EXPERIMENTAL ARTICLES

Biodiversity of Magnetotactic Bacteria in the Freshwater Lake Beloe Bordukovskoe, Russia

V. V. KoZIAeva^{a,*}, L. M. Alekseeva^{a,b}, M. M. Uzun^{a,b}, P. Leão^c, M. V. Sukhacheva^a, E. O.

Patutina^a, T. V. Kolganova^a, and D. S. Grouzdev^a

^a *Institute of Bioengineering, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, 119071, Russia*

^b *Moscow State University, Moscow, 199991, Russia,*

^c *Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, 21941-902, Brazil*

* *E-mail: veronikakoziaeva@gmail.com*

Received

Abstract---According to 16S rRNA gene- or genome-based phylogeny, magnetotactic bacteria (MTB) belong to diverse taxonomic groups. Here we analyzed the diversity of MTB in a sample taken from the freshwater lake Beloe Bordukovskoe near Moscow, Russia, by using molecular identification based on sequencing of the 16S rRNA gene and *mamK*, a specific marker gene for these bacteria. A protein encoded by the *mamK* gene is involved in magnetosome chain arrangement inside the cell. As a result, six operational taxonomic units (OTUs) of MTB were identified. Among them, OTUs affiliated with the phylum *Nitrospirae* were predominant. ‘*Ca. Etaproteobacteria*’ and *Alphaproteobacteria* represented the minor groups of MTB. We also identified a novel MTB belonging to the family *Syntrophaceae* of the *Deltaproteobacteria* class. Using a combination of fluorescence and transmission electron microscopy, the bacteria belonging to these new MTB groups were visualized. Electron microscopy revealed that *Syntrophaceae* MTB were rod-shaped and synthesized elongated magnetosomes, arranged as a disorganized cluster. Among the *Nitrospirae* group, two groups with vibrioid cell shape and one group of ovoid-shaped bacteria were identified, all of which had elongated magnetosome crystals consisting of magnetite.

Keywords: magnetotactic bacteria, magnetosomes, magnetotaxis, 16S rRNA gene, phylogenetic analysis, *Nitrospirae*, *Syntrophaceae*

Prokaryotes capable of directed active movement that is guided by geomagnetism are collectively called magnetotactic bacteria (MTB) (Blakemore, 1975). MTB are physiologically, morphologically and phylogenetically diverse, sharing only the ability to synthesize special organelles termed magnetosomes. Magnetosomes consist of nanometer-size magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystals surrounded by a lipid bilayer membrane containing proteins specific to the organelle. Magnetosomes are often assembled into chains inside the cell. MTB evolved the ability to conduct a special type of movement called magnetotaxis, which is based on orientation relative to magnetic field lines (Faivre and Schuler, 2008). Specific genes involved in magnetosome formation were found in the genomes of all MTB. There are currently nine highly conserved primary genes in magnetite- and greigite-producing MTB (*mamA*, *mamB*, *mamM*, *mamQ*, *mamO*, *mamI*, *mamP*, *mamK*, and *mamE*) (Lefèvre and Bazylinski, 2013). One of them, the *mamK* gene, encodes the actin-like protein MamK, which is responsible for the ordered arrangement of the magnetosome chain in the cell (Uebe and Schüler, 2016).

MTB are found within the phyla *Proteobacteria*, *Nitrospirae*, *Planctomycetes*, the candidate phyla ‘*Omnitrophica*’ and ‘*Latescibacteria*’ (Dziuba et al., 2016; Lefèvre and Bazylinski, 2013; Lin et al., 2017a; Lin and Pan, 2015). ‘*Ca. Etaproteobacteria*’ and *Alphaproteobacteria* are the most commonly observed and extensively characterized types of MTB (Lefèvre and Bazylinski, 2013; Monteil et al., 2018). Many of cultured marine and freshwater MTB belong to the class *Alphaproteobacteria*, including various vibrios and spirilla affiliated to the genera *Magnetospirillum*, *Magnetovibrio*, *Magnetospira*, and *Terasakiella* (Lefèvre and Bazylinski, 2013; Monteil et al., 2018). Numerous magnetotactic cocci and rods are affiliated to the order *Magnetococcales* of the class ‘*Ca. Etaproteobacteria*’ (Koziaeva et al., 2019). The two *Deltaproteobacteria* orders, *Desulfovibrionales* and the *Desulfobacterales*, also contain magnetotactic members. Cultured strains *Desulfovibrio magneticus* RS-1, FH-1, ML-1, AV-1, ZZ-1, and an uncultured bacterium WYHR-1 belong to the order *Desulfovibrionales* (Lefèvre and Bazylinski, 2013; Li et al., 2019). The order *Desulfobacterales* contains the strains *Desulfamplus magnetovallimortis* BW-1^T and SS-2, as well as several uncultured multicellular magnetotactic prokaryotes (MMP) (Abreu et al., 2007; Leão et al., 2017; Lefèvre and Bazylinski, 2013).

Axenic cultures of MTB of the *Nitrospirae* phylum have not yet been obtained. According to Parks et al., the most well-known members of this group form the candidate family ‘*Magnetobacteraceae*’. The family ‘*Ca. Magnetobacteriaceae*’ currently includes several candidate genera, which accommodate only MTB: ‘*Ca. Magnetobacterium*’, ‘*Ca. Magnetoovum*’, and ‘*Ca. Magnetominusculus*’ (Lefèvre and Bazylinski, 2013; Lin et al., 2011, 2017b). Most of MTB belonging to the *Nitrospirae* phylum were found in mesophilic freshwater habitats, with the exception of two representatives, WGC and BGC, the recently described giant cocci with multiple

chains of magnetosomes (Qian et al., 2019), and multiple sequences retrieved from the Yellow Sea in China (Xu et al., 2018). A thermophilic MTB named ‘*Ca. Thermomagnetovibrio paiutensis*’ HSMV-1 was also described. It is closely related to cultured *Thermodesulfovibrio* spp.

