the five OTUs obtained in this study. This database was reduced to one species per genus
221 keeping the highest quality sequences to draw a first tree with the BioNJ software [39] after
222 sequences were aligned using MAFFT 7 [40] and trimmed with the relaxed parameters of Gblocks
223 (Fig. S2) [41]. This tree was used to remove genetic redundancy by randomly selecting few
224 representative species only to get a final database of 45 sequences including 7 Acetobacteraceae
225 type strains as an external group. All sequences were aligned and trimmed following the same
226 method to get a final alignment of 1381 bp. A maximum-likelihood tree was built using IQ-TREE [42]
227 and a TIM3+F+R4 model for describing nucleotide evolution selected by Modelfinder [43] using the
228 Bayesian Information Criterion (BIC). Robustness of tree topology was tested using a non-parametric
229 bootstrap approach and 500 replicates.

230 ***Coupled Fluorescent* in situ *hybridization (FISH) and scanning electron microscopy (SEM)***

231 FISH was performed according to the procedure described by Pernthaler *et al*. [44]. First, an
232 ATTO488-labeled probe was designed, specific to the bacteria producing refractive inclusions that
233 were sorted from sediment samples (CCPp, 5'- GTCATTATCGTCGCGTGCGAAAGAGCTTTACAACCCG -3',
234 complementary to nucleotides 383 to 419 of the 16S rRNA molecule of OTU1, one mismatch with
235 OUT5 at position 414). This was based on the alignment of the most similar 16S rRNA gene
236 sequences of the Rhodospirillaceae family found in GenBank in August 2018. Probe specificity was
237 evaluated by using the PROBE_MATCH program in the RDP-II [45]. The nearest non-target hits
238 contain at least one mismatch with the specific probe CCPp. Oligonucleotide probes used in this
239 study were purchased from Eurofins Genomics.

240 Then, a 20 µL drop of magnetically concentrated cells was deposited on an 18 mm x 18 mm
241 coverslip covered with poly-L-lysine. After 10 min, the drop was removed and the cells adsorbed on
242 the coverslip were fixed overnight with 30 µL of a 4% paraformaldehyde solution. Fixed cells were
243 dehydrated by serial incubations in 50%, 80% and 100% ethanol during 5 min each. The hybridization
244 solution contained 10 ng/mL of the probe, 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 1 mM Na_2EDTA, and
245 0.01% sodium dodecyl sulphate (SDS), using the hybridization and washing stringencies
246 recommended for each probe (30% for CCPp). Hybridization was performed at 46 °C for 2 h. For
247 some samples, 10 µL of a solution of 1 µg/mL of 4,6-diamidino-2-phenyl- indole (DAPI) was added to
248 the coverslip, incubated 10 min at 4 °C and rinsed with water. Coverslips were stored at 4 °C in a
249 humidity chamber before observation.

250 Finally, hybridization assays were analysed using a Zeiss LSM780 confocal microscope
251 available on the ZooM platform at the Institute of Biosciences and Biotechnologies of Aix-Marseille.
252 Correlative fluorescence light and electron microscopy, combining the specificity of fluorescence
253 labelling with the high spatial resolution of scanning electron microscopy (SEM), was carried out with
254 a Zeiss LSM710 confocal laser scanning microscopy at the Institut de Minéralogie, de Physique des
255 Matériaux et de Cosmochimie in Paris. Correlation with SEM was performed using the KorrMik Life
256 Sciences sample holder and the correlative Shuttle and Find software implemented in ZEN as
257 previously described [46]. Images were collected in the backscattered and secondary electron modes
258 using a Zeiss Ultra 55 FEG-SEM operating at 10 kV, a working distance of 5 mm and an aperture of 30
259 µm.

260 ***Transmission electron microscopy (TEM)***

261 The ultrastructure of $CaCO_3$-producing MTB was observed by developing an alternative protocol to
262 fix cells on a TEM carbon-coated grid. Indeed, only few or no cell at all of $CaCO_3$-producing MTB were
263 fixed on TEM grids when using the standard protocol consisting simply of drying a droplet containing
264 the bacteria. Here, we pipetted 2 µL of liquid from the edge of the northern side of a hanging drop,
265 at the bottom of the drop, where magnetic cells aggregated and settled down due to their
266 magnetotactic behaviour and their density. Then, we slowly deposited these cells at the centre of the
267 TEM grid. The grid and the drop were kept in place during the fixation of the cells. Indeed, if the grid
268 was moved upside down as it is usually done for other MTB, cells with $CaCO_3$ granules did not adsorb
269 onto the carbon film of the TEM grid and instead settled down at the bottom of the drop likely
270 because of their high density. Once the drop started to dry, the grid was washed with filtered
271 deionized water. Although most of the $CaCO_3$-producing bacteria formed large aggregates of cells,
272 this technique facilitated their observation and enabled optimal adsorption of the MTB onto the
273 carbon film.

274 Electron micrographs were recorded with a Tecnai G^2 BioTWIN (FEI Company) equipped with
275 a CCD camera (Megaview III, Olympus Soft imaging Solutions GmbH) using an accelerating voltage of
276 100 kV. High-resolution transmission microscopy (HRTEM), z-contrast imaging in the high-angle
277 annular dark field (STEM-HAADF) mode, and elemental mapping by X-ray energy-dispersive
278 spectrometry (XEDS) were carried out using a JEOL 2100F microscope. This machine, operating at 200
279 kV, was equipped with a Schottky emission gun, an ultrahigh resolution pole piece, and an ultrathin
280 window JEOL XEDS detector. HRTEM images were obtained with a Gatan US4000 charge-coupled-
281 device (CCD) camera.

282 The sizes of magnetosomes and other mineral inclusions as well as the estimation of the area
283 occupied by these inclusions were measured from TEM images using the ImageJ software (1.48v).

284 ***Cryo-electron microscopy of vitreous sections (CEMOVIS)***

285 For CEMOVIS, a pellet of cells magnetically enriched from a Lake Pavin sediment sample was
286 harvested. The pellet was carefully mixed with an equal volume of 40% PBS. The suspension was
287 transferred in a gold coated cupper sample holder type A with a 200-µm diameter cavity and closed
288 with a flat sample holder type B (Wohlwend GmbH, Sennwald, Switzerland) before vitrification with a
289 HPM 100 (Leica microsystems, Vienna, Austria). Vitrified samples were placed in the cryo-chamber of
290 a Leica cryo-ultramicrotome at -150 °C. Ultrathin cryo-sections with a nominal feed of 40 nm were
291 obtained using a 35° cryo-diamond knife and were collected on quantifoil grids covered with a carbon
292 film. Grids were stored in liquid nitrogen before observations by cryo-TEM in the low dose mode with
293 a Tecnai G2 microscope equipped with a Gatan US 4000 CCD camera.

294 ***Shotgun genomic sequencing, assembly and functional annotation***

295 One single-cell of $CaCO_3$-producing MTB, named CCP1 (Calcium Carbonate Producing bacterium #1),
296 was sorted using a micromanipulator (as described above) from a sample collected on the shore of
297 Lake Pavin in May 2015. After WGA of this cell, gDNA was quantified using a QuBit Fluorimeter
298 (ThermoFisher Scientific) and quality was evaluated on a 1% TAE agarose gel.

299 The genome of CCP1 was sequenced on an Illumina MiSeq platform with a MiSeq® Reagent
300 Kit v2 (300 cycles, Illumina Inc.) using a Nextera® XT kit for the library preparation. A total of 1.9×10^6
301 paired-end reads was obtained, trimmed and filtered using Trimmomatic [47] and the default
302 settings. A draft assembly of 2.5 Mbp was assembled using SPAdes v.3.12.0 (with -k
303 21,33,55,77,99,127 —only-assembler —sc options) into 3365 contigs longer than 250 bp and
304 annotated using the fast method implemented in Prokka 1.14.0 [48]. The quality of genome assembly
305 was assessed using CheckM v1.1.2 [49].

