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Identification of novel species of marine magnetotactic bacteria affiliated with Nitrospirae phylum

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► **To cite this version:**

Xin-xin Qian, J Liu, Nicolas Menguy, Jinhua Li, François Alberto, et al.. Identification of novel species of marine magnetotactic bacteria affiliated with Nitrospirae phylum. *Environmental Microbiology Reports*, 2019, 11 (3), pp.330-337. 10.1111/1758-2229.12755 . hal-02333904

HAL Id: hal-02333904

<https://amu.hal.science/hal-02333904>

Submitted on 10 Feb 2020

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23 **Summary**

24 Magnetotactic bacteria (MTB) are a group of Gram-negative bacteria characterized by
25 synthesizing magnetosomes and swimming along geomagnetic field lines. Phylogenetically,
26 they belong to different taxonomic lineages including *Proteobacteria*, *Nitrospirae*,
27 *Omnitrophica*, *Latescibacteria*, and *Planctomycetes* phyla on the phylogenetic tree. To date,
28 six *Nitrospirae* MTB phylotypes have been identified from freshwater or low salinity
29 environments and described in the literature. Here we report the identification of two
30 *Nitrospirae* MTB phylotypes collected, for the first time, from the marine environment. Both
31 have a spherical morphology with a cell size of ~ 5 µM and similar motility but are different
32 colors (black-brown and ivory-white) under the optic microscope. They synthesized
33 bullet-shaped iron-oxide magnetosomes that were arranged in multiple bundles of chains.
34 Moreover, the cytoplasm of the black-brown *Nitrospirae* MTB contained sulfur inclusions
35 that conferred on cells a rough, granular appearance. Phylogenetic analysis based on their 16S
36 rRNA gene sequences revealed that they are two novel species and cluster with the previously
37 reported MTB affiliated with the phylum *Nitrospirae*, thus extending the distribution of
38 *Nitrospirae* MTB from freshwater to the marine environment.

39 **Originality-Significance Statement**

40 Magnetotactic bacteria (MTB) consist of a phylogenetic, physiological and morphologic
41 heterogeneous group of gram-negative microbes. They are distributed worldwide at the
42 oxic-anoxic interface in chemically stratified water columns or sediments. To date, despite
43 *Nitrospirae* being one of the major phyla of MTB, they have only been found in freshwater or

44 low-salinity environments. Here we report the identification of two novel species of
45 *Nitrospirae* MTB, from the marine habitat for the first time. They have a spherical
46 morphology and are relatively big in size approximately 5 x 3 μm . One contains abundant
47 intracellular sulfur inclusions. This study shows the great diversity of *Nitrospirae* phylum and
48 extends the distribution of *Nitrospirae* MTB into marine habitats.

49 **Introduction**

50 Magnetotactic bacteria (MTB) are a group of Gram-negative bacteria and exhibit the
51 unique capacity of aligning and swimming along the geomagnetic field lines, a behavior
52 referred to as magnetotaxis (Uebe and Schüler, 2016). This capacity is based on the
53 intracellular biomineralization of magnetite or greigite magnetic nanocrystal chains that give
54 MTB cells a net magnetic moment dipole. MTB present various morphotypes including cocci,
55 spirilla, rod-shaped, vibrio, and more complex multicellular aggregates, and have been found
56 in aquatic environments from freshwater to marine ecosystems (Blakemore, 1975; Bazylinski
57 and Frankel, 2004; Lefèvre and Bazylinski, 2013). Phylogenetically, magnetotactic bacteria
58 are members of several classes of the *Proteobacteria* phylum including the *Alpha*-, *Gamma*-,
59 *Delta*-, *Zeta*-, candidate *Lambda*-, candidate *Eta*-classes, the *Nitrospirae* phylum, the
60 candidate *Omnitrophica* phylum, the candidate *Latescibacteria* phylum and the
61 *Planctomycetes* phylum (Lin et al., 2017a; Lin et al., 2018).

62 Compared with the *Proteobacteria* phylum, few MTB have been found to be
63 phylogenetically affiliated with the phylum *Nitrospirae* and none of them have been cultured.
64 This is consistent with the general difficulty in cultivating MTB due to their fastidious

65 physiological requirements (Bazylynski and Frankel, 2004). The first to be discovered and the
66 most studied was *Candidatus Magnetobacterium bavaricum* (Spring et al., 1993). *Ca.*
67 *Magnetobacterium bavaricum* is a large, rod-shaped bacterium ranging from 8 to 10 μm in
68 length, and contains 600-1000 bullet-shaped magnetite magnetosomes in 3-5 bundles of
69 multiple chains (Jogler et al., 2010; Jogler et al., 2011). *Candidatus Magnetobacterium*
70 *casensis* shares similar morphology and 98.2% of the sequence identity of the 16S rRNA gene
71 with *Ca. Magnetobacterium bavaricum*. Analysis of the draft genome sequence of *Ca.*
72 *Magnetobacterium casensis* reveals an autotrophic lifestyle. Pathways involved in
73 denitrification, sulfur oxidation and sulfate reduction have also been predicted, which
74 indicates its considerable capacity to adapt to variable environments (Lin et al., 2014).

