Diversity of multicellular magnetotactic prokaryotes in the intertidal zone of Huiquan Bay, Qingdao

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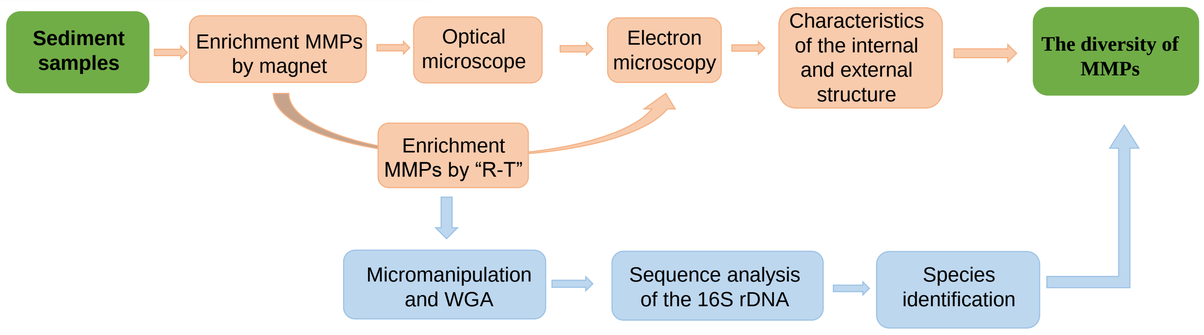
Introduction

Multicellular magnetotactic prokaryotes (MMPs) are a group of aggregates composed of 10-100 gram-negative cells synthesizing intracellular magnetic crystals. Two morphotypes of MMPs have been identified, including several species of globally distributed spherical mulberry-like MMPs (sMMPs), and ellipsoidal pineapple-like MMPs (eMMPs).

Experiment



Results

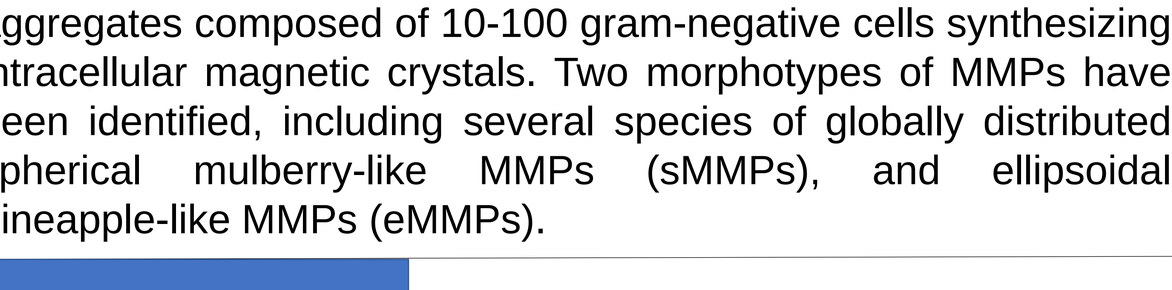


Fig. 1 Morphology and motion under optical microscope.
a and b The MMPs aligned along magnetic lines (the direction of the applied magnetic field was indicated at the bottom left) and accumulated at the north pole of the droplet. The red and black arrow sMMPs and eMMPs respectively. c and d showing magnifications of eMMPs and sMMPs respectively.
Bars = 20 μm in a, 10 μm in b and 2 μm in other panels.

Optical microscopy showed that there were two types of MMPs in the area, including sMMPs and eMMPs. The size of eMMPs was 9.25±0.79 × 7.48±0.79 μm (n = 24), and the average diameter of sMMPs was 5±0.66 μm (n = 24). And eMMPs consisted of more cells than sMMPs.