306 ***Scanning transmission X-ray microscopy (STXM)***

307 STXM analyses were performed on the HERMES beamline at the SOLEIL synchrotron (St. Aubin,
308 France) [50, 51]. Details on the STXM technique and its application to biomineralization can be found
309 in *e.g*. [52, 53]. This microscopy uses X-rays in the soft X-ray energy domain to provide images with a
310 ~25 nm spatial resolution and spatially-resolved speciation information of light elements such as C
311 based on X-ray absorption near edge structure (XANES) spectroscopy. For this purpose,

312 monochromated X-rays are focused to a ~25 nm spot by a Fresnel zone plate. An image is obtained
313 by positioning the sample at the focal point of the lens and raster scanning it in x and y, while
314 recording the intensity of the transmitted X-rays. After localizing cells of interest by optical
315 microscopy beforehand, we could easily localize them by STXM. A stack of STXM images of the cells
316 at a sequence of photon energies was acquired at the C K-edge. The step size was 75 nm and images
317 were obtained at 140 energy steps varying between 270 and 345 eV with an increment of 0.12 eV in
318 the 282-291.5 eV energy range, where most of the narrowest XANES peaks were present. The
319 imaged area also contained a sub-area free of cells to measure the incident flux (I0). This allowed to
320 convert images from transmitted intensity units to optical density units (OD). The XANES spectra at
321 the C K-edge of the $CaCO_3$ inclusions and the cells were extracted and mapped using the aXis 2000
322 software (McMaster University, http://unicorn.mcmaster.ca/axis/aXis2000-IDLVM.html).

323

324

Results

Observation of bacteria with electron dense granules and an unusual magnetotactic behaviour

Over the different sampling campaigns performed since 2015 on Lake Pavin, a large diversity of MTB morphotypes, mostly represented by cocci, was observed close to the oxic-anoxic boundary of the water column (45-60 m depth) [46]. MTB were also very diverse in the sediments collected from the shore down to a water depth of 40 m [54]. In most collected samples, one MTB morphotype escaped our first light and electron microscopy observations because of its atypical magnetotactic behaviour. Indeed, some slightly-curved rod-shaped MTB with large inclusions were only weakly magnetic, *i.e.* their rotation was slow compared to other MTB when the applied magnetic field was switched 180° to the initial direction. They did not, for the majority, accumulate at the edge of the hanging drop. Instead, they aligned along the magnetic field and slowly swam back and forth at the bottom of the drop without reaching the edge of the drop where light microscopy observations of MTB usually focus (Fig. 1a and Supplementary Video S1). Overall, these particular MTB cells were more efficiently concentrated by increasing the duration of the magnetic enrichment from 30 min to 3 h and applying a stronger magnetic field (neodymium, iron and boron magnet of 400 mT). As most of the other members of the MTB community, these bacteria were located in oxygen-depleted areas, *i.e*. below the oxycline [46]. The cell density of this new MTB morphotype in the water column was generally higher in autumn and its maximum was observed during the October 2018 campaign at a depth of 58 m, reaching 4.0×10^2 cells/mL (Fig. 1b). However, this cell density was probably underestimated since the used hanging drop technique likely biased the counting of such MTB downward. This new MTB morphotype was always observed in the hanging drop assay without previous magnetic enrichment in autumn. During the other seasons (spring and summer), they were observed after magnetic enrichment only (*i.e*. abundance <25 cells/mL). In sediment samples, these bacteria generally represented the dominant magnetotactic population and reached up to 5.8×10^5 cells/mL of porewater. In the sediments, the majority of the cells were detected below the oxic-anoxic interface as in the water column (Fig. 1c). After magnetic enrichment of sediment samples, aggregated cells formed a thin irregular whitish pellet measuring up to 4 cm in diameter and located against the magnet. The pellet was wider below the magnet, suggesting that the population was sensitive to gravity (Fig. 1d). In most samples collected on the shore, MTB pellets aggregated after 3 h against magnets were of similar size whenever the north or the south poles of the magnets were directed toward the sampling bottle. This suggests that there was an equal proportion of north- and south-seeking cells.

357 Bacteria with this atypical magnetotactic behaviour contained 2 to 4 characteristic large
358 refractive inclusions measuring 856 ± 95 nm in width (n = 147, Fig. 2). They also showed poor
359 adsorption onto the carbon film covering TEM grids and a specific protocol had to be developed for
360 their fixation on the grids (see Materials and Methods). Most of the cells formed large aggregates on
361 the TEM grids. Analysis of the cell ultrastructure revealed the presence of a single polar flagellum
362 (Fig. 2a). Inclusions were very dense to the electron beam and occupied the majority of the
363 cytoplasm, which significantly complicated the observation of the magnetosomes using conventional
364 TEM bright field mode (Fig. 2b and c). Magnetosomes overlapping with the inclusions were only
365 observed when using the HAADF mode in STEM (Fig. 2d and e). Some inclusions were observed to
366 form at the septum of dividing cells (Fig. 2f and Fig. S3). Shortly after cell division, each daughter cell
367 contained two inclusions of different sizes: a large one located at the flagellum pole and a small one
368 at the other pole (Fig. 2a, d- e and Fig. S3). When observed under the light microscope with an
369 applied magnetic field, recently divided cells always showed their smallest inclusion in the front and
370 their largest one in the back, at the flagellum pole (Fig. 2g). Based on the observation of 186 STEM-
371 HAADF images of cells from a water column sample, two scenarios linking cell division and the
372 formation of the inclusions could be proposed (Supplementary Information on cell division).
373 However, we cannot exclude the possibility that different species or sub-species of calcium
374 carbonate-producing MTB have different number and/or organization of inclusions.

375 **Chemical analyses indicate the presence of both calcium carbonate and magnetite inclusions**
376 **forming in environmental conditions undersaturated with Ca-carbonate phases**

377 The majority of cells, from sediment samples, producing the large electron-dense inclusions
378 contained a single chain of 14 ± 4 (n = 35) prismatic magnetite particles measuring 58 ± 4 nm in
379 length and 40 ± 3 nm in width (n = 223) (Fig. 3a and c). Similar cells were also observed with
380 cuboctahedral magnetite particles in the sediments (Fig. 3d and f). Moreover, cells producing such
381 large inclusions in the water column were always observed with cuboctahedral particles. TEM image
382 analysis revealed that these large electron-dense inclusions represent 73 ± 9% (n = 134) of the cell
383 area. Usually, extrapolation from 2D TEM images to 3D volumes is not straightforward. Here, we
384 assumed that both cell and electron-dense inclusions exhibited an ellipsoidal morphology, which is
385 fairly supported by the numerous images of randomly oriented cells. Based on this assumption, the
386 volume of the large electron-dense inclusions could be roughly estimated to 65% of the total cell
387 volume. X-ray energy-dispersive spectrometry (XEDS) and elemental mapping in the STEM-XEDS
388 mode indicated that the large inclusions contained mostly calcium, carbon and oxygen (Fig. 3b and e,
389 Fig. S3 and Supplementary Information), with weak peaks assigned to strontium and barium (Fig. 4).

390 Phosphorus-rich granules were also observed in some cells (Figs. 3b and 4b). Selected-area electron
391 diffraction patterns obtained on these inclusions showed that they were amorphous (Fig. S4).

392 STXM analyses were carried out on MTB cells collected in the water column and the
393 sediments to further characterize these amorphous Ca-, O- and C-containing inclusions. XANES
394 spectra at the C K-edge extracted from the cells showed peaks at 285.2, 286.6, 288.2 and 290.3 eV
395 which were interpreted as carbon 1s→π* electron transitions in aromatic, ketonic/phenolic, amide
396 carbonyl and carbonate functional groups, respectively [55]. Spectra measured on large inclusions
397 were very similar to those of reference amorphous calcium carbonate (ACC) particles and showed a
398 pronounced 1s→π* carbonate-C peak at 290.3 eV together with broader peaks at 296.2 and 301.6
399 eV that were attributed to a 1s→σ* electron transitions in carbonate C. Overall, this allowed to
400 unambiguously determine that inclusions in the MTB were composed of ACC [55] (Fig. 5).

401 In parallel, we measured the alkalinity (approximated to [HCO_3^-]), pH, and [Ca^{2+}] in 10
402 sampling bottles of onshore sediments to better understand the environmental conditions that led to
403 the formation of such inclusions. The pH of the pore water was between 6.88 and 7.64 ± 0.01, the
404 [Ca^{2+}] between 83.9 ± 0.7 and 634 ± 3.7 μM and the alkalinity between 725 and 3824 μM. Overall,
405 the ion activity product (IAP), (Ca^{2+}) × (CO_3^{2-}), varied between 3.32×10^{-11} and 1.23×10^{-9} and was
406 therefore systematically lower than the solubility products at 25°C of ACC (5.27×10^{-7}, [34]) and
407 calcite (3.31×10^{-9}). As a result, we could conclude that all solutions in contact with the sediments
408 and MTB were undersaturated with ACC, at least at the bulk scale. Available water chemistry data
409 indicate that ACC-producing MTB present in the water column are also in environmental conditions
410 undersaturated with ACC [56].