75 The third identified *Nitrospirae* MTB was a small rod-shaped bacterium collected from
76 sediment of Waller See, in Germany and named MHB-1 (Flies et al., 2005). This organism
77 contains a single bundle of multiple chains of magnetosomes with bullet-shaped magnetite
78 crystals. The fourth reported *Nitrospirae* MTB is a moderately thermophilic magnetotactic
79 bacterium, HSMV-1, which remained viable and motile in samples kept between 25 and 62°C
80 for several months (Lefèvre et al., 2010). HSMV-1 cells are small vibrios and biomineralize a
81 single chain of bullet-shaped magnetosomes. Two *Nitrospirae* MTB phylotypes named LO-1
82 and MWB-1 were identified from southern Nevada, USA and Beijing, China, respectively
83 (Lefevre et al., 2011; Lin et al., 2012a). The two species have 6% phylogenetic divergence
84 and possess a similar relatively big ($\sim 3 \times 2.5\mu\text{m}$) spherical morphology with bullet-shaped
85 magnetosomes and 4-7 bundles of magnetosome chains parallel to the long axis of cells.

86 Interestingly, the six described *Nitrospirae* MTB phylotypes have distinct cell

87 appearance and different magnetosome arrangement including single chain, multiple chains
88 with single bundle or multiple bundles. The obvious common feature is their bullet-shape
89 magnetite magnetosomes. Another common property of the reported *Nitrospirae* MTB is that
90 they dwell in low salinity habitats (< 3 ppt) (Spring et al., 1993; Flies et al., 2005; Lefevre et
91 al., 2011; Lin et al., 2012a; Lin et al., 2012b). Therefore, it has been proposed that salinity
92 might influence the distribution of MTB affiliated with *Nitrospirae* phylum (Lin et al., 2012b).
93 Here, we identified two novel *Nitrospirae* MTBs species and expand the distribution of
94 *Nitrospirae* MTB to marine ecosystems.

95 **Results and discussion**

96 **Morphology and swimming behaviour of black-brown cocci and ivory-white cocci**

97 Differential interference contrast (DIC) microscopic examination showed the morphology
98 of black-brown giant cocci (BGC) (Fig. 1 A, black arrows), ivory-white giant cocci (WGC)
99 (Fig. 1 A, white arrows) and small cocci of MTB collected from the sediments in Six-Fours
100 les Plages. BGC have a rough surface appearance with an average length of $5.39 \pm 0.72 \mu\text{m}$
101 and an average width of $4.04 \pm 0.33 \mu\text{m}$ (n=62). WGC have a smoother cell surface and a
102 similar cell size with an average length of $5.21 \pm 0.66 \mu\text{m}$ and an average width of 3.99 ± 0.30
103 μm (n=62) (B in Figure 1). During sample preparation for DNA amplification and sequencing,
104 we observed that BGC cells began to disintegrate 5 min following their transfer from seawater
105 to PBS buffer, and small granules were released from the cells (C1 in Figure 1). In contrast,
106 WGC cells shrunk and became smaller in PBS buffer (C2 in Figure 1). Further analysis
107 showed that approximately 50 % of the BGC and WGC cells remained motile and

108 accumulated at the north edge of the cell suspension droplets where the salinity was decreased
109 from 35 to 16 ppt by dilution with deionized water. The number of cells at the edge of the
110 droplets decreased as the dilution increased, and the BGC and WGC cells almost disappeared
111 completely from the droplets at 13 ppt salinity. This observation suggests a sensitivity of the
112 marine cells to the freshwater conditions. BGC and WGC also exhibited different fluorescent
113 properties. When excited with green light (510-550 nm), BGC emitted red fluorescence while
114 no auto-fluorescence signals were observed in WGC under these conditions (D2 in Figure1).
115 BGC emitted stronger yellow auto-fluorescence than WGC under blue light (450-480 nm)
116 excitation (D3 in Figure1). Similar intensity of green light was emitted by both BGC and
117 WGC when excited by violet light (400-410 nm) (D4 in Figure1). WGC emitted stronger blue
118 fluorescence under ultraviolet light (330-385 nm) compared to BGC (D5 in Figure 1).
119 Illumination at these wavelengths had no impact on the motility of these MTB cells. Lack of
120 culture samples prevents us from performing detailed spectrophotometry analysis to
121 characterize the nature of the fluorescent molecules.

122 Most of the BGC and WGC exhibited north-seeking magnetotaxis behavior, i.e.
123 swimming along the applied magnetic field lines, whereas 10% of cells were found to be
124 south-seeking. Cells demonstrated a dynamic motility in the hanging drops for approximately
125 30 min then began to slow down gradually and stopped swimming at the end of one hour. The
126 average swim velocities of BGC and WGC cells were $126.3 \pm 45.0 \mu\text{m}\cdot\text{s}^{-1}$ (n=52) and $148.9 \pm$
127 $28.0 \mu\text{m}\cdot\text{s}^{-1}$ (n=54), respectively (E in Figure 1). The corresponding maximal swim speeds of
128 BGC ($225.4 \mu\text{m}\cdot\text{s}^{-1}$) and WGC ($188.6 \mu\text{m}\cdot\text{s}^{-1}$) were slower than the closest freshwater relative
129 MTB MWB-1 ($260 \mu\text{m}\cdot\text{s}^{-1}$).

