Video Article

Collection, Isolation and Enrichment of Naturally Occurring Magnetotactic Bacteria from the Environment

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Abstract

Magnetotactic bacteria (MTB) are aquatic microorganisms that were first notably described in 1975[1] from sediment samples collected in salt marshes of Massachusetts (USA). Since then MTB have been discovered in stratified water- and sediment-columns from all over the world[2]. One feature common to all MTB is that they contain magnetosomes, which are intracellular, membrane-bound magnetic nanocrystals of magnetite (Fe_3O_4) and/or greigite (Fe_3S_4) or both[3, 4]. In the Northern hemisphere, MTB are typically attracted to the south end of a bar magnet, while in the Southern hemisphere they are usually attracted to the north end of a magnet[3,5]. This property can be exploited when trying to isolate MTB from environmental samples.

One of the most common ways to enrich MTB is to use a clear plastic container to collect sediment and water from a natural source, such as a freshwater pond. In the Northern hemisphere, the south end of a bar magnet is placed against the outside of the container just above the sediment at the sediment-water interface. After some time, the bacteria can be removed from the inside of the container near the magnet with a pipette and then enriched further by using a capillary racetrack[6] and a magnet. Once enriched, the bacteria can be placed on a microscope slide using a hanging drop method and observed in a light microscope or deposited onto a copper grid and observed using transmission electron microscopy (TEM).

Using this method, isolated MTB may be studied microscopically to determine characteristics such as swimming behavior, type and number of flagella, cell morphology of the cells, shape of the magnetic crystals, number of magnetosomes, number of magnetosome chains in each cell, composition of the nanomineral crystals, and presence of intracellular vacuoles.

Video Link

The video component of this article can be found at http://www.jove.com/video/50123/

Protocol

1. MTB Collection

1. When deciding on a freshwater site to collect magnetotactic bacteria (MTB), it is often best to start with a pond or slow-moving stream that has a soft muddy sediment layer. In this demonstration we collected a sample at the edge of the Olentangy River on the campus of The Ohio State University (OSU) in Columbus, Ohio (USA). While this was a convenient location for our demonstration, the protocol described here is applicable to any aquatic location. The materials used in this protocol can be found in **Table 1**. Find a location where the depth of the water is between 10 and 100 cm. At such a location, you should collect the upper-most layer of sediment using a clear, screw-top container. Scoop the sediment and water into the container until it is filled with one-third to one-half sediment and the remaining volume with water. Keep the container submerged until it is filled with water and then tightly cap the container with its screw-top lid. It's not necessary to mix the sediment. Wipe the outside of the container dry with a towel and then take the sample to your laboratory. It's not necessary to rush the sample back to your laboratory. We've left MTB samples in plastic containers in the field for several days before bringing them back to our laboratory. The MTB should be viable for several weeks to months as long as you store the samples in a cool, shaded place in the field.
2. Once the sample is in your laboratory, loosen the cap and leave it covering the container to reduce the amount of evaporation. Store the container at room temperature in a dark room, drawer, or completely cover the container with aluminum foil. Allow the sediment and fine particles to completely settle to the bottom of the container by leaving the sample undisturbed for several hours to several days. It is not

necessary to mix the sediment, MTB prefer an undisturbed environment. The clear walls of the plastic container will allow you to confirm that the particles have settled to the bottom. Depending on your sample, MTB can remain alive in the sample for many months.

2. MTB Isolation

1. When you are ready to isolate the MTB, place magnets on the sides of the plastic container approximately 1 cm above the sediment-water interface (**Figure 1A**). Be careful not to disturb the sediment in the bottom of the container. Place the south pole of a bar magnet on one side of the container and the north side of another bar magnet on the opposite side (**Figure 1A**). Almost any magnet can be used, such as a magnetic stir bar or large refrigerator magnet. Anything can be used to support the magnets at the correct height above the sediment-water interface. We've found that resting the magnets on the top of a cardboard or plastic box is best, however, the magnets can also be taped to the outside of the plastic container. Wait 30 min to several hours for the bacteria to swim to the magnet.
2. Use a sterile pipette to carefully collect the water from inside the container (**Figure 1A**) near the position of the south-pole bar magnet (for samples collected in the Northern hemisphere). This water should contain the MTB that have been attracted to the south-pole bar magnet. Next, a capillary racetrack should be used to further enrich the MTB.