411 **ACC-producing MTB represent a new genus within the Alphaproteobacteria and harbour an**
412 **atypical magnetosome gene cluster**

413 The identity of ACC-producing MTB isolated from the Lake Pavin sediments was investigated by
414 sequencing the 16S rRNA genes amplified from magnetically purified populations and sorted single-
415 cells (Fig. S1). We obtained 18 sequences (between 1450 and 1492 bp) composed of 5 operational
416 taxonomic units (OTUs) defined at a 99% identity level. These 5 OTUs shared 92.8-98.3% identity
417 indicating that this morphotype of ACC-producing MTB was composed of several species, likely
418 belonging to at least two genera based on a 95% sequence identity threshold [57]. Based on BLASTN
419 hits, the closest cultured species belonged to the *Azospirillum* and *Skermanella* genera of the
420 Rhodospirillaceae family of the Alphaproteobacteria class (following the current NCBI taxonomy).
421 However, sequence identities with these genera were low (between 91.7 and 93.8%) suggesting that

422 ACC-producing MTB belonged to at least two genomic undescribed genera. As a result, based on the
423 phylogeny inferred from the 16S rRNA gene sequences, these biomineralizing MTB formed a distinct
424 new monophyletic clade within Rhodospirillaceae, related to a group composed of the *Niveispirillum*,
425 *Rhodocista*, *Nitrospirillum*, *Azospirillum*, *Desertibacter* and *Skermanella* genera (Fig. 6a). However,
426 the tree topology was not well statistically supported and could not entirely resolve the evolutionary
427 relationships between Lake Pavin MTB and the closest Rhodospirillaceae genera. Indeed, the 16S
428 rRNA sequences harboured too much interspecific polymorphism with important genetic saturation
429 effects suggesting that the current Rhodospirillaceae family may actually gather very divergent
430 organisms.

431 Fluorescent *in situ* hybridization (FISH) using specific fluorescently-labelled oligonucleotide
432 probes and combining confocal microscopy with scanning electron microscopy observations further
433 validated the ACC-producing MTB identity (Fig. 7). As expected from similar observations on ACC in
434 cyanobacteria [22], the MTB ACC inclusions dissolved during the preparation of the FISH samples
435 because of the detrimental impact of fixation followed by incubation in buffers undersaturated with
436 ACC (Fig. 7e). A similar loss of ACC inclusions was observed in these bacteria when preparing
437 ultrathin TEM sections following conventional ultramicrotomy protocols (Fig. S5a-c). In contrast, ACC
438 inclusions were preserved by cryo-electron microscopy of vitreous sections (CEMOVIS) and showed
439 the existence of a lipid bilayer-delimited intracellular compartment enclosing ACC (Fig. S5d-f).

440 The genome of the representative ACC-producing MTB clone CCP-1 was tentatively
441 assembled. Single-cell sorting coupled with whole-genome amplification and sequencing allowed to
442 assemble a partial genome sequence of 2.5 Mb, in which a single 16S rRNA gene copy identical to the
443 OTU1 sequence was detected. Completeness was estimated to be lower than 22%, which was not
444 satisfactory enough to get a reliable reconstruction based on ribosomal proteins or to reconstruct full
445 metabolic pathways. This result indicates that ACC-producing MTB may have an atypical large
446 genome over 10 Mbp, compared to its closest relatives whose genomes size range between 5 and 6
447 Mb in average. Despite this low genome coverage, a full magnetosome gene cluster was retrieved
448 from the longest contig of 72.5 kbp using the BLASTP algorithm and the bank of *mam* sequences
449 publicly available for all Alphaproteobacteria (RefSeq non-redundant proteins database). Its
450 sequence was deposited in Genbank under the accession number MT411893. Indeed, up to 30 genes
451 were unambiguously identified with a high sequence homology degree with known magnetosome
452 genes (See Table S1 and Fig. S6). Comparative analysis of the magnetosome gene cluster (MGC) from
453 representative magnetotactic Rhodospirillaceae showed the presence of the main operons described
454 in *Magnetospirillum* species but with an atypical organization compared to both marine and

455 freshwater strains (Fig. 6b). For example, the *mamAB* operon was complete with the exception of
456 *mamJ*, which is a gene involved in ultrastructural organisation of the magnetosome chain specific to
457 the *Magnetospirillum* genus. However, gene synteny was not conserved as in the *mamXZ* operon in
458 which no *mamY* was detected while the canonical form of the *mamGFDC* operon is not present.

459

460

461 # Discussion

462 The ability to mineralize calcium carbonates has been described in very few prokaryotic taxa so far
463 and despite the tremendous importance that such a function may have had in the Earth's history,
464 very little is known about its distribution in the tree of Bacteria, its genetic basis and its ecological
465 role. This gap may be partially reduced by describing new organisms producing calcium carbonates in
466 very divergent phyla with different ecological niches. Here, we not only described a bacterium able
467 to perform two different types of biomineralization, but we also extended the record of
468 microorganisms able to perform intracellular Ca-carbonate biomineralization from two to three far-
469 related groups of Bacteria: Gammaproteobacteria, Cyanobacteria and Alphaproteobacteria. The MTB
470 performing both magnetic iron oxide and calcium carbonate biomineralization in the ferruginous
471 Lake Pavin undoubtedly form a monophyletic group composed at least of one or two genera that are
472 distinct from those currently described within the Alphaproteobacteria. The majority of the
473 phylogenetically closest genera, *i.e. Niveispirillum*, *Rhodocista*, *Nitrospirillum*, *Azospirillum* and
474 *Skermanella,* includes species isolated from the rhizosphere or freshwater environments. None of the
475 species from these genera has been reported to biomineralize ACC [58–62]. Most of these species
476 are chemoorganoheterotrophic and aerobic, but some can also be anaerobically phototrophic.
477 Future culture assays and genome sequencing will determine if ACC-accumulating MTB have similar
478 metabolisms and will help resolving the ancestry of both biomineralization processes in
479 Alphaproteobacteria.

480 MTB have often been used to illustrate how the cell functioning and compartmentalization
481 can be complex in prokaryotes through the description of numerous morphotypes with diverse cell
482 ultrastructures and physiologies in very chemically contrasted environments [63, 64]. Besides
483 magnetosomes, other large inclusion bodies storing elemental sulphur, polyphosphates, or
484 polyhydroxybutyrates (PHB) have been regularly observed in MTB [46, 65–71]. Here, we extend the
485 range of inclusions that MTB may form and give insights into the ultrastructure, chemistry and
486 formation of ACC inclusions during the cell cycle. TEM analyses showed that the inclusions were
487 amorphous and CEMOVIS confirmed the presence of a surrounding membrane. Interestingly, XEDS
488 analyses showed the presence of Sr and Ba in the ACC granules. The accumulation of the alkaline
489 earth elements such as Ca, Sr and Ba at a high level has been demonstrated for the ACC-
490 biomineralizing cyanobacterium *Gloeomargarita lithophora* [74]. For this reason, it has been
491 suggested that these prokaryotes could be used to remediate pollutions by alkaline earth elements
492 [26]. If such high accumulation abilities were proven for these ACC-producing MTB, they may
493 similarly be suitable for designing new remediation strategies or other applications under

494 physicochemical conditions different from those allowing the use of cyanobacteria [75]. In the light
495 of our data, it is still unclear how ACC-containing vesicles form and how cytosolic conditions initiate
496 the formation of ACC. However, similarly to what has been shown for three strains of cyanobacteria
497 [76] and for *Achromatium* [12], we showed that Alphaproteobacteria MTB also form Ca-carbonates
498 intracellularly in solutions undersaturated with respect to ACC phases. In other words, these
499 extracellular conditions are thermodynamically unfavourable to mineral precipitation, which
500 indicates that ACC formation in MTB is an active process and costs energy to the cell to maintain an
501 intracellular environment supersaturated with ACC, most likely within the membrane-enclosed
502 compartment observed by CEMOVIS.