130 **Phylogenetic analysis**

131 In the environmental samples analyzed, BGC cells were the dominant MTB while WGC
132 were approximately 4-fold less abundant as estimated by direct counting of the two
133 morphotypes in droplets (Experimental Procedures and Supporting Information Figure S1).
134 We collected 8 WGC cells using the magnetic micro-sorting approach, and performed Whole
135 Genome Amplification to get sufficient template DNA for further 16S rRNA gene
136 amplification and cloning. We obtained 24 sequences of 16S rRNA genes that shared >99.3%
137 identity between each other and thus formed one operational taxonomic unit (OTU-1,
138 GenBank number MK203828). In parallel, we obtained 40 full-length 16S rRNA gene
139 sequences of MTB prepared by a routine two-step magnetic collection (see Experimental
140 Procedures). Among them 31 sequences share more than 97.1% identity and form another
141 operational taxonomic unit (OTU-2, GenBank number MK073023). The nine other 16S rRNA
142 sequences consisted of 6 OTUs that were all affiliated with *Alphaproteobacteria*. The high
143 abundance of OTU-2 sequences detected is correlated with the dominant occurrence of BGC
144 in this sample (Fig. 1). A Blast search in NCBI indicated that the two OTUs sharing 92%
145 identity form a cluster that is affiliated with the phylum *Nitrospirae* (Fig. 2). They possess less
146 than 97% identity with all 16S rRNA genes in the GenBank. The most closely related
147 phylotypes are uncultured *Nitrospirae* MTBs clone MWB-1 (93% with OTU-1, 92% with
148 OTU-2) and *Candidatus* *Magnetoovum mohavensis* LO-1 (94% with OTU-1, 92% with
149 OTU-2). Therefore, the two OTUs represent a novel species of *Nitrospirae*.

150 Being one of the deep-branching MTB group, *Nitrospirae* MTB plays an important role
151 when analyzing the origin and evolution of magnetosome biomineralization and magnetotaxis.

152 Lin et al. suggested that the magnetosome biomineralization and magnetotaxis was present
153 before the separation of the *Nitrospirae* and *Proteobacteria* phyla, which was between ~3.38
154 and 3.21 Ga (Lin et al., 2017b). However, it remains unclear whether the acquisition of
155 magnetosome island (MAI), that encodes essential proteins for magnetosome biosynthesis in
156 the genome, occurred before or after the divergence between freshwater and marine
157 *Nitrospirae* MTB. It is equally possible that the spherical cells of *Nitrospirae* phylum got
158 MAI by horizontal gene transfer (HGT) then adapted into two different habitats or that they
159 diverged into different habitats then acquired MAI separately. Future genomic comparison
160 studies should provide answers to elucidate the evolutionary mechanism.

161 **Corroboration of authenticity of giant cocci by fluorescence *in situ* hybridization**

162 The BGC authenticity of OTU-2 was inferred from the fact that BGC is the most
163 abundant morphotype in the analyzed samples. To corroborate this inference we designed a
164 specific probe based on the conserved OTU-2 sequence and performed fluorescence *in situ*
165 hybridization (FISH) analysis. As shown in Figure 3 A2, the universal bacterial probe
166 hybridized with all the MTB, while the specific probe hybridized only with black-brown giant
167 cocci. The WGC cells only hybridized with the bacteria universal probe and were used as a
168 negative control. Therefore, the OTU-2 really corresponds to the abundant BGC morphotype.

169 We also confirmed the authenticity of the OTU-1 to the scarce WGC. A probe specific to
170 the conserved sequences of OTU-1 was designed. We found that the specific probe hybridized
171 only with the WGC morphotype while the universal probe hybridized with all bacterial cells
172 including BGC that served as the negative control (Fig. 3, B1). After FISH identification, the

173 same cover-slips were observed under scanning electron microscopy (SEM). As shown in B3
174 of Fig. 3, more than 8 bundles of multiple magnetosome chains aligned parallel to the long
175 axis and close to the outer surface in the WGC cell. Correlative FISH-SEM analysis not only
176 allowed identification of the OTU but also revealed characteristics of the magnetosome
177 chains.

178 **Magnetosome and element composition analyses**

179 After corroborating their authenticities, we analyzed the magnetosome chains and
180 element composition in BGC and WGC. Under DIC microscopy BGC appeared to contain
181 brown granules inside the cells while WGC had a smooth appearance (Fig. 4A). Observations
182 using High-angle annular dark-field scanning transmission electron microscopy
183 (HAADF-STEM) revealed that the BGC cells were filled with electron dense granules rich in
184 sulfur (Fig. 4B, top cell with white inclusions, and 4C in red color) in addition to the
185 magnetosomes chains (Fig. 4C green color). Further energy-dispersive X-ray spectroscopy
186 (XEDS) analysis confirmed that the granules were rich in sulfur, the most abundant element
187 in BGC cells but barely detectable in WGC (Fig. 4, D2 versus D1). The magnetosomes in
188 WGC were arranged in bundles of multiple chains as revealed by FISH-SEM analysis. We
189 attempted to identify the magnetosome crystals inside of BGC and WGC cells by electron
190 diffraction or high-resolution electron microscopy without success due to the thickness of the
191 cells. Although we identified isolated bullet-shaped crystals as magnetites on grids, but it is
192 uncertain if they were from BGC or WGC because environmental samples containing other
193 MTBs were used. We performed STEM-XEDS elemental map (Fig. 4C) and XEDS spectrum

194 analyses (Fig. 4B, E1, E2, F1 and F2), and unambiguously showed that the crystals in BGC
195 and WGC cells are iron-oxide minerals, which is consistent with the finding of only magnetite
196 minerals in *Nitrospirae* MTB magnetosomes in the literature.

