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Isolation and characterization of a marine magnetotactic spirillum axenic culture QH-2 from an intertidal zone of the China Sea

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Abstract

Magnetotactic bacteria (MTB) are ubiquitous in aquatic habitats. Because of their fastidious requirements for growth conditions, only very few axenic MTB cultures have been obtained worldwide. In this study, we report a novel marine magnetotactic spirillum axenic culture, designated as QH-2, isolated from the China Sea. It was able to grow in semi-solid or liquid chemically defined medium. The cells were amphitrichously flagellated and contained one single magnetosome chain with an average number of 16 magnetosomes per cell. Phosphate and lipid granules were also observed in the cells. Both rock magnetism and energy-dispersive X-ray spectroscopy characterizations indicated that the magnetosomes in QH-2 were single-domain magnetites (Fe_3O_4). QH-2 cells swam mostly in a straight line at a velocity of 20–50 μm/s and occasionally changed to a helical motion. Unlike other magnetotactic spirilla, QH-2 cells responded to light illumination. As a consequence of illumination, the cells changed the direction in which they swam from parallel to the magnetic field to antiparallel. This response appears to be similar to the effect of an increase in [O_2]. Analysis of the QH-2 16S rRNA sequence showed that it had greater than 11% sequence divergence from freshwater magnetotactic spirilla. Thus, the marine QH-2 strain seems to be both phylogenetically and magnetotactically distinct from the freshwater *Magnetospirillum* spp. studied previously.

Keywords: Magnetotactic bacteria; Response to light; Pure culture; Magnetosome; Phylogenetic analysis

1. Introduction

Magnetotactic bacteria (MTB) are a heterogeneous group of aquatic microorganisms which share the ability to orient themselves along magnetic field lines. The cell reaction to the magnetic field is due to the presence of magnetosomes, intracellular membrane-bound crystals of iron mineral which consist of either magnetite (Fe_3O_4) or greigite (Fe_3S_4) within the single domain (SD) size range (30–120 nm) (Bazylinski and Frankel, 2004). Because of their high abundance and their remarkable capacity for accumulating and precipitating iron minerals, MTB are assumed to have great impact on the biogeochemical cycling in natural sediments and are considered as an ideal model for understanding the mechanism of biomineralization. MTB comprise a variety of morphological types, such as coccoid, vibriod, rod-shaped, spiral-shaped, and multicellular aggregates. They are distributed worldwide and most of them are found at, or just below, the oxic–anoxic

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transition zone (OATZ) or redoxocline in aquatic habitats (Bazylinski and Frankel, 2004).

Considering their ubiquitous distribution and remarkable diversity with respect to physiology and biomineralization, axenic cultures of MTB are needed for a comprehensive understanding of the mechanisms of magnetosome biogenesis, magnetotaxis as well as of MTB ecological function. As a new type of bioresource, magnetotactic bacteria have also attracted much attention for their potential use in biotechnology, biomediation, and geobiology (Bazylinski and Frankel, 2004; Lang and Schüler, 2006). However, only very few strains are available in pure culture because of their fastidious growth requirements and strong metabolic diversity (Flies et al., 2005). Most cultivated MTB are affiliated with *Alphaproteobacteria* (Flies et al., 2005) except for the magnetotactic sulfate-reducing bacterium *Desulfovibrio magneticus* (Sakaguchi et al., 2002). Axenic marine cultures include two magnetic vibrios (MV-1, Bazylinski et al., 1988; MV-2, DeLong et al., 1993), one magnetic spirillum (MMS-1, formerly known as MV-4, Meldrum et al., 1993) and three magnetic cocci (MC-1, Frankel et al., 1997; MC-2, Devouard et al., 1998; MO-1, Lefèvre et al., 2009).

Here we report a novel marine magnetic spirillum axenic culture, designated strain QH-2, isolated from an intertidal zone of the China Sea. We will describe the growth features, cell structure, magnetic properties and novel motility characteristics.