503 TEM observations provided additional information on the biogenesis of these inclusions.
504 During cell division, each daughter cell systematically harboured one significantly smaller ACC
505 inclusion in the vicinity of the septum, that remained at one cell pole once cells detached.
506 Interestingly, a similar connection between ACC biomineralization and cell division was observed in
507 cyanobacteria species belonging to the *Thermosynechococcus elongatus* BP-1 clade [23]. These
508 observations raise questions about the formation, inheritance and maintenance of the polarity of the
509 cells, that of the the magnetosome chain and ACC inclusions over generations. Indeed, it is known
510 that magnetosomes formation is a complex process where chains of magnetosome particles are
511 concatenated, positioned and segregated by a multi-partite cytoskeletal network, called the
512 magnetoskeleton [77, 78]. The atypical MGC found in the genome of ACC-producing MTB could be an
513 indication of a specific adaptation of these bacteria to produce straight magnetosome chains in such
514 a crowded cytoplasm. Monotrichously flagellated MTB developed specific dividing strategies or
515 sensory apparatus to keep a sustainable magnetotaxis and produce two daughter cells with a similar
516 magnetic polarity, *i.e.* swimming in the same direction along magnetic field lines [79]. Here, in ACC-
517 producing Alphaproteobacteria, the flagellum was always observed at the oldest pole where the
518 largest and likely oldest ACC inclusion stands. This suggests that similarly to *Magnetovibrio*
519 *blakemorei* strain MV-1, they divide according to a scheme whereby the newly synthesized flagellum
520 emerges from the mother pole initially devoid of a flagellum [79]. This represents a most common
521 dividing scheme similar to that observed in *Caulobacter crescentus* [80]. In strain MV-1, all daughter
522 cells swim in the same direction (north-seeking), so it was suggested that the flagellum pushes the
523 cell body for one half of the population while pulling it for the other half of the population. In
524 contrast, magnetic enrichments of ACC-producing MTB populations collected from the shore of Lake
525 Pavin revealed a similar fraction of south-seeking and north-seeking cells. This observation suggests
526 that for these MTB, two daughter cells swim in opposite direction along magnetic field lines: the cell
527 inheriting the ancient flagellum will keep swimming in the same direction than the mother cell, while

528 the cell inheriting the newly synthetized flagellum will swim toward the opposite direction. This
529 observation is of ecological significance since it means that these population of MTB use the
530 magnetic field only as an axis of motility. Their chemotactic machinery is then responsible for their
531 stabilization in their optimal habitat [81].

532 Magnetotaxis is not the only strategy developed by microorganisms to facilitate their vertical
533 motility in the water column. For instance, some microorganisms living in anoxic environments
534 developed a negative phototactic response to the shorter wavelengths of visible light that penetrate
535 the deepest in the water column [82]. This behaviour, allowed by specific photoreceptors, drives
536 these microorganisms toward and into the anaerobic zone of aquatic habitats where they are found
537 at their highest abundance. Similarly, gas vesicles formed by some organisms are used as positive or
538 negative buoyant to perform vertical migrations in the water column [83]. For some cyanobacteria,
539 gas vesicles increase their residence time in the euphotic zone and are responsible for surface water-
540 blooms [84]. May ACC inclusions contribute to the vertical motility of these MTB in the water
541 column? The role of Ca-carbonate inclusions in the vertical motility of bacteria has been postulated
542 for *Achromatium* as well as cyanobacteria [13, 17, 19, 29]. Interestingly, the ACC-producing MTB
543 described here had a magnetotactic behaviour different from that usually observed for
544 environmental magnetically-responsive microorganisms. Indeed, they did not aggregate at the edge
545 of hanging drops after their magnetic enrichment from aquatic samples. Instead, cells settled down
546 at the bottom of the drops, swimming along the magnetic field lines applied and displaying a back
547 and reverse motility. Similarly, when deposited between a microscope slide and a coverslip, cells
548 were systematically observed swimming in the deepest part of the preparation. We showed that ACC
549 inclusions could occupy up to two-thirds of the cytoplasm, representing a considerable increase of
550 cell negative buoyancy. This indicates the potential of ACC to serve as ballasts and could represent an
551 adaptation to a benthic lifestyle, cells adjusting their vertical positioning using their flagellum. It is
552 possible that this process functions in coordination with magnetotaxis where the axis of motility and
553 orientation of the cell would be directed by the magnetosomes chain while the direction of motility
554 would be given by gravitaxis associated with ACC inclusions.

555 This unique behaviour, the potential ACC loss upon standard TEM sample preparation and
556 the difficulty to observe their magnetosomes due to the electron dense ACC inclusions filling the
557 cytoplasm, could explain why these microorganisms have been overlooked so far. In some samples,
558 these bacteria could reach a cell density above 10^5 cells/mL but yet remained invisible when standard
559 protocols were used to quantify MTB [63]. Recently, it was shown that the adaptation of new
560 protocols for the sampling, magnetic enrichment and observation allowed the discovery of new

561 magnetically-responsive microorganisms [85–87]. It is thus important to permanently reassess how
562 to observe magnetotactic organisms to unravel their whole diversity. Here, the new ACC-
563 accumulating microorganisms were discovered owing to the observation of an unusual
564 magnetotactic behaviour that gives the opportunity to concentrate rare organisms from complex
565 communities. This double biomineralization may be more widespread than we currently think.
566 Indeed, some Ba- and Ca-rich inclusions were observed in environmental MTB collected in the Seine
567 River (France) [88] and another study reported the presence of Ca-rich granules occupying most of
568 the intracellular volume of the giant freshwater magnetotactic rod-shaped strain GRS-1, affiliated
569 with the Gammaproteobacteria [89], although none of these studies confirmed or infirmed that
570 these inclusions are made of amorphous or crystalline carbonates. Presuming that the above-
571 mentioned inclusions were amorphous carbonate phases, all these observations suggest the
572 phylogenetic diversity of ACC-producing MTB may be even higher than what is reported here.
573 Indeed, although strain GRS-1 and *Achromatium* belong to the same order , *i.e.* Thiotrichales, both
574 strains remain phylogenetically distant [89]. Besides finding the origin of such processes, future
575 comparative genomics and molecular phylogenies will infer the ancestry and evolutionary forces
576 associated to their diversification.

577

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Conflicts of interest

The authors declare no conflict of interest

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824

825 # Figure Legends

826 **Figure 1. Observation of the refractive magnetotactic bacterium from Lake Pavin. a** Light
827 microscope images of north-seeking magnetotactic bacteria (MTB) sampled in the sediment of Lake
828 Pavin. The microscope was focused at the edge of the water drop closest to the south pole of the bar
829 magnet, producing a local field direction indicated by the black arrow (left panel). The microscope
830 was focused at 200 µm (middle panel) and 400 µm (right panel) from the edge of the water drop
831 showing the presence of MTB cells (seen as grey dots in the images) aligned with the magnetic field
832 but not aggregated at the edge of the hanging drop. **b** Vertical concentration profiles of oxygen (grey
833 dotted line), redox potential (black dotted line) and rods with large refractive inclusions (histogram)
834 through the water column of samples collected in Lake Pavin in October 17th, 2018. Note that the
835 measurements extend through the oxic-anoxic interface and the deeper regions of the anaerobic
836 zone of the water column. Cell counts are reported as the mean of triplicate measurements and line
837 extensions represent the positive standard deviation of the total number of MTB. Rods with
838 refractive inclusions were also observed above 52 m and below 60 m but at densities < 25 cells/ml
839 and thus escaped our counting assay. **c** Vertical concentration profiles of O_2 (grey dotted line) and
840 cell density of rods with large refractive inclusions (histogram) in a sediment sample collected from
841 the shore of Lake Pavin. Cell counts are reported as the mean of triplicate measurements and line
842 extensions represent the positive standard deviation. **d** Image of a one-litre sample collected from
843 the shore of Lake Pavin. The bottle was filled to about 1/3 of its volume with sediment (Sdt) and the
844 remainder of the bottle is filled to its capacity with water (Wtr) that overlaid the sediment. A large
845 whitish pellet (black arrows) of magnetotactic cells clustered against the magnet (Mgt) 2 h after
846 magnetic enrichment. Note the presence of black particles, in the centre of the pellet, composed of
847 magnetic sediments.

848 **Figure 2. Microscopy images of magnetotactic bacteria with electron dense inclusions isolated**
849 **from Lake Pavin sediment samples. a** Transmission electron microscopy (TEM) image of a cell
850 stained with 1% uranyl acetate, showing the presence of one single polar flagellum. **b-c** Bright field
851 TEM images of cells showing the electron dense granules that make the observation of the
852 magnetosomes difficult. **d-f** Scanning TEM-high-angle annular dark field (HAADF) images showing the
853 magnetosome chain partially overlapping the electron dense inclusions in the cytoplasm of the cells.
854 Panel **f** also shows the formation of small inclusion at the septum of a dividing cell. **g** Light
855 microscopy image of cells magnetically orientated in the same direction (north direction pointed by
856 the black arrow) showing that the smaller inclusions are always at the front of the cells. Scale bars
857 represent 1 µm (a-f) and 2 µm (g).