Despite the high diversity of MTB found in environmental samples, they are difficult to isolate in axenic culture. Therefore, culture-independent techniques are indispensable in research on these bacteria. Members of the phyla *Proteobacteria* and *Nitrospirae* have been studied extensively. However, analyses of metagenomic datasets revealed the presence of MTB within the phyla *Planctomycetes* and ‘*Ca. Latescibacteria*’ (Lin and Pan, 2015; Lin et al., 2017a). This finding indicates that the biodiversity of MTB is much broader than is currently known. Therefore, modifying and developing the strategies for investigation offers great promise towards identifying MTB groups. Another challenge in MTB biodiversity investigation is detailed characterization of cell morphology and crystallographic properties of their magnetosomes. A recently developed method of coordinated fluorescence *in situ* hybridization and electron microscopy (FISH-TEM) successfully linked the phylogeny with cell ultrastructure and magnetosome morphology of several uncultured MTB strains, including the Gammaproteobacteria strain SHHR-1, Deltaproteobacteria strain WYHR-1 and ‘*Ca. Magnetaquicoccus inordinatus*’ UR-1 (Li et al., 2017, 2019; Koziaeva et al., 2019).

Here we describe a diversity of MTB in the lake Beloe Bordukovskoe based on a novel isolation approach that does not depend on cell motility. For phylogenetic identification of collected cells, we used a universal primer system on the marker gene *mamK* and analysis of the 16S rRNA gene sequences. To characterize the morphology of cells and magnetosomes of bacteria of retrieved OTUs, the FISH-TEM method was applied.

MATERIALS AND METHODS

Sampling and MTB enrichment. Water and sediment samples were collected at the freshwater lake Belaye Bordukovskoe, Shatura District, Russia (55°37'56"N, 39°44'38"E). Samples with a 1 : 2 sediment : water ratio were used to create a microcosm (3 L) and incubated in the dark at room temperature for one year. The pH value and salinity were measured using an Acvilon pH meter (Russia) and a handheld portable refractometer, respectively. The elemental composition of organic carbon (C%) in the sediment was measured using a Flash 1112 elemental analyzer coupled with a Thermo-Finnigan Delta V Plus isotope mass spectrometer (Thermo Fisher Scientific, United States) at the Core Facility of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences.

Magnetic collection of MTB was performed using the MTB-CoSe (magnetotactic bacteria column separation) method developed in our laboratory (Fig. 1). Initially, sediment cores (15 mm in

diameter) were taken from three different sites of the microcosm and mixed. To desorb the cells from large clay particles, PBS buffer (final concentration 1.25×) and glass microbeads (2 g; 150–200 µm diameter; Sigma-Aldrich, United States) were added to a 50-mL tube containing 20 mL of the sample (sediment + water in approximately 1 : 4 ratio). The mixture was then treated on a ThermoShakerTS-100 (Biosan, Latvia) for 15 min at 100 rpm. The homogenate was transferred to a Bunsen flask and filtered under vacuum through a paper filter to remove large soil particles. The filtrate was transferred to 50-mL tubes and centrifuged (Eppendorf Centrifuge 5804R) for 10–15 min at 8000 rpm. Most of the supernatant was discarded, and 2 mL was retained for cell resuspension. Next, a miniMACS column (Miltenyi Biotec, Germany) was washed with 1.25× PBS buffer and placed on a magnet, and the cell mixture was applied to the column. MTB cells that adsorbed onto the column were washed 4–5 times with 2 mL 1.25× PBS buffer until no cells were present in the washing liquid (controlled by light microscopy). Next, the column was removed from the magnet, and the MTB cells were eluted twice with 100 µL 1.25× PBS buffer into a clean 1.5-mL tube. For samples with high cell concentrations, the eluate was reapplied onto the column, which was rinsed to completely remove nonmagnetic cells. The presence of MTB cells and nonmagnetic bacteria was assessed by microscopy of 5-µL aliquots of eluted liquid. The MTB sample was divided into two parts. One part was used to isolate DNA for metagenome sequencing and creating libraries of the *mamK* and 16S rRNA gene sequences. The second part was fixed for FISH/TEM studies. Genomic DNA was extracted using the DNeasy PowerSoil kit (Qiagen, Netherlands) according to the manufacturer's instructions.

Amplification, cloning and sequencing fragments of genes encoding 16S rRNA and *mamK*. A fragment of the *mamK* gene was obtained using nested PCR with two pairs of degenerate primers: external (mamK_79F and mamK_577R) and internal (mamK_86F and mamK_521R) (Table 1). The reaction buffer (25 µL) had the following composition: 5 µL Mas^{CFE}TaqMIX-2025 buffer (Dialat Ltd., Russia), 0.5 pmol/µL each primer, 0.04% BSA, and 10–50 ng of a DNA template. The temperature-time profile of the reaction was as follows: initiation, 3 min at 95 °C; 4 cycles of 30 s at 95 °C, 40 s at 58 °C, and 1 min at 72 °C; 36 cycles of 30 s at 95 °C, 40 s at 52 °C, 1 min at 72 °C; and a final cycle of 7 min at 72 °C. Amplification of the 16S rRNA gene sequences was performed using the universal primers 27F and 1492R (Lane, 1991). Clonal libraries of the *mamK* and 16S rRNA gene fragments were obtained and sequenced as previously described (Kozyaeva et al., 2017). The target insert of the *mamK* gene was sequenced using the primer M13F, and 16S rRNA gene was sequenced using the primers 341F, 530F, 1114F and 519R (Sambrook et al., 1989).

133 **Phylogenetic analysis.** Nucleotide and amino acid sequences were aligned using MAFFT
134 (Kato and Standley, 2013). Obtained MamK and 16S rRNA gene sequences were grouped in
135 OTUs using the identity threshold of 97%. Phylogenetic analysis was performed using the IQ-
136 TREE program (Nguyen et al., 2015) with selection of an evolutionary model using ModelFinder
137 (Kalyaanamoorthy et al., 2017) and estimation of branch supports using UFBoot2 (Hoang et al.,
138 2017). The MamK and 16S rRNA gene sequences representing OTUs identified in this study were
139 deposited in GenBank under the accession numbers MK636828-33 and MK63285-90, respectively.