197 One essential difference between BGC and WGC is that BGC contain numerous
198 sulfur-rich inclusions. Intracellular sulfur globules were also detected in *Nitrospirae*
199 magnetotactic bacteria *Ca. Magnetobacterium bavaricum*, LO-1, MWB-1 and MYR-1 (Jogler
200 et al., 2010; Li et al., 2010; Lefevre et al., 2011; Lin et al., 2012a; Li et al., 2016), and those in
201 the *Ca. Magnetobacterium bavaricum* are present in the form of cyclo-octa sulfur (S₈) (Eder et
202 al., 2014). Intracellular deposition of sulfur may serve as an electron donor or an electron
203 acceptor to provide energy for bacterial growth (Cox et al., 2002). We observed that BGC
204 became the dominant population during storage under laboratory conditions and were able to
205 survive for more than ten months while maintaining vivacious magnetotactic motility.
206 Therefore, it is plausible that the metabolism of intracellular sulfur-rich inclusions might
207 contribute to the long-term survival of BGC under laboratory conditions.

208 In conclusion, we reported the discovery of two novel marine species of *Nitrospirae*
209 MTB and their morphology and structural differences. Future genomic analysis would be
210 helpful to understand the evolution and adaptation mechanisms of *Nitrospirae* magnetotactic
211 bacteria to freshwater and marine environments.

212 **Experimental Procedures**

213 **Sediment collection and MTB enrichment**

214 Sediment samples were collected from Six-Four les Plages (43°07'N, 5°79'E) in the

215 Mediterranean Sea, Southern France. The site was characterized by a salinity of ~35 ppt, pH
216 of 7.5 and a temperature of 19°C at the time of sampling. Collected sediments were quickly
217 transferred to 1000 ml plastic bottles, covered with approximately 600 ml of *in situ* seawater,
218 transported to the laboratory, and incubated at room temperature without disturbance.

219 Black-brown giant cocci cells (BGC) in the sediment were magnetically enriched using
220 the race-track method (Wolfe et al., 1987; Zhu et al., 2010). The 16S rRNA genes were
221 directly amplified from the collections. White giant cocci cells (WGC) were further collected
222 by magnetic micro-sorting using a Transfer Man ONM-2D micromanipulator and an IM-9B
223 CellTram Oil manual hydraulic pressure-control system installed on an IX51 microscope
224 equipped with a 40 × LD objective (Zhang et al., 2014). WGC cells were directly transferred
225 to 3µl of PBS of the REPLI-g Single Cell Kit (QIAGEN). The PBS-suspended sample was
226 frozen and thawed repeatedly, and the whole genome amplification (WGA) reaction was
227 carried out using the REPLI-g Single Cell Kit (Chen et al., 2015). The 16S rRNA gene
228 sequences were obtained as previously described by Chen et al. (Chen et al., 2015).

229 **Phylogenetic analysis**

230 The 16S rDNA sequences were aligned using CLUSTAL W multiple alignment software.
231 Sequence identities were calculated with the BIOEDIT software. The phylogenetic tree was
232 constructed via the neighbor-joining method using MEGA VERSION (Kumar et al., 2016),
233 and boot-strap values were calculated from a 1000 replicates.

234 **Optical microscopy**

235 The morphologies and motilities of the enriched MTB cells were examined under DIC
236 microscopy (Olympus BX51 equipped with a DP71 camera; Olympus) using the
237 hanging-drop method in an applied magnetic field. The ratio between BGC and WGC was
238 estimated by counting the two morphotypes of cells at the edge of the droplets as shown in
239 Supporting Information Figure S1. To analyze the sensitivity to the osmolality the collected
240 cell suspensions were diluted with deionized water before observing their magnetotactic
241 behavior in the droplets. The trajectories of the cells were obtained using the dark-field mode
242 with an increased exposure time. To assess auto-fluorescence, fluorescence mirror units were
243 used to expose the cells to green light (510-550 nm), blue light (450-480 nm), violet light
244 (400-410 nm) and UV light (330-385 nm) with the corresponding filter sets of Olympus.