858 **Figure 3. Chemical identification of the mineral phase in the magnetosomes and the large**
859 **inclusions of bacteria isolated from sediment samples. a** STEM-HAADF image of two cells producing
860 four (left cell) and three (right cell) large inclusions (indicated by white arrows). **b** Composite XEDS
861 elemental mapping of calcium, iron and phosphorus. **c** High-resolution TEM image of a prismatic
862 magnetosome (shown by a red arrow in panel a) elongated along the [111] axis. **d** STEM-HAADF
863 image of three cells producing large and small inclusions). **e** composite XEDS elemental mapping of
864 calcium and iron. **f** HR-TEM image of a cuboctahedral magnetosome (shown by a red arrow in panel
865 d), an ideal model of a cuboctahedron with small {110} faces is superimposed. Detailed information
866 on the chemical and shape identification of the mineral phases present in these bacteria is given in
867 Supplementary information.

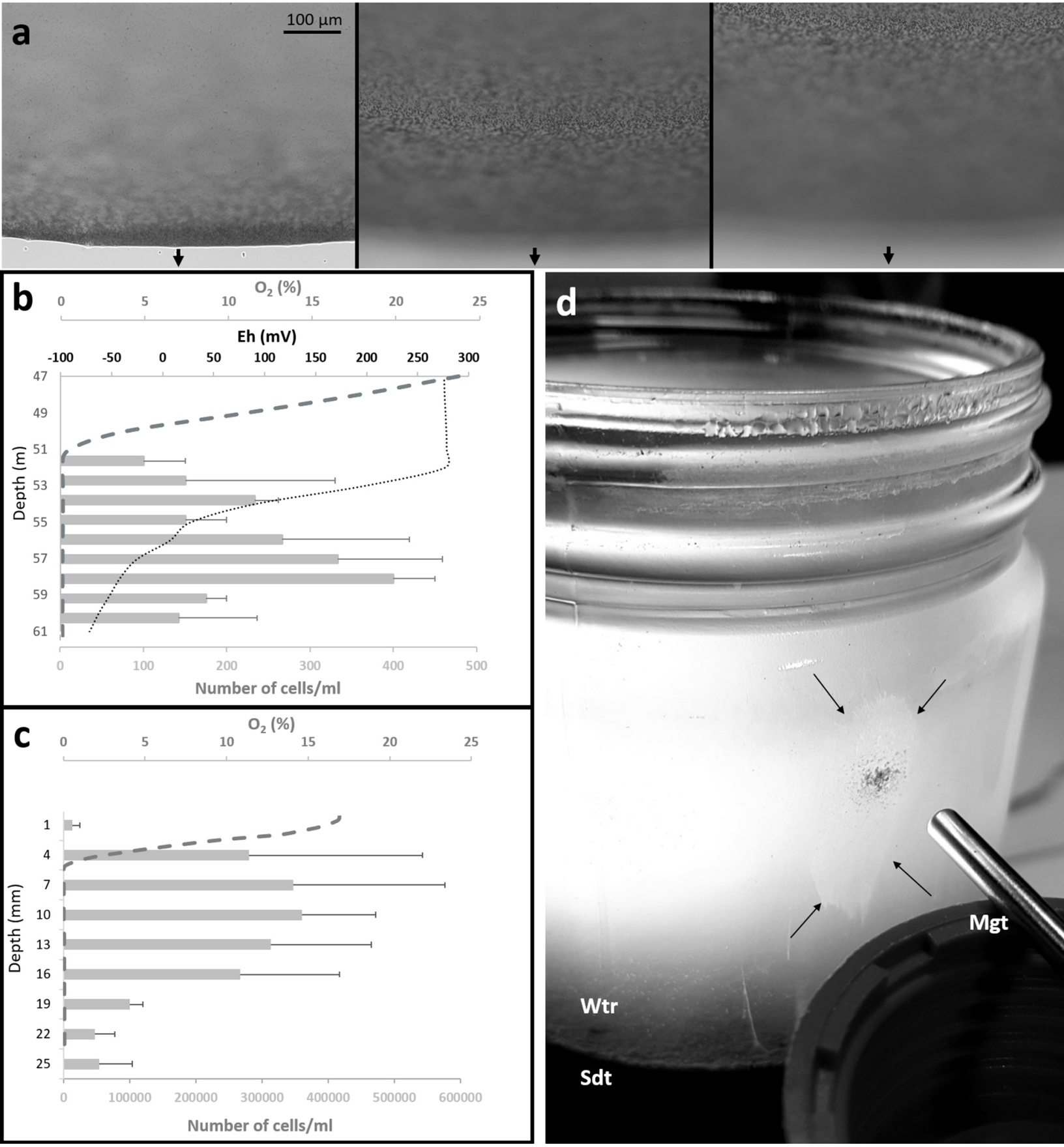
868 **Figure 4. Evidence of strontium and barium in the calcium-rich inclusions. a** STEM-HAADF image of
869 cells with calcium-rich inclusions, isolated from the sediment of Lake Pavin. **b** Composite XEDS
870 elemental mapping of calcium, iron and phosphorus. **c** XEDS elemental spectrum of an inclusion
871 showing the presence of calcium as well as traces of strontium and barium. Note that the coordinate
872 axis is in logarithmic scale.

873 **Figure 5. STXM analyses measured at the C K-edge on intracellular inclusions of magnetotactic**
874 **bacteria isolated from Lake Pavin. a** Bacterial cell isolated from the water column. From left to right:
875 STXM image at 320 eV; Map of two spectroscopically different compounds derived from the analysis
876 of a hyperspectral image: an ACC-like compound (red) and an organic, cell-like compound (blue);
877 XANES spectrum at the C K-edge of the ACC-like compound and of a reference synthetic ACC sample.
878 Y-axis shows optical density (OD) values. Peaks 1, 2 and 3 seen in both reference and MTB inclusions
879 are located at 290.3, 296.2 and 301.6 eV. **b** Bacterial cell isolated from the sediment. From left to
880 right: same as in panel a.

881 **Figure 6**. **Phylogenetic and magnetosome gene cluster (MGC) analyses of ACC-producing MTB**
882 **isolated from sediment samples of Lake Pavin. a** Maximum-likelihood tree of the Rhodospirillaceae
883 family (NCBI taxonomy) based on the 16S rRNA gene and rooted with representative members of the
884 Acetobacteraceae (grey group). Branch length represents the number of substitutions per site.
885 Bootstrap values were estimated with a non-parametric bootstrap approach and 500 replicates.
886 Representative MTB species and ACC producing MTB are in bold. The OTU to which the clone CCP-1
887 was affiliated is in dark blue. **b** MGC organization of the ACC-producing MTB clone CCP-1
888 representing OTU1. All genes homologous to any known magnetosome gene is coloured in dark
889 blue. Any gene with unknown function conserved in the vicinity of other MGCs is highlighted in light
890 blue. The map of the contig was drawn to scale and its sequences can be retrieved under the

891 Genbank accession number MT411893. Main operons organisation described in *Magnetospirillum*
892 species [5] are symbolized with arrows above the map. **c** Comparative analysis of the MGCs from the
893 ACC producing clone CCP-1 and different representative magnetotactic Rhodospirillaceae strains
894 previously reviewed in detail in Monteil *et al*. [90] (AMB-1: *Magnetospirillum magneticum*, MSR-1:
895 *Magnetospirillum gryphiswaldense*, LM-1: *Ca*. Magneticavibrio boulderlitore, MV-1: *Magnetovibrio*
896 *blakemorei*, PR-1: *Ca*. Terasakiella magnetica, QH-2: *Ca*. Magnetospira sp.). MGCs were organized
897 according to the phylogeny based on MTB 16S rRNA gene sequences, rooted with the sequence of
898 *Magnetococcus marinus* strain MC-1 (NR_074371.1). The arrows symbolize operons described by
899 Uebe and Schüler [5]; they were drawn to scale and the direction symbolizes the relative position of
900 genes within the operons in respect to that described in species of the *Magnetospirillum* genus.
901 Dotted lines symbolize continuous genomic regions and were not drawn to scale, while slashes
902 represent contigs edges.