140 **Light and electron microscopy.** Morphology of the cells collected after magnetic
141 separation using MTB-CoSe was examined using an Eclipse E200 light microscope (Nikon, Japan).
142 For conventional transmission electron microscopy (TEM), magnetically enriched cell suspensions
143 were deposited on Formvar-coated 300-mesh copper grids, washed with distilled water and imaged
144 using a Morgagni TEM (FEI, United States) operated at 80 kV.

145 **Fluorescence in situ hybridization/transmission electron microscopy (FISH-TEM).** For
146 fluorescence in situ hybridization (FISH), collected MTB cells were fixed in 3% paraformaldehyde
147 for 1.5 h. For the association of the retrieved 16S rRNA gene sequences with different MTB
148 morphotypes, a drop of sample was added to Formvar-coated center-marked or index copper grids.
149 A thin layer of carbon was sputtered onto the grids (Balzers CED-030/Baltec) atop each sample to
150 provide stabilization. FISH was performed on each grid using the conditions and buffers described
151 by Pernthaler et al. (2001) and 30% formamide in the hybridization buffer. After this procedure, the
152 grids were stained with 0.1 µg/mL 4,6-diamidino-2-phenylindole (DAPI) for 10 min, placed
153 between a glass slide and cover glass, and observed with a Zeiss AxioImager microscope equipped
154 with an AxioCam Mrc (Zeiss, Germany).

155 Probes used for FISH were designed based on the 16S rRNA gene sequences of retrieved
156 OTUs (Table 1). In addition, a mix of EUB388I, EUB388II and EUB388III bacterial universal
157 probes labelled with Alexa 488 was used as a control (Daims et al., 1999).
158 After performing FISH, the same grids were examined by TEM, and images were taken in the same
159 region where hybridization with the specific probe occurred.

160 **Metagenome binning and analysis.** The metagenome 3300021602 obtained from the IMG
161 database was binned using three different tools, MaxBin2 (Wu et al., 2014), MyCC (Lin and Liao,
162 2016), and Busy Bee Web (Laczny et al., 2017) prior to dereplication and refinement with the DAS
163 Tool (Sieber et al., 2018), which performs a consensus binning to produce the final bin set.
164 Completeness and contamination rates were assessed using CheckM v. 1.0.12 (Parks et al., 2015)

165 with the 'lineage wf' command and default settings. Magnetosome island genes were found using
166 local BLAST compared with reference sequences of magnetotactic bacteria.

167 RESULTS

168 **Collection of MTB from a sediment sample.** Lake Beloye Bordukovskoe is a round lake
169 with a maximum depth of 21 m and is possibly of glacial origin. The lake has transparent water
170 with a light brown color and visibility of up to 8 m. The brown color is due to water flow from the
171 nearby peat bog. The pH of the microcosm water was 6.3 and salinity was 1% during sampling. We
172 used an MTB-CoSe method to enrich MTB from the microcosm without inducing magnetotaxis.
173 Immediately after magnetic separation, light microscopy examination revealed the presence of 1.12
174 $\pm 0.06 \times 10^6$ MTB in 20 mL of the total sample. Vibrios were the most common MTB morphotype,
175 and ovoid cells, which accounted for about 30% of the magnetic fraction, were also detected.
176 Nonmagnetic cells were also present in small quantities (<1%).

177
178 **Phylogenetic analysis of the MamK sequences.** After the removal of chimeras and low-
179 quality reads, the clone library of the *mamK* gene fragments consisted of 346 sequences. Based on
180 the derived MamK sequences, phylogenetic analysis was conducted that yielded six OTUs (Table 2;
181 Fig. 2a). MamK_LBB_01, MamK_LBB_02, and MamK_LBB_03 were the most well-represented
182 OTUs. On the phylogenetic tree, these OTUs clustered together with the MamK sequences of
183 strains of the *Nitrospirae* phylum. The dominant OTU MamK_LBB_01 was represented by 87% of
184 all clones and formed a separate clade within the phylum *Nitrospirae*. The minor groups were
185 OTUs MamK_LBB_05 and MamK_LBB_06, which clustered with the MamK sequences of MTB
186 belonging to the *Magnetococcales* and *Rhodospirillales* orders.

187 One interesting finding was the presence of the OTU MamK_LBB_04, which was clearly
188 divergent from all other currently known MamK sequences. The level of similarity to the closest
189 MamK of the *Nitrospirae* bacterium MYbin3 was 69.2%, with 45% coverage. We could not
190 attribute this OTU to any of the known taxonomic groups containing MTB due to the lack of related
191 reference sequences. Therefore, we decided to conduct a search for possible homologues in the
192 metagenomic dataset available in the public databases IMG and NCBI. After searching for the
193 nearest homologues for the OTU MamK_LBB_04, a protein with a high sequence similarity
194 (97.0%) was found in the IMG database. This MamK was recovered from the metagenome
195 3300021602, which was obtained from a boreal shield lake in Ontario (IISD Experimental Lakes
196 Area). After binning of the metagenome, a bin (MAG_21602_syn32) containing the target *mamK*
197 sequence was obtained. In addition to *mamK*, other magnetosome genes (*mamI*, *mamE*, *mamQ-1*,

198 and *man2*) were found. The *man2* gene is located in the contig next to *mamK*. The OTU
199 MamK_LBB_04 together with MamK, which is derived from MAG_21602_syn32, formed a
200 phylogenetically distinct lineage between the *Nitrospirae* and *Deltaproteobacteria* branches.
201 Taxonomy analysis of the bin MAG_21602_syn32 sequence from Genome Taxonomy Database
202 showed that it belonged to the order ‘*Syntrophales*’ and, according to NCBI, to the order
203 *Syntrophobacterales*. Therefore the identified MamK could be associated with *Syntrophobacterales*
204 species. MTB of this taxonomic group have not been previously detected. The 16S rRNA gene
205 sequence of the received bin was also identified (acc. no. Ga0194060_100555694).

206

207 **Phylogenetic analysis of 16S rRNA gene sequences.** The assembled 16S rRNA gene clone
208 library contained 282 sequences, which were classified into 6 different OTUs (Table 3). A
209 phylogenetic tree was constructed and its topology was compared with the tree based on the MamK
210 sequences (Fig 2b).