3. MTB Racetrack

1. In order to enrich your sample with magnetotactic bacteria, a capillary racetrack is necessary (**Figures 1B and 1C**). These need to be made prior to isolating the cells from the clear-plastic container.
2. Use a 5.75 inch (146 mm) glass Pasteur pipette to make a racetrack. Use a diamond pen or file to cut off the top of the pipette, the length of the pipette is not crucial, but it should be able to contain approximately 1-2 ml of water. Next, use a Bunsen burner to melt the tip so that it becomes sealed (**Figure 1C**). The resulting pipette should have an open end and a sealed end.
3. Make several of these racetracks and then autoclave. Additionally, you will need to autoclave cotton and several long metal needles.
4. Add filtered sample water, collected from near the sediment water interface shown in **Figure 1A**, to an autoclaved racetrack using a long metal needle attached to a filtered syringe. The pore size of the filter should be 0.22 mm to eliminate debris and contaminants from the water. It is important to be absolutely sure that there are no air bubbles in the glass capillary.
5. Plug the bottom of the racetrack with sterile cotton (**Figure 1B**). Use the metal needle to push the cotton towards the sealed end of the racetrack so it is 0.5 - 1 cm away from the sealed tip (**Figure 1C**).
6. Using a sterile pipette, add the MTB-containing water (from section 2.2) to the sample reservoir (open end) of a prepared MTB racetrack (**Figure 1B**).

4. MTB Enrichment

1. Once the racetrack is filled with sample fluid, lay it on its side on a horizontal surface (*e.g.*, a benchtop) and place the south pole of a bar magnet (in the Northern hemisphere) next to the sealed tip of the racetrack (**Figures 1B and 1C**).
2. Wait 5 to 30 min for the MTB to migrate through the cotton. Then you should collect the fluid near the tip of the racetrack. Waiting too long can introduce contaminants, such as other motile bacteria, to the tip of the capillary. Optionally, you could use a light microscope to view the tip of the racetrack and watch the MTB collect at the racetrack's tip. This will allow you to determine how long it takes the MTB to migrate through the cotton plug.
3. Gently use the diamond knife to make a little scratch near cotton plug and snap off the end of the racetrack.
4. Use a 1 ml syringe with a narrow needle (25 or 27 gauge) to remove the fluid from the tip of the racetrack. This liquid sample should now contain the enriched MTB.

5. MTB Observation by Light Microscopy

1. Place a drop (10-20 µl) of the enriched MTB sample onto a coverslip.
2. Quickly flip the coverslip over so the drop is now facing down and hanging from the coverslip.
3. Place the coverslip onto an o-ring that is resting on a glass slide. The o-ring should have a slightly smaller diameter then the coverslip (about 1 cm; **Figure 2**).
4. Place this hanging drop onto a light microscope stage and focus on one edge of the drop. A 60X dry objective works very well because most have a high numerical aperture (NA; *e.g.*, 0.93) but do not require oil, which is difficult to use for the hanging drop method (**Figure 2**).
5. Place the south end of a bar magnet close to the hanging drop and MTB will begin to migrate towards the edge of the drop closest to the magnet (**Figure 3**). Within a few minutes many MTB should be at the edge of the drop (**Figure 3**). Prove to yourself that the bacteria are magnetic by reversing the pole of the magnet and then observe the bacteria swim in the opposite direction.