245 **FISH analyses**

246 BGC-specific probe (5'-ATACTTAATCTTCCGCAGACC-3') and WGC-specific probes
247 (5'-GGTTCCACACCGAAGCATT-3') were designed according to the conserved 16S rRNA
248 gene sequences of OTU-2 (GenBank number MK073023) and OTU-1 (GenBank number
249 MK203828), respectively and evaluated for their specificity by using the online probe
250 evaluation tools Ribosomal Database Project (RDP)
251 <https://rdp.cme.msu.edu/probematch/search.jsp> (Cole et al., 2009; Cole et al., 2014). The
252 BGC- and WGC-specific probes were synthesized and fluorescently labeled with the
253 hydrophilic sulfoindocyanine dye Cy3 at the 5' end. The universal bacterial probe EUB338
254 (5'-GCTGCCTCCCGTAGGAGT-3') was fluorescently labeled with fluorescein
255 phosphoramidite 200 FAM at the 5' end and used as a positive control for FISH (Amann et al.,

256 1990).

257 **Correlative FISH-SEM analyses**

258 The FISH and correlative FISH-SEM analyses were carried out as described by Li et al.
259 (Li et al., 2017). The same FISH coverslips of WGC were treated for SEM observations using
260 a Zeiss Ultra-55 field-emission gun SEM (Carl Zeiss) operating at 5 kV (Li et al., 2017).

261 **HAADF-STEM elemental composition analysis**

262 Magnetosome biomineralization was investigated using a JEM-2100F microscope
263 (JEOLLtd) operating at 200 kV equipped with a field emission gun, an ultra-high resolution
264 (UHR) pole piece and a Gatan energy filter (GIF2001) system (Gatan, Inc.). HAADF-STEM
265 was used for Z-contrast imaging. Chemical compositional analysis was performed by EDXS
266 elemental mapping in the HAADF-STEM mode. By using this method, nanometer-scale
267 spatial distribution of most elements can be determined, and the relative abundance of each
268 element at a certain location can be semi-quantitatively calculated (Li et al., 2017).

269 **Acknowledgments**

270 We are grateful to H. Zhang and F.X. Wang for their assistance in the coordinated
271 FISH-SEM experiments. X.X. Qian is grateful for her fellowship provided by the Chinese
272 Scholarship Council. This work was supported by grants 41776131 and U1706208 from
273 NSFC, grants 2016YFC0302502_02 and 2018YFC0309904 from National Key R&D
274 Program of China, a grant XDB06010203 from the Strategic Priority Research Program, by a

275 grant from the Excellence Initiative of Aix-Marseille University - A*Midex, a French
276 “Investissements d’Avenir” programme, and grant from CNRS for LIA-MagMC. The
277 FISH-SEM experiments were performed at the IGGCAS, Beijing, China and were supported
278 by a NSFC grant 41522402.

279 **Conflict of interest**

280 The authors declare that they have no conflict of interest

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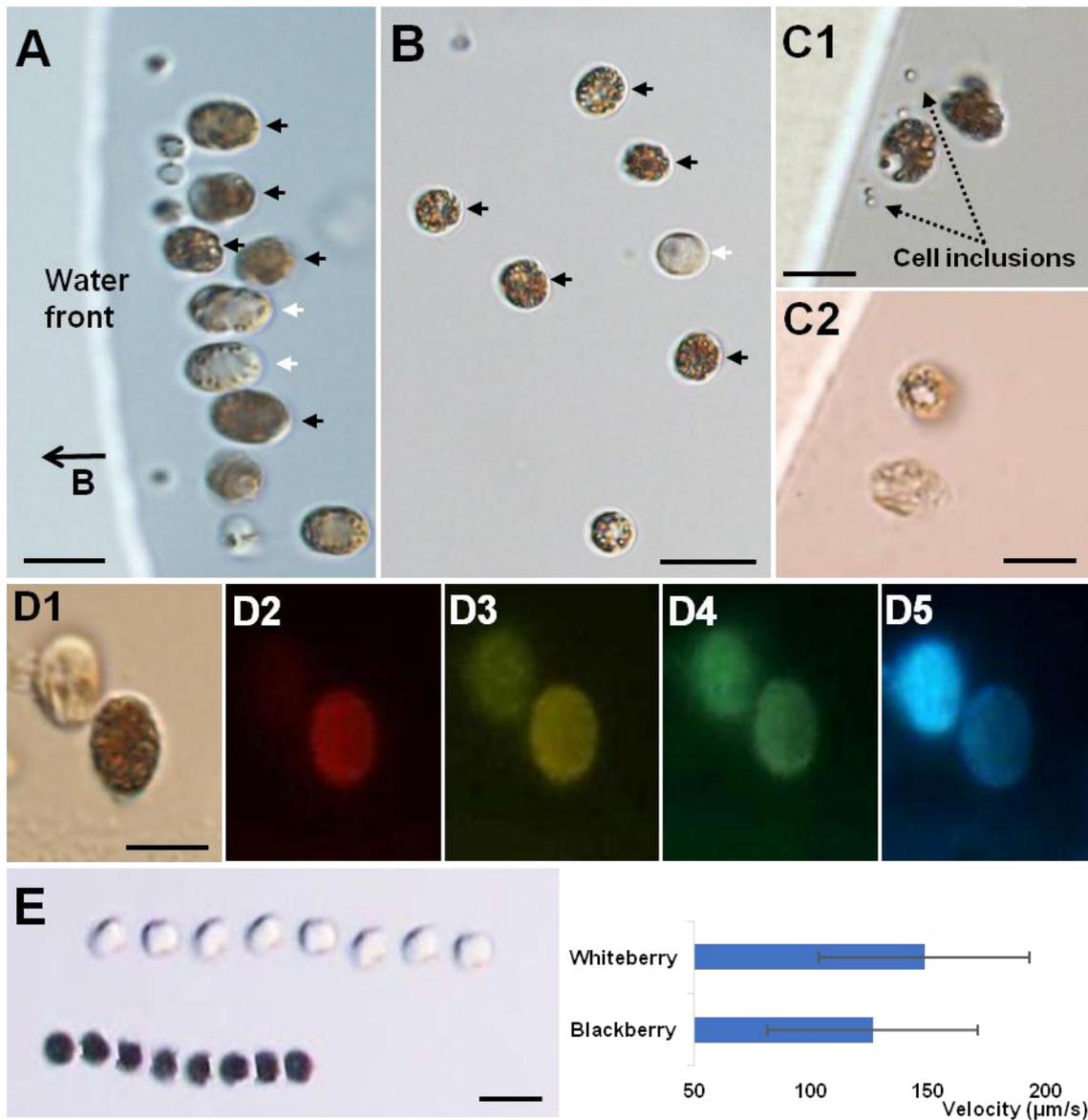
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360