211 Over 95% of the retrieved 16S rRNA gene sequences (OTU 16S_LBB_01–OTU
212 16S_LBB_03) clustered within the *Nitrospirae* phylum. A dominant group OTU 16S_LBB_01
213 formed a separate branch within the family ‘*Ca. Magnetobacteraceae*’ as well as on the
214 phylogenetic tree based on the MamK sequences. OTUs 16S_LBB_06 and MamK_LBB_05 formed
215 branches with bacteria of the *Magnetococcales* and *Rhodospirillales* orders, respectively, and
216 represented 3.9% of the 16S rRNA gene clonal library.

217 OTU 16S_LBB_04 had 96.8% similarity with the 16S rRNA gene identified in the bin
218 MAG_21602_syn32. On the 16S rRNA phylogenetic tree both sequences formed a separate branch
219 with representatives of the family *Syntrophaceae* (order *Syntrophobacterales*). The level of
220 similarity with the closest validly described species, *Syntrophus aciditrophicus* and *Smitella*
221 *propionica*, was 93.5% and 91.2%, respectively, which indicates that OTU 16S_LBB_04 belongs to
222 a novel genus within the family *Syntrophaceae*.

223 Topology of the trees was generally congruent, and both trees contained similar phylogenetic
224 groups. It is possible that both the *mamK* and 16S rRNA gene sequences correspond to the same
225 organism, but this hypothesis requires further verification. Due to the high level of similarity in 16S
226 rRNA gene and MamK sequences, it is possible that OTU 16S_LBB_05, bacterium LM-1, and
227 WMHbin7 are the same or closely related species of the *Rhodospirillales* order.

228

229 **Linking phylogeny to cell morphology by FISH-TEM.** To link phylogeny with the MTB
230 morphotype, FISH-TEM was carried out. Based on the target 16S rRNA gene sequences, the probes
231 were designed to identify the morphology of the matching MTB by FISH-TEM (Table 1).

232 The LBB_01 probe specific for OTU 16S_LBB_01 hybridized only with vibrioid-shaped
 233 bacteria (Figs. 3a–3d). This group of MTB possessed a thick chain of anisotropic magnetosomes
 234 organized along the long axis of the bacterial cell. Our morphology identification analysis showed
 235 that bacteria of OTU 16S_LBB_02 were small ovoids and synthesized two bundles of bullet-shaped
 236 magnetosomes (Figs. 3e–3h). The observed cell shape and magnetosome organization was similar
 237 to that of *Nitrospirae* MTB fosmid MY3-11A (Lin et al., 2011). The LBB_03 probe, which was
 238 specific for OTU 16S_LBB_03, hybridized to a vibrioid cell containing anisotropic magnetosomes
 239 in a single chain (Figs. 3i–3l).

240 To confirm that the identified OTU 16S_LBB_04 was related to magnetotactic bacteria,
 241 FISH-TEM analysis was conducted. The fluorescent probe specific to OTU 16S_LBB_04 identified
 242 a rod-shaped MTB with disorganized magnetosomes located close to the center of the bacterial cell
 243 (Figs. 3m–3p). Rod-shaped morphology is a common feature for bacteria of the *Syntrophaceae*
 244 family, which accommodates mesophilic bacteria inhabiting anoxic freshwater environments
 245 (Kuever et al., 2015).

246 For OTUs 16S_LBB_06 and MamK_LBB_05, FISH-TEM was not performed. However, a
 247 search of TEM images identified a coccoid cell with a single magnetosome chain (Fig. 4a). TEM
 248 images of spirilla with a chain of cubooctahedral magnetosomes were also observed (Fig. 4b).

249

250 DISCUSSION

251

252 Previous studies on the MTB diversity have mainly detected members of the
 253 *Alphaproteobacteria* and ‘*Etaproteobacteria*’ classes (Jogler et al., 2009; Lefèvre and Bazylinski,
 254 2013; Kozyaeva et al., 2017; Lin et al., 2017a). However, we found that these two types of MTB
 255 were in fact minority groups in the Lake Belye Bordukovskoe community. We found many
 256 representatives of the phylum *Nitrospirae*. Few studies have identified *Nitrospirae* as a dominant
 257 MTB phylum, including those at freshwater lakes Miyun (China) and Chiemsee (Germany) (Spring
 258 et al., 1993; Lin et al., 2011), as well as in the sediments from the continental shelf of the Yellow
 259 Sea (Xu et al., 2018). Differences between the studies may be due to a number of reasons. One
 260 main reason may be related to the procedure by which MTB are isolated from the environmental
 261 sample. *Alphaproteobacteria* and ‘*Ca. Etaproteobacteria*’ were usually the predominant classes or
 262 the only ones identified in the samples when a magnet was placed on the outside of the microcosm
 263 at the silt–water interface and then the cell suspension was purified using a “race-track” approach
 264 (Flies et al., 2005b; Lin et al., 2008; Postec et al., 2012; Dziuba et al., 2013; Pradel et al., 2016;
 265 Kozyaeva et al., 2017; Du et al., 2017;). Previous studies postulated the methods based on
 266 magnetotaxis to be highly dependent on the cell swimming ability of MTB (Lin et al., 2008; Lin

and Pan, 2009; Xu et al., 2018). The intensity of the magnetic field, distance to the magnet, and aerotaxis also affect the enrichment efficiency. Within the oxic–anoxic interface (OAI), different MTB species show different vertical distributions. Magnetotactic cocci usually concentrate at the OAI (Flies et al., 2005a; Lefevre et al., 2011) and occur closer to the magnet during the first step of magnetic separation. Therefore, cocci of the ‘*Ca. Etaproteobacteria*’ class gain a significant advantage with this enrichment method. In the present study, we obtained sludge samples from a microcosm using a vertical tube, and as a result a larger amount of the anoxic zone was captured. Since the movement of MTB is directed not only by magnetic field, but also by aerotactic signals, it is possible that some MTB may not leave their anoxic zone during magnetic extraction of the cells from the microcosm sediment.

We cannot rule out the possibility that the dominance of members of the phylum *Nitrospirae* shown in this study was due to a limitation of the MTB-CoSe method. Representatives of the *Nitrospirae* phylum contain larger numbers of magnetic particles than other MTB, and since the procedure requires several washes, this may favor cells containing a large number of magnetosome crystals.

