Research Article

Unexpected Diversity of Magnetococci in Intertidal Sediments of Xiaoshi Island in the North Yellow Sea

Haitao Chen,1,2 Jinhua Li,3 Xiang Xing,2 Zongjun Du,2 and Guanjun Chen2,4

1Deep-Sea Microbial Cell Biology, Department of Deep Sea Science, Sanya Institute of Deep-Sea Science and Engineering, Chinese Academy of Sciences, 62 Fenghuang Road, Sanya 572000, China
2College of Marine Science, Shandong University at Weihai, Weihai 264209, China
3Key Laboratory of Earth and Planetary Physics, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing 100029, China
4State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, China

Correspondence should be addressed to Guanjun Chen; guanjun@sdu.edu.cn

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Magnetotactic bacteria (MTB) are a group of prokaryotes that, despite their high morphological, phylogenetic, and ecological diversity, share one common capability of swimming along geomagnetic field lines in a process called magnetotaxis [1, 2]. This behavior is based on the dedicated intracellular organelle, the magnetosome, which consists of chain-structured nanocrystals of magnetite (Fe₃O₄) or greigite (Fe₃S₄), called magnetosomes, and swimming along geomagnetic field lines in a process called magnetotaxis. In this study, we investigated the MTB diversity within the intertidal sediments near Xiaoshi Island (Weihai) in the North Yellow Sea using a combination of restriction fragment length polymorphism (RFLP) analysis and 16S rRNA gene sequencing revealed seven new MTB genera affiliated with the Alphaproteobacteria class. Fluorescence in situ hybridization (FISH) analyses suggested that one magnetotactic coccus (designated as WHI-2) is the dominant species. TEM observations and energy dispersive X-ray analyses revealed that MTB cells mainly form magnetite magnetosomes that are organized into two chains of magnetosomes composed of e-prismatic magnetite crystals. This finding suggests the adaptation of a magnetotactic bacterial population to the marine tide. This is the first report of magnetotactic bacteria near Xiaoshi Island, which should be useful for studies of biogeochemical cycling and the geohistory of this area.

1. Introduction

Magnetotactic bacteria (MTB) are a group of prokaryotes that, despite their high morphological, phylogenetic, physiological, and ecological diversity, share one common capability of swimming along geomagnetic field lines, a process called magnetotaxis [1, 2]. This behavior is based on the dedicated intracellular organelle, the magnetosome, which consists of chain-structured nanocrystals of magnetite (Fe₃O₄) or greigite (Fe₃S₄) [3]. The chain structure of magnetosomes maximizes the total magnetic moment of the MTB cells, thus allowing MTB to sensitively follow geomagnetic field lines and to efficiently search for their favorite habitats within aquatic environments [4, 5]. Due to the unique biological and magnetic features of magnetosomes, MTB have long been of interest not only in biosciences and geosciences but also in nanomaterial science [6–8].

MTB are globally distributed and have been detected in various aquatic environments including lakes, rivers, ponds, estuary, lagoons, and marine sediments [9–17]. Petermann and Bleil discovered MTB in pelagic and hemipelagic sediments of the eastern South Atlantic Ocean, extending known MTB habitats to the deep sea [18]. Recently, MTB have also been found in various extreme environments [19, 20]. Morphologically, the MTB discovered so far include cocci, ovoid, bean-shaped, rod-shaped, spirilla, and multicellular bacteria [11–14]. Phylogenetically, most known MTB belong to the Proteobacteria phylum, including the Alpha-, Gamma-, and Deltaproteobacteria classes [21, 22]. Several uncultured MTB communities are affiliated with the Nitrospirae phylum, and one magnetotactic bacterium, strain SKK-01, is within Omnitrophica phylum [16]. Considering their worldwide distribution and highly phylogenetic diversity, MTB may
have played an important role in the past geochemical cycling of iron and may continue to do so [23], and their magnetic remains (i.e., magnetofossils) may have contributed significantly to the natural remnant magnetization of some marine and lake sediments [9, 10].