362 **Figure legends**

363 **Figure 1. Morphology and velocity of black-brown giant cocci (BGC) and ivory-white**
364 **giant cocci (WGC).**



365

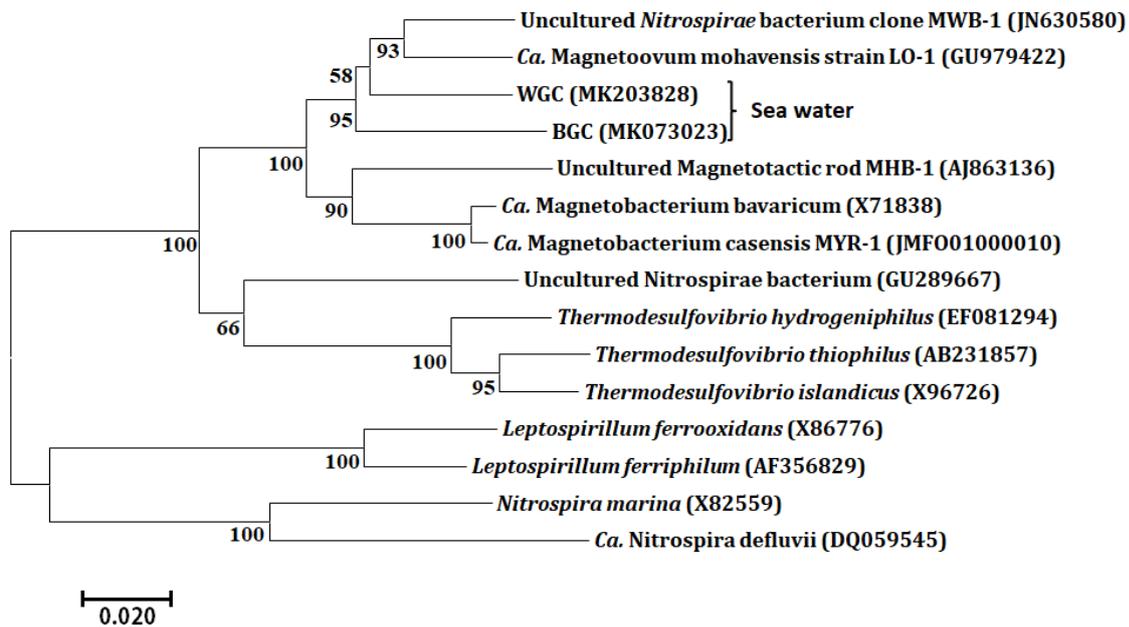
366 (A) Differential interference contrast (DIC) micrograph shows magnetotaxis and accumulation
367 of BGC (indicated by black arrows) and WGC (white arrows) at the north edge in a droplet. The

368 big black arrow B indicates the direction of the magnetic field. (B) DIC micrograph shows the

369 rough surface of BGC and smooth surface of WGC. (C) DIC micrograph shows both BGC

370 and WGC under low salinity conditions. (C1) Cell inclusions released from BGC. (C2)
 371 Shrunken shapes of WGC. (D) DIC micrograph (D1) and fluorescence micrograph (D1-D5)
 372 shows auto-fluorescence of BGC and WGC excited by light at different wavelengths. (D1)
 373 BGC and WGC under white light. (D2) BGC emitted red fluorescence while WGC had no
 374 fluorescence emission when exposed to green light. (D3) BGC emitted stronger yellow
 375 fluorescence than WGC under blue light. (D4) BGC and WGC emitted similar green
 376 fluorescence under violet light. (D5) WGC emitted stronger blue fluorescence than BGC under
 377 ultraviolet light. (E) Swim tracks and velocities of WGC (n=54) and BGC (n=52). The scale
 378 bars: 5µm in A, C, D. 10µm in B and E.