We also cannot exclude the possibility that during microcosm storage growth conditions were optimal for *Nitrospirae* MTB, rather than for magnetotactic cocci. The MTB community may change during incubation of the microcosm in the laboratory for a certain period of time (Flies et al., 2005b). It was previously shown that the numbers of magnetotactic cocci and spirilla significantly decreased over several months of the microcosm storage, whereas the numbers of the magnetotactic ovoid *Nitrospirae* bacterium LO-1 increased (Lefevre et al., 2011). Therefore, the distribution among the MTB phylogenetic groups may depend on the incubation time of the microcosm.

Magnetic separation methods represent robust tools for the investigation of MTB biodiversity and have been successfully used in numerous studies. However, any separation method can lead to a bias and thus may not reflect the real diversity of MTB in nature. As previously stated, the most acceptable approach for direct study of MTB biodiversity in natural samples is the one not involving magnetic separation (Lin and Pan, 2009). However, it is difficult to identify magnetotactic bacteria by direct amplification of their 16S rRNA gene sequences using universal primers. This is due to the fact that the ability to synthesize magnetosomes is not a taxonomic descriptor, and MTB and non-MTB may belong to the same taxonomic group (Lefèvre and Bazyliński, 2013). The absence of reference MTB strains in different taxonomic groups also makes it difficult to describe the diversity of MTB using the 16S rRNA gene sequence analysis. For example, the 16S rRNA gene sequences of magnetotactic ‘*Ca. Latescibacteria*’ and *Planctomycetes* have not been identified. Therefore, in this study we designed specific primers on the *mamK* gene

302 sequences to overcome the problem of 16S rRNA gene profiling of the MTB community. We
 303 analyzed these nine genes of all known MTB from *Proteobacteria*, *Nitrospirae*, and ‘*Ca.*
 304 *Omnitrophica*’ phyla for primer system design. Based on our analysis, only *mamK* had a suitable
 305 length and conserved regions within all taxonomic groups for the design of a universal primer
 306 system. It was previously shown that some non-magnetotactic bacterial genomes contain
 307 homologues of MamK (Lefèvre et al., 2013). However, the *mamK* genes belonging to
 308 magnetotactic bacteria have the highest level of similarity with each other compared to the genes of
 309 non-magnetotactic bacteria. Therefore, phylogenetic analysis must be carried out extremely
 310 carefully to exclude potential non-magnetotactic MTB.

311

312 Over the past several years, the information on MTB has increased significantly, leading to
 313 the recent critical discovery of magnetotactic protists (Leão et al., 2019; Monteil et al., 2019). As
 314 the amount of genomic data increased, new taxonomic groups of MTB have been found, indicating
 315 that the biodiversity of magnetotactic bacteria remains underestimated. We expect that continued
 316 improvements in detection and separation techniques will lead to the identification of novel MTB in
 317 the future.

318

319

ACKNOWLEDGMENTS

320 We thank Professor Josh D. Neufeld for permission to use metagenomic data (3300021602).
 321 We thank Dr. Svetlana Zhenilo for microscopy assistance and Dr. Vasil Gaisin for comments that
 322 improved an earlier version of this manuscript. We thank Jefferson Cypriano, CENABIO and
 323 Unimicro for helping with access to the TEM used in this work.

324

FUNDING

325 This study was funded by the Russian Foundation for Basic Research as research project no.
 326 18-34-01005 and by the Ministry of Science and Higher Education of the Russian Federation. This
 327 study was performed using scientific equipment at the Core Research Facility ‘Bioengineering’
 328 (Research Center of Biotechnology, Russian Academy of Sciences). The work conducted by the
 329 U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, was
 330 supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-
 331 AC02-05CH11231.

332

COMPLIANCE WITH ETHICAL STANDARDS

333 The authors declare that they have no conflict of interest. This article does not contain any
 334 studies involving animals or human participants performed by any of the authors.

335

REFERENCES

- 336 Abreu, F., Martins, J. L., Souza Silveira, T., Keim, C. N., Lins de Barros, H. G. P., Filho, F.
337 J. G., Lins, U., “Candidatus Magnetoglobus multicellularis”, a multicellular, magnetotactic
338 prokaryote from a hypersaline environment, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 1318–
339 1322. doi:10.1099/ijms.0.64857-0.
- 340 Blakemore, R. Magnetotactic bacteria, *Science*, 1975, vol. 190, pp. 377–379.
341 doi:10.1126/science.170679.
- 342 Daims, H., Brühl, A., Amann, R., Schleifer, K.-H., Wagner, M. The domain-specific probe
343 EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more
344 comprehensive probe set. *Syst. Appl. Microbiol.*, 1999, vol. 22, pp. 434–444.
345 doi:[https://doi.org/10.1016/S0723-2020\(99\)80053-8](https://doi.org/10.1016/S0723-2020(99)80053-8).
- 346 Du, H., Zhang, R., Zhang, W., Xu, C., Chen, Y., Pan, H., Zhou K., Wu L-F., Xiao T.
347 Characterization of uncultivated magnetotactic bacteria from the sediments of Yuehu Lake, China.
348 *Acta Oceanol. Sin.*, 2017, vol. 36, pp. 94–104. doi:10.1007/s13131-017-0980-8.
- 349 Dziuba, M., Koziaeva, V., Grouzdev, D., Burganskaya, E., Baslerov, R., Kolganova, T.,
350 Chernyadyev A., Osipov G., Andrianova E., Gorlenko V., Kuznetsov B. Magnetospirillum
351 caucaseum sp. nov., Magnetospirillum marisnigri sp. nov. and Magnetospirillum moscoviense sp.
352 nov., freshwater magnetotactic bacteria isolated from three distinct geographical locations in
353 European Russia. *Int. J. Syst. Evol. Microbiol.*, 2016, vol. 66, pp. 2069–2077.
354 doi:10.1099/ijsem.0.000994.
- 355 Dziuba, M. V., Kolganova, T. V., Gorlenko, V. M., Kuznetsov, B. B. Species diversity of
356 magnetotactic bacteria from the Ol’khovka River, Russia. *Microbiology.*, 2013, vol. 82, pp. 335–
357 340. doi:10.1134/s0026261713030028.
- 358 Faivre, D., and Schuler, D. Magnetotactic Bacteria and Magnetosomes. *Chem. Rev.*, 2008,
359 vol. 108, pp. 4875–4898.
- 360 Flies, C. B., Jonkers, H. M., De Beer, D., Bosselmann, K., Böttcher, M. E., Schüler, D.
361 Diversity and vertical distribution of magnetotactic bacteria along chemical gradients in freshwater
362 microcosms. *FEMS Microbiol. Ecol.*, 2005a, vol. 52, pp. 185–195.
363 doi:10.1016/j.femsec.2004.11.006.
- 364 Flies, C. B., Peplies, J., Schüler, D. Combined approach for characterization of uncultivated
365 magnetotactic bacteria from various aquatic environments. *Appl Env. Microbiol.*, 2005b, vol. 71,
366 pp. 2723–2731. doi:10.1128/AEM.71.5.2723.
- 367 Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., Vinh, L. S. UFB002:
368 improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.*, 2017, vol. 35, pp. 518–522.
369 doi:10.5281/zenodo.854445.