Despite their worldwide distribution and highly phylogenetic diversity, MTB are extremely fastidious and, therefore, difficult to isolate and grow. In the past 40 years, only a few strains have been isolated and purely cultured in the lab, and two Magnetospirillum strains (i.e., Magnetospirillum magneticum AMB-1 and Magnetospirillum gryphiswaldense MSR-1) have been extensively and deeply studied to understand the molecular mechanisms of magnetosome formation [24, 25]. Culture-independent studies on uncultured MTB within natural environments are still essential for better understanding the diversity and geological contribution of such intriguing microorganisms. In this study, we investigated the phylogenetic diversity and morphological features of MTB within the intertidal sediments near Xiaoshi Island (Weihai) using a combination of molecular ecology techniques and transmission electron microscopy (TEM). Seven new MTB genera affiliated with the Alphaproteobacteria class were detected by 16S rRNA gene sequencing, and one dominant magnetotactic coccus was characterized in detail using TEM.

2. Materials and Methods

2.1. Field Sampling and Set-Up of Microcosms. Marine sediments were collected from the surface layer (~3 to 5 cm) of the intertidalite near Xiaoshi Island (Weihai, north latitude 37°31’14.18” and east longitude 122°01’05.11” in the North Yellow Sea. The pH, temperature, and salinity of sediments measured at the moment of sampling were 8.1, 18.5°C, and 28.9‰, respectively. To set up microcosms, approximately 400 mL of sediments was transferred to 1-liter plastic bottles covered with ~100 mL of sea water from the sampling sites, covered loosely, and stored at room temperature in dim light. The quantity and community of MTB in the microcosms were periodically checked with the hanging drop method using an optical microscope (Olympus BX41) [26–28].

2.2. Magnetic Collection and Sample Preparation. Collection of MTB was performed by a two-step magnetic collection strategy as described previously [29]. Briefly, MTB cells were first collected from sediments in microcosms by attaching small magnets to bottles above the sediment surface with the magnetic S-pole toward the bottles. Collected MTB were then purified from nonmagnetic contaminations by a homemade “MTB trap” [30] or glass capillary [31]. The purified MTB cells were then washed with sterile distilled water twice and resuspended in 100 µL of sterile distilled water for TEM and PCR amplification.

2.3. Transmission Electron Microscopy (TEM). For TEM observations, approximately 10 µL of MTB cells was deposited onto a Formvar-supported, carbon-coated copper grid. After drying for 4–6 h at room temperature, the copper grids were cleaned with sterile distilled water approximately 5 times and dried in air. TEM experiments were performed on a JEM-2100 TEM with an accelerating voltage of 200 kV, equipped with an Oxford SDD detector (X-MaxN 80T). In that condition, the chemical composition and crystal structure of magnetosomes were also determined by energy dispersive X-ray spectroscopy (EDXS) and selected area electron diffraction (SAED), respectively [32].

2.4. PCR Amplification and RFLP Analysis. 16S rRNA gene sequences of magnetotactic bacteria were amplified as described previously by Lin et al. [13]. Polymerase chain reactions (PCR) were then carried out with the following cycle: a 6 min initial denaturing step at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 53°C, and 1.5 min at 72°C, and a final extension step of 10 min at 72°C. PCR products were checked by electrophoresis in 1% agarose gels and by ethidium bromide staining. PCR products were ligated into the pMD18-T cloning vector (Takara, Japan) and transformed into Escherichia coli DH5α to construct a 16S rRNA gene clone library. Positive clones were selected by blue-white screening and confirmed by PCR. PCR products containing the 16S rRNA gene sequences from positive clones were digested at 37°C withMspI and Rsal (Takara, Japan) for 3 h prior to RFLP analysis and were separated in 4% agarose gels. Clones with different band patterns were sequenced by Shanghai Sunny Biotechnology Co., Ltd.

2.5. Phylogenetic Analysis. The partial and full-length 16S rRNA gene sequences retrieved in this study were first analyzed using the BLAST search program on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The related sequences were preliminarily aligned with the default setting of CLUSTALW [33]. Phylogenetic tree analysis was performed with the software of MEGA 6.0 using the neighbor-joining method. To validate the reproducibility of the branching pattern of the tree, a bootstrap analysis of 1000 replicates was performed. The sequences retrieved in this study were submitted in GenBank database with accession numbers KT327168–KT327175.