903 **Figure 7 Fluorescence in situ hybridization (FISH) of magnetically concentrated MTB from the**
904 **sediments of Lake Pavin.** The specific oligonucleotide rRNA probe (CCPp) was used to authenticate
905 the 16S rRNA gene sequence of MTB with $CaCO_3$ inclusions. **a** Fluorescence microscope image of
906 three rods with large inclusions and four smaller vibrio (shown by white arrows) stained with 4',6-
907 diamidino-2-phenylindole (DAPI). **b** Fluorescence microscope image of the same cells hybridized with
908 the Bacteria specific probe Eubp. Note that both cell morphotypes fluoresce with this probe. **c**
909 Fluorescence microscope image of the same cells hybridized with the CCP-specific probe. Only cells
910 with large inclusions fluoresce with this probe. **d** Scanning electron microscope (SEM) image of the
911 same cells showing their ultrastructure and magnetosomes. White frame indicates the position of
912 the SEM image in panel **e**. Scale bar represents 2 µm.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O2 (%)
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Depth (m)
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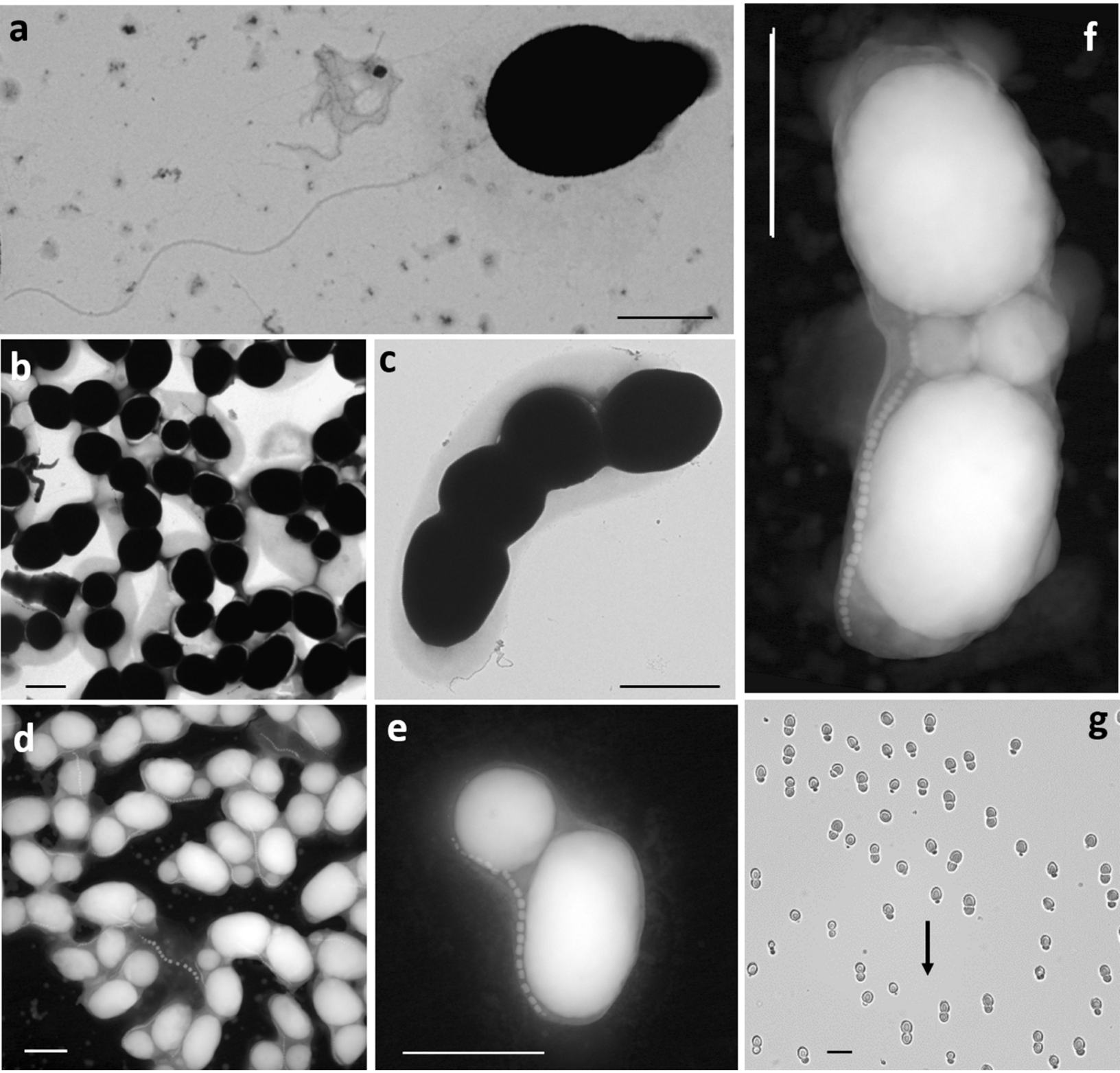
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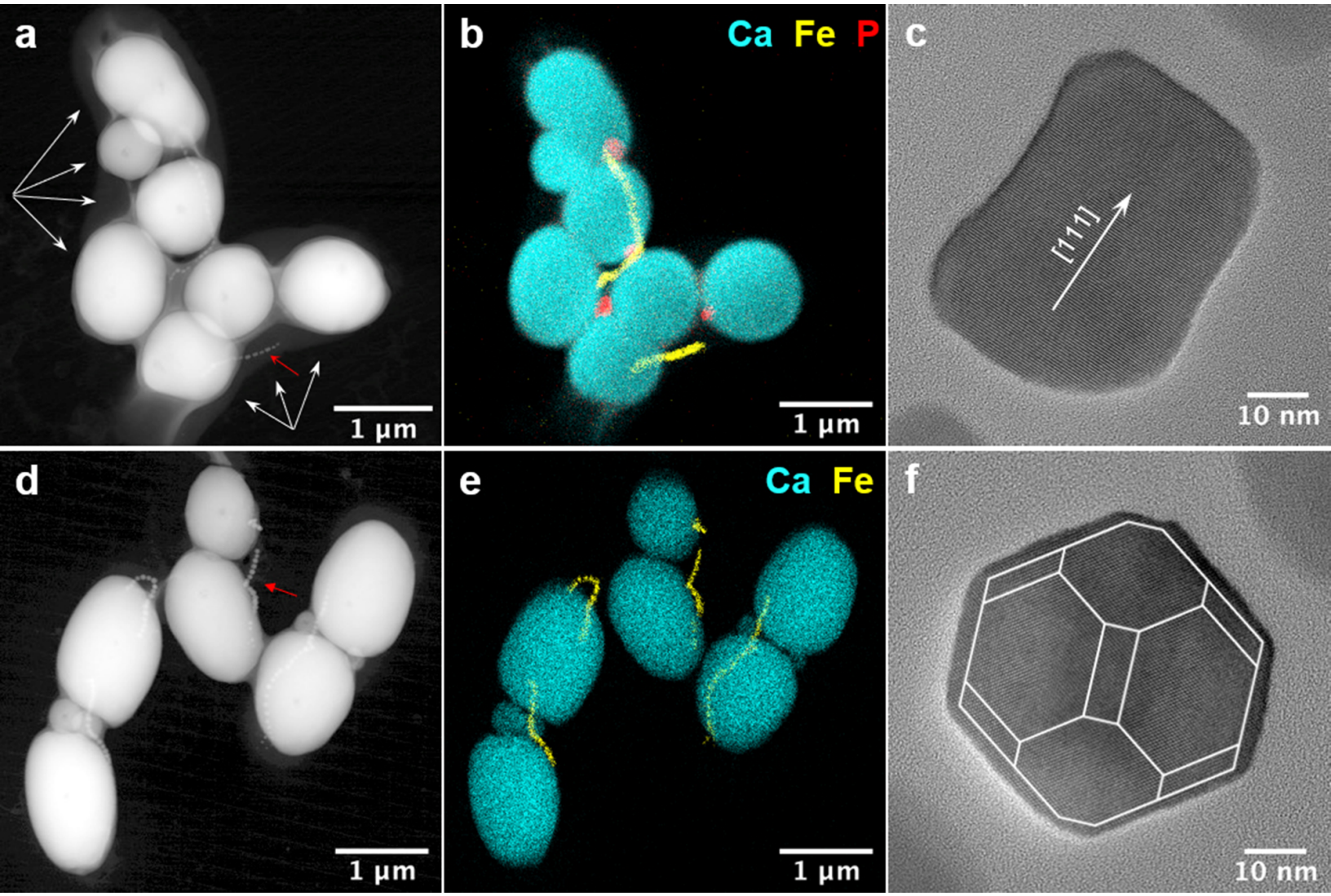
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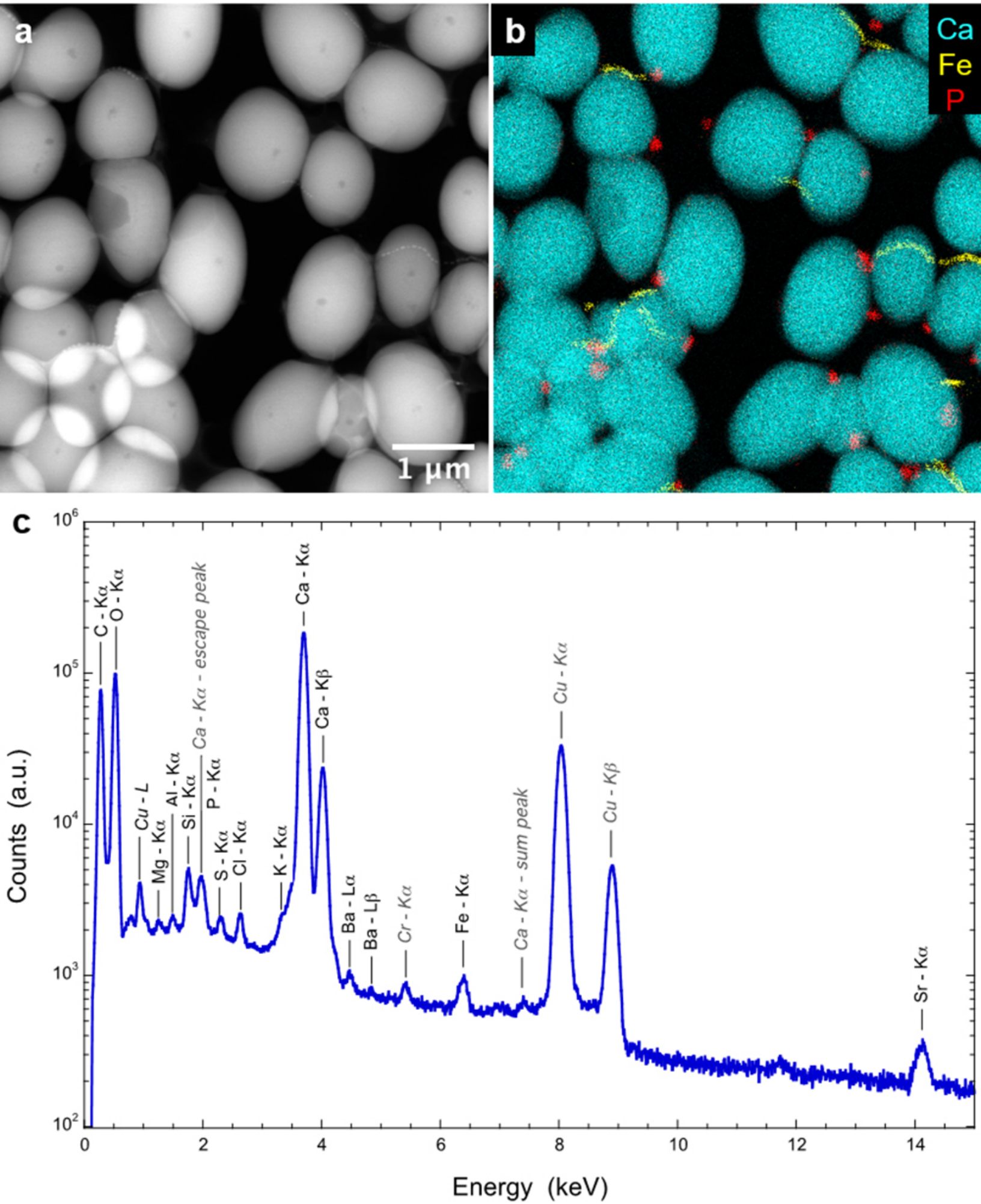