- 370 Jogler, C., Lin, W., Meyerdierks, A., Kube, M., Katzmann, E., Flies, C., Pan, Y., Amann, R.,
371 Reinhardt R., Schüler, D. Toward cloning of the magnetotactic metagenome: Identification of
372 magnetosome island gene clusters in uncultivated magnetotactic bacteria from different aquatic
373 sediments. *Appl. Environ. Microbiol.*, 2009, vol. 75, pp. 3972–3979. doi:10.1128/AEM.02701-08.
- 374 Katoh, K., and Standley, D. M. MAFFT multiple sequence alignment software version 7:
375 improvements in performance and usability. *Mol. Biol. Evol.*, 2013, vol. 30, pp. 772–80.
376 doi:10.1093/molbev/mst010.
- 377 Koziaeva, V., Dziuba, M., Leão, P., Uzun, M., Krutkina, M., Grouzdev, D. Genome-Based
378 Metabolic Reconstruction of a Novel Uncultivated Freshwater Magnetotactic coccus “Ca.
379 Magnetaquicoccus inordinatus” UR-1, and Proposal of a Candidate Family “Ca.
380 Magnetaquicoccaceae” Veronika. *Front. Microbiol.*, 2019, vol. 10, pp. 1–20.
381 doi:10.3389/fmicb.2019.02290.
- 382 Kozyaeva, V. V., Grouzdev, D. S., Dziuba, M. V., Kolganova, T. V., Kuznetsov, B. B.
383 Diversity of magnetotactic bacteria of the Moskva River. *Microbiology.*, 2017, vol. 86, pp. 106–
384 112. doi:10.1134/s0026261717010088.
- 385 Kuever, J., Rainey, F. A., Widdel, F. “Syntrophaceae fam. nov.,” in *Bergey’s Manual of*
386 *Systematics of Archaea and Bacteria.*, 2015, pp. 1–2. doi:10.1002/9781118960608.fbm00208.
- 387 Laczny, C. C., Kiefer, C., Galata, V., Fehlmann, T., Backes, C., Keller, A. BusyBee Web□:
388 metagenomic data analysis by bootstrapped supervised binning and annotation. *Nucleic Acids Res.*,
389 2017, vol. 45, pp. W171–W179. doi:10.1093/nar/gkx348.
- 390 Lane, D. J. 16S/23S rRNA sequencing In: Stackebrandt E, Goodfellow M (eds) Nucleic acid
391 techniques in bacterial systematics. *Wiley, New York.*, 1991, pp. 115–175.
- 392 Leão, P., Chen, Y.-R. Y., Abreu, F., Wang, M., Zhang, W. W.-J., Zhou, K., Xiao, T., Wu, L-
393 F., Lins U. Ultrastructure of ellipsoidal magnetotactic multicellular prokaryotes depicts their
394 complex assemblage and cellular polarity in the context of magnetotaxis. *Environ. Microbiol.*,
395 2017, vol. 19, pp. 2151–2163. doi:10.1111/1462-2920.13677.
- 396 Leão, P., Le Nagard, L., Yuan, H., Cypriano, J., Da Silva-Neto, I., Bazylnski, D. A.,
397 Acosta□Avalos, D., de Barros, H. L., Hitchcock, A. P., Lins, U., Abreu, F. Magnetosome magnetite
398 biomineralization in a flagellated protist: evidence for an early evolutionary origin for
399 magnetoreception in eukaryotes. *Environ. Microbiol.*, 2019. doi:10.1111/1462-2920.14711.
- 400 Lefèvre, C. T., and Bazylnski, D. A. Ecology, diversity, and evolution of magnetotactic
401 bacteria. *Microbiol. Mol. Biol. Rev.*, 2013, vol. 77, pp. 497–526. doi:10.1128/MMBR.00021-13.
- 402 Lefevre, C. T., Frankel, R. B., Abreu, F., Lins, U., Bazylnski, D. A. Culture-independent
403 characterization of a novel, uncultivated magnetotactic member of the Nitrospirae phylum. *Environ.*
404 *Microbiol.*, 2011, vol. 13, pp. 538–549. doi:10.1111/j.1462-2920.2010.02361.x.