2.6. Fluorescence In Situ Hybridization. In this study, we designed a specific oligonucleotide probe: 5’-CTTTCGGCTCTTGAGGCTTATGCC-3’ (positions 166 to 187), designated WH1-166. The bacterial universal probe EuB338 (5’-GCTGCCCTCCCGTAGGAGT-3’, positions 338 to 355, E. coli numbering) was used as a control [34]. The specificity of the probe was evaluated through the Ribosomal Database Project II (RDP-II) in the PROBE_MATCH program and through the NCBI website [34, 35]. The specific oligonucleotide DNA probe was labeled at the 5’ end with hydrophilic sulfoindocyanine Cy3, and the general probe was labeled at the 5’ end with phosphoramidite FAM. Both probes were synthesized and fluorescently labeled by Invitrogen Co., Ltd. Meimagnetically enriched MTB cells were fixed with 4% paraformaldehyde as described by Perntrather et al. Then, the cells were washed in 1×PBS and stored in ethanol-PBS (1:1) at −20°C. Approximately 20 µL of fixed cells was dropped onto a glass slide prepared by coating with gelatin. A second fixation (dehydration) was performed by applying increasing concentrations of ethanol at 50, 80, and 100%,
Table 1: Magnetotactic cocci morphotypes present in the sampled sediments, based on TEM.

<table>
<thead>
<tr>
<th>Size diameter (μm)</th>
<th>Number of magnetite chains</th>
<th>Number of crystals per cell</th>
<th>Crystals mean sizes (nm)</th>
<th>Number of magnetosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Length (σ)</td>
<td>Width (σ)</td>
</tr>
<tr>
<td>MC a</td>
<td>~2 to 3</td>
<td>2</td>
<td>~19</td>
<td>140 (46)</td>
</tr>
<tr>
<td>MC b</td>
<td>~1.5 to 3</td>
<td>2</td>
<td>~21</td>
<td>128 (35)</td>
</tr>
<tr>
<td>MC c</td>
<td>~2 to 2.5</td>
<td>2</td>
<td>~23</td>
<td>115 (37)</td>
</tr>
<tr>
<td>MC d</td>
<td>~1 to 2</td>
<td>2</td>
<td>~24</td>
<td>122 (28)</td>
</tr>
<tr>
<td>MC e</td>
<td>~1 to 2</td>
<td>2</td>
<td>~22</td>
<td>112 (31)</td>
</tr>
<tr>
<td>MC f</td>
<td>~1</td>
<td>2</td>
<td>15</td>
<td>118 (30)</td>
</tr>
<tr>
<td>MC g</td>
<td>~2</td>
<td>1</td>
<td>9</td>
<td>130 (37)</td>
</tr>
<tr>
<td>MC h</td>
<td>~1.5 to 2</td>
<td>Irregular</td>
<td>~22</td>
<td>82 (18)</td>
</tr>
<tr>
<td>MC i</td>
<td>~1.5</td>
<td>Irregular</td>
<td>19</td>
<td>68 (13)</td>
</tr>
</tbody>
</table>

Note: σ: standard deviation.

Figure 1: Morphologies of the enriched MTB cells. These MTB include nine morphotypes of magnetococci with elongated prismatic magnetosomes (designated MC a to MC i).

respectively. Then, 1 μL of probe (50 ng/μL) was mixed with 9 μL of hybridization buffer and used for fluorescence in situ hybridization, at 46°C for 3 h in hybridization buffer with 35% deionized formamide. The samples were washed with elution buffer for 25 min at 48°C, rinsed with water, and stained with 4′,6′-diamidino-2-phenylindole (Sigma), and the samples were washed and dried again. The results were recorded using a fluorescence microscope (Olympus BX51) [36–38].

3. Results and Discussion

3.1. Morphological and Chemical Features of MTB. As MTB are adapted to complex chemical gradients, it is very difficult to isolate and cultivate these cells in the laboratory. However, they were found to be of high abundance and ubiquitous in both marine and freshwater habitats. Using the hanging drop method, the MTB of sediment samples were detected and observed. The density of cells is up to 10^4–10^5 cells/cm³. The dominant morph type of the enriched MTB from
Figure 2: Continued.
Figure 2: Continued.
Xiaoshi Island was a coccid-to-ovoid cell shape of various dimensions. The morphotypes are defined principally on the basis of morphology, including cell size, magnetosome chains, magnetite crystals, and inclusion particles in cells. Statistical analysis revealed nine distinct groups (Table 1). This is in contrast to the intertidal MTB in the Qingdao Sea area, which have a single form [12, 17]; it is speculated that this is related to the particular ecological environment of Weihai.