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Cu - L
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P - Kα
S - Kα
Cl - Kα
K - Kα
Ca - Kα
Ca - Kβ
Ba - Lα
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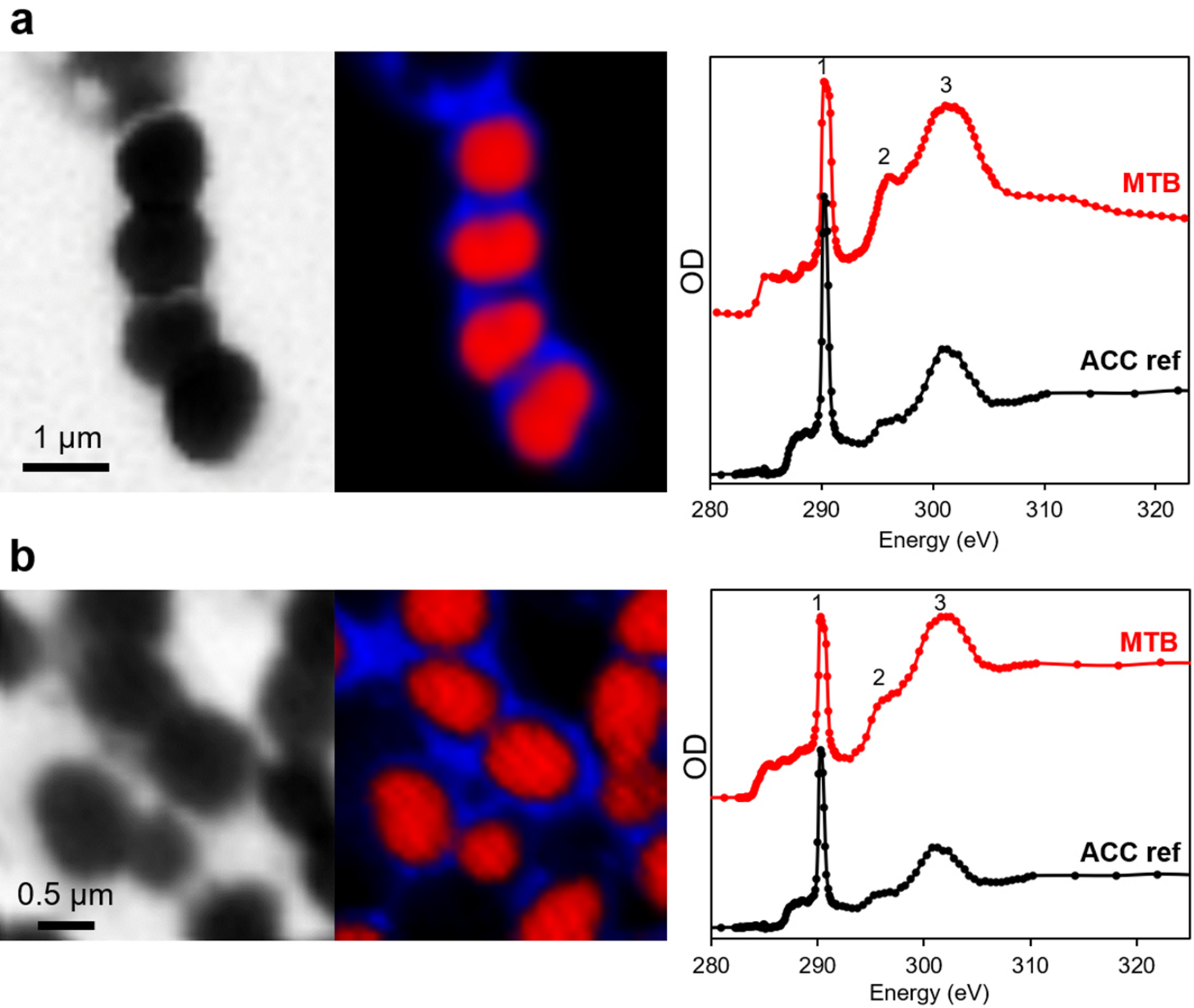
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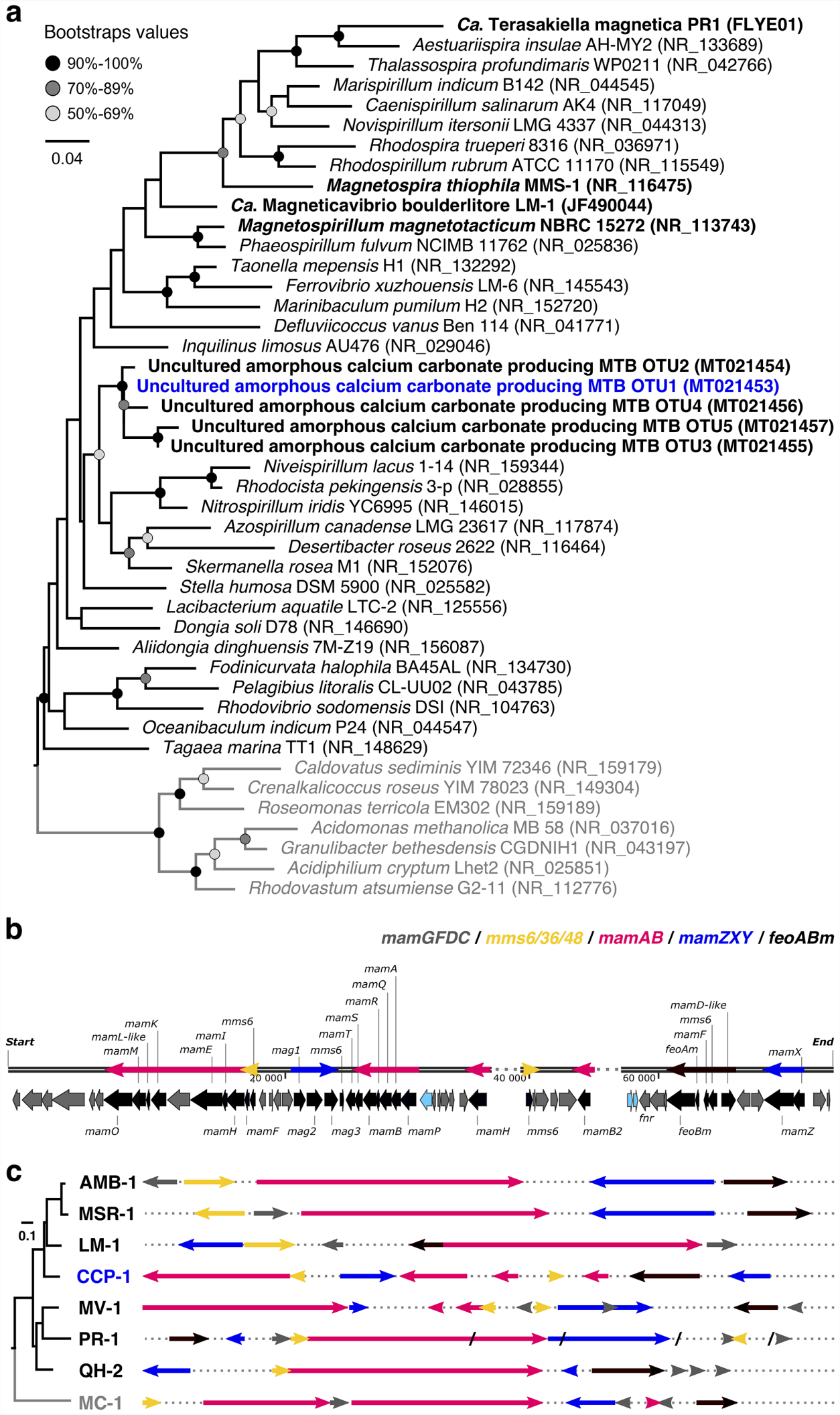
320
Energy (eV)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90%-100%
70%-89%
50%-69%
0.04
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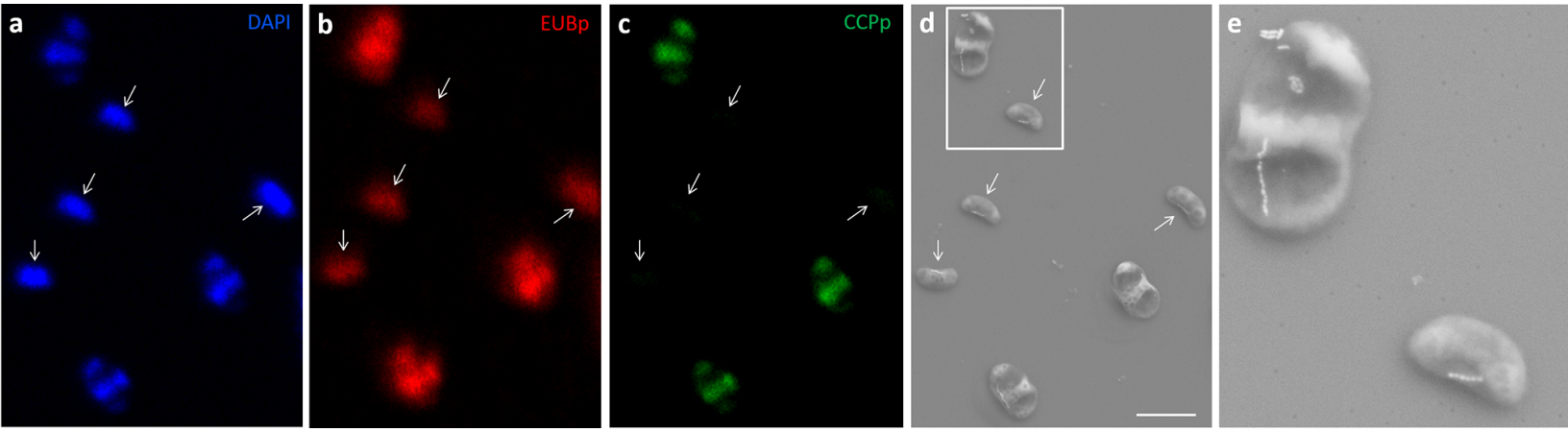
Aestuariispira insulae AH-MY2 (NR_133689)
Thalassospira profundimaris WP0211 (NR_042766)
Marispirillum indicum B142 (NR_044545)
Caenispirillum salinarum AK4 (NR_117049)
Novispirillum itersonii LMG 4337 (NR_044313)
Rhodospira trueperi 8316 (NR_036971)
Rhodospirillum rubrum ATCC 11170 (NR_115549)
Magnetospira thiophila MMS-1 (NR_116475)
Ca. Magneticavibrio boulderlitore LM-1 (JF490044)
Magnetospirillum magnetotacticum NBRC 15272 (NR_113743)
Phaeospirillum fulvum NCIMB 11762 (NR_025836)
Taonella mepensis H1 (NR_132292)
Ferrovibrio xuzhouensis LM-6 (NR_145543)
Marinibaculum pumilum H2 (NR_152720)
Defluviicoccus vanus Ben 114 (NR_041771)
Inquilinus limosus AU476 (NR_029046)
Uncultured amorphous calcium carbonate producing MTB OTU2 (MT021454)
Uncultured amorphous calcium carbonate producing MTB OTU1 (MT021453)