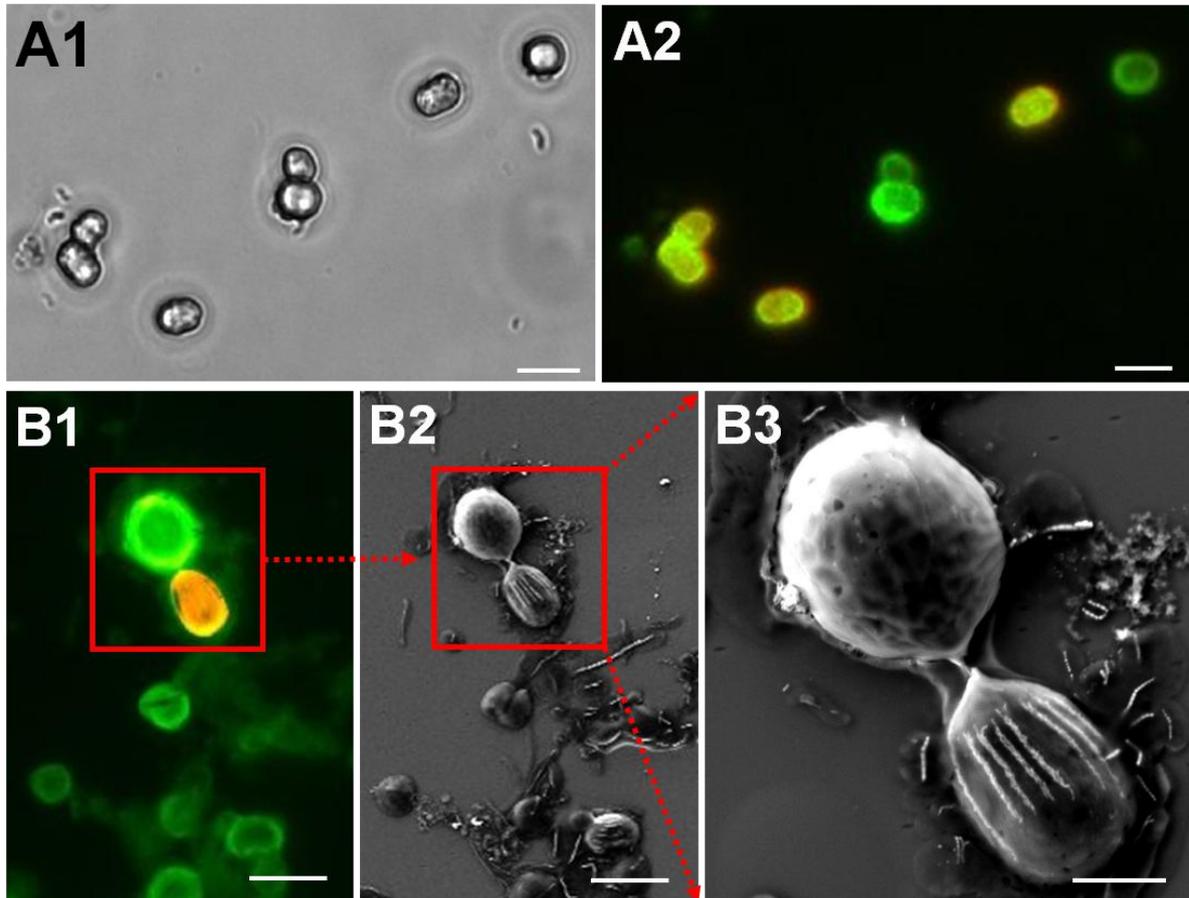
379 **Figure 2. Phylogenetic tree of 16S rRNA gene sequences from *Nitrospirae* MTB.**



380
 381 All currently characterized *Nitrospirae* MTB and some typical *Nitrospirae* non-MTBs were
 382 used to construct the phylogenetic tree. The *Nitrospirae* MTB from seawater clustered to
 383 closely related freshwater *Nitrospirae* MTB. Bootstrap values (higher than 50) at nodes are

384 given as percentages of 1000 replicates. GenBank accession numbers of the sequences are
385 given in parentheses. Scale bar represents 2% sequence divergence.