Optical microscopy observations show that magnetotactic cocci are dominant in the samples collected from the intertidalite near Xiaoshi Island. TEM observations revealed that these bacteria are highly diverse based on morphological features of cells and magnetosomes. At least nine distinct phenotypes (designated MC a–i) can be recognized on the basis of cell size, magnetosome chains, magnetite crystals, and inclusion particles in the cells. MC d is the most enriched in these groups under TEM observation. The size of all observed bacteria ranges between 1 and 4 µm. Two parallel or cross magnetosome chains are observed in MC a to f, whereas MC g has a single chain, and MC h and MC i have stacked magnetosomes (Figure 1). According to the recent reported references, MTB are found to display various cell morphologies, such as cocci, ovoid, bean-like shaped, rod shaped, spirilla, and a number of giant multicellular MTB [1–3]. Cell sizes are also very different, even in MTB with the same shape. For some magnetotactic cocci, the size of magnetooovoid MO-1 is about 1.3 by 1.8 µm [15], while the stain SKK-01 from the OP3 division, also ovoid-shaped, reaches 2.3 by 3.7 µm [16]. Among the collected magnetotactic cocci, we detected the MC g type, which is very similar to the previous descriptions of south-seeking magnetotactic cocci within the Alphaproteobacteria [39].

Magnetosomes in these cocci are all prismatic, with average shape factors from 0.71 to 0.94. The sizes of crystals range in length from 68 to 140 nm and in width from 51 to 122 nm. These crystals are likely to be single-domain magnetites according to the theoretical prediction described by Butler and Banerjee [40]. The size distribution of the magnetic crystals from MC a–i cells was broader compared with the crystals from Magnetococcus marinus MC-1 and Magnetovibrio blakemorei MV-1 (Figure 2) [41].

Furthermore, the SAED pattern and the high resolution transmission electron microscopy (HRTEM) images indicated that magnetosomes produced by magnetococci MC h are well crystallized magnetite with hexagonal prism crystal morphology (Figures 3(b) and 3(c)). Analyses by EDXS revealed the “iron-oxide” composition of the magnetosome crystals in MC h (Figures 3(a), 3(d), and 3(e)) and two types of particles in the MC a, MC c, and MC d cells (Figures 3(f), 3(g), and 3(h)); one type of particle contains O and S as in MC c (Figures 3(h) and 3(j)), while the other type contains O, Mg, P, and Ca as in MC d (Figures 3(f) and 3(i)). Interestingly, for the MC a bacteria, both particles are observed (Figures 3(g), 3(j), and 3(k)). X-ray spectroscopy analysis showed that magnetosomes from MTB of the intertidal area in Weihai were mainly composed of both Fe and O elements, which, combined with the diffraction SAED results, showed that the crystal type of magnetosome was magnetite. Furthermore, cells of these uncultured magnetotactic cocci contained three types of intracellular inclusions, a series of magnetosomes composed of magnetosome chains, that were crystals of magnetite. In addition, P-rich bodies and S-containing inclusions occupied the majority of the cell volume. The roles of these particles in the cell are not yet clear, and research in this area is relatively limited. According to Cox et al., the presence of these large P-rich and S-containing inclusions might be an indication of cell stress, or these inclusions may be important in cell buoyancy or in energy generation or useful as stored electron donors [42]. Because of their remarkable capacity of assimilation of iron, sulfur, phosphorus, and so on, MTB play an important role in the geochemical cycling in natural habitats. Moreover, considering their wide distribution in diverse aquatic and sedimentary ecosystems and their high intracellular iron content, MTB have global significance in bulk sedimentary magnetism and have numerous versatile applications in paleoenvironmental reconstructions and biotechnological and biomedical fields [6–9].

3.2. Phylogenetic Diversity of MTB. In this study, more than 70 positive clones were analyzed by RFLP, which can be defined as an operational taxonomic unit (OTU) and may often be inferred to be a single population within
a community. There are seven OTUs in total, of which one OTU, designated WHI-2, accounts for the main maps. One to two representative clones of each OTU were then selected for sequencing and phylogenetic analysis (Figure 4). The results indicated that seven of these sequences show similarity to the vast majority of reported MTB sequences, with more than 85–97% identity, whereas others are far from the known MTB (<75%), indicating that these sequences were likely derived from the environmental pollution; therefore, these sequences would not be used in the subsequent analysis. All of these eight sequences (designated WHI-2, WHI-8, WHI-9, WHI-14, WHI-17, WHI-18, WHI-27, and WHI-32) associate with the Alphaproteobacteria and are clustered closely to the unsequenced ovoid-coccoid magnetotactic bacteria. Through comparison to the NCBI nucleotide database using the BLAST algorithm, only WHI-32 had a high identity of 97% with the magnetotactic bacterium strain rj516, and the similarity of the other several strains was lower than 95% [43].
Because WHI-2 and WHI-18 share 99% identity with each other (based on ≥98% genetic similarity to sequences from just one species), therefore, the other identified strains might correspond to new genera and species; so our results identified seven MTB species in the ovoid-coccoid magnetotactic bacteria of Xiaoshi Island.