- 405 Lefèvre, C. T., Trubitsyn, D., Abreu, F., Kolinko, S., Jogler, C., de Almeida, L. G. P., de
406 Vasconcelos, A. T. R., Kube, M., Reinhardt, R., Lins, U., Pignol, D., Schüler, D., Bazylnski, D. A.
407 Ginét, N. Comparative genomic analysis of magnetotactic bacteria from the Deltaproteobacteria
408 provides new insights into magnetite and greigite magnetosome genes required for magnetotaxis.
409 *Environ. Microbiol.*, 2013, vol. 15, pp. 2712–2735. doi:10.1111/1462-2920.12128.
- 410 Li, J., Zhang, H., Liu, P., Menguy, N., Roberts, A. P., Chen, H., Wang, Y., Pan, Y.
411 Phylogenetic and Structural Identification of a Novel Magnetotactic Deltaproteobacteria Strain,
412 WYHR-1, from a Freshwater Lake. *Appl. Environ. Microbiol.*, 2019, vol. 85, pp. e00731-19.
413 doi:<https://doi.org/10.1128/AEM.00731-19>.
- 414 Li, J., Zhang, H., Menguy, N., Benzerara, K., Wang, F., Lin, X., Chen, Z., Pan, Y. Single-
415 cell resolution study of uncultured magnetotactic bacteria via fluorescence-coupled electron
416 microscopy. *Appl. Environ. Microbiol.*, 2017, vol. 83, pp. e00409-17 AEM.00409-17.
417 doi:10.1128/AEM.00409-17.
- 418 Lin, H.-H., and Liao, Y.-C. Accurate binning of metagenomic contigs via automated
419 clustering sequences using information of genomic signatures and marker genes. *Sci. Rep.*, 2016,
420 vol. 6, pp. 24175. doi:10.1038/srep24175.
- 421 Lin, W., Jogler, C., Schüler, D., Pan, Y. Metagenomic analysis reveals unexpected
422 subgenomic diversity of magnetotactic bacteria within the phylum Nitrospirae. *Appl. Environ.*
423 *Microbiol.*, 2011, vol. 77, pp. 323–326.
- 424 Lin, W., and Pan, Y. Specific primers for the detection of freshwater alphaproteobacterial
425 magnetotactic cocci. *Int. Microbiol.*, 2009, vol. 12, pp. 237–242. doi:10.2436/20.1501.01.103.
- 426 Lin, W., and Pan, Y. A putative greigite-type magnetosome gene cluster from the candidate
427 phylum Latescibacteria. *Environ. Microbiol. Rep.*, 2015, vol. 7, pp. 237–242. doi:10.1111/1758-
428 2229.12234.
- 429 Lin, W., Pan, Y., Bazylnski, D. A. Diversity and ecology of and biomineralization by
430 magnetotactic bacteria. *Environ. Microbiol. Rep.*, 2017a, vol. 9, pp 345–356. doi:10.1111/1758-
431 2229.12550.
- 432 Lin, W., Paterson, G. A., Zhu, Q., Wang, Y., Kopylova, E., Li, Y., Knight, R., Bazylnski, D.
433 A., Zhu, R., Kirschvink, J. L., Pan, Y. Origin of microbial biomineralization and magnetotaxis
434 during the Archean. *Proc. Natl. Acad. Sci.*, 2017b, vol. 114, pp. 2171–2176.
435 doi:10.1073/pnas.1614654114.
- 436 Lin, W., Tian, L., Li, J., Pan, Y. Does capillary racetrack-based enrichment reflect the
437 diversity of uncultivated magnetotactic cocci in environmental samples? *FEMS Microbiol. Lett.*,
438 2008, vol. 279, pp. 202–206. doi:10.1111/j.1574-6968.2007.01029.x.
- 439 Monteil, C. L., Perrière, G., Menguy, N., Ginét, N., Alonso, B., Waisbord, N., Cruveiller, S.,

- 440 Pignol, D., Lefèvre, C. T. Genomic study of a novel magnetotactic *Alphaproteobacteria* uncovers
441 the multiple ancestry of magnetotaxis. *Environ. Microbiol.*, 2018, vol. 12, pp. 4415–4430.
442 doi:10.1111/1462-2920.14364.
- 443 Monteil, C. L., Vallenet, D., Menguy, N., Benzerara, K., Barbe, V., Fouteau, S., Cruaud, C.,
444 Floriani, M., Viollier, E., Adryanczyk, G., Leonhardt, N., Faivre, D., Pignol, D., López-García, P.,
445 Weld, R. J., et al. Ectosymbiotic bacteria at the origin of magnetoreception in a marine protist. *Nat.*
446 *Microbiol.*, 2019, vol. 4, pp. 1088-1095. doi:10.1038/s41564-019-0432-7.
- 447 Nguyen, L.-T. T., Schmidt, H. A., Von Haeseler, A., Minh, B. Q. IQ-TREE: A fast and
448 effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.*,
449 2015, vol. 32, pp. 268–274. doi:10.1093/molbev/msu300.
- 450 Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., Tyson, G. W. CheckM²:
451 assessing the quality of microbial genomes recovered from isolates , single cells , and
452 metagenomes. *Genome Res.*, 2015, vol. 25, pp. 1043–1055. doi:10.1101/gr.186072.114.Freely.
- 453 Pernthaler, J., Glöckner, F.-O., Schönhuber, W., Amann, R. Fluorescence in situ
454 hybridization (FISH) with rRNA-targeted oligonucleotide probes. *Methods Microbiol.*, 2001, vol.
455 30, pp. 207–226. doi:https://doi.org/10.1016/S0580-9517(01)30046-6.
- 456 Postec, A., Tapia, N., Bernadac, A., Joseph, M., Davidson, S., Wu, L. F., Ollivier, B., Pradel,
457 N. Magnetotactic Bacteria in Microcosms Originating from the French Mediterranean Coast
458 Subjected to Oil Industry Activities. *Microb. Ecol.*, 2012, vol. 63, pp. 1–11. doi:10.1007/s00248-
459 011-9910-z.
- 460 Pradel, N., Fuduche, M., Ollivier, B. Magnetotactic bacteria population in a pristine French
461 Atlantic lagoon. *Environ. Sci. Pollut. Res.*, 2016, vol. 23, pp. 691–697. doi:10.1007/s11356-015-
462 5322-9.
- 463 Qian, X. X., Liu, J., Menguy, N., Li, J., Alberto, F., Teng, Z., Xiao, T., Zhang, W., Wu, L-F.
464 Identification of novel species of marine magnetotactic bacteria affiliated with Nitrospirae phylum.
465 *Environ. Microbiol. Rep.*, 2019, vol. 11, pp. 330–337. doi:10.1111/1758-2229.12755.
- 466 Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular cloning: a laboratory manual*. 2nd
467 ed. New York. 1989.
- 468 Sieber, C. M. K., Probst, A. J., Sharrar, A., Thomas, B. C., Hess, M., Tringe, S. G., Banfield
469 J. F. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy.
470 *Nat. Microbiol.*, 2018, vol. 3, pp. 836–843. doi:10.1038/s41564-018-0171-1.
- 471 Spring, S., Amann, R., Ludwig, W., Schleifer, K. H., Van Gernerden, H., Petersen, N.
472 Dominating role of an unusual magnetotactic bacterium in the microaerobic zone of a freshwater
473 sediment. *Appl. Environ. Microbiol.*, 1993, vol. 59, pp. 2397–2403.
- 474 Uebe, R., and Schüler, D. Magnetosome biogenesis in magnetotactic bacteria. *Nat. Rev.*

475 *Microbiol.*, 2016, vol. 14, pp. 621–637. doi:10.1038/nrmicro.2016.99.

