Novel Rod-Shaped Magnetotactic Bacteria Belonging to the Class Alphaproteobacteria

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Novel large, rod-shaped magnetotactic bacteria (MTB) were discovered in intertidal sediments of the Yellow Sea, China. They biominerlized more than 300 rectangular magnetite magnetosomes per cell. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that they are affiliated with the Alphaproteobacteria and may represent a new genus of MTB.

Magnetotactic bacteria (MTB) are ubiquitous in the water column and in sediments of freshwater and marine habitats (1, 2). MTB can form intracellular crystals termed magnetosomes, usually consisting of magnetite or greigite (3–5). MTB benefit from these magnetosomes, which confer an ability to orient and navigate along geomagnetic field lines, a unique form of motility referred as magnetotaxis (1, 3, 6, 7). MTB have been identified in Proteobacteria, Nitrospirae, and candidate division OP3 (5, 8–12), and a variety of morphological types have been found (6), of which coccoid is the dominant morphology (13–19). Here, we report a novel group of large, marine, rod-shaped MTB collected from Huiquan Bay (36°03’N, 120°21’E), China.

Sediments and water were collected and stored in 500-ml plastic bottles, and MTB collected using the capillary racetrack method were observed by optical microscopy (BX51; Olympus) using the hanging-drop method in an applied magnetic field (20, 21). Freshly collected MTB exhibited various cell shapes, including rods, vibrios, spirilla, and the dominant coccoid morphotype. After incubation in the dark at room temperature for 6 months (15), the MTB community varied greatly, and a group of large, rod-shaped MTB increased in numbers and became the dominant morphotype. Previous reports have described temporal variations in MTB communities in microcosms under laboratory conditions (2, 22).

The laboratory-enriched rod-shaped MTB cells from sediments were homogeneous in morphology, with an average length of 10.07 ± 1.87 μm and an average width of 3.51 ± 0.49 μm. Differential interference contrast (DIC) microscopy revealed that these cells usually possessed an interstice dividing the cell into two parts (Fig. 1B). An analysis of the ratio distribution of the lengths of the two parts showed that the lengths were usually unequal (n = 357; Fig. 1C). When exposed to an applied magnetic field, the rod-shaped MTB display north-seeking polarity (Fig. 1A).

Fluorescence microscopy of cells revealed membrane-like septa between the two parts (Fig. 1D), consistent with the morphology observed using DIC microscopy. When observed by transmission electron microscope (TEM; Hitachi H8100), two electron-dense structures were found around the center (Fig. 2A1). However, no obvious interstice was observed on the outer membrane of cells (Fig. 2A1), and energy-dispersive X-ray spectroscopy (EDXS) analysis showed no obvious differences in elemental composition. It may be that the membrane layer is not readily detected, possibly accounting for the observation of an interstice between the two parts when using DIC microscopy.

The large, rod-shaped MTB contained 320 to 567 magnetosomes per cell, arranged in a rope-like bundle formed by four to six parallel chains across the interface between the two parts of the cell (Fig. 2A2). A similar bundle of magnetosomes has been observed in large, freshwater, rod-shaped Nitrospirae strains (23) and large, watermelon-shaped MTB (12). Each magnetosome had a rectangular projected shape, with a length of 113 ± 16 nm and a width of 66 ± 11 nm (n = 370). This produced a shape factor of approximately 0.58 ± 0.07 (Fig. 2B). EDXS analysis indicated that the magnetosome crystals were composed of iron and oxygen (Fig. 2C). Consistently, the analysis by high-resolution TEM (HRTEM) identified magnetosome crystals as magnetite (Fig. 2D).

To determine the 16S rRNA gene sequence of the large, rod-shaped MTB, we extracted the DNA from purified samples, performed amplification by PCR using a pair of universal primers, 27f and 1492r (18, 24), and constructed a 16S rRNA gene library of 92 clones as previously reported (18). One dominant operational taxonomic unit (OTU) (86 clones, 93%) was identified by restriction fragment length polymorphism (RFLP) analysis and sequenced (13, 18).

The sequence obtained was analyzed by BLAST and CLUSTAL W multiple alignment (25), and a phylogenetic tree was constructed using MEGA 4.0 (26, 27), applying the neighbor-joining method. Analysis revealed that the 16S rRNA gene sequence showed maximum sequence identity (91.7%) with uncultured magnetococci collected from intertidal sediments of the China Sea (EF371486) and affiliated with the class Alp-
**haproteobacteria** (Fig. 3). This sequence exhibited a divergence of >8% from those of all previously reported bacteria. Therefore, the rod-shaped MTB described here may represent a new genus of MTB.

We further corroborated the authenticity of the novel sequence as being that of rod-shaped MTB by fluorescence in situ hybridization (FISH). The specific oligonucleotide probe p-774 (5'-CCA ACA ACC AGC ACT CAT CG-3'; positions 774 to 793)

**FIG 1** Morphology of large, rod-shaped MTB based on optical microscopy. DIC images of large, rod-shaped MTB are shown in panels A and B. The ratio distribution of the lengths of the two parts is shown in panel C. Panel D shows the fluorescence of large, rod-shaped MTB exposed to blue light (wavelength, 450 to 480 nm). Scale bars, 20 µm in panel A and 10 µm in panels B and D.

**FIG 2** Characteristics of large, rod-shaped MTB cells and intracellular features determined by TEM. (A1 and A2) Morphology of large, rod-shaped MTB cells (A1) and magnetosome chains (A2). (B1 and B2) Characteristics of magnetosomes: size histograms (B1) and shape factor distribution (B2). (C) EDXS analysis of magnetosomes. Top trace, magnetosomes (note the peaks of iron and oxygen); bottom trace, cell. a.u., arbitrary units. (D) High-resolution TEM images of a magnetosome (D1) and magnification of a selected area (D2). Scale bars, 2 µm in panel A1, 200 nm in panel A2, 5 nm in panel D1, and 1 nm in panel D2.
Nucleotide sequence accession numbers. The sequence obtained in this study was deposited in GenBank under accession number JX515337.

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REFERENCES


FIG 3 Phylogenetic tree showing the relationship between the novel, large, rod-shaped MTB and related magnetotactic bacteria. The tree was constructed based on neighbor-joining analysis using the sequence region from position 27 to position 1492 (E. coli numbering), and bootstrap values were calculated using 1,000 replicates. The description and accession number of the MTB whose sequence was determined in this study are shown in bold. GenBank accession numbers of the sequences used are indicated in parentheses. Scale bar, 0.01 substitution per nucleotide position.

FIG 4 FISH analyses of magnetically enriched, rod-shaped MTB cells. The same microscopic field is shown following hybridization with the 5′-FAM-labeled universal bacterial probe EUB338 (A) and with the 5′-Cy3-labeled probe (B). Scale bars, 5 μm.