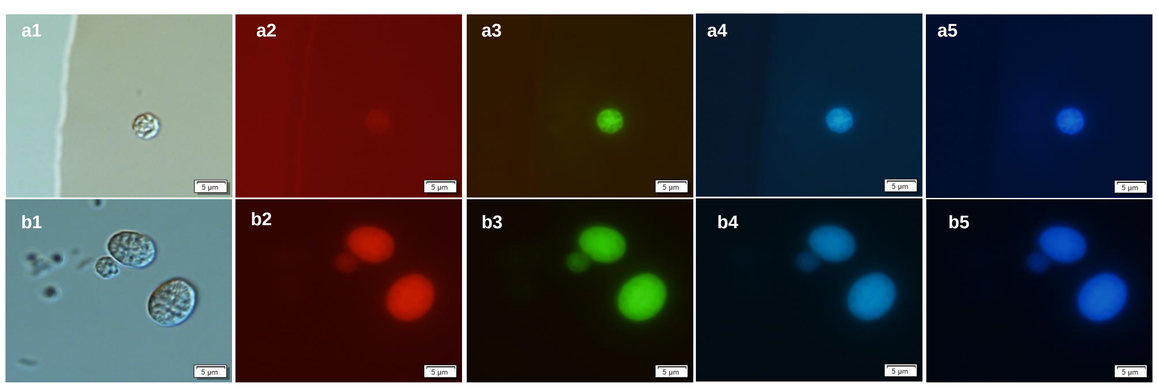


Fig. 2 Spontaneous fluorescence of MMPs stimulated by different wavelengths of light.
a2 – a5 and b2 - b5 was the spontaneous fluorescence of sMMPs and eMMPs stimulated by 510-550 nm (green light), 450-480 nm (blue light), 400-410 nm (violet light) and 380-400 nm（ultraviolet light). Bars = 5 μm.

These MMPs were observed by fluorescence microscope and it was found that they could emit spontaneous fluorescence when the MMPs were exposed to different wavelengths of light. Interestingly, the fluorescence intensity of sMMPs was weaker than that of eMMPs.

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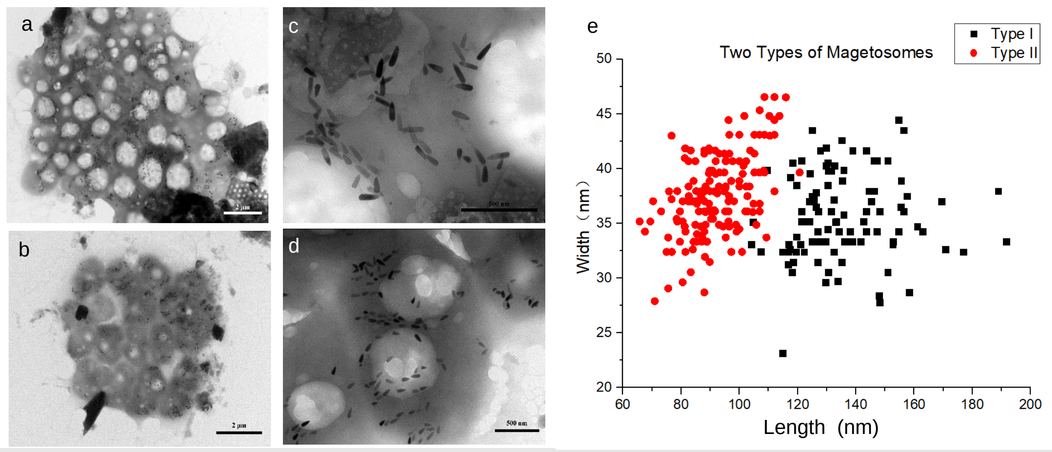

Fig. 3 Characteristics of the magnetosomes in MMPs
MMPs with abundant magnetosomes inside a and b. The magnified area of a showing the magnetosome chains the MMP c, the magnified of b showing the magnetosome chains the MMP d. e shows the length and width of two types magnetosomes. Bars = 2 μm in a and b, 500 nm in c and d.

Transmission electron microscopy showed that these MMPs contained two types of bullet-shaped crystals in parallel chains or clusters. The length and width ratios of these two types of magnetosomes were 3.81 (n=105) and 2.45 (n=168). It suggested that eMMPs tend to synthesize Type I magnetosomes, while sMMPs tend to synthesize Type II magnetosomes.