2. Materials and methods

2.1. Isolation and cultivation of the QH-2 strain

The samples were collected from a seawater pond located at Huiquan Bay in the city of Qingdao, China. The characteristics of the pond were previously described (Pan et al., 2008). The sediments together with interface water, with a ratio of 1:2, were collected and stored in 1-L glass bottles. Magnetotactic bacteria were enriched by attaching the south pole of permanent magnets (0.37 mT) outside the bottles placed at the water/sediment interface. After 20–30 min, cells accumulating as dark spots underneath the magnets were removed with a Pasteur pipette and saved as magnetically collected samples. These samples were further magnetically purified in Pasteur pipettes according to the racetrack purification method (Wolfe et al., 1987), and inoculated into 5 ml plastic tubes that were generally filled up to 4/5th of their volume with various media and sealed with parafilm and incubated at 22–26 °C in dim light. Bacteria grew and formed a sharp band or zone after several days of incubation.

The optimal growth medium for QH-2 (QH medium) was modified from that used for the *Magnetococcus* sp. MC-1 (Frankel et al., 1997). It contained 100 ml extract solution of seawater sediments (modified from the DSMZ, Medium 12: Soil Extract Medium); 5 ml modified Wolfe's mineral solution; 0.5 ml vitamin solution; 2.0 ml of 0.01 M ferric quinate; 1.0 g NH_4Cl; 2.5 g $Na_2S_2O_3 \cdot 5H_2O$; 1.5 ml of 0.5 M potassium phosphate buffer, pH 7.6; 1.3 g sodium lactate (50–60%); 2.4 ml of 0.8 M $NaHCO_3$; 0.05 g sodium thioglycolate; and 900 ml filtered seawater collected from the pond through a 0.45 μm filter membrane. Artificial seawater and 0.1 g/L peptone were used instead of natural seawater and sediment extract in order to make the chemically defined medium, called QH-C medium. The pH value of the growth medium was adjusted to 7.6–7.8. For semi-solid medium, 0.2–0.5 g agar was added to 1 L of growth medium. After pH adjustment, the medium was autoclaved at 120 °C for 20 min. Cultures were incubated at room temperature (between 22 and 26 °C).

2.2. Optical and electron microscopy observations

The swimming behavior of magnetotactic bacteria was analyzed by the 'hanging drop' method (Schüler, 2002) using a microscope (OLYMPUS BX51) connected to a Charge-Coupled Device (CCD, OLYMPUS DP71). Morphological examination of the cells was performed with fluorescence microscopy after staining with 1% acridine orange (AO). Nile red staining for lipid storage granules was performed according to Greenspan et al. (1985).

Fresh cells were deposited on formvar carbon-coated copper grids, either directly or after treatment with 0.1% uranyl acetate. The grids were dried in air. TEM observations were made using a Zeiss EM9 microscope at 80 kV. The size and shape factors of magnetosomes were estimated using (length + width)/2 and width/length, respectively. The chemical composition of magnetosomes was studied by EDXS–TEM.

2.3. Sequence analysis of the 16S rRNA gene

The 16S rRNA gene of the QH-2 was amplified between positions 27 and 1492 (*Escherichia coli* 16S rRNA gene sequence numbers), using primers 27F (5′-AGA GTY TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTI 'GTI' ACG ACT T-3′) by polymerase chain reaction (PCR) carried out with the following cycle: an initial denaturing step at 94 °C for 5 min, followed by 25 cycles of 1 min at 94 °C, 45 s at 50 °C and 1 min at 72 °C, and a final extension step of 10 min at 72 °C. Then the PCR products were sequenced directly by Sinogenomax Company in Beijing.

The sequences of the 16S rDNA gene were first analyzed using Advanced BLAST search program on the NCBI Website (http://www.ncbi.nlm.nih.gov/BLAST/). The related sequences were preliminarily aligned with the default setting of CLUSTALX (1.83). Phylogenetic analysis was performed with the default setting of MEGA 4 using the neighbor-joining method. Similarity was calculated using the BioEdit program.

The newly determined sequence is available from GenBank under accession number EU675666.

2.4. Magnetic measurements

For magnetic measurements, about 10^{10} QH-2 cells were collected by centrifugation from cultures. The centrifuged cells were washed once with distilled water, and then placed in a non-magnetic gelatin capsule. To avoid possible oxidization,