6. MTB Observation by Transmission Electron Microscopy (TEM)

1. Place a drop (~20 µl) of the enriched MTB onto a copper grid and allow the bacteria to settle for 10 min.
2. Wick off excess water with a piece of clean filter paper.
3. Optionally, the grid can be negatively stained with 2% uranyl acetate, 2% phosphotungstic acid pH 7.2, or 2.5% sodium molybdate[7, 8, 9]. This is done by placing the copper grid onto a drop of stain immediately after incubating the grid with the enriched MTB. Incubate the grid with the negative stain, the times will vary depending on the stain used, and then wick off the fluid with a piece of clean filter paper.
4. Observe the MTB using transmission electron microscopy (TEM, **Figure 4**). For the work described here MTB were adsorbed to Formvar stabilized and carbon coated 200 mesh copper grids (Ted Pella #01800). The grids were placed with the carbon side down on a drop of cell suspension for up to 10 min, then immediately washed one time by placing the grid on a drop of water for 30 sec. For staining, the grids were placed on a drop of 2% uranyl acetate (Ted Pella #19481) for 30 sec to 5 min and then dried completely using a piece of filter paper.

The grids were analyzed by TEM using either an FEI Tecnai Spirit at 80kV or a FEI Tecnai F20 using high angle annular dark field STEM at 200kV.

Representative Results

A magnet is an effective tool that can be used to isolate magnetotactic bacteria (MTB) contained in environmental samples (**Figure 1A**). A capillary racetrack (**Figure 1B**) uses the magnetic properties of MTB to attract them through a cotton plug where they can be separated from non-magnetotactic microorganisms also contained within the environmental sample.

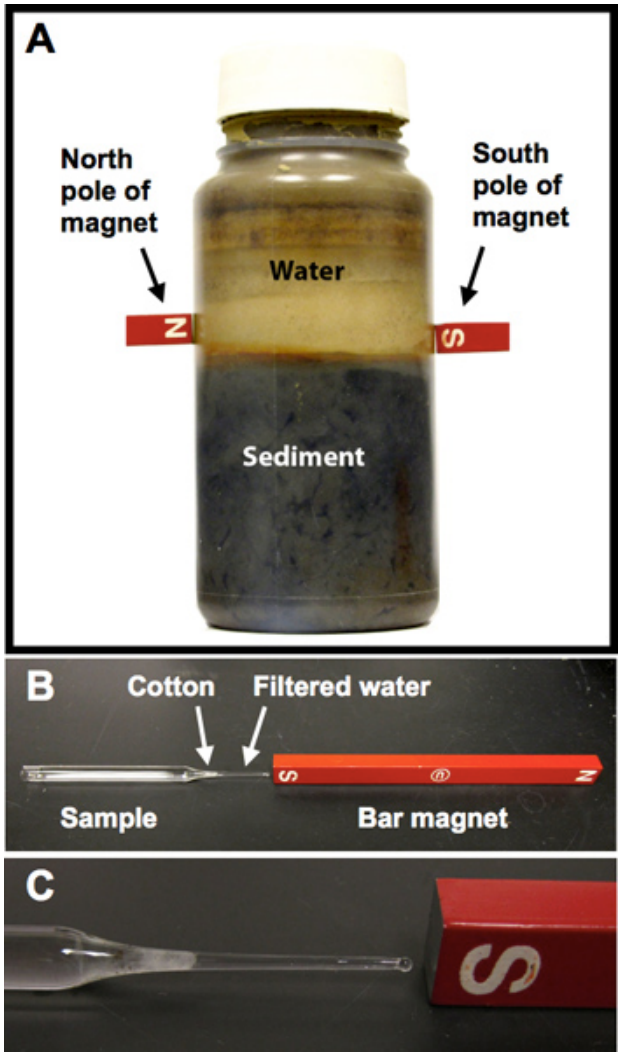

Figure 1. A clear plastic bottle containing a sediment and water sample collected from the Olentangy River in Columbus, Ohio (USA). The bottle contains approximately one-half sediment and one-half water. The south end of a magnet is placed approximately 1 cm above the sediment for up to several hours (A). After removing some of the fluid from near the magnet on the inside the container, it is placed inside of a capillary racetrack where the MTB swim through a cotton plug (arrow) towards the south end of a bar magnet (B). A close up view of the capillary racetrack showing the sample, cotton, filtered fluid, sealed end of the capillary tube and south end of a bar magnet (C).