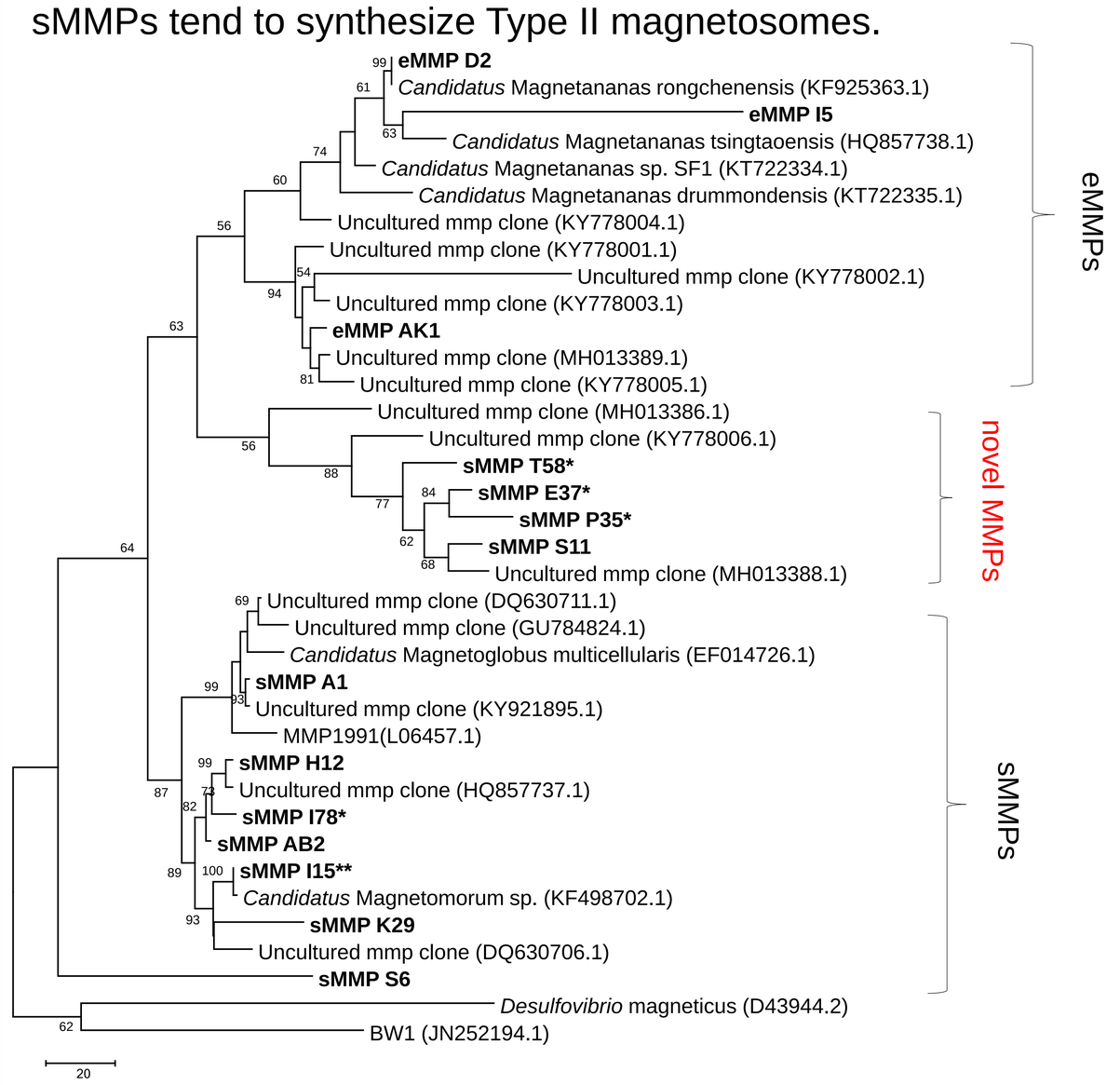

Fig. 4 Phylogenetic tree of MMPs based on 16S rRNA gene sequences. The tree was constructed based on the Maximum Parsimony method. The sequences determined in this study are shown in bold and * <97% sequence identity; ** <95% sequence identity. GenBank accession numbers are indicated in parentheses. Bars = 20 substitutions/nucleotide number.

We used micromanipulation to sort MMPs collected from the intertidal zone of Huiquan Bay, amplified and sequenced their 16S rRNA genes. In total, we obtained 14 OTUs (from 558 16S rRNA gene sequences), among them, four OTUs (T58, E37, P35 and I78) may belong to novel species and OTU I15 may belong to a novel genus, using a 97% and 95% sequence identity as the threshold for classifying species and genera respectively.

Phylogenetic tree of MMPs based on 16S rRNA gene sequences indicated that the diversity of MMPs was high in the intertidal zone of Huiquan Bay. Most sMMPs OTUs were clustered together, however, several sMMPs OTUs (T58, E37, P35 and S11) were clustered more closely to eMMPs. It may be a branch that is hardly distinguished from sMMPs in morphology but is more closely related to eMMPs based on the 16S rRNA sequence, and might represent a novel MMPs group. These observations provide a new perspective of the diversity of MMPs in general, however, their detailed classification needs further research.