Uncultured MTB in the environment are usually identified using fluorescence in situ hybridization analysis. It was confirmed that the sequences collected here originated from the enriched cocci. Based on the grouping of WHI-2 and WHI-18, a specific oligonucleotide probe, WHI-166, was designed to be complementary to these retrieved sequences and applied to fluorescence in situ hybridization of the MTB cells. Fluorescence microscopy revealed a strong signal, and the designed specific probes only recognized the magnetotactic cocci cells (Figure 5), whereas clones of E. coli could not be hybridized as controls (Figures 5(a)–5(c)). These results suggested that the sequences WHI-2 and WHI-18 did come from a population of environment sample because they could be specifically hybridized with the specific probe (Figures 5(d)–5(f)).

In our results for the phylogenetic tree, MTB belonging to the Alphaproteobacteria represent the dominant group in this marine environment, with magnetotactic cocci being the dominant type of MTB as reported in other studies worldwide [3]. The seven new strains of uncultured cocci being the dominant type of MTB as reported in other studies worldwide [3].

Figure 4: Phylogenetic tree showing the relationships between the 7 MTB OTUs in the Alphaproteobacteria. The tree was generated using neighbor-joining analysis. The numbers at nodes represent bootstrap values for the nodes (1000 times resampling analysis).
magnetotactic species are powerful approaches for obtaining further insight into genetic diversity. The results showed that WHI-2 is one of the main species, with a prevalence of approximately 35%. As expected, by designing probe WHI-166 for FISH analysis, we observed excellent results. The WHI-17 16S rRNA gene sequence showed 94% identity with the marine strain clone XSE-42 from the intertidal zone of Qingdao Huiquan Bay. The 16S rRNA gene sequences from all the other strains showed identity of 93%–88% with uncultured Magnetococcus sp. 1–9 collected from the intertidal zone in Huiquan Bay [12, 17]. At the same time, compared with the strains Magnetococcus marinus MC-1, Magnetovibrio blakemorei MV-1, and magnetoooid bacterium MO-1, among others, the maximum identity between the strains was only approximately 90%; therefore, with the exception of WHI-32, all of these strains represent new genus, demonstrating that the Weihai intertidal zone has rich MTB diversity [44–46]. These results show that there were differences between the bacterial diversity of different regions; geographic distances may be a major factor affecting the distribution of MTB communities. It is important that we understand more about the distribution, habits, and population dynamics of MTB and the characteristics of their magnetosomes. Indeed, Edwards and Bazylinski stated that even when population sizes are small relative to other prokaryotic groups in aquatic, soil, and sediments, the impact that MTB, and the tiny crystals they produce, can have in present-day biogeochemical cycling can be large; and the magnetosomes minerals that make it to the geological rock record may serve as better paleoenvironmental indicators [47].

4. Conclusions

As demonstrated in our study, the wide range of cell sizes, the different magnetosome arrangements and crystal shape, and the large number of different retrieved 16S rRNA gene sequences suggest that the MTB of Weihai represent several different magnetotactic cocci. These data also increase the view of Alphaproteobacteria as a major and diversified class of MTB in marine environments. Our work highlights the most important diversity of MTB in Xiaoshi Island and develops a better understanding of the molecular ecology and biology of natural MTB cells.

Both live and dead magnetotactic bacterial cells have proven useful in environmental, magnetic, and nanomedical applications [3]. After the death of the MTB, the magnetite particles settle through the ambient water column forming part of the bottom sediments; such magnetites in sediments, which are of biogenic parentage, are called magnetofossils [48]. The single-domain magnetofossils preserved in sediments are not only ideal carriers of magnetization for paleomagnetism but also potential biomarkers for retrieving paleoenvironmental information [23, 49]. More research involving the distribution and ecology of MTB in nature, especially in marine habitats, will help us better understand the conditions under which and to what extent MTB affect the biogeochemical cycle and the present-day biogeography. In addition, bacterial magnetite magnetosomes may prove to be useful and perhaps superior to chemically produced magnetite nanoparticles in the medical applications [50]. However, the research for magnetosomes from MTB as carriers of genes or drugs for cancer therapy or other diseases is limited, thus providing a challenge for follow-up studies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.
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