Uncultured amorphous calcium carbonate producing MTB OTU4 (MT021456)
Uncultured amorphous calcium carbonate producing MTB OTU5 (MT021457)
Uncultured amorphous calcium carbonate producing MTB OTU3 (MT021455)
Niveispirillum lacus 1-14 (NR_159344)
Rhodocista pekingensis 3-p (NR_028855)
Nitrospirillum iridis YC6995 (NR_146015)
Azospirillum canadense LMG 23617 (NR_117874)
Desertibacter roseus 2622 (NR_116464)
Skermanella rosea M1 (NR_152076)
Stella humosa DSM 5900 (NR_025582)
Lacibacterium aquatile LTC-2 (NR_125556)
Dongia soli D78 (NR_146690)
Aliidongia dinghuensis 7M-Z19 (NR_156087)
Fodinicurvata halophila BA45AL (NR_134730)
Pelagibius litoralis CL-UU02 (NR_043785)
Rhodovibrio sodomensis DSI (NR_104763)
Oceanibaculum indicum P24 (NR_044547)
Tagaea marina TT1 (NR_148629)
Caldovatus sediminis YIM 72346 (NR_159179)
Crenalkalicoccus roseus YIM 78023 (NR_149304)
Roseomonas terricola EM302 (NR_159189)
Acidomonas methanolica MB 58 (NR_037016)
Granulibacter bethesdensis CGDNIH1 (NR_043197)
Acidiphilium cryptum Lhet2 (NR_025851)
Rhodovastum atsumiense G2-11 (NR_112776)
b
mamGFDC / mms6/36/48 / mamAB / mamZXY / feoABm
Start
End
mamA
mamQ
mamR
mamS
mamT
mamK
mamL-like
mamM
mamI
mamE
mms6
mag1
mamD-like
mamF
feoAm
mamX
20 000
40 000
60 000
mamO
mamH
mamF
mag2
mag3
mamB
mamP
mamH
mms6
mamB2
fnr
feoBm
mamZ
c
0.1
AMB-1
MSR-1
LM-1
CCP-1
MV-1
PR-1
QH-2
MC-1


a
DAPI
b
EUBp
c
CCPp
d
e