476 Kalyaanamoorthy, S., Jermiin, L. S., Minh, B. Q., Wong, T. K. F., and von Haeseler, A.,

477 Jermiin, L. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods.*,

478 2017, vol. 14, pp. 587–589. doi:10.1038/nmeth.4285.

479 Wu, Y.-W., Tang, Y.-H., Tringe, S. G., Simmons, B. A., Singer, S. W. MaxBin: an

480 automated binning method to recover individual genomes from metagenomes using an expectation-

481 maximization algorithm. *Microbiome.*, 2014, vol. 2, pp. 26.

482 Xu, C., Zhang, W., Pan, H., Du, H., Xiao, T. Distribution and diversity of magnetotactic

483 bacteria in sediments of the Yellow Sea continental shelf. *J. Soils Sediments*, 2018, vol. 18. pp.

484 2634–2646. doi:10.1007/s11368-018-1912-8.

485

486 **Figure captions**

487 Figure 1. Scheme of MTB-CoSe separation procedure that allows collection of magnetotactic
488 components directly from the sediment samples.

489 Figure 2. Maximum-likelihood phylogenetic trees based on magnetosome associated protein MamK
490 (285 amino acid sites) reconstructed with evolutionary model LG+I+G4 (a); and 16S rRNA gene
491 sequences (1,399 nucleotide sites) reconstructed using the evolutionary model GTR+F+I+G4 (b).

492 Figure 3. FISH-TEM images of MTB from environmental samples. The circles and arrows indicate
493 the cells that did and did not, respectively, hybridize with the specific probe tested. Phase contrast
494 image of magnetically enriched environmental samples on Formvar-coated TEM grids (a, e, i, and
495 m); Bacteria present in the same region as imaged in a, e, i, and m, respectively, stained with DAPI
496 (b, f, j, and n); Image of the same area captured in images b, f, j, and n after hybridization with the
497 LBB_01, LBB_02, LBB_03, and LBB_04 probes, respectively (c, g, k, and o); TEM image of the
498 same cell that showed hybridization (circles) with the specific probes in c, g, k, and o (d, h, l, and
499 p).

500 Figure 4. TEM images of magnetotactic coccus (a) and magnetotactic spirilla (b) identified in
501 environmental samples. Bar 0.5 μ m.

502 Table 1. The list of primers and probes designed for *mamK* gene amplification and for FISH-TEM
503 analysis, respectively.

name of primer/probe	sequence 5' - 3'	Fluorescent label
mamK_79F	TNGGNDTHGAYYTNGGNACNTC	-
mamK_86F	GNATHGAYYTDGGNACNT	-
mamK_521R	CVACNNNRAANGGYTCNG	-
mamK_577R	GTNCCNGCVCCDATRTC	-
LBB_01	CCCCACAAAAGCGGTTTACGACCCGA	Cy3
LBB_02	GCCGTGGCTTATTCTTAAGGTACCGT	Cy3
LBB_03	CTCAGAGTCAGTCAAGACCCAGAA	Alexa 594
LBB_04	TGTCCAACCGAGGTAAAAACAGCA	Alexa 594

504 Table 2. Operational taxonomic units (OTUs) of MamK sequences retrived in this study and closest
505 representatives of MTB

OTU name	Number of clones	Taxonomy	Closest MamK in GenBank	Identity, %
MamK_LBB_01	301	<i>Nitrospirae</i>	' <i>Ca. Magnetobacterium bavaricum</i> ' TM-1	61.8
MamK_LBB_02	13	<i>Nitrospirae</i>	' <i>Ca. Magnetominusculus xianensis</i> ' HCH-1	85.4
MamK_LBB_03	25	<i>Nitrospirae</i>	Nitrospirae bacterium MYbin3	87.5
MamK_LBB_04	3	<i>Deltaproteobacteria</i>	MAG_21602_syn32*	97.0
MamK_LBB_05	1	<i>Alphaproteobacteria</i>	Alphaproteobacterium WMHbin7	100.0
			Fos002	100.0
MamK_LBB_06	3	' <i>Ca. Etaproteobacteria</i> '	HA3dbin1	98.4

506 *was obtained in this study from IMG metagenome 3300021602

507 Table 3. Operational taxonomic units (OTUs) of 16S rRNA gene sequences and closest
508 representatives of MTB

OTU name	Number of clones	Taxonomy	Closest 16S rRNA gene in GenBank	Identity, %
16S_LBB_01	251	<i>Nitrospirae</i>	' <i>Ca. Magnetobacterium bavaricum</i> ' TM-1	92.2
			' <i>Ca. Magnetominusculus xianensis</i> ' HCH-1	89.9
			' <i>Ca. Magnetoovum mohavensis</i> '	89.0
16S_LBB_02	16	<i>Nitrospirae</i>	' <i>Ca. Magnetominusculus xianensis</i> ' HCH-1	99.2
16S_LBB_03	3	<i>Nitrospirae</i>	Nitrospirae bacterium MYbin3	92.8
16S_LBB_04	1	<i>Deltaproteobacteria</i>	MAG_21602_syn32*	96.8
16S_LBB_05	2	<i>Alphaproteobacteria</i>	Alphaproteobacterium LM-1	98.5
			Alphaproteobacterium WMHbin7	98.3
16S_LBB_06	9	' <i>Ca. Etaproteobacteria</i> '	<i>Magnetococcus</i> sp. clone	98.9

			OTU2	
509	*was obtained in this study from IMG metagenome 3300021602			

sediment
homogenization

1

PBS
microbeads

filtration

2

cell concentration

3

MTB separation

4

MTB collection

5

A

B

A **B**