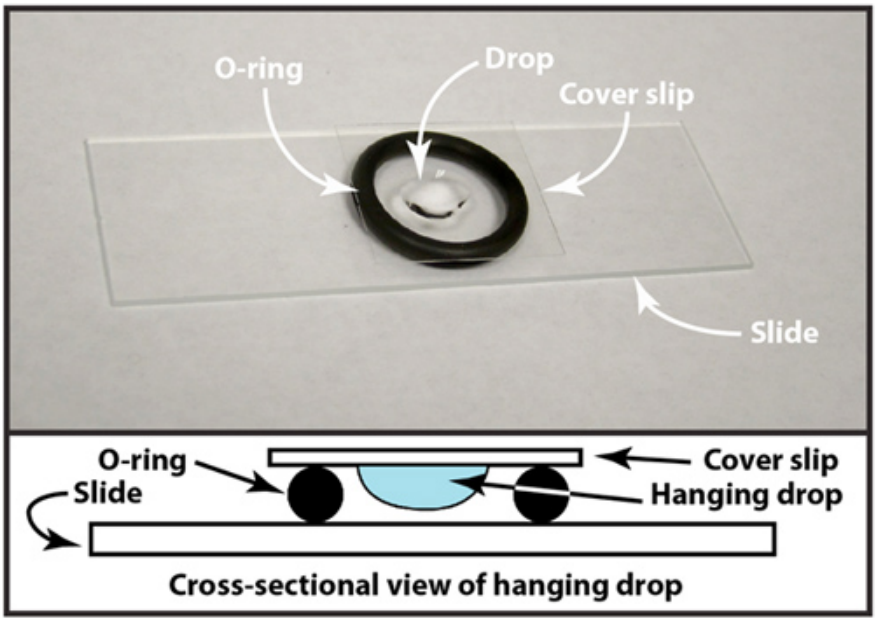

Figure 2. Once the MTB have been enriched from the racetrack, a small drop can be placed on a coverslip, which is then flipped upside down and placed on an o-ring that is resting on a slide. This slide-o-ring-coverslip sandwich can be placed on a light microscope stage and viewed using a 60X dry objective (oil lenses are inconvenient to use with a hanging drop).