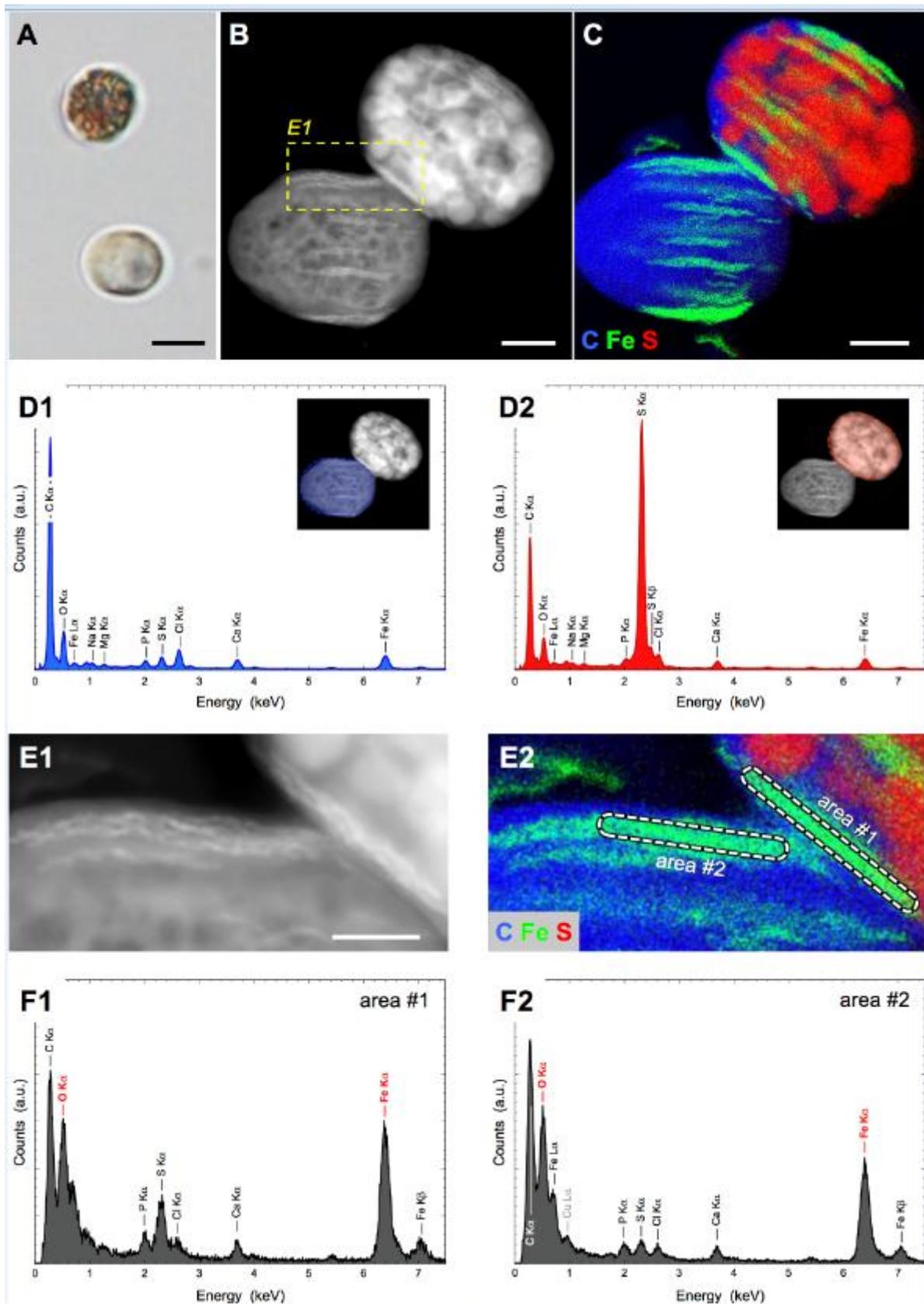
386 **Figure 3. FISH identification of magnetotactic giant cocci.**



387
388 Optical micrograph (A1) and fluorescence micrograph (A2) show BGC cells that were *in situ*
389 hybridized (FISH) with the 5'-FAM-labeled universal bacterial probe EUB338 and the
390 5'-Cy3-labeled BGC-specific probe. Green color indicates bacteria hybridized only with the
391 universal bacterial probe and yellow color shows the cell hybridized with both the universal
392 bacterial probe and the BGC-specific probe. (B) Correlative FISH - SEM microscopic analysis
393 of the WGC. (B1) In fluorescence micrograph, green color indicates bacteria hybridized only
394 with universal bacterial probe EUB338 and yellow color shows the cell hybridized with both
395 the universal bacterial probe and the 5'-Cy3-labeled WGC-specific probe. (B2) Correlated

396 SEM image of image B1. (B3) Correlated SEM image of the same red frame field in image B2
397 revealed the multiple magnetosome chains and bullet-shape magnetosome morphology of
398 WGS. The scale bars: 5 μ m in A1, A2, B1, B2. 2 μ m in B3.

399 **Figure 4. Element composition of BGC and WGC**



400

401 A) DIC image showed overall morphology difference between BGC (top cell) and WGC

402 (bottom cell). (B) STEM-HAADF image of BGC and WGC revealed multiple bundles of

403 magnetosome chains in both and granules filled in BGC, dashed rectangle is related to the
404 area shown in E1. (C) STEM-XEDS elemental map of BGC and WBC revealing the presence
405 of sulfur (red) and iron (green) overlaid with granules in BGC and iron-oxide crystals of
406 magnetosomes in both BGC and WGC. (D1) XEDS spectrum corresponding to WGC
407 showing that carbon is the most abundant element. (D2) XEDS spectrum corresponding to
408 BGC showing that sulfur is the most abundant element. (E1) STEM-HAADF image related to
409 the selected area in (A) and corresponding elemental map (E2). (F1) and (F2) represent XEDS
410 spectra of the magnetosome chains in the selected areas #1 and #2 (in E2) in WBC and BGC
411 cells, respectively. These spectra indicate the iron-oxide nature of magnetosome crystals. The
412 scale bars: 5 μm in A, 1 μm in B and C, 0.5 μm in E1.

413