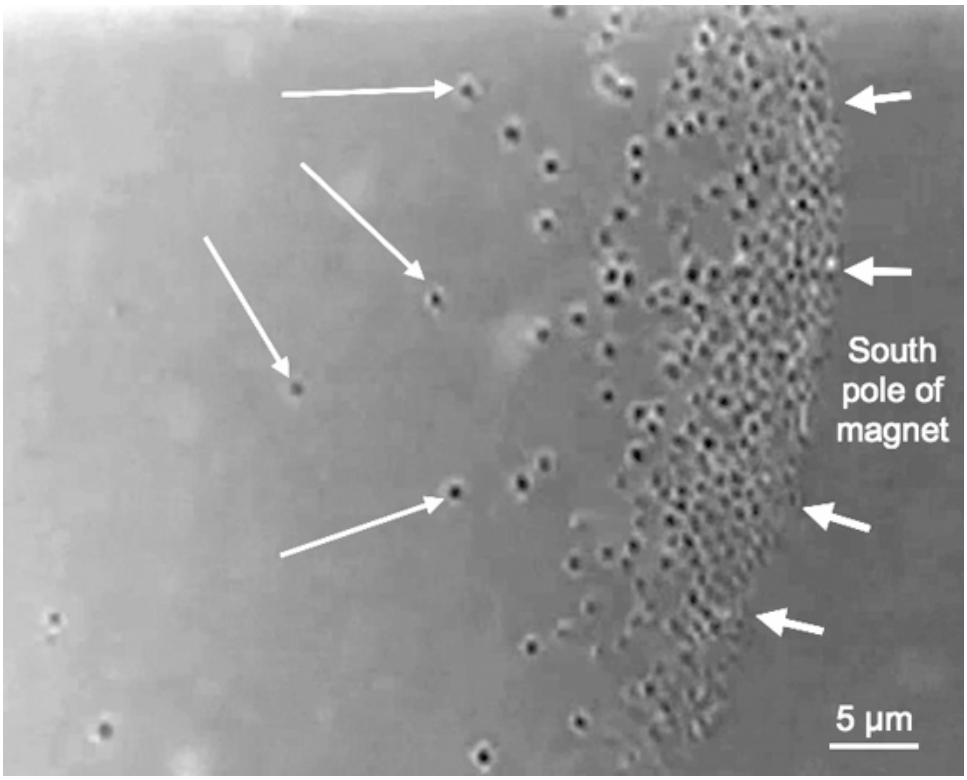

Figure 3. Bright field microscope image of MTB swimming (thin long arrows) and gathering at the edge of the hanging drop (short arrows) which is next to the south pole of a bar magnet.

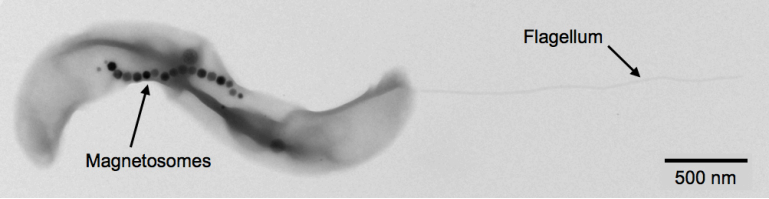

Figure 4. Transmission electron microscope image of a single magnetotactic bacterium enriched from an environmental sediment sample. The morphology of the cell (spirillum) and magnetosomes are clearly visible along with a single flagellum.

Discussion

Magnetotactic bacteria are not necessarily found in every aquatic environment[8] but when they do occur, they can be found on the order of 100 - 1,000 cells per milliliter[2]. In order to observe the MTB using optical microscopy, you will need approximately 50 bacteria/ml in your sample[8]. If there are no or few MTB in your sample, then you will either need to select a new environmental site to collect your samples or you will need to try one or more of the techniques discussed in the next section.

First, you should try collecting more sediment from the environment using a large plastic tub[8]. This is especially useful if large numbers of unculturable MTB are needed. Depending on the environmental sample, it may not be possible to isolate MTB samples having a concentration of 50 bacteria/ml immediately after collecting the sample. Therefore, when you bring your environmental sample back to the laboratory, it may be beneficial to wait for the sample to acclimate to laboratory conditions before trying to isolate the MTB using a bar magnet. This acclimation period will allow the bacterial community to mature and repopulate the culture leading to higher concentrations of MTB. Another simple technique that often produces more concentrated MTB samples is to leave the bar magnet on the side of the sample container (**Figure 1A**) for a longer period of time (*e.g.*, several days). This should allow the MTB more time to migrate to the magnet. One final technique that may be useful, is to use several racetracks (**Figure 1B**) at once and then combine the MTB from each racetrack into one sample. If you believe there is a problem with a racetrack or if there are too many contaminating microorganisms (*i.e.*, non-MTB) in your enriched sample, you can place the racetrack under a light microscope to observe the MTB as they swim through the cotton plug and into the tip. This will allow you to determine if contaminating microorganisms are also coming through the cotton plug and when to stop the enrichment process.

We should mention that there are more sophisticated ways to isolate MTB, but these methods require the use of more specialized equipment. One example involves the use of a magnetic coil, instead of a bar magnet, and customized glass vessels to isolate MTB from freshwater sediments[10, 11]. The protocol described here represents an inexpensive and effective method for determining whether an environmental site contains MTB. This isolation and enrichment protocol is straightforward enough that microbiology students can master and easily "fine-tune" so that higher yields of MTB can be achieved. Once the MTB have been isolated, other analyses such as fluorescence in-situ hybridization, 16S rRNA sequencing for community analysis, energy dispersive spectroscopy (EDS), TEM, optical microscopy and magnetic measurements can be conducted on the MTB[12, 13, 14].

Disclosures

No conflicts of interest declared.

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